

Scotland's Rural College

## **Bacterial 6-phytase supplementation can help improve bone mineralisation, phosphorus digestibility and growth performance of broilers fed corn-soyabean meal-based diet**

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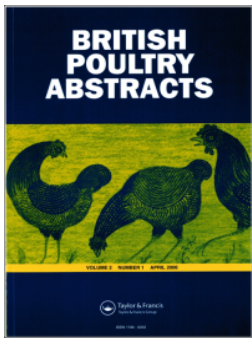
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## 2023 Abstracts

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## 2023 Abstracts

Oral communications and invited talks accepted for presentation at the WPSA UK Branch Meeting held on the 29<sup>th</sup> and 30 March 2023. These summaries have been reviewed and edited for clarity and style by the WPSA UK Programme Committee but have not been fully peer-reviewed.

K. Hawkey. Insects as animal feed.

B.A. Loureiro. Potential benefits of black soldier fly larvae ingredients in poultry nutrition and health.

D. V. Scholey, E., J. Burton and M. Sakkars. Post-harvest treatment of wheat with a mould inhibitor improves performance in meat poultry.

S. Galgano, L. Conway, N. Dalby, A. Fellows and J. G. M. Houdijk. Broiler gut microbiota responses to peracetic acid precursors.

V. Mulraney, D. V. Scholey, H. Warren and E. J. Burton. Viability of broiler diets free from mineral phosphate from day old chicks.

S. Struthers, I. C. Dunn, J. J. Schoenebeck, and V. Sandilands. Which beak is best: investigating the relationship between beak shape and pecking damage in commercial laying hens.

A. Šimić, G. González-Ortiz, S. C. Mansbridge, S. P. Rose, M. R. Bedford, M. Tukša and V. Pirgozliev. Effects of xylo-oligosaccharide supplementation with different dose and degree of polymerisation on short-chain fatty acid caecal production.

A. M. Thanki, S. Hooton, N. Whenham, M. Salter, M. Bedford, H. M. O'Neill and M. R. J. Clokie. Assessing the efficacy of bacteriophage therapy to reduce *Salmonella* colonisation in broiler chickens.

B. Tugnoli, A. Piva and E. Grilli. A microencapsulated blend of botanicals can support broiler chickens during an experimental model of coccidiosis.

C. L. Walk, P. Jenn, C. Chatelle, A. J. Cowieson, J. Schmeisser, and R. Aureli. Determining the impact of dietary phytate on the standardised ileal digestible calcium requirement of fast-growing male broilers from hatch to day 30 post-hatch.

M. E. E. Ball, C. C. Mulvenna, R. Ramsey, and Y. Devlin. The relationship between ammonia emissions and the dry matter of broiler litter and hen manure.

R. Hardy, C. Evans, A. Bello, S. Gilani, K. M. Venter, P. Plumstead and Y. Dersjant-Li. Production benefit of full matrix application for a novel consensus bacterial 6-phytase variant using a phased-dosing strategy in broilers.

A. Desbruslais, D. Gonzalez-Sanchez, and A. Wealleans. Supplementation of a combination of lysolecithin, a synthetic emulsifier and mono-glycerides, supports bird welfare and performance of broilers.

M. Naeem, E. J. Burton, D. V. Scholey, A. Alkhtib, S. Broadberry, and P. Garland. Effect of including oat hulls with varying particle size on amino acid digestibility of diets fed to broilers.

M. Naeem\*, E. J. Burton, D. V. Scholey, A. Alkhtib, and S. Broadberry. Effect of reduced protein diets diluted with the maize of differing particle size on nitrogen efficiency and litter characteristics in meat poultry.

J. G. M. Houdijk, S. Galgano, M. A. Hussein, A. Richmond, J. S. Bentley and L. M. Dixon. Reused litter and faba beans impact on growth and caecal microbiome in slow growing broilers.

