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Department für Agrarbiotechnologie, IFA-Tulln
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Plants for animal nutrition – Influencing digestion and nutrient use through bioactive compounds

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Abstract

Bioactive substances, that do not have a nutritional value, can directly or indirectly influence the digestion and metabolism of nutrients. Bioactive substances therefore include total dietary fibre and secondary plant compounds. While fibre plays a predominantly pre-absorptive role in providing nutrients to livestock, many secondary plant compounds have both pre-absorptive as well as post-absorptive properties. Furthermore, secondary plant compounds are partly transferred to products such as milk, eggs, meat and fish, and therefore have a direct impact on product quality and thus potentially also on human health. More information is needed before we routinely quantify the levels of secondary plant metabolites in feeds and relate these contents to effects on current nutrient digestibility and endogenous nutrient losses in the gut.

Einleitung

Per Definition können bioaktive Substanzen, die keine Nährstoffe sind, die Verdauung und Verstoffwechslung der Nährstoffe direkt oder indirekt beeinflussen. Zu den bioaktiven Substanzen gehören daher die Gesamtfaser der Ration sowie sekundäre Pflanzeninhaltsstoffe. Die Vielzahl an verschiedenen sekundären Pflanzeninhaltsstoffen erfüllen physiologische Funktionen, die für die Interaktionen zwischen Pflanzen und ihrer Umwelt unentbehrlich sind. Zu nennen sind Abwehrmechanismen (z.B. Tannine, Alkaloide), visuelle Signalvermittlung zwischen Pflanze und Tier (z.B. Flavonoide, Carotinoide), mechanische Festigung (Lignin), Photosynthese (Chlorophyll) und die Aromabildung (Terpenoide). Historisch betrachtet werden viele dieser sekundären Pflanzeninhaltsstoffe vom Menschen seit langem genutzt und gelangen u.a. in Form von Gewürzen, Getränken (Tee, Kakao, Kaffee) und natürlichen Farbstoffen in die Nahrungskette (Rempt & Gierus, 2018). Die biochemischen Prozesse, die diesen Wechselwirkungen zum Schutz des Pflanzen-Individuums zu Grunde liegen, sind für vielfältige Anwendungen entlang der Wertschöpfungskette der tierischen Produktion von Interesse.

Die Gesamtfaser und nicht wenige der sekundären Pflanzeninhaltsstoffe gehen Wechselwirkungen mit Nährstoffen ein, die für die Ernährung des Nutztieres essentiell sind. Somit beeinflussen sie synergistisch oder antagonistisch Absorption, Retention und Ausscheidung dieser Nährstoffe je nach Quelle und Einsatzmenge (Surai, 2014; Jha & Berrocoso, 2015; Shehata et al., 2022). Während Gesamtfaser überwiegend prä-absorptiv eine wesentliche Rolle bei der Nährstoffversorgung der Nutztiere einnehmen (Montagne et al., 2003), werden viele sekundäre Pflanzeninhaltsstoffe in Produkte wie Milch, Ei, Fleisch und Fisch übertragen, und haben einen unmittelbaren Einfluss auf die Produktqualität von Lebensmitteln und damit potentiell auch auf die Gesundheit des Menschen (z.B. Kumar et al., 2021). Aus dieser Perspektive nimmt ein prozess-orientierter Einsatz von Gesamtfaser und sekundären Pflanzeninhaltsstoffen in der Nutztierforschung an Bedeutung zu. Neben der Optimierung des Verdauungsprozesses bei Nutztieren und der Bereitstellung hochqualitativer Lebensmittel tierischer Herkunft, kann zudem ein Beitrag

zur Minderung von Emissionen klimarelevanter Gase aus der landwirtschaftlichen Produktion mittels gezieltem Einsatz von Gesamtfaser und/oder sekundärer Pflanzeninhaltsstoffe geleistet werden.
Mit Schwerpunkt auf sekundäre Pflanzeninhaltsstoffe ist das Ziel des vorliegenden Beitrages die Wechselwirkung zwischen sekundären Pflanzeninhaltsstoffen mit einer effizienten Nährstoffnutzung im Magen-Darmtrakt der Nutztiere, sowie der Transfer in Produkte tierischem Ursprungs, zu beschreiben. Der Schwerpunkt der sekundären Pflanzeninhaltsstoffe als potentielle Giftstoffe oder anti-nutritive Faktoren (z.B. Freisetzung von Blausäure, Wirkung von toxisch wirkenden Alkaloiden) wird allerdings nicht thematisiert.

Definition, Kategorisierung und analytische Erfassung sekundärer Pflanzeninhaltsstoffe

Sekundäre Pflanzeninhaltsstoffe entstehen zwangsläufig aus den Metaboliten des Primärstoffwechsels der Pflanzen. Für die Bildung der Sekundärmetaboliten gibt es zahlreiche Biosynthesewege. Es gibt >10.000 sekundäre Pflanzeninhaltsstoffe, die im Wesentlichen in drei Gruppen kategorisierbar sind: a) *Alkaloide*, als stickstoffhaltige Verbindungen, darunter z.B. Lupinine, Colchicin, Capsaicin, Kaffein, werden praktisch ausschließlich aus Aminosäuren gebildet; b) *Terpenoide*, darunter z.B. ätherische Öle, Pigmente (Carotin), Harze, entstehen aus dem Acetyl-CoA Stoffwechsel und weiters aus dem Mevalonsäureweg; und c) *Phenole*, darunter z.B. kondensierte oder hydrolisierbare Tannine, Chinone, Cumarine, Lignin, Isoflavone, entstehen aus dem Shikimisäureweg (Schlee, 1992).
In der vorliegenden Übersicht werden hauptsächlich Ergebnisse zur Wirkung sekundärer Pflanzeninhaltsstoffe, mit Fokus auf die Gruppe der Phenole diskutiert. Allein die große Anzahl an Sekundärmetaboliten dieser Gruppe stellt eine analytische Herausforderung dar; die Bestimmung in Nutzpflanzen, sowie in den daraus entstandenen Lebens- und Futtermitteln ist Schwerpunkt vieler Forschungsgruppen. Die Entwicklung und Standardisierung methodischer Ansätze zur Identifizierung und Quantifizierung relevanter Phenolverbindungen, wird zur Bestimmung kausaler Zusammenhänge häufig vorgenommen (Kardel et al., 2013; Ziegler et al., 2015). Allein der Aspekt der analytischen Erfassung der unterschiedlichen Sekundärmetaboliten aus der Gruppe der Phenole könnte in einer Literaturübersicht beschrieben werden, in diesem Beitrag spielt die biologische Wirkung der sekundären Pflanzeninhaltsstoffe jedoch die größere Rolle. Für weitere dokumentierte Erkenntnisse zur Analytik wird auf andere Literatur verwiesen (z.B. Salminen et al., 2011; Maugeri et al., 2022).

Wechselwirkungen zwischen Phenolverbindungen und Nährstoffen im Verdauungstrakt

Wechselwirkungen zwischen Phenolverbindungen (Flavonoide, Tannine) und für die Ernährung von Nutztieren essentiellen Nährstoffen (Aminosäuren, Fettsäuren, Mengen- und Spurenelementen) sind komplex und stark von der Menge an Einzelfuttermittel und Futtermittelzusatzstoffe, die als Quelle von Polyphenolen eingesetzt werden, abhängig. Diese wirken fördernd auf Futteraufnahme, modulieren die Fermentation in den Vormägen der Wiederkäuer und Dickdarm von Wiederkäuern und nicht-Wiederkäuern, beeinträchtigen die Absorption, Retention und Exkretion von Nährstoffen, und letztlich beeinflussen sie auch die Qualität tierischer Produkte u.a. dadurch, dass pflanzliche Sekundärmetaboliten übertragen werden (Gierus et al., 2012).
Die Betrachtung von Wechselwirkungen zwischen Phenolverbindungen und essentiellen Nährstoffen im Verdauungstrakt sind von zentraler Bedeutung. Kondensierte Tannine, also Phenole mit einem hohen Molekulargewicht, sind beispielsweise in der Lage Komplexe mit Proteinen einzugehen (Ziegler et al., 2015). Bei Wiederkäuern führt dies zur Bildung von stabilen Komplexen im Pansen (im pH Bereich von 3,5-7,0), sodass weniger Futterprotein mikrobiell zu Ammoniak abgebaut wird und daher mehr Futterprotein im Labmagen ankommt, wo die Komplexe bei niedrigerem pH-Wert wieder dissoziieren und in

Folge mehr Aminosäuren im Dünndarm aufgenommen werden können (Douglas et al., 2010). Da Tannine jedoch durch eine hohe strukturelle Variabilität gekennzeichnet sind, ist ihre biologische Wirkung unterschiedlich und folglich auch die Vorhersage ihrer Wirkung schwierig.
Bei Geflügel führt die Fütterung tanninhaltiger Futtermittel (z.B. Nebenprodukte der Weintraubenverarbeitung) auch zu einer Interaktion mit Proteinen. Kondensierte Tannine in hohen Konzentrationen können die Verdauung von Proteinen bei nicht-Wiederkäuern, also auch beim Huhn oder dem Schwein, einschränken, indem sie im Verdauungstrakt sowohl an Nahrungsproteine als auch an Verdauungsenzyme binden (Mehansho et al., 1987). Darauf ist bei der Auswahl der Einsatzmenge Bedacht zu nehmen. Da die meisten Polyphenole im Futter in Form von Estern, Glykosiden und anderen Polymeren vorliegen, die in diesen Formen nicht absorbiert werden können (Brenes et al., 2016), ist die Wirkung der kondensierten Tannine im prä-absorptiven Schritt des Verdauungsprozesses bei Geflügel (Schwarz et al., 2017; 2018) und Wiederkäuern (López-Andrés et al., 2013) zu erwarten. Dabei beeinflussen Mikroorganismen und Phenole sich im Darm gegenseitig, indem die Mikroorganismen phenolische Substanzen metabolisieren und die dabei entstehenden Stoffe wiederum die Population der Mikroorganismen verändern können (Brenes et al., 2016).
Die positive/negative Wirkung tanninhaltiger Futtermittel oder Nebenprodukte scheint mittels verschiedener Messgrößen nachweisbar zu sein, wie mehrere Studien des Instituts für Tierernährung, tierische Lebensmittel und Ernährungsphysiologie (TTE) der BOKU bisher zeigten (Wanka, 2016; Schabelreiter, 2016; Hermann, 2016; Brackmann, 2019; Rajkovic et al., 2021; Schwarz et al., 2021). Die in den Studien durchgeführten Messungen deuten auf eine positive Wirkung kondensierter Tannine hin. Dabei ist jedoch die Einsatzhöhe in der Ration entscheidend, sobald bestimmte Konzentrationen überschritten werden, sind Leistungseinbußen nicht auszuschließen (Lau & King, 2003; Schabelreiter, 2016). Zudem muss zwischen dem Einsatz der Phenolquellen als Extrakte oder als tanninhaltige Futtermittel unterschieden werden, da die Phenole bei zweiterem nicht isoliert, sondern im Verbund mit zahlreichen nicht-extrahierbaren Komponenten, allen voran Gesamtfaser, vorkommen und damit die Summenwirkung aller bioaktiver Substanzen zum Tragen kommt (Hanušovský et al., 2020).

Einfluss von Phenolverbindungen auf Tiergesundheit und Umwelt

Phenolverbindungen üben im intermediären Stoffwechsel des Tieres sehr spezifische Wirkungen aus, die gesundheitsfördernd bzw. -schädigend bei Überdosierung sein können. Gesundheitsfördernd sind die direkten oder indirekten antibakteriellen, entzündungshemmenden und/oder antioxidativen Eigenschaften, die z.B. von Polyphenolen (kondensierte Tannine) oder Flavonoiden (z.B. Quercetin und Catechin) ausgehen. Hierfür werden häufig Modell-Pflanzen zum besseren Verständnis der kausalen Zusammenhänge herangezogen, wie am Beispiel von Weintrauben und deren Nebenprodukte (Hassan et al., 2019; Fontana et al., 2013; Rajković et al., 2021).
Anders ist es bei Phytoöstrogenen (Isoflavone): Diese Sekundärmetabolite werden im Rahmen einer Therapie von Osteoporose und Herzerkrankungen beim Menschen positiv bewertet, stellen aber in der Ernährung von Säuglingen auf Basis von Sojaprodukten ein Problem dar (Gierus et al., 2012; Nile et al., 2021). In der Tierproduktion werden die Wirkungen von Phytoöstrogenen auf die tierische Leistung wenig berücksichtigt. In Soja enthaltene Isoflavone können an Östrogenrezeptoren binden und dosisabhängig östrogenartige oder antiöstrogene Wirkungen bei Legehennen entfalten (Cai et al., 2013). Insbesondere kann die Legeleistung und Eiqualität durch die Fütterung von Sojaprodukten an Hennen, die Entwicklung von Aufzuchtferkeln und Kälber durch die Übertragung von Phytoöstrogenen in die Milch, bzw. Milchaustauscher auf Sojabasis, je nach Gehalt und Quelle positiven und negativen Einfluss nehmen (Xiao et al., 2015; Reddy et al., 2020).
Die Pansenphysiologie wird von Phenolverbindungen wie Tanninen beeinflusst, insbesondere die Aktivität der Mikroorganismen ist hier betroffen. Eine Reduktion der Proteinabbaurate und Methanbildung im Pansen wurde bereits mit Tanninen nachgewiesen (Bueno et al., 2020; Durmic et al., 2021; Orzuna-

Orzuna et al., 2021). Allerdings wurden für einzelne Wirkstoffe noch kaum systematische Untersuchungen im Sinne einer Dosis-Wirkungsbeziehung der relevanten Phenolverbindungen in Futtermitteln durchgeführt. Weitere Informationen sind im Beitrag von Giller (2022) zu finden.

Übertragung sekundärer Pflanzeninhaltsstoffe in Lebensmittel tierischer Herkunft

Über die Fütterung der Nutztiere werden sekundäre Pflanzeninhaltsstoffe zum Teil in Lebensmittel tierischer Herkunft übertragen. Für einige Sekundärmetabolite ist bekannt, dass ihre Übertragung zu einer Wertsteigerung des Lebensmittels führt, mit der dann beim Verbraucher geworben werden kann. Klassisch ist die Anwendung von Pigmenten (Carotinoide, aus der Gruppe der Terpenoide) im Futter zur Färbung von Eidotter, Broilerhaut und -fleisch oder Lachsfilet. Auch die Übertragbarkeit von Isoflavonoide, welche als Phytoöstrogene wirken können, ist dokumentiert (Gierus et al., 2012). Mehr Studien sind für genauere Bestimmung der Übertragung von Phenolverbindungen notwendig (Höjer et al., 2012).
Durch die Übertragbarkeit von Sekundärmetaboliten in Lebensmittel tierischer Herkunft können Signaturen sekundärer Pflanzenstoffe wertvolle Informationen liefern, um die Bestimmung der Authentizität von tierischen Produkten einer bestimmten regionalen Herkunft mittels Elementanalysen, NMR, und chemometrische Methoden nachzuweisen (Prache et al., 2005; Karoui & De Baerdemaeker, 2007).
Es ist jedoch zu beachten, dass nicht alle sekundären Pflanzeninhaltsstoffe in der gleichen Rate ins tierische Lebensmittel übergehen können. Die chemische Struktur der Verbindungen spielt dabei eine große Rolle. Bei komplexen Strukturen wird bereits die Absorption in den tierischen Organismus verhindert. Damit können auch Veränderungen im tierischen Lebensmittel bestmöglich nur indirekt über die Wirkung auf das intestinale Milieu (antioxidative Wirkung, z.B. Chedea et al., 2019; Álvarez-Rodríguez et al., 2022) erreicht werden (Surai, 2014; Serra et al., 2021).

Schlussfolgerung

Weitere Informationen sind erforderlich, bevor wir routinemäßig den Gehalt an sekundären Pflanzeninhaltsstoffen in Futtermitteln quantifizieren und diese Inhalte mit Auswirkungen auf die tatsächliche Nährstoffverdaulichkeit und die endogenen Nährstoffverluste im Darm in Beziehung setzen können. Die chemische Zusammensetzung und die Interaktion der Nahrungskomponenten mit bioaktiven Substanzen sind komplexer als die reine Bestimmung deren Hauptinhaltsstoffe (Protein, Kohlenhydrate, Lipide, Mineralstoffe und Vitamine).

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Wirkung von Polyphenolen auf die effiziente Nährstoffnutzung in der Wiederkäuerernährung

Effects of polyphenols on efficient nutrient utilization in ruminant nutrition

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Abstract

To feed the growing world population in the future, a more efficient food production is required. In addition to the high resource input, the production of ruminant-derived foods is rather inefficient due to microbial nutrient degradation and gas production and excretion of non-utilized nutrients via urine and feces. These aspects may be modulated by dietary tannins, which are the most intensively investigated group of polyphenols in ruminant nutrition research. High dietary concentrations of tannins may have antinutritive effects by decreasing feed intake and reducing nutrient degradability. Both condensed (CT) and hydrolysable tannins (HT) have been shown to decrease methane formation, improve the transfer efficiency of dietary unsaturated fatty acids into milk and meat, and to possess a high nutrient-binding activity that particularly inhibits ruminal protein degradation. This decreases both the necessity for the liver to detoxify excess ammonia and the urinary nitrogen excretion. In addition, the protein supply at the duodenum is increased. However, tannin-protein complex formation is not always reversible and thus protein excretion via the feces can be promoted. Despite the overall potential of dietary tannins to improve the nutrient utilization efficiency in ruminants, results concerning their effects on ruminant productivity are contrasting. It can be assumed that effects vary not only between CT and HT but also between individual structures within each tannin group. It also has to be acknowledged that the effects of tannins on the nutrient utilization efficiency differ between ruminant species and may be affected by additional factors such as dose and diet composition.

Einleitung

Das starke globale Bevölkerungswachstum sowie die damit verbundene Abnahme der Ackerfläche pro Kopf begründen die Notwendigkeit einer Effizienzsteigerung in der Lebensmittelproduktion. Diese kann durch gesteigerten Ertrag bei gleichbleibendem Einsatz (Maximierungsprinzip) oder durch verringerten Einsatz bei gleichbleibendem Ertrag (Minimierungsprinzip) erreicht werden. Vor dem Hintergrund einer verbesserten Nachhaltigkeit der Lebensmittelproduktion besteht das Ziel, mit möglichst geringem Ressourceneinsatz möglichst viele hochwertige Lebensmittel zu produzieren. Der Ressourcenverbrauch ist bei der Produktion tierischer Lebensmittel aufgrund der beachtlichen Umwandlungs- und Energieverluste sowie dem hohen Bedarf an Trinkwasser jedoch besonders hoch. Im Gegensatz zu Monogastriern kommen bei Wiederkäuern zu der Ausscheidung nicht verwerteter Nährstoffe über die Exkremente auch noch der mikrobielle Nährstoffabbau im Pansen und die Produktion von Fermentationsgasen hinzu, weshalb insbesondere die Wiederkäuer als Nutztiere häufig in der Kritik stehen. Aus diesen Gründen wäre eine Effizienzsteigerung in der Ernährung von Wiederkäuern vorteilhaft.

Klassifizierung und Vorkommen von Polyphenolen

Die Gruppe der Polyphenole gehört zu den sekundären Pflanzenstoffen und umfasst tausende von Strukturen, die einen oder mehrere aromatische Ringe mit einer oder mehreren Hydroxylgruppen enthalten

(Crozier, 2003). Aufgrund ihrer Struktur lassen sich Polyphenole in die folgenden Untergruppen einteilen: Flavonoide, Phenolsäuren, Stilbene und Lignane. Zusätzlichen zu diesen eher niedrigmolekularen Nicht-Tannin Polyphenolen (NTP) finden sich auch oligo- und polymere Polyphenole, die eine deutlich komplexere Struktur aufweisen und als Tannine bekannt sind. Man unterscheidet drei große Gruppen von Tanninen: kondensierte Tannine (KT), hydrolysierbare Tannine (HT) und Phlorotannine, wobei Letztere nicht in terrestrischen Pflanzen vorkommen. Die KT bestehen aus bis zu 50 Flavonoideinheiten während die HT aus einem von Phenolsäuren umgebenen Polyolmolekül bestehen (Abbildung 1).

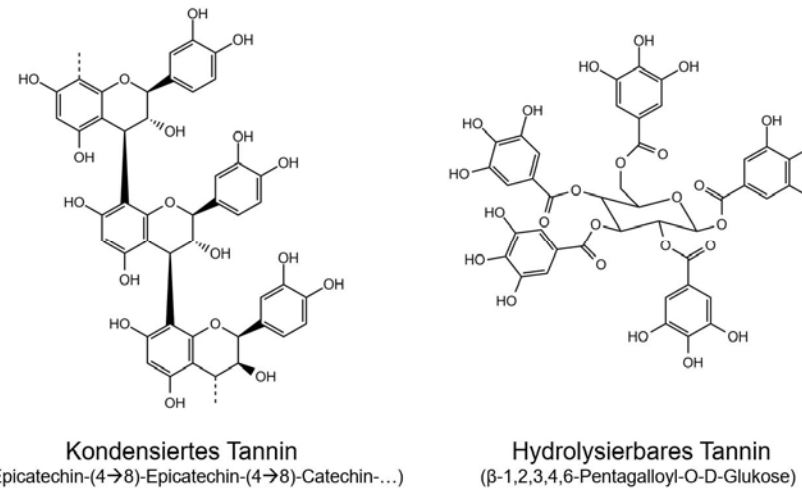

Abbildung 1. Beispielhafte Darstellung der Strukturen von in terrestrischen Pflanzen vorkommenden kondensierten und hydrolysierbaren Tanninen

Polyphenole finden sich in fast allen Pflanzen, jedoch variiert deren Konzentration in Abhängigkeit von Art und Alter der Pflanze und Umweltbedingungen wie Temperatur, Sonneneinstrahlung, Regenfall und Bodenbeschaffenheit (Manach et al., 2004). Trotz des ubiquitären Vorkommens ist die Gesamtaufnahme von Polyphenolen aus dem Futter als eher gering einzustufen. Für auf besonders polyphenolreichen Bergweiden grasende Milchkühe wurde die tägliche Polyphenolaufnahme auf etwa 500 g geschätzt (Fraisse et al., 2007). Allgemein liegen die Polyphenolgehalte in Grassilage etwa 4-fach höher als in Maissilage (Besle et al., 2010). Nebenprodukte der Lebensmittelindustrie wie Obst- und Gemüsetrester, die sich als Futtermittel für Wiederkäuer eignen, enthalten häufig höhere Konzentrationen an Polyphenolen als die üblichen Futterpflanzen (Giller et al., 2021a). Lignane sind die im Wiederkäuerfutter am häufigsten vorkommenden Polyphenole. Da sich die Forschung zu Polyphenolen und insbesondere zu deren Bioaktivität in der Wiederkäuernährung aber bisher hauptsächlich auf Tannine anstatt NTP fokussiert hat, werden im vorliegenden Beitrag (auch aus Platzgründen) ausschließlich die Wirkungen von Tanninen auf die Nährstoffeffizienz präsentiert.

Antinutritive Wirkungen von Tanninen

Für Tannine werden eine Reihe antinutritiver Wirkungen beschrieben, die auf ihrer Komplexbildung mit Makronährstoffen, insbesondere Proteinen, beruhen. Die hohe Anzahl an Hydroxylgruppen in Tanninen begünstigen diese Komplexbildung. Im Futter enthaltene Tannine präzipitieren Speichelproteine und haben daher eine adstringierende Wirkung, die die Futteraufnahme der Tiere vermindern kann (Piluzza et al., 2014). Dies wird jedoch meist durch eine Limitierung des Tanningehaltes im Futter auf maximal 5% verhindert (Frutos et al., 2004). In diesem Zusammenhang ist auch erwähnenswert, dass sich die Komplexbildung von Tanninen mit Speichelproteinen zwischen verschiedenen Wiederkäuerspezies unterscheidet und somit auch die weiteren Wirkungen der Tannine beeinflussen kann (Austin et al., 1989). Die Komplexbildung von Tanninen mit Proteinen im Pansen führt zum einen zu einer verminderten

Pansenabbaubarkeit von Futterprotein und zum anderen durch Komplexbildung mit Verdauungsenzymen zu deren Inhibierung (Crozier, 2003). In geringerem Ausmaß binden Tannine auch Faser und Mineralstoffe. Zusammen genommen kann also die Komplexbildung von Tanninen mit Nährstoffen die Verdaulichkeit des Futters reduzieren und zu einem Leistungsrückgang der Tiere führen. Im Hinblick auf HT muss auch erwähnt werden, dass bei einer Aufnahme von HT-haltigem Futter in höheren Mengen durch Wiederkäuer toxische Effekte bis hin zum Tod beobachtet wurden (Garg et al., 1992; Hawes and Gill, 2018).

Tannine, Pansenmikroben und Methanproduktion

Tannine können nicht nur mit den Futterinhaltsstoffen, sondern auch mit den Pansenmikroben wechselwirken. Während die HT zum Teil oder sogar vollständig durch die Mikroben abgebaut werden können, bleiben KT meist unverändert, passieren den übrigen Gastrointestinaltrakt und werden mit dem Kot ausgeschieden (Bhat et al., 1998). Aufgrund der geringen Abbaubarkeit sind für Mikroben im Gegensatz zu dem Wiederkäuer selbst eher die KT als die HT toxisch. Durch Bindung an bakterielle Zellwände hemmen die KT das Wachstum von zellulolytischen und proteolytischen Bakterien, was wiederum den Nährstoffabbau im Pansen beeinträchtigt (Bodas et al., 2012). Die unterschiedliche Abbaubarkeit der KT und HT beeinflusst also deren Interaktion mit den Nährstoffen ganz wesentlich.

Im Pansen können bis zu 12% des Kohlenstoffs aus dem Futter in Methan umgewandelt und so nicht mehr durch den tierischen Organismus genutzt werden (Blaxter, 1962). In einigen, aber nicht in allen Studien konnten methansenkende Wirkungen für Tannine gezeigt werden (Beauchemin et al., 2007; Staerfl et al., 2012; Giller et al., 2021a). Während dosisabhängige Effekte in Bezug auf die Methansenkung häufiger beobachtet wurden, sind systematische Struktur-Wirkungs-Beziehungen hier noch weitgehend unklar (Vasta et al., 2019; Giller et al., 2021a). Zudem ist fraglich, inwieweit sich die Methansenkung durch Tannine tatsächlich auf die Nährstoffeffizienz auswirkt.

Tannine und Stickstoffeffizienz

Die Stickstoffeffizienz in der Wiederkäuerernährung ist als eher niedrig einzuordnen. Häufig übersteigen das Rohprotein im Pansen und die mikrobielle proteolytische Aktivität die Proteinsynthesekapazität der Mikroben (Walker et al., 2005). In der Folge werden bis zu 50% des Futterproteins zu Ammoniak abgebaut und über den Urin ausgeschieden (Callaway et al., 2003). Dies führt nicht nur zu einem Nährstoffverlust für die Tiere, sondern darüber hinaus auch zu einer Umweltbelastung. Die vorgängig beschriebene Komplexbildung von Tanninen mit Proteinen im Pansen und der dadurch verminderte ruminale Proteinabbau können die Stickstoffeffizienz in der Wiederkäuerernährung beeinflussen. Die Struktur der Tannine hat dabei einen entscheidenden Einfluss auf die Proteinbindungsaktivität (Frutos et al., 2004). Durch den verminderten Proteinabbau verringern Tannine die Entstehung von Ammoniak im Pansen und führen dadurch letztlich zu einer geringeren Stickstoffausscheidung über den Urin und einer höheren postruminalen Proteinverfügbarkeit (Crozier, 2003). Um jedoch die Verdauung und Absorption des Proteins im Dünndarm zu gewährleisten, müssen die Tannin-Protein-Komplexe bis dahin abgebaut werden. Die Komplexbildung ist jedoch nicht immer reversibel und kann daher zu einer Erhöhung der Stickstoffausscheidung über den Kot führen (Scalbert, 1981). Diese Verschiebung der Stickstoffausscheidung vom Urin in den Kot ist zwar für die Umwelt vorteilhaft, führt allerdings nicht zu einer verbesserten Proteinversorgung der Wiederkäuer. Da so aber weniger Ammoniak von der Leber entgiftet werden muss, wird für diesen Prozess entsprechend weniger Energie aufgewendet, wenn der überschüssige Stickstoff dem tierischen Stoffwechsel gar nicht erst zugeführt wird. Zusammengenommen haben Tannine durchaus das Potenzial, die Stickstoffeffizienz in der Wiederkäuerernährung zu verbessern. Der Einfluss von Faktoren wie z.B. Art und Dosis der Tannine, Rationszusammensetzung, Spezies und physiologischem Status muss jedoch zunächst detaillierter untersucht werden.

Tannine und Überführungseffizienz ungesättigter Fettsäuren

Sowohl KT als auch HT können die Biohydrogenierung von ungesättigten Fettsäuren verringern und damit deren Absorption im Dünndarm erhöhen (Campidonico et al., 2016; Alves et al., 2017). Dies führt zu einer höheren Überführungseffizienz von ungesättigten Fettsäuren aus dem Futter in das Tier und damit auch in Milch und Fleisch (Toral et al., 2011; Giller et al., 2021b). So wurde in einigen Studien durch die Aufnahme von Tanninen auch der Anteil der für die Humanernährung als gesundheitlich vorteilhaft angesehenen Omega-3 Fettsäuren in Milch und Fleisch gesteigert (Moloney et al., 2008; Kalber et al., 2011). Die NTP scheinen diese Wirkung nicht zu haben (Lourenco et al., 2008).

Leistungssteigerung durch Tannine?

Ähnlich den variablen Ergebnissen in Bezug auf die Nährstoffeffizienz sind auch die Ergebnisse in Bezug auf eine potenzielle Leistungssteigerung von Wiederkäuern durch die Fütterung sowohl mit KT als auch HT widersprüchlich. Studien in diesem Bereich wurden bereits von Makkar (2003) und Waghorn (2008) ausführlich zusammengefasst und diskutiert. Es ist möglich, dass Beobachtungen von schnellerem Wachstum oder gesteigerter Milchleistung bei Wiederkäuern durch die Fütterung von Tanninen nicht (alleine) auf eine Steigerung der Nährstoffeffizienz durch Tannine zurückzuführen sind. Weitere bioaktive Wirkungen der Tannine und anderer Polyphenole wie z.B. antioxidative und antiinflammatorische Effekte sowie eine Modulation der Genexpression können den Stoffwechsel positiv beeinflussen und dadurch potenziell leistungssteigernd wirken.

Schlussfolgerungen

Tannine im Futter von Wiederkäuern haben grundsätzlich das Potenzial, die Nährstoff- und insbesondere die Stickstoffeffizienz zu verbessern. Inwieweit dies auch für NTP zutrifft, kann zum jetzigen Zeitpunkt aufgrund mangelnder Datendaten nicht beurteilt werden. Auch machen die variablen Ergebnisse der bisherigen Studien deutlich, dass die Struktur der Polyphenole einen entscheidenden Einfluss auf deren Bioaktivität hat und dass es nicht nur zwischen den Gruppen der KT und HT sondern wahrscheinlich auch zwischen individuellen Strukturen innerhalb jeder Gruppe große Unterschiede im Hinblick auf der Wirkungsweise und Wirksamkeit gibt. Zudem scheinen die Effekte auch zwischen verschiedenen Wiederkäuerspezies zu variieren. Entsprechend werden detailliertere Studien mit Wiederkäuern benötigt, die zum einen differenzierte Effekte von einzelnen HT und KT in verschiedenen Dosierungen analysieren und zum anderen die Effekte in unterschiedlichen Wiederkäuerspezies untersuchen. Aufgrund des Mangels an Studien zu NTP bei Wiederkäuern besteht insbesondere hier ein großer Forschungsbedarf.

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Modulation of the gastrointestinal tract of the pig with natural sustainable bioactive compounds

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Abstract

Weaning is a challenging time in commercial pig production systems. It involves complex dietary, social and environmental stressors that interfere with gut development of the piglet, and is characterized by a reduction in feed intake and growth, atrophy of the small intestinal architecture, up-regulation of intestinal inflammatory cytokines, dysbiosis and diarrhoea. Traditional measures to reduce weaning associated intestinal dysfunction have centred on dietary inclusion of antibiotic growth promoters in the weaning pig diet, or high concentrations of dietary minerals in the form of zinc oxide. As international health and environmental regulations increase the constraints on the use of in-feed antibiotics and minerals in swine production, the search for novel bioactives from sustainable natural resources increase. A spectrum of natural sustainable bioactives from plant and animal sources have been identified that show promise. These compounds show a diverse range of activities, with many having multifunctional bioactivity. Modes of action vary from commensal microbial stimulants, targeted antimicrobial activity, gut barrier repair, maturation of the mucosal structure (villous architecture, absorptive capacity, nutrient transporters) and mucosal anti-inflammatory activity. While it is beneficial to supplement the pig with these bioactives at weaning, there may be substantial advantages to supplementing the sow during gestation/lactation on the gut microbiome and maturation of the gut of the piglet at weaning time. The overall aim of our research program is to provide dietary support to ensure an appropriate level of immune reactivity in the gut to accommodate the presence of beneficial and dietary microorganisms, while allowing effective immune/inflammatory responses to eliminate pathogens.

Early development of the gastrointestinal tract in the pig

The mammalian gastrointestinal tract is a dynamic environment, where a symbiotic relationship exists between the digestive system, the immune system and the resident microbiota. The development of the immune system begins in-utero and is further developed following the colonization of the GIT with microbiota during birth and postnatal life. The early establishment of this relationship is fundamental to the development and long-term maintenance of gut homeostasis, with unfavourable alterations in the composition of the microbiota, dysbiosis, being implicated in many conditions.

Weaning is a crucial event on commercial pig farms. Weaning is currently performed abruptly, between 21 and 28 days of age. The newly weaned pig not only transits from milk to a solid and more complex diet, but is also subjected to additional stressors including separation from sow and littermates, comingling with unknown pigs, adaptation to new environmental settings and increased pathogen exposure (Campbell and Crensha, 2013). All these stressors result in reduced feed intake, lasting up to 48 hours post-weaning, which is the main driver of the observed gastrointestinal dysfunction, poor performance, post-weaning diarrhoea (PWD) (McCracken et al., 1999) that is evident for 10-15 days post-weaning. It should be a key goal of any production system to have animals to be as mature as possible at weaning time. This would mean that the gastrointestinal tract would be able to deal with the abrupt change in diet which would then ensure that the animal would have the energy to cope with the social challenges. Some of our current unpublished data suggests that up to 25% of piglets are weaned at less that 6kg at an average of 26 days of age and hence would be particularly challenged by abrupt weaning.

Traditional measures to reduce weaning associated intestinal dysfunction

Traditional measures to reduce weaning associated intestinal dysfunction have centred on dietary inclusion of antibiotic growth promoters (AGP) in weaning pig diets, or high concentrations of dietary minerals in the form of zinc oxide at doses well above nutritional requirements. The direct purpose of these additives is to suppress the growth of pathogenic bacteria such as Escherichia coli and Salmonella enterica subsp. enterica serotypes. However, owing to the possible contribution of in-feed antibiotics to the development of antibiotic resistant strains of bacteria, the European Union implemented a full ban on AGP usage in livestock diets in January 2006. Zinc oxide (ZnO) was a successful alternative to deal with the negative impact of weaning on growth and gastrointestinal dysfunction (including dysbiosis) in pigs, but ZnO will also be banned in the EU by 2022 due to its association with environmental contamination and antimicrobial resistance ((Commission Implementing Decision of 26.6.2017, C(2017) 4,529 Final). Furthermore, the use of antimicrobials in farm animals will be subjected to additional restrictions in the EU from 2022 (Regulations (EU) No. 2019/6 and No. 2019/4). Thus, there is an increasing urgency for alternative dietary supplements that can support growth and gastrointestinal health and functionality in the post weaned pig.

Table 1. Potential modes of action of bioactives and their physiological effects that could be measured as indicators of positive effects

Mode of action of bioactives	**Physiological effects**
Stimulate digestive function	Stabilize pH Increase pancreatic and digestive enzymes Increase beneficial short chained fatty acids
Improve nutrient digestibility/absorption	Enhance villous architecture Increase expression of nutrient transporters
Support the immune system	Promote tight junction formation and mucin production Reduce pro-inflammatory cells/cytokines Prime B and T cells Increase immunoglobulin conc. in colostrum
Support beneficial bacteria/ Prevent establishment of pathogens	Maturation and stabilization of microbial environment

The search for natural sustainable sources of plant bioactives

A wealth of chemodiversity has arisen in nature, as plants have evolved to develop an array of anabolic and protective molecules to thrive in the varying complex biosystems of the biosphere. These molecules include: primary metabolites that are primarily required for growth; secondary metabolites that primarily mediate plant-environment interactions; and hormones that regulate cellular processes and metabolism. Primary metabolites are fundamental to the growth, development and reproduction of an organism and these include carbohydrates, lipids, nucleotides, and amino acids. Amazingly, more than 50,000 secondary metabolites have been identified in the plant kingdom alone. Some of the more commonly known classifications include terpenes, phenols and nitrogen-containing compounds. These molecules can be multifunctional and have a spectrum of biological properties in the plant that contribute to flowering, colour, fragrance, abscission, control deciduous behaviour, provide defence, etc. When these primary and secondary metabolites are extracted from plants, they exhibit an array of biological activities including analgesic, apoptotic, anti-allergic, anti-inflammatory, anti-oxidant, antimicrobial, antitumour, cytotoxic, prebiotic, etc properties in mammalian cell and systems. Such molecules are referred to as 'bioactives' and form the basis of the fact that mammals benefit from consuming a diet rich in plants. Interestingly, mammals do not benefit from eating numerous uncultivated plants in their whole state as these plants contain significant plant defence molecules that have negative consequences

on the animal. Hence the need to extract the bioactives which are beneficial and discard other molecules that are harmful. Potential modes of action of bioactives and their physiological effects in the gastrointestinal tract are presented in Table 1.

Marine Macroalgae as a source of bioactives

There is considerable interest in the utilisation of marine macroalgae as a underexploited resource for feed for agricultural animals. Marine macroalgae, broadly classified into brown, red and green seaweeds, are a major source of novel bioactives. Macroalgae contain varying concentrations of non-digestible polysaccharides, polyphenols, minerals, vitamins, proteins and lipids. Several studies have identified that the feeding of whole seaweed biomass has negative effects on pig performance (Satessa et al., 2020; Michiels et al., 2020). This is likely due to a spectrum of defence molecules in the whole seaweed that are designed to deter grazers. Hence there is a need to extract the beneficial molecules and remove the harmful molecules. It must be noted that the extraction methodologies and conditions used to extract polysaccharides (i.e. combination of parameters such as solvent, pH, temperature, time, solvent to seaweed ratio) are important contributing factors to the quantitative, structural and functional variability of seaweed polysaccharides, as is the species of seaweed and season of harvest (Garcia-Vaquero et al., 2017). Of particular interest to the quest to find alternatives to zinc oxide are extracts that are rich in the non-digestible polysaccharides of brown seaweeds, namely, laminarin and fucoidan.

Laminarins

Laminarins are storage glucans (a polysaccharide of glucose). They accumulate, as a carbohydrate food reserve, in the vacuoles during summer and early autumn to support survival and growth during the winter and early spring. Structurally, they are low molecular weight β-glucans consisting of a linear backbone of (1,3)-β-linked glucopyranose residues with a varying level of β-(1,6)-branching (Kadam et al., 2015). Seaweed species such as *Laminaria digitata* and *Laminaria hyperborea* are rich sources of Laminarins. Several studies have demonstrated the benefits of laminarin-rich extracts as a dietary supplement during the post-weaning period in pigs. Performance parameters such as final bodyweight, daily gain, feed intake and gain to feed ratio were positively influenced in weaned pigs supplemented with crude or highly purified laminarin-rich extracts (McDonnell et al., 2010; Walsh et al., 2013; Heim et al., 2014a; Rattigan et al., 2020a). These performance effects were likely underpinned by improved villus architecture mainly characterised by increased villus height (VH) and VH: Crypt depth ratio and increased expression of nutrient transporter genes, indicating enhanced nutrient digestion and absorption in the small intestine, as well as increased numbers of Lactobacillus spp. in the colon (Heim et al., 2014; Rattigan et al., 2020a;). Indeed, the potential for laminarin rich extracts to replace zinc oxide was confirmed, as a laminarin-rich extract reduced the incidence of diarrhoea and improved daily gains in weaned pigs maintained in unsanitary husbandry conditions (Rattigan et al., 2020b).

Fucoidans

Fucoidans are a complex and heterogenous group of water-soluble sulphated fucose-rich polysaccharides that contain small quantities of other monosaccharides (e.g. xylose, mannose, galactose, rhamnose, glucose) as well as glucuronic acids and acetyl groups. The backbone structure of fucoidan consists of (1,3)-α-linked fuco-pyranose residues or alternating (1,3)-α- and (1,4)-α-linked fuco-pyranose residues with sulphate groups occurring mainly at C-2 and C-4 positions and rarely at C-3 (Ale and Meyer, 2013). The chemical structure of fucoidans varies between different seaweed species. Ascophyllum nodosum is among the fucoidan-rich seaweed species and, is commonly used as a source of this polysaccharide. The effects of dietary fucoidan-rich extracts on performance parameters in pigs are less pronounced than in laminarin-rich extracts and is probably due to the non-digestible nature of the compounds resulting in a lack of effect in the small intestine. However the ability of fucoidan to modulate the gastrointestinal microbiota and its metabolic products in the large intestine has been highlighted. In

particular, the ability to reduce Enterobacteriace as well as Salmonella numbers. *In vitro* studies identified various fucoidan-rich seaweed extracts which inhibit the growth of the pathogenic *S.* Typhimurium or stimulate the growth of the commensal *Lactobacillus* spp. and *Bifidobacterium* spp. strain (Venardou et al., in press). Furthermore, in both an experimental infection (Bouwhuis et al., 2017) and a natural infection with *S.* Typhimurium (Venardou et al., in press), dietary supplementation of pigs with a fucoidan-rich seaweed extract was associated with improved performance, reduced *Salmonella* shedding and colonisation and reduced intestinal inflammation.

Supplement the diet of the piglet or the sow?

While many studies focused on supplementing the piglet after weaning, an alternative strategy is to supplement the sow during gestation/lactation. Supplementing the sow has a number of potential benefits including modulating the sows gut microbiome and hence the environmental microbiome that the piglet will be exposed to after birth, and secondly advancing the maturation of the digestive tract of the piglet at weaning time. Both Leonard et al. (2012) and Heim et al. (2014b) identified that supplementing pregnant sow diets with seaweed extracts containing laminarin and fucoidan during late gestation reduced the Enterobacteriaceae population in the sow's faeces, while also reducing colonic Escherichia coli numbers in the piglets at weaning. Improved resistance to infection and reduced pathogen shedding post-weaning were also observed in piglets suckling SWE-supplemented sows following an ETEC challenge (Heim et al., 2014b) and a S. Typhimurium challenge (Bouwhuis et al., 2017). This indicates that manipulation of the microflora of the sow through seaweed extract has the potential to reduce the abundance of pathogenic bacteria in the intestinal tract of her offspring.

Conclusion

Traditional practices have focused on supplementing the piglet diet in the post-weaning period with antibiotics and ZnO to alleviate post-weaning complications. Our research suggests that there are other natural, sustainable alternatives that could be exploited. While benefits are observed with post-weaning supplementation, further research exploring the potential of maternal supplementation during fetal and postnatal life to support homeostasis in the piglet GIT throughout the weaning period is warranted.

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Wirkung von Faser in der Fütterung von Schweinen

The role of fibre in feeding pigs

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Abstract

The importance of fibre in pig nutrition has been underestimated for a long time. Apart from its negative impacts, i.e. reduced energy concentration and decreasing nutrient use efficiency, fibre plays an important role in physiology, health and wellbeing of pigs. The term dietary fibre (DF) summarises all carbohydrates that are not degraded by mammalian enzymes but can be degraded by microbes in the gastro-intestinal-tract (GIT). The challenge still is to measure and evaluate DF in a reliable and thorough way. Different methods are used and discussed, like the detergent-fibre method or the total-dietary-fibre method. Furthermore, DF needs to be evaluated in a functional way which means that besides the chemical fractions also parameters like solubility, viscosity and water-holding-capacity need to be assessed. Depending on age and production stage the effects of DF in pig rations differ, but this is also strongly influenced by the composition and source of DF. However, DF is essential to sustain the microbiota in the lower GIT of pigs and the importance of the microbiome, not only for gut health, but for the overall wellbeing and health of pigs attracts notice in pig nutrition science. An additional positive effect of DF supplemented rations is the shift from highly volatile nitrogen excretion with urine to the excretion of more stable nitrogen compounds with faeces. Because the importance of DF in pig nutrition is well known, it is of high importance to standardise analytical methods to enable a functional evaluation of feed stuffs. Finally, recommendations for the supply of pigs with DF are needed.

Hintergrund

Die Bedeutung von Faser in der Ernährung von Schweinen, wurde lange Zeit unterschätzt bzw. galt Faser lange Zeit als anti-nutritiver Stoff, der die wertgebenden Nährstoffe der Ration und damit den Energiegehalt verdünnt. Dieser Zusammenhang ist eindeutig belegt (Wenk 2001), sowie auch die Tatsache, dass Faser neben diesen negativen Auswirkungen, auch wichtige physiologische Funktionen hat und sich auf die Gesundheit und das Wohlergehen der Tiere auswirken (Bach Knudsen et al. 2012). Die einseitige Beurteilung von Faser als anti-nutritiver Substanz, hängt auch damit zusammen, dass die allgemein genutzte Weender Analyse zur Beschreibung von Futtermitteln, die Faser als „Rohfaser" nur unzureichend beschreibt und viele Faserfraktionen nicht unter die Definition „Rohfaser" fallen. Zur umfassenden Beschreibung der Faser in Futtermitteln und Rationen bedarf es daher einer anderen Methodik, die eine funktionale Beschreibung von Faser ermöglicht und damit auch eine Beschreibung der Wirkung von Faser im Gastro-Intestinal-Trakt (GIT) von Schweinen. Hier gibt es bereits seit langem Ansätze, wie z.B. die Analyse von Nicht-Stärke-Polysacchariden (NSP) (Englyst und Hudson 1996) oder die Bestimmung von „Dietary Fibre" (Total Dietary Fiber, TDF; Prosky et al. 1988). Auch die für Futtermittel für Wiederkäuer entwickelte Detergenzien-Analyse (NDF/ADF; Van Soest, 1967) wird zur Quantifizierung von Faser in Futtermitteln für Schweine genutzt (z.B. DLG, INRA). Da die Wirkung von Faser durch ein Zusammenspiel zahlreicher komplexer Vorgänge bestimmt wird, ist es bisher nicht gelungen, eine allgemein akzeptierte Methode zu entwickeln, die eine umfassende funktionale Beschreibung von Faser erlaubt. Ziel dieses Beitrags ist es darzustellen, wie Faser definiert ist und welche Unterschiede zwischen den gängigen Messmethoden bestehen. Die Wirkung von Faser im GIT wird beschrieben,

sowie die Effekte von Faser in Rationen für Schweine auf Leistung, Gesundheit, Tierwohl und Umwelt zusammengefasst.

Faser – worüber wir eigentlich reden

Für die Humanernährung wurde der Begriff „Dietary Fibre" (Nahrungsfaser) im *Codex alimentarius* (2008) wie folgt definiert: Kohlenhydrate, mit 10 oder mehr Monomeren, die nicht durch endogene Enzyme im Dünndarm hydrolysiert werden können. Aufgrund der physiologischen Nähe zwischen Mensch und Schwein, erscheint es sinnvoll diese Definition auch für das Schwein zu nutzen. Abbildung 1 gibt einen Überblick über die Zusammensetzung von Nahrungskohlenhydraten, ihr Verhalten im GIT und gängige Methoden zu ihrer Erfassung. Nachfolgend wird „Dietary Fibre" mit dem Begriff Gesamtfaser (DF) bezeichnet.

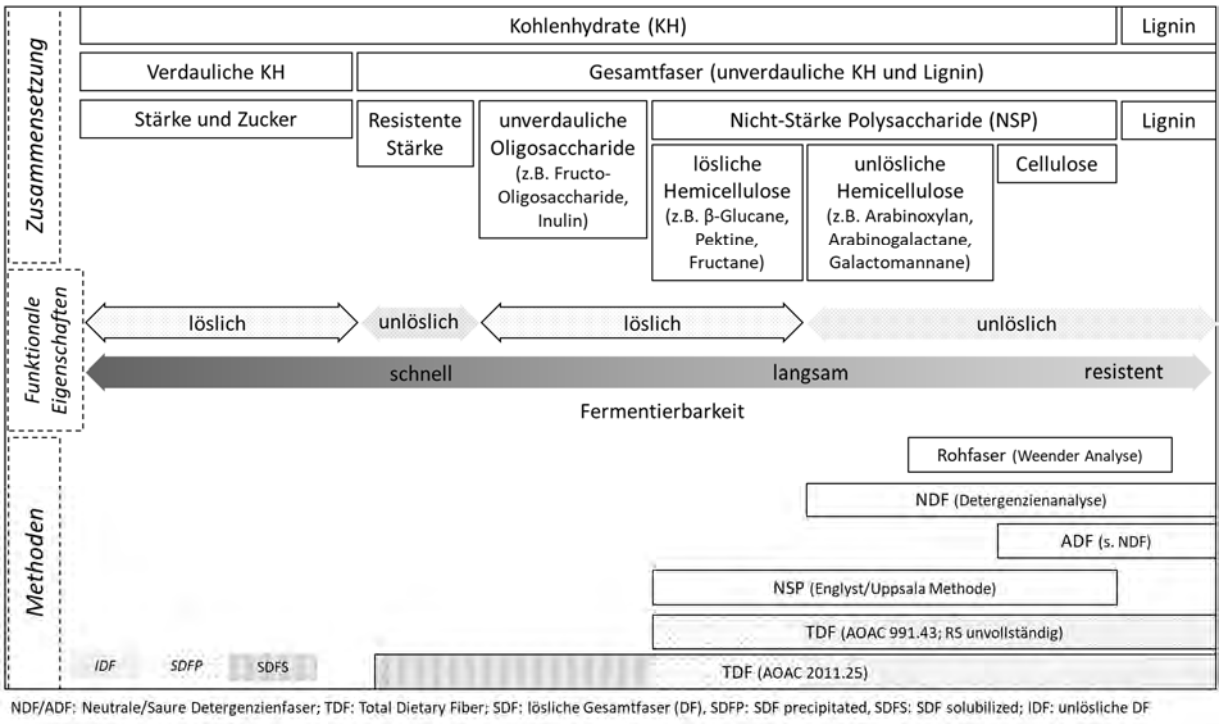

Abbildung 1: Überblick Nahrungskohlenhydrate, analytische Methoden und funktionelle Eigenschaften

Es wird deutlich, dass es sich bei der Gesamtfaser um eine chemisch sehr komplexe Gruppe handelt. Eine funktionale Beschreibung allein anhand der chemischen Fraktionen bzw. ihrer Zusammensetzung, ist kaum möglich, und die Bestimmung z.T. sehr aufwendig. Daher wird durch die Einteilung in lösliche (soluble, SDF) und unlösliche (insoluble, IDF) Gesamtfaser, bzw. durch eine Abschätzung der Fermentierbarkeit der einzelnen Faserfraktionen versucht zu einer funktionalen Beschreibung zu kommen. Neben den in Abb.1 dargestellten Zusammenhängen, müssen auch physico-chemische Eigenschaften von Fasern bzw. Faserfraktionen berücksichtigt werden, da sie die Passage und den Abbau von Faser im GIT und damit die Nährstoffabsorption insgesamt, maßgeblich beeinflussen können. Hier sind vor allem die Wasserhalte- (WHC) oder –bindungskapazität (WBC) sowie das Quellvermögen, die Viskosität und auch die Pufferkapazität zu nennen (Slama et al. 2019). Diese Eigenschaften korrelieren häufig mit der Einteilung in lösliche und unlösliche Gesamtfaser, so hat SDF i.R. eine höhere Wasserhaltkapazität und ein größeres Quellvermögen als IDF. Auch die Viskosität korreliert mit dem SDF-Anteil am Gesamtfasergehalt. Bisher werden die physico-chemischen Eigenschaften nicht in die Bewertung von Futtermitteln einbezogen und auch methodisch steht eine Standardisierung noch aus.

Die Wirkung von Gesamtfaser im Gastro-Intestinal-Trakt

Die Übersicht in Tab. 1 zeigt, dass die Gesamtfaser eine Substanz mit hoher Bioaktivität ist und wesentliche physiologische Funktionen im GIT beeinflusst.

Tabelle 1: Überblick Effekte von Gesamtfaser auf die Verdauung und ihre physiologische Wirkung (Capuano 2017, verändert)

Verdauung	**Effekte von Gesamtfaser (DF)**	**Physiologische Effekte von DF**
Mund (<1 min) Speichelamylase u. -lipase	Komplexe zw. DF und Phenolen	Veränderung der Verfügbarkeit von Phenolen
Magen (2-3 h) Pepsin; pH 1-3 nüchtern, pH 5-7,5 nach Nahrungsaufnahme	Steigerung der Viskosität	Magenentleerung/Nährstoffabsorption ↓; Sättigung ↑
	Hemmung Pepsin	Proteinverdauung ↓
	Auflösung DF-Phenol-Komplexe (variabel)	Veränderung der Verfügbarkeit von Phenolen
Dünndarm (3-5 h) Bauchspeicheldrüsenenzyme Mikroflora im Ileum pH 6-8	Strukturelle Barriere der Pflanzenzellwände	Verfügbarkeit interzellulärer Substrate ↓
	Steigerung der Viskosität	Nährstoffabsorption ↓, Sättigung ↑
	Enzymhemmung	Hydrolyse Nährstoffe ↓
	Bindung von Metallionen	Verfügbarkeit im Dünndarm ↓, Bildung Ca-Seifen Fettsäuren (FS) ↓→ Fettverdauung↓
	Bindung von Phenolen	Verfügbarkeit Phenole, Einfluss auf antioxidative Aktivität
	Bindung von Gallensäuren	Ausscheidung Gallensäuren ↑, Emulsionsbildung ↓ → Fettverdauung ↓
Dickdarm (12-24 h) Darmmikrobiom pH 5-6	Produktion kurzkettige FS	Selektives Wachstum „positiver" Mikroben → prebiotische Effekte; Schutz vor Pathogenen; Effekte auf Fett- und Glucosestoffwechsel; Proliferation von Colonozyten ↑
	gesteigertes mikrobielles Wachstum	Fäcale Masse ↑ → Verdünnung toxischer Substrate, Passagerate ↑,
	Stimulation Darmperistaltik und Mukusproduktion	Passagerate ↑ → Kontaktzeit mit toxischen Substraten ↓
	Freisetzung Phenole	Verfügbarkeit im Dickdarm ↑

Einfluss von Gesamtfaser auf Leistung, Gesundheit & Tierwohl sowie die Umwelt

Die negativen Effekte von Faser auf Nährstoffverdaulichkeit, Energiekonzentration und Wachstumsleistung sind vielfach beschrieben worden, wobei der Effekt sowohl mit der Dosierung als auch der Faserquelle bzw. der Zusammensetzung der Faserquelle variiert. Nicht in allen Studien wirken sich Faserzulagen negativ auf die Leistung wachsender Schweine aus (Agyekum und Nyachoti 2017). In einem Wahlversuch konnten Pichler et al. (2020) zeigen, dass Mastschweine ihre Ration aus einem energiearmen (faserreichen) und einem energiereichen (faserarmen) Futter so wählen, dass sie gegenüber den Kontrolltieren, die nur die energiereiche Ration gefressen hatten, bei gleicher Futteraufnahme, gleiche Zuwachsleistungen erzielten. Dabei selektierten die Tiere so, dass der Gehalt an DF 20-25% höher war als in der faserarmen Ration. Bei den Kontrolltiere, die nur die faserreiche Ration zu fressen bekamen, verringerte sich die Zuwachsleistung. Dieser Versuch zeigt, dass Gesamtfaser sich nicht immer negativ auf die Wachstumsleistung auswirkt, und dass die Tiere einen gewissen Anteil an Gesamtfaser freiwillig in ihre Ration integrieren. Ein Vergleich von Studienergebnissen ist oft schwierig, da die verschiedenen Studien verschiedene Gesamtfasern einsetzen, z.T. reine Quellen oder als Bestandteil von faserreichen Futtermitteln. Die Einsatzmengen variieren stark und hinzukommen methodische Un-

terschiede in der Beschreibung der eingesetzten Gesamtfaser. Der Einsatz von Faser in der Sauenfütterung hat vor allem positive Effekte auf die Leistung ergeben, wobei die Leistungsparameter hier andere sind: so konnte z.T. das Wurf- und Absetzgewicht gesteigert und die Körperkondition der Tiere stabilisiert werden (Li et al. 2021), ein übermäßiger Gewichtszuwachs in der Trächtigkeit wirkt sich negativ auf die Gesundheit der Sau und der Ferkel sowie die Reproduktionsleistung aus.
Die Wirkung von Gesamtfaser auf Gesundheit und Wohlbefinden von Schweinen ist ebenfalls nicht eindeutig zu beantworten. Sie ist z.B. von der Art und Menge der Faser, der Vermahlung des Futters und vor allem dem Alter der Schweine abhängig. Bei Absetzferkeln hatte der Zusatz von unlöslicher Gesamtfaser positive Effekte auf die Darmgesundheit(Gerritsen et al. 2012), was auf die erhöhte Passagerate und die dadurch reduzierte Verweildauer im GIT zurückgeführt wird, die Pathogenen die Proliferation erschwert. Auch der Einsatz von löslicher Gesamtfaser beim Absetzferkel hat sich in Studien positiv gezeigt (prebiotische Effekte;(Molist et al. 2009)), aber es wurden auch negative Effekte in Form von vermehrten Durchfällen beobachtet. Dies wird als Effekt erhöhter Viskosität, und damit erhöhter Verweildauer und Proliferationszeit für Pathogene, beschrieben. Bei Mastschweinen wird der Einsatz von Nahrungsfaser als positiv im Hinblick auf Reduzierung von Magengeschwüren und Verhaltensstereotypien beschrieben (Wenk 2001) und auch positive Effekte auf die Darmgesundheit konnten mehrfach nachgewiesen werden (Zijlstra et al. 2012). Es gibt auch Berichte, dass der Einsatz von größeren Mengen löslicher, also fermentierbarer Gesamtfaser, das Auftreten von Dysenterie beim Mastschwein begünstigt (Pluske et al. 1996), allerdings zeigen andere Studien auch gegenläufige Ergebnisse (Thomsen et al. 2007). Bei trächtigen Sauen wird der Einsatz von Gesamtfaser hauptsächlich positiv bewertet: Verstopfungen können reduziert werden, die besonders um die Geburt herum große gesundheitliche Probleme auslösen. Des Weiteren konnte gezeigt werden, dass eine faserreiche Ration in der Trächtigkeit, die Futteraufnahme in der Laktation erhöht, was sich positiv auf das Ferkelwachstum auswirkte (Quesnel et al. 2009). Darüber hinaus wurde eine Reduzierung der Geburtsdauer beobachtet (Li et al. 2020).
Gesamtfaser hat außerdem eine essentielle Bedeutung für die Mikrobiota im GIT (Metzler-Zebeli et al. 2010; Williams et al. 2001). Die Bedeutung der „Darmbewohner" wird beim Menschen intensiv beforscht und auch in der Nutztierforschung gewinnt das Gebiet immer mehr an Bedeutung (Pu et al. 2020; Ndou et al. 2018). Insbesondere mit Blick auf das Thema Tierwohl wird deutlich, dass neben den bekannten positiven Effekten von faserreichen Futtermitteln auf das Verhalten von Schweinen (Reduzierung von Aggressionen und Stereotypien) durch Beschäftigung und Befriedigung von Verhaltensmustern wie Futtersuche, es auch einen Einfluss des Darmmikrobioms auf das Gehirn („Microbiota-gut-brain axis") und damit auf Stressreaktion, Verhalten und Wohlbefinden gibt (Kobek-Kjeldager et al. 2022).
Die Aktivität der Mikrobiota im Dickdarm von Schweinen hat einen direkten Einfluss auf die Stickstoff (N) -Ausscheidung des Schweins. Unverdaute Proteine aus der Nahrung und endogene N-Ausscheidungen werden im Dickdarm von Darmbakterien zum Aufbau mikrobieller Masse genutzt. Beim Einsatz von faserreichen Rationen stehen im Dickdarm auch ausreichend Energiequellen zur Verfügung, die eine effiziente Fermentation ermöglichen (Bindelle et al. 2008). Es wird weniger Ammonium ausgeschieden und somit N-Emissionen in den Stall und aus der Gülle verringert. Darüber hinaus wird auch Harnstoff aus dem Blut im Dickdarm zum Aufbau mikrobieller Masse genutzt, immer unter der Voraussetzung, dass genügend Energiequellen vorhanden sind. Dadurch sinkt der Blutharnstoffgehalt und es wird weniger N über den Urin ausgeschieden. Auch das führt zu einer Reduzierung von N-Emissionen. Des Weiteren tragen die bei intensiver Fermentation entstehenden kurzkettigen Fettsäuren zu einer Absenkung des pH-Werts im Kot und damit in der Gülle bei, dadurch entstehen weniger flüchtige N-Verbindungen. Der Einsatz von löslicher Gesamtfaser hat sich dabei als besonders wirksam gezeigt (Jha und Berrocoso 2016).

Ausblick

Die Gesamtfaser hat einen festen Platz in jeder Ration für Schweine, als „unvermeidlicher" Bestandteil vieler Futtermittel. Die Rolle als „notwendiges Übel" hat die Gesamtfaser mittlerweile abgelegt, wir wissen um ihre Bedeutung bei physiologischen Vorgängen sowie für Gesundheit und Wohlbefinden der

Tiere. Die Herausforderung liegt nun in der Beschreibung und Bewertung von Gesamtfaser. Ziel muss es sein, Gesamtfaser anhand ihrer funktionalen Eigenschaften zu beschreiben, nur so lassen sich die komplexen Eigenschaften und ihre vielfältige Zusammensetzung in einer Art bewerten, die dann auch für die Rationsgestaltung genutzt werden kann. Dazu bedarf es einer Weiterentwicklung bzw. Standardisierung bekannter Methoden, die die Gesamtfaser als Ganzes erfassen können. Ebenso wichtig ist die Entwicklung bzw. Weiterentwicklung von Methoden zur Bestimmung funktionaler Eigenschaften wie physico-chemische Parameter und Fermentationsvermögen. Eine Kombination solcher Parameter könnte in Zukunft eine funktionale Bewertung von Gesamtfaser in Futtermitteln ermöglichen, die auch für die praktische Rationsformulierung genutzt werden kann. Dazu bedarf es der Ableitung von Versorgungsempfehlungen für Schweine, die aber nur anhand einer standardisierten Methodik erfolgen kann. Der gezielte Einsatz von Gesamtfaser in Rationen für Schweine würde die Optimierung von Rationen im Hinblick auf Gesundheit und Wohlbefinden sowie auf Umweltwirkungen erlauben, und eine Balancierung gegenüber leistungsmindernden Effekten ermöglichen.

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Anti-nutritional components in European soybeans: Plant breeding options

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Abstract

Domestic soybean production has increased during previous years in many European countries, as soybean is the major source of protein for food and feed industries. Genetic variation in soybean seed composition has recently been explored in more detail, which enables plant breeding to develop new cultivars for specific needs and nutritional requirements. For the livestock feed industry, soybeans with reduced content of protease inhibitors have been introduced. New cultivars with a reduced concentration of allergenic protein components, lectins and other proteins could be developed from respective breeding populations. Furthermore, soybean cultivars with low accumulation of toxic components such as heavy metals or reduced levels of bioactive ingredients including isoflavones could be selected from germplasm available at present. This might be similarly interesting to both the soy-food and livestock-feed industries.

Background

Soybean (*Glycine max* [L.] Merr.) is the major protein crop grown for livestock feed and human food production. In Austria, over 75.500 ha of soybeans were grown in year 2021 yielding a total harvest of about 232.000 t. On the European level, although about 8.7 to 10.2 mio t of soybeans were produced annually during the last five years in Europe, a considerable amount of either whole soybeans or defatted soy-meals for livestock feeding is still imported from overseas (Rittler and Spreitzer, 2022). The high demand of feed and food industries for soybeans and soy-components is due to its unique seed composition which is different from most other legumes, oilseeds and cereal crops: On average, soybean contains 40% (range: 30-49%) protein, 21% oil and about 34% carbohydrates as major seed constituents (Medic et al., 2014). In contrast to pea, faba bean, chickpea or lentil, which are lower in protein and rich in starch content instead, soybean does not contain significant amounts of starch (Vollmann, 2016). The high seed protein content of soybean is due to biological (symbiotic) fixation of atmospheric nitrogen through rhizobial bacteria in soybean root nodules. As about 30 to 70% of soybean nitrogen uptake is originating from biological nitrogen fixation, no additional mineral nitrogen fertilizer is required in soybean production. This represents a highly attractive feature of soybean both in terms of environmentally friendly production avoiding nitrogen leakage into the ground water as well as in reducing costs for fertilizers.