F. Khattak, M. Jlali, T. Rougier, J. Houdijk and C. Hincelin. Bacterial 6-phytase supplementation can help improve bone mineralisation, phosphorus digestibility and growth performance of broilers fed corn-soyabean meal-based diet.

F. van Immerseel. Nutritional sensitivity of the poultry gut microbiome.

P. W. Wilson, I. C. Dunn\*, and H. A. McCormack. An *in vivo* bone phenotype for use in genetic selection and welfare monitoring in laying hens.

E. Putyora, S. Brocklehurst, F. Tuytens and V. Sandilands. Laying hens display resilience to sleep disruption.

M. B. M. Maidin, J. Liang, H. A. McCormack, P. W. Wilson, B. Andersson, M. Schmutz and I. C. Dunn. Heritability and genetic correlation of keel bone morphometric phenotypes and injury grading in laying hens.

R. Preisinger. Practical application of sexing embryos before hatch.

C. Hagan and V. Sandilands. Practicalities of sexing embryos and rearing males in practice.

A. Cerniauskaite, D. V. Scholey, A. D. Kemp, and E. J. Burton. Exploring effect of housing system on skeletal development in laying hens throughout lay.

H. Kang, M. Haskell, S. Jarvis and V. Sandilands. Using a live vaccine as a model for respiratory disease in hens: inducing clinical symptoms.

V. Pirgozliev, A. Jozwik, I. M. Whiting, S. C. Mansbridge, J. Rollinger, A. G. Atanasov, and S. P. Rose. Dietary black pepper and supplementary xylanase improve blood lipids profile in broilers.

M. Tuksa, S. C. Mansbridge, A. Šimić, P. Rose, M. R. Bedford and V. R. Pirgozliev. The effect of xylanase on growth performance and nutrient digestion in broiler chickens when 7.5% of maize-based diet is replaced with cereal brans with different non-starch polysaccharide composition.

## Insects as animal feed

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The global insect protein market is expected to increase dramatically over the next 10 years. There is growing interest in the potential use of insects as novel feed ingredients due to a generally good nutritional profile and potential other benefits. Nutritionally insects are often described as a novel protein source to replace traditional protein sources, such as soybean meal and fishmeal. In the six major species protein levels range from 41% to 63% on dry matter basis (Hawkey, Brameld, Parr, Salter, & Hall, 2021), therefore represent an important potential source of protein. Furthermore, insect protein digestibility has been shown to be similar to fishmeal in broilers (Hall et al., 2018) and the amino acid profile is comparable to soybean meal and fishmeal (Hawkey et al., 2021). Alongside protein, insects represent a potentially useful energy, fibre and mineral source. Depending on dietary requirements, extraction of fat may be needed for some uses. Other components of insects which may be of use are chitin and anti-microbial proteins (AMP's). There is also expanding evidence that the nutritional composition of insects can be manipulated as a result of using additives, hormones or genetic modification. The potential hazards from insects include bioaccumulation and allergenicity. Depending on location the legality of use

of insects varies. In 2021, the European Union published an act which authorised the use of farmed insects PAP's in poultry and pig feed. However, use of insects in the UK is restricted by current regulations.

Novel proteins are more expensive than traditional feed sources due to supply and cost of production, and to see insects used more widely in feed formulation for monogastric diets further formulation parameters may be needed. The correct use of insect meal in feed formulation may be an opportunity to overcome some of the limitations of plant-based materials. Though, the backing of retailers and consumer perception are going to be vital to drive change to allow insects to be utilised in the UK market.

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## Potential benefits of black soldier fly larvae ingredients in poultry nutrition and health

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According to projections, human population may reach 9 billion in 2050. With this continuous growth, demand for animal protein is expected to increase in the near future. The consumption of poultry products is expected to increase more than other types of animal protein, because of production cost and lowest carbon and water footprint, drawing a lot of attention to this production chain. Quality and quantity of animal feedstuff such as soybean meal, may be influenced by global warming and climate change, as well as increasing feed and energy costs, thereby influencing global food security. At this context, the investigation for alternative feed resources is necessary (Abd El-Hack et al., 2020).

Recent studies have shown interesting findings regarding the appropriateness of insect meals as feed ingredients in poultry production. Among these insects, black soldier fly larvae (BSFL), *Hermetia illucens*, is one of the most promising insect species for commercial production. BSFL has the ability to grow on a wide range of low value organic residues, able to grow with very low demand of water and land. Considering the poultry production dimension worldwide, even small inclusions (0.5–2%) of this alternative feed source in poultry diets, this replacement has potential to reduce production environment impact significantly with we consider a large scale.

Beyond the perspective of sustainability, BSFL have unique nutritional composition with high levels of good quality protein and fat. Besides macronutrients, BSFL contains bioactive peptides, lauric acid derivatives and chitin (the main component of the arthropod exoskeleton) which are associated with several health benefits. Bioactive peptides and lauric acid derivatives present in BSFL meal are known to exhibit antimicrobial activity against wide range of pathogenic microorganisms (Veldkamp, Dong, Paul, & Govers, 2021). According to literature, a large majority of antimicrobial peptides in BSFL belong to defensins and cecropins families. Both these families have very strong activity against some gram-negative bacteria (Moretta et al., 2020). Whereas lauric acid present in BSFL is also known to have antimicrobial activity against gram-negative bacteria, such as *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella*

*pneumoniae* and *Serratia marcescens* (Matsue et al., 2019). Additionally, peptides present in BSFL derivatives seems protect animal cells from oxidative damage as a consequence of immune response (Mouithys-Mickalad et al., 2020)

BSFL also contain chitin, a linear polysaccharide of  $\beta$ -(1-4) N-acetyl-D-glucosamine units, reported to have immunostimulatory effect and similar effects to antibiotics (Benzertiha et al., 2020). It also affect cecal fermentation and short-chain fatty acid production acting as a probiotic by modulating the intestinal microbiota, promoting intestinal development and may improve the growth performance of poultry (Swiatkiewicz, Swiatkiewicz, Arczewska-Wlosek, & Jozefiak, 2015).

In this perspective, the use of BSFL ingredients can potentially low the footprint of poultry production, modulate gut microbiota and their metabolic products, promoting poultry performance and may reduce the use of antimicrobial additives and the cost of including prebiotics in poultry diet.

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## Post-harvest treatment of wheat with a mould inhibitor improves performance in meat poultry

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### Application

Post-harvest treatment of wheat with mould inhibitors may improve broiler performance and meat yield.

### Introduction

Mycotoxins are produced by moulds and both can contaminate grain and feed, resulting in detrimental effects on bird performance and health. They have been shown to decrease palatability (and hence feed intake), as well as nutrient digestibility (Xu, Kiarie, Yiannikouris, Sun, & Karrow, 2022).

Mycotoxins are generated more rapidly in warm, humid conditions, so may become even more prevalent as global warming continues (Adegbeye et al., 2020). Mould inhibitors are commonly used to decrease contamination and inhibit the growth of moulds, thereby reducing the potential production of mycotoxins during storage. They are typically organic acid based, which may also have a positive effect on gut health. This study investigates the effect of pre-treating Irish (IE) wheat with an acidic mould inhibitor (containing organic acids and antioxidants) on the subsequent performance and meat yield of broilers.