Anti-nutritional soybean components

While superior seed protein content and high availability of illeally digestible lysine are favorable characteristics of soybean for livestock feeding, a number of anti-nutritional factors are present as well which have a negative impact on overall feed quality. This limits the utilization of soybean in feed applications or makes additional steps of processing necessary. An overview of soybean anti-nutritional factors and other critical components representing potential threats to food / feed safety is presented in Table 1.
Anti-nutritional proteins such as protease inhibitors are most prominently present in soybean. As they reduce protein digestibility in non-ruminant animals particularly, heat treatments such as toasting of

defatted soy meal are required in order to inactivate protease inhibitors and enhance protein digestibility. Other proteins such as allergens or lectins may have adverse effects in sensitive human individuals or in piglets. However, they cannot be inactivated easily by processing.
In addition to proteins, fatty acids, heavy metals and various other components of soybeans may exhibit anti-nutritional or toxic effects as well. Cadmium taken up from contaminated soils is highly toxic and accumulates in the kidney. The oligosaccharides raffinose and stachyose are present in soybean seeds as well causing flatulence and thus represent a loss of energy in the feeding ratio. Bioactive components such as isoflavones (also termed phytoestrogens) might influence the reproductive cycle of female animals if present in high concentration.

Table 1: Soybean seed fractions and individual seed constituents with anti-nutritional potential or negative effects on food/feed safety (from Watanabe et al., 2018)

Seed fraction	Class	Individual constituents
protein	allergens	Gly m Bd 30K (P34)
		7S globulin (β-conglycinin) α, α', β sub-units
		Gly m Bd 28K
		Gly m 1, 2, 3, 4, 2S albumin, oleosin, glycinin A3
	protease inhibitors	Kunitz trypsin inhibitor
		Bowman-Birk inhibitor
	other proteins	lectins (hemagglutinins)
		lipoxygenase
oil	fatty acids	linolenic acid
		palmitic acid
		stearic acid
metals	heavy metals	cadmium
		mercury
		chromium
other	oligosaccharides	raffinose
		stachyose
	isoflavones	
	saponins	
	tannins	
	phytic acid	

Breeding for improved nutritional quality

Genetic variation in the concentration of anti-nutritional constituents is present mainly in soybean germplasm accessions (genetic resources, landraces) which can be utilized in breeding for improved nutritional quality by eliminating undesirable constituents in progeny from dedicated crosses. This requires high-throughput analytical methods such as near-infrared reflectance spectroscopy (NIRS) or genetic markers for screening large numbers of genotypes during generations of selection.
In case of the soybean Kunitz trypsin inhibitor, a null allele had previously been identified in a landrace and was subsequently introgressed into soybean varieties for reducing trypsin inhibitor activity (TIA) of raw soybeans (Vollmann et al., 2003). Protein electrophoresis (SDS-PAGE) can be utilized for examining the presence or absence of the Kunitz protein. Thus, in a recent study (Haberlandt project, unpublished results) nine out of 77 European elite soybean cultivars were found to be Kunitz-null cultivars, i.e. the Austrian cultivar Josefine, and the Italian cultivars Pepita, Amma, Ananda, Bahia, Adonai, Avatar, Buenos and Guru. Similarly, for reducing soy allergenicity in food and feed materials, null-variants of the immuno-dominant soybean allergen P34 (Gly m Bd 30 K) were introgressed into modern varieties, and P34-null lines were selected using either immunological methods or genetic markers (Watanabe et al., 2017). There are about 15 soybean proteins known to cause allergenic reactions in sensitive humans. For targeted selection approaches towards optimizing soybean protein properties for pig feeding, more information would be necessary about which of the allergenic proteins are most relevant and should therefore be reduced primarily.

Genetic variation has also been detected in cadmium (Cd) accumulation of soybean. An efficient genetic marker discriminating between high and low Cd uptaking cultivars has been developed for rapid classification of individual soybean genotypes (Vollmann et al., 2015). This is of similar relevance to soybean breeding for both human food as well as animal feed production, when soybean is grown in regions with Cd contamination of soils or low soil pH.
Soybean germplasm variants differing in soluble carbohydrate concentrations have mainly been utilized in specialty soy-food products so far. For reducing flatulence and loss of energy, soybeans with a lower content of the oligosaccharides raffinose and stachyose are available (Hou et al., 2009) and might be of interest in non-ruminant feeding as well.
Isoflavones have been controversely discussed for over the last two decades. While soy-foods rich in isoflavones may deliver additional health benefits to consumers such as a reduced cancer risk or decreases in LDL cholesterol, they also may negatively affect the taste of soy-products (adstringency) or might impact thyroid and reproductive functions in animals fed with high concentrations of soy meals (Xiao, 2008). Although soybeans have rarely been selected for high or low isoflavone content, quantitative genetic variation for the major isoflavones has been described among soybean cultivars (Seguin et al., 2004). In addition, however, isoflavone concentration is highly influenced by environmental conditions as well. Recently, mass spectrometry imaging has revealed that isoflavones are mainly present in the germ (particularly the root parenchyma) regions of the soybean seed rather than in the cotyledons. This applies to isoflavone aglycones daidzein, glycitein and genistein as well as their glycoside, malonyl and acetyl derivatives (Sagara et al., 2020). Thus, milling techniques separating cotyledons from germs and seed coats would also reduce isoflavone concentration of soy products.
Apart from genetic variation present in breeding materials, the availability of efficient analytical techniques for screening large numbers of samples is a prerequisite for breeding progress in seed quality traits. While NIRS-based determination of seed moisture, protein and oil content is widely practised, a new range of calibrations for parameters such as trypsin inhibitor activity (TIA), protein solubility and various other characteristics relevant in feed analysis has become available recently (https://www.legumehub.eu/de/neuigkeiten/nirs/, accessed 21 Feb. 2022), which might support plant breeding as well.
In general, genetic variation found in seed quality characters is opening plant breeding options for further optimization of soybean seed composition based on the specific requirements of animal nutrition. However, for successful utilization of such variation in the development of new cultivars, biological functions of individual components, nutritional properties as well as market needs in the processing and feed industries need to be considered and discussed.

Conclusion

Genetic variation in soybean seed composition could be utilized for tailoring soybeans to better meet the requirements of the livestock feed sector. This includes protein composition as well as toxic and bioactive components.

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The evolution of the formulation of aquaculture feeds

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Abstract

Aquaculture production is a very dynamic field encompassing a very diverse number of species produced under a wide variety of conditions. The animals are generally fed feeds of high quality composed with many ingredients of different origins and manufactured to very high standards of quality. The formulation of aquaculture feeds has evolved very significantly. They are increasingly based on ingredients of plant origins and the contribution of ingredients of marine origins (e.g. fish meals and fish oils) has been decreasing rapidly.
The evolution in aquaculture feed formulation and production was made possible by massive research and development (R&D) efforts invested by a plethora of private and public organizations over the past five decades. R&D efforts have focused on the precise definition of the nutritional requirements of aquaculture species, the better characterization of the nutritive value of feed ingredients and the assessment of the value of functional feed additives. Commercial aquaculture feeds are now formulated to contain minimal levels of fish meal and fish oil and other costly marine ingredients. More work is required to optimize and improve the cost-effectiveness of aquaculture feeds.

The aquaculture production & feed landscape

Aquaculture is one of the fastest growing food production sectors in the world. Annual aquaculture production currently amounts to more than 85 million tonnes (MT) of which about 50% is the production of "fed" species (FAO, 2021). Aquaculture is an extremely diverse and complex sector with more than 350 fish and crustacean species cultivated worldwide. More than 50% of fish and crustaceans consumed around the world are farmed products (FAO, 2021). These animals are reared using a wide variety of production systems, feeds and feeding strategies.
Like most other agricultural enterprises, aquaculture operations are confronted to similar challenges: Stagnant or decreasing marketable product prices, increasing production cost, and increasing scrutiny about the composition, wholesomeness and sustainability of their products. The long-term sustainability of aquaculture operations is, therefore, highly dependent on how well they can improve their productivity, manage their production cost, improve the yield, quality, perceived wholesomeness of their marketable products, as well as reduce their waste outputs and environmental footprint.
Nutrition and feeding have a determinant effect of the production cost, waste outputs, and final product quality of aquaculture operations. Consequently, like in all other animal production sectors, feed manufacturers play a central role in providing cost-effective inputs and assisting aquaculture operations address their challenges.
The aquaculture feed industry is one of the fastest growing animal feed industries in the world. Aquaculture feed production grew from approximately 10 million metric tons (MMT) in 1995 to more than 40 MMT today (Alltech, 2022). The aquaculture feed market is expected to increase significantly over the next decades.
Many aquaculture feed manufacturers serve a large client-base cultivating several fish and invertebrate species in very different production systems (ponds vs. cages, marine vs. freshwater environment, etc.) and socio-economical contexts (small scale farmers vs. medium-scale regional operations vs. large multinational corporations). Aquaculture feeds are characterized by the wide nutritional specification to which they are formulated to. This is expected given the very large number of fish and crustacean species produced around the world using feed-based production systems. However, the protein, lipid, starch

and digestible energy contents of feeds can significantly vary not only as a function of species and life stages for which they are formulated (trout vs. tilapia feed, starter vs. grower vs. finisher feed), but also as a function of a myriad of other factors, such as production systems, farmers' or feed manufacturers' preferences, environmental constraints, and socio-economical conditions (e.g., fish price, access to credit, degree of risk).
Most aquaculture feeds are generally formulated to high protein (> 28%) and high lipid (> 6%), levels using a wide variety of ingredients. Aquaculture feeds generally need to be produced to very high standard in terms of physical characteristics. Virtually all aquaculture feeds need to be fed in particulate forms (pellets, crumbles) of appropriate sizes. Most feeds are manufactured by extrusion or steam-pelleting. Very stringent quality control is applied since the tolerance of the market for over- or under-size feed particles is very low. Feeds containing more than 1% fines are generally considered to be unacceptable by aquaculture producers. Undersized feed particles are not consumed by the animals and can contribute to water quality degradation and increase in production cost. Physical quality of the feed is almost as important as nutritional quality for aquaculture feeds. This emphasis of physical characteristic of the feeds adds to the cost of the feed. Many aquaculture feed manufacturing plants are amongst the most modern and sophisticated of the entire animal feed milling sectors.

Shifts in aquaculture feed formulations

Approximately 25 years ago, fish meal and fish oil represented about 70% of the weight of most commercial salmon and marine fish species feeds sold worldwide. There is a finite amount of fish meal (5 to 6 MMT) and fish oil (ca. 1 MMT) produced per year. The growth of the aquaculture feed industry has led to an ever-increasing demand for these ingredients resulting in very significant increase of their price. The price of fish meal (Fair average quality (FAQ) basis 65 percent protein, FOB Peru) has surged from about $500 to more than $1,500/MT over the past two decade. The price of fish oil ($1500-2000/MT) is also roughly 4-fold higher than it was 25 years ago. The limited production volumes of these ingredients and the high demand often results in supply chain issues in some regions.
These increases in prices and supply chain issues have created a strong incentive to reduce the reliance on marine-derived resources for aquaculture feed manufacturing. Feed manufacturers have had to progressively decrease fish meal and fish oil levels in their feeds. They have learned to increasingly rely on the use of an increasingly diverse array of alternative feedstuffs of plant, terrestrial animal or microbial origins.
Fish meal is a complex ingredient. It is an ingredient that is highly palatable to most fish species and has excellent nutritional properties. It is a source of highly digestible protein containing high levels of most essential amino acids in proportions that quite closely correspond to the requirements of fish. It is an ingredient rich in essential nutrients, such as long chain omega-3 polyunsaturated fatty acids (eicosapentanoic acid (EPA), docosahexanoic acid (DHA)), vitamins (A, D, K, etc.) and minerals (phosphorus, selenium, etc.).
Formulation of feed containing lower levels of fish meal required accurate and detailed information on essential nutrient requirements of aquaculture species. Decreasing fish meal levels and increased reliance on proteins with poorer amino acid profiles in feed meant paying greater attention to the essential amino acid requirements of animals. It became clear in the early 2000s that estimates of requirements of fish proposed by the National Research Council (NRC) in 1993 (NRC, 1993) for certain amino acids were too low and that these values should be revised upward. In 2011, the NRC revised several estimates of essential amino acid requirements upward and also estimated the requirements of several commercially important species.
Since the NRC (2011) was published, studies have shown significant benefits from supplementing plant-based fish and shrimp feeds with certain nutrients, such as taurine and cholesterol, abundant in fish meals. Replacing fish meal therefore also means paying attention to once overlooked nutrients present in fish meals and other animal products. Progress was highly dependent on a "balanced" understanding of the nutritional requirements of the animals and the nutritional composition of feed ingredients. This is discussed in the following section of this short paper.

The use of costly marine fisheries ingredients (fish meal, fish oil, squid meal, krill, etc.) is still considered essential in some feeds (e.g. salmon, marine fish and shrimp feeds) to ensure good growth performance and health of the animals. However, these ingredients are now used at relatively low levels (3-15%). They are considered mainly as functional ingredients and not major sources of nutrients.
There is also considerable interest by the industry to reduce the level of fish meal and fish oil derived from capture fisheries in order to reduce the perceived environmental footprint of aquaculture operations. Certification programs, such as the Global Seafood Alliance's Best Aquaculture Practices (BAP) or the Aquaculture Stewardship Council (ASC) require that certified feed manufacturers and aquaculture operations achieved a forage fish dependency ratio (FFDR) or a Fish In : Fish Out ratio (FIFO) below a certain imposed level (often below 1).
Consequently, there is also a strong appetite in "alternative" ingredients that could further reduce the reliance on fish meal, fish oil and other ingredients of marine origin. Despite their high costs, algal biomass and oils are increasing finding a role as "sustainable" sources of long chain omega-3 polyunsaturated fatty acids (DHA, EPA) in high quality feeds (e.g. shrimp, marine fish and salmon feeds) sold to "certified" aquaculture operations. There is hope that novel ingredients, such as fermentation products (e.g. single cell proteins) and insect biomass, could help further reduce fish meal levels in feed formulations. The main issue is that these ingredients are costly to produce and these need to be sold at prices that are higher than what high-quality fish meals are sold for. The use of these novel ingredients currently only makes sense as "functional" feed ingredient with low level of incorporation (1 to 5%) in high-end feeds. The market for high-end feeds is limited in volume since these only represent about 10% of the market, especially in Asia where the bulk of aquaculture production occurs. There is great hope for the wide adoption of these novel ingredients in mainstream aquaculture feeds but the reality in the field is far more contrasted.

The role of research and development (R&D) efforts

Aquaculture nutrition is a very dynamic field of research with hundreds, if not thousands, of academic research groups having contributed to the field over the past 50+ years. Despite the considerable progress made, more work is required to improve the cost-effectiveness of aquaculture feeds. The large number of papers published each year, the marked diversity in species cultivated and in the production conditions and the demands of end-users make it very difficult to develop a wholesome understanding of the state-of-the-art and what next steps are most critical.
Many aquaculture feed manufacturers, large or small, have invested heavily in R&D activities and have established own research facilities to test their commercial feed formulations, determine the effect of nutritional specifications and feed ingredients on performance of their species of interest grown under commercial-like conditions representative of their own market. This has resulted in improvement in the cost-effectiveness of the feeds available to aquaculture producers globally. However, limited amount of information from these efforts trickles down to the global aquaculture nutrition community since the information generated is generally proprietary and is closely guarded from public disclosure.
Publicly funded efforts have significantly contributed to integration of available information in a form usable by different industry stakeholders. The International Aquaculture Feed Formulation Database (IAFFD.com) is one such efforts. It is a free, online, resources developed by a consortium composed of the University of Guelph's Fish Nutrition Research Laboratory, Wittaya Aqua International (wittayaaqua.ca) and the United States Soybean Export Council (USSEC.org). The IAFFD contains tow main databases. A Feed Ingredient Composition Databases (FICD) with detailed information on the chemical composition and nutritive value of over 650 feed ingredients commonly used in the formulation of aquaculture feeds and an Aquaculture Species Nutritional Specifications Databases (ASNS) with nutritional specifications for 30+ commercially important aquaculture species at different life weight ranges. The databases were developed based on a review of the scientific and technical literature and the use of cutting-edge mathematical nutritional models developed at the University of Guelph over the past four decades.

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Transfer von phytogenen Stoffen in die Milch — Gibt es Grenzen?

Transfer of phytogenic substance into milk – are there limits?

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Abstract

Phytogenic substances (i.e. botanical preparations rich in secondary plant metabolites) have a long history in human and animal nutrition. Besides their beneficial use as, for example, feed additives in order to maintain animal health and/or improve performance, plant secondary metabolites, which constitute a health risk for humans, may unintentionally enter the food chain via transfer from feed into food of animal origin. Although case reports exist demonstrating such transfer of some harmful secondary plant metabolites, sound knowledge about the toxikokinetics of their transfer as a basis for quantitative risk assessment and conclusions regarding feed safety is scarce. This also includes knowledge about (de-)toxification of plant metabolites in the animal. However, quantification of the feed to food transfer is crucial to derive and recommend maximum levels in feed that would ensure food safety. In the past years, we and others have performed transfer studies with dairy cows focusing on the transfer of certain phytogenic substances from feed into milk. Here a few examples will be discussed focusing on a transfer of toxic secondary plant metabolites from feed in to milk, recent insights into their metabolism in the animal, attempts to set regulatory limits as well as future analytical and ethical challenges.

Einleitung

Kräuter und sogenannte Botanicals (i.e. aus Pflanzen gewonnene pflanzliche Stoffe und Zubereitungen) sind seit Jahrtausenden bekannt für ihre Wirkung auf die menschliche und tierische Gesundheit. Einerseits ist die positive Wirkung einer Vielzahl dieser Substanzen auf das Immunsystem, das Darmmikrobiom und die Verdauung, Futteraufnahme und Leistung bei z. B. landwirtschaftlichen Nutztieren durch eine große Zahl an wissenschaftlichen Untersuchungen belegt. Im europäischen Register der Futtermittelzusatzstoffe gemäß V (EG) 1831/2003 findet man eine lange Liste an Einträgen von pflanzlichen Sekundärmetaboliten als „Aromastoffe" und „zootechnische Zusatzstoffe". Andererseits ist auch die toxische Wirkung vieler phytogener Stoffe bei Mensch und Tier schon lange bekannt. Eine besondere Herausforderung besteht dann, wenn landwirtschaftliche Nutztiere diese toxischen phytogenen Stoffe mit der Nahrung aufnehmen und ein Teil davon in die tierischen Lebensmittel übergeht. Hier sind Grenzen erforderlich, um Risiken für die Gesundheit von Mensch und Tier zu minimieren. Dies erfordert jedoch neben Kenntnissen über das Vorkommen der toxischen Substanz selbst und ihre toxische Wirkungsweise im Organismus auch Kenntnisse zur Toxikokinetik (Absorption im Darm, Verteilung in Gewebe, Metabolisierung, Ausscheidung (z. B. über die Milch)). In den seltensten Fällen ist die Datenlage bei landwirtschaftlichen Nutztieren hierfür jedoch ausreichend. Am Beispiel des Transfers von potenziell gesundheitsschädlichen pflanzlichen Sekundärmetaboliten aus dem Futter in die Milch von Kühen soll dies im Folgenden näher beleuchtet werden.

Transfer von Pflanzentoxinen in die Milch

Der Transfer von potenziell gesundheitsschädlichen pflanzlichen Sekundärmetaboliten aus Futtermitteln in die Milch ist keine neue Erkenntnis. So trat beispielsweise bereits Anfang des 19. Jahrhunderts im mittleren Westen Nordamerikas die sogenannte „milk sickness" bei Siedlern auf, verursacht durch die

Aufnahme und den Transfer in die Milch von den in *Ageratina altissima* vorkommenden Benzodihydrofuran-Derivaten (Tremeton) durch laktierende Rinder (Liener, 2002). Es dauerte mehrere Jahrzehnte ehe die Substanz(gruppe) als Ursache identifiziert wurde, deren chemische Charakterisierung offenbar aber auch heute noch nicht abgeschlossen ist (Lee et al., 2010). Ein anderes Beispiel für ein erst seit neuerem Bekanntes Pflanzentoxin ist der Transfer des im Adlerfarn (*Pteridium aquilinum*) vorkommenden Ptaquilosid bzw. dessen Metabolit in die Milch von Kühen, Schafen und Ziegen (Alonso-Amelot et al., 1996, Virgilio et al., 2015, Aranha et al., 2019). Dabei ist wichtig, dass die in der Pflanze vorkommende Substanz zunächst nicht direkt das toxikologische Risiko darstellt, sondern ein instabiles Intermediärprodukt (in dem Fall das genotoxisch-kanzerogen wirkende Dienon). In den zuvor genannten Studien wurde analytisch das aus dem Dienon entstehende, stabile Abbauprodukt Pterosin B in der Milch analysiert. Eine quantitative Abschätzung des Transfers des Dienons in die Milch ist bislang nicht erfolgt, so dass eine tatsächliche Risikobewertung auf der Basis einer Expositionsschätzung nicht möglich ist. Dieses Beispiel verdeutlicht ein gewisses Dilemma bei der Ableitung von gesundheitlichen Risiken durch potenziell toxische pflanzliche Sekundärmetabolite in der Milch, wenn die eigentlich wirksame Substanz nicht analysiert werden kann bzw. ierst m Organismus entstehende Metabolite toxisch wirken. Ein weiteres Beispiel ist der Transfer von Tropanalkaloiden (z. B. aus *Datura stramonium, Hyoscyamus niger*) in die Milch (Lamp et al., 2021). Für die Alkaloide Scopolamin und Atropin wurden in dieser Studie zwar relativ niedrige Transferraten (Verhältnis von Gesamtausscheidung/Gesamtaufnahmemenge/Tag) von 0,007 % bzw. 0,037 % festgestellt; angesichts vermehrter Berichte zum Vorkommen von *Datura stramonium* z. B. in Maisbeständen/-silage könnte dies jedoch zu nicht unerheblichen Konzentrationen in der Milch führen (Aboling et al., 2019). Anderseits verdeutlicht die Studie von Lamp et al. (2021) auch die Herausforderungen im Bereich der Analytik: Atropin liegt in der Pflanze als racemisches Gemisch aus D-/L-Hyoscyamin vor, wobei nur das L-Hyoscyamin eine pharmakologische Wirkung hat. Eine Beurteilung des Transfers auf der Basis des Gesamt-Atropins ohne Unterscheidung der Racemate kann daher schnell zu einer Überschätzung des Transfers und des Gesundheitlichen Risikos für Verbraucher*innen führen. Hier sind zukünftig (wie bei vielen anderen Pflanzentoxinen auch) die analytischen Nachweismethoden von großer Bedeutung. Es existieren aber auch Beispiele, die den Transfer eines gut charakterisierten Pflanzentoxins (i.e. Colchicin) in die Milch analytisch klar nachwiesen, jedoch aufgrund der offenbar gleichzeitig stark hemmenden Wirkung auf die Milchbildung im Nutztier eine signifikante Verbraucherexposition mit kontaminierter Milch unwahrscheinlich ist (Hamscher et al., 2005). Das Bundesinstitut für Risikobewertung und andere Einrichtungen, die sich mit der Sicherheit von Lebens- und Futtermitteln beschäftigen, haben in den vergangenen Jahren eine Reihe von gezielten Transferstudien mit Milchkühen zum Transfer von potentiell toxischen sekundären Pflanzeninhaltsstoffen in die Milch und *in vitro* Untersuchungen durchgeführt. Drei sekundäre Pflanzeninhaltsstoffe und neueste Forschungserkenntnisse stellen wir hier kurz vor:

Pyrrolizidinalkaloide

Pyrrolizidinalkaloide (PA) und deren *N*-Oxide umfassen eine Gruppe von ca. 600 Verbindungen und werden von einer Vielzahl unterschiedlicher Pflanzenarten gebildet. Bekannt sind hier vor allem die Kreuzkräuter (z. B *Senecio jacobaea, S. vernalis, S. inaequidens, S. vulgare*), welche in den letzten Jahren und in einigen Regionen teilweise massenhaft im Grün- und Ackerland aufgetreten sind. Toxikologisch für Mensch und Tier relevant sind die 1,2-ungesättigten PA, welche aufgrund der genotoxisch-kanzerogenen Potenz als Gruppe äquipotenter Stoffe mit additiver Wirkung angesehen werden (BfR, 2020). Kenntnisse über die toxische Wirkung bei Nutztieren sowie den möglichen Transfer von PA aus dem Futter in die Milch basierten lange Zeit entweder auf vereinzelten Fallberichten oder auf alten Dosisversuchen. Die Ergebnisse zeigten eine derart hohe Variabilität, dass eine Dosis mit negativen Effekten bei Wiederkäuern nicht abgeleitet werden konnte (Wiedenfeld & Edgar, 2011). Gezielte Transferstudien holländischer Kollegen haben jedoch gezeigt, dass einige PA aus *S. jacobaea* und anderen PA-bildenden Pflanzen, wenn diese unter experimentellen Bedingungen direkt in den Pansen von Milchkühen verabreicht werden, in die Milch übergehen können (Hoogenboom et al., 2011, Mulder et al., 2020). Trotz der für die meisten PA sehr niedrigen Transferraten (ca. 0,05 % für alle untersuchten PA), konnte gezeigt werden, dass diese für einzelne PA (i.e. Jacolin) deutlich über 1 % lagen. Aufgrund der genotoxisch-kanzerogenen Potenz dieser Substanzen sollte dem möglichen Transfer unter praktischen

Bedingungen eine verstärkte Aufmerksamkeit zukommen. Untersuchungen über das Vorkommen in Grundfuttermitteln (Gottschalk et al., 2015), den Abbau während der Silierung (Klevenhusen et al., 2019), sowie die Biotransformation im Pansen *in vitro* (Tänzer et al., 2020) sind dabei ein erster Ansatz.

Chinolizidinalkaloide

Chinolizidinalkaloide (englisch: Quinolizidinalkaloides, QA) sind eine Gruppe von Pflanzentoxinen, die vor allem in Leguminosen vorkommen. In der Tierernährung sind vor allem die in Lupinen vorkommenden QA von Bedeutung. Obwohl die heute in der Tierernährung eingesetzten Süßlupinen einen geringen QA-Gehalt aufweisen (0,02 – 0,5 %) im Vergleich zu den bitteren Varianten (5-8 % QA-Gehalte), können Einflüsse wie Sorte, Anbaujahr und (Hitze-)Stress zu deutlichen Variationen im QA-Gehalt führen. Höchstgehalte existieren nicht, es gibt lediglich Empfehlungen für maximale QA-Gehalte in Süßlupinen für den Einsatz in der Human- und Tierernährung (0,02 bzw. 0,05 %). QA zeichnen sich durch ihre hemmende Wirkung auf Acetylcholin-Rezeptoren aus und können als klinische Symptome kardiovaskuläre und Verdauungsprobleme hervorrufen (EFSA, 2019). Obwohl die Wirkung insbesondere für das Alkaloid Spartein beschrieben ist, wird in der toxikologischen Bewertung, ähnlich wie bei den PA, ein sogenannter Gruppenansatz verwendet, der allen anderen QA ein äquipotentes toxisches Potenzial wie dem des Sparteins zuschreibt (EFSA, 2019). Das erschwert in gewisser Weise die toxikologische Bewertung von z. B. QA in blauen Süßlupinen, welche nur sehr geringe Konzentrationen von Spartein, dafür jedoch andere QA (Lupanin, 13-Hydroxylupanin, Angustifolin) enthalten. Ein Transfer von QA aus Lupinen in die Milch war bis vor kurzem noch nicht beschrieben; indirekte Hinweise aufgrund ihrer chemischen Eigenschaften sowie aus Fallberichten ließen jedoch einen Transfer vermuten (EFSA, 2019). Eine Studie am Bundesinstitut für Risikobewertung mit Milchkühen, welche unterschiedliche Mengen an blauen Süßlupinen (mit einem Gesamt-QA-Gehalt von ca. 0.2 %) in der Ration erhielten zeigte, dass ein für diese Stoffgruppe nicht unerheblicher Transfer von 1 bis zu 5 % in die Milch möglich ist, ohne dass dabei negative Effekte auf die Tiergesundheit oder Leistung beobachtet wurden (Engel et al., 2021). Da für das Spartein ein für die menschliche Gesundheit toxikologischer Referenzwert abgeleitet wurde, ermöglicht dies bei Berücksichtigung der mittleren Transferraten und einem unterstellten Verzehr von Milch durch Verbraucher*innen die Abschätzung maximal zulässiger täglicher Aufnahmemengen von QA für Milchkühe.

Cannabinoide

Die ursprünglich aus Mittelasien stammende Hanfpflanze (*Cannabis sativa*) ist eine der ältesten Nutzpflanzen der Welt. Ihr Anbau erfolgte in der Geschichte hauptsächlich zur Gewinnung von Hanffasern aus dem Stängel der Pflanze sowie zur Produktion von Hanfsamen für die unterschiedlichsten Einsatzzwecke einschließlich der Verfütterung an Tiere. Die Hanfpflanze produziert circa 100 verschiedene Cannabinoide, darunter auch Δ9-Tetrahydrocannabinol (Δ9-THC), Cannabidiol (CBD), sowie deren Säurevorstufen enthalten sind. Legal angebauter, sogenannter „Nutzhanf" darf einen Δ9-THC-Gehalt von maximal 0,3 % aufweisen und muss aus zertifiziertem Saatgut stammen. Nicht zuletzt aufgrund von (über)regionaler Futterknappheit wurde in den letzten Jahren vermehrt über den Einsatz von Nutzhanf-Ganzpflanzen als Futtermittel für Milchkühe berichtet. Aufgrund eingeschränkter bzw. fehlender Daten zum Übergang von Δ9-THC und anderen Cannabinoiden aus dem Futter in Lebensmittel tierischer Herkunft und der oftmals fehlenden Differenzierung zwischen Δ9-THC und seiner Vorstufe Δ9-THCA war bislang keine reale Risikobewertung des Einsatzes von Nutzhanf in der Fütterung landwirtschaftlicher Nutztiere möglich (EFSA, 2015). Schätzungen auf der Basis von Fallberichten ließen auf eine Transferrate von ca. 0,15 % für Δ9-THC schließen (EFSA, 2011, 2015). Am Bundesinstitut für Risikobewertung wurde aus diesem Grund eine Fütterungsstudie mit Milchkühen durchgeführt, welche unterschiedliche Mengen an Nutzhanf (Gesamt-Δ9-THC-Gehalt: < 0,12%) in der Ration erhielten (Wagner et al., 2020). Dabei konnte nicht nur die Transferrate von ca. 0,2 % für Δ9-THC festgestellt werden, sondern es wurde auch deutlich, dass bereits die Verfütterung von 1-2 kg TM der Nutzhanfsorte an Milchkühe massive Effekte auf die Tiergesundheit hat (Wagner et al., 2020). Diese Erkenntnis und die Bestimmung einer Transferrate für Δ9-THC aus dem Futter in die Milch lassen unter Zuhilfenahme der bereits für die menschliche Gesundheit abgeleiteten akuten Referenzdosis (ARfD) von 1µg/kg KGW und Annahmen für den Milchverzehr Rückschlüsse auf maximal zulässige Gehalte in der Ration für Milchkühe zu.

Fazit

Der Transfer von toxischen pflanzlichen Sekundärmetaboliten aus dem Futter in die Milch stellt aktuell und zukünftig angesichts klimatischer Veränderungen in Mitteleuropa eine größer werdende Herausforderung an die Wissenschaft und landwirtschaftliche Praxis dar. Neben der Identifikation und quantitativen Bewertung möglicher Risiken stehen wir auch vor großen Herausforderungen hinsichtlich der Analytik dieser Substanzen. Interessant ist in diesem Zusammenhang das im Lebensmittelbereich bereits etablierte und ständig aktualisierte „Compendium of botanicals", einer Datenbank mit Botanicals, welche Substanzen mit möglichen negativen Auswirkungen auf die menschliche Gesundheit enthalten (EFSA, 2012). Allerdings können nur dann Grenzen im Sinne von Orientierungswerten bzw. toxikologisch abgeleiteten Höchstgehalten in Futtermitteln gesetzt werden, wenn Kenntnisse zur Toxizität und Toxikokinetik im Nutztier, einschließlich des Transfers in die Milch, sowie zum Vorkommen in Futtermitteln existieren, denn die Exposition (also die aufgenommene Dosis) ist entscheidend für das Risiko. Angesichts der toxischen Wirkung unterschiedlicher Pflanzeninhaltsstoffe im Tier, sollten neben dem klassischen Fütterungsversuche Alternativen zu *in vivo* Untersuchungen vermehrt genutzt werden (Klevenhusen et al., 2021b).

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Effect of enriching cows' diet with microalgae *Chlorella Vulgaris* on the protozoal community in the rumen

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Abstract

The rumen is a complex ecosystem where feeds consumed by animals are digested with the help of rumen ciliates, which play an important role in contributing nutrients to the host animal. This study focused on investigating the effect of microalgae Chlorella vulgaris dietary inclusion on the protozoal community in the rumen and qualitative parameters of the ruminal fluid. This study tested dietary treatments during three 21-d experimental periods, with the first 14d for an adaptation period. Each period contains a different amount of microalgae supplement. In the first trial period in the cows' diet was added 30g (3,14g/kg of DM) of Chlorella, in the second period-90g (9,6g/kg of DM) of algae and third period-170g (18,7 g/kg of DM). The rumen content of each animal was collected for pH measurement as well as enumeration of rumen protozoa. As a result of this study, ciliates of 10 genera were identified and detected. The number of protozoal families and the total amount of ciliates were counted. Besides, the motility of ruminal ciliates was observed. The rumen pH level was affected by the period of sampling, but there was no significant impact of algal diet treatment on rumen pH seen. The pH values the same as physical characteristics (color, odor, consistency, sedimentation time) in rumen fluid samples were within the normal range. In conclusion, dietary Chlorella supplementation affects both the total number of rumen protozoa and the distribution of particular genres in the protozoal population in the rumen.

Introduction

The rumen hosts a large scale of microorganisms that play an essential role in maintaining the stability of rumen ambiance and the health of the animals (Castillo-González et al., 2014). Rumen ciliates represent approx. 50 – 80 % of the vial ruminal biomass and are significantly contributed to the digestion of ruminants; in particular, they degrade around 20 % of the total amount of proteins received by the host animal (Purevtsogt et al., 2016). The total number of ciliates in rumen fluid varies from 10^4 to 10^7 ml^{-1} and depends on the composition of the feeding ration and fermentation level in the rumen(Newbold et al., 2015) . Microalgae are a rich source of many vitamins, minerals, antioxidants, proteins, and fats. Their biomass contains almost all of the essential amino acids, such as valine, leucine, lysine, phenylalanine (Ramos-Suárez et al., 2014). Therefore, microalgae have a significant potential for application in humans and animal nutrition. In addition, microalgae are able to convert solar energy efficiently, are not affected by external environmental conditions, and produce more per unit area than traditional crops (Priyadarshani & Rath, 2012). Ruminants are suitable animal models for feeding with microalgae since they possess the ability to digest an unprocessed microalgae cell wall (Madeira et al., 2017).

Material and methods

Our study used four fistulated cows of the beef breed to evaluate the effect of the enriched basal ration with microalgae Chlorella Vulgaris on ruminal fermentation. The cows were fed once daily in the morning with a basic feed ration consisting of hay (11±5kg), and cereals concentrate "Biostan" (1.2 kg/pc) (BD – basal diet). Water and trace mineral salt was available free choice. The experimental setup involved 14 days without algal supplementation to harmonize the microflora of the rumen and 21 days of dietary algal supplementation repeated in 3 experimental regimes for all cows. Tested cannulated cows were

divided – the first cow was the control (CON) and had diet without microalgae supplementation; the second three animals were the experimental group. Experimental diets consisted of BD with lyophilized microalgae Chlorella Vulgaris, which was supplemented in different amounts. The first diet (ALG - 1) included 30g (3,14g/kg of DM) of algae, in the second diet (ALG - 2) was added 90g (9,6g/kg of DM) of Chlorella, and in the third diet (ALG - 3) was added 170g (18,7 g/kg of DM) of Chlorella. Rumen liquor samples were collected two times per week, three hours after morning feeding via a rumen cannula with a probe connected to a vacuum pump. The samples were immediately transported in a water bath (39°C) to the laboratory for analysis. Samples were filtered through the synthetic cloth (119µm, Uhelon 59 S, Silk & Progress). Sample filtration helps to remove larger particles from the sample and makes the visualization of the ciliate's protozoa easier than without filtration (Dehority, 2017). Immediately after collection, the ruminal fluids were examined for physical characteristics such as color, odor, consistency. Also, sedimentation activity tests were carried out by putting the sample of rumen fluid into a test tube and allowing it to stand. The sedimentation time of fine particles in all samples ranged between 8 – 9 minutes throughout the study. The protozoal activity was examined by placing one drop of ruminal fluid on a pre-warmed microscope slide, and a coverslip was placed. The motility of the protozoa was tested in a fresh film of the rumen liquor under low magnification and was graded in four categories: 4- high motility and very crowded: >10 mobile protozoa per one field in the Bürker chamber; 3- medium motility: 6-9 mobile protozoa per chamber square; 2- subnormal: 3-5 mobile protozoa per square; 1- very low motility: <3 mobile protozoa per square.

Table 1. Grading of protozoal motility observed under a microscope

Diet treatment	**Motility score**
Control (basal diet)	+++
Chlorella 30 g	+++
Chlorella 90 g	++++
Chlorella 170 g	++++

Our results showed that the highest motility of the protozoa in the cow's rumen was observed during feeding animals with treatment included Chlorella 90 g, which correlates with results achieved during microscopic counting of the total amount of ciliates. The fixing rumen content samples were preserved in 18,5% formaldehyde solution and stained with Brilliant Green Dye, then samples were mixed and allowed to stand overnight. The density of the rumen protozoa per mL was obtained using a Bürker counting chamber in an optical microscope at magnification 40x. The microscopic examination of protozoa is widely accepted as a golden standard for analyzing ciliate community structure. It can provide useful information about the physiological processes associated with animal nutrition in the rumen. The density and generic composition of the protozoa in samples were identified according to criteria described by Imai & Ogimoto (Ogimoto & Imai, 1981), Baraka T.A. (Baraka, 2012), and Burk A. Dehority (Dehority, 2017). The pH of ruminal fluid was measured immediately after collecting the samples (EUTECH CyberScan PC510 pH/Conductivity Bench Meter, USA). A small amount of rumen fluid was measured on ammonia concentration, and 50 ml of liquor was stored and frozen for the following analysis of VFA using a two-capillary isotachophoresis analyzer (EA 102, Villa Labeco, SK).

Results and discussion

The mean of the ruminal pH, as shown in Figure 1, ranged between 6.26 to 7.08 and was affected by the period of sampling. Different treatment diets had no significant impact on pH in the cow's rumen. However, the ALG fed group had a slightly lower rumen pH value pattern than the control animal. Nevertheless, the pH values in all dietary treatments were within the normal range of ruminal pH values. Krause and Oetzel (Krause & Oetzel, 2006) suggested that minimum ruminal pH values measurement may improve the probability of detecting treatment differences over mean ruminal pH values when continuous pH measurement is used. The effect of dietary microalgae supplementation on the rumen pH did not differ significantly among the treatments and ranged from 6.26 to 7.08 throughout the study. This result suggests that the rumen microbial population can adapt to the diet given, regardless of the addition and difference in composition of the algae supplemented.

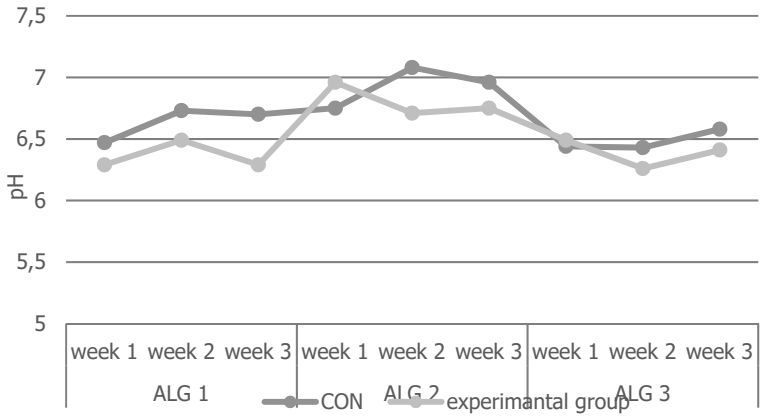

Figure 1. Effect of pH value in rumen fluid of cows supplemented with dietary microalgae Chlorella Vulgaris at different weeks. (CON-basal diet without algae included. ALG 1 – basal diet + 30 g (3,14 g/kg of DM – dry matter) of Chlorella included; ALG 2 – basal diet + 90 g (9,6 g/kg of DM) of Chlorella included; ALG 3 – basal diet + 170 g (18,7 g/kg of DM) of Chlorella included)

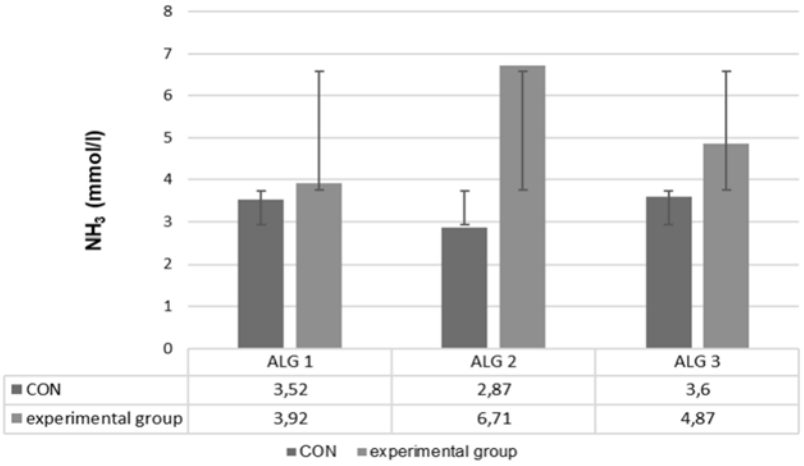

Figure 2. The amount of ammonia (NH3) in the ruminal fluid of cows supplemented with microalgae Chlorella Vulgaris at different experimental periods. (CON-basal diet without algae included. ALG 1-Chlorella 30 g diet treatment; ALG 2- Chlorella 90 g diet treatment; ALG 3- Chlorella 170 g diet treatment)

Figure 2 shows the average amount of NH_3 in rumen fluid of the control animal and experimental group, which were fed with the basal diet included a different amount of Chlorella supplemented. To evaluate rumen fermentation and multiplication of microorganisms themselves, the content of ammonium ions is an important measure.

The total protozoal counts in rumen fluid in cows fed with a diet ALG 2 and ALG were significantly higher than the control group, which means that the total number of ciliates was considerably affected by the algal dietary treatments. During this study, the following genres of ruminal ciliates were observed and also their percentage composition was counted: genera of Entodinium (CON:51,5 %; ALG1:59%; ALG2:35%; ALG3:44%), g.Dasytricha (CON:30,3%; ALG 1:21%; ALG 2:33%; ALG 3:26%), g.Charonina (CON:6%; ALG 1:9,3%; ALG 2:7,7%; ALG 3:5%), g.Isotricha (CON:5%; ALG 1:3,7%; ALG 2:12%; ALG 3:12,2%), g.Ostrachodinium (CON:3%; ALG 1:3,7%; ALG 2:4%; ALG 3:3,4 %), g.Diplodinium (CON:4,6%; ALG 1:1,8%; ALG 2:3,7%; ALG 3:8,2%), g.Buetschlia (CON:1%; ALG 1:1,1%; ALG 2:3,2%; ALG 3:2%), g.Epidinium (CON:0,4%; ALG 1:0,1%; ALG 2:0,6%; ALG 3:0,9%), g.Ophrioscolex (CON:0,1%; ALG 1:0,2%; ALG 2:1,5%; ALG 3:1,65%).

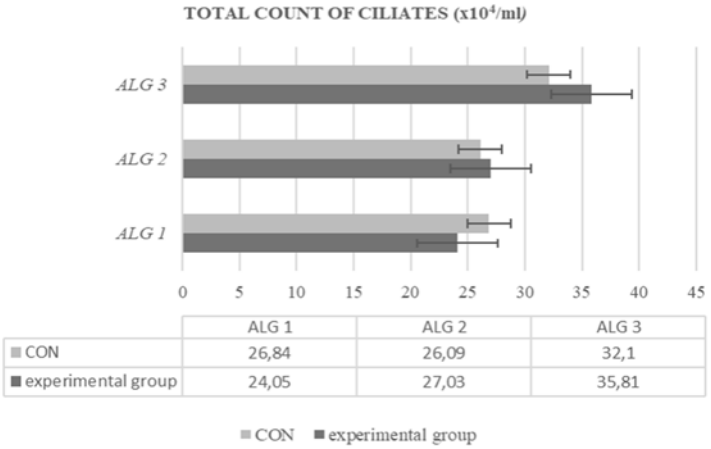

Figure 3. Effect of algal diet inclusion on protozoal density in the rumen (left side)

Conclusion

The analysis of the rumen protozoa population shows a visible effect of the presence of microalgae in cows' diet. In particular, the density and motility of ciliate protozoa of the cows treated with 90 and 170 g of the algal supplement were higher than the control diet. The amount of ammonia in rumen fluid was also increased during feeding with Chlorella inclusion, which can be explained by the contribution of particular protozoal genera to ammonia formation. Microalgae-based supplement diet had a stimulative effect on the ruminal protozoa population and caused increasing in many protozoa families such as Isotricha, Charonina, Buetschlia, Ostrachodinium, Ophryoscolex, Epidinium. It appears that the algal diet supplementation somehow created a more favorable environment in the rumen for efficient microbial growth and thus increased nutrient ability to the host.

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Natural betaine versus betaine hydrochloride as dietary supplement in broiler chickens

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Abstract

Betaine acts as an osmolyte and as a methyl donor in animal nutrition. The present study aimed to compare the effect of dietary betaine (natural and synthetic) on gut integrity and permeability in broiler chickens. Birds were randomly assigned into three treatments: control, natural betaine and synthetic betaine-HCL (1 kg active Betaine /ton of feed). Overall, the supplementation of natural betaine improved the general performance of broilers. At the end of the experiment (d 35), birds supplemented with the natural betaine had a greater ($P < 0.05$) body weight compared to the other two groups. In addition, betaine supplementation increased villus height, apparent surface area and villus height: crypt depth ratio at different time points. Finally, betaine supplementation did not compromise the barrier function in the gut, thereby supporting gut integrity and health. Altogether, the dietary inclusion of natural betaine had a positive effect on performance and had no negative impact on gut paracellular permeability.

Introduction

Betaine is a multi-functional nutrient, acting as the most efficient methyl group donor and as an organic osmolyte, with direct influence on the functionality and health of the gastrointestinal tract (GIT). Betaine, as a methyl group donor- provides methyl groups for synthesis of numerous substances, intermediates in protein and energy metabolism, and it is involved in DNA methylation. Furthermore, as an organic osmolyte it contributes maintain the intestinal/ mucosal barrier reduce epithelial permeability. It also protects proteins (e.g. tight junctions) and enzymes against inactivation and is involved in restoring and maintaining the cellular integrity. Natural betaine is primarily obtained from sugar beet molasses, but other sources of betaine produced by chemical processes, such as betaine hydrochloride (HCl), are also available.

In poultry production, betaine can play a role in improving performance and carcass composition, reducing litter moisture as well as helping to overcome coccidiosis and stress (Kettunen et al., 2001). Similar to unfavorable conditions (infections and heat stress), betaine may reduce digestive disorders and mortality – thereby improve production efficiency. Furthermore, it was reported that dietary betaine supplementation is capable to improve the productive performance and reduce the negative impact of heat stress on viability and immune response by improving cell osmoregulation (Wang et al., 2004; Attia et al., 2005; Santosa et al., 2019). Several studies showed that betaine had the ability to improve bird performance, however, how betaine exert this positive effect is not known. Furthermore, there is limited data in scientific literature comparing betaine-HCl and natural betaine. Therefore, an animal experiment was conducted to ascertain the effects of betaine supplementation (natural and synthetic) on performances and intestinal physiological responses of broilers.

Material and methods

Hundred and five 1-day-old broiler chicks were randomly assigned into three groups (35 birds/group): control, natural betaine (1 kg active Betaine/ton of feed, respectively 1000 mg of Betaine / kg of feed) and synthetic betaine-HCL (1 kg active Betaine /ton of feed, respectively 1000 mg of Betaine/ kg of feed). Body weight (BW) was determined at first day of life and then every week until 35 days of age. The body weight gain (BWG) was calculated as the difference between the final and initial bird weight during each of the weighing periods. Furthermore, feed intake over the course of the experiment was measured for control and infected birds and consequently feed conversion ratio (FCR) was calculated. At 21, 28 and 35 days of age, five birds from each group were euthanized by injection of thiopental (20 mg/kg) into the wing vein and by bleeding of the jugular veinfor necropsy and sampling. Furthermore, six birds/ group were killed between 21-28, 28-35, and 35-42 days of age, respectively, for Ussing chamber measurements.

For histomorphological examination, tissue samples were taken from jejunum close to the junction of Meckel's diverticulum and cecum. The samples were fixed in 4% formaldehyde. Tissue cross-sections (five per bird, 5 µm thick) from each of five birds per treatment were cut by a microtome and fixed on slides. A routine staining procedure was carried out using hematoxylin and eosin. The sections were inspected under Olympus BX53 microscope and documented with an Olympus DP72 camera (Olympus Corporation, Tokyo, Japan).

For Ussing chamber analysis, intestinal segments were taken from the mid-jejunum and cecum immediately after killing of birds (6 birds/group). The intestinal segments were directly placed into ice-cold buffer solution. After tissues preparation, the epithelial sheets were mounted in Ussing chambers. Flux rates of mannitol (J_{man}), were measured at a bilateral concentration of 10 mM. The radioactive tracer, ^{14}C-mannitol, was added to the mucosal solution. This allows calculating J_{man} ms from the time-course of serosal appearance of tracer measured by scintillation counting. Changes in the transepithelial flux of the small hydrophilic mannitol largely reflect changes in the permeability of the paracellular pathway.

Results and discussion

The initial BW of chicks did not differ ($P > 0.05$) between the groups. At the end of the experiment (d 35), there were no significant difference between control birds and birds supplemented with betaine-HCL. However, the body weight and weight gain were significantly ($P < 0.05$) increased by the dietary inclusion of natural betaine compared with other treatments. Histological assessment showed lower jejunal crypt depth and villi height/crypt depth ratio in betaine-HCL group compared with natural betaine fed birds, whereas jejunal crypt depth and villi height/crypt depth ratio were bigger. Furthermore, it was found that betaine-HCL negatively affects the integrity of the intestine by an increased intestinal paracellular permeability as evidenced by a higher mannitol flux in both jejunum and cecum.

Conclusion

Altogether, it can be concluded that natural betaine, versus betaine hydrochloride (chemical synthesis), has the potential to improve the productive performance and health status of broiler chickens. The results suggest that natural betaine could be a promising feed additive to promote the intestinal health by supporting intestinal integrity and modulating microbial fermentation, as it serves as a methyl donor and a direct substrate for gut microbes (Santos et al., 2019).

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Impact of processed wheat bran on meat quality of broiler chickens

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Abstract

The aim of this study was to evaluate the influence of extruded and fermented by *Lactobacillus casei* and *Lactobacillus paracasei* wheat bran in the diet on productivity and production quality of broiler chickens (BC). The BC were reared up to 40 days of age. The BC were randomly distributed to 2 groups: control group (CON group) – was fed by basic compound feed. The 3% of basal were replaced by extruded and fermented WB in the diet of treatment group (WB group) and fed for 15 days of fattening. After 15 days, the broilers of WB group received basic compound feed. The following productivity parameters were evaluated: body weight (BW), feed conversion ratio (FCR) and mortality of broiler chickens. The carcass yield and meat quality parameters, such as dry matter (DM) content, pH, colour, drip loss (DL), water-holding capacity (WHC), cooking loss (CL), tenderness, protein, ash, and intramuscular fat content of breast meat were determined. In addition of fermented and extruded WB, the tendencies of the broiler's BW and FCR increasing (by 4%) were established ($p \geq 0.05$). It was found, that in WB group, the carcass yield by 5%, thigh and drumstick by 2% was improved, compared with control group ($p \geq 0.05$). Also, tendencies of the breast meat WHC by 3 %, redness by 4% and fat 1 % increasing was found, compared with control group ($p \geq 0.05$). Finally, extruded and fermented WB in diet of BC showed positive tendencies on poultry production and meat quality, however, more research is needed.

Introduction

Wheat bran (WB) is a cereal by-product produced by wheat grain processing and consists of the botanical bran and the aleurone with some residual endosperm attached to it (Roye C. et al., 2020). WB is a complex composed mainly of dietary solube and insolube fiber, proteins, enzymes, phytic acid, betain, choline, minerals and trace elements, antioxidants and vitamins (Annalisse B. et al., 2020; Semjon B. et al., 2020). Despite the physiological and nutritional benefits of WB, bioactive compounds are entrapped inside rigid aleurone cell walls which withstand digestion in the gastrointestinal tract (GIT), limiting their absorption (Roye C. et al., 2020). Only the unbound fraction is absorbed in the GIT and can exert its beneficial effects to the host. High fiber content of WB limits their use in poultry feed as it reduces the digestibility of nutrients, increase intestinal viscosity, reducing the passage rate of the digesta through the GIT (Tejeda J. and Kim K., 2021). WB can be converted into value-added products and animal feeds by using the process of extruction and fermentation. Extrusion process involves a combination of high shear, temperature and pressure, leading to physico-chemical, structural and microbial alterations of food materials (Ayua O., 2021). It results in the opening of aleurone cells, denaturation of proteins, oxidation of lipids, destruction of bacteria and solubilisation of arabinoxylans

(Bender A.B.B. et al., 2019; Roye C. et al., 2020). In previous studies, the extent effects of extrusion were shown to depend on the applied process parameters such as moisture contents, set temperature, screw speed and configuration design (Roye C. et al., 2021). Fermentation with selected lactic acid bacteria (LAB) strains is commonly processing technique for wheat bran, leading to increased content and bioavailability of nutrients and degradation of anti-nutritive compounds (Verni M. et al., 2019; Spaggiari et al., 2020; Zokaityte et al., 2021). Moreover, the fermentation with selected LAB and extrusion leads to lower mycotoxin and biogenic amines content as well as microbial contamination of WB (Zokaityte E. et al., 2021). Studies about extruded or fermented WB are mainly focus on the digestive processes, intestinal morphology, microbiota and production parameters of poultry (Feng Y. et al., 2020; Wanzenböck E., 2020). However, there are no data on the effects of dietary supplementation of extruded and fermented WB on meat quality of broiler chickens. This study aimed to determine the effect of dietary supplementation of extruded and fermented with selected LAB WB on carcass yield and meat physico-chemical parameters of broiler chickens.

Material and methods

All animal procedures were conducted according to the EU Directive 2010/63/EU (The Protection of Animals Used for Scientific Purposes). The study was performed at the facilities of the Institute of Animal Rearing Technologies of Lithuanian University of Health Sciences (Kaunas, Lithuania). A total of 42,000-day-old *Ross 308* broiler chickens were fattened for 40 days. Birds were randomly assigned to 2 groups: control group (CON group) – was fed by basic compound feed. The 3% of basal were replaced by extruded (at 130 °C by two different speeds of the extruder screw (20 and 25 rpm)) and fermented by *Lactobacillus casei* and *Lactobacillus paracasei* WB in the diet of treatment group (WB group) and fed for 15 days of fattening. After 15 days, the broilers of WB group received basic compound feed. Unprocessed and extruded WB were obtained from SME "Ustukiu malunas" (Pasvalys, Lithuania). A phase feeding (starter, grower and finisher) was applied. A soybean meal and corn-based diet (basic compound feed) and was formulated according to NRC (1994) and the *Ross* nutrition specification (2019). The broiler chickens (BC) were kept on deep litter. Drinking water and compound feed were available *ad libitum* throughout the trial.

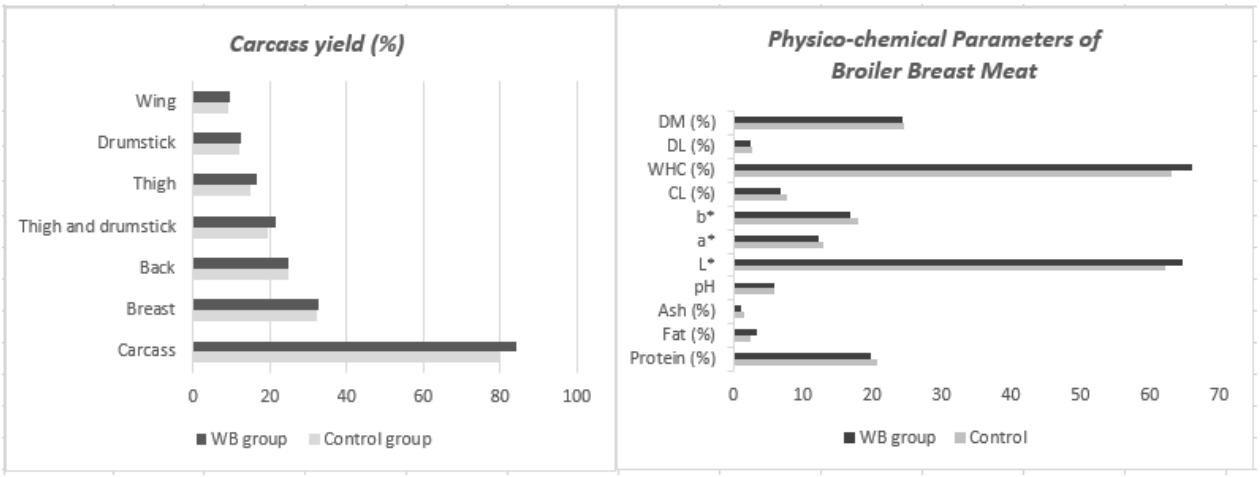
Body weight (g), average daily feed intake (g) and mortality (%) were recorded for the entire period for each treatment; the feed conversion ratio (FCR) (g/kg) was calculated on the basis of feed intake to body weight gain.

At the end of the trial, 10 chickens with similar body weights from each group were selected for carcass trait evaluation as described by Marché (2000). The chemical, physical and technological characteristics of breast meat samples were evaluated by methods described by Mozuriene et al. (2016).

SPSS software version 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. The analysis of variance was used to determine whether significant differences existed between means. Results were considered statistically significant at $p \leq 0.05$.

Results

The results of broiler chicken's production performance showed, that the BW and FCR of broilers was increased by 4% in supplementation of extruded and fermented WB, compared with control group ($p \geq 0.05$). The addition of processed WB had no effect on mortality of BC. There were not any significant differences due to application of WB, but slight effect on yield of carcass (by 5%), thigh and drumstick (by 2%) evaluated, compared with control group (Figure 1). Quality parameters of the broiler chicken's breast meat was not significantly influenced by WB supplement, but WHC by 3 %, redness by 4% and fat 1 % was increased, compared with control group ($p \geq 0.05$).



Abbr.: DM-dry matter; DL - drip loss; WHC - water-holding capacity; CL - cooking loss; L*- lightness; a*-redness; b*-yellowness

Figure 1. Effect of extruded and fermented WB on meat quality of broiler chickens

Discussion

The introduction of extruded and fermented by *Lactobacillus casei* and *Lactobacillus paracasei* contributed to increasing the BW of BC. This may be related to better development of GIT. Wu S.Q. et al. (2020) observed an enhancement in nutrient digestibility by improving antioxidant status, gizzard development, intestinal digestive enzyme activities and morphology in broilers fed extruded WB. The growth performance and gut morphology was ameliorated in broiler chickens after extruded WB administration for 35 days (Li B. et al., 2018). We found, that WB had no beneficial effect on FCR – it increases by 4%. Semjon B. et al. (2020) found, that addition of 10% of fermented WB showed both a lower average daily feed intake and total feed consumption. The slight effect of WB on meat chemical, physical technological attributes as well as carcass yield was found in our study. As it was stated by Kim C.H. and Kang H.K. (2016), the addition of fermented WB by *Lactobacillus plantarum* KCTC 1048 and *Bacillus subtilis* ATCC 21322 in the diet of BC had no effect on breast meat DL and WHC, content of moisture, crude ash, crude fat, crude protein. Semjon B. et al. (2020) noted that supplementation of the broiler diet with fermented feed positively influenced the quality, increased the nutritional value of broiler chicken meat, via the positive modification of the fatty acid profile, without affecting sensory quality.

Conclusion

Addition of extruded and fermented by *Lactobacillus casei* and *Lactobacillus paracasei* WB in compound feed of broiler chickens could reveal some tendencies of improved body weight, carcass yield and breast meat quality (water holding capacity and meat redness as well as fat content), however, more research is needed, e.g., detailed chemical analysis, lipid oxidation level and antioxidant status of meat of broiler chickens.

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Thyme oil and thymol as ZnO alternatives to control *E. coli* K88/F4 infection on *in vitro* cultured enterocytes

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Abstract

Escherichia coli (*E. coli*) K88/F4 is the main responsible for the onset of post-weaning diarrhea (PWD) in piglets. The utilization of pharmacological doses of zinc oxide (ZnO) has led to an effective control PWD symptoms, but European institution opted for a complete ban of medicinal ZnO from June 2022 due to environmental and resistance issues. Plant essential oils (EO) and their bioactive principles are considered potential candidates to substitute ZnO for their antimicrobial and anti-inflammatory activity. This study aimed to evaluate the effectiveness of ZnO, thyme EO (ThyEO), and thymol (Thy) to control bacterial growth and prevent damages exerted by *E. coli* K88/F4 during an *in vitro* challenge on intestinal cells. While ZnO did not show strong antimicrobial effects, ThyEO and Thy could successfully inhibit bacterial growth at 500 ppm and 1.87 mM, respectively. The two alternative treatments could also protect intestinal cells against an *E. coli* infection by keeping intestinal integrity values higher than the challenged control and reducing bacterial translocation across the cellular monolayer. The mechanism of action of both ThyEO and Thy was dual: not only they improved the expression of tight-junction proteins while, but also they reduced bacterial adhesion to target cells, mimicking the action of ZnO.
In conclusion, ThyEO – and especially its main bioactive component Thy – can be considered powerful candidates to control PWD and *E. coli* K88/F4 in weaning piglets without ZnO utilization.

Introduction

Post-weaning diarrhea (PWD) is one of the striking issues of the pig breeding industry because of its heavy impact on piglets (Luppi, 2017). The main causative agents of PWD are Enterotoxigenic E. coli strains, in particular E. coli K88/F4. The pathogen colonizes the small intestine where it produces heat-labile and heat-stable toxins that target the weaners' intestinal mucosa. Toxins impair epithelial integrity, cause inflammation, and trigger the onset of diarrhea (Dubreuil et al., 2016).
Historically, PWD symptoms are controlled with pharmacological doses (2000-3000 ppm) of zinc oxide (ZnO), which reduce diarrhea incidence while maintaining performance parameters (Grilli et al., 2015). However, the utilization of such medicinal doses of ZnO will no longer be allowed in the European Union, because of environmental issues and risks of antibiotic-resistant bacteria selection (Bonetti et al., 2021). Plant essential oils (EO) represent promising alternatives. They are recognized for their antimicrobial and anti-inflammatory properties, even if they suffer from an intrinsic variability in composition. For this reason, nature identical compounds – the synthetic analogues of active principles contained in EO – are acquiring a growing attention to overcome standardization issues and reduce costs (Rossi et al., 2020). The aim of this study was to investigate the effects of ZnO, thyme EO, and its main active principle thymol in preventing the damages exerted by a field strain of E. coli K88/F4 in an infection model of intestinal cells in vitro.

Material and methods

For the determination of the antimicrobial activity, a minimal inhibitory concentration (MIC) test with microdilution method was performed according to Bonetti et al., 2020. Briefly, the field strain of *E. coli*

K88/F4 (10^5 CFU/mL) was incubated with serial dilutions of ZnO (100-2000 ppm), ThyEO (Thy content 52%; 100-2000 ppm), and Thy (0.12-7.5 mM) at 37°C for 24 h. After incubation, turbidity was measured at the spectrophotometer (OD 630 nm) with MIC defined as the lowest concentration capable to give null absorbance.
For trans-epithelial electrical resistance (TER) and bacterial translocation (BT) determination, Caco-2 cells were differentiated on 3.0 µm-pore Transwell® inserts with basal culture medium (BM). On the infection day, cells were maintained in BM (CTR) or infected with *E. coli* K88/F4 $5x10^7$ CFU/mL in BM (CTR+) or BM containing 20 ppm ThyEO, 10 ppm Thy or 0.2 mM ZnO. After 2 h and 4 h, TER was measured, while aliquots of basolateral media were seeded on agar plates for BT assessment. For gene expression analysis, cells were harvested after the infection, RNA was extracted, quantified, and reverse-transcribed. The cDNA obtained was used for qPCR analysis. Gene expression was normalized using two reference genes. Relative changes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).
For adhesion assay, Caco-2 cells were differentiated in 24-well plates and infected for 1 h as described for TER and BT. After 1 h, cells were washed, lysed, and samples seeded on agar plates to quantify bacterial adhesion.
Data were analyzed with One-way (BT, adhesion, qPCR) or Two-way (TER) ANOVA, with each treatment having 6 independent replicates (n=6). Differences were considered significant with $p < 0.05$.

Results

MIC results are shown in Table 1. ZnO did not show an antimicrobial activity up to the highest dose tested. Conversely, the MIC for ThyEO was registered at 500 ppm, which is consistent with the results obtained by its main active principle Thy, whose MIC was equal to 1.87 mM.

Table 1: Minimal inhibitory concentration (MIC) of the tested compounds against a field strain of *E. coli* K88/F4

Strain	ZnO	ThyEO	Thy
E. coli K88/F4	>2000 ppm	500 ppm	1.87 mM

Figure 1 shows results of TER measurements during the *E. coli* K88/F4 challenge on Caco-2 cells. Both ThyEO and Thy are capable to maintain TER at values higher than the challenged control (CTR+), showing a protective effect higher or equal to ZnO (Figure 1A and 1B). Moreover, both ThyEO and Thy proved effective in reducing BT at both 2 h and 4 h after infection start, displaying performances to the same extent of ZnO (Figure 2A and 2B). Compared to infected control (CTR+), the three treatments could also significantly reduce bacterial adhesion to Caco-2 cells, as demonstrated by Figure 2C.

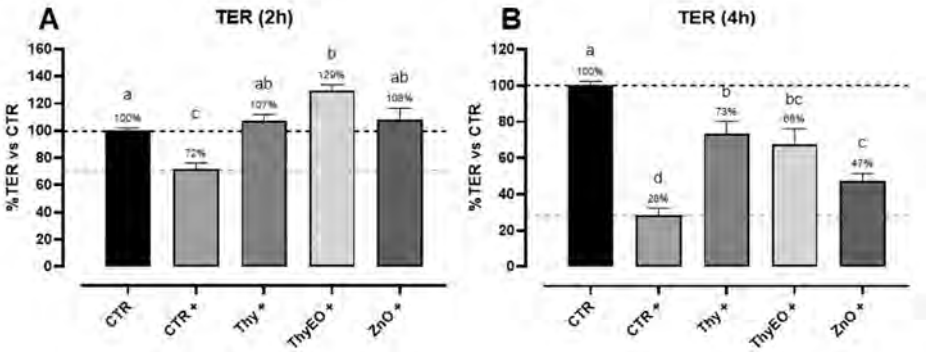

Figure 1: Transepithelial Electrical Resistance (TER, panels A and B) results of Caco-2 cells infected with *E. coli* K88/F4 ("+" groups) and treated with thymol 10 ppm (Thy), thyme EO 20 ppm (ThyEO), or ZnO 0.2 mM

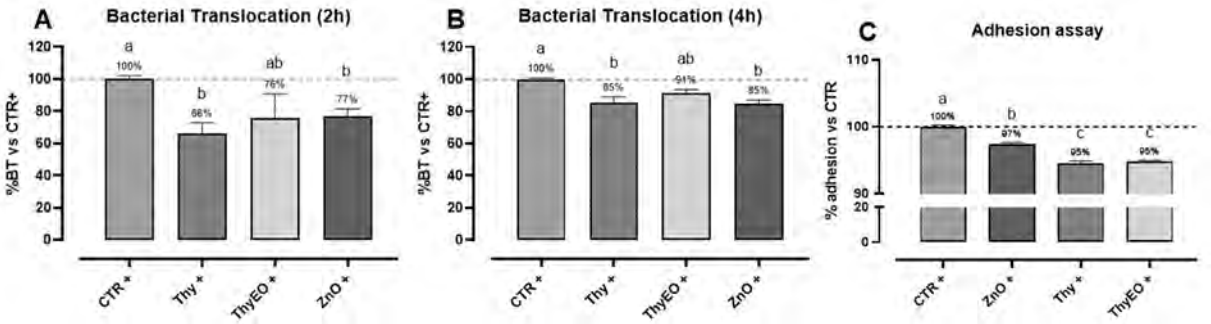

Figure 2: Bacterial Translocation (BT, panels A and B) and Adhesion assay (panel C) results of Caco-2 cells infected with *E. coli* K88/F4 ("+" groups) and treated with thymol 10 ppm (Thy), thyme EO 20 ppm (ThyEO), or ZnO 0.2 mM

Figure 3 reports the results of the gene expression of Caco-2 cells harvested after 4 h infection with *E. coli* K88/F4 and the selected treatments. The addition of ZnO could significantly improve the expression of two markers of tight-junctions if compared to the infected group (CTR+). Thymol performed equally to ZnO, while Thyme EO proved less efficient in keeping a higher expression of both ZO-1 and ZO-2.

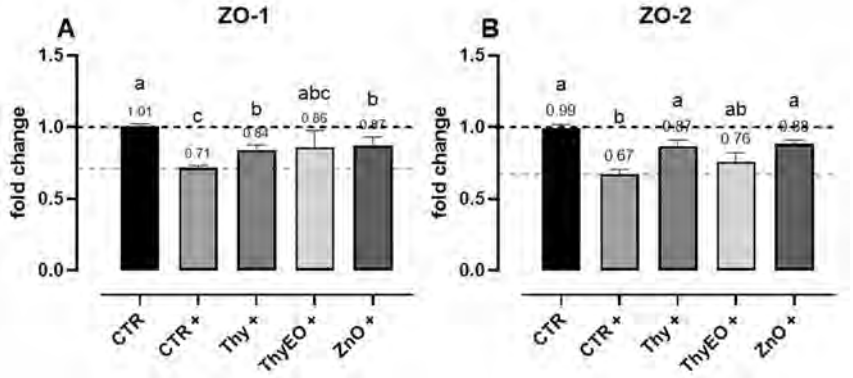

Figure 3: Expression of Zonula Occludens 1 (ZO-1, panel A) and Zonula Occludens 2 (ZO-2, panel B) in Caco-2 cells infected 4 h with *E. coli* K88/F4 ("+" groups) and treated with thymol 10 ppm (Thy), thyme EO 20 ppm (ThyEO), or ZnO 0.2 mM

Discussion

Despite being recognized as an efficient way to control PWD, our data show that ZnO cannot efficiently control *E. coli* K88/F4 growth, thus supporting that ZnO does not exert a direct antimicrobial activity, but rather it acts on the intestinal mucosa (Bonetti et al., 2021). This observation is further proved by data obtained from the *in vitro* tests of this study, where ZnO proved able to improve barrier integrity by maintaining TER at values higher than the challenge alone. ZnO could also reduce the translocation of bacteria across the Caco-2 cell layer at both the tested timepoints, thanks to an improvement in tight-junction markers gene expression, as showed by the increased amount of zonula-occludens 1 (ZO-1) and zonula-occludens 2 (ZO-2) with ZnO treatment during the challenge. These results are in agreement with the findings of Roselli et al., 2003, where ZnO could protect cultured enterocytes from the drop in TER exerted by the *E. coli* challenge, also reducing paracellular permeability and improving tight-junction expression.

However, the continuous utilization of pharmacological doses of ZnO in the pig breeding industry has led European institutions to a complete ban of such high concentrations, putting in danger the efficient control of PWD in piglets. Novel alternatives such as EO and their synthetic bioactive compounds are progressively gaining attention as more environmental-friendly solutions. They are well recognized for their antimicrobial, anti-inflammatory, and anti-oxidant activity, with particular interest in nature identical compounds, that overcome standardization issues related to EO utilization (Rossi et al., 2020).

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In our study, ThyEO not only could control *E. coli* K88/F4 growth, but also maintain TER at high levels during bacterial challenge, showing performance equal to or better than ZnO. Thy, the main active principle of ThyEO, employed at a concentration equal to its amount in the EO, protected cells from the drop in TER exerted by the challenge, keeping values in line with the unchallenged control, ZnO, and ThyEO at 2h, and performing even better than the other treatments at 4h. The increased tightness of the Caco-2 monolayer when botanicals are used was further confirmed by BT results: with Thy or ThyEO supplementation to the infection medium, bacterial passage is reduced. The improvement of Caco-2 cells integrity during the challenge is probably due to the capacity of ThyEO and Thy to increase the expression of tight-junctions related components, ZO-1 and ZO-2, if compared to the challenged control. Moreover, another mechanism of action of both ThyEO and Thy can be related to their ability to reduce bacterial adhesion to Caco-2 cells, to an exent even greater than ZnO. The lower ability of bacteria to interact with epithelial cells is probably related to the recognized action of botanicals, such as Thy, to modulate the expression of bacterial virulence genes (Bonetti et al., 2020).

Conclusion

To conclude, the present study proved how thyme EO can represent a valid alternative to manage *E. coli* K88/F4 infections on intestinal cells *in vitro*. Its efficacy is mainly driven by thymol, whose employment is able to reduce pathogen adhesion to target cells, while simultaneously protecting enterocytes by improving the monolayer integrity. Nature identical compounds such as thymol therefore represent powerful candidates to control PWD and *E. coli* K88/F4 infections also *in vivo*, without pharmacological ZnO utilization.

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Metabolomic approach to explore the anthelmintic activity of sesquiterpene lactones from bioactive crops

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Abstract

The increasing development of drug-resistance in livestock parasites has motivated the search for novel control tools, including bioactive plant compounds. Sesquiterpene lactones (SL) are phytochemicals synthesized by bioactive crops and increasingly investigated for their effects against helminths of ruminants and monogastrics. However, the anthelmintic mode of action of SL remains unknown. Here, an untargeted metabolomic approach was developed to explore the metabolic pathways affected by SL in the nematode model *Caenorhabditis elegans*. Three SL were isolated from chicory (*Cichorium intybus*): lactucin (LAC), 8-deoxylactucin (8-DOL) and lactucopicrin (LCP). Synchronized first-stage larvae of *C. elegans* were incubated with either LAC, 8-DOL or LCP at 50 µg/mL for 24 h. Post-treatment, worm metabolites were analysed by GC-MS. Metabolite identification was performed by matching with metabolomic libraries. GC-MS analyses of exposed worms detected 302 distinct metabolites, with 81 metabolites being significantly different between treatments. All SL significantly impacted the glyoxylate and dicarboxylate metabolism of nematodes, but with different effects on certain metabolites: while 8-DOL and LCP reduced the concentration of glyoxylate in treated worms, LAC treatment increased glyoxylate, whereas 8-DOL and LAC enhanced glycine in exposed nematodes. Further research is needed to confirm the affected metabolic pathways by SL in parasitic nematodes, particularly the impact on the glyoxylate cycle and its enzymes, which have not been identified in mammals but are present in *C. elegans* and parasitic nematodes. Integrating *in vitro* studies and untargeted metabolomics is a promising approach to explore the mode of action of bioactive antiparasitic compounds for livestock.

Introduction

Globally, current parasite control strategies in livestock rely almost entirely on the use of antiparasitic drugs. However, the rising development of drug-resistance in parasitic helminths (e.g. nematodes and trematodes) of ruminants and monogastrics has motivated an intense exploration for novel parasite control tools, including the study of bioactive plant compounds (Charlier et al., 2022). Sesquiterpene lactones (SL) are bioactive molecules naturally synthesized by several plant crops (e.g. chicory, lettuce), with numerous studies reporting the antibiotic, anti-protozoal, trematocidal and anti-cancer activities of these compounds (Chadwick et al., 2013). SL-containing bioactive crops like chicory (*Cichorium intybus*) have been investigated for their direct antiparasitic effects in livestock, with studies consistently reporting that animals consuming chicory-rich diets have lower worm burdens of gastrointestinal nematodes (Peña-Espinoza et al., 2018). Recently, the antiparasitic role of pure SL against nematodes of ruminants and pigs has been confirmed (Valente et al., 2021). However, the antiparasitic mechanisms of SL and their molecular target(s) in nematodes remain unknown. Here, an untargeted metabolomic-based approach was developed with the aim to explore the metabolic pathways targeted by SL in the nematode model *Caenorhabditis elegans*.

Material and methods

Three pure SL were isolated from chicory leaves: lactucin (LAC), 8-deoxylactucin (DOL) and lactucopicrin (LCP). Preliminary *in vitro* assays revealed that the three SL had different anthelmintic activities against

C. elegans (LAC EC_{50}=385.2 µg/mL; DOL EC_{50}=27.9 µg/mL; LCP EC_{50}=104.9 µg/mL). Synchronised first-stage larvae of *C. elegans* CB6147 strain (n=5,000 worms/well) were incubated in triplicates with either LAC, 8-DOL or LCP at 50 µg/mL for 24 h. Worms incubated in DMSO (0.1%) were used as negative controls. Post-treatment, worms were snap-frozen and metabolites were extracted with 80% methanol, derivatized and analysed by untargeted metabolomics using GC-MS. Obtained MS data were processed for compound identification by matching with metabolomic libraries. Statistical analyses and mapping of affected metabolic pathways were performed in MetaboAnalyst and the KEGG database for *C. elegans*

Results

Untargeted metabolomic analyses of worms not-exposed and exposed to SL by GC-MS identified a total of 302 metabolites, with 81 metabolites being significantly different between treatments. All three SL induced a distinct metabolite profile in exposed worms, in comparison with the negative controls (Fig. 1A). However, the identity of only 23 metabolites could be confirmed in metabolomic reference libraries. All three SL significantly impacted the glyoxylate and dicarboxylate metabolic pathway in exposed nematodes, but with different effects on specific metabolites within the pathway: while DOL and LCP significantly reduced the concentration of glyoxylate in treated worms, LAC treatment increased glyoxylate, whereas DOL and LAC significantly enhanced glycine in exposed nematodes (Fig.1B). In addition, all three SL affected the glycine, serine and threonine metabolic pathway, while DOL and LCP significantly impacted the glycerolipid metabolism in treated worms.

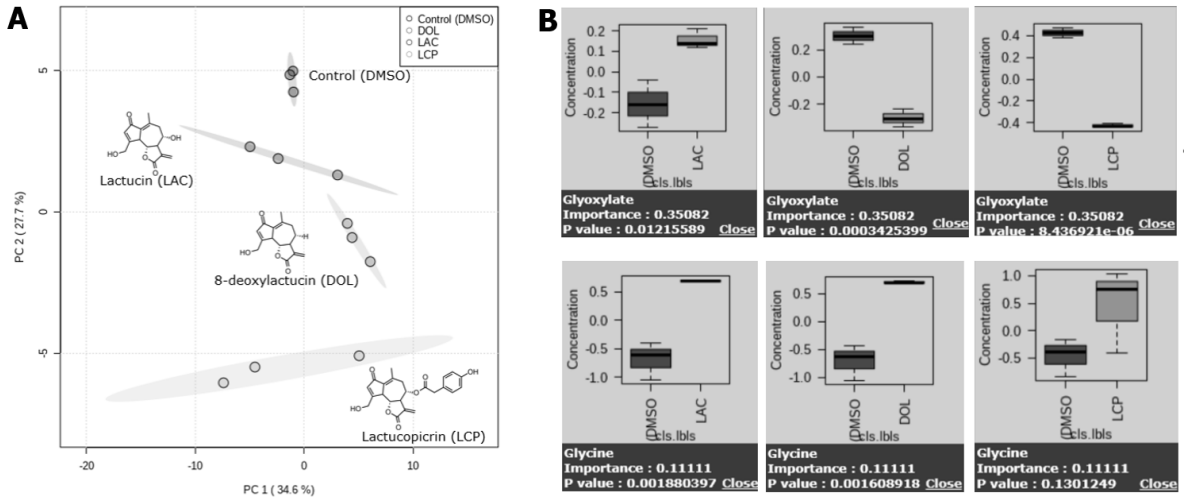

Figure 1. A. Principal component analysis (PCA) of total metabolite profiles of *Caenorhabditis elegans* exposed or not-exposed to pure sesquiterpene lactones from chicory (*Cichorium intybus*). B. Concentration of identified metabolites from the glyoxylate and dicarboxylate metabolic pathways of of *Caenorhabditis elegans* exposed or not-exposed to pure sesquiterpene lactones from chicory (*Cichorium intybus*). LAC: lactucin; DOL: 8-deoxylactucin; LCP: lactucopicrin

Discussion

In the present study, SL from chicory induced marked changes in the metabolomic profiles of exposed nematodes. All SL significantly impacted the glyoxylate and glycine metabolic pathway, but with differences in their effects on individual metabolites within the pathway. Further research should confirm this affected metabolic pathway by SL in parasitic nematodes, particularly the impact on the glyoxylate cycle and its enzymes, which have not been identified in mammals but are present in *C. elegans* and parasitic

nematodes (Salinas and Risi, 2017). Only 23 detected metabolites were conclusively identified in this study due to the still limited databases of metabolites from free-living and parasitic helminths in metabolomic libraries. Further analyses are being conducted to uncover the metabolites not yet identified. Furthermore, transcriptomic analysis are underway to complement the metabolomic results here presented and to confirm the molecular targets of SL in *C. elegans*.

Conclusion

Sesquiterpene lactones (SL) induced marked changes in the metabolome of the nematode model *C. elegans*. All tested SL significantly affected the glyoxylate and glycine metabolism of exposed worms. Further research should confirm the affected metabolic pathways by SL in parasitic nematodes, particularly the impact on the glyoxylate cycle and its enzymes, which have not been identified in mammals but are present in *C. elegans* and parasitic nematodes. The integration of *in vitro* studies and untargeted metabolomics is a promising approach to explore the anthelmintic mode of action of bioactive compounds for the development of novel parasite control approaches in livestock.

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Praxistest eines phytogenen Futterzusatzstoffes bei Milchkühen mit hohen Zellzahlen

Field test of a phytogenic feed additive in dairy cows with high somatic cell counts

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Dr. Eckel Animal Nutrition GmbH & Co. KG, Niederzissen, DE

Abstract

In high-yielding dairy cows, the goal of decreasing somatic cell counts (SCC) without the widespread use of antibiotics seems to be contrary to each other. In this challenging situation, anti-microbial and immune-modulatory effects of secondary plant-compounds could offer the way out, as they promise to improve animal health and performance. After all, it has been shown that feed additives containing secondary plant compounds not only support the animals' health status by reducing SCC, but also increase the cows' productivity.

To gain more experience regarding the practical benefits of feeding secondary plant compounds on dairy farms, we tested a phytogenic feed additive (PFA) in 85 Simmental cows with high SCC. After a milk sampling without feeding PFA, the cows received PFA for a test period of two months. During the test period, SCC, milk yield and milk composition were determined once a month. While feeding PFA, we observed that the amount of cows with low SCC (< 100.000/mL) increased by 29%. Furthermore, cows produced 2.01 kg more energy corrected milk/d as compared to the period before feeding PFA.

This field test indicates that PFA ameliorates performance and udder health in dairy cows with high SCC and open ups the field for research to confirm these observations with robust scientific data.

Einleitung

Nicht zuletzt unter dem Druck einer stetig ansteigenden Weltbevölkerung (FAO, 2009) hat sich die Milchproduktion von Hochleistungskühen in den letzten 50 Jahren mehr als verdoppelt (Oltenacu und Broom, 2010). Die gesteigerte Milchleistung ist zwangsläufig mit einer intensiveren Fütterung und einer extremen Stoffwechselbelastung verbunden und zieht damit eine erhöhte Krankheitsanfälligkeit der Kühe nach sich (Knaus, 2008). So haben sich beispielsweise Eutergesundheit und Zellzahlgehalte (SCC) unter den intensiven Produktionsbedingen der letzten 30 Jahre sichtlich verschlechtert (Oltenacu und Broom, 2010).

Um die Gesundheit von Milchkühen auch bei steigender Leistung zu erhalten, ist der Einsatz von therapeutischen und leistungsfördernden Antibiotika weit verbreitet. Die Therapie von Eutererkrankungen (Kumar et al., 2019) und die Verbesserung des Pansenstoffwechsels (Wall et al., 2014) sind dabei wichtige Gründe, um Antibiotika bei Milchkühen einzusetzen. Obwohl sich Antibiotika in der Vergangenheit bewährt haben, um die Gesundheit und Leistung von Kühen zu verbessern, steht ihr flächendeckender Einsatz aufgrund potentieller Rückstände und der Entwicklung antibiotischer Resistenzen zunehmend in der Kritik (Wall et al., 2014).

Stattdessen rücken vermehrt die antibakteriellen und immun-modulatorischen Wirkungen sekundärer Pflanzenstoffe in den Fokus, um Tiergesundheit und Leistung positiv zu beeinflussen. So wurde in zahlreichen Studien nachgewiesen, dass sich die Fütterung sekundärer Pflanzenstoffe positiv auf die Milchleistung auswirkt (Wall et al., 2014). Des Weiteren wurde der positive Einfluss von sekundären Pflanzenstoffen auf die SCC gezeigt (Möddel et al. 2019). Die von Möddel et al. (2019) untersuchten

sekundären Pflanzenstoffe sind in Form eines Futterzusatzstoffes kommerziell erhältlich, wurden allerdings in einer Herde mit relativ niedrigen SCC (117 Tsd./mL) getestet.
Um mehr Erkenntnisse über die Wirkung und den praktischen Nutzen des Futterzusatzstoffes zu erlangen, wurde daher nun ein Praxistest bei Milchkühen mit hohen Zellzahlen (257 Tsd./mL) durchgeführt.

Material und Methoden

Der Fütterungsversuch wurde auf einem deutschen Milchviehbetrieb mit 85 melkenden Fleckviehkühen durchgeführt. Die Milchleistung lag bei durchschnittlich 27,4 kg/Kuh/Tag mit 4,43 % Fett und 3,74 % Eiweiß. Der SCC lag durchschnittlich bei 257 Tsd./mL. Während des Versuchs wurden die Tiere in einem Boxenlaufstall gehalten. Der Versuch dauerte drei Monate und umfasste einen Kontrollzeitraum ohne Behandlung (August 2020) sowie einen Behandlungszeitraum (September und Oktober 2020). Während des gesamten Versuchs wurden die Kühe mit einer Teil-TMR basierend auf Grassilage, Maissilage und Biertreber gefüttert. Zusätzlich erhielten die Tiere leistungsbezogen 2 bis 7 kg Milchleistungsfutter und Rapsextraktionsschrot pro Kuh und Tag über Transponderfütterung. Im Behandlungszeitraum erhielt die gesamte Herde den phytogenen Futterzusatzstoff (PFA; Anta®Phyt MO, Dr. Eckel Animal Nutrition). Dieser wurde mit einer Dosierung von 10 g/Kuh/Tag über die Vormischung der Teil-TMR verabreicht. Milchmenge und Milchqualität (Fett-, Eiweiß- und Zellzahlgehalt) wurden im Rahmen der monatlichen Milchkontrollen durch das Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V. am 26.8.2020 (Kontrollzeitraum) sowie am 28.9. und am 30.10.2020 (Behandlungszeitraum) ermittelt. Anschließend wurde die energiekorrigierte Milch (EKM) nach der Formel EKM = Milchmenge (kg) * [0,38 * (Fett %) + 0,21 * (Eiweiß %) + 1,05] / 3,28 (Spiekers et al., 2009) berechnet. Zudem wurde der Anteil der Kühe in der Herde mit SCC <100 Tsd./mL, mit 100–300 Tsd./mL sowie mit >300 Tsd./mL berechnet.

Ergebnisse

Im Versuchszeitraum stieg der Anteil der Kühe mit einem SCC von <100 Tsd./mL von 38 auf 49 % an. Gleichzeitig reduzierte sich der Anteil der Kühe mit 100–300 Tsd. Zellen/mL um 18 %. Auch der Anteil der Kühe mit >300 Tsd. Zellen/mL reduzierte sich um 17 % (Abb.1).
Tabelle 1 zeigt die Milchleistung, den Milchfettgehalt sowie den Milchproteingehalt im Kontroll- und Behandlungszeitraum. Dabei war die Milchmenge im Behandlungszeitraum 0,53 kg höher als im Kontrollzeitraum. Auch der Milchfett- und Milchproteingehalt waren numerisch um 0,35 und 0,14 Prozentpunkte erhöht. Dies führte insgesamt zu einer 2,01 kg höheren EKM-Produktion.

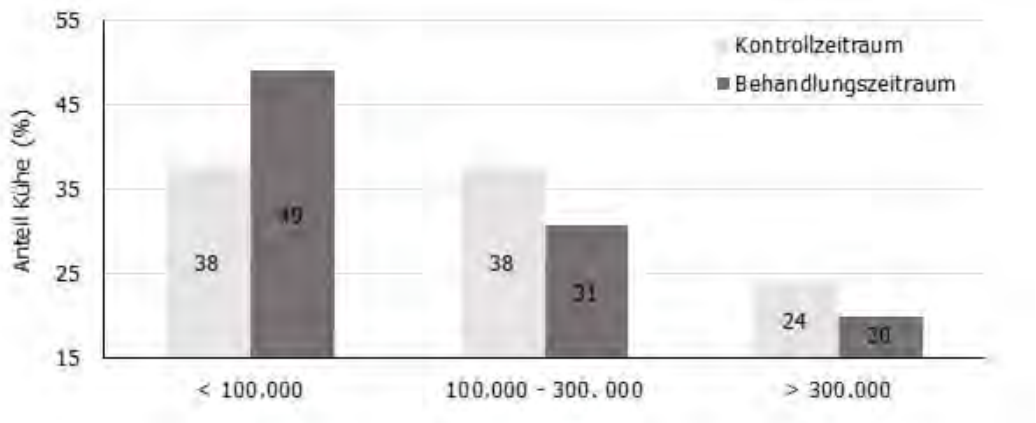

Abbildung 1: Vergleich der Zellzahlklassen im Kontroll- und Behandlungszeitraum

Tabelle 1.: Vergleich der Milchleistung und Milchinhaltsstoffe im Kontroll- und Behandlungszeitraum

Parameter	Kontrollzeitraum	Behandlungszeitraum
Laktationstag (Ø)	150 ± 93	145 ± 92
Milchmenge (kg/Tag)	29,33 ± 7,6	29,86 ± 7,4
Milchfett (%)	4,25 ± 0,64	4,60 ± 0,73
Milchprotein (%)	3,63 ± 0,42	3,77 ± 0,36
EKM, (kg/Tag)	30,66 ± 7,1	32,67 ± 8,4

Diskussion

Ziel dieser Studie war es, mehr Erkenntnisse über die Effekte von PFA unter praktischen Bedingungen zu gewinnen. Dabei stand besonders die Frage im Fokus, ob PFA auch bei Milchkühen mit hohen Zellzahlen zu reduzierten Zellzahlen und einer verbesserten Milchleistung beitragen kann. Tatsächlich wurde der Anteil der Kühe mit einem SCC im Normbereich (<100 Tsd./mL) um 29% erhöht. Dies bestätigt die Versuchsergebnisse von Möddel et. al (2019), welche die zellzahlreduzierende Wirkung von PFA bei Kühen mit niedrigerem Zellzahlgehalt nachweisen konnten. Die zellzahlreduzierende Wirkung ist vermutlich die Folge anti-mikrobieller und immun-modulatorischer Effekte von PFA, die bereits bei Kühen gezeigt wurden (De Nardi et al., 2014; De Nardi et al., 2016).
Übereinstimmend mit bisherigen Ergebnissen, die die positiven Effekte sekundärer Pflanzenstoffe und von PFA auf die Milchleistung belegen (Wall et al., 2014; Möddel et al., 2019), erhöhten sich während dieser Studie sowohl die Milchleistung als auch die Milchinhaltsstoffe der Kühe im Behandlungszeitraum. Da erhöhte Zellzahlen und subklinische Mastitiden die Milchleistung um bis zu 20 % reduzieren (Kumar et al., 2019), könnte die bessere Milchleistung eine Folge der verbesserten Zellzahlen gewesen sein. Allerdings wäre es auch denkbar, dass die verbesserte Milchleistung durch eine direkte Beeinflussung des Pansenmikrobioms und der Pansenfermentation durch PFA erzielt wurde. So wurde bereits in einer früheren Studie gezeigt, dass PFA das Potenzial hat, den Pansen-pH-Wert zu stabilisieren und die Diversität und den Reichtum des Pansenmikrobioms zu steigern (De Nardi et al., 2016). Insbesondere stabilisierte Pansen-pH-Werte führen zu einer verbesserten Faserverdauung, einer erhöhten Produktion kurzkettiger Fettsäuren und damit zu einem verbesserten Fettgehalt der Milch. Auch die Erhöhung von Bypass-Protein wurde für sekundäre Pflanzenstoffe beschrieben (Rodrigues et al., 2019). Dies könnte neben den verbesserten Bedingungen im Pansen zum verbesserten Proteingehalt der Milch beigetragen haben.
Insgesamt stimmen die Beobachtungen, die während dieses Praxistests gemacht wurden, mit bisherigen wissenschaftlichen Erkenntnissen zur positiven Wirkung von PFA auf Milchleistung und SCC überein. Diese Effekte scheinen auch bei Kühen mit hohen Zellzahlen zum Tragen zu kommen. Daher sollte die Wirkung von PFA bei Kühen mit hohen SCC durch wissenschaftliche Studien weiter untersucht werden.

Schlussfolgerung

Die Beobachtungen aus dem durchgeführten Praxistest weisen darauf hin, dass der Phytogene Futterzusatzstoff Anta®Phyt MO die Eutergesundheit und die Produktivität von Kühen mit erhöhten Zellzahlen unterstützt. Dieser Effekt sollte in wissenschaftlichen Studien genauer untersucht werden.

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Hydrolysierte Hefe *Kluyveromyces fragilis* im Milchaustauscher führt zu positiven Effekten auf Gesundheits- und Leistungs-parametern bei Mastkälbern

Hydrolyzed yeast Kluyveromyces fragilis *in the milk replacer leads to positive effects on health and performance parameters in fattening calves*

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Abstract

A feeding trial was conducted to investigate the effect of hydrolyzed yeast *Kluyveromyces fragilis* on health and performance parameters of fattening calves. 228 Holstein Friesian bull calves (14d, Ø 48kg) have been divided into two homogeneous trial groups. From day 1-59 of the trial period, which was 29 weeks, both groups received a milk replacer (MR) either with an inclusion of 12g hydrolyzed yeast (treatment) or without (control). The hydrolyzed yeast was calculated into the MR, which means that 2% of the daily MR ration of one calf, consisting of whey protein concentrate, were exchanged by 1.2% of the hydrolyzed yeast and 0.8% of sweet whey powder. Both diets were isonitrogenous and isoenergetic. Measured parameters were body weight (d1, d31, d59, d203), calculated average daily gain (ADG) after the first (d1-d31), second (d32-d59) and third (d60-d203) period and for the whole trial period (d1-203). Additionally, a fecal scoring was done from day 1-35, which was structured in 4 grades. In period 1 (d1-d31) ADG in the treatment group was significantly higher compared to the control group ($P<0.05$). Total ADG, ADG in period 2 (d32-d59) and ADG in period 3 (d60-d203) were numerically improved by the yeast supplementation. Body weights in the treatment group were numerically higher compared to the control group at the end of period 1, 2 and 3. The results of the fecal scoring show that treated calves had a better feces consistency (less diarrhea) during the trial, especially on d15 ($P<0.004$) and d20 ($P<0.079$).

Einleitung

Junge Kälber haben in ihrer frühen Lebensphase ein schwaches Immunsystem, weswegen sie anfällig für Krankheitserreger sind, oft unter Durchfall und/ oder Atemwegserkrankungen leiden und nicht selten Wachstumseinbußen zu den Folgen zählen. Typische Stressoren wie Transport, Futterumstellungen und das Zusammenführen mit fremden Artgenossen in einer neuen Umgebung stellen eine große Herausforderung für die Jungtiere dar und begünstigen die zuvor genannten Gesundheitsprobleme (Brade und Flachowsky, 2007; Irimia et al., 2020). Ein erhöhter Einsatz an Medikamenten wie Antibiotika, der gesellschaftlich und politisch kritisch diskutiert wird, kann beobachtet werden (von Inger Klatt, 2019). Neben einem schwach entwickelten Immunsystem ist auch das Verdauungssystem junger Kälber noch nicht ausreichend entwickelt, weshalb pflanzliche Nährstoffe von diesen nur begrenzt genutzt werden können. Die Stimulierung der Futteraufnahme, die Unterstützung der Darmgesundheit und das Bereitstellen leicht verdaulicher Proteinquellen ist daher von großer Bedeutung für das Jungtier (Osorio, 2020). Hydrolysierte Hefen basierend auf dem Hefestamm *Kluyveromyces fragilis* (TechnoYeast, Biochem Zusatzstoffe) werden als Einzelfuttermittel klassifiziert und enthalten nicht nur das proteinreiche Zellextrakt, sondern auch die prebiotisch wirkenden und immununterstützenden Bestandteile der Hefezellwand wie die Mannan-Oligosaccharide (MOS) und ß-1,3-1,6-Glukane. Die positive Wirkung hydrolysierter Hefen zur Stabilisierung von Leistungs- und Gesundheitsparametern sind bei abgesetzten

Ferkeln bereits umfangreich dokumentiert (Keimer, 2019). Inwieweit positive Effekte auch bei Mastkälbern zu beobachten sind, wurde in der vorliegenden Studie untersucht.

Material und Methoden

228 männliche Mastkälber der Rasse Holstein Friesian (14 Tage alt, Ø 48 kg Körpergewicht) wurden in zwei homogene Behandlungsgruppen (Versuch, Kontrolle) mit jeweils 114 Kälbern aufgeteilt. Der Fütterungsversuch, der auf einem modernen, konventionellen Kälbermastbetrieb durchgeführt wurde, erstreckte sich über eine Dauer von 29 Wochen (203 Tage). Während der ersten 31 Versuchstage wurden alle Kälber einzeln gehalten und gefüttert. In den darauffolgenden Wochen wurden die Kälber beider Gruppen jeweils auf 19 Buchten á 6 Tiere aufgeteilt (Spaltenboden). Die Kälber in beiden Gruppen erhielten einen konventionellen Milchaustauscher (MAT) auf Basis von Molkenproteinkonzentrat. Von Versuchstag 1 bis 59 wurde ein hydrolysiertes Hefeprodukt in den MAT der Versuchsgruppe ergänzt. Dazu wurden 2% des MAT, bestehend aus Molkenproteinkonzentrat, durch 1,2% hydrolysierte Hefe (HY) und 0,8% Süßmolkenpulver ausgetauscht. Die 1,2% HY entsprechen damit einer Tagesdosierung von 12g/ Kalb. Die HY wurde durch diese Optimierung kostenneutral und trotzdem isoenergetisch sowie isonitrogen in den MAT der Versuchsgruppe integriert. Bei dem getesteten Produkt handelte es sich um eine hydrolysierte Hefe, die auf dem Hefestamm *Kluyveromyces fragilis* (TechnoYeast, Biochem Zusatzstoffe) basiert. Von Tag 60 bis zum Versuchsende wurden beide Behandlungsgruppen dann mit einem identischen MAT weiter gefüttert. Die Fütterung des MAT erfolgte in beiden Gruppen über die gesamte Versuchsdauer restriktiv. Gemäß den gesetzlichen Vorgaben wurden ab dem 14. Lebenstag Kraft- und Strukturfutter zusätzlich zum MAT angeboten. Das Körpergewicht wurde individuell an Tag 1, 31, 59 und 203 ermittelt, woraus dann die durchschnittlichen täglichen Zunahmen für die erste (Tag 1-31), zweite (Tag 32-59), dritte (Tag 60-203) und für die gesamte Periode (Tag 1-203) errechnet wurden. Zusätzlich wurde eine Kotbonitur von Tag 1-35 durchgeführt, um Rückschlüsse auf die Darmgesundheit treffen zu können. Die Kotbonitur erfolgte in 4 Stufen (0=optimaler Kot, 1=weicher Kot, 2=flüssiger Kot (leichter Durchfall), 3=wässriger Kot (schwerer Durchfall). Für die Berechnung signifikanter Unterschiede zwischen den Behandlungsgruppen wurde eine einfaktorielle Varianzanalyse angewendet. Die statistischen Analysen wurden mit dem Softwarepaket SPSS (IBM SPSS, Version 24) durchgeführt. Unterschiede mit $P<0,05$ wurden als signifikant definiert.

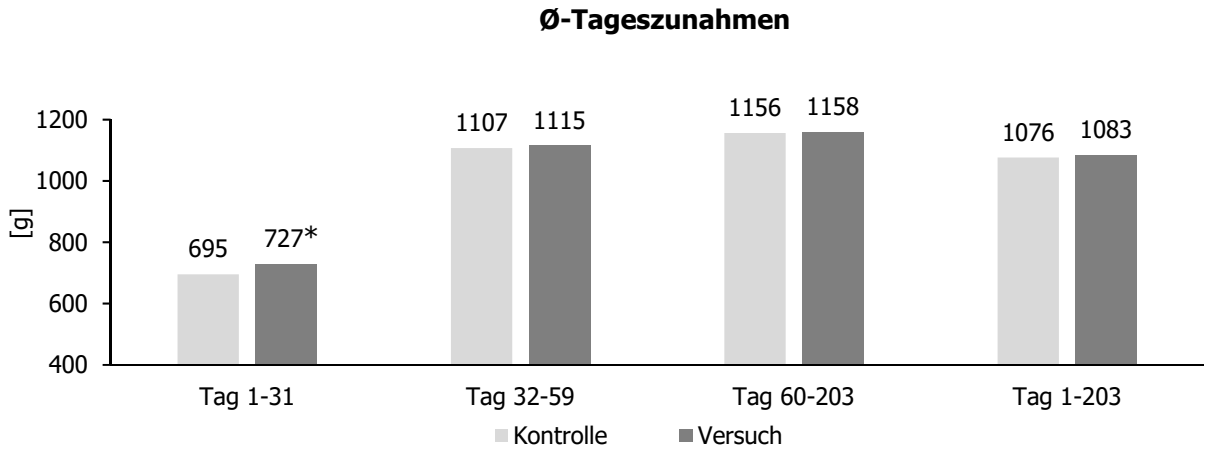

Abbildung 1: Der Einfluss von TechnoYeast (Versuchsgruppe) supplementiert von Tag 1-59 auf die durchschnittlichen Tageszunahmen im Vergleich zur Kontrollgruppe, * $P<0,05$

Ergebnisse

In Periode 1 (Tag 1-31) lagen die Ø-Tageszunahmen in der Versuchsgruppe mit 727g signifikant oberhalb der der Kontrollgruppe mit 695g (P<0,05). Der Unterschied zwischen den Gruppen in der ersten Periode umfasste demnach 32g. Die Ø-Tageszunahmen in Periode 2 (Tag 32-59), in Periode 3 (Tag 60-203) und in der Gesamtperiode (Tag 1-203) waren in der Versuchsgruppe numerisch höher als in der Kontrollgruppe (Abb. 1). Die Körpergewichte in den einzelnen Perioden waren in der Versuchsgruppe numerisch höher als in der Kontrollgruppe (Abb. 2).

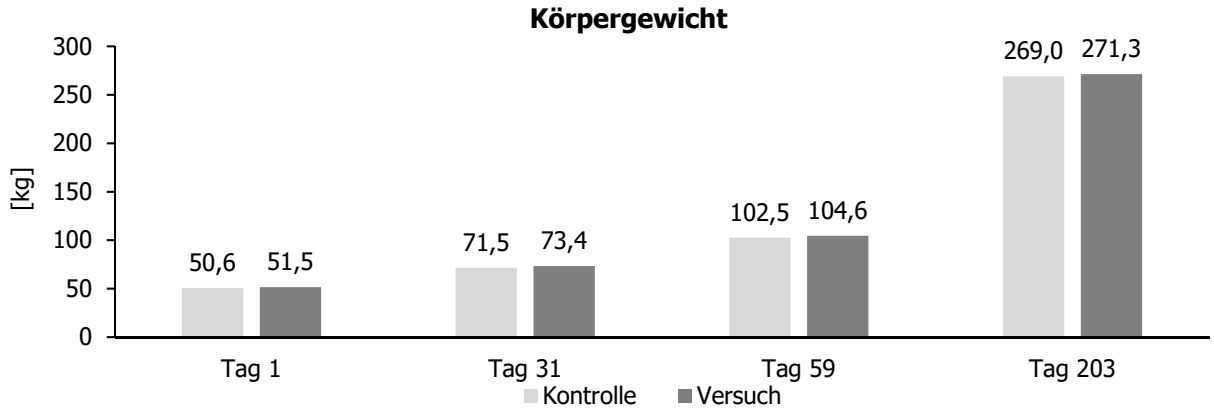

Abbildung 2: Der Einfluss von TechnoYeast (Versuchsgruppe) supplementiert von Tag 1-59 auf das Körpergewicht im Vergleich zur Kontrollgruppe

Die Kotbonitierung, wie Abbildung 3 zu entnehmen, zeigte, dass im Verlaufe des Versuches die Tiere in der Versuchsgruppe im Vergleich zur Kontrollgruppe eine bessere Kotkonsistenz aufwiesen. Besonders an Tag 15 (P=0,004) und 20 (P=0,079). In der Gesamtbetrachtung litten in der Versuchsgruppe weniger Kälber an Durchfall.

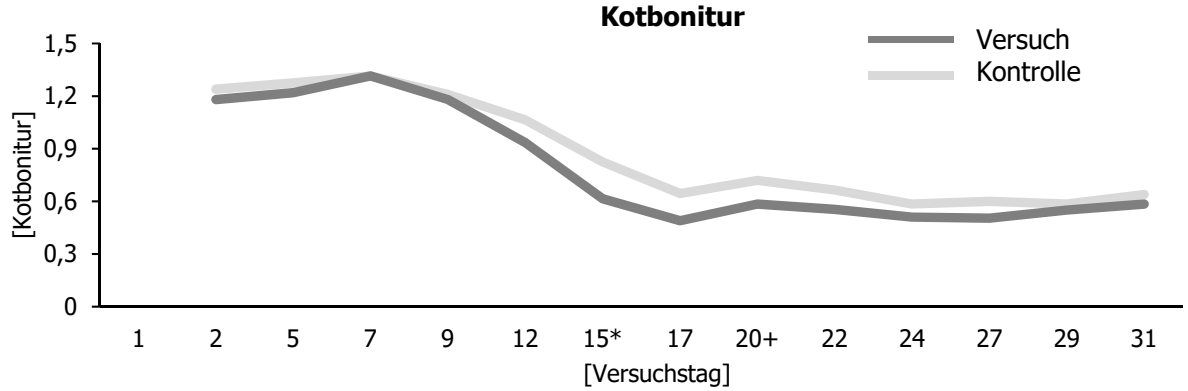

Abbildung 3: Der Einfluss von TechnoYeast (Versuchsgruppe) auf die Kotbonitur im Vergleich zur Kontrollgruppe; *P<0,05; $^{+}$P<0,1 (Trend), dargestellt als gleitender Durchschnitt der Vorperiode

Diskussion

Die Tageszunahmen der Versuchsgruppe konnten im Vergleich zur Kontrollgruppe besonders in der ersten Phase des Versuches (Tag1-31), d.h. Lebenstag 15-46, positiv durch die hydrolysierte Hefe beeinflusst werden. Es ist bekannt, dass besonders in den ersten Lebenswochen eine Unterstützung von

Verdauungsgeschehen und Immunität bei Kälbern von großer Bedeutung ist (Brade und Flachowsky, 2007; Irimia et al., 2020). Es kann daher angenommen werden, dass insbesondere in dieser schwierigen Zeit für die Kälber eine Unterstützung durch die hydrolysierte Hefe von Vorteil ist. Die hydrolysierte Hefe wurde von Versuchstag 1-59 supplementiert, doch auch im Zeitraum nach der Supplementierung bis Versuchstag 203 konnten positive Effekte der Hefe auf die Gewichtsentwicklung festgestellt werden. Daraus ist zu schließen, dass die hydrolysierte Hefe durch ihren beschriebenen positiven Effekt auf Darmfunktion und Immunität einen langfristig positiven Effekt auf die Gesundheit und Leistung von Kälbern ausübt. Die Kotbonitur wurde ebenfalls in den ersten kritischen Lebenswochen der Kälber durchgeführt. Nach einem annähernd gleichen Niveau der Kotbeschaffenheit zu Versuchsbeginn im Vergleich zur Kontrollgruppe, konnte für die Kälber aus der Versuchsgruppe auch in Bezug auf diesen Parameter ein positiver Effekt auf die Darmentwicklung durch die hydrolysierte Hefe festgestellt werden. Insgesamt fiel die Bewertung des Kotes in beiden Gruppen relativ positiv aus. Die Ergebnisse dieser Studie stehen somit im Einklang zu weiteren Studienergebnissen, die zur getesteten hydrolysierten Hefe bereits durchgeführt wurden (Keimer, 2019).

Schlussfolgerungen

Bei Mastkälbern zeigte die hydrolysierte Hefe basierend auf dem Hefestamm Kluyveromyces fragilis (TechnoYeast, Biochem Zusatzstoffe) mit einer Dosierung von 12g pro Tier und Tag im Vergleich zu einer Kontrollgruppe (keine Hefesupplementierung) verbessernde Effekte auf die Darmgesundheit, was sich in einem reduzierten Durchfallauftreten bemerkbar machte. Zudem konnten Verbesserungen in Bezug auf die Wachstumsleitung festgestellt werden. Das getestete Hefeprodukt kann somit durch eine Steigerung von Gesundheit und Leistung sowie einem kostenneutralen Einsatz zu einem erhöhten ökonomischen Gewinn beitragen.

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Effect of fermented milk permeate on blood profile, growth and fecal bacterial community of young calves

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Abstract

This paper examines the effect of fermented milk permeate (FMP) supplement on blood parameters, growth performance and fecal microbiological profile in newborn calves. Twenty female Holstein calves were involved in this 14-day experiment. Half of calves (control group - Co) was offered colostrum and a standard milk replacer from day 2 to 14 of life. Other half (treated group – TrFMP) was offered the same diet supplemented with 50 mL of the milk permeate fermented with *Lactobacillus uvarum* LUHS245. No significant differences were observed in blood parameters and growth performance between tested calves' groups at the end of experiment. The higher count of LAB (by 17.02%) and a lower percentage of enterobacteria (by 10.38%), a higher overall number of probiotic bacteria and a 1.7-fold higher species variety were found in feces of TrFMP group after 14 days of experiment. Significant differences in bacterial strains profile between the feces of Co and TrFMP groups were found after 14 days. *Lactobacillus* and *Bifidobacteria* were predominant strains in TrFMP group, while *Blautia* and *Tyzzerella* were prevalent in Co group. The obtained results of this study demonstrated that FMP inclusion in calves feeding had a significant impact on the microbial profile of calves feces by increasing the counts of important *Lactobacillus* and *Bifidobacterium*. However, blood parameters or growth performance of calves were not affected by FMP supplement.

Introduction

Neonatal calf diarrhoea and other inflammations of the gastrointestinal tract are frequent health issues in dairy cattle herds and usually causes significant economic casualties for farmers worldwide. In order to improve gut microbiome and animal health, various supplements with probiotics and prebiotics could be included in animal feed. Probiotics help to manage the beneficial gut flora and protect the intestine from proliferation of pathogenic bacteria (Mingmongkolchai & Panbangred, 2018; Stefanska et al., 2021). Individual lactic acid bacteria (LAB) strains or combination of them have been studied and used for livestock to prevent and facilitate intestinal disorders (Li et al., 2020; Sandes et al., 2017; Maldonado et al., 2018). Prebiotics, the nondigestible compounds, also manage activities of the gastrointestinal microbiota (Adhikari & Kim, 2017). Galactooligosaccharides (GOS), which belong to nondigestible oligosaccharides, are promising antimicrobial growth promoters and help to create a healthier microbiota in calves with the domination of lactobacilli (Azcarate-Peril et al., 2018). However, the greatest influence of probiotic and prebiotic is mostly seen in the first weeks or months of a calf's life (Maldonado et al., 2018; Uyeno et al., 2015). Previous study of Zokaityte et al. (2020) showed that fermentation of milk

permeate with certain LAB strains led to functional supplements with antimicrobial properties. Milk permeate is a dairy industry by-product and fermentation with selected LAB strains could add the additional value for this product by modifying its sensory, antimicrobial and physicochemical properties, including production of GOS (Zokaityte et al., 2020). For this reason, fermented milk permeate could be a promising ingredient of feed for newborn calves. Therefore, this paper examines the effect of fermented milk permeate (FMP) supplement on blood parameters, growth performance and fecal microbiological profile in newborn calves.

Materials and methods

Fermented Milk Permeate and Feeding Scheme

Fermented milk permeate (FMP) was prepared as described by Zokaityte et al. (2020). The calves were housed indoors and were individually tethered and cared for in accordance with the Lithuanian State Food and Veterinary Service Requirements. Research was carried out in accordance with both the Republic of Lithuania Act (6 November 1997) regulating animal care and maintenance and its subsequent legal amendment (Act 8-500).
Twenty female Holstein calves were divided into two equal groups: control (Co) and treated (TrFMP) groups (on the day of birth). All the calves received the first colostrum from their dams during day 1 and were included in the study on day 2. Each calf in Co group was offered colostrum and a standard milk replacer (22.5% crude protein, 18% fat, 9% ash, 1.75% lysine, 0.55% methionine, and 0.5% cysteine on a dry matter basis) once a day, at 7:00 a.m., for 14 days. Each calf in TrFMP group was offered the same diet supplemented with 50 mL of the FMP (FMP was mixed with the milk replacer (130 g/L reconstituted in hot water at 65 °C)). Each calf was placed in an individual outdoor box (2.00 m 1.25 m), with free access to warm water. They were fed from a bucket with 8–10 l of unmedicated milk replacer at 39 °C—either with or without the FMP.

Blood Analysis and Assessment of Growth Performance

Blood samples were taken and growth performance was recorded at 2 and 14 days of the experiment. The detailed procedures are given by Vadopalas et al. (2021). The content of lactate and aspartate aminotransferase (AST) in blood was determined with an automatic biochemical analyser in an accredited laboratory (Kaunas, Lithuania). Electronic weighing scales (model BF/E 1425E, Technosystem, Via Toscana, Certaldo FI, Italy) were used for the assessment of calves' body weight.

Microbiological and Metagenomic Analysis of Fecal Samples

Feces samples were collected on days 2 and 14 (kept at +4 C with a medium (Faecal Enteric Plus, Oxoid, Basingstoke, UK)), and analysed on the same day. The total counts of aerobic and facultative anaerobic microorganisms (TCM), lactic acid bacteria (LAB), total bacteria (TBC), enterobacteria (TCE), and yeast/mould (Y/M) were evaluated as described by Zavistanaviciute et al. (2020). Metagenomic analysis was done in an independent service laboratory (Baseclear, Leiden, The Netherlands).

Statistical Analysis

Z-Test Calculator for Two Population Proportions (Social Science Statistics) was used to analyse differencesbetween the most prevalent bacterial genera among the tested groups of calves. The rest of data was analysed using SPSS package (Version 15.0, SPSS, Chicago, IL, USA). All results were considered statistically significant at $p \leq 0.05$.

Results and discussion

Blood Metabolites and Growth Performance of Calves

No significant differences were observed between Co and TrFMP groups in blood parameters and growth performance at the beginning or at the end of the experiment (Table 1). Changes in calves' AST values

were also not found by Dar et al. (2018) when prebiotic and probiotic dietary treatments were applied. However, other studies about the effect of probiotics and prebiotics on the growth performance of calves show inconsistent data.

Table 1. Blood metabolites and growth performance measured in newborn calves fed milk replacer (Co group) or supplemented with fermented milk permeate (TrFMP group)

Parameter	Day	Co	TrFMP	P-Value Day x treat Int
AST(µkat/L)	Baseline	82.70±54.07$^{A;a}$	95.10±31.23$^{A;a}$	0.299
	14	43.57±4.57$^{A;a}$	55.57±27.58$^{A;a}$	
Lactates (mmol/L)	Baseline	5.27±2.05$^{A;a}$	4.83±0.94$^{A;a}$	0.411
	14	4.04±2.34$^{A;a}$	2.69±1.91$^{A;a}$	
Growth performance	Baseline	39.10±3.93$^{A;a}$	39.70±3.77$^{A;a}$	0.0001
	14	45.00±5.27$^{B;a}$	46.00±5.09$^{B;a}$	

The data are presented as mean standard error (n = 10/group). Baseline measurements were done on day 2 before the start of the feeding experiment. AST, aspartate aminotransferase; Treat. Int., treatment interaction. ^{A,B} different capitals indicate significant time-related differences ($p < 0.05$). ^{a,b} different letters indicate differences among treatments ($p < 0.05$)

Microbiological Profiles of the Calves' Feces

Significant differences in microbiological parameters of the feces of both tested groups were not determined (Table 2). The higher count of LAB (by 17.02%) and a lower percentage of enterobacteria (by 10.38%) were found in feces of TrFMP group after 14 days of experiment. Lower faecal levels of coliform, Escherichia, Clostridium spp. and higher population of bifidobacterial and lactobacilli were found in prebiotic- or probiotic-fed calves (Dar et al., 2018; Alawneh et al., 2020; Khaziakhmetov et al., 2020). In other cases, no influence of probiotic treatment on counts of beneficial bacteria in feces was found (Heinrichs et al., 2009). Metagenomic profile of feces of Co and TrFMP groups is given in Figure 1. At the beginning of experiment, 39.676 and 41.469 bacterial reads were found from the feces of Co and TrFMP groups, respectively. The determined bacteria strains were similar between tested groups of calves. Clostridium, Escherichia, Enterococcus, Terrisporobacter, Klebsiella and Streptococcus composed more than 93% of the total bacterial reads. After 14 days, 28.265 bacterial reads were found in Co group and 26.968 in TrFMP group (Figure 2). Significant differences in bacterial strains profile between groups were found. Lactobacillus and Bifidobacteria were predominant strains in TrFMP group, while Blautia and Tyzzerella were prevalent in Co group. These bacteria strains composed for 45.2% and 53.5% of all bacterial counts in the TrFMP and Co groups, respectively. Moreover, Erysipelatoclostridium, Bacteroides, Butyricicoccus, Escherichia, Faecalibacterium and Ruminococcus were also predominated in both tested groups. The count of strains with an occurrence of at least 0.01% of the total bacterial reads was significantly different between the groups: 234 species were found in the TrFMP group and 138 species in the Co group. The most predominant species in the TrFMP group were probiotic, including Lactobacillus amylovorus, Lactobacillus johnsonii, Bifidobacterium longum, and Tyzzerella nexilis. The most predominant species in the Co group were Ruminococcus torques, Butyricicoccus pullicaecorum, Blautia wexlerae, and T. nexilis. The results of this study showed that FMP inclusion in calves feeding had a significant impact on the microbial profile of calves feces and increased the counts of important Lactobacillus and Bifidobacterium. The positives effect of these bacteria on human and animal health were already demonstrated in many studies (Deaver, Eum & Toborek, 2018; Fernández et al., 2018; Sugahara et al., 2015). Studies about the most predominant bacteria strains of Co group are still scarce.

Table 2. Microbiological parameters of calves' feces fed milk replacer (Co group) or supplemented with fermented milk permeate (TrFMP group)

Parameter	Day	Co	TrFMP	P-Value Day x Treat. Int.
LAB	Baseline	5.29±1.89$^{A;a}$	6.14±1.46$^{A;a}$	0.004
	14	6.14±1.46$^{A;a}$	7.40±0.43 $^{B;b}$	
TCE	Baseline	6.15±0.04$^{A;a}$	7.23±1.35 $^{B;a}$	0.200
	14	7.71±0.55$^{A;b}$	6.91±0.64$^{A;a}$	
TCM	Baseline	6.89±1.54$^{A;a}$	7.55±1.25$^{A;a}$	0.076
	14	8.26±0.93$^{B;a}$	7.71±0.63$^{A;a}$	
Enterococcus faecalis	Baseline	5.86±1.12$^{A;a}$	6.20±0.97$^{A;a}$	0.053
	14	5.04±0.93$^{A;a}$	5.41±0.78$^{B;a}$	
Y/F	Baseline	4.77±1.08$^{A;a}$	5.40±0.92$^{A;a}$	0.764
	14	5.06±0.80$^{A;a}$	5.35±1.20$^{A;a}$	

The data are presented as the mean standard error (n = 10/group). Baseline measurements were done on day 2 before the start of the feeding experiment. CFU, colony-forming units; LAB, lactic acid bacteria count; TCE, total count of enterobacteria; TCM, total count of aerobic and facultative anaerobic microorganisms; Treat. Int., treatment interaction; Y/F, yeast/fungi. ^{A,B} different capitals indicate significant time-related differences ($p < 0.05$). ^{a,b} different letters indicate differences among treatments ($p < 0.05$)

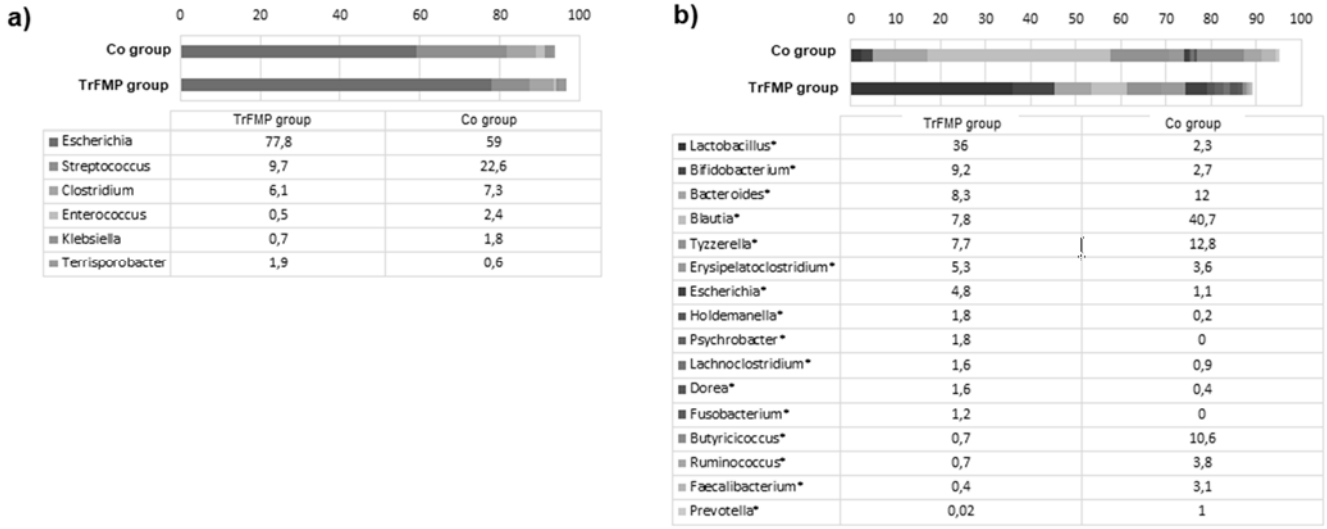

Figure 1. Bacterial strains percentage occurrence in the feces of calves before the experiment (a) and after (b). The strains are included in the list only if the occurrence was at least 1% of the total bacterial reads in the feces of both groups combined. Co, calves fed milk replacer; TrFMP, calves fed with milk replacer and supplemented with fermented milk permeate. * Significant differences between the groups

Conclusion

The supplement of milk permeate fermented with *Lactobacillus uvarum* LUHS245 had a significant influence on the fecal microbiological profile in newborn calves. The higher count of LAB (by 17.02%) and a lower percentage of enterobacteria (by 10.38%), a higher overall number of probiotic bacteria and a 1.7-fold higher species variety were found in feces of TrFMP group after 14 days of experiment. *Lactobacillus* and *Bifidobacteria* were predominant strains in TrFMP group, while *Blautia* and *Tyzzerella* were prevalent in Co group. The obtained results of this study demonstrated that FMP inclusion in calves feeding had a significant impact on the microbial profile of calves feces by increasing the counts of important *Lactobacillus* and *Bifidobacterium*. However, no significant differences were observed in blood parameters and growth performance between tested calves' groups at the end of experiment.

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Influence of a rumen-protected grape extract around vaccination on antioxidant defenses and humoral response in young cattle

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Abstract

Vaccination failures represent an economic burden for the farmer. Polyphenol supplementation, known for its antioxidant properties, could help reduce oxidative damage and improve vaccination success. The purpose of this study was to examine the effect of a rumen-protected grape extract (RPGE) supplementation around vaccination on the immune response in young ruminants.
22 young female cattle (aged 6 to 8 months), born in the same farm, were randomly divided into 2 groups. One group (BP-O, n = 11) was supplemented with a RPGE (Nor-Grape® BP-O, Nor-Feed, France), whilst a control group (CTL, n = 11) was not. All animals were vaccinated (D14) with an inactivated vaccine against PI3V and BRSV. A booster was given 3 weeks later (D35). Supplementation began 15 days before vaccination (D0) and ended 15 days after the last injection (D49). Antibody titers and total antioxidant status (TAS) were performed on blood samples drawn on D0, D35 and D56.
Results show that the BP-O group tended to a greater overall antibody response to BRSV and PI3 V on D56 ($P < 0.10$) and PI3V titer was significantly higher in the BP-O group on D35 ($p < 0.05$). A greater total antioxidant capacity ($P<0.05$ at D56) was observed in the supplemented group. Results showed a strong correlation between PI3V antibody titers and TAS ($p < 0.001$). Thus, since supplemented animals became seropositive faster and long-term immunity appeared to be improved, this supplementation strategy could be interesting to enhance the immune response during a vaccination episode by reducing oxidative stress.

Introduction

Vaccination is an important pillar for animal health and limits therefore the economic impacts of different infectious agents (Roth, 2011). However, vaccination success is limited by several exogenous and endogenous factors (Richeson *et al.*, 2019). It has been shown that stress and involved oxidative disbalance can impact the immune system of the vaccinated animal (Amadori & Zanotti, 2016).
To counteract oxidative stress, several antioxidant compounds are used also from natural sources, such as polyphenols. Several studies reported the intricate relationship among polyphenols' antioxidant, anti-inflammatory and immuno-modulating effects in different animal species including cows (Pauletto *et al.,* 2020). However, no published research has been carried out on the effects of grape polyphenols supplementation in cattle adaptive immunity. Therefore, the aim of this work was to evaluate the effect of a supplementation with a low dose of rumen-protected grape extract (RPGE) around a vaccination in young cattle on their humoral response.

Material and methods

22 healthy, 6 to 8 months-age Prim'Holstein heifers were recruited. The mean estimated weight at the beginning of the experiment was 161 ± 17 kg and both groups did not differ significantly. They have

never been vaccinated against the pathogens targeted in the study. All animals were born and raised on the farm and thus shared the same environmental, feed and herd management conditions. The basal diet of the animals was constituted as follows: *ad libitum* straw and 4kg per day of a mixture of 49% corn grain, 27.5% rapeseed cake, 7% wheat straw, 7% hay, 7% molasses, 2% minerals and 0.5% salt. The heifers were randomly divided into 2 groups: a control group (CTL, n=11) and a supplemented group (BP-O, n=11) and received the same basal diet. The animals from the BP-O group were additionally fed 670mg (contained >60% total polyphenols by spectrophotometric method) of a commercial rumen-protected grape extract (RPGE) per animal and per day (Nor-Grape® BP-O, Nor-Feed, France) for 7 weeks. The vaccination protocol was as follows: The first injection of a combination of inactivated Para Influenza 3 Virus (PI3V) and inactivated Bovine Respiratory Syncytial Virus (BRSV, BOVALTO RESPI 3®, Merial, France) on Day 15 and the second booster doses of both on Day 35 of the supplementation period. Blood samples were taken from the heifers' coccygeal vein on Day 1, Day 35 and Day 56 for analyzing the specific antibody titration and total antioxidant status. The reagents used were Metmyoglobin and ABTS (2, 2'-amino-di-[3-ethylbenzthiazoline sulphonate]) present in the chromogen reagent, peroxide hydrogen (substrate). Statistical analyses were carried out using R studio (version 1.4.1106) with multcomp (multiple comparisons) and nlme (nonlinear mixed-effects models) packages. The data of neutralizing antibody titer and total antioxidant status were analyzed using a linear mixed effects model. This model for studying the effect of time and groups on the observed value was as follows: Observed values$_{ij} \sim \mu + (\text{Time})_i + (\text{Group})_j + (\text{Time} * \text{Group}) + \varepsilon_{ij}$; where: μ=average; Time = fixed effect of time (i= D1, D35 or D56); Group= fixed effect of group (j= BP-O or CTL); Time*Group = interaction between Time and Group; ε_{ij}= the residuals. When the interaction of time and group did not significantly affect the studied criterion, a multiple comparison test was performed with Tukey's test, allowing for a pairwise comparison of means.

Results

No difference of BRSV-specific antibody titers and the very low average antibody titer (0.5±0.2 and 0.6±0.3 for CTL and BP-O group respectively) were found before the beginning of the experiment (Figure 1), signifying an absence of exposure to the BRSV virus before the beginning of the experiment. On D35, no statistical difference was observed between groups for their BRSV-specific circulating antibody titers. However, On D56, the average BRSV-specific antibody titer from BP-O heifers tended to be higher than that from the CTL group (3.9±0.5 vs. 2.9±0.3, respectively, P<0.10). In both groups, antibody titers were significantly higher on D56 than on D35 or D1 (p<0.001).

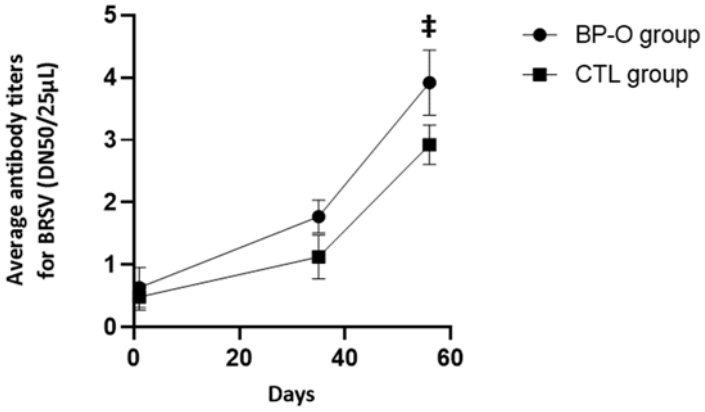

Figure 1: Comparison of BRSV neutralizing antibody titers between groups after vaccination (‡ 0.05≤P≤0.1)

No difference of PI3V-specificantibody titers were observed, but both groups showed a medium average antibody titer (2.9±0.3 and 3.8±0.3 for CTL and BP-O group, respectively) signifying the existence of a

previous exposure to the PI3V virus before the beginning of the experiment (Figure 2). On D35 the average PI3V-specific antibody titer from BP-O heifers was significantly higher than that from the CTL group (7.6±0.4 vs. 5.3±0.6, respectively, P<0.05). Furthermore, on D56, the average PI3V-specific antibody titer from BP-O heifers also tended to be higher than that from the CTL group (7.1±0.5 vs. 5.1±0.5, respectively, P<0.10). Additionally, on D35 and D56, the antibody titer of both groups was significantly higher than the D1 one (p<0.05).