**Table 1.** Bodyweight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and breast meat yield in broilers fed standard and acidified wheat to day 35. <sup>a-b</sup> Means within the same column with no common superscript differ significantly ( $P < 0.05$ )

Diet	BWG D0-35 (g)	FI D0-35 (g)	FCR D0-25	Breast yield (g)	Litter DM (%)
Standard wheat	1670 <sup>c</sup>	3075 <sup>b</sup>	1.84 <sup>b</sup>	316 <sup>c</sup>	69
Acidified wheat	1968 <sup>a</sup>	3287 <sup>a</sup>	1.68 <sup>a</sup>	408 <sup>a</sup>	65.4
50:50 wheat mix	1788 <sup>b</sup>	3166 <sup>b</sup>	1.78 <sup>b</sup>	346 <sup>b</sup>	66.5
SEM	27	39.3	0.028	9.5	1.35
p value	<0.001	0.002	0.002	<0.001	0.09

## Material and methods

288 male Ross 308 broiler chicks were randomly allocated into groups of 6 birds in 48 floor pens in an environmentally controlled room. Mash diets were formulated based on wheat (50% inclusion) and soyabean meal with added oil, vitamins, minerals and amino acids, to meet the requirements of the age and strain of the birds. Dietary treatments were as follows: standard wheat (A), acidified wheat (B) and a 50:50 blend of the standard and acidified wheats (C) and each diet was fed to 16 pens. Feed and water were provided ad libitum. Acidified wheat was pre-treated with Myco CURB<sup>®</sup> ES liquid as a mould inhibitor and recovery was <0.1. 1.77 and 1.07 kg/t in wheats A, B and C respectively. Analysis showed undetectable mycotoxin levels in all wheats. Bird body weight gain (BWG) and feed intake (FI) were measured at D10, 28 and 35, and litter moisture measured on D35. Pens were thinned to 5 birds per pen at D10. Post-euthanasia by cervical dislocation on d35, breast meat was removed from two average sized birds per pen. The trial received a favourable opinion from the University's ethical review group prior to starting. One-way ANOVA was used to determine the effect of dietary treatment on all parameters measured using R.

## Result

Birds fed the acidified wheat were significantly larger, with higher breast meat yield than birds fed standard wheat, with the 50:50 diet between the two (Table 1). FCR was also improved in the birds fed acidified wheat.

## Conclusion

Pre-treatment of wheat with mould inhibitor improved bird growth, FCR and also meat yield, even though there was no mycotoxin challenge present, so the treatment appears to be acting as an acidifier in this case. It is notable that the birds were small in this study: this may be due to the fine particle size of the mash diets or a subclinical disease issue with the chicks, although birds were healthy throughout. Both wheats were analysed, showing similar geometric mean diameters (700µm), so any particle size issues were consistent across treatments. This suggests that pre-treatment may be an economic benefit to the industry. However, further investigations using pelleted diets need to be carried out, to remove any issues with particle size. Pelleting can also enhance the effectiveness of organic acids, so this may increase the benefits of pre-treatment (Tabib, Jones, & Hamilton, 1984).

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## Broiler gut microbiota responses to peracetic acid precursors

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## Application

The antimicrobial alternative peracetic acid (PAA), derived from precursor hydrolysis, qualitatively affects microbiota targeting different gut locations according to the precursors formulation and mode of delivery.

## Introduction

We previously demonstrated performance benefits from PAA-precursors provision, both in-water during trial 1 (T1, Galgano et al., 2023a) and in-feed during trial 2 (T2, Galgano, Conway, Dalby, Fellows, & Houdijk, 2023b), with impact on total bacterial concentration in the crop and more distal jejunum,

respectively. Here, we present the assessment of microbiota composition, from which gut location specific relationships with broiler performance could be deduced.