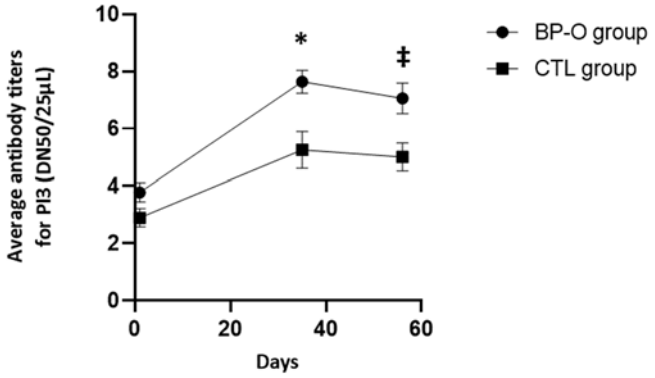

Figure 2: Comparison of PI3 neutralizing antibody titers between groups after vaccination (* P<0.05; ‡ 0.05≤P≤0.10)

No difference was observed between groups for their total antioxidant status (TAS) at the beginning (Figure 3). However, on D56, the average TAS from BP-O heifers was significantly higher than in CTL animals (2.13±0.23 mmol eq. Trolox/L vs. 1.60±0.11 mmol eq. Trolox/L, respectively, p<0.05). Also, whilst BP-O TAS was significantly increased by D56 (p<0.05), no difference between D0 and D56 TAS could be observed in the CTL group.

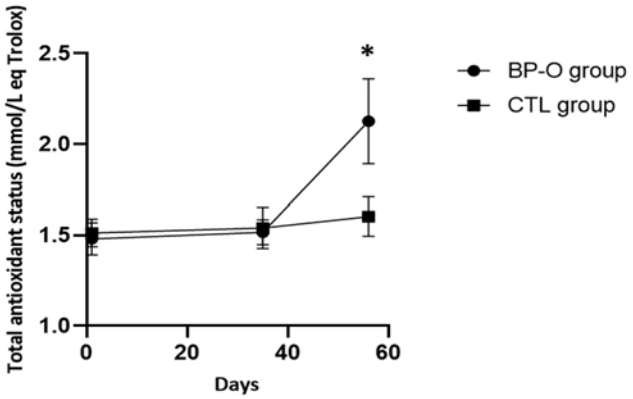

Figure 3: Comparison of total antioxidant status between groups after vaccination (* P<0.05)

Discussion

The increase in total antioxidant status observed in heifers supplemented with rumen-protected grape extract are in accordance with the extensive literature on the antioxidant effect of grape polyphenols in different species (Brenes et al., 2016; Gessner et al., 2017). Interestingly, the inclusion level used in this study (0.67 g/head/day) was significantly lower than that used in the discussed literature but induced a significant effect. This suggests that the rumen-protection was able to protect the polyphenols'

activity from degradation by the ruminal fluid evidenced described elsewhere (Chedea et al., 2016) and to increase TAS in supplemented cattle. Furthermore, whilst a wide diversity of phenolic compounds exists within grape extracts, the low molecular weight of the polyphenols from the present extract (oligomeric and monomeric flavanols, anthocyanins) are known to be small enough to be absorbed at the intestinal level (Brenes et al., 2016). Thus, good bioavailability of the phenolic compounds found in the present grape extract, associated with a rumen-protection, could explain why beneficial effects could be observed despite a very small dose.

Findings moreover indicate that grape extract polyphenols not only have an antioxidant effect, but also could play a role in down-regulating inflammatory pathways and stimulate the immune system and the production of defence substances (e.g. immunoglobulins) (Gessner et al., 2017). The present results support this hypothesis, as heifers with higher antioxidant levels showed a higher humoral response. A strong correlation between the TAS of heifers and their BRSV-antibody titres further reinforced this hypothesis.

Conclusion

The findings of the present study underline the interest of a supplementation with rumen-protected grape extract (RPGE) in young cattle around the vaccination events in order to promote a good antioxidant protection and humoral response and, in turn, a successful immunization associated with a better immune protection due to higher circulating antibody levels.

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Einfluss der Schusskonditionierung in Farmwildgehegen auf die Wildfleischqualität

Influence of shot conditioning before slaughtering on the meat quality of red deer calves

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Abstract

The general slaughter performance of the red deer calves and the differences in meat quality were investigated. There are numerous studies on conventional farm animals, which show that stress before slaughter has a negative impact on meat quality. The study at hand investigates whether this effect also applies to farmed deer. For the slaughter performance of red deer calves, the carcasses were cut seven days after slaughter. All 12 animals achieved a high slaughter yield (on average over 63%). To deter-mine the meat quality, a 16 cm long sample of the back muscle was taken from each animal during cut-ting. There were also no significant differences in meat colour and tenderness. To observe the effects of stress before slaughter, pH was measured one hour and 48 hours after maturation. Again, there was no significant difference to this parameter. In general, the hypothesis that shot conditioning influences meat quality cannot be confirmed, as no parameter of the study at hand shows significant differences.

Einleitung

Im Jagdjahr 2019/2020 wurden in Österreich insgesamt 407.000 Stück an Schalen-wild erlegt, darunter 278.000 Stück Rehwild, 57.500 Stück Rotwild, 47.300 Stück Schwarzwild und 19.100 Stück Gamswild. Dies entspricht im Vergleich zum vergangenen Jagdjahr einen leichten Anstieg von 2,8 % (Statistik-Austria, 2021). Die beiden bedeutendsten Wildarten in Österreich sind demnach Reh- und Rotwild. Im Gegensatz zu diesem freilebenden Wild gibt es in Österreich ungefähr 1.870 Farmwildhalter, welche insgesamt 16.500 Stück Rotwild und circa 30.000 Stück Damwild in Gehegen halten. Die Anzahl der Betriebe mit landwirtschaftlicher Wildhaltung steigt von Jahr zu Jahr, da diese extensive Grünlandbewirtschaftung auch die Bewirtschaftung von Steilflächen zulässt und sich zudem für den Nebenerwerb anbietet. Die Hauptwildarten in Farmwildgehegen sind Rot-, Dam-, Sika- und Muffelwild. Wildfleisch ist jedoch ein Nischenprodukt mit geringem Marktanteil. Der durchschnittliche jährliche Pro-Kopf-Verbrauch an Fleisch beläuft sich auf 94,8 kg, wovon Schweinefleisch mit 52,7 kg mehr als die Hälfte ausmacht. Der Geflügelfleischkonsum steigt in den Jahren kontinuierlich und beträgt derzeit 21,2 kg und der Wildfleischverbrauch pro Kopf in Österreich liegt bei 0,7 kg (BMNT, 2019, S. 160). Die Nachfrage nach Wildbret steigt aber tendenziell und die Nachfrage nach Wildfleisch ist größer als das Angebot. Wildfleisch ist aufgrund seiner Zusammensetzung ein sehr hochwertiges und wertvolles Lebensmittel, und ergänzt den immer wichtiger werdenden gesunden Lebensstil der Bevölkerung. Wildfleisch ist feinfaserig, zart, fett- und cholersterinarm.

Der Konsument der Zukunft richtet seine Prioritäten immer mehr auf die Regionalität und ist sehr sensibi-lisiert auf die Art und Weise, wie das Lebensmittel entsteht beziehungsweise produziert wird. Wildfleisch aus freier Wildbahn gehört zu den am tierschutzgerechtesten gewonnenen Fleischarten, und ist somit auch aus ethischer Sicht ein Lebensmittel von höchster Qualität. Dadurch aber die Nachfrage nach

Wild-fleisch höher ist als das Angebot, ist die Farmwildhaltung eine nachhaltige landwirtschaftliche Nische, um die Lücke zwischen Angebot und Nachfrage zu verkleinern. Auch in der Gatterhaltung wird das Wild sehr naturnah bewirtschaftet und Fleisch aus der Gatterhaltung ist durchaus mit Wildfleisch aus der Natur vergleichbar. Dennoch handelt es sich bei Gatterwild um landwirtschaftliche Nutztiere, welche geschlach-tet werden. Die Schlachtung von landwirtschaftlichen Nutztieren, im Speziellen beim Schwein und beim Rind, wird in den letzten Jahren immer genauer tierschutzrechtlich beobachtet und auch der Konsument verlangt eine stressfreie Schlachtung mit kurzen Transportwegen. Es gibt zahlreiche Studien, welche belegen, dass sich der Stress vor der Schlachtung, bei Schweinen und Rindern, negativ auf die Fleisch-qualität auswirkt. Ausschlaggebend für diese Wertminderung ist unter anderen der pH-Wert und der damit verbundenen Glykogengehalt in der Muskulatur. Ist das Tier gestresst, so baut der Körper schon vor der Schlachtung Glykogen ab und somit ist in weiterer Folge für die Fleischreifung nicht mehr genug in der Muskulatur vorhanden, um den pH-Wert zu senken (Gierus et al., 1997; Friedrich et al., 2015). Fleischfehler wie bei DFD- (dark firm dry) und PSE- (pale soft exsudativ) Fleisch sind ebenso auf erhöh-ten Stress vor der Schlachtung zurückzuführen. Zusätzlich zu diesen Wertverlusten des Fleisches können längere Transportwege und gestresste Tiere auch einem höheres Hygienerisiko ausgesetzt sein. In der Farmwildhaltung fallen diese kritisch beäugten Lebendtransporte weg, da die Tiere im Gehege ge-schlachtet werden und somit eine stressfreie Schlachtung möglich ist, denn die Tiere werden in gewohn-ter Atmosphäre geschlachtet.
Die vorliegende Arbeit beschäftigt sich mit der Frage, ob es auch bei Gatterwild (Rotwild) zu Fleischqualitätsverlusten und -unterschieden kommen kann, wenn die Tiere vor der Schlachtung (sprich vor dem Schuss) gestresst sind. Es wird erwartet, dass die schusskonditionierten Tieren keine negativen Auswirkungen auf die Fleischqualität, welche durch Stress hervorgerufen werden können, aufweisen. Es werden drei Farmwildbetriebe mit unterschiedlicher Schusskonditionierung untersucht, die Konditionierung erfolgte für den vorliegenden Versuch und wurde in Absprache mit den Betriebsleitern durchgeführt. Ein weiterer Aspekt dieser vorliegenden Arbeit ist die Schlachtleistung (Ausschlachtung, Schlachtkörperzu-sammensetzung, Fleischanteil, etc.) von Rotwildkälbern.

Material und Methoden

Der Versuchsplan sah den Vergleich dreier Schusskonditionierungen bei gleicher Tierkategorie beziehungsweise Fütterung vor. Die Schusskonditionierungen waren: keine Konditionierung (B1), eine ferne Konditionierung (B2) und eine nahe Konditionierung (B3), wobei je eine Konditionierung auf einem Farmwildbetrieb stattfand. Somit wurde der Versuch an drei unterschiedlichen Betrieben durchgeführt und die Fleischkühlung und -reifung fanden dann in einem zugelassenen Schlacht- und Verarbeitungsraum statt. Die Fleischanalyse wurde an der HBLFA Raumberg-Gumpenstein durchgeführt. Die zu entnehmenden Tiere, Rotwildkälber, wurden alle im Mai-Juni 2018 geboren und an den jeweiligen Betrieben bis zur Entnahme gefüttert und betreut. Der Versuch wurde am 17. November 2018 beim Betrieb 1 (keine Konditionierung) gestartet und am 16. Dezember 2018 wurde am Betrieb 3 (nahe Konditionierung) das letzte Rotwildkalb geschlachtet.

Tabelle 1. Versuchsplan

Konditionierung	Entnahme 1		Entnahme 2 (14 Tage später)	
	Tier Nr.1	Tier Nr.2	Tier Nr.3	Tier Nr.4
Nein (B1)	weiblich	männlich	männlich	männlich
Fern (B2)	männlich	männlich	männlich	weiblich
Nah (B3)	männlich	männlich	weiblich	männlich

Insgesamt werden für den vorliegenden Versuch zwölf Tiere analysiert. Es wurden pro Betrieb vier Kälber erlegt, wobei die Entnahme der Tiere pro Betrieb auf zwei Zeitpunkte aufgeteilt wurde. In Tabelle 1 wird der Versuchsplan dargestellt, pro Entnahme wurden drei Schüsse abgegeben. Schuss 1 in den Boden, so hat jedes Tier mindestens einen Schuss gehört, mit Schuss 2 wurde das erste Tier getötet

und mit Schuss 3 das zweite Tier. Diese Schussabfolge wurde bei allen Entnahmen bei allen drei Betrieben gleich gehandhabt und auch die Zeitabstände zwischen den einzelnen Schüssen wurden, so weit als möglich, gleichgehalten. Die zweite Entnahme pro Betrieb wurde vierzehn Tage nach der ersten Entnahme durchgeführt, da ansonsten die Kapazitäten des Schlacht- und Verarbeitungsraumes nicht ausgereicht hätten. Die Entnahme an sich erfolgte identisch.

Ergebnisse und Diskussion

Wie in Tabelle 2 ersichtlich, war die durchschnittliche Lebendmasse der Tiere, von allen drei Betrieben, annähernd gleich, womit dem Ziel bei gleicher Lebendmasse zu schlachten entsprochen wurde. Das schwerste Rotwildkalb wog 71,60 kg und das Leichteste 51,80 kg, dies entspricht einer Standardabweichung von ± 7,04 kg. Die Ausschlachtung betrug bei jedem Tier über 60%, im Mittel erreichten die Rotwildkälber eine Ausschlachtung von 63,7%.

Tabelle 2. Schlachtleistung der Rotwildkälber

Merkmal		Mittelwert				SD	P-Werte
		B1	B2	B3	gesamt		
Tiere	n	4	4	4	12		
Lebendmasse Schlachtung	kg	57,85	65,33	60,23	61,13	7,04	0,339
Schlachtkörper $_{warm}$	kg	38,90	43,50	39,68	40,69	4,98	0,415
Schlachtkörper $_{kalt}$	kg	37,20	41,63	38,15	38,99	4,84	0,437
Ausschlachtung $_{kalt}$	%	64,22	63,64	63,34	63,73	1,32	0,676
Schlachtkörper $_{Zerlegung}$	kg	32,05	36,15	33,10	33,77	4,43	0,437

Tabelle 3. Einfluss der Schusskonditionierung auf die pH-Wert Absenkung im Muskelfleisch von Rotwildkälbern nach der Schlachtung

pH-Werte	Mittelwert				SD	P-Werte
	B1	B2	B3	gesamt		
Keule 1 h p.m.	6,50	6,53	6,55	6,52	0,20	0,957
Keule 48 h p.m.	5,77	5,78	5,82	5,79	0,09	0,694

Der pH-Wert hat direkten Einfluss auf die Qualitätsfaktoren: Farbe, Zartheit, Geschmack, Wasserbindungsvermögen und Haltbarkeit des Fleisches (Hofmann, 1986). Im lebenden Muskel liegt dieser Wert nahe dem Neutralpunkt (7), nach der Schlachtung sinkt der pH-Wert durch die im toten Muskel ablaufende Glykogenolyse innerhalb von 24 Stunden zu einem End-pH-Wert ab, um nach Ende der Glykogenolyse, bei der Reifung des Fleisches, wieder leicht anzusteigen (ca. 0,1 pH-Einheiten) (Hofmann, 1986; Bykowska et al., 2018). Gibt es Unregelmäßigkeiten in der Glykogenolyse, führt dies zu Fehlern in der Fleischbeschaffenheit. Bei Rindfleisch ist das DFD-Fleisch ein markanter Fleischfehler, welcher nicht selten vorkommt, denn ein zu hoher End-pH-Wert (nach über 24 Stunden nach der Schlachtung über 6,2) führt es zu einem dunklen, festen und trockenen Fleisch (Binke, 2003). Übernimmt man die Richtwerte vom Rindfleisch auf das Wildfleisch so erkennt man in der Tabelle 3, dass der End-pH-Wert deutlich unter 6,2 liegt, folglich lag bei keinem Tier ein Fleischfehler vor. Es gab keine signifikanten Unterschiede in den pH-Werten der drei Konditionierungsbetriebe.

Im Hinblick auf die Hypothese des vorliegenden Versuches wird eine Signifikanz in den Parametern pH-Wert, Wasserbindungsvermögen, Fleischfarbe und der Zartheit erwartet. Die Ergebnisse zeigen jedoch, dass es bei diesen Parametern zu keinen signifikanten Unterschieden der drei Konditionierungen gab (Daten nicht dargestellt). Deutz (2014) hat End-pH-Werte, bei vor dem Erlegen gehetztem oder gestresstem Wild, von 6,6 bis 6,95 festgestellt. Die Tiere für diese Erhebung wurden auf einer Treibjagd erlegt. Es wurde in freier Wildbahn mit Treibern und Hunden gejagt, welche die Wildtiere aus ihrem

Einstand trieben und diese wurden dann von Schützen geschossen. Dieser Stress kam von vielen verschiedenen Richtungen und hielt die ganze Treibjagd an (ca. drei Stunden), da die eingesetzten Jagdhunde darauf spezialisiert sind, das Wild den Schützen zuzutreiben. Dieser besagte Stress, welcher zu Fleischqualitätsverlusten führt, ist quantitativ aber nur sehr schwer messbar. Man kann die Zeit als Hauptfaktor nehmen, dennoch ist auch die Intensivität der Beunruhigung maßgebend. Bei lebenden Tieren (zum Beispiel Rindern) kann man den Stress anhand von Messungen, wie zum Beispiel die Herz- oder Atemfrequenz und Körpertemperatur, quantifizieren. Jedoch sind diese Messungen bei Wildwiederkäuern nicht durchführbar. Die Stresssituation, welche diesen Stressfaktoren vorangeht, ist aber nur subjektiv bewertbar, und sehr schwierig in Zahlen zu fassen.

Schlussfolgerungen

Die Schusskonditionierung zeigt keine Effekt auf die pH Veränderung und Schlachtleistung, was auf einen geringeren Stresseinwirkung hindeutet. Die Tiere am Betrieb 1 (ohne Schusskonditionierung) hatten zwar Stress, da sie noch nie einen Schussknall hörten, jedoch waren sie nicht stark gehetzt, sondern lediglich durch den Schussknall beunruhigt. Diese Beunruhigung war nicht groß genug für Qualitätsverluste, somit wurden keine signifikanten Unterschiede gefunden.

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Einfluss einer verkapselten Fettsäurekombination und phytogener Zusatzstoffe auf ausgewählte Parameter bei der Zuchtsau

Influence of an encapsulated fatty acid combination and phytogenic additives on certain parameters of lactating sows

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Abstract

Due to legally required antibiotic reduction in livestock farming, the application of organic acids have increasingly come into focus. Especially medium-chain fatty acids play an important role, due to their antibacterial effects as well as their energy providing properties (Jackman et al., 2020).
The aim of this study was to evaluate the effect of a certain fatty acid combination and phytogenic additives on selected parameters of lactating sows. This feeding trial was set up on a practical farm with 1800 sows (Topigs TN70 x Topigs TN Select) in Germany. Both groups, trial group and control group, received the standard lactation feed of the farm. In the trial group the product BEWI-FATRIX® SynerG+ was added as a top dressing, from five days before farrowing until weaning (12 g per sow and day). The number of life born piglets, losses of suckling piglets and number of weaned piglets were recorded. By addition of BEWI-FATRIX® SynerG+ the losses of suckling piglets could be reduced by 8.2% compared to the control group and number of weaned piglets increased by 0.6 piglets per sow.
The trial clearly shows that the addition of a matrix-encapsulated combination product based on a certain fatty acid combination and phytogenic additives was able to decrease piglet losses. The application of a pure plant-based product in a targeted combination of ingredients creates synergistic effects that lead to a significant reduction of medication on the farm. At the same time number of weaned piglets has been increased, contributing to the success of the farm.

Einleitung

Mittelkettige Fettsäuren (MCFA) sind bekannt für ihre ausgeprägten Effekte gegen grampositive Bakterien wie Clostridien und Streptokokken. Streptococcus suis gilt im Saugferkel- und Aufzuchtbereich mittlerweile als wichtigster bakterieller Krankheitserreger. Zu den Krankheitsbildern einer Infektion mit Streptococcus suis zählen unter anderem Entzündungen von Hirnhaut, Gelenken, Nabel, Mittel- und Innenohr. Darüber hinaus kann es zu erhöhter Neugeborenensterblichkeit kommen. Problematisch und ebenso kostspielig sind für die Betriebe aber oftmals auch die Folgen einer Streptokokkeninfektion, die für den Landwirt nicht immer direkt sichtbar sind.
Der Infektionsdruck auf die Tiere, ausgelöst durch schweinespezifische, pathogene Keime wie Streptokokken und Clostridien, ist ein Hauptgrund für den Einsatz von antibiotischen Arzneimitteln. Ziel des gesetzlich verankerten Minimierungskonzeptes ist eine dauerhafte Reduzierung des Antibiotikaeinsatzes in der Nutztierhaltung. Viele Betriebe suchen daher besonders im Bereich der Futtermittelzusätze nach Alternativen, um dieses Ziel zu erreichen. Hier haben sich pflanzliche Produkte mit MCFA in den vergangenen Jahren etabliert (Ferrara, 2012). Synergistische Effekte von MCFA können jedoch nur durch eine gezielte Kombination der Wirkstoffe erzielt werden.

Das breite Wirkungsspektrum von MCFA trägt nachweislich zur Hemmung von grampositiven Bakterien im Verdauungstrakt der Tiere bei. Die antibakterielle Wirkung von MCFA auf schweinespezifische Bakterien ist dabei unterschiedlich. Laurinsäure gilt hierbei als besonders aktiver und antibakterieller Wirkstoff (Kabara, et al., 1972; Batovska, et al., 2009; Schemmer, 2020). Das Ziel ist es, durch die Ergänzung von MCFA im Mischfutter die unerwünschten Bakterien zu unterdrücken und dadurch die Entwicklung von erwünschten Bakterien im Darm zu fördern. Ein ausgereiftes und stabiles Immunsystem des Tieres bildet die Grundlage für ein optimales Wachstum und zur Ausschöpfung des maximalen Wachstumspotenzials.
Das in der Literatur beschriebene antimikrobielle Potenzial von MCFA wurde bereits in der Praxis erprobt. Die Einsatzbereiche und die Wirkungen der jeweiligen Säuren sind jedoch sehr verschieden, so dass durch eine gezielte Auswahl und Kombination von Säuren synergistische Effekte hervorgerufen werden können. Verstärkt werden kann die Wirkung zudem durch den Einsatz von phytogenen Zusatzstoffen wie ätherische Öle und deren Extrakte. Vor diesem Hintergrund war die Zielsetzung den Einfluss einer verkapselten Fettsäurekombination und phytogene Zusatzstoffe auf ausgewählte Parameter bei der laktierenden Sau zu untersuchen.

Material und Methoden

Durchgeführt wurde die Studie auf einem konventionell wirtschaftenden Praxisbetrieb mit 1800 Sauen im Nord-Westen Deutschlands. Insgesamt wurden 75 Sauen (Genetik Topigs TN70 x Topigs TN Select) einer Kontroll- und Versuchsgruppe zugeordnet. Für den Versuch wurden sowohl primipare als auch multipare Sauen eingestallt. Das Umstallen in die Abferkelbucht erfolgte etwa 7 Tage vor dem kalkulierten Abferkelungstermin. Beiden Gruppen wurde ein mehlförmiges Standard-Laktationsfutter nach der betriebseigenen Futterkurve gefüttert. Hergestellt wurde das Laktationsfutter in der eigenen Futtermischanlage des Betriebes. Die verfütterten Rationen unterschieden sich zwischen Versuchs- und Kontrollgruppe nur in der Zulage des Futterzusatzstoffs.
In der Versuchsgruppe wurde den Sauen ab 5 Tage vor dem kalkulierten Absetztermin täglich 12 g BEWI-FATRIX® SynerG+ als Topdressing gegeben. Vorversuche haben gezeigt, dass die Zulage ohne negative Folgen auf die Futteraufnahme als Einmaldosis gegeben werden kann. Dies verringerte den Arbeitsaufwand deutlich. Die Zulage erfolgte ohne Nährstoffausgleich. Die Ferkel wurden nach durchschnittlich 28 Tagen abgesetzt. Die Untersuchungsparameter während der 4-wöchigen Säugeperiode waren die Anzahl lebend geborener Ferkel, Verluste während der Säugephase und die Anzahl der abgesetzten Ferkel.

Ergebnisse

Während der Versuchsphase wurde in beiden Gruppen die zugeteilten Futtermengen und die Zulage bei allen Fütterungen restlos aufgenommen. Die Ergebnisse des Versuchs sind in Tabelle 1 dargestellt. Da das Produkt erst ab 5 Tage vor dem kalkulierten Abferkeldatum gegeben wurde ist nicht davon auszugehen, dass die Anzahl der lebend geborenen Ferkel hierdurch beeinflusst wurde. Durch die Zulage von BEWI-FATRIX® SynerG+ konnten die Saugferkelverluste um 8,2 Prozentpunkte im Vergleich zur Kontrollgruppe reduziert werden. Mit lediglich 6,72 % waren die Saugferkelverluste auf einem sehr niedrigen Niveau. Die Anzahl der abgesetzten Ferkel lag in der Versuchsgruppe mit 13,3 abgesetzten Ferkeln pro Wurf um durchschnittlich 0,6 Ferkel je Sau höher.

Tabelle 1: Ergebnisse der zootechnischen Leistungen

	Sauen	**Leb. geb. Ferkel je Sau**	**Saugferkel- verluste**	**abgesetzte Ferkel je Sau**
	[n]	[n]	[%]	[n]
Versuchsgruppe	38	14,3	6,72	13,3
Kontrollgruppe	37	14,9	14,9	12,7

Diskussion

Viele *in vitro*-Studien zeigen beim Einsatz von MCFA ausgeprägte antibakterielle Effekte sowohl gegen gramnegative als auch grampositive Keime (Kabara et al., 1972; Batovska, et al., 2009). In der vorliegenden Studie wurde dieser Effekt einer speziellen matrixverkapselten Fettsäurekombination auf ausgewählte Parameter bei der Sau überprüft. Die Ferkelverluste in der Säugephase konnten in der Versuchsgruppe deutlich reduziert werden. Durch die Zulage des MCFA Produkts vor der Abferkelung wird der Gesundheitszustand der Sauen bereits vor der Geburt verbessert. Der Infektionsdruck der Sauen wird deutlich reduziert, was auch die Gefahr einer Infektion für das Saugferkel während der Geburt deutlich verringert. Eine Infektion der Saugferkel mit Streptococcus suis geschieht oftmals schon während der Geburt über kleinste Wunden wie den Nabel. Über das Blut können sich die Erreger dann in den bestimmten Stellen des Körpers ansiedeln und vermehren.
Durch den Einsatz von MCFA werden nachweislich diese unerwünschten Bakterien im Verdauungstrakt der Tiere gehemmt. Die eingesetzte Laurinsäure wirkt dabei als aktivierte Komponente, welche jedoch als freie Fettsäure nur bedingt in die Bakterienzelle eindringen kann. Die spezielle Kombination der Wirkstoffe, hilft dabei, die grampositiven Bakterien zu öffnen (Türöffner). Die Laurinsäure kann in die Zelle eindringen und eine Übersäuerung der Zelle herbeiführen, wodurch diese abstirbt. Besonders die grampositiven Bakterien, die durch eine stabile und dicke Mureinschicht in der Zellwand äußerst robust sind, werden von dieser speziellen Fettsäurekombination erfolgreich reduziert. Hierdurch wird die Entwicklung erwünschter Bakterien in der Darmflora gefördert und die Besiedelung des Darms wird so auf natürliche Weise positiv beeinflusst. Die positiven Effekte einer MCFA Zulage bereits im Sauenfutter und die damit verbundenen verringerten Saugferkelverluste werden mehrfach in der Literatur beschrieben (Newcomb et al. 1991; Azain 1993; Jean und Chiang 1999)
Synergistische Effekte können zudem durch den kombinierten Einsatz von MCFA und phytogenen Zusatzstoffen wie ätherische Öle und deren Extrakte (Baltic et al. 2017). Des Weiteren wird durch die Matrixverkapselung des Produktes erreicht, dass es zu keinen negativen Effekten kommt, wie sie teilweise in der Literatur bei zu hohen Dosierungen von MCFA Produkten beschrieben wird. Außerdem werden direkte und indirekte Leistungseffekte auf die Epithelfunktion auch in den hinteren Abschnitten des Dünndarms beschrieben.
Durch die systemische Wirkungsweise werden die aktiven Wirkstoffe von der Sau über die Milch auf die Ferkel übertragen (Kour et al., 2020). Die verbesserte Kolostrum- und Milchqualität wirkt sich wiederum positiv auf Gesundheit, Entwicklung und Leistungsparameter beim jungen Saugferkel aus. Auch vorausgegangene Untersuchungen zeigen die reduzierten Saugferkelverluste (Schemmer, 2021) und zudem einen signifikant erhöhten Wurfzuwachs (Schemmer & Hovenjürgen, 2021). Dies belegen auch weitere *in vivo*- und *in vitro*-Studien in der Literatur (Zentek et al. 2011).

Schlussfolgerungen

Vor allem in kritischen Phasen wie der Laktation und dem Absetzen von der Sau haben sich verschiedene Produkte auf Basis MCFA bewährt. Im vorliegenden Versuch konnten durch die Zulage eines matrixverkapselten Kombinationsprodukt auf Basis einer speziellen Fettsäurekombination und phytogenen Zusatzstoffen (BEWI-FATRIX® SynerG+) im Laktationsfutter die Saugferkelverluste deutlich reduziert und die Anzahl abgesetzter Ferkel je Wurf erhöht werden.
Durch den positiven Einfluss auf die Darmentwicklung und die Mikrobiota beim Ferkel und ihre antimikrobiellen Eigenschaften wird der Gesundheitsstatus der Tiere positiv beeinflusst. Der Einsatz rein pflanzlicher Produkte in einer gezielten Wirkstoffkombination kann das Risiko einer Infektion der Saugferkel mit Streptokokken, Clostridien wie auch weiteren Infektionen durch den Einsatz im Laktationsfutter bereits im Voraus effektiv verringern und durch die daraus resultierenden Synergieeffekte zu einer deutlichen Reduzierung des Medikamenteneinsatzes auf landwirtschaftlichen Betrieben beitragen.

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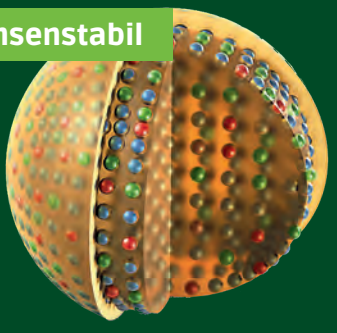

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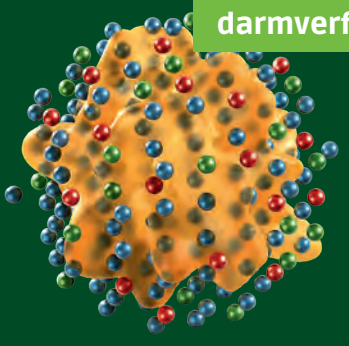

- Cholin
- Lysin
- Methionin
- B-Vitamine
- Pflanzenextrakte
- Ätherische Öle
- Organische Säuren
- Fettsäuren etc.

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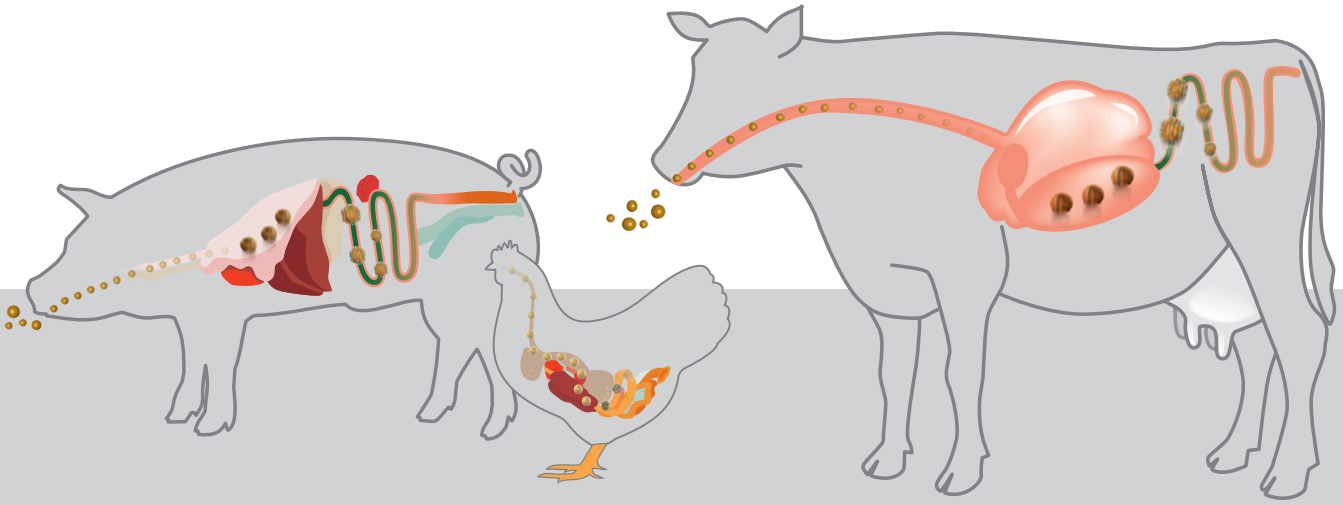

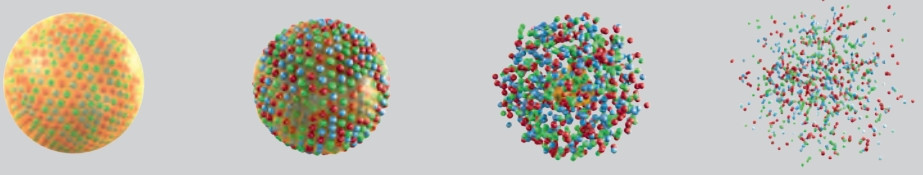

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Autorenanschrift

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Auswirkungen der Zugabe einer Kombination antioxidativ wirksamer Zusätze während des Absetz-Östrus-Intervalls auf die Reproduktionsleistung von Sauen

Effects of the addition of a combination of antioxidant additives during the weaning-estrus interval on the reproductive performance of sows

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Abstract

Genetic selection of hyper fertile sows has led to an increase in litter sizes, along with an increased incidence of underdeveloped piglets and heterogeneous litters. This causes major problems for producing farms. Hyper productive sows are exposed to oxidative stress due to their high reproductive output, which increases the production of reactive oxygen species (ROS) and causes cellular damage. Supplementation of a concentrated melon extract (MELOFEED; Lallemand Animal Nutrition), naturally rich in the primary antioxidant superoxide dismutase (SOD), and a highly available organic selenium source (ALKOSEL; Lallemand Animal Nutrition) is an effective way to reduce the effects of oxidative stress on sows. The present results have shown that feeding an antioxidant combination of melon extract and organic selenium together with vitamins, during the weaning estrus period positively affected several reproductive parameters such as the number of piglets born (+4.4%), born alive (+5.6%), more litters per sow per year (2.45 vs. 2.41), weaning rate (1.5% vs. 5.3% in the control), and farrowing rate (93.7% vs. 85.1%) had an impact. Short-term application increased farm profitability and could be a promising approach to increase farm efficiency

Einleitung

Die genetische Selektion hyperfruchtbarer Sauen hat zu einer Zunahme der Wurfgrößen geführt, zusammen mit einem vermehrten Auftreten unterentwickelter Ferkel und heterogenen Würfen. Dies verursacht große Probleme für die Erzeugerbetriebe. Hyperproduktive Sauen sind aufgrund ihrer hohen Reproduktionsleistung oxidativem Stress ausgesetzt, wodurch die Produktion reaktiver Sauerstoffspezies (ROS) gesteigert wird und Zellschäden verursacht werden (Berchieri-Ronchi et al., 2011). Diese Schäden können negative Auswirkungen auf die Einnistung des Embryos haben (Aurousseau et al., 2004). Die Ergänzung eines konzentrierten Melonenextraktes (MELOFEED; Lallemand Animal Nutrition), das von Natur aus reich an dem primären Antioxidans Superoxiddismutase (SOD) ist, und einer hochverfügbaren organischen Selenquelle (ALKOSEL; Lallemand Animal Nutrition) ist ein wirksamer Weg, um die Auswirkungen von oxidativem Stress auf Sauen zu verringern. Eine frühere Studie zeigte eine vorteilhafte Wirkung einer antioxidativen Lösung im Absetz-Östrus-Intervall der Sauen auf den Prozentsatz unterentwickelter Ferkel pro Wurf und auf die Homogenität der Geburtsgewichte innerhalb des Wurfs (Le Treut et al., 2013). In der vorliegenden Studie sollten die Effekte einer Fütterung mit einer spezifischen Kombination antioxidativ wirksamer Nährstoffe, die die natürliche Abwehr während des Intervalls zwischen Absetzen und Östrus stärken sollen, auf die Reproduktionsleistung von Sauen sowie die Betriebsprofitabilität untersucht werden.

Material und Methoden

In der durchgeführten Studie wurden auf einem kommerziellen Betrieb in Deutschland 429 Sauen in 6 aufeinander folgenden Durchgängen in zwei Gruppen aufgeteilt: 188 Sauen bekamen beim Absetzen einen Bolus aus einer antioxidativer Wirkstoffkombination (ANTIOX), 241 erhielten keinen Bolus (KONTROLLE). Die Sauen in der Versuchsgruppe erhielten täglich einen 8 g-Bolus an 5 aufeinander folgenden Tagen, passend zum Absetz-Östrus-Intervall (Abb.1).

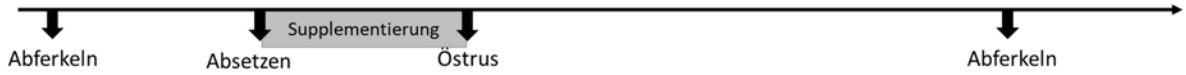

Abbildung 1: Eingabeschema der Boli

Der Bolus enthielt 200 mg MELOFEED, 0,24 mg organisches Selen aus ALKOSEL sowie Vitamine.
Neben der Umrauschrate wurde die Abferkelrate sowie die Ferkelanzahl (gesamt geborene, lebend geboren, tot geboren und abgesetzte Ferkel) in dieser Studie näher betrachtet. Alle Daten wurden mittels ANOVA unter Verwendung des T-Testverfahrens oder Mann-Whitney-Test mit SAS (SAS Inst. Inc., Cary, NC, USA) analysiert.

Ergebnisse

Die Kombination antioxidative Zusätze steigerte signifikant den Besamungserfolg bei den Versuchssauen. Die Abferkelrate verbesserte sich um 8,6 Prozentpunkte ($p<0,01$; Abb. 2). Ebenso wurden die nicht erfolgreichen Besamungen um 3,9 Prozentpunkte reduziert ($p<0,01$; Abb. 2).

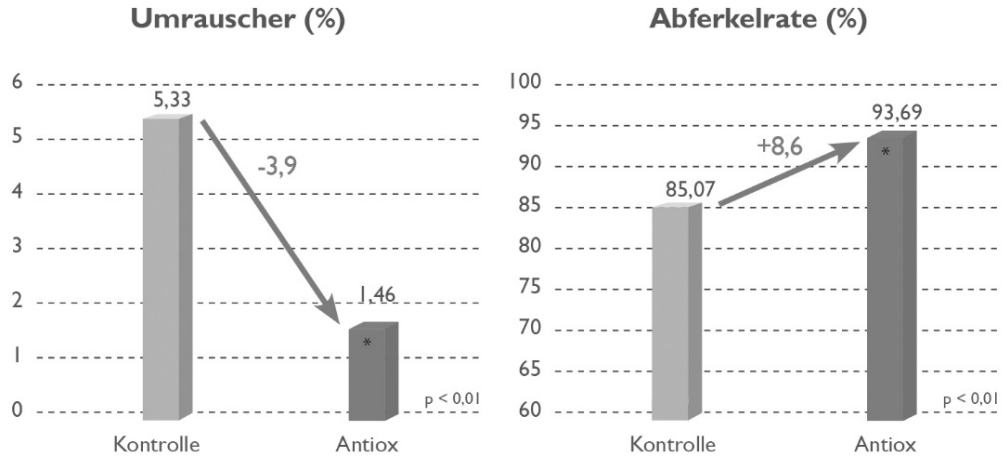

Abbildung 2: Einfluss der antioxidativen Kombination auf die Fruchtbarkeit der Sau ($p<0,01$)

Bereits eine 5-tägige Gabe des antioxidativen Bolus zeigte eine signifikante Verbesserung der Abferkelleistung. Die folgende Tabelle zeigt eine um 4,4 % gesteigerte Anzahl an gesamt geborenen Ferkeln im Vergleich zur Kontrolle (Tab. 1). Auch die Zahl der lebend geborenen Ferkel zeigt einen signifikanten Unterschied (Tab.1). Der prozentuale Anteil an tot geborenen Ferkeln konnte durch den Einsatz der antioxidativen Kombination um 1,3 Prozentpunkte gesenkt werden (Tab. 1).

Tabelle 1: Effekt der antioxidativen Kombination auf die Abferkelleistung

FERKEL/WURF	KONTROLLE	ANTIOX	Abweichung
Gesamt geborene	15,8^{A}	16,5^{B}	+ 4,4 %
Lebend geborene	14,2^{a}	15,0^{b}	+ 5,6 %
Tot geborene (%)	10,0	8,7	- 1,3 Punkte

Die vorliegende Studie konnte zudem zeigen, dass bei Sauen mit den Paritäten 3 bis 6 der verringerte Mittelwert (%) an tot geborenen Ferkeln am deutlichsten ausgeprägt war (p<0,05; Abb.3).

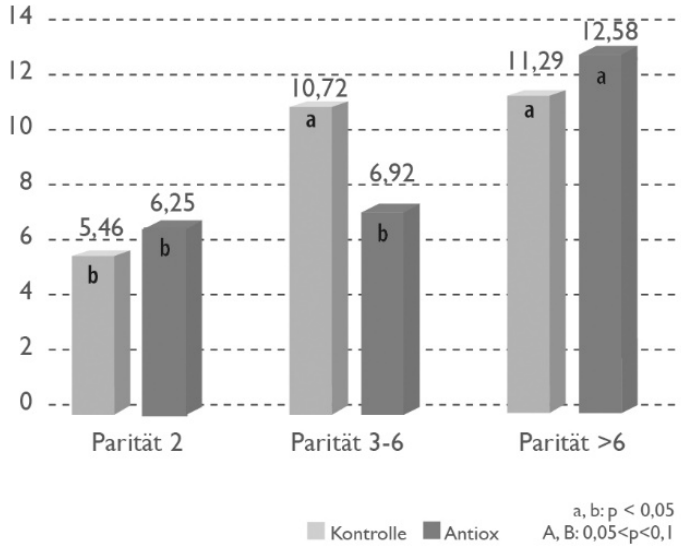

Abbildung 3: Tot geborene Ferkel pro Wurf nach Paritäten (%) (a,b:p<0,05; A,B: 0,05<p<0,1)

In einem weiteren Aspekt dieser vorliegenden Studie sollte die Entwicklung der Betriebsprofitabilität unter dem Einsatz eines antioxidativen Bolus näher betrachtet werden. Der Versuchsbetrieb verzeichnete in den Versuchsgruppen eine geringere Umrauschrate (1,5 % vs. 5,3 % in der Kontrolle), eine verbesserte Abferkelrate (93,7 % vs. 85,1 %), mehr lebend geborene Ferkel (15 vs. 14,2), mehr Würfe/Sau/Jahr (2,45 vs. 2,41) bei geringeren Kosten sowohl je Sau/Jahr (653 € vs. 687 €) als auch je Ferkel (20,7 € vs. 23,3 €). In einem Betrieb mit 1.000 Sauen betragen die jährlichen Kosten für den Einsatz der antioxidativ wirksamen Kombination 6.000 €. Der erwirtschaftete Vorteil beträgt 75.000 €, dies entspricht einem ROI von 11,5:1 (Quelle: Cost simulator designed by SIP Consultors (www.3tres3.com).

Diskussion und Schlussfolgerungen

In den letzten zwei Jahrzehnten haben Schweineproduzenten zunehmend mit genetisch hochfruchtbaren Sauen gearbeitet, um eine Steigerung der Ferkelanzahl pro Sau und damit eine höhere Betriebsprofitabilität zu erreichen. Oft führt diese Zunahme der Wurfgrößen zu heterogenen Würfen mit teils unterentwickelten, häufig schwächeren Ferkeln. Hochleistungsfähige Sauen sind zudem aufgrund ihrer hohen Reproduktionsleistung oxidativem Stress ausgesetzt. Eine Folge der durch oxidativen Stress bei

Sauen verursachten Schädigung ist die intrauterine Wachstumsretardierung (IUWR). Bei den leichten Ferkeln eines Wurfs leiden einige an einer fetalen Wachstumsverzögerung und sind bei der Geburt unterentwickelt. Die IUWR ist mit hohen Morbiditäts- und Mortalitätsraten vor dem Absetzen, einer geringen Effizienz der Futterverwertung, dauerhaften Auswirkungen auf Wachstum und Entwicklung sowie einer schlechten Schlachtkörperqualität verbunden (Chevaux et al., 2010).
Die vorliegenden Ergebnisse haben gezeigt, dass sich die Fütterung einer antioxidativen Kombination aus Melonenextrakt und organischem Selen zusammen mit Vitaminen, während der Absetz-Östrus-Periode positiv auf verschiedene Fortpflanzungsparameter wie die Anzahl der gesamt geborenen, lebend geborenen und abgesetzten Ferkel, der Umrauschrate sowie der Abferkelrate auswirkte. Die kurzfristige Anwendung steigerte die Rentabilität des Betriebs und könnte ein vielversprechender Ansatz zur Effizienzsteigerung in der Schweineproduktion darstellen.

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Influence of polyphenol antioxidants supplementation in gestation and lactation diets on reproductive parameters, productive performance and blood parameters of sows and their offspring

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Abstract

The present experiment was carried out to assess the effects of supplementation in gestation and lactation feed with natural water- and fat-soluble polyphenol antioxidants mixture (MiaPhenol, MIAVIT GmbH, Robert-Bosch-Straße 3, 49632 Essen (Oldenburg), Germany), in sow diets on their performance and the effects on their offspring. Previous studies have proven the effect of polyphenolic compounds on the antioxidant defense ability and oxidative stress levels in sows (Chen et al., 2016; Hu et al., 2015; Lykkesfeldt and Svendsen, 2007) and their progeny (Wang et al. 2017). A total of 200 sows were randomly allocated to two experimental diets. The sows of the control group (CG) had no MiaPhenol supplementation, whereas the MiaPhenol group (MPH) was supplemented with 200 mg MiaPhenol per sow and day. Diets were iso-nutritive and iso-energetic. Performance parameters and blood analyses for sows and offspring were recorded together with blood Performance data of the sows and piglets were recorded. Whereas the results shown no statistical effects on the performance parameters, a statistically significant improvements of the enzymatic antioxidants (SOD, GSH-Px), a higher recovery of phenolic compounds and a lower malondialdehyde value both in supplemented sows and their offspring which could lead to an improvement on supplemented polyphenol antioxidants sows and offspring endogenous antioxidant defense capacity, as well as, a successful carry-over of the antioxidant compounds from the sows to their offspring.

Introduction

With the increase in reproductive capacity, it can be deduced that, a decade ago, the nutritional requirements of sows were different from current ones of hyperprolific sows (Lázaro, 2013; Panzardi, 2009). Considering that nutrient levels must be provided at each stage of pregnancy, failures in the reproduction process can have variable consequences on growth rate, development of fetuses in uterus, weight of piglet at birth, body reserves and subsequent performance (Close and Cole, 2001).
In this sense, it is also believed that oxidative stress in sows negatively influences the weight of piglets at birth, mainly due to the high metabolic activity observed in sows (Tan et al., 2015). In addition, oxidative stress has been suggested as causal agent in disorders related to human and animal pregnancy, such as embryonic resorption, recurrent pregnancy loss, preeclampsia, intrauterine growth restriction and fetal death, which are predictive of high risk of metabolic syndrome in postnatal life and may be a common pathway in metabolic development programming (Pereira and Martel, 2014; Sanchez-Aranguren et al., 2014). In piglets, inflammatory stress is usually accompanied by increased oxidation products, resulting in oxidative stress, and is related to increased severity of effect on lactating animals. The addition of antioxidant compounds in pig diets may improve endogenous antioxidant defense capacity, which has been considered a plausible way to prevent oxidative stress. In sows, previous studies have shown that dietary antioxidant supplementation, such as naturally occurring polyphenols in plants, enhance the antioxidant defense ability and relieve oxidative stress effectively, which is beneficial for

litter size and piglet growth (Chen et al., 2016; Hu et al., 2015; Lykkesfeldt and Svendsen, 2007), even having effects on the newly born piglets (Wang et al. 2017).

Material and methods

A total of 200 gilts and sows from Topigs Norsvin TN70 strain (1-6 parities) maintained under the same conditions, were sorted into two treatment groups during the whole gestation and lactation period to evaluate the effects of supplementation in the feed with natural water- and fat-soluble polyphenol antioxidants mixture (MiaPhenol, 200 mg/sow/day; MIAVIT GmbH, Robert-Bosch-Straße 3, 49632 Essen (Oldenburg), Germany; MPH group), remaining a control group of sows untreated (Control group).
At the beginning of the experiment, the sows were weighed and measured the backfat thickness which was measured at P2 point, 6.5 cm from the dorsolumbar line and 6.5 cm from the last rib in the cranial direction. From the confirmation of pregnancy on, the sows were housed in individual cages, and at 110 days of gestation were transferred to maternity sheds with feeders and drinkers for sows and piglets, as well as a shelter with heat source and cooling system for the sow. Diets were formulated to meet the minimum nutritional requirements in accordance with the recommendations of Topigs Norsvin commercial strain manual for gestation and lactating sows. During pregnancy, sows received 1.7 kg of feed and 2.8 kg of feed were provided at pre-lactation period. Throughout lactation the sows were subjected to a feeding management, with gradual increase of feed supply until reaching 6 kg of diet at the 5th postpartum day with four portions per day. From the 6th day postpartum ration was offered considering 2 kg per sow and 0.5 kg per suckling pig, remaining constant until weaning. The quantities and leftovers were weighed daily, and the MiaPhenol supplementation was performed on top in the first daily feed, ensuring 200 mg of MiaPhenol/sow/day in the MPH group.
The total number of piglets, number of stillborn piglets, number of mummified piglets and number of live born piglets were recorded. Live piglets were individually identified with earrings and weighed at birth and weaning. The uniformity of the litter was carried out among piglets of females of the same treatment until the third day of life of the piglets, to maintain 13-14 piglets per sow. Also, the piglets received prestarter feed from day 7 until weaning.
Blood samples at 15 days postpartum from 20 sows per treatment were taken, together with blood samples of 40 piglets per treatment (20 litters x 2 piglets each) at 7 days of age for analysis of blood count, leukogram, serum enzymatic antioxidants (superoxide dismutase, glutathione peroxidase), total phenolic compounds, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, lipid peroxidation (Malondialdehyde - MDA) and C-reactive protein.
After 24 days of lactation, the piglets were weaned and weighed, the sows were weighed and measured again. Data were analyzed using the GLM-procedure of the statistical package SAS. Differences with a p-value of less than 0.05 were considered statistically significant.

Results and discussion

No statistical differences were observed in the performance parameters of sows and piglets. The sows had a similar feed intake and body weight loss. Nevertheless, MPH group sows obtained numerically higher litter weight at birth compared with Control group (21.13 vs. 20.18 kg; p=0.125) and at weaning (79.61 vs. 78.01 kg; p=0.598).
Blood analyses results of sows and piglets are shown in table 1. There were no differences observed on hemo- and leukogram blood parameters among treatments, whereas enzymatic antioxidants and biochemical parameters did. Superoxide Dismutase resulted significantly higher in MPH group sows (127.79 vs. 112.36 U/ml; p=0.012) and piglets (115.69 vs. 105.36 U/ml; p=0.023), in the same way, Glutathione Peroxidase was significantly increased in MPH group sows compared with Control group (38.75 vs. 29.36 U/ml; p=0.001) and marked a statistical tendency in their offspring too (29.45 vs. 26,55 U/ml; p=0.083). Phenolic Compounds (Gallic Acid Equivalent) shown both in sows and piglets MPH group a numerically higher recovery tendency compared with Control group ones (26.47 vs. 23.88; p=0.072 and 25.63 vs.

22.66; p=0.071 respectively). Additionally, Malondialdehyde value was significantly decreased in MPH group sows compared with Control group ones (2.63 vs. 2.85 µmol/ml; p=0,038), and tending to be lower on their offspring too (2.46 vs. 2.69; p=0.084).

Table 1. Average serum parameters of lactating sows and piglets according to sows' diet

Parameters	Sows 15 days p.p.			Piglets		
	Control	MiaPhenol	p-value	Control	MiaPhenol	p-value
n	20	20		40	40	
Hemogram						
Red cells (10^6/µl)	5.53	5.81	0.254	5.91	6.05	0.131
Hemoglobin (%)	12.09	12.63	0.246	10.43	10.68	0.156
Hematocrit (%)	35.02	34.86	0.457	34.65	35.39	0.236
MCV[1] (µm³)	66.93	68.62	0.315	58.61	58.40	0.647
MCHC[2] (%)	32.45	33.10	0.546	30.13	30.21	0.546
Leukogram						
Leukocytes (10^3/µl)	10.95	11.01	0.349	7.80	7.03	0.355
Neutrophils (%)	46.36	48.96	0.689	53.33	52.57	0.665
Lymphocytes (%)	41.37	44.36	0.354	40.92	39.88	0.316
Eosinophils (%)	1.09	0.96	0.215	2.15	1.95	0.642
Monocytes (%)	5.31	4.02	0.775	4.18	5.55	0.565
Enzymatic antioxidants						
SOD[3] (U/ml)	112.36b	127.79a	0.012	105.36b	115.69a	0.023
GSH-Px[4] (U/ml)	29.36b	38.75a	0.001	26.55	29.45	0.083
Biochemical parameters						
Triglycerides (mg/dl)	28.35	28.57	0.321	44.50	45.63	0.156
Cholesterol (mg/dl)	67.53	67.02	0.348	141.27	138.56	0.654
HDL (mg/dl)	38.12	41.57	0.456	13.62	14.66	0.345
LDL (mg/dl)	35.36	34.02	0.754	119.37	118.02	0.364
PC[5] (GAE µg/ml)	23.88	26.47	0.072	22.66	25.63	0.071
MDA[6] (µmol/ml)	2.85b	2.63a	0.038	2.69	2.46	0.084
CRP[7] (mg/l)	14.56	15.36	0.656	8.66	8.69	0.449

[1]Mean Corpuscular Volume. [2]Mean Corpuscular Hemoglobin Concentration. [3]Superoxide Dismutase. [4]Glutathione Peroxidase. [5]Phenolic compounds (Gallic Acid Equivalent). [6]Malondialdehyde. [7]C-reactive protein. [a,b] Means followed by different letters on the same line differ by Tukey's test ($p < 0.05$)

Conclusion

The current trial indicates that the supplementation with natural water- and fat-soluble polyphenol antioxidants (MiaPhenol) did not affect statistically performance parameters of sows and their offspring except numerically higher litter weight at birth and at weaning. In addition, it was observed a statistically significant improvements of the enzymatic antioxidants (SOD, GSH-Px), a higher recovery of phenolic compounds and a lower malondialdehyde value both in supplemented sows and their offspring. Further studies are needed to clarify mode of action and efficacy of MiaPhenol.

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Novel *Ascophyllum nodosum* feed additive for pig nutrition

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Abstract

The mycotoxin binding ability and adhesion properties of processed seaweed meal from *Ascophyllum nodosum* (MAXASCO) were analyzed utilizing radioactive labelling and an ex-vivo intestinal mucosa model. The material showed better mycotoxin binding abilities compared to bentonite and was inhibiting the adhesion of *Escherichia coli* (*E. coli*) to mucus of the small intestine of pigs more effectively than Mannan-oligo-saccharide (MOS). Furthermore, feeding trials with fattening pigs and rearing piglets were conducted to assess the effects of MAXASCO in pig nutrition. The supplementation of MAXASCO led to improvements of average daily weight gains and feed conversion in both, piglets and fattening pigs.

Introduction

The reduction of antibiotic use in agricultural animal husbandry in order to prevent antimicrobial resistance is a recurring topic in both scientific and social debates. Furthermore, the use of zinc oxide in piglet feeding will be increasingly restricted around the world and completely banned in some places mainly due to environmental pollution. These are only two examples of rapid changes in the framework conditions pig farmers and piglet producers have to deal with. Animal nutrition and the feed industry are called upon here in the search for natural, innovative solutions to positively influence the gut microbiome and thus the gut integrity of farm animals and the efficiency of feeding. Algae can be part of this solution as they comprise a wide variety of photosynthetically active organisms that generate their biomass from carbon, water and minerals. They draw an unprecedented spectrum of macro and micronutrients from the sea. For this reason, a wide variety of algal meals and algal extracts are already used in the feeding of farm animals (Bikker et al. 2020). The brown algae *Ascophyllum nodosum*, which is harvested in the coastal waters of the North Atlantic, is also rich in secondary plant constituents, such as the sulphated polysaccharides fucoidan and ascophyllan, the β-glucan laminarin and alginate, which are known to have prebiotic and various other beneficial feeding properties. However, research on the use of the dried and ground brown algae Ascophyllum nodosum could not always show positive effects (Michiels 2012). Hence, a digestion processing technology was developed in order to transfer as high a proportion of the polysaccharides as possible from the non-soluble to the soluble fraction and to potentiate the positive effects, such as toxin binding, germ-inhibition (Ford et al. 2020), prebiotic activity and formation of a protective film on the intestinal mucosa thanks to improved gelling properties. Viscosity measurements using various methods (including Brookfield viscometer) were able to demonstrate a clear influence of the processing on the rheological properties of the seaweed meal (Data not shown, available on request). Numerous trials were conducted both in-vitro and in-vivo to assess, how the processed material (MAXASCO) performs in terms of mycotoxin binding capacity (in comparison with bentonite as a conventional binding agent), adhesion properties of E. coli (in comparison with MOS) and zootechnical parameters in practical feeding.

Material and methods

Toxin binding: The mycotoxin binding capacity of MAXASCO was investigated in a study in comparison with a conventional toxin binder (bentonite) (CON). The products were tested in different supplements

in order to obtain statements on the dose-response relationship and for the efficiency of the binding or adhesion properties. The binding efficiency was tested under both acidic and neutral conditions, simulating the stomach (pH 2.5) as well as the intestine (pH 6.5). Radioactively labelled deoxinivalenol (DON) mycotoxin was transferred in defined concentrations into a buffer/test material suspension at pH 2.5 and 6.5. After incubation at 38 °C for two hours and subsequent centrifugation, the amount of unbound mycotoxin was determined by measuring the radioactivity using liquid scintillation counting.
Evaluation of the adhesion properties: The effect of MAXASCO supplementation on the adhesion of *E. coli* to the mucus of the small intestine was assessed. In the present study, the effect of MAXASCO in comparison to MOS as an adhesion inhibitor was investigated in an ex vivo intestinal mucus model (pig) using radioactively labelled bacterial cultures (E. coli).
Feeding trials fattening pigs: In two fattening trials (in cooperation with the Christian-Albrechts-University of Kiel), 144 animals (DanAvl x Duroc) were divided into control and experimental groups and housed in pairs. The animals weighed an average of 29.3 kg at housing. Both groups received the same pre-fattening and finishing feed (Phase I: trial days 1 - 49 and Phase II: trial days 50 - 84) ad libitum. The experimental group received an additional 500 ppm MAXASCO for the entire duration of the trial. The feed intake and weight of the pigs were determined weekly.
Feeding trial rearing piglets: In another study, the effect of MAXASCO on the biological performance of rearing piglets was investigated. In this trial, 200 weaned piglets (DanAvl x Pietrain) with an average age of 26 days were divided into two groups. They were divided into an experimental and a control group directly after weaning and subsequently fed nursery feed. The experimental group received an additional supplement of 500 ppm MAXASCO. Animals were fed ad-libitum via gruel dispensers. During the 42-day trial, live weight of the piglets and feed intake were determined every 14 days.

Results

The test evaluation showed that bentonite could only bind DON with moderate success (Figure 1). In contrast, MAXASCO was able to achieve a significantly higher binding of DON overall and reached over 25% at a dosage of 2000 ppm. Binding was also more efficient at neutral pH (gut).

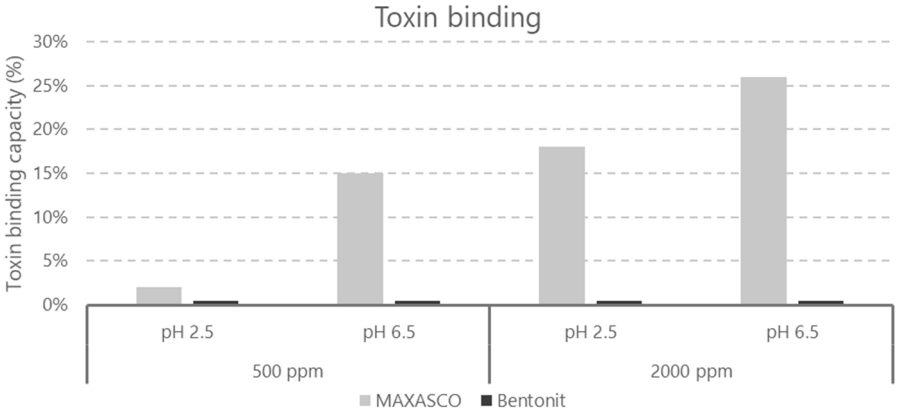

Figure 1: Comparison of deoxinivalenol (DON) binding capacities of MAXASCO and bentonite compared at acidic (2.5) and neutral (6.5) pH and at two different dosages

The results (Figure 2) clearly indicate that MAXASCO is effectively inhibiting the adhesion of *E. coli* to the small intestines of piglets. Depending on the dosage efficacy ranges from 13% (500 ppm) to 55% (2000 ppm) and there is a clear advantage when compared with the inhibition efficacy of MOS.

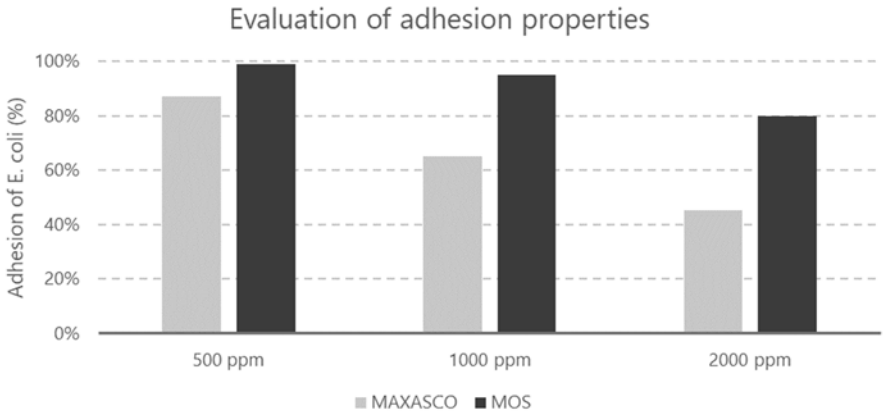

Figure 2: Influence of different doses of MAXASCO and mannan-oligo saccharides (MOS) on adhesion of E. coli to small intestinal mucus of piglets

Feeding trials fattening pigs:

Table 1: Effect of MAXASCO on daily weight gain and feed conversion ratio (FCR) in fattening pigs

Study	Dosage (ppm)	Daily weight gain Phase I [g/Day]	Daily weight gain Phase II [g/Day]	Daily weight gain Total [g/Day]	FCR Phase I	FCR Phase II	FCR Total
Trial 1	0	981[b]	1162	1055	2.36[a]	3.00	2.63[a]
	500	1018[a]	1177	1083[a]	2.27[b]	2.97	2.58[b]
Trial 2	0	933	1167	1030	2.38	2.75[a]	2.55[a]
	500	936	1202	1048	2.36	2.62[b]	2.48[b]

Higher daily weight gains and improved feed conversion could be achieved by supplementing MAXASCO in both trials (Table 1). The improvement of FCR was significant in both trials over the entire period (trial I 1.9%, trial II 2.7%). In the first trial, daily live weight gains were also significantly improved by 27.88 g per animal over the entire trial period.

Feeding trial rearing piglets:

Table 2: Effect of MAXASCO on daily weight gain and feed conversion ratio (FCR) in weaned piglets

	Daily weight gain [g/Day]				Feed conversion ratio			
Dosage (ppm)	Days 1 - 14	Days 14 - 28	Days 28 - 42	Total	Days 1 - 14	Days 14 - 28	Days 28 - 42	Total
0	255[c]	497	742[a]	499[a]	1.31	1.46	1.56	1.48
500	273[d]	521	785[b]	527[b]	1.23	1.46	1.60	1.49

Over the entire duration of the trial, a very high overall performance level was observed for both the experimental and the control group (Table 2). The average daily weight gains were 499 g (control) and 527 g (MAXASCO). The MAXASCO group showed an increased mean live weight gain per day in all rearing phases compared to the control group. For the phases day 28 - 42 as well as over the entire rearing period (days 1 - 42), the differences were significant ($p<0.05$). In the first phase from day 1 - 14, the average live weight gains were also significantly better ($p<0.1$). Thus the experimental group had an increased average daily weight gain of 5.8% (day 28 - 42) and 5.6% (day 1 - 42) compared to the control group.

Discussion

MAXASCO showed a higher binding capacity for DON then bentonite, which is remarkable as DON is known to be difficult to bind. This effect might be explained by an improved chemical-physical binding capacity of the brown algae and an increased ion exchange capacity, due to the processing. Influential

for the capacity of the binding properties of Ascophyllum nodosum are the contents of soluble mannuronic acid (M) and guluronic acid (G). M and G are the characteristic monomers of alginic acid. Processing increases the ratio of available mannuronic to guluronic acid and this shift brings about an increase in binding capacity (Sterner & Edlund 2015). MAXASCO was also more efficiently binding DON under neutral pH compared to acidic conditions. This is significant because the intestine (pH 6.5) anatomically follows the stomach (pH 2.5) and nutrients, but also toxins such as mycotoxins, are absorbed here. In further trials, MAXASCO has also shown to bind aflatoxin B1 and zearalenone efficiently (Data not shown, available on request). Mycotoxins from field and storage fungi pose problems in animal nutrition, such as mycotoxicosis, impaired animal performance and welfare.
Apart from mycotoxins, endotoxins such as *E. coli* are a recurring threat for productivity, especially in young animals. At all tested dosages, MAXASCO was showing an inhibitory effect on the adhesion of *E. coli*. This might be partly explained by the release of a higher amount of reactive groups due to the processing which leads to an improved *E. coli* binding capacity and by its content of phlorotannins. These polyphenols, which are mainly found in brown algae, can have a germ-inhibiting and -regulating effect. Studies have shown effects against *E. coli* (Ford et al. 2020) so far. The results can be considered meaningful for practical feeding as the chosen experimental approach offers an advantage over simple binding experiments as it simulates the potential attachment of *E. coli* to the intestinal mucus.
The positive results of the feeding trials might be explained by the binding effects above. Furthermore, prebiotic properties of the material and protection of the intestinal mucosa by formation of a protective film are possible contributors to the improved zootechnical parameters.

Conclusion

The advantages of the process for digesting seaweed meal lie in the improvement of the swelling behavior, the increase in the binding capacity and the protection of the gastro-intestinal tract of the animals resulting in higher zootechnical performances. Due to these improvements, MAXASCO can make a decisive contribution to meeting the constantly growing demands of modern animal production and feeding. MAXASCO not only reduces the adhesion of pathogens (*E. coli*) in the digestive tract, but also effectively binds various mycotoxins such as DON. The nutritional, prebiotic properties in line with the effective binding properties of MAXASCO, led to improved feed efficiency and growth performance of piglets and fattening pigs. Furthermore, because of the improved gel-forming properties, a protective effect in the gastro-intestinal tract can be expected. MAXASCO is also suitable for use in organic farming.

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Hydrolysierte Hefe *Kluyveromyces fragilis* in der Ferkelfütterung: Erfolgreicher Austausch von Blutplasma führt zu reduzierten Futterkosten

Hydrolyzed yeast Kluyveromyces fragilis *in piglet nutrition: Successful exchange of blood plasma leads to feed cost savings*

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Abstract

A feeding trial was conducted to investigate whether the hydrolyzed yeast Kluyveromyces fragilis can replace porcine blood plasma in diets of weaned piglets. 1404 piglets, weaned at day 27 of age, were homogeneously allocated into two treatment groups. One group was supplemented with porcine blood plasma (PBP) and the other with hydrolyzed yeasts (HY). The diets in the 3-phase feeding program were mainly based on processed cereals, soy protein-concentrate, whey powder, toasted soybeans, and soybean meal. The total trial period lasted 51 days. Based on the low weaning weights it was decided to prolong pre-starter phase until day 17 post weaning. Following parameters were measured for the whole trial period (d1-51): body weight (BW), average daily weight gain (ADWG), average daily feed intake (FI), feed conversion ratio (FCR), mortality and feces consistency. Evaluation of fecal scores was done three times a week for the first 14 days after weaning (1=well-formed, 2=soft, 3=pasty, 4=watery). Piglets of both groups started with 5.55kg (±0.96) BW and finished with 26.31kg (±2.24). Additionally, no differences in ADWG, FI or FCR between the PBP and HY group could be observed either in total trial period nor in one of the different feeding phases. Diarrhea did not occur neither in the PBP group nor in HY group. Mortality rates averaged 1.07% without differences between treatment groups. Due to an equal performance level within both groups and higher product costs of PBP, the total replacement of PBP led to feed costs savings for the HY group.

Einleitung

Das Absetzen stellt eine der kritischsten Phasen im Leben eines Ferkels dar. Die abrupte Trennung von der Sau, die Futterumstellung und die Zusammenführung mit anderen Ferkeln in einer neuen Umgebung gehören zu den großen Herausforderungen eines Absetzferkels. Reduzierte Futteraufnahmen, unvollständig entwickelte Verdauungssysteme und ein erhöhter Erregerdruck in Verbindung mit einem schwachen Immunsystem führen häufig zu Leistungseinbußen und gesundheitlichen Problemen wie z.B. Durchfall (Pluske et al. 1997; Dong et al. 2007; van Beers-Schreurs et al. 1992). Zunehmende politische Rahmenbedingungen und gesellschaftliche Diskussionen lassen das Interesse an alternativen, medikamentenfreien Fütterungskonzepten für ein erfolgreiches Absetzmanagement steigen. Eine nicht unwesentliche Rolle zur Reduzierung der zuvor genannten Absetzprobleme spielt die Rationsgestaltung. Porzines Blutplasma ist aufgrund seiner hohen Schmackhaftigkeit, Funktionalität und Proteinverdaulichkeit eine häufig verwendete Komponente in Rationen für Absetzferkel. Es ist bekannt, dass Blutplasma bei Dosierungen von mehr als 2,5% die Futteraufnahme, die Darmentwicklung, die Verdauung und die Wachstumsleistung bei Aufzuchtferkeln verbessert (Lallès et al. 2007, van Dijk et al. 2001), jedoch auch ein hochpreisiges Futtermittel darstellt. Die hydrolysierte Hefe des Hefestammes *Kluyveromyces fragilis* (TechnoYeast, Biochem) ist nachweislich in der Lage die Futteraufnahme sowie die Darmentwicklung bei Absetzferkel zu verbessern (Keimer et al., 2018). Ziel der vorliegenden Studie war es, zu prüfen inwieweit das Produkt TechnoYeast die zootechnische Leistung und das

Durchfallgeschehen im Vergleich zu Blutplasma bei gleichzeitiger Optimierung der Futterkosten beeinflusst.

Material und Methoden

1404 Ferkel (DanBreed x Pic 408), mit ca. 4 Wochen abgesetzt (ø 5,55kg), wurden unter Berücksichtigung von Körpergewicht und Geschlecht gleichmäßig auf zwei Behandlungsgruppen verteilt (n=23 Futterautomaten). Jeweils zwei Buchten teilten sich einen Futterautomaten. Die Rationen der 3-phasigen Fütterung bestanden hauptsächlich aus aufbereitetem Getreide (Weizen, Gerste, Mais), Soja-proteinkonzentrat, Molkenpulver, gerösteten Sojabohnen und Sojaschrot und unterschieden sich zwischen den beiden Gruppen im Anteil an Blutplasma (PBP) bzw. TechnoYeast (HY). Die Diäten waren innerhalb der beiden Gruppen isonitrogen und isoenergetisch. Nährwerte der einzelnen Futter und Fütterungsphasen sowie die Dosierinformation zu den beiden geprüften Produkten (PBP, HY) sind Tab. 1 zu entnehmen. Zwischen den Phasen (um Tag 17 und 35 nach dem Absetzen) wurden die Rationen praxisüblich verschnitten. Der gesamte Versuch dauerte 51 Tage. Die erhobenen Versuchsparameter umfassten die Gewichtsentwicklung (Körpergewicht und Tageszunahmen), die Futteraufnahmen, die Futterverwertung, die Mortalität und das Durchfallgeschehen. Ebenfalls wurde die Kotkonsistenz anhand eines subjektiven Bewertungsschlüssels pro Bucht beurteilt (1=gut geformt, 2=weich, 3=pastös, 4=wässrig). Für die Statistik wurde eine einfaktorielle Varianzanalyse mittels SPSS (IBM SPSS, Version 24) angewendet. Unterschiede mit P<0,05 gelten als signifikant.

Tabelle 1: Nährwertgehalte der 3-phasigen Fütterung und Dosierinformation von Blutplasma und der hydrolysierten Hefe TechnoYeast.

Phase	**Prestarter** (Tag 1-17)		**Starter I** (Tag 18-35)		**Starter II** (Tag 36-51)	
Gruppe	PBP	HY	PBP	HY	PBP	HY
Energie (MJ ME/ kg)	14,6		13,8		13,4	
Rohprotein (%)	17,5		17,0		17,0	
Lysin (%)	1,48		1,35		1,27	
Blutplasma (%)	4,5	-	1,8	-	-	-
Hydrolysierte Hefe (%)	-	2,2	-	1,5	-	-

Ergebnisse

Die Ferkel aus beiden Gruppen starteten mit einem durchschnittlichen Körpergewicht von 5,55kg (±0,96) in den Versuch und beendeten den Versuch mit einem durchschnittlichen Körpergewicht von 26,31kg (±2,24). Es bestanden keine signifikanten Unterschiede zwischen den beiden Behandlungsgruppen (P>0,1). Wie Abbildung 1-3 zu entnehmen, bestanden desweiteren keine signifikanten Unterschiede in Bezug auf die Futteraufnahme, die durchschnittlichen täglichen Zunahmen oder den Futteraufwand zwischen den beiden Gruppen, weder in Hinblick auf die gesamte Versuchsperiode noch in Anbetracht der einzelnen Fütterungsphasen.
In den ersten zwei Wochen nach dem Absetzen, konnte weder in der PBP- noch in der HY-Gruppe Durchfall diagnostiziert werden, weswegen auch keine Behandlungen gegen Durchfall notwendig waren. Das Auftreten von Durchfall, d.h. Bonitur 1 oder 2, lag in der ersten zwei Woche nach dem Absetzen bei 3,55% (P>0,1). Die Mortalitätsrate lag im Durchschnitt bei 1,07% in beiden Gruppen (P>0,1). Der vollständige Austausch von Blutplasma durch TechnoYeast führte bei gleichbleibenden Leistungsparametern zu Einsparungen bezüglich der Futterkosten um -8 % (Abb. 6). Dazu wurden die Gesamtfutterkosten durch den Zuwachs geteilt. Dies entspricht einer Kostensenkung von ~5 Cent pro kg Gewichtszunahme. Unter Annahme einer Gewichtszunahmen von 20kg (von 5 auf 25kg) ermöglicht die HY 1,00 € weniger Futterkosten pro Ferkel in der Aufzuchtperiode.

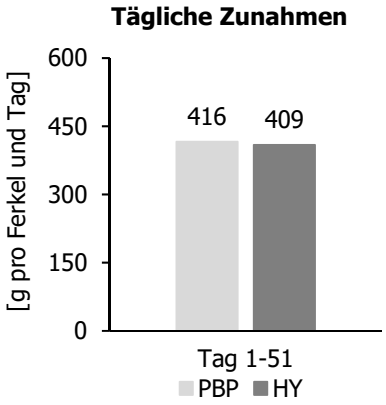

Abb.1: Einfluss von HY und PBP auf die täglichen Zunahmen von Absetzferkeln, (P>0.1)

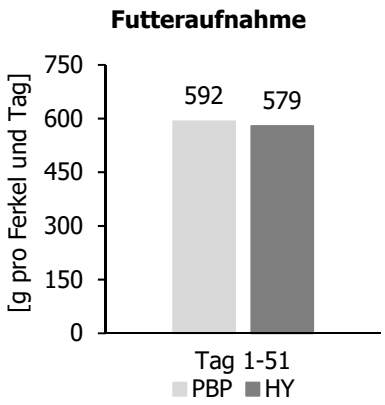

Abb. 2.: Einfluss von HY und PBP auf die Ø Futteraufnahme von Absetzferkeln, (P>0,1)

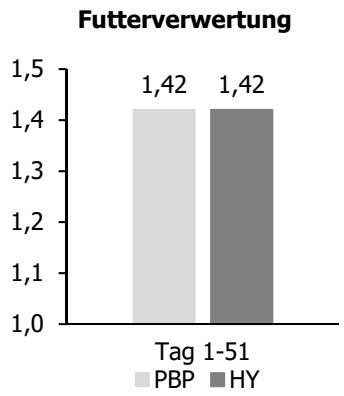

Abb. 3.: Einfluss von HY und PBP auf die Futterverwertung von Absetzferkeln, (P>0.1)

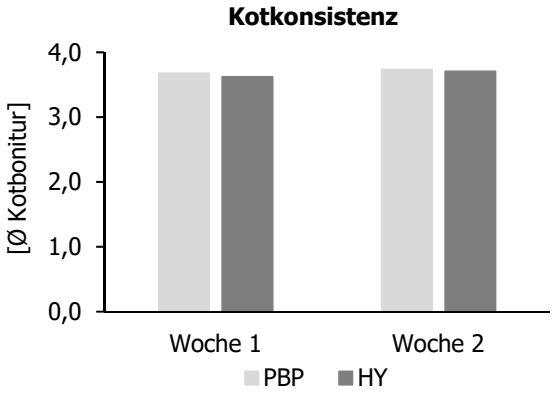

Abb. 4: Einfluss von HY und PBP auf die Kotbonitur von Absetzferkeln, (P>0,1)

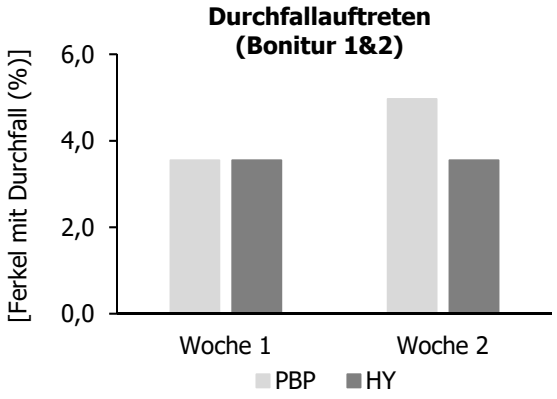

Abb. 5: Einfluss von HY und PBP auf das Auftreten von Durchfall (Bonitur 1&2) von Absetzferkeln, (P>0,1)

Diskussion

Hinsichtlich der Leistungsfähigkeit und des Gesundheitszustands (Durchfallauftreten) wurden zwischen der PBP und HY-Gruppe keine Unterschiede festgestellt, was beweist, dass die TechnoYeast mit einer Dosierung von 1,5-2,2% Blutplasma mit einer Dosierung von 1,8-4,5 % in der Ferkelaufzucht ersetzen kann. Grundsätzlich war das Leistungsniveau in diesem Versuch in beiden Gruppen auf einem moderaten Niveau.

Wirtschaftlich betrachtet ergab sich ein klarer Vorteil der HY-Gruppe aufgrund niedrigerer Futterkosten pro kg Gewichtszunahme (-8%) bei Aufrechterhaltung der Leistung im Vergleich zur PBP-Fütterung. In diesem Versuch sind die Effekte der hydrolysierten Hefe TechnoYeast auf die Ferkelleistung durchaus vergleichbar mit denen von Blutplasma, was in Ferkelabsetzfuttern als hochqualitative Proteinquelle für gute Effekte auf Futteraufnahmen und Darmgesundheit weitläufig eingesetzt wird (van Dijk et al. 2001).

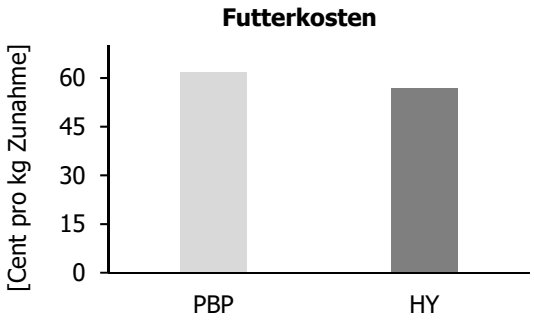

Abb. 6: Einfluss von HY und PBP auf die Futterkosten

Schlussfolgerung

Das Produkt TechnoYeast basierend auf dem Hefestamm *Kluyveromyces fragilis* zeigt im Vergleich zu der hochwertigen und funktionellen Proteinquelle Blutplasma in gleicher Weise die Fähigkeit ein Ferkelfutter aufzuwerten. Dies kann durch die Leistungsfähigkeit und den Gesundheitszustand, die in beiden Gruppen auf einem gleichen Niveau lagen, bestätigt werden. Das getestete Hefeprodukt konnte die gleiche Leistung zu geringeren Kosten erreichen, was zu einem ökonomischen Vorteil in der Ferkelaufzucht führen kann.

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INNOVATION IST UNSERE MOTIVATION

Hohe Gehalte an Rohfaser im Ferkelfutter mit und ohne energetischem Ausgleich: Auswirkungen auf die Futteraufnahme und Leistung

High levels of crude fibre in diets of weaning pigs with and without energetic adjustment: effects on feed intake and performance

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Abstract

In the present feeding experiment different crude fiber levels in diets of weaning pigs (4.0, 4.5 and 5.0%) with and without energy adjustments were applied and the effects on feed intake and performance were studied. As result, there was no effect of feeding strategy on average daily gain and average feed intake ($p>0.05$). In feeding phase 1 - 21 dafter weaning, the high inclusion level of crude fiber improved feed conversion ratio as well as metabolizable energy utilization ($p<0.05$). Based on performance data of the whole feeding experiment, there was no effect on feed conversion ratio, but metabolizable energy utilization was improved due to higher crude fiber levels in the diets. These results illustrate that energy adjustment using oil in diets with high levels of crude fiber (up to 5.0%) it is not necessary to improve piglet performance.

Einleitung

Die Bedeutung der Versorgung von Schweinen mit Faserstoffen hat Eingang in Beratung und Praxis gefunden und ist im DLG-Merkblatt 463 „Fütterung und Tierwohl beim Schwein" dokumentiert (DLG, 2021). Gemäß diesem Merkblatt und weiterer Literaturstellen wirkt sich eine optimale Versorgung mit Faser positiv auf das Fressverhalten, das Sättigungsgefühl, die Verdauungs- und Stoffwechselvorgänge, die Mikrobiota im hinteren Verdauungstrakt sowie den Immunstatus aus (Lindberg, 2014; Jha and Berrocoso, 2016).Während in früheren Publikationen der DLG für Ferkel ab 12 kg Lebendmasse (LM) 35 g und für Ferkel ab 20 kg LM nur 30 g Rohfaser pro kg Futter empfohlen werden (DLG, 2008), werden im Merkblatt 463 ausschließlich Rohfasergehalte von mehr als 35 g pro kg Futter angeführt. In Versuchen aus Schwarzenau wurden bereits vor Jahren höhere Rohfasergehalte im Ferkelfutter getestet und dabei positive Effekte auf die Aufzuchtleistung beschrieben (Preißinger et al., 2013; 2015). Demgegenüber wird durch die Anwendung der Schätzgleichung (GfE, 2008) zur Berechnung des Energiegehaltes von Futtermischungen verdeutlicht, dass sich der Gehalt an Rohfaser negativ auf die Energiedichte auswirkt. In Vorversuchen wurde bei Rohfasergehalten von etwa 50 g pro kg Futter ein energetischer Ausgleich mit Futteröl durchgeführt, wodurch die Futterkosten erheblich anstiegen. In vorliegender Untersuchung sollte u.a. geprüft werden, ob sich die positiven Effekte eines hohen Rohfasergehaltes auch ohne energetischen Ausgleich nachweisen lassen.

Material und Methoden

Der Versuch wurde am Staatsgut Schwarzenau der Bayerischen Staatsgüter durchgeführt. Dazu wurden 96 Absetzferkel der Rasse Pi x (DL x DE) nach LM, Geschlecht und Abstammung ausgewählt und gleichmäßig auf folgende Versuchsgruppen aufgeteilt:

- A: 40 g Rohfaser pro kg Futter
- B: 45 g Rohfaser pro kg Futter (mehr Gerste, weniger Weizen)
- C: 50 g Rohfaser pro kg Futter (Einsatz eines Fasermix)
- D: 50 g Rohfaser pro kg Futter (Einsatz eines Fasermix, energetischer Ausgleich mit Pflanzenöl)

Die Ferkel wurden in 8 Buchten zu je 12 Tieren auf Kunststoffspalten ohne Einstreu gehalten. Sie waren zu Beginn des Versuches im Durchschnitt 27 Tage alt und wogen bei der Aufstallung ca. 7,5 kg. Die Futterzuteilung erfolgte über Abrufstationen mit integrierter Futterverwiegung für das Einzeltier (Schauer Agrotronic GmbH). Die LM wurden wöchentlich am Einzeltier erfasst. Der Versuch gliederte sich in zwei Fütterungsphasen. Sowohl Phase I als auch Phase II dauerten 21 Tage wobei in der Phase 1 zusätzlich 5 Tage Adaption an die Abrufstationen erfolgten.
Die Rationen (Tabelle 1) wurden mit dem Programm Zifo2 (Zielwertfutteroptimierung, LfL) kalkuliert und in der Versuchsmahl- und Mischanlage Schwarzenau hergestellt. Diese wurden in der Schraubmühle Volkach pelletiert und im Labor der Bayerischen Landesanstalt für Landwirtschaft in Grub nach Vorgaben des VDLUFA (2012) analysiert. Die Gehalte an umsetzbarer Energie (ME) wurden nach der Mischfutterformel (GfE, 2008) bestimmt. Der verwendete Fasermix setzte sich laut Deklaration aus 30 % Apfeltrester, 30 % Trockenschnitzel, 24,5 % Sojabohnenschalen, 15 % Weizenkleie sowie 0,5 % Pflanzenöl zusammen. Laut eigenen Analyseergebnissen wies der Fasermix 210 g Rohfaser, 398 g Neutral-Detergenzien-Faser nach Amylasebehandlung und Veraschung (aNDFom) sowie 288 g Säure-Detergenzien-Faser nach Veraschung (ADFom) pro kg Futter bei 88 % TM auf.
Die statistische Auswertung wurde mit Hilfe des Statistikprogramm SAS 9.4 (SAS Institute Inc., Cary, NC, USA) unter Anwendung der Prozedur GLM durchgeführt. Im Modell wurden als fixe Effekte die Behandlung, das Geschlecht und die Abstammung sowie deren Interaktionen berücksichtigt.

Tabelle 1: Versuchsmischungen und kalkulierte Nährstoffgehalte (Angaben bei 88 % TM)

	Ferkelaufzuchtfutter I				Ferkelaufzuchtfutter II			
	A	B	C	D	A	B	C	D
Weizen, %	41,5	26,5	22,5	21	44,5	29,5	25,5	24
Gerste, %	30	45	45	45	30	45	45	45
Sojaöl, %	1,5	1,5	1,5	3	1,5	1,5	1,5	3
Sojaextr.-Schrot, LP, %	22	22	22	22	19	19	19	19
Fasermix, %	-	-	4	4			4	4
Fumarsäure, %	1	1	1	1	1	1	1	1
Mineralfutter[1)], %	4	4	4	4	4	4	4	4
ME, MJ	13,0	12,8	12,6	13,0	13,0	12,8	12,6	13,0
Rohprotein, g	169	169	168	167	159	159	159	157
Rohfaser, g	42	45	52	52	40	43	49	50
Rohfett, g	33	34	34	48	33	33	33	48
Lysin, g	12,5	12,5	12,6	12,5	11,8	11,8	11,9	11,8
Calcium, g	7,0	7,1	7,2	7,2	7,0	7,0	7,1	7,1
Phosphor, g	4,8	4,8	4,7	4,7	4,6	4,7	4,7	4,6

[1)] mit 15 % Ca, 3 % P, 11 % Lysin; 3 % Methionin; 4,5 % Threonin; 0,4 % Tryptophan

Ergebnisse und Diskussion

In Tabelle 2 sind die Ergebnisse der analysierten Gehalte ausgewählter Nährstoffen dargestellt. Insgesamt kann eine gute Übereinstimmung mit den kalkulierten Nährstoffgehalten nachgewiesen werden.

Die anvisierte Erhöhung des Rohfasergehaltes wurde wie geplant realisiert. Die erhöhten Rohfasergehalte im Futter der Gruppen B und C spiegelten sich erwartungsgemäß in verminderten Gehalten an ME wider. Ein höherer Gehalt an ME in Gruppe D wurde realisiert. Die Analysenwerte von Rohfaser, aNDFom und ADFom lagen bei nahezu allen Versuchsrationen im Bereich der Orientierungswerte der DLG von 2021. Nur knapp außerhalb dieser Vorgaben lag der ADFom-Gehalt des Ferkelaufzuchtfutters I von Gruppe C.

Tabelle 2: Analysierte Nährstoffgehalte der Versuchsrationen (Angaben bei 88 % TM)

	Ferkelaufzuchtfutter I				Ferkelaufzuchtfutter II			
	A	B	C	D	A	B	C	D
ME, MJ	13,2	13,0	12,7	12,9	13,2	13,1	12,7	13,1
Rohprotein, g	164	164	161	159	148	149	154	153
Rohfaser, g	39	47	53	53	40	42	51	48
Rohfett, g	36	36	36	47	36	34	37	47
aNDFom, g	111	134	136	138	115	121	145	135
ADFom, g	52	64	73	69	54	58	69	66
Lysin, g	13,3	13,6	12,9	13,1	12,2	12,3	12,4	11,8
Methionin, g	3,6	3,4	3,4	3,4	3,4	3,5	3,2	3,2
Threonin, g	7,7	7,8	7,6	7,7	7,4	7,9	7,0	6,9
Tryptophan, g	2,1	2,1	2,0	2,0	1,9	2,0	1,9	1,9
Calcium, g	8,2	6,6	6,5	7,5	6,9	6,7	7,3	7,3
Phosphor, g	4,8	4,9	4,6	4,6	4,5	4,4	4,7	4,4

Aus Tabelle 3 gehen die Leistungsdaten hervor. In beiden Fütterungsphasen sowie im Versuchsmittel konnte kein signifikanter Einfluss der Rohfasererhöhung auf die Tageszunahmen festgestellt werden, wenngleich auch in Phase 1 unmittelbar nach dem Absetzten in den Gruppen B, C und D im Vergleich zur Gruppe A durchgehend numerisch höhere tägliche Zunahmen festgestellt werden konnten.

Tabelle 3: Aufzuchtleistungen, Futterverbrauch und Futteraufwand (LSQ-Werte)

	A	B	C	D	sign. p[1)]
			Tageszunahmen, g/Tier		
Phase 1	323	338	366	357	0,099
Phase 2	726	729	704	731	0,653
gesamt	498	508	513	519	0,721
			Futterabruf, g/Tier und Tag		
Phase 1	458	450	481	471	0,556
Phase 2	1040	1040	1053	1064	0,903
gesamt	711	707	730	729	0,766
			Futteraufwand, kg/kg Zuwachs		
Phase 1	1,42[b]	1,34[a]	1,32[a]	1,31[a]	0,019
Phase 2	1,44[b]	1,42[b]	1,50[a]	1,46[ab]	0,039
gesamt	1,43	1,39	1,43	1,40	0,159
			Kalkulierte ME-Aufnahme, MJ/Tier, Tag		
Phase 1	6,1	5,8	6,1	6,1	0,798
Phase 2	13,7	13,6	13,4	13,9	0,736
gesamt	9,4	9,2	9,3	9,5	0,869
			ME-Aufwand, MJ/kg Zuwachs		
Phase 1	18,8[b]	17,4[a]	16,8[a]	16,9[a]	<0,001
Phase 2	19,0	18,6	19,1	19,1	0,478
gesamt	18,9[b]	18,1[a]	18,2[a]	18,2[a]	0,025

[1)] Irrtumswahrscheinlichkeit

Hinsichtlich des Futterabrufs gab es ebenfalls keine signifikanten Unterschiede, sowohl in den beiden Fütterungsphasen, als auch über den gesamten Versuchszeitraum. Demgegenüber zeigten sich signifikante Unterschiede beim Futteraufwand pro kg Zuwachs in den einzelnen Fütterungsphasen. So war der Futteraufwand in Phase I in den Gruppen B, C und D signifikant niedriger als in Gruppe A. Der höhere Rohfasergehalt im Futter dieser Gruppen hatte scheinbar insbesondere im Zeitraum nach dem

Absetzten eine positive Wirkung auf die Verdauungsvorgänge und führte zu einer verbesserten Futtereffizienz (DLG, 2021). Im weiteren Versuchsverlauf (Phase 2) zeigte sich in Gruppe C der höchste Futteraufwand. Die Unterschiede zu den Gruppen A und B konnten statistisch abgesichert werden. Im Versuchsmittel ließ sich kein Effekt der Rohfasererhöhung auf den Futteraufwand pro kg Zuwachs feststellen. Betrachtet man die kalkulierte Aufnahme an ME, so waren trotz der niedrigeren ME-Gehalte im Futter von Gruppe C in beiden Versuchsphasen und im Mittel des Versuchs keine signifikanten Unterschiede zu erkennen. Der Aufwand an ME pro kg Zuwachs war in den Gruppen B, C und D signifikant niedriger als in Gruppe A und deckte sich damit sehr gut mit den Werten des Futteraufwands. Etwas anders war es hingegen in Phase 2. Hier zeigte sich kein signifikanter Effekt. Im Versuchsmittel war der Aufwand an ME pro kg Zuwachs in Gruppe A signifikant höher als in den Gruppen B, C und D. Zwischen den Gruppen C und D konnten bei allen berücksichtigten Parametern weder in den einzelnen Fütterungsphasen noch im Mittel des Versuchs statistisch absicherbare Unterschiede festgestellt werden. Daraus lässt sich ableiten, dass ein energetischer Ausgleich mit Öl bei Rohfasergehalten von 5,0% nicht notwendig ist. Dieser Umstand kann vielleicht darin begründet sein, dass der verwendete Fasermix einen gesteigerten Anteil an löslicher Faser aufwies (Apfeltrester und Trockenschnitzel) dessen Fermentierbarkeit größere Energiemengen freisetzte.

Zusammenfassung und Schlussfolgerung

Im Versuch zeigten sich keine negativen Effekte des gesteigerten Rohfasereinsatzes auf die Tageszunahmen und den Futterabruf. Numerisch höhere Tageszunahmen waren jedoch in der Fütterungsphase 1. nach dem Absetzten der Ferkel festzustellen. Die Rohfasererhöhung wirkte sich insbesondere bei jüngeren Ferkeln (Phase 1) positiv auf den Futteraufwand bzw. den Aufwand an ME pro kg Zuwachs aus. Dies zeigte sich unabhängig von der Höhe des Einsatzes (45 bzw. 50 g/kg) oder der Anwendung des energetischen Ausgleichs mittels Öl. Die Erhöhung des Fasergehaltes ist somit vor allem in der Phase um das Absetzen empfehlenswert. Zusätzlich kann auf einen energetischen Ausgleich mit Futteröl nicht nur in Zeiten hoher Futtermittelpreise verzichtet werden.

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Roggen in Ferkel- und Schweinemastrationen: Auswirkung auf die Aufzucht-, Mast- und Schlachtleistungen

Rye in diets of weaning and fattening pigs: Effect on zootechnical and slaughter performance

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Abstract

It was hypothesized that hybrid rye may replace wheat and barley in diets for weaning and fattening pigs without impacting performance. A total of 96 weaning pigs (9.3 kg) were allocated to four treatment groups with two replicate pens per group. Within each phase, pigs were fed a control wheat-barley and soybean meal-based diet or a diet in which wheat and barley were replaced with increasing levels of rye (up to 20% in phase 1 and 30% in phase 2, respectively). In the end of the feeding experiment pigs were used in a following 3-phase feeding experiment (30-60; 60-90 and 90-118 kg BW) applying diets with increasing levels of hybrid rye (up to 50 and 70% in phase 1, 2 and 3). The substitution of wheat and barley showed no effect on the pigs' BW, average daily gain and average daily feed intake ($p>0.10$) during the weaning and fattener period. Moderate inclusion level of hybrid rye (up to 15% in phase 1 and 25% in phase 2) resulted in best feed conversion ratio (FCR) in weaning pigs while there was a decrease in FCR using hybrid rye in diets of fattening pigs regarding the whole experiment.

Einleitung

Roggen rückt wieder stärker in den Fokus der Tierernährung, was auf züchterische Bemühungen (Hybridroggen und PollenPlus®-Technologie) und pflanzenbauliche Vorteile zurückzuführen ist. Neben gesteigerten Erträgen (Wiersma et al., 2018) und reduzierter Anfälligkeit auf Mutterkorn (Miedaner and Geiger, 2015) können positive Effekte auf die Leistungsfähigkeit, die Tiergesundheit sowie das Wohlbefinden genannt werden (Schwarz et al., 2015; 2016; Chuppava et al., 2020; McGhee and Stein, 2021). Zusätzlich weist Roggen unter den Getreidearten die höchste intrinsische Phytaseaktivität auf (Rodehutscord et al., 2016) welche in gesteigerten Nährstoffverdaulichkeiten resultieren kann. Darüber hinaus verfügt Roggen über gesteigerte Gehalte an Nicht-Stärke-Polysaccharide die im Zuge der mikrobiellen Fermentation im hinteren Verdauungstrakt positiven Einfluss auf die Darmgesundheit üben kann (Chuppava et al., 2020). Im folglich dargestellten Fütterungsversuch soll der Effekt ansteigender Anteile an Roggen in Ferkel- und Schweinemastrationen auf die Aufzucht-, Mast- und Schlachtleistungen der Tiere untersucht werden.

Material und Methoden

Der Versuch wurde am Staatsgut Schwarzenau der Bayerischen Staatsgüter durchgeführt. Dazu wurden 96 Absetzferkel (9,3 kg LM) der Genetik Pi x (DL x DE) nach Wurf, Geschlecht und Lebendmasse (LM) den 4 Versuchsgruppen (VG) zugeordnet. In der Versuchsgruppe A wurde sowohl in der Ferkelaufzucht- als auch in der Mastphase kein Roggen angewendet, wohingegen bei den weiteren Gruppen ansteigende

Anteile verwendet wurden. Die Rationen wurden mit dem Programm Zifo 2 (Zielfutteroptimierung, LfL) laut der Empfehlungen (GfE, 2006) kalkuliert (Tabelle 1).

Tabelle 1: Anteil an Roggen in den jeweiligen Rationen (%) sowie die kalkulierten Nährstoffgehalte

	Fütterungsphase				
Versuchsgruppe	FAF 1	FAF 2	AM	MM	EM
A	-	-	-	-	-
B*	10	20	35	50	50
C	15	25	40	60	60
D	20	30	50	70	70
Kalkulierte Nährstoffgehalte (88 % TM)					
Energie, MJ ME	13,0	13,0	13,0	12,9	12,9
Rohprotein, g	178	169	157	140	123
Lysin, g	12,6	12,0	10,7	9,5	8,4
Phosphor, g	4,8	4,7	3,8	3,6	3,3

*laut DLG (2006); FAF, Ferkelaufzuchtfutter; AM, Anfangsmast; MM, Mittelmast; EM, Endmast

Die Tiere wurden sowohl in der Phase der Ferkelaufzucht wie auch in der Mast in 8 Buchten zu je 12 Tieren gehalten und die Zuteilung der pelletierten (Ferkelaufzucht) und schrotförmigen (Schweinemast) Versuchsfuttermischungen erfolgte über Abrufstationen mit integrierter Futterverwiegung für das Einzeltier. Nach einer Adaptionsphase (5 Tage) folgte der Ferkelaufzuchtversuch, welcher sich in 2 Abschnitte zu jeweils 3 Wochen gliederte (FAF 1 und 2). Die anschließende Mast (30,3 kg LM) gliederte sich demgegenüber in 3 Fütterungsphasen (30-60, 60-90 und 90-120 kg LM). Die LM der Tiere wurde wöchentlich erfasst und zur Berechnung der täglichen Zunahmen herangezogen. Die tierindividuelle Futteraufnahme pro Tag wurde wöchentlich aufsummiert und zur Kalkulation des täglichen Futterabrufes sowie der Futterverwertung genutzt. Die Analyse der Futtermischungen erfolgte nach Vorgaben des VDLUFA (2012), die Kalkulation der umsetzbaren Energie nach GfE (2008).

Ergebnisse

In der Tabelle 2 und 3 werden die Ergebnisse der analysierten Gehalte an ausgewählten Nährstoffen der Versuchsrationen veranschaulicht. Sowohl bei den Rationen der Ferkelaufzucht- als auch bei jenen der Schweinemast kann eine gute Übereinstimmung mit den kalkulierten Nährstoffgehalten nachgewiesen werden. Hinsichtlich des Gehaltes an umsetzbarer Engie traten Abweichungen von 0,2 bis 0,5 MJ ME zwischen den kalkulierten und analysebasierten Energiegehalten auf.

Tabelle 2: Analysierter Gehalt an ausgewählten Nährstoffen der Ferkelaufzuchtfutter

	FAF 1				FAF 2			
	A	B	C	D	A	B	C	D
TM, g	900	899	899	899	903	900	899	899
Rohprotein, g	163	169	174	165	159	153	148	150
Rohfaser, g	40	37	34	38	35	37	44	42
Phosphor, g	4,9	4,8	5,0	4,8	4,8	4,9	4,8	4,5
[1]Energie, MJ ME	13,2	13,4	13,5	13,3	13,4	13,3	13,0	13,1
Lysin, g	11,0	12,0	12,2	11,7	11,0	11,8	11,0	10,4

[1]kalkuliert nach Mischfutterformel (GfE, 2008); FAF, Ferkelaufzuchtfutter

Tabelle 3: Analysierter Gehalt an ausgewählten Nährstoffen der Schweinemastrationen

	AM				MM				EM			
	A	B	C	D	A	B	C	D	A	B	C	D
TM	893	898	900	899	889	891	889	893	892	897	901	899
Rohprotein, g	146	142	141	138	131	127	122	125	117	111	108	107
Phosphor, g	4,3	4,0	4,0	3,9	3,6	3,6	3,8	3,8	3,6	3,8	3,6	3,7
Rohfaser, g	37	35	36	34	39	32	32	34	35	33	32	31
[1]Energie, MJ ME	13,2	13,2	13,2	13,3	13,1	13,3	13,2	13,1	13,1	13,1	13,1	13,0
Lysin, g	9,5	8,2	9,4	10,0	9,1	9,6	11,9	9,5	8,9	7,7	7,6	7,5

[1]kalkuliert nach Mischfutterformel (GfE, 2008); AM, Anfangsmast; MM, Mittelmast; EM, Endmast

In Tabelle 4 werden die Leistungsdaten aus dem Ferkelversuch veranschaulicht. Sowohl zum Start des Versuches als auch am Ende der Ferkelaufzuchtphase wurden keine Unterschiede in der LM zwischen den VG festgestellt (p>0,10). Ferkel der VG C (FAF 1: 15 % und FAF 2: 25 % Roggenanteil) zeigten in der Phase 1 die höchsten täglichen Zunahmen und unterschieden sich von VG 1 und 2 (p<0,05). Demgegenüber kehrte sich dieser Trend in der Phase 2 um, die Tiere der VG 1 zeigten die höchsten täglichen Zunahmen (p<0,05), was über den gesamten Versuchszeitraum keine Unterschiede zwischen den VG nachweisbar machte (p>0,05). Hinsichtlich des Futterabrufs unterschied sich die VG 1 von jenen Gruppen denen Rationen mit Roggenanteilen gefüttert wurde (VG 2, 3 und 4) signifikant in der Phase 1, wobei VG 2, 3 und 4 eine gesteigerte Futterverwertung zeigten (p<0,05). Über den gesamten Versuchszeitraum der Ferkelaufzucht konnten keine Unterschiede im Futterabruf festgestellt werden, wobei VG B und C eine gesteigerte Futterverwertung im Vergleich zu VG A und D zeigten (p<0,05).

Tabelle 4: Leistungen der Tiere in der Ferkelaufzuchtphase

	A	B	C	D	p-Wert
			Tageszunahmen, g/Tag		
Phase 1	352[b]	366[b]	407[a]	376[ab]	0,022
Phase 2	692[a]	686[ab]	650[bc]	613[c]	<0,001
Gesamt	518	522	526	492	0,092
			Futterabruf, g/Tier und Tag		
Phase 1	603[a]	515[b]	536[b]	501[b]	0,002
Phase 2	965	993	987	983	0,841
Gesamt	780	748	756	736	0,343
			Futterverwertung, kg/kg		
Phase 1	1,76[b]	1,42[a]	1,31[a]	1,32[a]	<0,001
Phase 2	1,39[a]	1,45[a]	1,52[b]	1,61[c]	<0,001
Gesamt	1,51[b]	1,43[a]	1,44[a]	1,50[b]	0,016

[ab]signifikante Unterschiede (p<0,05)

Auch in der Schweinemast (Tabelle) übte der ansteigende Roggenanteil keinen Effekt auf die Lebendmasse aus (p>0,10). Die Tageszunahmen unterschieden sich in der Phase der Mittelmast, in der die Tiere der VG 1 höhere Leistungen im Vergleich zu VG 3 und 4 erzielten (p<0,05). In der Anfangsmast zeigten die Tiere der VG 2 und 3 den höchsten Futterabruf und unterschieden sich von der VG 1 (p<0,05). Der Anteil von 60 % Roggen (VG 3) resultierte in der Endmast in dem höchsten Futterabruf im Vergleich zu den weiteren Versuchsgruppen (p<0,05). Über den gesamten Versuchszeitraum konnten keine Unterschiede nachgewiesen werden (p>0,10). Hinsichtlich der Futterverwertung wiesen die Tiere der VG 1 in der Anfangs- und Mittelmast sowie über den gesamten Versuchszeitraum der Schweinemast die besten Ergebnisse auf (p<0,05). Zusätzlich sind die bedeutendsten Schlachtleistungsparameter angeführt. Der Einsatz von Roggen ließ unabhängig von der Einsatzmenge nur geringe Effekt im Vergleich zur VG 1 erkennen.

Tabelle 5: Leistungen der Tiere in der Schweinemast

	A	B	C	D	p-Wert
			Lebendmasse, kg		
Beginn	30,3	30,7	30,8	29,5	0,281
Ende	123,5	121,6	122,3	120,1	0,623
			Tageszunahmen, g/Tag		
Anfangsmast	805	822	855	806	0,338
Mittelmast	954^{a}	923^{ab}	854^{bc}	828^{c}	0,005
Endmast	767	730	782	763	0,609
Gesamt	828	820	828	794	0,433
			Futterabruf, g/Tier und Tag		
Anfangsmast	1,66^{b}	1,83^{a}	1,86^{a}	1,75^{ab}	0,021
Mittelmast	2,34	2,42	2,29	2,34	0,494
Endmast	2,60^{b}	2,58^{b}	2,80^{a}	2,53^{b}	0,023
Gesamt	2,19	2,26	2,31	2,19	0,170
			Futterverwertung, kg/kg		
Anfangsmast	2,07^{a}	2,22^{b}	2,17^{b}	2,18^{b}	0,003
Mittelmast	2,46^{a}	2,64^{b}	2,70^{bc}	2,85^{c}	<0,001
Endmast	3,46	3,64	3,63	3,35	0,212
Gesamt	2,65^{a}	2,76^{b}	2,80^{b}	2,77^{b}	0,020
			Schlachtleistungsparameter		
Schlachtgewicht, kg	101,1	99,2	98,5	97,4	0,300
Rückenmuskelfläche, cm²	60,1^{a}	56,6^{b}	55,4^{b}	55,7^{b}	<0,001
Fettfläche, cm²	14,8	15,5	14,3	14,1	0,305
MFA, %	60,7	60,0	59,7	59,8	0,222

^{ab}signifikante Unterschiede (p<0,05); MFA, Muskelfleischanteil

Diskussion

Mit den dargestellten Ergebnissen konnte nachgewiesen werden, dass Einsatzmengen von bis zu 30 % Roggen in der Ferkelaufzucht und 70 % in der anschließenden Schweinemast keine negativen Effekte auf die täglichen Zunahmen wie auch den Futterabruf und die Schlachtleistungen ausüben. Diese Ergebnisse wurden auch von weiteren Studien bestätigt, wenngleich diese entweder die Ferkelaufzucht- oder Mastphase in die wissenschaftliche Betrachtung zogen (McGhee und Stein, 2021; Wilke, 2020; Schwarz et al., 2015 und 2016; McGhee et al., 2021; Thacker et al., 1991). Als Grundvoraussetzungen hierfür sind zum einen die minimale Belastung mit Ergotalkaloiden sowie die optimale nährstoffliche Charakterisierung der Einzelfuttermittel im Zuge der Rationsoptimierung zu nennen.

Schlussfolgerungen

Auf Grund pflanzenbaulicher Verbesserungen und nährstofflicher Vorteile des Roggens (z.B. gesteigerte intrinsische Phytaseaktivität, hohe Konzentration an Lysin pro 100 g Rohprotein, hoher Gehalt an Nicht-Stärke-Polysacchariden) ist dessen gesteigerter Einsatz sowohl in Ferkel- als auch Schweinemastrationen empfehlenswert. Auch Rationsanteile über den Einsatzempfehlungen der DLG (2006) können Anwendung finden, ohne die Leistungsfähigkeit negativ zu beeinflussen.

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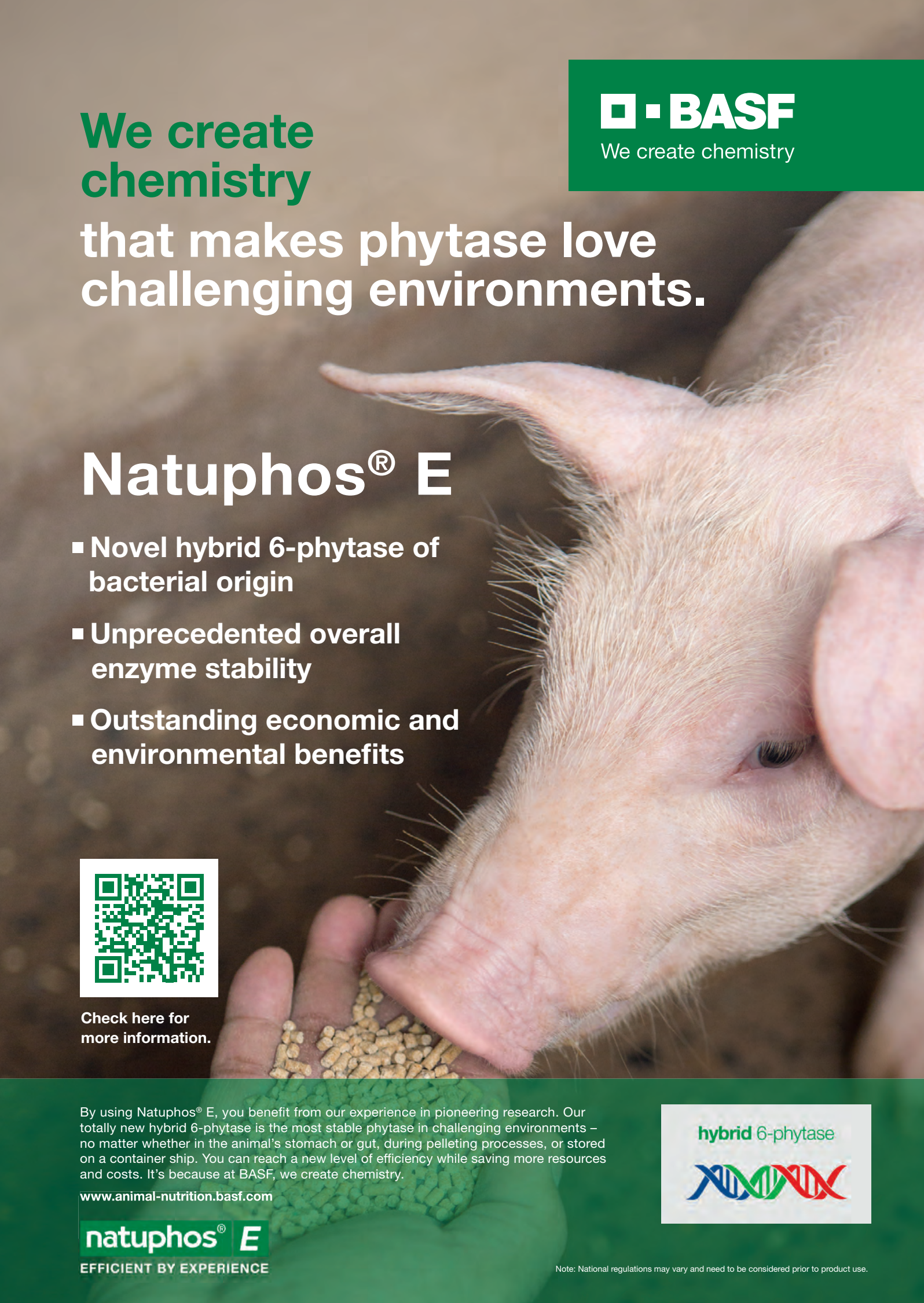
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Effect of a clinoptilolite variety on zootechnical performance of fattening pigs

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Abstract

A feeding trial with 32 fattening pigs was performed to evaluate the impact of 2% clinoptilolite (IPUSagro F) on feed intake, daily weight gain and feed conversion as well as slaughter performance and specific health parameters. Results showed that a use of 2% IPUSagro F in diets of fattening pigs did not cause any health impairments due to specific blood parameters. Fattening pigs of experimental group had a higher average daily weight gain and indicated an improved feed conversion ratio compared to control group. Furthermore, no negative effects occurred for feed intake, slaughter performance and parameters of meat quality.

Introduction

From the perspective of today's animal nutrition, next to productivity the focus is on animal welfare, efficient use of resources, minimal food competition to humans, and low impact on environment. To achieve these goals the inclusion of feed additives is a common and widely used strategy. Several studies underline the positive effects of e.g. phytogenic compounds, phenolic compounds or organic acids (Windisch *et al*. 2008; Mahfuz *et al*. 2021, Pluske *et al*. 2021). Also related to swine, several feed additives with different mode of action are promoted due to age, performance level and specific external conditions (Radzikowski and Milczarek, 2021). Some studies have already been carried out to investigate the impact of natural minerals and especially clinoptilolite (e.g. Raj *et al*. 2021; Herc *et al*. 2021). Special properties of clinoptilolite such as composition, porosity, storage capacity and binding capacity might initiate beneficial effects also in diets of swine. Only few research has already been conducted to understand the mechanism beyond the integration of clinoptilolite. A previous study of Wetscherek *et al.* 2014 indicates positive effects of a clinoptilolite variety (IPUSagro F) on top of a diet for fattening pigs on zootechnical performance. To validate mentioned impact of clinoptilolite, a dosage of 2% in experimental diet of fattening pigs should be tested and compared to a negative control group. Therefore, the aim of the present study was to evaluate the effects of the clinoptilolite variety (IPUSagro F) on feed intake, daily weight gain and feed conversion as well as slaughter performance and health.

Material and methods

The feeding trial was performed under standardised conditions with 32 fattening pigs, crossbred of German Large White × Piétrain at an experimental station in the southeast of Styria (Hatzendorf, Austria). Pigs were distributed randomly according to weight, sex and litter to four pens, containing eight animals each. Half of the animals were assigned to feeding group 1 and half to feeding group 2 (table 1). All pens offered fully slatted floor and were equipped with dry-feeders as well as nipple drinkers. Water and feed were provided *ad libitum* during the whole study period. The experimental trial started with an average body weight of 37.5 kg and ended up with individual slaughtering when pigs reached

119.5 kg. Experimental diets of both feeding groups based on corn, barley and soybean meal and were calculated iso-energetic and iso-caloric. Pigs fed experimental diet 1 had no addition of IPUSagro F while for experimental group 2 diets included 2% of IPUSagro F (table 2). IPUSagro F is a clinoptilolite of sedimentary origin from a Slovakian deposit of IPUS near Kucin. Products based on it are sold under the brand 'Migulators'. It is already approved for the functional group of binders with an amount of 10 g/kg of feed. Proximate analyses of representative diet samples were performed according to VDLUFA (2012). Bodyweight was determined individually at the beginning of the trial and then continuously in a three weeks interval until the end of experiment to calculate average daily gain (ADG). Feed consumption per pen was recorded weekly to verify average daily feed intake (ADFI) and feed conversion ratio (FCR). Blood samples were collected during the slaughtering process and analysed for specific immune parameters (Labordiagnostik, Vienna). Statistical analyses were performed by ANOVA (GLM procedure) of SAS (SAS Inst., Inc., Cary, NC, USA).

Table 1: Experimental study design

	FG 1	FG 2
IPUSagro F, %	0	2
pens, n	2	2
Animals per pen, n	8	8
Animals per feeding group, n	16	16

FG 1, control group; FG 2, feeding group with IPUSagro F

Table 2: Composition of experimental diets (based on 88% DM)

feed, %	FG 1	FG 2
corn whole grain silage, 72% TS	43.51	43.51
barley	6.23	4.67
wheat	8.90	6.90
soybeanmeal-42	24.72	25.71
rapeseed oil	-	0.99
pigfaser	1.68	1.26
IPUSagro F	-	2.00
trace element and vitamin premix	2.97	2.97

FG 1, control group; FG 2, feeding group with IPUSagro F

Results and discussion

In table 3 analysed content of crude nutrients and metabolisable energy of experimental diets are shown. Accordingly, it was achieved to use well comparable feeds with 173 to 175 g/kg crude protein and 13.18 to 13.44 MJ/kg metabolisable energy. During the experiment, all pigs stayed healthy and both groups showed a very high performance level, with the exception of two outliers. The supplementation of 2% IPUSagro F resulted in increased (+122 g) average daily gain in the first half of the fattening period. Subsequently, both groups had the same very high level of about 900 g per day.

Table 3: Analysed content of crude nutrients and calculated metabolisable energy of experimental diets (88% DM)

	FG 1	FG 2
dry matter, g/kg	880	880
metabolisable energy, MJ/kg	13.44	13.18
crude protein, g/kg	175	173
ether extract, g/kg	24	32
crude fibre, g/kg	34	34
crude ash, g/kg	42	64
starch, g/kg	446	408
sugar, g/kg	30	31

FG 1, control group; FG 2, feeding group with IPUSagro F

Considered over the entire fattening period, the group with 2% IPUSagro F also achieved higher average daily gain compared to control group (+47 g). The result over the whole trial period also confirmed an

improvement in feed conversion ratio from 2.82 *vs.* 2.59. Table 4 presents summarised findings of zootechnical performance. These results are confirmed by another feeding trial by Wetscherek (2014), which was conducted with the same level of 2% IPUSagro F but on top, without energy compensation. Between 35 to 75 kg body weights, the performance of the experimental groups was identical. In the final fattening period up to 117 kg weight, the group with the migulators IPUSagro F had a higher performance compared to the control group. Considered for the entire fattening period, performance resulted in 5.6% higher daily gains due to the addition of IPUSagro F.

Table 4: Zootechnical performance of fattening pigs fed diets with or without IPUSagro F

Body weight (BW), kg	FG 1	FG 2	SEM	P-Value
initial BW	37.3	37.5	0.63	0.9007
42. day of experiment	69.2[b]	74.4[a]	1.18	0.0014
BW at the end of experiment	120.2	119.0	1.11	0.4651
Average daily gain, g				
37 to 72 kg BW	758[b]	880[a]	16	<0.0001
72 to 120 kg BW	906	894	21	0.6558
37 to 120 kg BW	841[b]	888[a]	15	0.0193
Feed conversion ratio, kg	FG 1	FG 2	-	-
37 to 72 kg BW	2.72	2.22	-	-
72 to 120 kg BW	2.89	2.91	-	-
37 to 120 kg BW	2.82	2.59	-	-

FG 1, control group; FG 2, feeding group with IPUSagro F

Regarding slaughter performance, no differences were associated with the supplementation of 2% IPUSagro F (table 5). Pigs showed desired similar weight at slaughter with around 120 kg and a uniform dressing by around 78%. In addition, the meat quality parameters did not differ between feeding groups. These results can also be confirmed by the findings of Wetscherek (2014), where 2% IPUSagro F (on top of a diet) did not negatively affect the slaughter performance or meat quality. Furthermore, all analysed values of specific blood parameters were in physiological range of fattening pigs without exceeding or falling below common limits. Summarising the variety of analyses, pigs fed diets containing 2% IPUSagro F differed only numerically from the control group with regard to white blood cell differentiation (subtypes of leukocytes), haematology and chemical parameters of blood.

Table 5: Slaughter performance and meat quality parameters of fattening pigs fed diets with or without IPUSagroF

	FG 1	FG 2	SEM	P-Value
BW at slaughter, kg	120.2	119.0	1.11	0.4651
BW after slaughter, kg	94.7	93.4	0.94	0.3322
dressing, %	78.8	78.4	0.31	0.3734
back fat depth (a), mm	11.9	9.3	0.95	0.0770
loin meat depth (b), mm	79.7	78.5	1.36	0.5086
lean*, %	61.5	62.4	0.54	0.2562

FG 1, control group; FG 2, feeding group with IPUSagro F; BW, body weight,
*lean, calculated as 48.7719 – 0.48330 x a + 0.23127 x b according to BGBl. II Nr. 23/2019, § 5

Conclusion

Results of present study showed that a use of 2% clinoptilolite (IPUSagro F) in diets of fattening pigs did not cause any health impairments due to specific blood parameters and not affected feed intake. Furthermore, fattening pigs fed diets containing migulators had a higher average daily gain and indicated an improved feed conversion ratio. Slaughter performance and parameters of meat quality did not differ due to the inclusion of 2% clinoptilolite (IPUSagro F).

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On-farm evaluation of plant-based feed additives for reducing faecal ammonia-N and ammonia emissions in laying hens and fattening pigs

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Abstract

Plant bioactives such as tannins and saponins can improve the intestinal health of monogastrics and contribute to reduce the environmental impact of the livestock sector. Here, we evaluated on-farm the effects of plant-based feed additives (Silvafeed®) on the performance, faecal ammonia-nitrogen, ammonia emissions and faecal microbiota composition in laying hens and fattening pigs. In Exp 1, 60000 laying hens were placed into two barns and fed a basal diet or a basal diet + 750 g/ton of Silvafeed® for 120 days. The chemical analysis and microbiota composition of dry excreta samples were done on a monthly basis. Additionally, ammonia emissions from excreta was assessed using static chamber at days 60 and 120. In Exp 2, more than 300 fattening pigs from two different barns and fed a basal diet or a basal diet + 1000 g/ton of Silvafeed®. In Exp 1, excreta from laying hens fed with Silvafeed® were drier (+6% of DM), with less total nitrogen (-11%), less ammonia-nitrogen (-26%), and emitted less ammonia gas (-32%) as compared with the control group. In Exp 2, pigs fed with Silvafeed® had daily gain and feed efficiency (+6%), and reached the target weight 6 days before the control group. Faeces of pigs fed with Silvafeed® had constantly less ammonia-nitrogen (-28%) and less nitrate (-55%) as compared with the control group. Dietary inclusion of plant-based feed additives Silvafeed® (750-1000 ppm) reduces faecal ammonia-nitrogen by 26-28% in monogastrics, and proved to be a promising tool to reduce ammonia emissions in livestock production.

Introduction

Livestock production contributes to ammonia emissions, which is a concern for environmental pollution as well as the health of livestock animals and farm workers. Plant-based bioactives such as tannins (as found in chestnut and quebracho extracts) and saponins have been shown to improve the digestive health and metabolism in monogastrics (Liu et al. 2020; Redondo et al., 2022), and reduce nitrogen excretion and ammonia emissions (Schiavone et al, 2008). Therefore, we conducted on-farm trials to evaluate the effects of plant-based feed additives Silvafeed® on the performance, faecal ammonia-nitrogen, ammonia emissions and faecal microbiota in laying hens and fattening pigs.

Material and methods

In experiment 1, 60000 seventeen-week-old laying hens were placed into two free-range barns from the same farm and fed a basal diet or a basal diet + 750 g/ton of Silvafeed® for 120 days. Every month, five samples of dry excreta were taken from different places in each barn, then pooled together for the chemical analysis (dry matter; total Kjedhal nitrogen; and ammonia-nitrogen) and microbiota composition analysis. Briefly, total genomic DNA was extracted using QIAGEN kit, and V3-V4 hypervariable regions of the 16S subunit gene of bacterial rRNA were amplified by PCR reaction and sequenced using Illumina MiSeq System platform. Taxonomic groups with a relative abundance >2% were used for graphical output using RStudio 6.3. Additionally, ammonia emission from poultry excreta was measured

at days 60 and 120 using static chamber method and a photoacoustic gas monitor (INNOVA, Lumasense). In experiment 2, 327 female and entire male pigs (Landrace × Large White) were from two different barns were fed a basal diet or a basal diet + 1000 g/ton of Silvafeed for the entire fattening period (from 20 up to 110 kg of bodyweight). Pig performance and carcass characteristics were obtained at the end. Every month, ten faecal samples from different places in each barn were pooled for chemical analysis (dry matter, total nitrogen, ammonia-nitrogen, nitrate).

Results

In experiment 1, the high-productive performance of laying hens and egg quality remained satisfying in both barns according to the historical results of the farm. The chemical analysis of the excreta showed that laying hens fed with Silvafeed® were drier (+6% of DM), with less nitrogen (-11% of total nitrogen), less ammonia-nitrogen (-26%). Moreover, ammonia gas emitted from the excreta was reduced in Silvafeed® group (-32%) as compared with the control group (Figure 1).

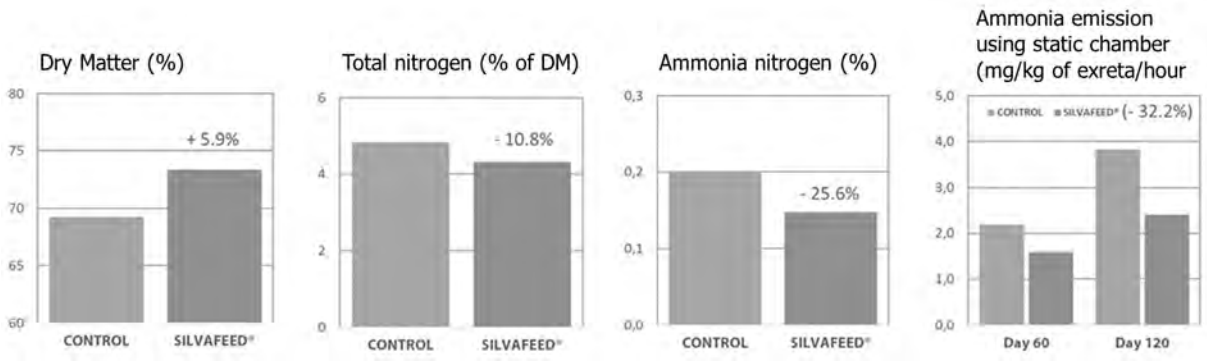

Figure 1. Average values for the chemical analysis (DM, Total N and NH3-N) of poultry excreta over 120-day feeding trial and the emissions of ammonia gas using static chamber

The analysis of the gut microbiota composition showed that Firmicutes and Bacteroidetes were dominant phyla of bacteria in poultry excreta (>75% of bacterial abundance) in both group, with the genus *Lactobacillus* representing approximately 12% (Figure 2).

Table 1. Performance and carcass characteristics of fattening pigs

Performance and carcass characteristics	Control	Silvafeed® 1000 ppm	% change (treatment vs control)	P-value Student's t-test
Average daily gain (kg/day)	0.64	0.68	+6.1%	<0.01
Feed conversion	2.56	2.41	-5.9%	n.d.
Mortality (%)	6.3	4.5	-27.9%	n.d.
Carcass weight (kg)	89.15	90.32	+1.3%	<0.1
Carcass score (SEUROP)	49.2	49.9		>0.1
Fat thickness (mm)	8.58	9.13	+6.4%	<0.1
Muscle thickness (mm)	57.5	57.2		>0.1

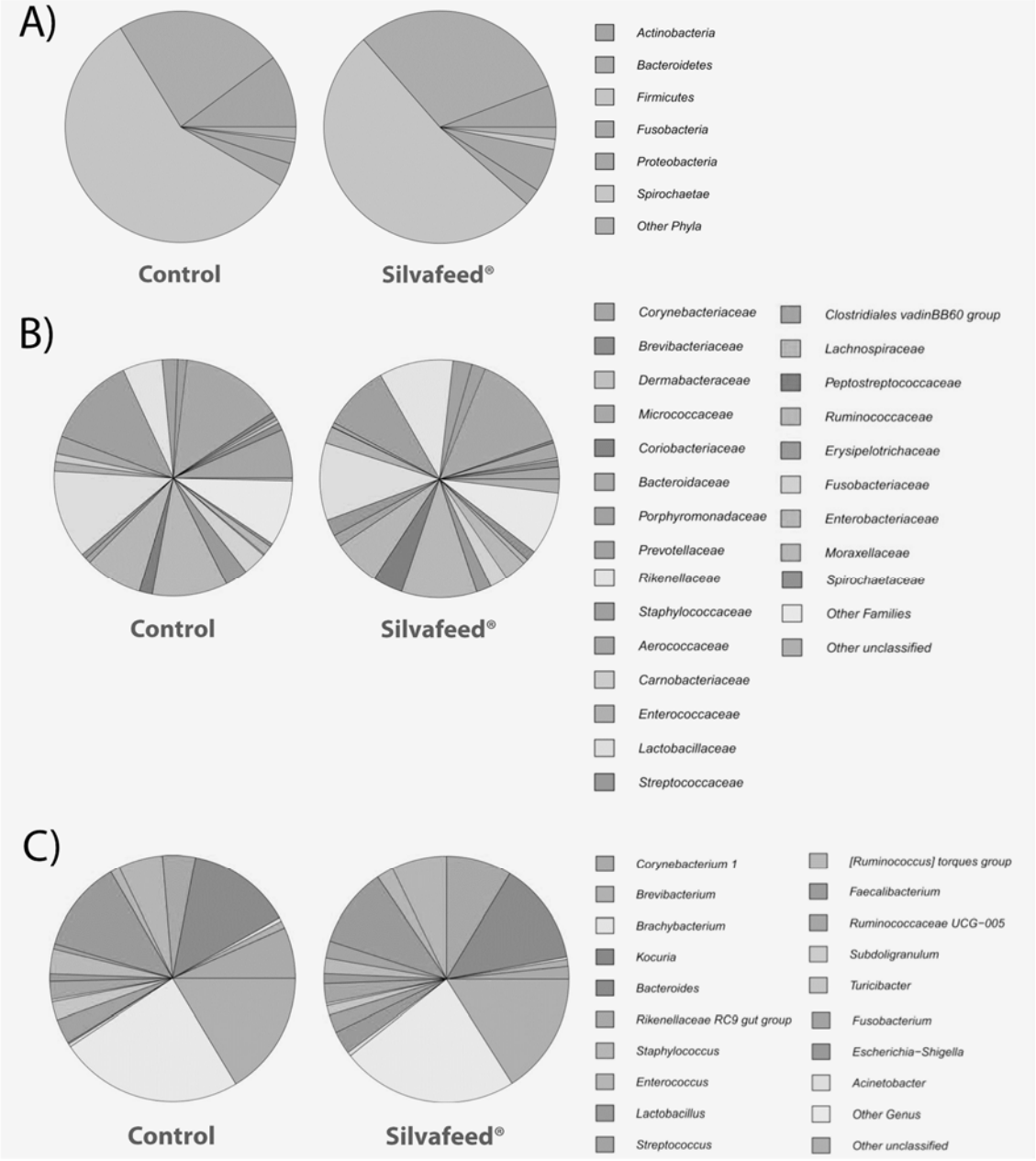

Figure 2. Average relative abundances of bacteria in hen excreta at phyla (A), family (B) and genus (C) levels. Time 0 was included only in Control group

In experiment 2, pigs fed with Silvafeed® reached the final weight (target ≈ 110 kg) 6 days before the pigs in control group on average. Higher daily gain (+6%) and improved feed conversion (-6% FCR) were observed in Silvafeed® group as compared with the control group (Table 1).
We showed that pig faeces in Silvafeed® group contained less ammonia-nitrogen (-28% on average) as compared with the control group. The level of nitrate in faeces remained < 50 mg/kg in Silvafeed® group while it increased in control group in two occasions.