## Material and methods

Both T1 and T2 study design and protocol were approved by SRUC Animal Welfare and Review Body. During T1, six equidistant in-water PAA levels were tested from day 7 to day 14 (daily administration) to 96 Ross 308 male broilers (4 birds/pen, 4 pens/treatment; including a 0ppm control). During T2, three levels (0, 30 and 80ppm) were tested on broilers housed on re-used litter, with clean and re-used litter particle-free controls. T2 treatments were administered from day 0 to day 28 to 375 Ross

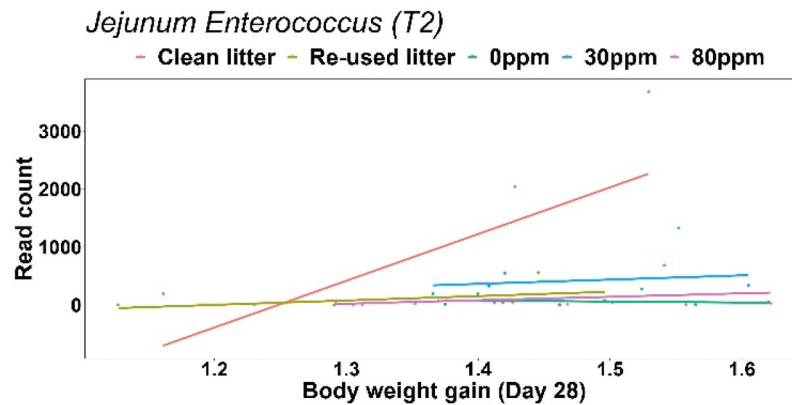


Figure 1. Jejunal enterococcus abundance Vs body weight gain at day 28 (T2).

308 male broilers (5 birds/pen, 15 pens/treatment). Region V4 of the 16S rRNA gene was targeted for sequencing (a515F-a806R) of crop, jejunum and caecal content collected at the end of the trials. Bioinformatic analysis was carried out using QIIME2, and metabolic pathway prediction was performed via PICRUS2. We assessed the treatment effect on the measured variables via linear mixed model, outputting both P and Q values, the latter adjusting for the former's false discovery rate.

## Results

During T1, we observed a PAA driven reduction of *Lactobacillus* ( $P < 0.05$ ,  $Q < 0.05$ ) and *Flectobacillus* ( $P < 0.05$ ,  $Q < 0.05$ ) in the crop of birds also showing better performance (Galgano et al., 2023a) and greater abundance of the predicted 4-aminobutanoate degradation (V) pathway. During T2, birds on 30ppm showed improved body weight gain (BWG, Galgano et al., 2023b), a decreased jejunal *Firmicutes* and an increased *Proteobacteria* relative abundance compared to the control ( $P < 0.05$ ,  $Q = 0.05$ ). At genus level, some of the most noticeable changes were in the caeca of the 30ppm group, where the relative abundance of the lactate producer *Bacillus* and of the butyrate producer *Flavonifractor* increased compared to control ( $P < 0.05$ ,  $Q = 0.1$ ),

Moreover, jejunal *Enterococcus* relative abundance and BWG at day 28 tended to be positively correlated ( $P = 0.09$ , Figure 1), whilst its relative abundance was greater for 30ppm compared to control on re-used litter ( $P < 0.05$ ,  $Q = 0.05$ ) although its maximum levels were observed in the group on clean litter.

## Conclusion

PAA driven probiotic effects likely relate to the gut location in which the species are viable, *Lactobacillus* increase in the upper gut could have detrimental effects due to decreased lipid-derived energy levels available for the host, whilst butyrate and acetate producers have a beneficial effect when increased in the lower gut. *Lactobacillus* is a known lactate producer, highly represented in the crop of broilers and used successfully as a probiotic, when viable in the lower intestine. However, our findings could point towards possible beneficial effects following its decreased abundance in the crop, likely due to a decreased intestinal activity of *Lactobacillus*-bile salt hydrolase, likely leading to an increased host lipid metabolism and energy harvest.

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## Viability of broiler diets free from mineral phosphate from day old chicks

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### Application

Mineral phosphate supply in poultry starter diets is not essential for bone health.