Table 2. Average values of faecal characteristics from pigs during fattening period

Chemical analysis of faecal samples	Control	Silvafeed® 1000 ppm	% change (treatment vs control)
Dry matter (%)	26.0	25.7	
Total Nitrogen (% of DM)	5.29	3.51	-33.7%
Ammonia-nitrogen (% of DM)	2.33	1.68	-27.6%
Nitrate NO3 (mg/kg)	110.8	<50	-54.9%

Discussion

The performance of laying hens and egg quality remained highly satisfying according to the historical results of the farm. The effects of Silvafeed® on the excreta of laying hens is in line with Schiavone et al. (2008), which showed higher dry matter and lower nitrogen content in broiler faeces. No clear differences of gut microbiota composition were observed between the groups, which may be due to the limited numbers of replicates. The improvement of performance for pigs fed Silvafeed® confirm promising results obtained in piglets (Liu et al., 2020). Interestingly, the deposition of fat was higher in pigs fed Silvafeed®, especially in entire male pigs, which could reflect a better energy utilisation efficiency.

Conclusion

Plant-based feed additives Silvafeed® (750-1000 ppm) reduces the level of faecal ammonia-nitrogen by 26-28% in laying hens and fattening pigs, and proved to be a promising natural tool to reduce ammonia emissions in livestock production.

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Antioxidative properties of eubiotic lignocellulose contribute to maintaining performance and eggshell stability in laying hens at a late laying period

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Abstract

A prolongation of the hens' laying cycle whilst stabilizing laying persistency is a major aim in egg producing industry. One crucial challenge is the negative correlation of egg size and bird's age. A thinner eggshell in bigger eggs increases the risk of breakage and thus, represents a limiting factor of the duration of a laying cycle. The objective of the present study was to determine the effects of a supplementation of an eubiotic lignocellulose on the performance and oxidative status of commercial laying hens during the late stage of their production cycle. Due to the specific composition of the eubiotic lignocellulose (LC), a beneficial impact on performance, eggshell quality as well as on health status of layers in a critical phase of their production cycle was hypothesized.

Introduction

Although historically considered as antinutritive ingredient, scientific studies of recent years contributed to our knowledge about the positive effects of dietary fibre in monogastric nutrition (Jarrett & Ashworth 2018, Kheravii et al. 2018). Still discussed, it is widely accepted that a balanced inclusion of fibre contributes directly and indirectly to proper intestinal functions and promotes gut health. The use of lignocellulose (LC) as a highly concentrated source of insoluble fibre turns out to be an efficient tool in optimizing diets of swine and poultry.

When focussing on poultry production a supplementation of LC affects the birds' gut functions on different levels: LC is potent to modulate gut morphology by stimulation of villi growth leading to an increased absorptive area (Röhe et al. 2020) and to stimulate the development of the proventriculus as the pacemaker of intestinal motility. Further, LC supplementation is discussed to influence microbiome in ileum and caecum (Kheravii et al. 2018), which taken together may lead to an improved digestion of poultry diets. Consequently, performance of broilers as well as laying hens may be affected beneficially (Sozku and Ipek 2020). Nevertheless, it is too short-sighted to consider an eubiotic LC simply as a functional fibre source only. Contrary to standard LC products, eubiotic LC contains a considerable amount of fermentable parts (Youssef and Kamphues 2017) as well as wood derived bio-active molecules and hence, also acts as a nutritional additive influencing, for example, the oxidative status of the birds. Moreover, eubiotic LC was found to exhibit anti-inflammatory and gut protective effects that were absent in the standard LC (Zeitz et al. 2019). Therefore, we assumed that the supplementation of an eubiotic LC is a helpful tool in prolonging the laying cycle while maintaining a high laying persistency. According to the farm and region, laying hens are housed in the production sites for about 80 to 85 weeks of age until they are replaced. Consequently, the longer this substitution of birds can be delayed, the higher is the economic output per hen. The economically limiting factor in the challenge of a prolonged laying cycle is keeping the laying persistency at a constant level and simultaneously stabilizing eggshell quality: the older the birds, the bigger the eggs – thus, the ratio eggshell to egg weight changes to a disadvantageous level as the relative thickness of the eggshell declines with increasing size of the egg (Roberts 2004), i.e. eggshell quality negatively correlates with bird's age and the duration of the laying cycle.

Our assumption was, that due to the above-mentioned functional and nutritional impact, an eubiotic LC will help to maintain egg-shell stability in diets of laying hens at a late stage of the production cycle. To test this hypothesis, we run a feeding trial under controlled environment with laying hens between 60 to 75 weeks of age. Considering a plausible mode-of-action, we further evaluated the influence of eubiotic LC supplementation on antioxidant parameters.

Material and methods

48 laying hens (Lohmann LSL) were allocated randomly to 2 treatments á 6 replicates comprising 4 hens per pen under controlled environment. For comparison, laying hens were offered the laying diet without supplementation of LC (control group) or with a laying diet supplemented with 8 g LC/kg feed (LC; OptiCell® agromed Austria GmbH). Diets were formulated to contain equal quantities of crude protein, amino acids and metabolizable energy (11.6 MJ/kg), see table 1.

Table 1: Analysed values of the experimental layer diet (as fed)

Treatment groups		Control	Lignocellulose
Dry matter	g/kg	890.4	890.6
Crude protein	g/kg	167.2	167.5
Neutral-detergent fibre aNDFom	g/kg	159.8	165.2
Acid-detergent fibre ADF om	g/kg	33.3	38.8
Crude fibre	g/kg	31.3	32.9
Crude fat	g/kg	40.9	43.7
Crude ash	g/kg	118.3	118.9
Starch	g/kg	425.2	418.6
Sugar	g/kg	31.4	30.9
Calcium	g/kg	36.1	36.5
Total phosphorus	g/kg	5.3	5.4
Sodium	g/kg	1.8	1.9

Laying hens were 60 weeks of age at the begin and 75 weeks of age at the end of the trial. Individual body weight was recorded in a two-weeks interval from day 1 to day 112 of the experiment. In the same manner data on feed intake, laying rate, egg mass output, egg weight, feed-to-egg mass ratio were obtained. 6 eggs per treatment (1 egg per pen) collected at d 28, d 56, d 70, d 84, d 98 and d 112 of trial were used for determination of eggshell stability. To gain information on the antioxidative status, blood samples were taken at the end of the trial an analysed for superoxide-dismutase (SOD) activity as well as thiobarbituric acid-reactive substances (TBARS), calculated as malondialdehyde concentration via a commercial kit (Sigma, Deisenhofen, Germany). Differences were considered significant when $p<0.05$, whereas $p<0.10$ was considered a near-significant trend. Analysis was performed SigmaStat vers 4.0 (Systat software Inc.).

Results

Hens fed the diet supplemented with LC consumed significantly more feed compared to the control diet. Nevertheless, egg production and feed to-egg-mass ratio were improved significantly. The application of the LC product did on average numerically enhance egg mass output (+4.3%), egg number (+3.1%) and mean egg weight (+1.0%) in comparison with the control group (table 2).

The TBARS, calculated as malondialdehyde, in hens fed diets containing LC were on average significantly reduced as compared with the control group, while the antioxidative capacity characterized by superoxide dismutase was significantly enhanced. Moreover, the supplementation of LC reduced the total bilirubin content in hens' serum at the end of the trial.

Table 2: Performance data of laying hens according to feeding group

	Control	Lignocellulose
Body weight start (g)	1,724 ± 127	1,736 ± 59
Body weight end (g)	1,739 ± 117	1,772 ± 55
Body weight change (g)	15 ± 23[a]	36 ± 33[b]
Cumulative feed intake (kg)	13.5 ± 0.14[a]	13.9 ± 0.91[b]
Daily feed intake (g)	120.5 ± 1.3[a]	123.7 ± 0.8[b]
Egg number (n)	102.7 ± 7.5[a]	107.1 ± 2.8[b]
Egg weight (g)	62.0 ± 1.1[a]	63.6 ± 1.1[b]
Total egg mass (g)	6,368 ± 452[a]	6,812 ± 172[b]
Broken egg rate (%)	1.30 ± 0.48	1.10 ± 1.11
Feed conversion ratio (rel. egg mass)	2.13 ± 0.15[a]	2.04 ± 0.05[b]
Egg shell stability (N)	42.0 ± 2.0	44.5 ± 3.0

Table 3: Antioxidant relevant parameters and bilirubin content in serum of laying hens at the end of the feeding trial

	Control	Lignocellulose
SOD activity (U/g Hb)	1715 ± 114[a]	1978 ± 241[b]
TBARS (nmol/ml)	4.7 ± 0.1[a]	4.2 ± 0.2[b]
Total bilirubin (mmol/l)	1.13 ± 0.71[a]	0.52 ± 0.23[b]

Discussion

A prolongation of the laying cycle while maintaining a high laying persistency can only be achieved when providing birds with diets, which fully match their nutritional requirements. This is true throughout a hen's life, starting directly after hatch and ends with the death of the animal. Besides the declining laying performance with progressing life age, an impaired eggshell stability in old hens is a limiting factor that forces egg producers to replace laying hens early due to economic reasons. Eggshell stability cannot simply be improved by providing more calcium for calcification of the eggshell, as the calcium retention decreases with calcium consumption. Instead, the maintenance of the whole gastrointestinal tract is a key to success, which implies a well-balanced fibre profile, which is confirmed by the results of the present study: Although egg mass and egg weight were clearly increased, lignocellulose supplementation did not negatively affect eggshell stability, but on the contrary even slightly improved eggshell stability (Table 2). Consequently, even though egg size was increased, the frequency of broken eggs was slightly reduced, which suggests a beneficial influence of the eubiotic LC on eggshell quality compensating a higher risk for broken eggs with increased egg size.

The stability of the eggshell is not only related to the extent of calcification, but moreover the elasticity reflects the risk of getting damaged. The liver is the site of biosynthesis of proteins responsible for the degree of elasticity. A high bilirubin level in blood is an indicator for dysfunction of liver tissue (Lumeji 1994). Especially in laying hens in the later laying period impaired liver functions are a limiting factor for laying performance. The reduced bilirubin level due to lignocellulose supplementation give proof of a healthy and fully operational liver tissue.

A long-term egg production causes an accumulation of oxidative stress and is a main cause for a decline of zootechnical performance and egg quality during the late laying period (Liu et al. 2018): the balance between reactive oxygen species generation and antioxidants systems can be disrupted because of gradually decreased levels of antioxidants during the aging process (Subramanian and James, 2010). In the present study, the blood profile measured at the end of the trial (75 weeks of age) revealed, that the supplementation of eubiotic LC caused a significant reduction of TBARS, which are substances formed as by-products during the oxidative degradation of fat and lipids. The reduction of TBARS in layers fed on LC supplemented diet indicates an improved oxidative status of the animals and suggest

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a higher stress resilience compared to birds of the control group (Table 3). These findings are in concordance with a significantly improved enzyme activity of SOD. This group of enzymes is capable to neutralize superoxide molecules, which in turn are highly reactive oxygen species and can damage intestinal tissues irreversibly. Thus, data indicate that the supplementation of the used LC compensated for stress dependent impairment of performance and eggshell stability and suggests that the used LC beneficially affects the physiology of old laying hens via both, nutritional and functional properties.

Conclusion

Data of this feeding trial clearly proof the beneficial influence of a specific LC product supplemented in layer diets on the performance of old laying hens as well as on eggshell stability. Hence, LC turns out to be crucial in optimizing the fibre profile of layer diets and contributes via nutritional and functional properties and should be considered as an important tool when aiming at a prolonged and economically reasonable use of laying hens for egg production.

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Sicherstellen der Arginin-Versorgung in proteinreduzierten Broiler-Rationen durch Supplementierung von Guanidinoessigsäure

Guanidinoacetic acid guarantees Arginine supply in protein reduced diets for broiler chickens

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Abstract

Creatine (Crea) plays a vital role in energy metabolism. The endogenous synthesis of its single precursor - guanidinoacetic acid (GAA) - requires Arginine (Arg). If GAA is directly added to broiler diets, dietary Arg can be spared, which can be of advantage in low protein diets. The objective of this study was to determine to which extent GAA can spare Arg in low crude protein (CP) diets. A total of 480 male twenty-day-old Ross 308 chickens received an adequate starter diet. During the grower (d10-24) and finisher (d25-42) phase all birds were assigned to six dietary treatments: a control diet, a low CP diet (-15 g/kg) deficient in Arg, a low CP diet sufficient in Arg and three low CP diets, where 0.1% L-Arg was replaced by GAA at 50, 100 and 150% of Arg-equivalence. Deficiency in dietary Arg led to 7.8% lower body weight gain, 10 points higher FCR, 8.5% lower breast meat yield and 27.2% lower breast meat Crea concentration compared to the control diet. Arg sufficient, low CP diets were comparable to the control diet. Performance results and breast meat Crea concentration of birds that were offered the diet where GAA spared Arg at 150% were comparable to the control or the Arg sufficient low CP group. Birds that received the diets where GAA spared Arg at 100% and 50% had lower FCR (-3 and -5 points, respectively) than the control birds. In conclusion, supplementation of GAA to low CP broiler diets can spare dietary Arg at 150%.

Einleitung

Kreatin (Krea) spielt eine zentrale Rolle im zellulären Energiestoffwechsel. In seiner phosphorylierten Form Kreatinphosphat stellt es jene Phosphatgruppe bereit, die benötigt wird, um energiearmes Adenosindiphospat (ADP) in energiereiches Adenosintriphosphat (ATP) umzuwandeln (Balsom et al., 1994). Die Synthese von Krea umfasst einen zweistufigen enzymatischen Prozess. Der erste Schritt in der Niere umfasst die Übertragung einer Aminogruppe von Arginin (174,2 g/mol) auf Glycin durch eine Transaminase, wodurch Ornithin und Guanidinoessigsäure (GAA; 117,1 g/mol) entstehen. In der Leber wird durch eine Transmethylase eine Methylgruppe auf GAA irreversibel übertragen. Als Produkt dieses Syntheseweges entsteht Krea, welches hauptsächlich im Skelettmuskel gespeichert wird.
(Wyss und Kaddurah-Daouk, 2000).
Kreatin findet man lediglich in Futtermitteln tierischen Ursprungs. Da Krea jedoch nur bedingt hitzestabil ist, wird es durch die notwendige Temperatureinwirkung bei der modernen Futterherstellung zum Großteil zu Kreatinin irreversibel abgebaut. Überdies ist Broilerfutter heutzutage hauptsächlich pflanzenbasiert und enthält somit keinerlei Krea. Eine direkte Quelle für Krea in der Tierernährung ist dessen endogene Vorstufe - GAA. Der Futtermittelzusatzstoff Creamino® (min. 96% GAA, Alzchem Trostberg GmbH) ist stabil unter Futterproduktionsbedingungen (van der Poel et al., 2018) und zeichnet sich durch eine sehr gute

Verdaulichkeit (>99%; Tossenberger et al., 2016) aus. GAA wird nach intestinaler Absorption in der Leber zu Krea synthetisiert und steht so dem Energiestoffwechsel des Tieres zur Verfügung. Durch die Absorption der endogenen Vorstufe von Krea kann im Metabolismus der erste Syntheseschritt umgangen werden. So kann das dafür benötigte Edukt, d.h. Arg, „gespart" werden und steht für andere Prozesse im Stoffwechsel zur Verfügung.
Zahlreiche Studien haben gezeigt, dass die Zugabe von GAA (Creamino®) die Supplementierung von L Arg ersetzen kann und darüber hinaus die Krea-Synthese steigert (DeGroot et al., 2018 und 2019). Gerade vor dem Hintergrund, Stickstoffausscheidungen zu reduzieren und Ressourcen effizienter zu verwerten, werden Rohprotein (XP)-reduzierte Futterrationen bei gleichzeitiger Supplementierung mit freien Aminosäuren immer relevanter.
Vor diesem Hintergrund sollte diese Studie überprüfen, inwieweit GAA (Creamino®) L-Arg in XP-reduziertem Broilermastfutter ersetzen kann und welchen Effekt dies auf die Krea-Synthese im Metabolismus hat.

Material und Methoden

Der Fütterungsversuch wurde an der Universität New England, Australien, durchgeführt. An Tag 0 wurden insgesamt 480 männliche Ross 308 Küken zufällig auf 48 Abteile aufgeteilt. Alle Tiere erhielten während der Starterphase ein bedarfsgerechtes Futter (23,3% XP; 2975 kcal AME_n/kg; Aviagen 2019). An Tag 10 wurden alle Tiere gewogen und gleichmäßig (< 3% Abweichung vom Mittelwert) auf 48 Abteile aufgeteilt (10 Tiere/Abteil mit 8 Abteilen/Wiederholung).
Die insgesamt sechs Fütterungsgruppen umfassten eine Kontrollgruppe (bedarfsgerecht nach Aviagen, 2019; 21,5% XP/3100 kcal AME_n/kg und 19,7% XP/3200 kcal AME_n/kg in Grower- und Finisher-Phase; dArg:dLys 1,05; CON), eine XP-reduzierte, Arg defizitäre (-1,5% XP in beiden Phasen; RED - L-Arg) sowie eine Arg bedarfsgerechte (+0,2% L-Arg; RED + L-Arg) Gruppe. Die RED + L-Arg Gruppe wurde zusätzlich in drei Stufen modifiziert, indem 0,1% Arg durch GAA mit unterschiedlichen Arg-Äquivalenten ersetzt wurde: 50% (RED + GAA 50; 0,2% GAA), 100% (RED + GAA 100; 0,1% GAA) und 150% (RED + GAA 150; 0,067% GAA). Das Aminosäuremuster der proteinreduzierten Gruppen wurde durch Zulage freier Aminosäuren ausgeglichen.
Das Körpergewicht und der Futteraufwand wurden an Tag 0, 10, 24 und 42 erhoben. Mortalitäten wurden täglich erfasst. Futteraufnahme, Zuwachs und mortalitäts-korrigierte Futterverwertung (FCR) wurden phasenweise kalkuliert. Bei der Schlachtung wurden Brustmuskelproben entnommen, gewogen und auf Krea (Alzchem Trostberg GmbH, Deutschland) untersucht.

Ergebnisse

Tabelle 1 zeigt die Futteraufnahme, den Zuwachs sowie die Futterverwertung der sechs Behandlungsgruppen. Die Reduzierung des XP-Gehalts bei gleichzeitigem Arg-Defizit (RED + L-Arg) hatte einen Rückgang des Zuwachses um 7,8% (3194 g vs. 2946 g) und eine Erhöhung der Futterverwertung um 10 Punkte im Vergleich zu CON-Gruppe zur Folge. Ein Ausgleich der Arg-Versorgung (RED + L-Arg) führte zu vergleichbarem Wachstum und vergleichbarer Futterverwertung wie die CON-Gruppe. Die Behandlungsgruppe, welche GAA mit einer Arg-Äquivalenz von 150% erhielt, zeigte vergleichbare Wachstumsleistungen sowie Futterverwertung wie die CON-Gruppe und die RED+L-Arg-Gruppe. Wurde GAA ein L-Arg Spareffekt von 100% (RED + GAA 100) und 50% (RED + GAA 50) zugewiesen, konnte eine niedrigere Futterverwertung als in der CON-Gruppe (-3 und -5 Punkte) erzielt werden.
Bei den Brustmuskel-Krea Konzentrationen wies die RED - L-Arg Gruppe signifikant geringere Werte auf als die CON-Gruppe (2,392 g *vs.* 3,287 g; Tabelle 2). Auch der Anteil des Brustmuskels am Lebendgewicht war signifikant verringert (173,5 g/kg *vs.* 189,6 g/kg). Die mit L-Arg supplementierte Gruppe (RED + L-Arg) hatte wiederum vergleichbare Brustmuskel-Krea Konzentrationen (3,109 g/kg) und auch einen vergleichbaren Anteil an Brustmuskelfleisch am Schlachtkörper (183,8 g/kg) wie die CON-Gruppe (189,6 g/kg). Auch die Tiere der RED + GAA 100 und RED + GAA 150 Gruppen wiesen einen vergleichbaren Anteil des Brustmuskels am Lebendgewicht (187,1 g/kg und 187,0 g/kg) verglichen mit der CON-Gruppe auf. Brustmuskel der RED + GAA Gruppen (Arg-Äquivalenz von 100% und 150%) waren im Mittel um

27g schwerer als jene der RED + L-Arg Gruppe. Die Brustmuskel-Krea Konzentration der RED + GAA 150 Gruppe (3,155 g/kg) war vergleichbar mit der CON-Gruppe. Wurde GAA eine 100% L-Arg Äquivalenz zugewiesen (RED + GAA 100), konnte die Brustmuskel-Krea Konzentration um 27,5% im Vergleich zur RED + L-Arg Gruppe erhöht werden. Bei einer zugewiesenen Äquivalenz von 50% (RED + GAA 50) konnte analog eine Steigerung um 45,2% und im Vergleich zur CON-Gruppe eine Steigerung um 37,3% beobachtet werden.

Tabelle 1: Futteraufnahme, Zuwachs und FCR während des Versuchszeitraums (Tag 10 bis 42) der sechs Behandlungsgruppen.

Behandlungsgruppe	Futteraufnahme (g/Tier)	Zuwachs (g/Tier)	Futterverwertung
CON	4843^{a}	3194^{a}	1,517^{bc}
RED - L-Arg	4749^{abc}	2946^{c}	1,612^{a}
RED + L-Arg	4744^{abc}	3142^{ab}	1,510^{bcd}
RED + GAA 50	4650^{c}	3179^{ab}	1,463^{e}
RED + GAA 100	4735^{abc}	3184^{ab}	1,488^{d}
RED + GAA 150	4781^{ab}	3132^{ab}	1,527^{b}
P-Wert	<0,05	<0,05	<0,05

Tabelle 2: Brustmuskel-Kreatin-Konzentration, Anteil Brustmuskel am Lebendgewicht sowie Brustmuskel-Gewichte der sechs Behandlungsgruppen am Tag 42.

Behandlungsgruppe	Brustmuskel-Kreatin (g/kg Frischmasse)	Anteil Brustmuskel (g/kg)	Brustmuskel (g)
CON	3,287^{cd}	189,6^{a}	685,3^{a}
RED - L-Arg	2,392^{e}	173,5^{d}	595,6^{c}
RED + L-Arg	3,109^{d}	183,8^{abc}	647,7^{b}
RED + GAA 50	4,514^{a}	179,1^{cd}	649,0^{b}
RED + GAA 100	3,964^{abc}	187,1^{ab}	673,5^{ab}
RED + GAA 150	3,155^{d}	187,0^{ab}	675,1^{ab}
P-Wert	<0,05	<0,05	<0,05

Diskussion

Arginin ist eine essentielle Aminosäure für Geflügel, da Vögel diese aufgrund des fehlenden Harnstoffzyklus nicht endogen synthetisieren können (Khajali und Wideman, 2010). Speziell in XP-reduzierten Futterrationen für Geflügel kann Arg schnell eine limitierende Aminosäure werden und muss deshalb in kristalliner Form zugegeben werden. Arginin fungiert nicht nur als Baustein für die Körperprotein-Synthese, sondern dient auch als Edukt der Krea-Synthese via des intermediären Metaboliten GAA. Durch die direkte Supplementierung von GAA zum Futter kann Arg in seiner Funktion als Krea-Synthese-Edukt „gespart" werden. Stöchiometrisch kann 1 Molekül GAA mit 117,1 g/mol, Arg, mit einer molaren Masse von 174,2 g/mol, zu 149% sparen. Zudem ist die Gabe von GAA effektiver in der Synthese von Krea als die Zugabe von L-Arg (DeGroot et al., 2019).

Die Ergebnisse dieser Studie zeigen, dass durch die Rohprotein-Reduzierung und ein Defizit an Arg in der Ration das Wachstum sowie die Krea-Synthese stark limitiert wurden. Das Endgewicht war um 248 g geringer und die Brustmuskel-Krea Konzentrationen um 27,2% reduziert im Vergleich zu adäquat versorgten Tieren der Kontrollgruppe. Das Leistungsdefizit konnte durch die Supplementierung von L-Arg

wieder kompensiert werden und die Leistungsdaten glichen sich jenen der bedarfsgerecht versorgten Tiere der Kontrollgruppe an.
Die zentrale Fragestellung dieser Studie bestand darin, zu überprüfen, ob die positiven Effekte einer L-Arg Supplementierung in einer XP-reduzierten Ration durch den Ersatz mit GAA erhalten bleiben oder verbessert werden können. Durch den metabolischen Spareffekt kann Arg für andere Prozesse, wie z.B. Proteinsynthese, Hormonfreisetzung und „cell signaling" genutzt werden und muss nicht mehr als Edukt für die Krea-Synthese verwendet werden. Dazu wurde 1 kg L-Arg/t Futter durch GAA mit drei verschiedenen Äquivalenzwerten von 50 % (2 kg GAA/t), 100% (1 kg GAA/t) und 150% (0,067 kg GAA/t) ersetzt. Die Hypothese, dass GAA bei 150% Äquivalenz zu L-Arg die Leistung in XP-reduzierten Rationen erhalten kann, konnte durch die Ergebnisse dieser Studie deutlich bestätigt werden. Die Futterwertung der GAA-Gruppen 50 und 100 war um 5 und respektive 3 Punkte geringer als die der bedarfsgerechten CON-Gruppe. Das nutritive Arg-Defizit zeigte sich im Speziellen auch am Gewicht des Brustmuskels (RED – L-Arg). Durch L Arg Zulage (RED + L-Arg) konnte das Gewicht zwar um 8,7% erhöht werden, jedoch konnte eine GAA-Zulage (Arg-Äquivalenz von 150%) eine Steigerung um 13,3% bewirken, was die besondere Relevanz von Krea für den Muskel unterstreicht.
Freies Arg im Blut wird schnell für metabolische Prozesse verwendet und ist nicht der effizienteste Weg, um Krea zu synthetisieren (DeGroot et al., 2019). So zeigten auch die Ergebnisse der Studie, dass die Krea-Synthese deutlich durch die Supplementierung von GAA im Vergleich zur alleinigen Supplementierung von L-Arg gesteigert werden konnte. Zwar wurde die Brustmuskel-Krea Konzentration durch Zugabe von L-Arg zur Arg-defizitären Ration erhöht, jedoch hatte der Ersatz von L-Arg durch GAA mit Äquivalenz 100% eine Steigerung der Brustmuskel-Krea Konzentration um weitere 27,5% zur Folge.

Schlussfolgerungen

Guanidinoessigsäure kann L-Arg zu 150% in moderat XP-reduzierten Futterrationen für Broiler ersetzen, um vergleichbare Leistungen und eine Krea-Synthese wie in Norm-Protein Rationen oder mit L-Arg supplementierten XP-reduzierten Rationen zu erzielen. Wird beim Einsatz von GAA eine Äquivalenz für L-Arg von 50 oder 100% angesetzt, bietet dies die Möglichkeit einer weiteren Verbesserung der Futterverwertung.

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Lysolecithin im Broilerfutter steigert die Effizienz

Lysolecithin supplementation in broiler feed improves efficiency

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Abstract

At broiler production efficiency is most important. Since decades feed conversion efficiency is seen only regarding cost efficiency. The discussion on sustainability lead to a different view. Consumption of water, energy, land use and many others gaining more and more importance. This culminates in CO2 requirements to produce 1 kg of broiler meat. Energy supply can be improved with fat supplementation and additional use of nutritional emulsifiers, like hydrolysed lecithin (lysolecithin). In growing broiler, they have shown to improve energy digestibility, improve body weight gain and reduce feed conversion ratio. The aim of this study was to compare the effects of several emulsifiers as a supplement in broiler feed with reduced energy content. A total of 360 one-day-old broilers were fed with an energy rich diet (positive control, PC), 3% reduced energy and amino acids levels (negative control, NC), NC + FRA® LeciMax Dry or one of three other diets based on NC + emulsifier (product A, B or C). The reduction in energy (NC) caused reduced feed intake and growth with subsequent increase in feed conversion ratio. Only FRA® LeciMax Dry could keep all production parameters on PC level. The other emulsifiers used in NC diets successfully kept feed conversion ratio at PC level but overall production was reduced due to lower body weight gains.
Supplementation of emulsifiers in broiler feed can improve feed conversion ratio of low energy diets on level of standard diets. However, only the lysolecithin FRA® LeciMax Dry was able to keep production level as high as for energy rich broiler diets.

Einleitung

Eine ökonomische Geflügelfleischerzeugung setzt voraus, dass die Tiere schnell wachsen und das Futter effizient verwerten. Die dafür benötigten Rationen müssen eine hohe Energiekonzentration aufweisen, was durch die Zugabe pflanzlicher Fette erreicht wird. Der Umfang der Fettverdauung ist abhängig von der Art und Herkunft der Fette, der Zusammensetzung der Rationen und dem Alter der Tiere. Aufgrund der unreifen Physiologie und einer geringen natürlichen Lipaseproduktion ist die Verdauungs- und Aufnahmefähigkeit von Lipiden bei jungen Broilern gering (Al-Marzooqi und Leeson, 1999). Die Emulgatoren unterstützen die Phospholipide der Galle beim Emulgieren der Fette und fördern in weiterer Folge die Bildung kleiner, stabiler Mizellen, die einfach absorbiert werden können. Sie fördern den Einbau von Fettsäuren in Mizellen und erhöhen die Fettverdaulichkeit und Wachstumsleistung von Küken (Polin, 1980).
Der Grad der hydrophilen und lipophilen Eigenschaften eines Emulgators bestimmt die Art der geförderten Emulsion. Dies wird durch das hydrophile-lipophile Gleichgewicht (HLB) angezeigt. Ein niedrigerer HLB-Wert zeigt einen lipophileren Emulgator an, der eine Wasser-in-Öl-Emulsion fördert, wohingegen ein höherer HLB-Wert einen hydrophileren Emulgator anzeigt, der eine Öl-in-Wasser-Emulsion fördert. Da die Fettverdauung in einer wässrigen Umgebung stattfindet, ist für eine effiziente Fettverdauung eine Öl-in-Wasser-Emulsion erforderlich. Aufgrund ihrer Struktur und des HLB-Wertes im idealen Bereich von 8-12 fördern Lysolecithine Öl-in-Wasser-Emulsionen und somit die Fettverdauung.

Die gesteigerte Fett-Emulgierung reduziert deren Neigung die Enzyme des Protein- und Kohlenhydratabbaus zu hemmen. Somit kann die Verdauung insgesamt verbessert werden.
FRA® LeciMax Dry (FRAMELCO BV, The Netherlands) ist ein Emulgator auf Basis von hydrolysierten Lecithinen. Das Ziel dieser Studie war es, die Wirkung von FRA® LeciMax Dry in Broilerdiäten mit reduziertem Energie- und Aminosäurengehalt im Vergleich zu weiteren Emulgatoren zu untersuchen. Aufgrund der verbesserten Verdauungsleistung als Folge des Zusatzes von Lysolecithinen kann es möglich sein, den Energie- und Aminosäurengehalte im Futter zu reduzieren, bei Erhalt der Leistung und gleichzeitiger Senkung der Futterkosten.

Material und Methoden

Dieser Versuch wurde in einer unabhängigen Forschungseinrichtung in Taiwan durchgeführt. Der Versuch dauerte 35 Tage. Je 180 weibliche und männliche Eintagsküken (Ross 308) wurden eingestallt und am Tag der Ankunft im Versuchsbetrieb zufällig auf die 6 Behandlungen mit je 6 Abteilen und je 6 Tieren verteilt. Die Raumtemperatur betrug bei Ankunft der Küken 32° C und wurde im Versuchsverlauf kontinuierlich auf Außentemperaturniveau abgesenkt.
Im Versuch wurden eine Starter- (Tag 0-10), eine Mittel- (Tag 11-24) und eine Endmastdiät (Tag 25-35) verfüttert. Es wurden zwei Grundrationen formuliert: Rationen PC (positive control) mit normalen Gehalten an Energie und Aminosäuren (Tabelle 1) sowie Rationen NC mit um 3% abgesenkten Gehalten. Die Rationsbestandteile waren für alle Rationen gleich: Mais, Sojabohnen, Sojaextraktionsschrot, Mineralien, Vitamine und Aminosäuren. Zusätzlich zu den Behandlungen PC und NC gab es vier Fütterungsgruppen mit Emulgatoren FRA® LeciMax Dry (Lysolecithin), Produkt A und B (je Lysolecithin & synthetischer Emulgator) sowie Produkt C (synthetischer Emulgator), die mit je 500 g/t „on top" zu den Rationen NC zugelegt wurden.
Wägungen der Tiere erfolgten an Tag 0, Tag 10, Tag 24 und Tag 35. Die Futteraufnahme je Bucht wurde zu diesen Zeitpunkten erhoben und die Futterverwertung (FCR) aus der gemessenen Gewichtszunahme und Futteraufnahme berechnet.
Die Daten wurden einer Varianzanalyse mit dem Statistikprogramm SAS unterzogen. Das Niveau der statistischen Signifikanz wurde auf $P \leq 0,05$ festgelegt.

Tabelle 1: Nährstoffgehalte in der Versuchsration (g/kg bzw. MJ/kg)

	PC			NC		
Parameter	Starter	Grower	Finisher	Starter	Grower	Finisher
Rohprotein	232	216	199	228	216	195
Rohfett	77	81	86	59	66	69
Rohfaser	38	36	34	38	36	34
TDF[1]	176	163	158	173	167	159
sDF[2]	11	10	10	12	11	10
Calcium	9,5	8,5	8,0	9,5	8,5	8,0
Phosphor	7,6	7,3	6,9	7,6	7,3	6,9
Natrium	1,3	1,3	1,3	1,3	1,3	1,3
Lysin	13,2	12,1	10,9	12,5	12,0	10,6
Methionin	6,3	5,5	5,0	6,1	5,0	4,8
Umsetzbare Energie (ME)	11,5	12,0	12,4	11,2	11,6	12,0

[1]TDF: total dietary fibre; [2]sDF: soluble dietary fibre

Ergebnisse

Tabelle 2 zeigt die Leistungen der Broiler im Versuch für die jeweiligen Mastabschnitte. Die Nährstoffreduzierte Fütterung der Gruppe NC ergab ein vermindertes Wachstum über alle Mastabschnitte hinweg. Zu keinem Zeitpunkt erfolgte eine kompensatorische Futteraufnahme. Demzufolge waren Mastendgewichte (1,38 kg) und Futterverwertung (1,556) deutlich gegenüber der Gruppe PC vermindert (2,19 kg, 1,409; Tabelle 3).

Tabelle 2: Leistungen der Broiler im Versuch je Mastabschnitt

Emulgator, g/t	PC (positive control)	NC (negative control)	FRA® Leci-Max Dry 500	Produkt A 500	Produkt B 500	Produkt C 500
Tag 1-10						
Futteraufnahme, g/Tag	23,3^{ab}	23,2	21,9	20,1	19,7	24,7
Wachstum, g/Tag	21,1^{a}	14,5^{c}	20,4^{ab}	12,9^{c}	13,3^{c}	18,6^{b}
Futteraufwand, kg/kg	1,104^{cd}	1,602^{a}	1,072^{d}	1,555^{ab}	1,485^{ab}	1,328^{bc}
Tag 11-24						
Futteraufnahme, g/Tag	92,5^{a}	59,8^{c}	88,0^{a}	57,7^{c}	72,4^{b}	73,8^{b}
Wachstum, g/Tag	68,7^{a}	40,7^{c}	65,4^{a}	44,5^{c}	57,1^{b}	55,0^{b}
Futteraufwand, kg/kg	1,346^{ab}	1,470^{a}	1,346^{ab}	1,298^{b}	1,269^{b}	1,341^{ab}
Tag 25-35						
Futteraufnahme, g/Tag	136,2^{a}	91,9^{c}	135,9^{a}	112,9^{b}	125,0^{ab}	118,4^{ab}
Wachstum, g/Tag	88,3^{a}	56,3^{b}	86,2^{a}	78,9^{a}	80,6^{a}	83,1^{a}
Futteraufwand, kg/kg	1,542	1,633	1,576	1,432	1,551	1,424

^{ab} signifikante Unterschiede $p \leq 0,05$

Von den zugelegten Emulgatoren konnte nur FRA® LeciMax Dry das Wachstum der Broiler trotz Nährstoffreduktion auf das Niveau der PC-Tiere heben, wenngleich 3,7% im Mastendgewicht zur PC-Gruppe fehlten und der Futteraufwand um 1,1% erhöht war, beides $p>0,05$. Die Produkte A, B und C haben Wachstum und Futteraufwand gegenüber den Broilern der NC-Gruppe verbessert und rechtfertigen ihrem Gebrauch. Allerdings waren diese dem FRA® LeciMax Dry unterlegen. V.a. in den ersten beiden Mastabschnitten ergaben sich große Unterschiede. Es wird deutlich, dass während der intensivsten Wachstumsphase „Grower" nur FRA® LeciMax Dry den Nährstoffmangel im Vergleich zur PC-Gruppe ausfüllen konnte. Interessanterweise erzielten die Produkte A und B beide eine deutlich geringere Wachstumsleistung als FRA® LeciMax Dry. Produkt A schnitt schlechter ab als Produkt C und im Vergleich zu FRA® LeciMax Dry erzielte Produkt C ein um 9,7 % geringeres Wachstum.

Tabelle 3: Leistungen der Broiler über den gesamten Mastabschnitt

Emulgator, g/t	PC (positive control)	NC (negative control)	FRA® Leci-Max Dry 500	Produkt A 500	Produkt B 500	Produkt C 500
Mastendmasse, kg/Tier	2,19^{a}	1,39^{e}	2,11^{ab}	1,66^{d}	1,86^{cd}	1,91^{bc}
Futteraufwand, kg/kg	1,409^{b}	1,556^{a}	1,424^{b}	1,388^{b}	1,421^{b}	1,380^{b}

Diskussion

Die im vorliegenden Versuch eingesetzten Emulgatoren konnten Wachstum und Futterverwertung der negativen Kontrolle (NC) deutlich verbessern. Es bleibt aber zu bemerken, dass die NC-Broiler ihr Potential bei weitem nicht ausschöpfen konnten. Demzufolge erscheint ein Vergleich mit der positiven Kontrollgroppe (PC) sinnvoller. Hier erzielten alle Emulgatoren einen der PC entsprechenden Futteraufwand. Dieses sehr positive Ergebnis wird durch ein geringeres Wachstum der Produkt A, B oder C versorgten Broiler geschälert. Nur mit FRA® LeciMax Dry entsprachen Zunahmen und Futteraufwand der Gruppe PC. Die in der Literatur beschriebenen Auswirkungen von Emulgatoren auf das Wachstum von Broilern sind widersprüchlich.

Einerseits wird die Wachstumsleistung durch Zulage von Emulgatoren als verbessert beschrieben (Emmert et al., 1996; Huang et al., 2007; Zhang et al., 2011; Zulkifli et al., 2019), wohingegen andere das Gegenteil berichteten (Blanch et al., 1996; Azman & Ciftci, 2004).

Grundsätzlich unterstützen Emulgatoren nicht nur die Verdaulichkeit von Fetten, sondern auch weiterer Nährstoffe (Zulkifli et al., 2019; Wealleans et al., 2020). Mögliche Ursachen widersprüchlicher Ergebnisse werden im Fettsäurenmuster (Dierick & Decuypere, 2004), aber vor allem in der Art des Emulgators gesehen. Hier sind Lysolecithine wirksamer als Lecithine (Wealleans et al., 2020). Wealleans et al. (2020) sehen die Funktionsweise der Lysolecithine in einer verbesserten Fetthydrolyse sowie gesteigerten Absorption aller Nährstoffe.

Für eine effektive Fettverdauung und -verwertung und damit für die Leistung der Tiere ist eine richtige Fettemulgierung der Schlüssel und daher ist die Wahl des Emulgators wichtig. Dieser Versuch zeigt, dass nicht nur die Art des Emulgators, sondern auch der Gehalt an Lysolecithinen eine wichtige Rolle bei der Bestimmung der Wirksamkeit von Emulgatoren spielt. Diese Versuchsergebnisse zeigen, dass FRA® LeciMax Dry der wirksamste Emulgator der vier in diesem Versuch verwendeten Produkte ist. Dies erklärt sich aus dem höheren Gehalt an Lysolecithinen und den Vorteilen, die Lysolecithine gegenüber der Verwendung von synthetischen Emulgatoren haben.

Schlussfolgerungen

Die Zugabe von Lysolecithinen zum Broilerfutter führt zu einer höheren Nährstoffverfügbarkeit und -verwertung. So ist es möglich, den Energie- und Aminosäurengehalt zu senken, die Tierleistung zu erhalten und Futterkosten zu sparen.

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Drinking water supplementation of a mixture based on star anise essential oils alleviates heat stress in broilers

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Abstract

Heat stress is a common issue in poultry production, which leads to reduced performance and productivity, thereby increasing mortality and decreasing animal welfare in the flock. This study aimed to evaluate the effect of a commercially available mixture of star anise essential oils, vitamin C and electrolytes (LOVIT Granule Anilyte+C, Kaesler Nutrition GmbH, Cuxhaven, Germany) on the negative consequences of heat stress in broilers. One thousand day-old ROSS broilers were randomly allocated into two groups. During two natural heat phases with temperatures above 30°C the control group received no supplementation, while the trial group received LOVIT Granule Anilyte+C via drinking water with an inclusion rate of 1 kg 1000 litres. During the experimental period, birds in the LOVIT group had evidently higher final body weight along with average daily weight gain ($p<0.001$). Feed conversion ratio was reduced in the LOVIT group compared to the control group. Moreover, supplementation of the mixture markedly decreased the mortality rate and reduced cases of leg weakness in the LOVIT group. Results of this study indicated that supplementing the mixture based on star anise essential oil improved performance and vitality of broilers exposed to heat stress.

Introduction

Heat stress is a common issue in poultry production, which hampers growth, animal welfare, and vitality of the flock[1]. Heat stress occurs when the heat produced by the chickens' body cannot be dissipated to the environment. In consequence, poultry behaviour during heat stress includes measures to avert heat production such as minimising body movement, suspended wings and panting. Panting carries the risk of alkalosis, which inhibits growth and could be life threatening. At cellular level, heat stress leads to an increase in superoxide radicals, highly reactive molecules that can trigger a chain reaction and thus cause enormous tissue damage (oxidative stress)[3]. The apparent consequence of oxidative stress in broilers is a poor meat quality[2]. The general physiological consequences of heat stress include reduced feed intake and the associated reduced performance, weakened immune response, and increased mortality rate in the flock[1,2].

Hence, to counteract heat stress and simultaneously reduce the danger of alkalosis, the application of antioxidants together with electrolytes is advisable[4].

Star anise has long been used in medicine and food due to its antimicrobial, anti-inflammatory and antioxidative properties[5]. The relaxation of muscles is beneficial during intestinal issues to increase the well-being[6]. Star anise stimulates digestion and appetite, which can counteract reduced feed intake in poultry[7]. The main active ingredient of the essential oil is anethole (70 – 92%), followed by estragole (~2%), and limonene (~2%)[8]. The conjugated double-bound system of these molecules stabilises free radicals and contribute for their antioxidant properties[9].

The current trial aimed to evaluate the impact of a commercially available drinking water application containing star anise essential oil, vitamin C, and electrolytes (LOVIT Granule Anilyte+C, Kaesler Nutrition GmbH, Cuxhaven, Germany) on performance and vitality of broilers under environmental heat stress.

Material and methods

The trial was conducted at the Faculty of Agrobiotechnical Sciences at the University of Osijek, Croatia. One thousand ROSS 308 broilers were randomly allocated into two groups and located in the trial farm of the University to be kept under field conditions. The LOVIT group received the star anise essential oil based mixture (LOVIT Granule Anilyte+C) in their drinking water at a rate of 1 kg per 1000 litres daily on day 7 until 16 and day 25 until 34 depending on temperature and humidity (figure 1). The control group (CON) received no supplementation.

Body weight was measured at day 1, 18 and 38 (i.e. the end of the fattening period). Monitored parameters were body weight (BW), average daily weight gain (ADWG), average daily feed intake (ADFI), feed conversion ratio (FCR), mortality and general health status of the flock (animals showing lameness/leg weakness). Each bird was weighted individually for the determination of body weight. Statistical analysis was performed by TIBCO Software Inc. (2020). Data Science Workbench, version 14. http://tibco.com. Significance was declared at $p \leq 0.05$. Data are expressed as the mean ± standard deviation.

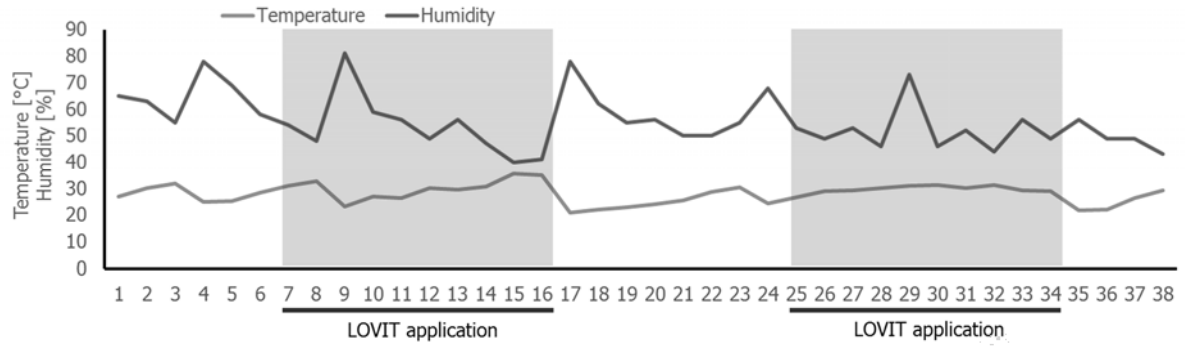

Figure 1: Temperature and humidity during the trial as well as the application period of the star anise essential oil based mixture (LOVIT Granule Anilyte+C) on days 7 until 16 and days 25 until 34. Dark and light grey lines depict humidity (%) and temperature (C), respectively

Results

At the start of the trial, both groups showed a high flock uniformity with an average weight of 49.86 and 49.88 g, respectively. After 18 days and the first heat period with temperatures above 30°C, body weight and ADWG of the LOVIT group were significantly higher than the control (table 1). While the ADFI was similar in both groups, the FCR was numerically different, indicating a positive trend in the LOVIT group.

Birds in the LOVIT group had a higher final BW as well as ADWG at the end of the fattening period in comparison with their counterparts in the control group ($p \leq 0.001$). The FCR of the LOVIT group remained to be lower than the one of the control, although the difference did not reach the significant level.

Mortality was recorded during the whole trial for both groups. While the control developed a mortality rate of 11 %, the LOVIT group showed a reduced mortality of only 2 % (table 1). In addition, the birds in the LOVIT group showed a better condition regarding lameness. In the control group, 4.2 % of the birds developed leg weakness during the heat stress; however, in the LOVIT group merely 0.4 % showed the same condition (table 1).

Table 1: Impact of drinking water supplementation of the star anise essential oil based mixture (LOVIT) during heat stress on body weight, average daily weight gain (ADWG), average daily feed intake (ADFI), and FCR of broilers as well as mortality and leg weakness. Means within a row with different letters indicate significant difference ($p<0.05$)

	CON	**LOVIT**
Body weight – day 1, g	49.86 ± 0.82	49.88 ± 0.82
Body weight – day 18, g	752.63[a] ± 54.77	809.87[b] ± 73.91
Body weight – final, g	2,694.75[a] ± 135.72	2,892.54[b] ± 182.34
ADWG – day 18, g	41.34[a] ± 3.22	44.74[b] ± 4.35
ADWG – final, g	71.48[a] ± 3.67	76.83[b] ± 4.93
ADFI – day 18, g	51.01	51.51
ADFI – final, g	109.21	110.24
FCR – day 18, kg/kg	1.249	1.156
FCR – final, kg/kg	1.532	1.510
Mortality, %	11.0	2.0
Leg weakness, %	4.2	0.4

Discussion

A decreased feed intake at high temperatures has implications on the intake of vitamins and electrolytes, which play important roles in the performance, immune function and cardio-vascular activity of poultry[3]. Under normal conditions, the chicken's endogenous vitamin C (ascorbic acid) synthesis is sufficient, but not under stressful conditions. In heat stress situations, the application of vitamin C has shown various positive effects on physiology like promoting the energy generation from storage fats and supporting the immune system[10]. In addition, vitamin C is a powerful natural antioxidant, protecting the cells against oxidative – and heat stress – damage[3,4]. The positive effect of vitamin C under heat stress conditions is enhanced by electrolytes[4,10]. Sodium, potassium and chloride play a crucial role in maintaining body acid/base balance as well as osmotic pressure in body fluids[11]. During heat stress, hyperventilation disturbs the blood acid/base balance and results in respiratory alkalosis, leading to growth depression. This suppression can be counteracted by the supplementation of electrolytes[11], which are part of LOVIT Granule Anilyte+C.

Diverse studies report about the appetite increasing effect of star anise essential oil[7], which was confirmed in this trial. In contrast to the results of previous works[12,13], the current trial was not able to show a significant improvement of FCR, although BW and ADWG were significantly higher in the LOVIT group, which received the star anise based supplement. Moreover, the star anise mixture was able to shield the broilers against performance depression and higher mortality, which is most probably due to the improved antioxidative status star anise oil had induced in the broilers as reported previously[7,9].

During the trial, the control group developed leg weakness. Due to their rapid growth and lower activity rate during heat periods, leg problems often occur in broilers. Long-time sitting on the floor may cause leg skin lesions and deformities, further reducing the activity and to some degree vitality[15]. The supplementation of the star anise based mixture was able to vitalize the birds during heat stress and to reduce the number of leg problems to a minimum.

It is known that heat stress significantly increases mortality in poultry flocks[16]. Increased mortality does not only reduce production, it also is an animal welfare issue. In this trial, the control group showed a mortality five times higher than the LOVIT group, which demonstrates the importance of cardio-vascular and antioxidative support during heat stress to reduce losses in the flock.

Conclusion

The star anise essential oil based mixture (LOVIT Granule Anilyte+C) was shown to successfully reduce mortality and leg weakness during heat stress and thereby enhanced the vitality of the birds. The improved animal welfare was reflected by an increased final body weight and ADWG under heat stress

conditions. A drinking water supplementation with the star anise mixture seems to enable the birds to adjust appropriately under the periods of heat stress.

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Investigation of the active metabolites of *Bacillus subtilis* DSM 29784 and their effects in broiler chickens

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Abstract

The probiotic microorganism of the genus Bacillus appeared to be an attractive candidate to replace antibiotics. The Bacillus subtilis DSM 29784 (Bs29784) is one of these strains. To date, the probiotic effect has not been completely understood, but it is supposed that the effect depends on metabolites of the microorganism. Although different modes of action have been proposed, studies showing effects on what metabolites the bacteria produce in a test tube, and whether these can also be found in the intestine of animals that were given these strains as feed additives, are lacking. In the current study, we showed that Bs29784 changes the microbial composition in the gut of chickens by reducing opportunistic pathogenic bacterial families and promoting beneficial bacterial families. We show that two active molecules, hypoxanthine and nicotinic acid, are produced by the Bs29784 and are elevated in the intestinal tract of these animals. We hypothesize that nicotinic acid can be used by beneficial microbes and is essential for their intestinal colonization, and that both molecules can have a positive effect on the intestinal wall. Imaging high-performance thin-layer chromatography (HPTLC) was also use to visualize differences in the metabolite profile of bacteria with high genetic similarity to allow a better understanding of the probiotic effect. In comparison to other bacteria, Bs29784 produced a higher level of antioxidants or bioactive substances such as surfactin. Different in vitro approaches enabled to better explain the probiotic effect of Bs29784.

Introduction

Probiotics are used in both human and animal nutrition for their health benefits. In animal diets, probiotics are included as feed additives to create a healthy and resilient intestinal microbial environment. Maintaining a beneficial intestinal microbial composition helps in improving the overall health of the animal and thereby positively affects body weight gain (BWG) and feed conversion ratio (FCR). Many different microorganisms are used as probiotics in poultry production. Bacillus spp. are the most commonly used probiotic microorganisms because of their ability to form endospores. This enables them to survive the feed manufacturing process and the passage through the stomach. Moreover, spores allow easy administration, storage and prolonged shelf-life. One frequently used species, Bacillus subtilis, is considered to be safe for consumption. A variety of B. subtilis strains are available as feed additives for animals with each having their own strain specificity. One example is B. subtilis strain 29784 (Bs29784), for which beneficial effects on growth performance are consistently reported in broilers, turkeys and layer pullets. In addition, the strain reduces IL-8 expression and improves intestinal barrier integrity by upregulating tight junction protein expression, as was shown in a cell culture model. Although effects of the administration of Bacillus strains on intestinal health parameters have been observed, insights in the exact modes of action of these probiotic strains are often limited. Different modes of action have been suggested in literature, including vitamin and nutrient production, enzyme production, antagonistic effects on pathogens, pH reduction due to short-chain fatty acids (SCFA) and lactate production,

amongst others, but causal relationships between the produced metabolites and the observed effects are generally not proven. Studies investigating the metabolites produced by probiotic strains have focused mainly on fermentation products such as lactic acid and SCFA, while, to the best of our knowledge, none have carried out a metabolome analysis and verified whether the metabolites produced in vitro could also be detected in the intestinal tract. Therefore, the aim of the current study was to identify metabolites that are produced by the probiotic B. subtilis strain Bs29784 in vitro, elucidate whether these metabolites are also produced in the chicken intestinal tract after in-feed supplementation of Bs29784, and how Bs29784 affects the intestinal microbiome.

Material and methods

The study was undertaken following the guidelines of the ethics committee of the Faculty of Veterinary Medicine, Ghent University, in accordance with the EU Directive 2010/63/EU. One-day-old Ross 308 broiler chicks were obtained from a local hatchery and divided into 2 groups of 5 birds consisting of (1) a control group that received a standard commercial diet and (2) a group that received a standard commercial diet supplemented with the commercial Bs29784 probiotic at a dose of 10^{10} CFU/kg feed (FARM 1&2 mash, Versele-laga, Deinze, Belgium). Animals were housed on a solid floor covered with wood shavings at a density of 5 birds/m^2. Animals were subjected to a light schedule of 12 h light and 12 h dark. All broilers were given water and feed ad libitum. At 13 days of age, all birds were weighed, the birds were euthanized, and digestive content from the jejunum, ileum and cecum was collected. These samples were frozen in liquid nitrogen directly after sampling and stored at −20 ◦C until further processing. The material from the 3 sections was used for metabolomic analysis and Bacillus quantification, while the ileal and cecal content was used for 16S sequencing. At 13 days of age, no differences in bodyweight could be observed, with an average bodyweight of 273.4 g ± 19.54 g (mean ± SD) for the control group and 254.3 g ± 38.37 g for the Bs29784-supplemented group ($p = 0.358$). The detection of metabolites via derivatization was done using the HPTLC. The corresponding chromatogram was dipped (immersion time 2 s, immersion speed 3.5 cm/s, Chromatogram Immersion Device 3) in the primuline reagent (250 mg primuline in 50 mL water and 200 mL acetone) and dried in a cold stream of air (hairdryer) for 1 min; or in the diphenylamine aniline o-phosphoric acid reagent (2 g diphenylamine in 100 mL i-propanol and 2 mL aniline in 100 mL i-propanol, mixed 1:1, V/V, and slowly added 20 mL o-phosphoric acid, 85%); or in the ninhydrin reagent (500 mg ninhydrin in 230 mL ethanol with 20 mL acetic acid); or in the anisaldehyde sulfuric acid reagent (1.5 mL 4-anisaldehyde in a mixture of 210 mL methanol, 25 mL acetic acid, and 13 mL sulfuric acid). The latter three plates were heated at 110 °C (Plate Heater 3) for approximately 10 min.

Results

The total number of bacteria, as well as the number of Bacillus spp. in the jejunum, ileum and cecum were determined using qPCR. Supplementation of the diet with the probiotic B. subtilis strain Bs29784 did not introduce alterations in the total bacterial load, but significantly increased the number of Bacillus spp. in the ileum ($p = 0.005$), jejunum ($p = 0.008$), and cecum ($p = 0.014$). To further assess whether this increase in Bacillus spp. was reflected in an increase in Bs29784 metabolites, the levels of hypoxanthine and nicotinic acid were determined. Overall, broilers fed a Bs29784-containing diet showed higher levels of hypoxanthine and nicotinic acid in the intestinal content. The increase in hypoxanthine was most pronounced in the ileum ($p = 0.0003$) but did not reach significance in the jejunum ($p = 0.095$) or cecum ($p = 0.171$). In-feed supplementation of Bs29784 tended to increase the level of nicotinic acid in the ileum ($p = 0.051$), as compared to birds fed the control diet, but had no effect on nicotinic acid levels in the jejunum ($p = 0.223$) or cecum ($p = 0.306$).

Discussion

Hypoxanthine is a breakdown product of nucleic acids and can be taken up and incorporated by intestinal bacteria or the host via the nucleotide salvage pathway. Additionally, hypoxanthine from the microbiota is salvaged for energy and nucleotide. biosynthesis in intestinal epithelial cells, thereby supporting wound healing, mucus generation and intestinal barrier function. Notably, hypoxanthine has also been shown to act as a substrate for the antimicrobial function of the enzyme xanthine oxidoreductase (XOR) which is located on the outer surface of epithelial cells. XOR is responsible for the conversion of hypoxanthine to xanthine and from xanthine to uric acid. During both reactions, oxygen is reduced, generating hydrogen peroxide (H2O2) and reactive oxygen species (ROS). XOR-generated H2O2 has been shown to act as an effective antimicrobial agent against commensal microorganisms and anaerobes, although pathogenic bacteria could be more resistant. Moreover, XOR-generated ROS have been hypothesized to initiate neutrophil infiltration in response to pro-inflammatory mediators. These neutrophils can then help to combat infections. In chickens, XOR is mainly expressed in the intestine, liver and pancreas. It is thus possible that hypoxanthine produced by Bs29784 contributes to intestinal health through enhancing epithelial barrier function and mucus production, while protecting the intestinal epithelial cells against microorganisms through H2O2 production. This could be one of the reasons a reduction in several genera of the Enterobacteriaceae, such as Enterobacter and Escherichia-Shigella, is seen in the ileum of broilers fed Bs29784-supplemented feed. Nicotinic acid, or niacin (pyridine-3-carboxylic acid), is a form of vitamin B3, an essential nutrient for animals, including broilers. In humans and rodents, nicotinic acid is known to bind on the GPR109A receptor (aka HCA2 or HM74a in humans and NIACR1 in rodents), which is also one of the receptors for butyrate. GPR109A has been shown to act as an anti-inflammatory mediator via the β-arrestin signaling pathway, protecting epithelial cells against inflammation and oxidative stress. It is unclear whether nicotinic acid induces similar effects in birds, since an equivalent homologous receptor has not yet been identified. Nevertheless, nicotinic acid shows comparable effects on the regulation of the lipid transport apolipoproteins apoA and apoB in broilers as in humans which is mediated by GPR109A in the latter. Furthermore, nicotinic acid is an important precursor for the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) that play an essential role in, among others, antioxidant protection. This suggests that nicotinic acid, produced among others by Bs29784, may be taken up by the epithelial cells, protecting the cells from oxidative stress, while at the same time H2O2 is generated outside the cell by the action of the cell-surface xanthine oxidoreductase on hypoxanthine, also produced among others by Bs29784.

Conclusion

This study identified mainly hypoxanthine and nicotinic acid as two important metabolites produced by B. subtilis strain 29784. The probiotic was shown to be metabolically active, producing these two metabolites in the intestine of broilers. These metabolites contribute, at least in part, to the interaction of Bs29784 with both the host and the microbiome, either through direct anti-inflammatory or anti-bacterial properties or by increasing the abundance of beneficial butyrate-producing bacteria in the cecum, potentially through cross-feeding.

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Investigation and localization of individual tight junction proteins in the context of intestinal permeability in broiler chickens

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Abstract

Connections between epithelial cells lining the gut intestine are essential to maintaining a protective intestinal mucosal barrier to the gut lumen. This barrier function restricts access of toxins, antigens and pathogens, while preserving ability to absorb nutrients via the paracellular pathway. Tight junctions (TJs) are intercellular complexes that seal the space between individual cells and regulate paracellular permeability. The main components of TJs are claudins (CLDN), occludin (OCLN), tight junction associated MARVEL-domain proteins (TAMPS), the scaffolding zonula occludens (ZO) proteins and junction-adhesion molecules (JAMs). Disruptions to paracellular permeability, known as "leaky-gut" phenomenon, caused by either pathogens (e.g. *Campylobacter*) or their toxins can promote the translocation of luminal bacteria to the underlining tissue and internal organs. We hypothesise that changes to TJ could have a major impact on the destructive effects of pathogens on the intestinal barrier in chickens.
Unfortunately, little is known about structure, function and dynamics of TJs in the chicken gut. Optimising Freeze-Fracture-Immunogold labelling as well as Immunofluorescence for selected TJs in the chicken gut offers a new possibility to investigate their dynamics and morphological constitution during (patho-) physiological conditions.

Introduction

Tight junctions (TJ) are small dynamic protein complexes localised in the apical area between epithelial cells. Tight junctions are typically composed of several components such as claudins (CLDN), tight junction associated MARVEL-domain proteins (TAMPS), the scaffolding proteins zonula occludens (ZO) and the junction adhesion molecules (JAMs). In the intestine these intercellular complexes play a major role in controlling paracellular permeability and are crucial in order to maintain homeostasis (Umeda et al. 2006; Krause et al. 2008; Van Itallie and Anderson 2014).
Several studies confirmed that the regulation of the intestinal barrier can be disturbed by toxins, antigens and pathogens such as *Campylobacter* (*C.*) *jejuni.* The consequence of such disturbances is a so-called "leaky gut" leading to the translocation of luminal bacteria to internal organs (Molnár et al. 2015; Awad et al. 2017, 2018; Awad et al. 2020).
To analyse mRNA expression of TJ, nine different TJ proteins probe based RT-qPCRs were established. Their analysis revealed changes in expression during age development as well as during *C. jejuni* infection (von Buchholz et al. 2021). However, studies on the composition of TJ-proteins in chickens are limited. This study aimed to establish new methods to benchmark the composition and distribution of TJ proteins in chicken gut.

Material and methods

Samples from jejunum and caecum were collected from euthanized broiler chicken and immersion fixed in 2% PFA and 15% picric acid in 0.1 M PB for SDS-digested Freeze-Fracture-Immunogold-labelling (FRIL). The next day intestines were cut in 120µm thick slices with a vibratome (Linear-Pro7, Dosaka, Japan) in ice-cold 0.1M PB. After incubation in 30% glycerol in 0.1 M PB O/N for cryo-protection, samples were placed into gold or copper carriers and frozen under high pressure (>300 bar) using an HPM010 (Leica, Wetzlar, Germany). Fracturing was performed at -120 °C und high vacuum (approx. 2.0 x 10^{-7} mbar) using Leica ACE 900. To digest the tissue, replica were placed in 2.5% SDS, 20% sucrose in 15 mM Tris buffer (pH 8.3) and incubated for 20hrs at 80°C under gentle agitation (40rpm). On digested replica immunolabelling with commercial antibodies against OCLN (Invitrogen), CLDN3 and CLDN10 (Sigma-Aldrich) was applied. These antibodies were then localised with 10 nm colloidal gold secondary antibodies. The structure and distribution of TJ immunolabelling was visualized by transmission electron microscopy.

Results and discussion

Little is still known about the exact structure, quantity and dynamics of TJs in the chicken gut. It was possible to apply and optimise FRIL method on chickens' jejunum and caecum. All tested antibodies (OCLN, CLDN3, CLDN10) gave strong signal. In addition, these same antibodies were used for immunofluorescence on cryosections of intestinal samples of the same animals to visualise the TJ-network of singular TJs through the epithelial cell lining. Overall, we succeeded to establish different real time PCRs together with immunofluorescence and the FRIL method on chicken gut samples, offering new options to investigate the morphological constitution during (patho-) physiological conditions.
In an ongoing experiment the visualising methods described above are used to display the dynamics and precise localization and density of component TJ proteins during the developmental growth of broiler chickens. Establishing these TJ benchmarks will enable subsequent challenge infections to understand pathophysiological changes and the consequences on intestinal permeability in chickens.

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Effects of dietary sodium diformate in turkey – a weight gain performance analysis

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Abstract

Dietary sodium diformate (Formi NDF, ADDCON) has been tested in poultry production since 2006 and numerous publications and conference contributions on its use have been published. A word-wide data analysis on its impact on poultry performance is available, however those data cover only the performance enhancement in broiler production. Its use in turkey production has not yet been well documented. This study analyzed the average impact from all studies carried out in Europe on the effect of the additive on the weight gain till slaughter in turkey toms and hens. The final dataset contained the results of 7 trials with NDF-inclusion, which ranged from 0.3% to 0.4%. Results are expressed as percentage difference from the negative control. The average level of dietary NDF from the dataset in all treated turkeys was 0.34%. The performance of turkey based on final weight was highly significantly increased by 3.1% (P=0.0005). If results are separated between toms and hens, data show a similar picture. NDF-fed toms are by 3.0% heavier than their negative controlled counterparts (21.65 kg vs. 21.01 kg; P=0.001), while hen weight differed also significantly (P=0.035) between the groups (10.79 kg and 10.48 kg for NDF- and Control-hens, respectively). It is therefore concluded that dietary sodium diformate can improve turkey production under European conditions.

Introduction

Gastrointestinal diseases pose a serious threat to commercial poultry production. Infections with pathogenic bacteria and their subsequent translocation to other organs and tissues, cause deterioration of feed conversion, increase mortality and reduce productivity. On the other hand, it is generally agreed that good gut health is effective against intestinal pathogens and is the cornerstone to competitive poultry production, especially in broilers, where treating intestinal borne pathogens is too time consuming to be considered cost-effective. In the past, this strategy was only made possible through the routine use of antibiotic growth promoters (AGPs) in the feed. However, the realisation that creating and maintaining a healthy intestinal environment can produce comparable, if not better performance without using AGPs means that understanding and achieving gut health is essential to poultry productivity. Gut health requires not only the absence of intestinal pathogens, as can be achieved using AGPs, but encompasses questions of effective digestibility and absorption of nutrients, a normal, stable microbiota and a healthy, functional gut mucosa, without inflammation. To this end, it is important to see the gut environment as a living system, influenced by environmental factors, especially those delivered via the feed. The judicious use of feed additives can also support gut health through dietary means.

Improving broiler performance or hygienic conditions with the aid of organic acids has been reported by many sources, as reviewed by Desai et al. (2007). An important limitation, however, is that organic acids are rapidly metabolised in the fore-gut (crop to gizzard) of birds, which will reduce their potential impact on growth performance. Dietary sodium diformate, traded as Formi NDF, ADDCON, Germany – hereafter abbreviated as NDF) has been proven to be effective against pathogenic bacteria along the whole gastro-intestinal tract (Lückstädt and Theobald, 2009). The reduced impact of pathogenic bacteria on the broiler, as well as the improved gut microflora, leading to a state of eubiosis in treated chickens, due to the inclusion of sodium diformate (NDF) in broiler diets resulted in improved bird performance. Several trials have been carried out over the last decade world-wide which have documented the positive effects on broiler performance – and were additionally confirmed using a meta-

analysis (Lückstädt, 2013). A performance analysis of 17 trials with broilers, fed diets with NDF, resulted in a highly significant (P=0.0001) average increase of weight gain by 5.2%. The overall productivity – calculated as a combination of weight gain, survival rate and feed efficiency (European Broiler Index), was even improved by more than 12% (P=0.0005).
On the other hand, only limited published information is available on the usage of acidifier in turkey diets. Mikulski et al. (2008) reported on increased body weights of male turkey until slaughter at day 140, while also numerical improvements on feed efficiency were noticed (Table 1 and 2). In that study the acidifier was used throughout the whole growth period at a dosage of 0.5%.
Furthermore, Glawatz et al. (2011) mentioned improved performance parameters, as well as gut and feet health in male turkey fed with 0.4% NDF (Table 3).
However, more data on the use of dietary acidifier in turkey are not available. Thus, due to the scarcity of published data on the use of dietary organic acids in turkey – and especially of sodium diformate, this study was carried out.

Table 1: Effect of diets containing an organic acid blend on performance parameter body weight (BW), feed conversion ratio (FCR), mortality (%) and productivity index of male turkey (modified after Mikulski et al., 2008)

	Control	Acidifier
BW at d 84 [kg]	9.07[a]	9.67[b]
BW at d 140 [kg]	18.51	19.23
FCR at d 84	2.34	2.21
FCR at d 140	2.91	2.76
Mortality at d 56 [%]	3.0	3.0
Mortality at d 140 [%]	5.0	6.0
Productivity index PI	60.2	65.3

*PI = weight gain [kg] × survival rate [%] / (10 × FCR)
Means in a row not sharing the same superscript are significantly different (P<0.05)

Table 2: Effect of diets containing an organic acid blend on carcass characteristics and gastro-intestinal parameters of male turkey (modified after Mikulski et al., 2008)

	Control	Acidifier
Carcass dressing [%]	82.0	82.8
Breast muscles [%]	25.0	25.2
Thigh muscles [%]	10.4[a]	11.2[b]
pH of the crop digesta	5.0[a]	4.6[b]
pH of the ileal digesta	6.3[a]	6.1[b]
SCFA pool concentration of caeca digesta [µmol / kg body weight]	221	273

Means in a row not sharing the same superscript are significantly different (P<0.05)

Table 3: Performance parameters of male turkeys fed with or without the dietary acidifier Formi NDF (modified after Glawatz et al., 2011)

	Control	0.4% FORMI NDF	Difference [%]
Initial weight [kg]	5.78	5.63	-2.6
Weight after P4 [kg]	10.70	10.99	+2.7
Final weight [kg]	19.90	20.43	+2.7
ADG [g]	137	141	+2.9
Mortality [%]	12.46	12.09	-3.0

Material and methods

This study analyzed the average impact from all studies and trials available on the effect of the additive on the performance parameter slaughter weight. The final dataset contained the results of 7 negatively controlled studies which tested NDF-inclusion, whose dosages ranged from 0.3% to 0.4%. Those studies were carried out between 2010 and 2016 under commercial and semi-commercial conditions in Germany and included more than 72,000 turkey hens and toms.
The above mentioned performance parameter is expressed as percentage difference from the negative

control. The results are given as means and were statistically analysed using the t-test. A confidence level of 95% was defined for these analyses.

Results and conclusions

The average level of dietary NDF from the dataset in all treated turkey (toms and hens) was 0.34% (Table 4). The typical dosage for NDF in turkey ranges from 3-4 kg/tonne feed, depending on age (dietary protein level) and hygienic status of the farm.

As seen in Table 4, the usage of dietary sodium diformate led in both sexes to a significant increase of the final weight at slaughter. Neither toms nor hens showed a difference in the impact of the acidifier, which gave in both cases an average significant increase of 3% of the final weight. This is in full agreement with the other available published studies on this topic (Mikulski et al., 2008 and Glawatz et al., 2011). In poultry, improved zootechnical performance is thought to stem from both improvements of the intestinal microflora, because of suppressing pathogenic bacterial species, and improved protein digestion. This seems to be also the case for turkey production – since Glawatz et al. (2011) speculated on the improved gut health in diformate fed male turkey.
It can therefore be concluded that dietary sodium diformate (Formi NDF) can play an important role in improving turkey nutrition under European conditions, leading to a sustainable turkey production while supporting further the EU-wide antibiotic reduction initiatives.

Table 4: Meta-analysis on the performance parameter "slaughter weight in kg" of 7 European trials with male and female turkeys, fed diets with or without sodium diformate (Formi NDF)

	Toms	Hens	All turkey
N	35,528	36,480	72,008
Control [kg]	21.01±0.91[a]	10.48±0.32[a]	16.49[a]
Formi NDF [kg]	21.65±0.92[a]	10.79±0.20[b]	17.00[b]
Difference [%]	3.0	3.0	3.1
P-level	0.0011	0.035	0.0005

Means in a column not sharing the same superscript are significantly different (P<0.05)

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Application of beneficial strain *Enterococcus faecium* EF 412 in horses of Slovak warm-blood breed

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Abstract

Fecal horses strain *E. faecium* EF 412 possessing probiotic and bacteriocin potential was applied in horses of Slovak warm-blood breed. One g of freeze-dried EF 412 strain, 10^9 CFU/ml was applied to horses (for 21 days) in a small feed ball. Twelve clinically-healthy horses of various ages (5-13 years) were involved in a 35- days long experiment, also functioning as control for themselves. They were stabled in separate boxes, fed twice a day (hay, whole oats or grazed) with water access *ad libitum.* Sampling was performed at days 0/1, 21 (3 weeks of EF 412 application) and at day 35, (2 weeks of EF 412 cessation). EF 412 colonized GIT of horses with 3.54 ± 0.75 CFU/g (log 10) at day 21. Total enterococci and lactic acid bacteria significantly increased ($P < 0.001$) checking by the use of standard microbiological method. Using next-generation sequencing, the phyla Bacteroidetes and Firmicutes dominated; the families detected were Bacteroidales BS11, S24-7 gut goups, and Lentisphaere. Increasing tendency in phagocytic activity (PA) was noted after EF 412 application. Biochemical parameters were in the physiological range. Total protein value was significantly decreased at day 21 compared with day 0/1 and day 35 ($P < 0.05$). Cholesterol, triglycerides were decreased at day 21 compared with day 0/1 and day 35. Fecal strain *E. faecium* EF 412 showed a promising application potential.

Introduction

Optimizing the host microbiota in host organism is important regarding the maintenance of its health status. Beneficial (probiotic) bacteria are used widely as nutritional supplements for livestock or other animals (Cooke et al. 2021). The use of beneficial enterococci has been already reported in various animals (Pogány Simonová et al. 2020a; Vargová et al., 2020); showing their impact on stimulation of phagocytic activity (PA, unspecific immunity parameter) or influencing tissue repair ability of enterocytes in broiler rabbits (Pogány Simonová et al. 2020b.) Many enterococcal species strains have been also found to produce antimicrobial substances of proteinaceous character, enterocins (Nes et al. 2014). Also enterocins can act beneficially in animals (Lauková et al. 2012, 2018, Revajová et al, 2020). E. g. administration of Ent M in horses led to reduction of coliforms, campylobacters ($P < 0.05$) and *Clostridium* spp. ($P < 0.001$); also significant increase in phagocytic activity (PA) was noted ($P < 0.0001$). Biochemical blood parameters were not negatively influenced (Lauková et al. 2018). Antimicrobial activity of *E. faecium* AL41 and Ent M was noticed in feces and caecum of broiler rabbits against coliforms ($P < 0.05$), pseudomonads and staphylococci. In experimentally infected hens, Revajová et al. (2020) reported the immune response prompted by *E. faecium* AL41 against *Campylobacter jejuni* CCM 6191. EF AL41 acted predominantly 24 h *post*-infection in the case of *C. jejuni.* It displayed a distinct manner of T and B cell activation. However, there is still limited information regarding the efficacy of probiotics in horses. Mostly non-autochthonous bacterial species were used for this purpose. Administration of multistrain bacterial formulations to increase stamina in exercising horses was promising (Cooke et al. 2021). However, new beneficial strains are always being studied for their use in horse breeding. Therefore, in this study autochthonous strain *E. faecium* EF 412 was applied in horses analyzing the following parameters:

microbiota/microbiome, phagocytic activity, and biochemical parameters. *E. faecium* EF 412 belongs in the genus *Enterococcus*, in the family Enterococcacae. It contains *Enterocin* genes for Enterocin A and B production. This strain with probiotic potential shows susceptibility to antibiotics, adhesion ability to human and canine mucus, and it produces a thermo-stable bacteriocin (Lauková et al. 2008, 2020).

Material and methods

Six mares and six stallions, Slovak warm-blood breed owned by private clients (who agreed with application) were experimented. The animals were placed in the stables (CE SK 339004) of the Slovak Agricultural University (Nitra, Slovakia) respecting the Guide for Animal Practice approved by the Ethics Commissions of both institutions participating in the experiment (Apr.no. 339004). The animals of various ages (5-13 years) were stabled in separate boxes. They were fed twice a day with hay and whole oats or grazed and had access to water *ad libitum*. The experiment lasted for 35 days. Sampling was performed at day 0/1, at day 21 (3 weeks of EF 412 application) and at day 35 (2 weeks of EF 412 cessation, end of experiment). Feces were sampled immediately after each horse`s defecation. Blood was sampled from the *vena jugularis.* Fecal samples were stored and transported for analyses. Each horse itself served as control animal for each separate sampling; their status at the day 0/1 was compared with their status after application of EF 412 strain and after two weeks of its cessation. After initial sampling, the animals were administered EF 412 at one g per animal per day (10^9 CFU/ml) in a small feed ball to ensure that it was eaten by the specified horse. EF 412 strain was applied for three weeks (21 days). The animals had a standard drinking regimen. Microbiome analyses using next-generation sequencing (ngs) method was performed as previously described by Lauková et al. (2022). But to enumerate EF 412 strain, enterococci and lactic acid bacteria (LAB), feces from horses were also treated using the standard microbiological dilution method (in Ringer solution, ratio 1:9, Merck, pH 7. 0). After homogenization (Stomacher Masticator IUL, Instruments, Spain) according to the International Organization for Standardization (ISO) and dilution, 100 µl of appropriate dilution was plated on M-*Enterococcus* agar (ISO 7889, Difco, Detroit, USA) to enumerate the enterococci. M-*Enterococcus* agar enriched with rifampicin (100 µl) was used for *E. faecium* EF 412 (marked with rifampicin to differentiate it from the other enterococci). De Man-Rogosa-Sharpe broth enriched with agar (1.5%, MRS, Merck, Germany) was used to enumerate lactic acid bacteria (LAB) after incubation at 37 ºC for 24-48 h. Bacterial counts were expressed in (log 10) colony-forming unit per gram (CFU/g) ± SD. Blood was sampled into Eppendorf tubes containing microspheric hydrophilic particles (MSHP) and heparin. Ingestion of MSH particles by polymorphonuclear cells (PMN) was determined using a modified test according to Větvička et al. (1982). The percentage of phagocytic cells was evaluated using an optical microscope, by counting PMN up to 100. Subsequently the index of PA was calculated. In blood samples were analyzed a total protein, albumin, cholesterol, triglycerides, alanine aminotransferase (ALT), bilirubin, calcium (Ca in mmol/l), and magnesium (Mg in mmol/l). For analysis a DiaSys kit (Diagnostic Systems GmBH, Holzheim, Germany) was used and Randox RX Monza semi-automatic analyzer according to Kováčik et al. (2017) as well. Minerals were analyzed using an EasyLite analyzer *via* ion-selective electrode (Kolesárová et al. 2008). Statistical evaluation was performed using one-way analysis of variance (ANOVA), followed by Tukey post test. The results are quoted as means ± SD and were compared among groups within the same days of samples collection to check the changes during the experiment. Differences between mean values were considered statistically significant at $P < 0.05$. All statistical analyses were performed using GraphPad Prism statistical software (GraphPad Prism version 6.0, GraphPad Software, San Diego, California, USA).

Results

In GIT of horses, *E. faecium* strain EF 412 reached 3.54 ± 0.75 CFU/g (log 10) at day 21; two weeks after cessation (at day 35) its count decreased to 1.72 ± 0.43 CFU/g ($P < 0.001$). Application of EF 412 contributed to the total enterococci increase; at day 21 enterococci were in significantly higher amount

compared with day 0/1 ($P < 0.001$), and compared with the total enterococci at day 35 (5.57 ± 0.36 and 4.40 ± 1.11 CU/g log 10, $P < 0.001$). At day 35 a lower count of total enterococci was determined, but still significantly higher compared with the EF 412 count at day 35 ($P < 0.001$). The LAB were also increased at days 21 and 35 comparing with day 0/1, ($P < 0.001$, $P < 0.001$). Significant increase in LAB was also at day 21 compared with the total enterococci at day 21 ($P < 0.001$). LAB were found to be almost the same at day 35 and at day 21 (7.79 ± 0.18 and 7. 63 ± 0.62 CFU/g with day 35 ($P < 0.001$). Using ngs analysis, the phylum Bacteroidetes dominated in the feces of horses at day 0/1 with abundance 40.2 ± 6.3%, followed by the phylum Firmicutes (27.0 ± 5.2%), Lentisphaerae (8.3 ± 2.9%), Spirochaetae (6.06 ± 2.5 %), Fibrobacteres (6.3 ± 2.5 %), Proteobacteria (3.7 ± 1.9 %) and Euryarchaeta (1.3 ± 0.2%). At day 21, the phylum Firmicutes was significantly increased compared with day 0/1 ($P < 0.05$) and compared with day 35 ($P < 0.05$, abundance 34.5 ± 5.9%). Increase was also noted at day 35 compared with day 0/1 ($P < 0.05$) and abundance 35.4 ± 6.0%; however, the phylum Bacteroidetes still dominated (36.0 ± 6.3%). It looks, that EF 412 application led to an increase in the phylum Firmicutes, and both phyla mentioned had almost the same count at days 21 and 35. Lower abundance of the phylum Bacteroidetes at day 21 can indicate that EF 412 probably contributed in competitive interaction between the phyla Firmicutes and Bacteroidetes, and the consequence was Bacteroidetes decrease at day 21 compared with day 0/1 ($P < 0.05$). At day 35, two weeks after EF 412 cessation, higher abundance of Firmicutes was noted (35.4 ± 6.0%), and Bacteroidetes were almost unchanged. It indicated still competitive effect of EF 412. The phyla Lentisphaerae, Spirochaetae, Fibrobacteres, Proteobacteria, Euryarchaeta were detected with low abundance at day 21 and day 35 (abundance 1.1 - 11.7), and their counts were not reduced. Regarding the family level, Bacteroidetes BS11 gut group was detected with the highest abundance, followed with Lentisphaere gut group, and Bacteroidales S24-7 group. The other families were detected in very low %, involving also Enterococcacae. Although PA in horses was not significantly influenced after EF 412 application, and after its cessation (at day 35), increasing tendency regarding PA was noted (at day 21 it was 58.3 ± 7.63%, and the highest value of PA was measured at day 35 (59.2 ± 7.69 %). Regarding biochemical parameters and minerals, values of albumin, cholesterol, triglycerides and Mg were not significantly influenced. However, a total protein value was significantly decreased at day 21 compared with day 0/1 ($P < 0.05$) and also at day 21 compared with day 35 ($P < 0.05$). Cholesterol level and triglycerides were not significantly influenced at day 21 compared with day 0/1 and day 35, although they decreased. Alanine-aminotransferase (ALT) was decreased at day 21 compared with day 35 ($P < 0.01$) and day 0/1. Higher count of ALT was noted at day 35 compared with day 0/1 ($P < 0.01$). Bilirubin was significantly decreased at day 21 compared with day 35 ($P < 0.001$). Calcium (Ca) was significantly increased at day 21 compared with day 0/1 ($P < 0.05$) and also compared with day 35 ($P < 0.05$). All biochemical parameters were not negatively influenced.

Discussion

There is no information about the use of autochthonous enterococci in horses. Some commercial probiotic strains were applied, but their low colonization was noted, no alteration of intestinal microbiome, no effect on health status, immune parameters, and/or enzymatic activity. However, horse farmers and owners have still been looking for supporting health condition in horses. In our previous study, non-autochthonous, bacteriocin-producing, and probiotic strain *E. faecium* AL41=CCM 8558 and its Ent M were applied in horses with benefits (Lauková et al. 2018, 2020). Theelen et al. (2021) found similar abundance % of microbiota regarding the phylum level in horses in the Netherlands as we found. Bacteroidetes dominated at the start of the experiment; at day 21 they decreased, but Firmicutes increased. Spirochaetae and Fibrobacteres were also reduced. Families Bacteroidetes BS11 gut group dominated, followed with Lentisphaere gut group, and Bacteroidales S24-7 group and the other families were detected in very low %, involving also Enterococcacae. Administration of EF 412 probably produces rearrangements in the gut microbiota. Many factors can influence the fecal microbiome of horses, e.g. age, gender, diet, horse type, pasture access, even season of sampling (Theelen et al. 2021). *E. faecium* CCM 8558 strain similarly as EF 412 showed a tendency to increase PA, and an even higher value of PA

was reached at day 14 (75.11 ± 8.66%), after 14 days application of CCM 8558 strain. Minerals play a critical role in the health of horses. Mainly Ca resorption through the GIT was influenced in our study. Utilization of Mg decreases with age, so the age of the horse is also important for this parameter (Gálik et al. 2012). Total protein decrease indicated a decline in protein synthesis. Enzymes values were not changed; decrease in cholesterol indicated the repair process going on in possibly damaged cells. The hypocholesterolemic effect of probiotic strain EF 2019=CCM 7420 was e.g. noted in broiler rabbits (Pogány Simonová et al. 2020). EF 412 strain produced rearrangements in horses microbiota at the phylum and families levels; PA was influenced showing a tendency to increase, which can support immunity. It appears that in horses the benefit intensity of probiotic strains used does not depend on the autochthonous or non-autochthonous character of the strains (Lauková et al. 2020). However, all the time the safety of the applied strains has to be kept in mind. The European Food Safety Authority (EFSA) recommends assessing enterococci based on individual strain considerations and health-risk exclusions for their potential use as feed additives and supplements (Cooke et al. 2021). The results obtained contribued to the limited research published to date on probiotic bacteria efficacy, safety and tolerability in equids (Cooke et al. 2021). The results obtained here are a new contribution to the limited research published to date on probiotic bacteria efficacy, safety and tolerability in equids with aim of supporting their health (Cooke et al. 2021).

Conclusion

Fecal horses strain *E. faecium* EF 412 colonized increased the total enterococcal and LAB counts in horses GIT. It produced shifts in horses microbiota using the standard microbiological method and next-generation sequencing at the phylum and family levels. A tendency to stimulate an unspecified immunity parameter (PA) was indicated. Biochemical parameters remained in the physiological range. Results indicate that autochthonous, faecal strain *E. faecium* EF 412 showed a promising application potential.

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Dipeptide enterocin A/P, a promising feed additive in rabbit farms

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Abstract

The aim of this study was to examine the effect of dipeptide enterocin (Ent) A/P on growth, phagocytic activity and meat characteristics in rabbits. Tested Ent A/P was applied during 14 days for both, preventive and medicinal purposes. To simulate the spoilage/pathogenic environment, methicillin-resistant *Staphylococcus epidermidis* SE P3/Tr2a strain was applied to rabbits (positive control) for 7 days after resp. before Ent A/P addition; achieved results were compared to control data (without any additives; negative control). The additives were administered in drinking water. Administration of Ent A/P with preventive effect lead in increase of weight gain; the feed conversion ratio was also reduced. During Ent A/P application (both preventive and medicinal), the phagocytic activity was stimulated in rabbits, also after its withdrawal. Ent A/P addition did not influence the tested rabbit meat properties. Based on results, beneficial effect of Ent A/P was achieved mostly on growth and immunity of rabbits and reflect great potential of Ent A/P as feed additive in rabbit nutrition to improve health of animals.

Introduction

The increased use of antibiotics has led to the emergence of antibiotic-resistant bacteria. Current research is focusing on alternative antimicrobial compounds for use in animal production. Bacteriocins are antimicrobial proteins with broad antibacterial spectrum produced by Gram-positive and Gram-negative bacteria, mostly by lactic acid bacteria (LAB), including enterococci (producing bacteriocins named mostly enterocins; Franz et al. 2007). Bacteriocins are used mostly in food industry as bio-preservatives, but they have been applied in several fields: human health and medicine, animal production and veterinary medicine, because of their great antimicrobial potential in prevention and treatment of bacterial infections. They are also safe to consumption, without altering the food/product quality and safety, showing a lower tendency to develop resistance than conventional antibiotics and possess antimicrobial, anti-cancer, antioxidant and immuno-modulatory effect (Bemena et al. 2014; Hernández-González et al. 2021). Rabbits as food animals, provide nutrients essentials for human health; rabbit meat is an excellent source of easily digestible proteins, minerals and trace elements - potassium, calcium, phosphorus, selenium and the highest concentration of iron among all types of meat. It is rich mainly in vitamins B3, B6, B12, E, in omega 3 and 6 fatty acids, and for its low sodium, fat and cholesterol level is advised for children, pregnant women and people with cardiovascular diseases (Dalle Zotte 2002). Therefore, bacteriocins present a promising alternative as feed additives in rabbit farms to improve health of animals and maximized production.

Material and methods

A total of 88 rabbits, (meat lines M91 and P91, weaned at age of 35 days), were divided into 3 experimental groups (E, S, E+S) and one control group (C). The experiment lasted 42 days. Animals fed a

commercial pelleted diet for growing rabbits (KV, Tekro-Nitra, Ltd., Slovakia) during whole experiment with access to water *ad libitum*. The animals in group E were administered enterocin (Ent) A/P (prepared according to Mareková et al. 2007), a dose 50 µl/animal/day, with activity 25,600 AU/mL (tested by the agar spot test according to De Vuyst et al. 1996) during first 14 days of the treatment period (between days 0 and 14), to control the preventive effect of tested Ent. Rabbits in group S received the methicillin-resistant *Staphylococcus epidermidis* SE P3/Tr2a strain (1.0×10^5 CFU/ml) in their drinking water at a dose of 500 µl/animal/day for 7 days, between 14 and 21 days, to simulate a pathological agent resp. attack (positive control). The strain was marked by rifampicin to differ it from the total staphylococci and prepared as described previously by Strompfová et al. (2006). Rabbits in E+S group firstly consumed the Ent A/P for 14 days (between 0-14 days) and after it the SE P3/Tr2a strain was applied to animals for 7 days, starting at day 14 (when the Ent A/P application was cecessed) and finishing at day 21 of the trial. After a one-week break, at day 28, rabbits in E+S group administered Ent A/P for 14 days (between 28 and 42 days) to detect the medicinal effect of tested Ent. The additives were applied in drinking water. Control rabbits (group C) had the same conditions, but without additives being applied to their drinking water, and they were fed a commercial diet (negative control). Drinking water was provided through nipple drinkers. Body weight (BW) and feed consumption (FC) were measured every week during the experiment; average daily weight gain (ADWG) and feed conversion ratio (FCR) were calculated mathematically. Phagocytic activity (PA) was measured by direct microscopic counting procedure, using yeast cell method (Šteruská 1981). Meat samples of *Longissimus thoracis et lumborum* (n=8) were tested for selected parameters. Total water, protein and fat contents were estimated using an INFRATEC 1265 spectroscop and expressed in g/100g; from these values, the energy value was calculated [EC (kJ/100g) = 16.75 x Protein content + 37.68 x Fat content]. The results were quoted as the mean value ± standard deviation (SD), statistical evaluation of the results was performed by the one-way ANOVA and the Tukey test. Significant differences were considered at $p < 0.05$.

Results

The animals were in good health throughout the experiment. Higher BW and ADWG were recorded in all experimental groups during additives application compared to control data between 0-21 days (E: by 20.5 %; S: by 17.1%; E+S: by 25.6 %; Table 1). Between 22-42 days, higher BW was noted only in E group (by 13.0%). The highest body weight and ADWG (E; $p < 0.0001$) and the lowest feed conversion ration (E: by 10.2 %) were noted during Ent A/P preventive application (between 0-21 days). Lower FCR (by 1.5 %) was also noted in E+S group at day 21, compared to C. Ent A/P addition increased the PA values compared to C (day 14; E; E+S vs. C: $p < 0.001$; Table 1), with a tendency to increase to the end of the experiment (days 21 and 42; E vs. C: $p < 0.001$). Reduced PA value in E+S was noted after the SE P3/Tr2a strain application (between 14-21 days), but the repeated application of Ent A/P with therapeutic aim increased PA value again (day 42; E+S vs. S, C: $p < 0.01$). PA value was elevated also in group S, after SE P3/Tr2a application (positive control; S vs. E, E+S, C: $p < 0.001$). Tested meat parameters were not influenced during additives application (Table 2).

Table 1. Effect of dipeptide Ent A/P on growth parameters and phagocytic activity of rabbits

Tested parameters	Day	E	S	E+S	C
BW (g)	0	1153.0 ± 178.2^{a}	1140.2 ± 149.3^{ab}	1060.9 ± 150.8^{ab}	1012.9 ± 118.8^{b}
	14	1735.0 ± 260.0^{a}	1734.5 ± 152.3^{ab}	1691.5 ± 213.1^{ab}	1487.8 ± 203.9^{b}
	21	2052.7 ± 266.1^{a}	2014.8 ± 186.1^{ab}	1999.0 ± 212.2^{ab}	1759.7 ± 218.6^{b}
	42	2854.1 ± 330.7^{a}	2711.8 ± 243.9^{ab}	2696.7 ± 272.3^{ab}	2469.2 ± 285.5^{b}
ADWG (g/day/rabbit)	0 - 14	41.58 ± 5.85^{a}	42.45 ± 8.67^{ab}	45.04 ± 9.54^{ab}	33.92 ± 8.53^{b}
	14 - 21	45.39 ± 8.13^{a}	40.04 ± 8.82^{ab}	43.93 ± 7.80^{ab}	38.85 ± 10.61^{b}
	21 - 42	38.16 ± 3.05^{a}	33.19 ± 4.75^{b}	33.22 ± 4.18^{b}	33.79 ± 3.19^{b}
FCR (g/g)	0 - 21	2.37 ± 0.44	2.78 ± 0.92	2.60 ± 0.67	2.64 ± 0.20
	21 - 42	4.17 ± 0.89	4.26 ± 0.75	4.38 ± 0.54	3.90 ± 0.38
PA (%)	0	63.75 ± 3.33	63.75 ± 3.33	63.75 ± 3.33	63.75 ± 3.33
	14	69.63 ± 2.13^{a}	61.63 ± 2.77^{b}	71.00 ± 2.14^{a}	59.63 ± 3.66^{b}
	21	70.25 ± 1.39^{a}	81.13 ± 1.60^{b}	53.88 ± 3.60^{c}	58.25 ± 2.82^{d}
	42	70.88 ± 2.17^{a}	57.75 ± 4.10^{b}	62.00 ± 2.51^{c}	58.25 ± 3.60^{b}

E – Ent A/P application between 0-14 days; S – SE P3/Tr2a application between 14-21 days; E+S – Ent A/P preventive application for 2 weeks (between 0-14 days) before SE P3Tr2a strain addition for 1 week (between 14-21 days) and therapeutical application for 2 weeks (between 28-42 days); C – control group (without additives); a, b, c, d – mean values marked with different letters differ significantly at $p \leq 0.05$

Table 2. Effect of dipeptide Ent A/P on tested meat parameters in rabbits

Tested parameters	Day	E	S	E+S	C
Total water content (g/100g)	21	74.96 ± 0.37	74.97 ± 0.15	74.40 ± 0.37	74.56 ± 0.22
	42	73.82 ± 0.53	74.19 ± 0.79	73.75 ± 0.32	74.49 ± 0.64
Total protein content (g/100g)	21	23.05 ± 0.19	22.84 ± 0.37	23.07 ± 0.37	23.04 ± 0.40
	42	23.62 ± 0.21	23.70 ± 0.24	23.78 ± 0.46	23.67 ± 0.48
Total fat content (g/100g)	21	1.43 ± 0.20	1.16 ± 0.20	1.38 ± 0.12	1.32 ± 0.12
	42	1.12 ± 0.22	0.84 ± 0.11	1.28 ± 0.13	0.95 ± 0.15
Energy value (kj/100g)	21	439.8 ± 8.8	426.2 ± 11.1	438.4 ± 2.2	434.3 ± 10.7
	42	437.8 ± 10.0	428.7 ± 6.3	446.3 ± 8.1	432.0 ± 13.3

E – Ent A/P application between 0-14 days; S – SE P3/Tr2a application between 14-21 days; E+S – Ent A/P preventive application for 2 weeks (between 0-14 days) before SE P3Tr2a strain addition for 1 week (between 14-21 days) and therapeutical application for 2 weeks (between 28-42 days); C – control group (without additives); a, b – mean values marked with different letters differ significantly at $p \leq 0.05$

Discussion

Good health status, higher BW and ADWG of rabbits recorded in all experimental groups during additives application indicate the beneficial effect of Ent A/P on one hand, and on the other hand no negative impact of SE P3/Tr2a strain on animals' growth was noted. Reduced FCR in E and E+S groups reconfirms the positive influence of Ent A/P on rabbits' growth performance, which can be explained by better feed intake and consumption. Previous studies also confirmed beneficial effect of enterocins on rabbits growth (Lauková et al. 2012; Pogány Simonová et al. 2020). Medicinal effect of Ent A/P on growth parameters was not recorded. In addition to the positive „growth impact" of bacteriocins, their immuno-stimulatory effect is also important; they can stimulate/modulate host innate (increased PA, cytokine production) and specific immune response (elevated immunoglobulins, including secretory IgA; Maldonado et al. 2015). Significant increase of PA after 14 days application of Ent A/P showed the immuno-stimulatory, including both, preventive and therapeutic effect of the tested bioactive compound. Moreover, higher PA values noted one/four weeks after its cessation repeatedly confirmed the prolonged immuno-stimulative effect of Ent A/P, as it was previously described during several enterocins treatment in rabbits (Pogány Simonová et al. 2015, 2020a). Rabbit meat is highly

recommended because of its high nutritional and dietetic properties. Till now, several bioactive compounds, including bacteriocins and herbal extracts have been already tested in rabbits with aim to improve meat quality (Pogány Simonová et al. 2020a, b). During these treatments, mostly improved fatty acid and mineral content of rabbit meat was noted, without any adverse effect on the physico-chemical meat characteristics, similarly to our present results. Nevertheless, further investigations are needed to assess the efficacy of dipeptide Ent A/P in rabbit farms, to expand the knowledge of the other meat components.

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Experimental application of mundticin-like substance EM41/3 in broiler rabbits

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Abstract

In broiler rabbits husbandry is weaning a stressful period accompanying with weakening and easier infection attack of organism. Therefore, farmers and breeders have looked forwards some steps which can prevent and/or reduce a risk of infection. Promising solution is use of beneficial microbiota and/or by them produced antimicrobial substances-bacteriocins. In this study bacteriocin, mundticin-like substance EM41/3 was applied in broiler rabbits. It is thermo-stable peptide originated from non-autochtonous horses fecal strain *Enterococcus mundtii* EM41/3. Mundticin-like substance EM41/3 showed anti-staphyloccoccal effect in broiler rabbits. Rabbits were absent of *Eimeria* spp. oocysts. At the end of the experiment, higher weight gain was noted in rabbits with mundticin-like substance compared to control. Mundticin has never been tested for this effect in broiler rabbits. It is original contribution in its application ability. The evaluation of the other results is processing.

Introduction

Animal farming is mostly addressed for human consumption taking into consideration the health and welfare of animals (Zommiti et al. 2020). In broiler rabbits husbandry is weaning a stressful period accompanying with weakening and easier infection attack of organism. This status can threaten husbandry. Therefore, farmers and breeders have looked forwards some steps which can prevent and/or reduce a risk of infections. Promising way and solution is use of beneficial microbiota and/or by them produced antimicrobial substances-bacteriocins (Franz et al. 2007). Benefits of probiotic enterococci and their enterocins has been already published in our several studies (Lauková et al. 2012, Pogány Simonová et al. 2020, 2021a,b, Szabóová et al. 2021, Chrastinová et al. 2021). When dipeptide Ent A/P was applied in rabbits, the highest daily weight gain was noted and quality of rabbit meat was not negatively influenced (Chrastinová et al. 2021). Antimicrobial activity of Ent M and durancin ED26E/7 against coliforms and pseudomonads in feces of rabbits was reported by Pogány Simonová et al. 2021a, $p<0.x$ et al. (2021) reported that Ent M showed the potent stimulatory effect on mucus production in the rabbit small intestine. Fecal coliforms and staphylococci were reduced ($p<0.05$) in broiler rabbits after Ent M administration and also higher daily weight gain was noted (Lauková et al. 2012). However, never effect of bacteriocin-mundticin was studied in rabbits. Therefore, the aim of this work was to map microbial community, *Eimeria* spp. oocysts control and growth parameters in broiler rabbits after mundticin-like EM41/3 application. It is thermo-stable peptide originated from non-autochtonous horses fecal strain *Enterococcus mundtii* EM41/3 (Focková et al. 2022).

Material and methods

A total 48 *post*-weaning rabbits (meat lines M91 and P91) aged 35 days, both sexes were divided into 2 groups, the control and experimental. The experiment lasted 42 days. Rabbits were kept in standard cages supplied by the Kovobel company (Domažlice, Czech Republic), two animals per cage. The cages

allowed feces separation. A cycle of 16 h of light and 8 h of the dark was used throughout the experiment. Temperature and humidity were recorded continually. Animals care and experimental procedures followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals and approved by the Ethical Committee of both institutions. Rabbits fed a commercial pelleted diet for growing rabbits (KV, Tekro-Nitra, Ltd., Slovakia) during whole experiment with access to water *ad libitum*. Rabbits in experimental-E group were administered mundticin-like substance EM41/3 prepared according to Focková et al. (2022) in a dose 50 µl/animal/day, with activity 25 600 AU/ml (tested by the agar spot test according to De Vuyst et al. 1996) for 21 days. Rabbits in group C received only diet. Mundticin-like substance was applied in drinking water. Drinking water was provided through nipple drinkers. Microbiota were determined using the standard microbiological dilution method on media according to ISO. At day 21 and 42, rabbits were slaughtered (n=4) and microbiota of caecum and appendix was determined. To check *Eimeria* spp. oocysts occurrence, the quantitative Mac Master method (1986) was used. Body weight (BW) and feed consumption (FC) were measured every week during the experiment; average daily weight gain (ADWG) was calculated mathematically. The results were quoted as the mean value ± standard deviation (SD), statistical evaluation of the results was performed by the one-way ANOVA and the Tukey test.

Results

The total fecal counts of enterococci, staphylococci, lactic acid bacteria, streptococci, coliforms and pseudomonads were not influenced by mundticin-like substance EM41/3. In E group were enterococcal counts higher at day 21 than in C rabbits and they were decreased only with difference one log cycle at the end of experiment (day 42, 3 weeks of mundticin cessation). Almost the same amount was determined for the other bacteria (LAB, staphylococci, streptococci). Pseudomonads reached up to 10^6 CFU/g on average and coliforms as well. It seems that mundticin-like EM41/3 in E rabbits lead to reduction of staphylococci at day 21 with mathematical difference 0.11 log, but with significant decrease at day 42 ($p<0.05$). The other selected bacterial group in caecum were not influenced by EM41/3. Also in appendix staphylococci were reduced at both days 21 and 42 with mathematical differences 0.29 and 0.50 log cycle. At day 21, in appendix also clostridiae were drecrease with difference 2.15 log cycle. The rest of selected bacteria were not influenced. During whole experiment broiler rabbits were *Eimeria* oocysts absent. Higher average weight gain was noted in E rabbits at day 42 compared to C rabbits (2753 ±200 g: 2671 ± 335 g). Also higher daily weight gain was noted in E rabbits in the period 35-42 days (45.05 g) compared to C (39. 84 g). Regarding mortality, in E rabbits one animal was lost and in C rabbits four animals.

Discussion

The species *E. mundtii* belongs in the *E. faecium* group based on gene similarity analysis (16S rRNA, Franz et al. 2011). Some representatives of this species were found to produce bacteriocins-mundticins (Kawamoto et al. 2002, Ferreira et al. 2007). However, there are still limited information regarding mundticins especially those exerted by horses strain. As formerly mentioned fecal horses strain *Enterococcus mundtii* EM41/3 produces mundticin-like substance with a broad antimicrobial spectrum *in vitro* (Focková et al. 2022). However, we would like to know its effect *in vivo*. Broiler rabbits are very good model for *in vivo* testing. They also are food-producing animals; so testing a new type of mundticin – like substance in rabbits GIT could be useful also for finding its application benefit. Mostly, mundticins seem to have predominantly anti-listerial effect. It was also noted in our previous *in vitro* study (Focková et al. 2022). However, *in vivo* conditions can act differently. In this study, in rabbit feces no antimicrobial effect was noted; however, in caecum and appendix anti-staphylococcal effect was noted by mathematical and significant reduction of staphylococci. Also another enterocins, e.g. those produced by *E. faecium* strains (Ent M or Ent 2019) showed anti-staphylococcal effect in rabbits (Lauková et al. 2012, Pogány Simonová et al. 2020, 2021). Mundticin also lead in increase of weight gain in rabbits at the end of experiment comparing E rabbits with those in C group. This benefit was also mentioned with the

other enterocins (Pogány Simonová et al. 2020, 2021). Based on these preliminary results, it looks that mundticin EM41/3 could be a promising additive. In processing is also evaluation of microbiota using next-generation sequencing as well as assessment of biochemical and immunological parameters and quality of meat as well. For each case, it is original contribution in bacteriocin-enterocin application studies.

Conclusion

Mundticin-like substance produced by the fecal horses strain *E. mundtii* EM41/3 showed anti-staphyloccoccal effect in broiler rabbits. Husbandry was absent of *Eimeria* oocysts spp. At the end of the experiment, higher weight gain was noted in rabbits with mundticin-like substance compared to control. It looks that mundticin EM41/3 could be a promising additive. Therefore, the evaluation of the other results is processing.

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Benefit of Enterocin 55 application in rabbit husbandry

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Abstract

Ent 55 is produced by poultry strain *Enterococcus faecium* EF55. Because producer strain has shown beneficial effect in poultry and rabbits as well; we decided to apply enterocin individually in rabbits. Ent 55 had a relatively broad antimicrobial range *in vitro*. This was reflected in a reduction of staphylococci, *Clostridiae*, *Pseudomonas* spp. and coliforms. The beneficial effect of Ent 55 was manifested by stimulation of phagocytic activity as well as by reduction *Eimeria* spp. oocysts. Ent 55 produced by not-autochtonous strain can also have protective and beneficial effect in broiler rabbits.

Introduction

From the aspect of probiotic properties and bacteriocins, *Enterococcus faecium* belongs to the most frequently studied species among enterococci. Enterocin 55 (Ent 55) is produced by poultry strain *Enterococcus faecium* EF55 (Strompfová and Lauková, 2007). It belongs to Class II enterocins, thermo-stable, small peptides. Enterocins are broad-spectrum bacteriocins that inhibit both Gram-positive and Gram-negative bacterial species (Strompfová et al., 2003; Ščerbová and Lauková, 2016). Levkut et al. (2009) reported inhibition activity of *E. faecium* EF55 against *Salmonella Enteritidis* PT4 in chicks. So far, the strain *E. faecium* EF55 was tested *in vivo* in different kind of poultry where its effect was studied at the level of antimicrobial activity, immunomodulatory effecs; the strain *E. faecium* EF55 also demonstrated beneficial impact on intestinal morphometry in the jejunum (Levkut et al., 2016; Ševčíková et al., 2016). Rabbits represent not only a suitable animal model, but after weaning they have problems with digestive disorders. The aim of every breeder is to maintain a healthy farm and thereby to obtain healthy and safe food. Following our previous studies regarding bacteriocin-produicng strain *E. faecium* EF55, this study was focused on the application Ent 55 in rabbit farming. The aim was to spread information concerning proteinaceous substances with antimicrobial effect – Ent 55, its interaction with *in vivo* environment, and its benefits in rabbit husbandry.

Materials and methods

The experiment was performed in co-operation with National Agriculture and Food Centre-NAFC (Nitra, Slovakia). The rabbits were kept in the standard cages (0.61 m x 0.34 m x 0.33 m; Kovobel company, Czech Republic), 2 animals per cage in the accordance with the guidelines stated in the Guide for the Care and Use of Laboratory Animals approved by the Slovak State Veterinary and Food Administration and the Ethical Committees of both institutions (permission code: SK CH 17,016 and SK U 18,016). A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Temperature 22±4°C and humidity 70±5% were maintained throught the experiment by heating and ventilation systems. The rabbits had access to feed and water *ad libitum*. The commercial feed mixture for broiler rabbits (pellets 3.5 mm) was fed. A total of 48 weaned rabbits (aged 35 days) Hyplus breed (both sexes) were used. The animals were divided into 2 groups in each 24 animals; control group (CG), experimental group (EG) receiving Ent 55 (semi-purified) prepared as previously reported Strompfová and Lauková (2007). Its activity was checked by agar diffusion method (De Vuyst, 1996) against the indicator strain *Enterococcus faecium* EA5. Ent 55 was applied in the water at a dose 50 µl per animal per day for 21 days (3 weeks). The experiment duration was 42 days.Faecal sampling was performed at day 0-1 (the start of

experiment; 10 mixture samples from 48 rabbits), at day 21 application (3 weeks of Ent 55 application; 5 mixture samples from each group) and at day 42 (the end of experiment, 3 weeks after Ent55 termination of administration; 5 mixture samples from each group). At day 21 and 42, three animals (n=3) from each group were slaughtered. The microbial counts were checked using the standard microbiological method (ISO, International Organisation for Standardization). The samples of appendix content (1g), caecal content (1g) and faecal were diluted in Ringer solution (pH 7.0; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and were plated onto media according to ISO. The plates were incubated according to the bacterial genera (30 °C and/or 37 °C for 24-48 h). The bacterial counts were expressed in colony-forming units (log10) CFU per gram ± SD. Blood samples (n=6) were taken from rabbits´ *vena auricularis.* At day 0-1 mixed blood samples including all groups, at day 21 and 42 from each group separately. Phagocytic activity (PA) was measured using the direct counting procedure with microspheric hydrophilic particles (MSHP). Ingestion of MSHP by polyporponuclear cells (PMNs) was determined using a modified test described by Vetvicka et. al (1982). The quantitative Mac Master method was used for detection *Eimeria* spp. oocysts (1968) expressed in OPG/g (detected oocysts per gram of faecal samples). Statistical evaluation of the results was performed using one-way ANOVA test with Tuckey`s post hoc test (the level of significance set at $p<0.05$ ± standard deviation – SD).

Results

Antimicrobial activity of Ent 55 in faeces was recorded by significant reduction of coagulase-negative staphylococci (CoN)S in EG at day 21 compared to EG at day 42 ($p<0.001$). Pseudomonas spp. in EG were reduced at 21 day compared to day 0-1 ($p<0.05$). A reduction was also observed for coliform bacteria in EG at day 42 compared to day 0-1 ($p<0.05$). At day 21 were reduced coliforms in EG compared to CG ($p<0.05$). Microbial counts were generally lower in the caecum and appendix than in the faeces. Similarly as in faeces, the counts of CoNS were reduced in caecum at day 21 compared to day 42 ($p<0.01$). In caecal samples, slightly (numerical, not significant) reduced counts of coliforms, Pseudomonas spp. and Clostridia spp. were recorded during Ent 55 addition. On the other hand, coagulase-positive staphylococci (CoPS), enterococci and lactic acid bacteria (LAB) were neither influenced by Ent 55 in faeces, nor in caecum and appendix. There was a significant reduction in coliform bacteria in the caecum (EG) at day 21 compared to day 42 ($p<0.05$). The lower counts of Clostridium spp., Pseudomonas spp. were observed at day 42 with differences 0.87 log cycle, 1.07 log cycle. Phagocytic activity was significantly stimulated in EG compared to CG at day 21 ($p<0.01$), as well as at day 42 ($p<0.001$). Blood serum parameters indicated no influence, only a slight effect was noted within the reference values for the individual parameters.The counts of Eimeria oocysts have been reduced at day 21 and 42 in EG compared to CG rabbits.

Discussion

Antibiotic resistance is rising worldwide, so there is a need to look for "substitutes" for antibiotics; in this case, natural substances are promising. The bacteriocins are ribosomally synthetized antimicrobial peptides, produced by Gram-positive and Gram-negative bacteria, mostly by lactic acid bacteria. The genus *Enterococcus* belong to the phylum Firmicutes. A large number of bacteriocins produced by enterococci (Belguesmia et al., 2011) was noted, mostly enterocins, proteinaceous substances with antimicrobial effect (Franz et al., 2007). Previously, bacteriocins were characterized that they act against closely related bacteria but many studies have spread last decade this statement because e.g. enterocins can inhibit more or less related bacteria, spoilage bacteria including. Enterocins are known to have frequently a broad antimicrobial spectrum. Many *in vitro* studies were related to this statement (Nes et al., 2002; Franz et al., 2007; Perez et al., 2014; Ščerbová and Lauková, 2016). However, concerning the application abilities in animals to protect husbandry has been still reported only limited information. Our laboratory has focused on the study of enterocins for several years (Lauková et al., 2012; Szabóová et al., 2011; Ščerbová and Lauková, 2016). Ent 55 is produced by poultry strain *E. faecium* EF55 as formerly mentioned. *In vitro* testing of Ent 55 showed a broad-spectrum antimicrobial effect against indicator strains of various origin (Strompfová et al., 2003) which was confirmed in our *in vivo* study

with broiler rabbits by a significant reduction of CoNS and *Clostridium* spp.; Gram-negative bacteria (coliforms and *Pseudomonas* spp.) were similarly inhibited by significant reduction or by bacteriostatic effect of their growth. Similar inhibition was recorded using other enterocins; e.g. Ent 2019 (produced by *E. faecium* CCM 7420) also reduced staphylococci including *Staphylococcus aureus*, *Clostridium spp.* and *Pseudomonas spp.* or Ent M (produced by *E. faecium* AL41-CCM8558) also showed reduction of staphylococci, pseudomonads, Clostridiae but also coliforms (Simonová et al., 2008; Lauková et al., 2016). Although amino sequence analysis of Ent 55 has not been tested, their properties correspond to the Class II enterocins. As mentioned above, this group includes thermo-stable, small peptides. The host gut is a complex ecosystem in which interactions between intestinal microbiota, pathogens and the immune system take place. All these components interact with each other and contribute to the maintenance of homeostasis of the organism. Therefore, it is important to monitor interaction between organisms, the immune response, inflammatory processes, but also pathogens in in vivo experiments. The reduction of clostridiae, *Pseudomonas* spp. and coliforms in appendix is very interesting. Appendix could be a unique niche for commensal bacteria in the body and appendix has importance because of differentiation and forming of lymphoid cells which after migration in the secondary lymphatic organs are multiplied (Fortun-Lamothe et al., 2007). The appendix could play role in eliminating of pathogenic microbiota. Phagocytic activity is important parameter, there polymorphonuclear leucocytes are responsible for non-specific immune response and in first line share of phagocytosis introdefence of the host to infectious and inflammatory actions (Escribano et al., 2005). In the case of testing our enterocins, stimulation of phagocytic activity was repeatedly monitored, e.g. in rabbits was recorded after administration of Ent M and Ent 2019 (Lauková et al., 2012; Pogány Simonová et al., 2013) as well as in Ent 55 in the current study. We could assume that the prolonging stimulating effect on phagocytic activity was caused by these natural substances, enterocins. In addition, this may be related to the overall stimulating as well as protective effect of enterocins. Very important and original finding is that *Eimeria* spp. oocysts were reduced in EG (with Ent 55) compared to CG. The probable possibility of explaining the effect of Ent 55 is in the already mentioned stimulation of the immune system. Higher immunity means less sensitivity to agents such as *Eimeria* spp.. The reduction of *Eimeria* oocysts was observed also by (Simonová et al., 2008 and Szabóová et al., 2011). Our results are significant in terms of basic research to know the range of effects of the studied enterocin-Ent 55 in its experimental application in the digestive tract of rabbits.

Conclusion

The beneficial effect of Ent t55 was found in rabbit husbandry. The application of Ent 55 showed antimicrobial effect, especially in the observed potentially pathogenic mirobiota such as staphylococci, Clostridiae, pseudomonads and coliforms in faeces, appendix and caecum as well. The beneficial effect of Ent 55 was manifested by stimulation of phagocytic activity as well as by reduction of Eimeria spp. oocysts. Our study suggested that Ent 55 produced by non-autochtonous strain can have beneficial effect in rabbit husbandry.

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Thymol sustained administration to rabbits, its effect on microbiota in caecal content and faeces

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Abstract

This study could give us answer to question about whether thymol as a major constituent of *Thymus vulgaris* is efficiently absorbed in the rabbit organism after its sustained administration and if it is also able to exhibit its beneficial properties on microbiota from caecal content and faeces. The main objective of this study was to evaluate the concentration of thymol in plasma, intestinal wall, caecal content and faeces after its sustained administration (21 d; dose 250 mg/kg feed) and then withdrawn for 7 d. Its effect on microbiota in caecal content and faeces was also evaluated. Thymol content in faeces ($P < 0.001$) and caecal content ($P < 0.05$) was significantly higher than in plasma during the thymol addition and withdrawal. Lactic acid bacteria (LAB) in caecal appendix ($P < 0.01$) and faeces ($P < 0.05$) were significantly higher and were still present after withdrawal of thymol. Thymol was sufficiently absorbed from the rabbit intestine, and was also detected in faeces even after its withdrawal from the diet as a consequence of caecotrophy. Thymol in the applied concentration did not reduce pathogenic bacteria, but was able to increase the number of beneficial LAB in the caecal appendix during its addition to the diet.

Introduction

One of the major components of thyme essential oil is phenolic compound thymol which is mainly responsible for its beneficial properties like antimicrobial and antioxidant (Abdel-Gabbar et al., 2019; Placha et al., 2019). The phenolic compounds (e.g. thymol and carvacrol) are mainly effective against intestinal colonization by undesirable pathogenic bacteria, and could positively affect the growth of beneficial bacteria such as lactobacilli (Christaki et al., 2020). To our knowledge, no study has been carried out on thymol absorption and distribution in the rabbit organism, only small number of studies has been published about thymol distribution in animal tissues. Mason et al. (2017) detected thymol in liver, kidney and fat of cattle, Zitterl-Eglseer et al. (2008) in kidneys of piglets, Haselmeyer et al. (2015) in plasma, muscle, liver and kidneys of broiler chickens; Oceľová (2017) in intestinal wall and gut content of broiler chickens. The main objective of this study was to evaluate the effect of sustained application of thymol and its withdrawal on thymol absorption from the gastrointestinal tract in connection with its effect on microbiota of the large intestine.

Material and methods

A total of 48 rabbits at five weeks of age were randomly divided into two dietary treatment groups (C/control, T/thymol addition) of 24 animals in each with six replicates (two cages - one cage with two rabbits/one replicate). The rabbits were fed with thymol addition (250 mg/kg feed) for three weeks and for the following week the thymol was withdrawn. Eight rabbits from each group at the age of eight

(with thymol) and nine (without thymol) weeks were sacrificed. Plasma for thymol analysis was obtained after centrifugation of blood at 1180 g for 15 min. Samples of plasma, small intestine wall, caecum and appendix content and hard faeces (freshly-voided, collected using nets mounted under the cages) for thymol analyses were immediately frozen in liquid nitrogen and stored at -70°C until analysed. To test the microbiota, samples (approximately 1 g) of appendix and caecum content were collected from the same animals, and faeces (n=6, one sample from one replicate) on the same days of age. Detection of thymol in plasma, intestinal wall, caecum and faeces was performed using headspace solid-phase microextraction followed by gas chromatography coupled with mass spectrometry (GC/MS) described by Bacova et al, (2020). The samples of faeces, caecal and appendix content (1 g) were treated using the standard microbiological dilution method proposed by the International Organization for Standardization (ISO) and cultivated as was described by Pogány Simonová et al. (2020). The statistical analyses were performed using GraphPad Prism version 5.0 for Windows. The Kolmogorov-Smirnov test evaluated normality or non-normality of distribution. Statistical analysis of the results used analysis of variance as a 2 x 2 factorial design that represents two main factors: time of measurements (8 and 9 weeks) and treatment (with and without thymol). Three main objectives were examined: the effect of time, the effect of thymol and the interaction between time and thymol addition. Differences between diets with and without thymol addition were analysed by two-way analysis of variance (ANOVA). When interaction between time and treatment was statistically significant, the simple Mann–Whitney U test was performed. For comparison of thymol concentrations between plasma, intestinal wall, caecal content and faeces Kruskal-Wallis test with post hoc Dunn´s Multiple Comparison test was used. Correlations of thymol concentration between plasma and intestinal wall were analysed using nonparametric Spearman's Rank Correlation and expressed as Spearman's correlation coefficient (r_s). Results are presented as the mean ± standard error of mean (SEM). Significant differences were considered at $p < 0.05$.

Results

Thymol content in faeces ($P < 0.001$) and caecal content ($P < 0.05$) revealed significantly higher concentrations in comparison with plasma during the thymol addition and withdrawal (2.442 ± 0.451, 0.881 ± 0.231 µg/g DM and 0.046 ± 0.028 µg/mL, respectively and 0.150 ± 0.041, 0.046 ± 0.012 µg/g DM and 0.003 ± 0.0005 µg/mL, respectively). Only the correlation between thymol content in plasma and intestinal wall was observed on statistical significant differences during the period of thymol addition ($r_s = -1.0$, $P < 0.01$). It is important to notice, that after withdrawal thymol was detected only in trace amounts except the faeces. *Enterococcus* sp. in faeces was lower in 9 weeks old rabbits comparing to 8 weeks old ($P < 0.05$). Lactic acid bacteria (LAB) in caecal appendix ($P < 0.01$) and faeces ($P < 0.05$) were higher in the treatment groups, in faeces also higher in the 9 weeks old ($P < 0.05$). Coagulase-negative staphylococci (CoNS) in all collected site was lower at 9 weeks old comparing to 8 weeks old ($P < 0.05$) and higher in faeces of groups fed thymol than the control group ($P < 0.05$). Coagulase-positive staphylococci (CoPS) in caecal appendix was lower in the treatment group ($P < 0.01$). The statistical significance on interaction between studied factors without differences on main effects was presented for CoPS in faeces. The treatment group was lower on CoPS in faeces at 8 weeks old, however was higher in this group at 9 weeks old. None difference was found between time and groups in all collected sites for Coliform bacteria ($P < 0.05$).

Discussion

After biotransformation processes of thymol in intestinal wall, thymol or its metabolites can be transported back into the intestinal lumen or are distributed within the organism through the blood and systemic circulation (Bacova et al., 2020). As thymol metabolites can be converted back into parental compounds by microbial enzymes in the caecum, we assume that this process together with consumption of cecotrophs containing thymol (metabolised or non-metabolised) could explain the higher amount

of thymol in the caecal content. As cecotrophy is the process of redigestion and absorption of previously-undigested nutrients, this together with processes of biotransformation could explain our detection of thymol in the faeces also after its withdrawal. Even though we expected the inhibitory effect of thymol on pathogenic bacteria in our study, we could not confirm this expectation either during thymol addition or after its withdrawal. Surprisingly, only beneficial LAB increased significantly in the caecal appendix during the period with thymol addition. The same tendency to colonize the rabbit gut with beneficial bacteria after supplementation of thyme essential oil in their diet was demonstrated by Placha et al. (2013). Rhouma et al. (2018) also found a stimulatory effect of thymol on LAB in the distal gut, which is beneficial in preventing the proliferation of undesirable microorganisms. Based on Iqbal et al. (2020), the strong antioxidant properties of thymol could protect the intestinal epithelial cells and prevent inflammation, resulting in suppression of pathogenic bacteria on the one hand and supporting beneficial bacteria such as LAB on the other. We detected approximately 5-times higher amounts of thymol in the faeces than in the intestinal wall during thymol addition. From this, we assume that thymol was present in adequate amounts in the caecal appendix as well, and was able to affect the microbiota in that area. Increased concentration of microbiota, particularly of pathogenic Gram-positive bacteria like CoPS in the faeces after thymol withdrawal, indicate that its concentration during this period was unable to exert such antimicrobial effect as during the thymol addition.

Conclusion

Even though phenolic compounds are able to improve gut health due to their strong antioxidant and antimicrobial potential, further studies are needed to understand how the processes of thymol biotransformation in the gastrointestinal tract can affect the gut microbiota, because of the specificities of rabbit digestion.

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Microbiota and phagocytic activity in broiler rabbits infected with *Enterococcus hirae* and treated with Enterocin M

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Abstract

Broiler rabbits are susceptible to bacteria causing gastrointestinal diseases. This food-producing animals are also a suitable research animal model. The aim of this study was to check *in vivo* the interaction of Ent M with biofilm-forming fecal strain *Enterococcus hirae* B^+Kr8 possessing *GelE* gene to find out whether Ent M can reduce *E. hirae* B^+Kr8, and how the host organism deals with it. *Post*-weaning rabbits (96), aged 35 days of both sexes (meat - line breed M91) were divided into four groups (24 animals in each group). They were fed a commercial diet for growing rabbits with access to water *ad libitum*. Rabbits in E1 were also administered 10^8 CFU/ml of *E. hirae* B^+Kr8 (500 µl per animal per day), Ent M (50 µl per animal per day) in E2, and a combination of both additives in E3 (in their drinking water for 21 days). The experiment lasted 42 days. Rabbits were kept in standard cages, two animals per cage. Sampling of feces was performed at day 0-1 (mixtures from 96 animals in ten samples (n=10); at day 21 (21 days of additive applications, mixtures from each group of 24 animals (n=5); and at day 42 (21 days of additives cessation, with five fecal mixtures from each group (n=5) being sampled. At days 21 and 42, four rabbits from each group (n=4) were slaughtered. Ent M probably interacts with bacteria in the detected phyla, causing their reduction. *E. hirae* B^+Kr8 did not have any pathogenic influence on rabbits; Ent M induced prolonged PA increase, probably supporting the immune response in rabbits against the B^+Kr8 strain. The prospective importance of this study lies in the indication and support of the administration of enterocins to reduce/prevent diseases by reducing their causative agents.

Introduction

Young rabbits are susceptible to harmful bacteria, especially to those causing gastrointestinal diseases. Acute enterococcosis caused by *Enterococcus hirae* strains is more associated with poultry; however, this species has also been detected in rabbits (Dicpinigaitis et al. 2015, Bino et al. 2018). Enterococci are controversial bacteria. On the one hand, clinical strains can cause infection in humans and animals; on the other hand, they are known to have probiotic character and produce antimicrobial substances - bacteriocins (Franz et al. 2007, Hanch et al. 2018). The species *E. hirae* occurs as a common inhabitant in the gut microbiota of animals (Bino et al. 2018). However, those strains which contain virulence factors, and they are biofilm-producing, or they possess antibiotic resistance genes, they can stimulate enterococcosis (Costerton and Stewart 2001). Increase in antibiotic resistance in particular has been noted in bacteria, also involving biofilm-forming bacteria; the consequence of which is insufficient elimination of bacterial disorders. One promising approach to dealing with this problem is based on bacteriocins - antimicrobial proteinaceous substances with inhibition effect against more or less related bacteria (Nes et al. 2014). They have already been used in rabbits breeding with beneficial effect; enterocin M (Ent), for example, a thermo-stable enterocin with a broad inhibition spectrum produced by *E. faecium* AL41 (Mareková et al. 2007, Lauková et al. 2012) has shown benefits in rabbits (Lauková et al. 2012, 2018, Pogány Simonová et al. 2020). Lauková et al. (2012) and Pogány Simonová et al. (2020) reported that coliform bacteria and *Staphylococcus aureus* were reduced in broiler rabbits ($p <$

0.05) after Ent M administration and higher average daily weight gain was also measured. Broiler rabbits are food-producing animals. They are also a suitable research animal model (respecting the 3R principle in EU2010/63). The aim of this study was to check *in vivo* the interaction of Ent M (characterized in our laboratory) with biofilm-forming fecal strain *E. hirae* B^+Kr8 (isolated in our laboratory), which possesses virulence factor gene for gelatinase production (*GelE)*, to find out whether Ent M can reduce *E. hirae* B^+Kr8, and how the host organism deals with it. This aim was conceptualized following the strategy: healthy food-producing animals lead to safe animal-derived products.

Material and methods

The experiment was performed at the National Centre of Agriculture and Food, Nitra-Lužianky (Slovakia) using *post*-weaning rabbits (96), aged 35 days of both sexes (meat - line breed M91). Animals care and experimental procedures followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals and approved by the Ethical Committee of both institutions and by the Slovak Veterinary and Food Administration in the certified stall (SK CH 17,016 and SK U 18, 016). Rabbits were divided into four groups with 24 animals in each group. They were fed a commercial diet for growing rabbits during the experiment, with access to water *ad libitum*. Rabbits in group E1 were additionally administered 10^8 CFU/ml of biofilm-forming strain *E. hirae* B^+Kr8 at a rate 500 µl per animal per day. Rabbits in group E2 were administered Ent M (50 µl per animal per day), and rabbits in group E3 received a combination of both additives in their drinking water for 21 days. From day 22 onwards, all animals were fed only the standard diet. The experiment lasted 42 days. Rabbits were kept in standard cages supplied by the Kovobel company (Domažlice, Czech Republic), two animals per cage. The cages allowed feces separation. A cycle of 16 h of light and 8 h of the dark was used throughout the experiment. Temperature and humidity were recorded continually. The average live weight of the rabbits at the start of the experiment ranged from 994.17 to 1006.25 g. Sampling of feces was performed at day 0-1 (mixtures from 96 animals in ten samples (n=10); at day 21 (21 days of additive applications, mixtures from each group of 24 animals (n=5); and at day 42 (21 days of additives cessation, with five fecal mixtures from each group (n=5) being sampled. At days 21 and 42, four rabbits from each group (n=4) were slaughtered. Caecum and appendix were sampled for standard microbial analysis. *E. hirae* B^+Kr8 was prepared as described by Strompfová et al. (2003). Ent M was prepared according to Mareková et al. (2007) (activity 12 800 AU/ml). *E. hirae* B^+Kr8, enterococci and lactic acid bacteria (LAB) were determined using the standard microbiological dilution method on media according to ISO. The plates were incubated at 37 °C for 48 h. Bacterial count was expressed in colony-forming units per gram (CFU/g, log 10) ± SD. Fecal samples from day 21 were analyzed also using next-generation sequencing as described by Lauková et al. (2022). Blood from the *vena auricularis* at days 0-1, 21 and 42 was analysed for phagocytic activity (PA) according to Vetvicka et al. (1982). Statistical evaluation was performed using one-way analysis of variance (ANOVA), followed by Tukey *post*-test. The results are quoted as means ± SD and were compared among groups within the same days of samples collection to check the changes during the experiment. Differences between mean values were considered statistically significant at $p < 0.05$. All statistical analyses were performed using GraphPad Prism statistical software (GraphPad Prism version 6.0, GraphPad Software, San Diego, California, USA).

Results

E. hirae was the highest in the feces of E1 (3.81 ± 0.91 CFU/g log 10). Its high count was also in E3 rabbits. At day 21, a significant decrease was found ($p < 0.01$) in E3 compared with E1. It indicates that Ent M has shown inhibition effect against *E. hirae.* At day 42, in E1 and E3, *E. hirae* reached up to 10^1 CFU/g. At day 21, enterococci were almost at the same level in all rabbits. Significant increase of enterococci was found at day 21 comparing with day 0/1 ($p < 0.001$), and with day 42 as well. LAB were not influenced at days 21 and 42. However, comparing days 0-1 and 21, a significant increase of LAB was noted ($p < 0.01$). In the caecum, at day 21, *E. hirae* was almost at the same level in both groups which was also in balance with enterococci. LAB in the caecum at day 21 reached up to 5.29

CFU/g. At day 42, lower counts of *E. hirae* as well as enterococci were noted. LAB reached almost 10^4 CFU/g. Only in E3, a mathematical decrease in LAB was found comparing with rabbits in E1, E2, and C. In the appendix, *E. hirae* reached up to 1.02 CFU/g (log 10) at day 21. Enterococci were the highest in E1; at day 42, enterococci were the lowest in E1. LAB were not influenced in appendix. Using ngs, the phylum Firmicutes had the highest abundance in all rabbits; 61.9% in E2 (Ent M), 57% in E1 (*E. hirae*) and the lowest abundance in E3 (both additives, 47.4%). In C, abundance for Firmicutes was 56.9%. The other phyla, such as Verrumicrobia, Bacteroidetes, Tenericutes, Proteobacteria, Cyanobacteria, Saccharibacteria, and Actinobacteria were detected in low abundance. Verrumicrobia were the highest in C (17.2 %); in E1 they reached 12.4 %; in E2 and E3, it was 8.6% and 5.6%. Ent M appears probably to interact with these bacteria; they were reduced. Bacteroidetes, Tenericutes, Cyanobacteria, and Saccharibacteria were the lowest in E3 indicating their possible interaction with Ent M. Actinobacteria were not influenced. Proteobacteria in E3 reached abundance 30.1%-higher compared with C, E1 and E2; in E2 the lowest were found Proteobacteria (1.3 %). Phagocytic activity (PA) in C maintained almost the same value at days 21 and 42. At day 21, PA was higher in all E groups than in C. Comparing E groups with C at day 21, significant increase was found in E1 and E2, ($p < 0.001$), indicating that *E. hirae* did not attack PA. At day 42, higher PA was again in E groups. Significant increase in PA was found comparing E1 with C ($p < 0.001$) and comparing E3 with C ($p < 0.001$). In E1 and E2 lower PA was found, while in E3 a higher PA was measured than at day 21;it was the highest value out of all E groups. This indicates that the stimulating effect of Ent M started later; it had a prolonged effect.

Discussion

In *post*-weaning period bacterial infections in rabbits have more chance to affect them. It is necessary therefore to know how the rabbits` organism can respond to this kind of attack, and how to deal with it. Although the highest count of *E. hirae* B^+Kr8 was reached in feces of E1, and it featured highly in E3; at day 21 it was found to be significantly decreased in E3 compared with E1 indicating that Ent M probably had an inhibiting effect against the B^+Kr8 strain. Ent M is known to have broad antimicrobial effect against more or less related bacteria (Lauková et al. 2012, 2016, 2018). The species *E. hirae* represents a group of related bacteria which belongs in the same *E. faecium* cluster as also Ent M-producing *E. faecium* CCM8558 strain. The B^+Kr8 count in our rabbits reached a level comparable with autochthonous probiotic enterococci counts such as *E. faecium* EF2019 (Pogány Simonová et al. 2020a). In the caecum and appendix, low counts of B^+Kr8 strain were found; however, in general, the caecum and appendix are locations where applied strains have typically reached up to 1.0 CFU/g (log 10) (Pogány Simonová et al., 2020a,b, 2021). Using ngs analysis, high and well-balanced abundance % was noted in C, E1 and E2 rabbits with the lowest count in E3, indicating inhibition of *E. hirae* B^+Kr8 strain by Ent M. Inhibition activity of Ent M has been noticed *in vitro* for example against virulence factor genes residing in fecal strains of *E. hirae* from ostriches (Lauková et al. 2016). Representatives of the phylum Firmicutes are mostly isolated from the GIT of animals, including rabbits (Lauková et al. 2022). Pogány Simonová et al. (2021) reported significant decrease in coliforms and pseudomonads in feces of broiler rabbits after application of Ent M or durancin ($p < 0.001$ or $p < 0.05$). *E. hirae* apparently did not infect our rabbits and prolonged effect especially for PA was noted as it is usually noted after enterocin application (Lauková et al. 2021). Based on our previous results, we suppose that Ent M can support the GALT by stimulating the IgA system (Pogány Simonová et al. 2021).

Conclusion

Ent M probably interacts with bacteria in the detected phyla, causing their reduction. *E. hirae* B^+Kr8 did not have any pathogenic influence on rabbits; Ent M induced prolonged PA increase, probably supporting the immune response in rabbits against the B^+Kr8 strain. The prospective importance of this study lies in the indication and support of the administration of enterocins to reduce/prevent diseases by countering their causative agents.

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Steirischer Kürbiskernpresskuchen als teilweiser Fischmehlersatz: Einfluss auf die Filetqualität von Salmoniden und Welsen

Pumpkin seed cake as partial fishmeal substitute in fish nutrition: Effects on fillet quality of salmonids and catfish

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Abstract

For ecologic and economic reasons, the inclusion of fishmeal in aquaculture diets has been gradually reduced. As soybean meal also faces limitations, other plant-based alternatives are currently under investigation in fish nutrition. In addition to performance traits, also fillet quality should be considered, for a thorough evaluation. In four feeding trials the effects on fillet quality of rainbow trout (*Oncorhynchus mykiss* W.), brook trout (*Salvelinus fontinalis* M.), African sharptooth catfish (*Clarias gariepinus* B.), and wels catfish (*Silurus glanis* L.) were evaluated with a diet in which 60% of the fishmeal protein of a reference diet were substituted by pumpkin seed cake protein (PSC). Apart from major differences in fillet quality depending on the selected fish production system, results show no changes in physical fillet quality owing to the different diets. However, fillets of African sharptooth catfish had higher ether extract concentrations when fed PSC. Independent of the fish species the concentration of n6 fatty acids was higher and n3 fatty acids reduced when the diet contained PSC. In conclusion, PSC can partly substitute fish meal in diets of salmonids and catfish without negative effects on physical fillet quality, however, a decrease in valuable n3 fatty acids has to be expected.

Einleitung

Fisch, vor allem aus Aquakultur, spielt eine immer bedeutendere Rolle für die Ernährung des Menschen weltweit (FAO, 2020). Eine wichtige, wenn auch in den letzten Jahren etwas rückläufige, Proteinquelle in Fischfuttermitteln ist Fischmehl. Dieses setzt sich zwar vermehrt aus Nebenprodukten der Fischproduktion zusammen, nichtsdestotrotz hat der Wildfang weiterhin den größten Anteil am Fischmehl, wodurch der Druck auf Wildbestände immer noch gravierend ist (FAO, 2020). Aus ökologischen aber auch ökonomischen Gründen wird Fischmehl daher vermehrt nur noch in ausgewählten Produktionsstadien (Brut, Elterntiere, Endmast) eingesetzt, wohingegen bei wachsenden Tieren auf pflanzliche Alternativen gesetzt wird (FAO, 2020). Im Vergleich zu Fischmehl weisen diese aber eine geringere Nährstoffverdaulichkeit und geringere Anteile an den ernährungsphysiologisch bedeutenden n3 Fettsäuren auf (De Francesco et al., 2004; FAO, 2020). Daher kann die veränderte Fütterung auch Einfluss auf die Qualität des tierischen Lebensmittels nehmen. Eine klassische Alternative ist Sojaextraktionsschrot, das aber aufgrund der hohen Gehalte an antinutritiven Faktoren (ANF) und Gesamtfaser nur begrenzt in der Fischfütterung eingesetzt werden kann (Glencross et al., 2020). Im Vergleich zu vielen anderen Ölpresskuchen weist der Steirische Kürbiskernpresskuchen (KPK) geringere Konzentrationen an ANF und Rohfaser auf (Zdunczyk et al., 1999; Greiling et al., 2018), weshalb er im vorliegenden Versuch als Fischmehlalternative eingesetzt werden soll. Dabei wird erwartet, dass der partielle Ersatz

von Fischmehl durch KPK den Gehalt an n3 Fettsäuren im Filet reduziert, die Filetqualität sich jedoch bei allen Fischarten gleichförmig verändert. Ziel dieser Arbeit ist es, den Einfluss von KPK auf die Filetqualität von zwei Kaltwasser- und zwei Warmwasserfischarten zu betrachten, um dessen Eignung als heimische und nachhaltige Alternative zu Fischmehl zu evaluieren.

Material und Methoden

Die Fütterungsversuche wurden am Institut für Fischerei der Bayerischen Landesanstalt für Landwirtschaft in Starnberg (Deutschland) durchgeführt. Es kamen vier verschiedene Fischarten in den jeweils für ihre Produktion gängigen Produktionssystemen zum Einsatz (jeweils acht Becken bei Regenbogenforelle, Bachsaibling und Afrikanischem Wels (n=4) und sechs Becken beim Europäischen Wels (n=3)). Versuchsgruppen wurden randomisiert angeordnet. Im Vergleich zu einer Kontrollration (KON) wurde in der Versuchsration KPK 60% des Fischmehlproteins in KON durch KPK-Protein auf Basis des verdaulichen Proteins ersetzt (Tabelle 1). Die Fische wurden händisch einmal täglich bis zur scheinbaren Sättigung gefüttert. Details zu Haltung und Fütterung sind bei Greiling et al. (2018) nachzulesen, Details zum Einfluss der Fütterung auf die Darmmorphologie der Fischarten bei Humer et al. (2018).

Tabelle 1. Zusammenstellung der Futtermittel für Salmoniden und Welse

	Regenbogenforelle, Bachsaibling		Afrikanischer bzw. Europäischer Wels	
	KON	KPK	KON	KPK
Komponenten [g kg^{-1}]				
Fischöl	110,0	110,0	115,8	115,8
Sojaschrot	247,4	247,4	260,4	260,4
Blutmehl	90,0	90,0	94,7	94,7
Weizengluten	90,0	90,0	94,7	94,7
Fischmehl (Anchovis)	200,0	80,0	210,5	84,2
Kürbiskernpresskuchen	-	141,0	-	148,5
Rapsöl	50,0	46,0	-	-
Weizen	200,0	183,0	210,5	188,3
L-Lys HCl	2,0	2,0	2,1	2,1
DL-Met	5,6	5,6	5,9	5,9
Mono-Calcium-Phosphat	3,0	3,0	3,2	3,2
Vit-Min-Prämix	2,0	2,0	2,1	2,1
Chemische Analysen [g kg^{-1} TM]				
Trockenmasse [g kg^{-1}]	928	916	921	924
Rohprotein	514	488	515	503
Rohfett (hydrolysiert)	138	148	109	116
Rohfaser	18,1	22,1	18,9	22,4
Rohasche	67,5	61,4	69,8	63,9
Fettsäuremuster [g/kg TM]				
SFA	22,1	24,1	22,0	24,1
MUFA	71,9	73,9	55,7	58,3
PUFA	52,1	57,9	45,4	50,4
n6	28,9	37,1	23,7	31,8
n3	22,4	20,0	20,7	17,8

KON = Kontrollration; KPK = Ration für Salmoniden und Welse in der 60% des Fischmehlproteins in KON durch Kürbiskernpresskuchenprotein auf Basis des verdaulichen Proteins ersetzt wurde; SFA = gesättigte Fettsäuren, MUFA = einfach ungesättigte Fettsäuren, PUFA = mehrfach ungesättigte Fettsäuren

Am Ende des Fütterungsversuches (Tag 63 bei Salmoniden, Tag 54 bei Welsen) wurden vier Fische je Becken nach 24-stündiger Nüchterung fachgerecht betäubt, getötet, zerlegt und die Filets gewonnen. Wasserhaltekapazität mittels Zentrifugation (Castellini et al., 2002), Garverlust (2 min bei 90°C im Vakuumbeutel), Rohnährstoffgehalte (Naumann and Bassler, 2012) sowie Fettsäuremuster (Sukhija and Palmquist, 1988) wurden in den Filetproben ermittelt. Für die statistische Auswertung (SAS 9.4, SAS Institute Inc., Cary, USA) wurde ein 2*4 faktorielles Design mit zwei Futtermitteln und vier Fischarten sowie deren Interaktion gewählt.

Ergebnisse und Diskussion

Einfluss Fütterung: Der teilweise Ersatz von Fischmehl durch KPK im Futter hatte keinen Einfluss auf die physikalische Zusammensetzung der Filets (p>0,05; Tabelle 2). Es gab lediglich einen Trend zu einem höheren Garverlust in den KPK-Gruppen (p<0,10). Auswirkungen des Futters auf die Rohnährstoffe im Filet waren vor allem beim Afrikanischen Wels zu beobachten, wo ein Anstieg von Trockenmasse und Rohfett mit einem Rückgang von Rohasche und Rohprotein verbunden war. Veränderungen im Fettgehalt des Filets wurden auch in anderen Studien beobachtet (De Francesco et al., 2007), vor allem, wenn keine Anpassung der essentiellen Aminosäuren in der Ration durchgeführt wurde.

Tabelle 2. Auswirkung von Kürbiskernpresskuchen im Fischfutter auf die physikalische und chemische Filetqualität ausgewählter Fischarten

	Regenbogenforelle		Bachsaibling		Afrik. Wels		Europ. Welse		SEM	p-Wert		
	KON	KPK	KON	KPK	KON	KPK	KON	KPK		FM	FI	FM*FI
WHC, %	60,3	59,8	59,9	60,6	60,4	60,1	67,5	67,7	0,50	NS	***	NS
GV, %	10,03	11,20	7,14	8,16	6,83	7,16	9,06	8,61	0,19	(*)	***	NS
TM, %	$29,8^{a}$	$28,9^{A}$	$30,4^{a}$	$29,7^{A}$	$26,4^{bY}$	$29,9^{AX}$	$21,7^{c}$	$22,4^{B}$	0,39	NS	***	*
XA, % TM	$4,32^{b}$	$4,57^{A}$	$4,35^{b}$	$4,37^{A}$	$4,15^{bX}$	$3,40^{BY}$	$5,43^{a}$	$4,93^{A}$	0,07	*	***	*
XP, % TM	$76,0^{b}$	$77,8^{B}$	$74,1^{b}$	$72,5^{C}$	$74,9^{bX}$	$68,6^{CY}$	$87,9^{a}$	$86,1^{A}$	0,67	*	***	***
XL, % TM	$20,9^{a}$	$20,5^{B}$	$23,1^{a}$	$23,8^{B}$	$23,6^{aY}$	$30,2^{AX}$	$8,44^{b}$	$10,3^{C}$	0,69	***	***	***
Fettsäuremuster, % FAME												
SFA	$19,6^{b}$	$19,4^{C}$	$17,1^{c}$	$17,3^{D}$	$28,9^{aX}$	$28,3^{AY}$	$20,6^{bY}$	$21,6^{BX}$	0,42	NS	***	***
MUFA	$50,8^{bX}$	$48,9^{BY}$	$52,3^{aX}$	$51,1^{AY}$	$43,5^{c}$	$42,9^{C}$	$44,2^{c}$	$44,3^{C}$	0,36	***	***	*
PUFA	$29,6^{bY}$	$31,6^{BX}$	$30,7^{b}$	$31,5^{B}$	$27,6^{c}$	$28,5^{C}$	$35,3^{a}$	$34,5^{A}$	0,25	***	***	***
n6	$14,8^{bY}$	$18,5^{AX}$	$15,3^{abY}$	$18,0^{ABX}$	$13,6^{cY}$	$17,5^{BX}$	$15,7^{aY}$	$17,5^{BX}$	0,17	***	***	***
n3	13,8	12,0	14,4	12,6	13,3	10,4	18,3	15,8	0,21	***	***	NS
n6:n3	$1,09^{aY}$	$1,49^{BX}$	$1,06^{aY}$	$1,43^{BX}$	$1,03^{aY}$	$1,67^{AX}$	$0,86^{bY}$	$1,12^{CX}$	0,02	***	***	***

KON = Kontrollration; KPK = Ration für Salmoniden und Welse in der 60% des Fischmehlproteins in KON durch Kürbiskernpresskuchenprotein auf Basis des verdaulichen Proteins ersetzt wurde; FM=Futtermittel; FI=Fischart; n.a. = nicht analysierbar; WHC = Wasserhaltekapazität; GV = Garverlust; TM = Trockenmasse; XA = Rohasche; XP = Rohprotein; XL = Rohfett; FAME = Fettsäuremethylester; SFA = gesättigte Fettsäuren; MUFA = einfach ungesättigte Fettsäuren; PUFA = mehrfach ungesättigte Fettsäuren; p<0,10(*); p<0,05*; p<0,01***; NS=nicht signifikant; ^{abc} = Unterschiede Fischart innerhalb von KON (p<0,05); ^{ABC} = Unterschiede Fischart innerhalb von KPK (p<0,05); ^{XY} = KON vs. KPK (p<0,05) innerhalb der Fischarten

Die Proteinquelle KPK reduzierte in allen Fischarten den relativen n3 Fettsäuregehalt und erhöhte die n6 Fettsäuren in den Filets. Infolgedessen stieg auch das n6:n3-Verhältnis in allen Fischarten, die mit KPK gefüttert wurden. Diese Veränderungen, wenn auch immer noch in einem sehr günstigen Bereich (Empfehlung Verhältnis ≤5:1 in der Gesamtaufnahme (DACH, 2021)), konnten zwar auch bei Fütterung von KPK an Seesaibling (Murray et al., 2014) beobachtet werden, sie sind jedoch kein Spezifikum für KPK. Allgemein bringt der Austausch fischbasierter Rohstoffe mit pflanzlichen Alternativen diese Veränderung mit sich (De Francesco et al., 2004). Um dem entgegenzuwirken, könnte gezielt eine Finisherration mit höherem Fischmehl/-ölanteil verwendet werden, die am Ende der Mast die ernährungsphysiologisch günstigen n3 Fettsäuren im Filet anreichert (Zajic et al., 2016). Interessanterweise war der Gehalt an gesättigten Fettsäuren (SFA) bei afrikanischen Welsen verringert, bei Europäischen Welsen jedoch erhöht, wenn sie mit KPK im Vergleich zu KON gefüttert wurden. Nur bei Salmoniden verringerte die Fütterung von KPK die einfach ungesättigten Fettsäuren (MUFA) in den Filets. Ein Anstieg der mehrfach ungesättigten Fettsäuren (PUFA) war nur bei Regenbogenforellen zu beobachten, die KPK erhielten. Auch zeigte die Auswertung der absoluten Fettsäuregehalte, dass die Welsarten die n3 Gehalte bei KPK Futter besser als die Salmoniden im Vergleich zu KON Futter halten konnten (Daten nicht gezeigt) und damit die Notwendigkeit einer Finisherfütterung mit Fischmehl/-öl bei Welsen geringer ist.

Einfluss Fischart: Die Filets vom Europäischen Wels wiesen die höchste Wasserhaltekapazität auf, während Filets der Regenbogenforelle den höchsten Garverlust verzeichneten. Im Vergleich zu anderen Fischarten hatten Filets des Europäischen Welses fütterungsunabhängig den höchsten Rohasche- und

Rohproteingehalt und gleichzeitig einen geringeren Anteil an Rohfett. Darüber hinaus wiesen Afrikanische Welsfilets die höchsten SFA-Gehalte und gleichzeitig niedrigere Konzentrationen an MUFA, PUFA und n6- und n3-Fettsäuren auf. Bei Europäischen Welsfilets waren bei niedrigstem Fettgehalt die PUFAs am stärksten vertreten, dementsprechend war bei dieser Fischart das n6:n3 Verhältnis am niedrigsten und damit am ernährungsphysiologisch günstigsten (Haupteffekt Fischart: $p<0,001$).

Schlussfolgerungen

Die Ergebnisse dieser Arbeit zeigen, dass die physikalische Filetqualität unverändert bleibt, jedoch auch bei der Fütterung von Steirischen Kürbiskernpresskuchen als Fischmehlalternative die beschriebene Reduktion von n3 Fettsäuren im Filet verzeichnet wird. Da sich bei geringem Fettgehalt diese Veränderung weniger stark ausprägen, scheint der Europäische Wels am günstigsten auf die Fütterung zu reagieren.

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Strategy for wheat processing by-products pre-treatment to produce safer feed stock

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Abstract

The aim of this study was to evaluate the influence of combining extrusion and fermentation processes on the biogenic amines (BA) and mycotoxin (My) concentration of wheat bran (WB). Extrusion was performed by testing two different temperatures (115 and 130°C) and three different speeds of extruder screw (16, 20, and 25 rpm). The lactic acid bacteria (LAB) strains (*Lactobacillus casei* and *Lactobacillus paracasei*) were used for WB fermentation. High-Performance Liquid Chromatography Coupled to Triple Quadrupole Mass Spectrometry was used for My analysis and High-Performance Liquid Chromatography with Ultra Violet detector was used for BA evaluation in WB. It was found, that in extruded and extruded/fermented WB, the total BA content was by two times lower than that in non-treated ones. The lowest My content in fermented WB extruded at 130°C using a screw speed of 20 and 25 rpm was established. It was concluded, that the combination of extrusion and fermentation can be prospective pre-treatment for WB, potentially capable to reduce BA and My concentrations.

Introduction

Most of the bioactive compounds in cereal are concentrated in the outermost tissues. On the other hand, undesired compounds (mycotoxins, etc.) also occur in these fractions. For these reasons, pre-treatment technologies to improve safety of WB are being studied. Fermentation with selected technological strains could increase bioavailability of WB functional compounds (Verni, Rizzello & Coda, 2019). Also, extrusion technology is known as causes many physicochemical and microbial transformations of stock, however, the data about the WB extrusion mainly describe the hydration properties or the solubility of WB dietary fibre (Bender, Goulart, Silva & Penna, 2019). Despite that LAB having GRAS (Generally Recognized as Safe) status, it should be pointed out, that during the technological fermentation process BA could be formed. For this reason, evaluation of BA formation in fermented stock must be included. Another safety aspect which should be analysed when technologies for by-product valorization are developed, is related to the occurrence of mycotoxins (My). Approximately 455 My have been identified that may be relevant to the food/feed supply chain, and approximately 65 have been evaluated in an international risk assessment; of the 390 unevaluated My, about 270 are reported to occur in one or more food/feed products, in addition to the evaluated My and their modified forms (van den Brand & Bulder, 2019). For this reason, the My concentration during the technological processes must be controlled, and the safest processes should be selected. The aim of this study was to evaluate the influence of combining extrusion and fermentation processes on the BA and My concentration of WB.

Material and methods

Wheat Bran and Lactic Acid Bacteria Strains Used in Experiments

WB (unprocessed and extruded at different temperatures in a Twin Screw extruder (Jinan Shengrun Machinery Co., Ltd., Jinan, China)) were obtained from the SME 'Ustukiu malunas' (Pasvalys, Lithuania). The temperature in the different extrusion zones was I – 60–61 °C, II – 70 °C, and III – 90 °C; moisture content was 20%, feed rate F was 8.2 ± 0.3 kg h^{-1}, and nozzle diameter was 6 mm. Extrusion experiment was performed by testing two different temperatures (samples were extruded at 115 °C and at 130 °C) and three different speeds of extruder screw (16, 20, and 25 rpm). A non-extruded WB were analysed as a control (W_{Con}). Four different treated WB samples were prepared (W_{ex115} – extruded at 115 °C with a screw speed of 16 rpm; $W_{ex130/screwspeed16}$ – extruded at 130 °C and 16 rpm; $W_{ex130/screwspeed20}$ – extruded at 130 °C and 20 rpm; $W_{ex130/screwspeed25}$ – extruded at 130 °C and 25 rpm). The LAB strains *L. casei* and *L. paracasei* were used for WB fermentation. The LAB strains were obtained from the Lithuanian University of Health Sciences (Kaunas, Lithuania). Pure LAB strains were multiplied in MRS broth (de Man–Rogosa–Sharpe, CM 0359, Oxoid Ltd, Hampshire, UK) at 30 ± 2 °C for 48 h, before use for the fermentation of WB. The WB, water, and a suspension of LAB strain (3% of dry matter of the wheat bran mass) containing 8.9 $\log_{10}$ CFU mL^{-1} were fermented at 30 ± 2 °C for 24 h. For 100 g of WB, 60 mL water was used.

Evaluation of Biogenic Amines and Mycotoxins concentration in Wheat Bran

The extraction and determination of BA in WB samples followed the procedures developed by Ben-Gigirey, Vieites Baaptista de Sousa, Villa, and Barros-Velazquez (1999). For My analysis High-Performance Liquid Chromatography Coupled to Triple Quadrupole Mass Spectrometry (HPLC-MS/MS) was used (Vadopalas et al., 2020).

Statistical Analysis

Results were expressed as the mean (n = 3). In order to evaluate the effects of the different treatments, the data were analysed by multivariate analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) procedure, as post-hoc tests. The results were recognized as statistically significant at $p \leq 0.05$.

Results and discussion

Biogenic Amine Formation in Wheat Bran Samples

The BA concentrations in WB are shown in Table 1. No PHE, TYR, or SPRMD was found in untreated or treated WB.

CAD and HIS were found only in W_{Con} samples. Comparing non-fermented WB, the highest concentration of PUT was established in $W_{ex130/screwspeed25}$ samples. In other non-fermented WB (W_{Con}, W_{ex115}, $W_{ex130/screwspeed16}$, $W_{ex130/screwspeed20}$, and $W_{ex130/screwspeed25}$) the PUT concentration was, on average, 33.6%, 29.1%, 9.4%, and 20.8% lower, respectively. Comparing the PUT concentration in non-fermented and fermented WB groups, the PUT concentration was increased in W_{ConLc}, $W_{ex115Lc}$, $W_{ex115Lpa}$, and $W_{ex130/screwspeed20Lc}$, compared with non-fermented ones (on average by 15.9%, 15.0%, 16.5%, and 18.0%, respectively). In other fermented WB, the PUT concentration was reduced compared with that in non-fermented WB. In non-fermented WB, the highest SPRM concentration was found in W_{Con}; in the other samples analysed the SPRM concentration was, on average, 3.5 times lower, and fermentation with both LAB strains reduced the SPRM concentration in non-extruded WB. However, after fermentation, the SPRM concentration increased in $W_{ex115Lc}$ and $W_{ex115Lpa}$ (on average 27.3 and 36.7% higher, respectively). There was a significant effect of the type of LAB applied for the fermentation and of extrusion, as well as interaction of these factors, on the PUT, CAD, HIS, and SPRM concentration in WB ($p \leq 0.001$). BA are generally synthesized through microbial decarboxylation of the corresponding amino acids by decarboxylases, and decarboxylase activity is related to bacterial strain rather than to species

or genus Barbieri et al. (2019). BA at low concentrations occur naturally in cells and have many important physiological functions; however, at high concentrations they may have an undesirable impact on mammals health (Flasarová et al., 2016).

Table 1. The BA (mg kg^{-1}) concentration in treated and nontreated WB

	BAs concentration, mg kg^{-1}						
	PHE	PUT	CAD	HIS	TYR	SPRMD	SPRM
W_{Con}	nd	102.4	41.3	63.6	nd	nd	111.9
W_{ConLc}	nd	121.8	nd	nd	nd	nd	33.2
W_{ConLpa}	nd	77.6	nd	nd	nd	nd	29.5
W_{ex115}	nd	109.2	nd	nd	nd	nd	26.1
$W_{ex115Lc}$	nd	128.4	nd	nd	nd	nd	35.9
$W_{ex115Lpa}$	nd	130.7	nd	nd	nd	nd	41.2
$W_{ex130/screwspeed16}$	nd	139.6	nd	nd	nd	nd	34.8
$W_{ex130/screwspeed16Lc}$	nd	106.2	nd	nd	nd	nd	32.5
$W_{ex130/screwspeed16Lpa}$	nd	112.2	nd	nd	nd	nd	35.2
$W_{ex130/screwspeed20}$	nd	122.1	nd	nd	nd	nd	31.5
$W_{ex130/screwspeed20\ Lc}$	nd	148.9	nd	nd	nd	nd	37.7
$W_{ex130/screwspeed20Lpa}$	nd	95.9	nd	nd	nd	nd	26.3
$W_{ex130/screwspeed25}$	nd	154.1	nd	nd	nd	nd	32.5
$W_{ex130/screwspeed25Lc}$	nd	106.9	nd	nd	nd	nd	29.5
$W_{ex130/screwspeed25Lpa}$	nd	121.2	nd	nd	nd	nd	38.7

W – wheat bran; Con – not extruded wheat bran; Lc – fermented with *Lactobacillus casei*; Lpa –fermented with *Lactobacillus paracasei*; $_{ex115}$ – extruded at 115°C, screw speed 16 rpm; $_{ex130/screwspeed16}$ - extruded at 130°C, screw speed 16 rpm; $_{ex130/screwspeed20}$ - extruded at 130°C, screw speed 20 rpm; $_{ex130/screwspeed25}$ - extruded at 130°C, screw speed 25 rpm. BAs – biogenic amines; PHE – phenylethylamine; PUT – putrescine; CAD – cadaverine; HIS – histamine; TYR – tyramine; SPRMD – spermidine; SPRM – spermine; nd – not determined. Data are represented as means (n = 3) ± SE

Influence of the Different Treatments on Mycotoxin Concentration in Wheat Bran

Mycotoxin concentration in treated and nontreated WB is shown in Table 2. After extrusion, different tendencies were established for changes in AOH concentration: in W_{ex115} it was reduced, on average by 2.1 times, in $W_{ex130/screwspeed16}$ it was reduced, on average by 56.8%, in $W_{ex130/screwspeed20}$ it remained similar, and in $W_{ex130/screwspeed25}$ it increased, on average by 1.9 times, compared with W_{Con}. Comparing the AOH in fermented and non-fermented samples, a lower AOH concentration was found in most of the fermented samples. Comparing the AME concentration in non-fermented and fermented samples, different tendencies were found, and according to Janic Hajnal et al. (2020), reduction of *Alternaria* toxins is different in different steps in wheat processing, and better results are found for LAB fermentation compared to extrusion.

Comparing non-fermented and fermented WB, fermentation with *L. paracasei* reduced the 17-DMAG concentration in all WB extruded at 130 °C, and fermented with *L. casei* reduced the 17-DMAG concentration in $W_{ex130/screwspeed25Lc}$ (on average, by 12.2%). Comparing the 15-DON concentration in non-fermented WB, a higher concentration of 15-DON was established in all extruded WB, compared with W_{Con}, and comparing non-fermented WB, the highest concentration of DON was found in non-extruded WB (58.8 μg kg^{-1}), and in most cases (except in $W_{ex115Lc}$), DON concentration showed tendencies to reduce after fermentation with both LAB strains. In most of the fermented WB no D3G was established. DON is a *Fusarium graminearum* and *F. culmorum* metabolite; however, 15-DON, D3G, and 3-ADON are produced during biosynthesis of DON (Wipfler et al., 2019). After fermentation, no fusarenon X (FUSX) remained in $W_{ex130/screwspeed16Lpa}$, $W_{ex130/screwspeed20Lc}$, $W_{ex130/screwspeed20Lpa}$, $W_{ex130/screwspeed25Lc}$, or $W_{ex130/screwspeed25Lpa}$ samples. FUSX belongs to the B trichothecene group; however, in most cases, no FUSX is found or it is found at a low concentration in livestock feed (Nualkaw et al., 2020). The meleagrin (MEL) concentration was increased by 3.7 times in non-extruded WB fermented with *L. casei* compared with non-fermented ones. The 15ACS concentration in WB was increased after fermentation. Comparing non-fermented and fermented WB, in most cases, fermentation reduced the T-2 concentration. It was reported that T2 toxin concentration can be reduced during cereal based substrates fermentation and LAB strains has affinity for the 12,13-epoxy ring responsible for the toxicity of T-2 (Adhikari et al., 2017). After fermentation, HT-2 was established in W_{ConLc}, $W_{ex115Lpa}$, and $W_{ex130/screwspeed16Lpa}$ samples.

In opposite, the NEO concentration after fermentation showed tendency to reduce. The enniatin A (ENN A) concentration was increased after fermentation of extruded WB.

Table 2. Mycotoxin concentration in treated and nontreated WB

My	Cereal by-product samples														
	W_{Con}	W_{Con} Lc	W_{Con} Lpa	W_{ex115}	$W_{ex115Lc}$	$W_{ex115Lpa}$	$W_{ex130/screwspeed16}$	$W_{ex130/screwspeed16Lc}$	$W_{ex130/screwspeed16Lpa}$	$W_{ex130/screwspeed20}$	$W_{ex130/screwspeed20Lc}$	$W_{ex130/screwspeed20Lpa}$	$W_{ex130/screwspeed25}$	$W_{ex130/screwspeed25Lc}$	$W_{ex130/screwspeed25Lpa}$
	Mycotoxins, µg kg^{-1}														
AOH	1.76	1.01	0.82	0.85	0.64	0.72	1.00	0.65	0.75	1.80	1.51	3.55	3.29	1.34	1.44
AME	nd	1.40	nd	nd	2.53	0.87	0.85	0.91	nd	0.51	nd	1.13	3.45	2.45	1.51
17-DMAG	nd	nd	nd	nd	nd	nd	0.34	0.30	0.23	0.79	0.87	0.71	0.82	0.56	0.72
15-DON	50.1	70.4	32.0	75.2	77.8	84.3	106	19.0	15.8	85.6	nd	nd	83.4	nd	25.2
MEL	0.30	1.11	0.14	0.43	0.18	0.25	0.19	0.05	0.04	0.16	0.02	nd	0.05	nd	0.03
Neo	nd	nd	nd	0.10	0.05	0.03	0.08	nd	0.06	0.06	0.04	0.05	0.07	nd	nd
15ACS	15.2	11.6	13.40	1.20	1.50	1.24	nd	1.32	1.53	1.31	1.83	3.09	nd	2.17	1.58
ENN A	5.31	1.63	1.96	1.61	65.7	66.2	1.34	14.7	33.7	1.29	1.97	4.31	1.29	1.42	1.42
ENN A1	1.24	0.47	0.38	0.35	19.1	16.1	0.25	3.22	6.77	0.25	0.31	0.89	0.26	0.32	0.27
FB1	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.91	0.72	1.16	0.07	0.06	0.05
FB2	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.07	0.04	0.28	nd	nd	nd
DON	58.8	55.6	48.8	45.1	46.8	42.5	32.9	29.4	27.1	24.4	21.4	15.2	23.2	16.2	18.0
STC	nd	0.11	nd	0.11	0.37	0.26	0.70	0.73	1.09	1.76	1.65	1.94	1.96	1.47	1.66
OTB	nd	nd	nd	nd	0.14	0.11	nd	nd	nd	nd	nd	nd	nd	nd	nd
FUSX	7.66	1.93	2.43	4.05	2.98	4.94	4.54	1.99	nd	4.90	nd	nd	4.35	nd	nd
T-2	0.18	0.22	0.19	0.89	0.68	0.88	0.98	0.68	0.85	0.85	1.10	0.78	1.48	0.91	0.87
HT-2	3.76	1.99	nd	2.85	nd	7.25	nd	nd	4.01	nd	nd	nd	nd	nd	nd
OTA	1.81	0.66	0.61	1.68	4.32	3.17	0.63	1.11	1.39	0.29	0.37	0.37	0.54	0.44	0.44
D3G	3.93	nd	nd	2.79	nd	0.89	1.83	nd	nd	1.05	nd	nd	1.11	nd	nd
AFB1	3.20	1.55	1.72	2.55	2.98	3.18	1.70	1.84	1.76	0.91	0.99	2.42	0.92	0.76	0.87

My – mycotoxins; W – wheat bran; Con – not extruded wheat bran; Lc – fermented with *Lactobacillus casei*; Lpa – fermented with *Lactobacillus paracasei*; $_{ex115}$ – extruded at 115°C, screw speed 16 rpm; $_{ex130/screwspeed16}$ - extruded at 130°C, screw speed 16 rpm; $_{ex130/screwspeed20}$ - extruded at 130°C, screw speed 20 rpm; $_{ex130/screwspeed25}$ - extruded at 130°C, screw speed 25 rpm. AOH – Alternariol; AME - Alternariol monomethyl ether; 17-DMAG - 17-dimethylaminoethylamino-17-demetoxygeldanamycin; 15-DON - 15-Acetyldeoxynivalenol; MEL – Meleagrin; Neo – Neosolaniol; 15ACS - 15-Acetoxyscirpenol; ENN A - Enniatin A; ENN A1 - Enniatin A1; FB1 - Fumonisin B1; FB2 - Fumonisin B2; DON – Deoxynivalenol; STC – Sterigmatocystin; OTB - Ochratoxin B; FUSX - Fusarenon X; T-2 - T-2 Toxin; HT-2 - HT-2 toxin; OTA - Ochratoxin A; D3G - Deoxynivalenol-3-glucoside; AFB1 - Aflatoxin B1. Data are represented as means (n = 3). nd – not determined

However, fermentation with both LAB strains tested reduced the ENN A1 concentration in non-extruded fermented WB (on average by 1.5 times), the opposite to that observed for extruded fermented WB, in which the ENN A1 concentration after fermentation was increased (except $W_{ex130/screwspeed25Lpa}$). ENN A and ENN A1 are secondary non-ribosomal *Fusarium* metabolites which are synthesized by the multifunctional enzyme enniatin synthetase (ESYN1) (Liuzzi et al., 2017). FB1 was found in the $W_{ex130/screwspeed20}$ and $W_{ex130/screwspeed25}$. Fumonisin B2 (FB2) was established only in the $W_{ex130/screwspeed20}$ group of WB, with the highest concentration in $W_{ex130/screwspeed20Lpa}$ (0.28 µg kg^{-1}). Fumonisins are carcinogenic and genotoxic mycotoxins and they can have a potential regenerative cell proliferation effect in animals (Ostry, Malir, Toman & Grosse, 2017). No sterigmatocystin (STC) was established in W_{Con} or W_{ConLpa}. Fermentation with both LAB strains reduced the AFB1 concentration in W_{Con}, on average by 2 times. Also, fermentation reduced the AFB1 concentration in $W_{ex130/screwspeed25Lc}$ and $W_{ex130/screwspeed25Lpa}$ (on average by 17.4% and 5.4%, respectively). However, in other extruded WB, fermentation showed a tendency to increase the AFB1 concentration. STC is considered a carcinogen, and its molecules are stable during thermal processes (Viegas, Nurme, Piecková, & Viegas, 2020). Comparing non-fermented WB, in all cases a lower OTA concentration was found in extruded WB. Ochratoxin B (OTB) was only found in two fermented samples, $W_{ex115Lc}$ and $W_{ex115Lpa}$. ANOVA indicated that there was a significant

effect of the type of LAB applied for the fermentation and of extrusion, as well as interaction of these factors, on all the mycotoxin concentrations analysed in WB samples ($p \leq 0.001$), and the lowest total indicated mycotoxin concentration was found in $W_{ex130/screwspeed20}$ and $W_{ex130/screwspeed25}$ samples fermented with both LAB strain.

Conclusion

Extrusion, as well as extrusion in combination with fermentation reduces total BA content (on average, 2 times). The lowest mycotoxin concentration was found in $W_{ex130/screwspeed20}$ and $W_{ex130/screwspeed25}$ samples fermented with both LAB strains. Finally, the combination of extrusion and fermentation can be confirmed as a prospective pre-treatment for WB, potentially capable of enhancing its safety characteristics.

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Using *Pediococcus pentosaceus* as ensiling agent in grain silage

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Abstract

Lactic acid bacteria (LAB) are a group of bacteria that has lactic acid as the major metabolite resulting after sugar fermentation. Prevention of mycotoxigenic fungi growth and detoxification of mycotoxins by LAB inoculants and limiting the development of pathogenic bacterial microflora through controlled fermentation with the participation of LAB and the presence of their metabolites is the main goal. Although mycotoxins are present in a range of livestock feeds including grains, green forages, hays, and silages, studies on mycotoxins formation or inhibition pattern during ensiling formed the basis of ruminant nutrition for decades. Due to the ban of antibiotics as growth in the European Union in 2006, growing interest for the fermentation process and the improvement expected on the nutritive value of ensiled grains increased considerably to reduce mycotoxigenic fungi growth. As presented in this work, the LAB *Pediococcus pentosaceus* LBM18 has important antifungal activity against several common fungi of silage, showing its potential to be used as inoculant for silage, acting as a biocontroller of the growth of mycotoxigenic fungi and their mycotoxins.

Introduction

In agricultural systems, silages are artificial ecosystems, mostly carried out as solid-state fermentation process, aiming to keep or improve the nutritive value and to avoid deterioration of the conserved biomass (grass, maize), with interactions among physical (e.g. environment temperature), chemical (e.g. silage composition) and biological (e.g. epiphytic microorganism) agents. In general, the process of fermentation, aiming biomass conservation, focuses on pH decrease in shortest time. Mycotoxigenic fungi growth is a problem as they exhibit a high capacity of adaptation and have acquired the ability to resist chemical treatments and some preservatives. Epiphytic or inoculated LAB dominate and displace the rest of the flora through acid formation and competition for nutrients, stabilizing the silage for the purpose of conservation of feed. LAB has lactic acid as the major metabolite resulting after sugar fermentation, and most of the LAB strains in silage, which are able to exhibit such capacities, belong to the genera *Lactobacillus*, *Pediococcus* and *Lactococcus* (Müller et al., 2001). *P. pentosaceus* is one of the promising LAB to be used as ensiling agent, since this species was found to fight against several fungi, especially *Aspergillus*, *Penicillium*, *Fusarium* and *Candida albicans* (Jiang et al., 2021), suppressing or inhibiting the production of mycotoxins (Sellamani et al., 2016). In this context, this work aimed to evaluate *P. pentosaceus* LBM18 as a ensiling agent against fungi.

Material and methods

Ensiling: corn grains was ensiled at SAZ Animal Nutrition, São Paulo, Brazil. The additive used in this trial was a lyophilized inoculant containing 2×10^{11} CFU/g of *P. pentosaceus* LBM18 (Azevedo et al., 2020). The inoculant was diluted in sterile deionized water and applied using a manual sprayer. Three silos were done per treatment (control and treated silage).

Microbiological assay: Approximately 300 g of samples (control and treated silos) were collected and vigorously homogenized for viable counts measurement. Afterwards, 10 g of samples (control and treated silos) were diluted with 90 mL of sterile deionized water and homogenized. Serial dilutions were pour-plated (10^{-1} to 10^{-10}; three replicates per dilution) on Di-Clorine Rose Bengal Chloramphenicol agar and Sabouraud agar supplemented with chloramphenicol (0.04 g/L). The fungal colonies were isolated from the Petri dishes of the control silage (without inoculant treatment) and treated silage for further *in vitro* analyses.

Results

In vitro experiments were performed to evaluate the antifungal effect of the inoculant *P. pentosaceus* LBM18. After fungi isolation from corn grains silage in Petri dishes, different filamentous fungi were cultivated in the presence (treated sample) or absence (control sample) of the inoculant, aiming to demonstrating qualitative results. The results clearly showed inhibition of fungi growth and morphological changes in pigmentation, sporulation and aerial mycelium. First, the inoculant was evaluated against *Acremonium* spp. (Figure 1) isolated from Austria corn grains silage (Bueno et al., 2018) and also against *Penicillium* spp. and *Aspergillus* spp. (Figure 2) isolated from Brazilian corn grains silage. To observe the antifungal effect of the inoculant on fungi of silages from different origins (Austria and Brazil), *Acremonium* spp., isolated from Austria silage, was used to compare de antifungal effect of the inoculant under study between fungi isolated from Brazilian silage (*Penicillium* spp. and *Aspergillus* spp.). Significant morphological changes on *Penicillium* spp. (Figure 3), isolated from Brazilian corn grains silage, was also observed when the inoculant was tested at three different concentrations (0.2 mg/mL, 2 mg/mL and 20 mg/mL).

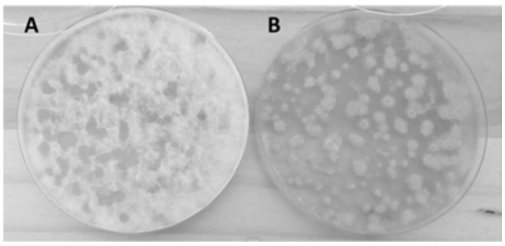

Figure 1. Antifungal effect against *Acremonium* spp. (10^{-3}), isolated from Austrian corn grains silage, after 24 h of cultivation in the absence (A) or in the presence (B) of the inoculant *P. pentosaceus* LBM18. Plates were incubated at 25°C for 30 days

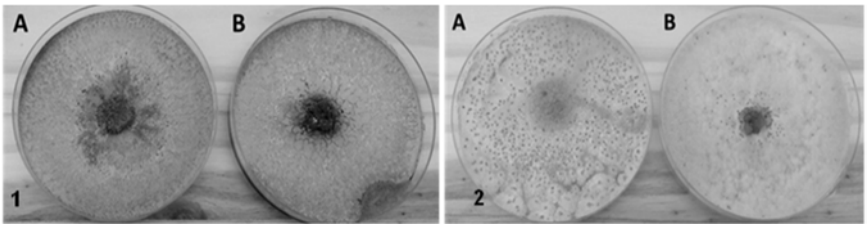

Figure 2. Morphological changes on the (1) *Penicillium* spp. and (2) *Aspergillus* spp. colonies isolated from Brazilian corn grain silage after 7 days of ensiling. Fungi were isolated from (A) control silo (absence of the inoculant *P. pentosaceus* LBM18) and treated silo (presence of the inoculant *P. pentosaceus* LBM18)

To assess the effect of the inoculant on the production of mycotoxins, two species of mycotoxins-producing fungi were used: *Aspergillus nomius* and *Alternaria alternata*. Focusing on *A. nomius*, as shown in Figure 4, the greatest reduction in aflatoxins (B1, B2, G1 e G2) production (32.9, 50, 63 and 40%, respectively) was at the lowest concentration (0.2 mg/mL) of the inoculant compared with control. At 2 mg/mL, there was reduction of aflatoxins B1 and G1 of 15.1 and 48.7%, respectively and, at the highest concentration (20 mg/mL), there was no reduction in the production of these aflatoxins. Another example is the *A. alternata* and the tenuazonic acid (TeA) production. As a pathogen, it is relevant in cereals (black fungi). Regarding the TeA production by *A. alternata* (Figure 5), a significant reduction

was observed in all tested concentrations (0.2, 2 and 20 mg/mL). However, at the lowest concentrations (0.2 and 2 mg/mL), the reduction of TeA production was the highest, representing 75.1 and 73% reduction in the production of this mycotoxin, respectively. At the highest concentration (20 mg/mL), the reduction was about 19% compared with control.

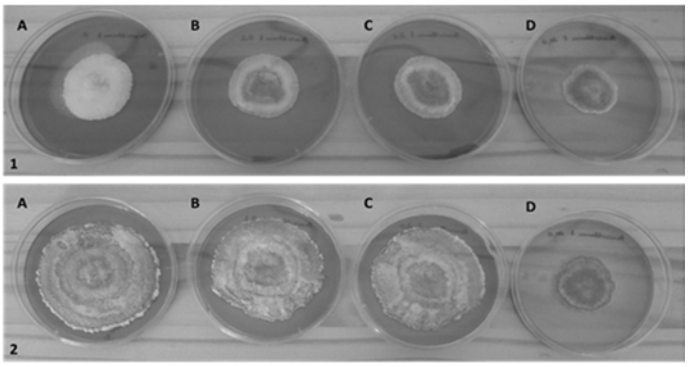

Figure 3. Antifungal effect against *Penicillium* spp., isolated from Brazilian corn grains silage, after 24 h of cultivation in the absence (A) or in the presence of the inoculant *P. pentosaceus* LBM18 at 0.2 mg/mL (B), 2 mg/mL (C) and 20 mg/mL (D). Plates were incubated at 25ºC for 13 days (1) and 21 days (2)

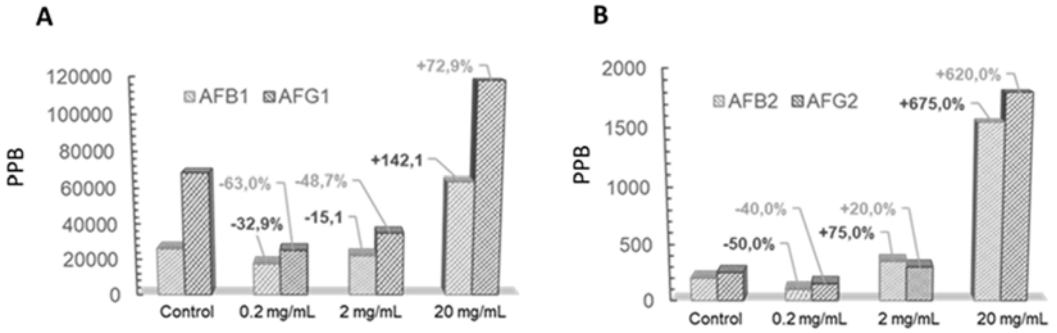

Figure 4. Aflatoxins B1, B2, G1 and G2 production by *Aspergillus nomius* after cultivation at 25ºC for 07 days in the presence of the inoculant *P. pentosaceus* LBM18 at different concentrations (0.2, 2 and 20 mg/mL)

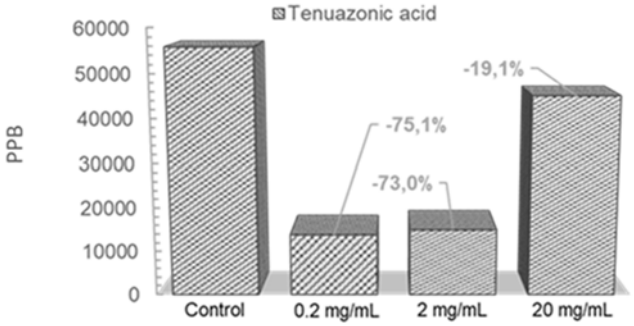

Figure 5. Tenuazonic acid production by *Alternaria alternata* after cultivation at 25ºC for 07 days in the presence of the inoculant *P. pentosaceus* LBM18 at different concentrations (0.2, 2 and 20 mg/mL)

Discussion

The application of LAB strains are particularly effective for grain silage preservation, whereas they are substrates naturally susceptible to fungal and mycotoxin contamination. Taking into account the production of aflatoxins by *A. nomius*, results suggest that the inverse dose-dependent (Figure 4) response to the treatment with the inoculant was due to the disrupting quorum signaling (Martín-Rodríguez et

al., 2014) and it may also be by the consumption by fungi of some metabolite produced by *P. pentosaceus* LBM18. Specifically for *Aspergillus nomius*, the inoculant at the highest concentration (20 mg/mL) may work as substrate for fungi growth modulating mycotoxin production, since high content of carbohydrates (e.g. glucose, ribose, sucrose, xylose and glycerol), as occurring in grain silage, is more favorable to mycotoxin production. Production of mycotoxins not always depends on fungal growth, however, biochemical factors (e.g. C and N content) and environmental factors (e.g. humidity, temperature, water deficit) usually correlates with the induction of fungal growth and mycotoxin accumulation (Kumar et al., 2021). These results suggests that the inoculant *P. pentosaceus* LBM18 affects both mycelium growth and the general fitness of the fungi evaluated.

The anti-mycotoxigenic effect of *P. pentosaceus* LBM18 was confirmed by a significant reduction of tenuazonic acid (TeA) by *A. alternata* in all tested concentrations (Figure 5). Several studies have reported the potential of LAB to control mycotoxin production; however, there is no data about the effect of LAB on TeA producing species. In recent review of LAB as antifungal and anti-mycotoxigenic agent, the authors highlight the scarcity and the need for research on the potential of these microorganisms to eliminate emerging mycotoxins. According to Sadiq et al. (2019), there are several ways that LAB can affect mycotoxin production or availability, including direct influence on fungal growth leading to inhibition of mycotoxin production, chemical modification of the growth media or the environment, mycotoxin removal through adsorption or degradation process. Therefore, it is possible to suggest that *P. pentosaceus* LBM18 affected the TeA production of *A. alternata* by reducing the mycelium growth and removing the toxin through an adsorption mechanism.

Conclusion

P. pentosaceus LBM18 seems to be a promising inoculant for corn grains silage, since it is able to promote important morphology changes of fungi (i.e. sporulation, decrease aerial mycelium), and also by suppressing the growth of the most prevalent mycotoxigenic fungi found in silage (e.g. *Aspergillus* spp., *Penicillium* spp.), reducing mycotoxins contamination and maintaining the quality and nutritional value in silages, generating improved animal performance after silage intake.

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In-vitro evaluation of phytogenic formulation: antimicrobial effects

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Abstract

Clostridium perfringens belongs to the normal gut microbiota of vertebrates (1); its overgrowth in the intestine is linked to diseases, like necrotic enteritis in poultry (2). Most antimicrobial formulations used for gut health do not discriminate between beneficial and pathogenic bacteria (3). This *in-vitro* study shows strong antimicrobial activity of a phytogenic formulation (PFA) against *C. perfringens*, with less effect on *Lactobacilli spp.*

Material and methods

Experiments were conducted at EWNI, using the agar dilution method. Agar was prepared by adding PFA to: (a) MRS Agar (*Lactobacillus* spp.) and (b) Robertson's cooked meat (RCM) agar (*C. perfringens*) at: 0 µg/mL – control; 750 µg/mL; 1000 µg/mL and 1250 µg/mL. Subsequently, *Lactobacillus* spp. were cultured microaerophylically in MRS broth, and *C. perfringens* anaerobically in RCM broth, at 37°C. Cultures were adjusted to OD 1 and serial diluted. 100 µl were spread on the surface of the prepared agar. MICs were defined after 24 hours.

Results

The PFA decreased *C. perfringens* in a dose-dependent manner. At 750 µg/mL, scarce colonies were observed; and at 1000 µg/mL, no growth was observed. Concerning *Lactobacillus* spp., even at the highest concentration (1250 µg/mL) the effects were minimal for *L. agilis* and *L. acidophilus*, while *L. casei and L. plantarum* were unaffected.

Conclusions

The PFA impaired *C. perfringens* growth, with negligible effect on *Lactobacillus spp.* These *in-vitro* results indicate that an optimal combination of phytogenics can be effective in modulating gut microbiota in the right direction, contributing to the gut health and performance of the animals.

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Possibilities for the reduction of ASF virus in feed through bioactive substances

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Abstract

Combating the spread of African Swine Fever Virus (ASFV) is currently a top priority worldwide. The spread of the virus poses a serious challenge to pig production, with the threat of major losses. Detection of the virus is becoming more frequent in various countries around the world. Measures such as culling, the establishment of quarantine zones and restrictions on the movement of pigs lead to serious problems that affect animal welfare, productivity and marketability.
To prevent the spread of the virus, various biosecurity concepts must be followed. One of these concepts is the prevention of transmission via feed. Several studies have already shown that the use of feed additives can help to reduce the viral load. Based on this knowledge, prevention of virus transmission through feed is possible.
So far, most studies which have been published dealt only with chemical substances and their transmission-inhibiting properties. However, it has already been shown in the past that plant-based ingredients are active against viruses or bacteria. For this study, therefore, the positive effect of specifically selected bioactive substances from plants on the spread of ASFV in feed was investigated. Two different plant extracts, were examined for their effect, both individually and in combination. These results were then compared with the mode of action of SCFA. The results showed that the plant extracts exhibited significantly higher efficacy against African Swine Virus than SCFAs, suggesting that they could reduce the spread of the virus through feed.

Introduction

Since the introduction of porcine epidemic diarrhoea virus (PEDV) into the United States in 2013, it has become apparent that swine diseases can be both introduced and transmitted through feed and feed ingredients. Due to the transregional sale of feed for pig production, diseases can spread quickly. The ASFV poses a significant menace to the global protein supply, with an extremely negative impact on pork production and consumption (Woonwong et al., 2020).

As already described by EFSA, this virus can be transmitted via different ways, such as infected live animals or swill feeding. Feed or feed ingredients are other possible modes of transmission. Though the possibility has been categorised as low by EFSA, it would be too risky to exclude it completely. The virus can be transmitted via mash as well as pelleted compound feed. In feed matrices the virus is stable for up to 30 days. (EFSA AHAW Panel, 2021). Because ASFV is a very complex virus with unknown protective antigens, controlling the infection or developing a vaccine is very difficult (Rock, 2017).

For this reason, it is very important to stop the virus quickly and prevent it from spreading widely, as it poses a huge risk to pig production. Apart from comprehensive biosecurity measures, a look at improving feed safety via suitable feed additives seems promising. While organic acids and other chemical additives are commonly used to control bacterial contamination, e.g. *Salmonella ssp.*, in feed and have recently also been studied for their efficacy against viral infections including ASFV (Isabel Rodriguez Amado, 2013; Trudeau et al., 2016), antiviral properties of secondary plant metabolites in feed are less known.

The aim of the current study therefore was to verify the effects of two specific plant extracts on ASFV infectivity in feed and thus their suitability as a means of controlling infection.

Material and methods

A screening study was conducted in which the effect of bioactive plant substrates against ASFV in feed was tested against short chain fatty acids (SCFA). In the following, the bioactive substances are referred to as plant extract 1 (PE-1) and plant extract 2 (PE-2). For the analysis, aliquots of 100 g of feed were placed in paper bags (similar to commercial feed bag). The amount of additive PE-1 and PE-2 was adjusted to the 100 g feed sample. Another set of bags was used as paired controls without the addition of any feed additive (Positive Control) and another without virus and feed additives (Negative Control). After the additives were added to the appropriate bags, 10 ml of ASFV solution with a viral concentration of 10^8 HAD_{50}/ml were added to the positive control and the treatments followed by shaking to mix well. All bags were incubated at room temperature. After incubation for one, three, or seven days, the surviving viruses were eluted from the samples with a medium solution. The obtained samples were then analysed by cell culture method for observation of cytopathogenic effect. The importance of this test is based on the ASFV specificity to haemadsorb in leukocyte cultures, a property that is unique among swine viruses.

Table 1: Treatment design

No.	Treatment	Additive	Dosage
1	Positive Control (Virus + Feeds)	-	-
2	Treatment 1	SCFA	3.0 [kg/to]
3	Treatment 2	PE-1	0.4 [kg/to]
4	Treatment 3	PE-2	0.1 [kg/to]
5	Treatment 4	PE-2	0.5 [kg/to]
6	Treatment 5	PE-1 + PE-2	0.4 + 0.5 [kg/to]
7	Treatment 6	PE-1 + PE-2	0.4 + 0.1 [kg/to]
8	Negative Control (no Virus)	-	-

PE-1: plant extract 1; PE-2: plant extract 2, SCFA: short chain fatty acid

Results

The ASFV titration assay showed that SCFA, PE-1, and PE-2 reduced viral load of ASFV in the feed from day one to seven (Figure 1). A marked decrease in viral HAD_{50} titers could be observed for all treatments already at day one with treatment 6 having the most effect followed by treatment 5, 2, 4, 3 and 1 respectively. The viral HAD_{50} titers in all samples treated with either the plant extracts or combinations thereof were below the detection limit of 1.5 log10HAD_{50}/ml by day three, while it took seven days for the SCFA treatment.

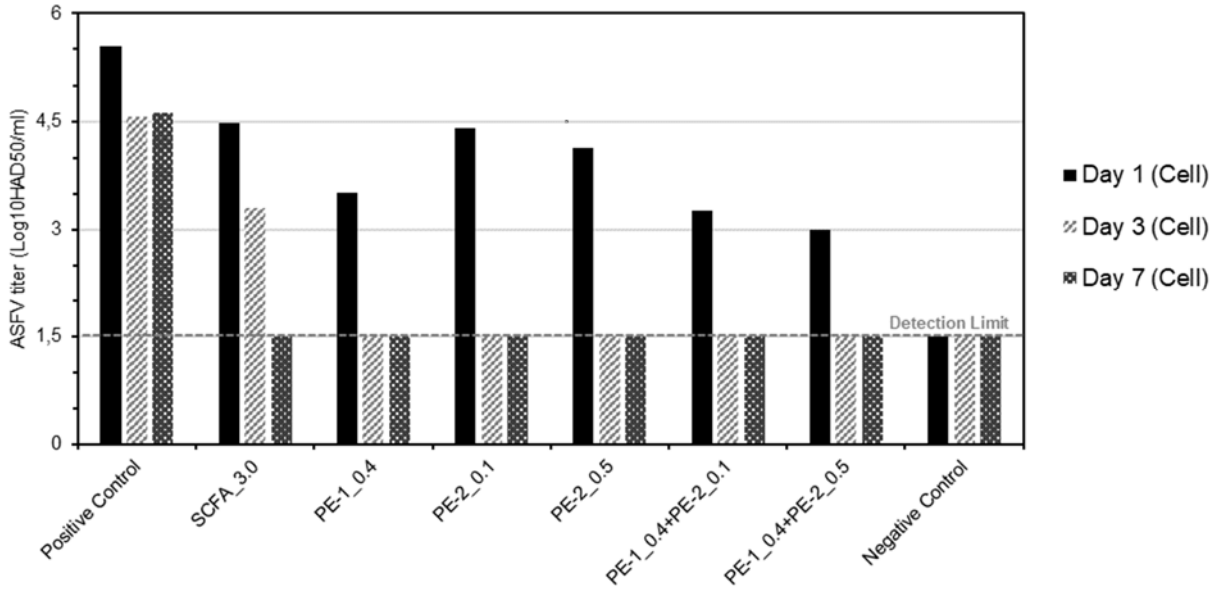

Figure 2: Assessment of ASFV kinetics by Log10HAD$_{50}$

Discussion

African Swine Fever (ASF) presents one of the greatest risks to global swine production today. It is a highly contagious, generalised disease of pigs caused by an iridovirus of the *Asfarviridae* family (FAO, 22.2.2022). This exhibit different virulence depending on the strain. The ASFV is an enveloped double-stranded DNA virus (Galindo & Alonso, 2017). It is also characterised by a very high resistance to physical and/or chemical inactivation. The pathogen can remain viable for a long time in blood, faeces, and tissues. These properties make it particularly important to combat the virus quickly and prevent it from spreading. Control of ASFV is mainly focused on eradication policies and strict enforcement of quarantine. Although feed is not the main route of transmission, it is still a risk (EFSA AHAW Panel, 2021) and should not be neglected. The fact that the virus can survive in feed for several days was confirmed in the present study in which a high virus concentration could still be detected in the feed after seven days in the positive control.

For this reason, several researchers have already been looking for ways to improve feed safety by using chemical additives like formaldehyde, benzoic acid or other SCFA (Niederwerder et al., 2021; Zhai et al., 2021).

The present trial could also show a reduction of viral load in feed with the application of SCFA. At the same time, the trial demonstrated that the two selected plant extracts had an even better mitigating effect. Bioactive substances from plants like essential oils have been extensively studied concerning their antibacterial, antifungal and anti-inflammatory properties. Antiviral studies have focused on enveloped virus because of the described mode of action via interaction with the envelope (di Sotto et al., 2018; Ma & Yao, 2020). Since ASFV is also an enveloped virus, a similar mode of action could be proposed for the selected plant extracts in the present study. Their effect was more pronounced than that of SCFA in this study.

The results of the trial showed that all three additives SCFA, PE-1 and PE-2 had an effect to decrease the survivability of ASFV in feed and demonstrated rapid viricidal activity against ASFV. In this case, our study could prove that SCFAs have a negative effect on ASFV and that its concentration in feed can be reduced. However, the study also showed that plant extract 1 and plant extract 2 have a much higher effect against the ASFV. In this case, a reduction of the virus concentration below the detection limit could already be achieved after three days compared to seven days with SCFA. The combination of both

plant extracts (PE-1 + PE-2) proved to be particularly effective. A strong reduction in infectivity compared to the positive control (from Log10 HAD_{50} 5.5 to 3.0) could be observed. This indicates that feed which has been treated with the bioactive substances used in this study is less liable to transmit ASFV and that an infection through feed is no longer possible after three days.

Conclusion

The study showed that by adding certain bioactive substances to the feed, the risk of ASFV transmission through the feed can be mitigated. Phytogenic additives can potentially reduce the viral load in feed and improve feed security. Suitable plant extracts can therefore be part of a comprehensive biosecurity concept to control the spread of AFSV.

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