### Introduction

A fundamental mineral of concern in the production of broilers is phosphorus (P). Phosphorus that occurs naturally in the plant-derived raw materials regularly used in the commercial

production of broiler feed is not always bioavailable to the birds. The majority of dietary P is primarily stored in the form of phytates. These phytates are poorly bioavailable to poultry due to the lack of the endogenous intestinal enzyme phytase which is a phytate-degrading enzyme. (Humer, Schwarz, & Schedle, 2014). Common practice in commercial diets is to supplement with mineral P and exogenous phytase enzymes. Scholey, Morgan, Riemensperger, Hardy, and Burton (2018) found that mineral P could be excluded from the diets of broilers from day

**Table 1.** Impact of diet and age on bone strength, BWG, FCR & FI of broilers at varying time points

Age	Diet	BWG (g) ( $\pm$ SE)	FCR ( $\pm$ SE)	FI ( $\pm$ SE)	Bone Strength (N) ( $\pm$ SE)
Day 7	High dig P Control (0.45% dig P)	122 (2.6) <sup>a</sup>	1.32 (0.039)	162 (5.1)	50.56 (17.75) <sup>a</sup>
	Low dig P Control (0.35% dig P)	116 (1.7) <sup>a</sup>	1.50 (0.054)	174 (5.6)	52.98 (17.28) <sup>a</sup>
	Phytase only P (0.35% dig P)	109 (2.4) <sup>b</sup>	1.60 (0.049)	172 (5.1)	47.01 (16.23) <sup>b</sup>
P value		<0.001	0.28	0.28	<0.002
Day 14	High dig P Control (0.45% dig P)	202 (2.6) <sup>a</sup>	1.21 (0.022) <sup>a</sup>	246 (4.5) <sup>a</sup>	174.28 (59.23) <sup>a</sup>
	Low dig P Control (0.35% dig P)	190 (3.2) <sup>b</sup>	1.21 (0.021) <sup>b</sup>	229 (4.5) <sup>b</sup>	123.85 (53.23) <sup>b</sup>
	Phytase only P (0.35% dig P)	174 (3.5) <sup>c</sup>	1.19 (0.019) <sup>b</sup>	204 (4.2) <sup>c</sup>	133.84 (59.83) <sup>b</sup>
P value		<0.001	<0.001	<0.001	<0.001
Day 21	High dig P Control (0.45% dig P)	560 (7.4) <sup>a</sup>	1.53 (0.041)	854 (20.5)	249.73 (56.71) <sup>a</sup>
	Low dig P Control (0.35% dig P)	514 (14.6) <sup>b</sup>	1.57 (0.060)	845 (25.8)	222.21 (62.88) <sup>b</sup>
	Phytase only P (0.35% dig P)	503 (9.1) <sup>b</sup>	1.62 (0.057)	824 (32.1)	211.46 (65.04) <sup>b</sup>
P value		<0.001	0.433	0.711	<0.01

<sup>a,b</sup>Diets with differing subscripts within an age boundary are significantly different.

10 with no negative effects. This study excluded mineral P from mash starter diets of day-old chicks. A high dose of exogenous phytase was added to maximise the release of P from phytate sources within the feed.

### Materials and methods

The trial received a favourable opinion from the university's ethical review group prior to starting. There were 816 male Ross308 broilers used in this study and they were fed a specially formulated mash diet designed for the age and strain of the broiler bird in 2 phases: 0–10 days starter diet and 10–21 days grower diet. The study was designed as a randomised block trial with three treatment groups: high digestible P Control – 0.45% digestible P from a mineral source with 500FTU phytase; low digestible P Control – 0.35% digestible P from mineral source with 500FTU phytase added and phytase only P – 0.35% digestible P provided by adding 2000FTU phytase. Birds were offered *ab libitum* access to all diets and water throughout the trial. Birds were randomly allocated pens and housed in groups of 17 per pen from day one with sixteen replicates per treatment diet. Birds were euthanised at day 7 (7 birds per pen), day 14 (4 birds per pen) and day 21 (6 birds) for sampling. For each time point, BWG, FCR, FI and bone strength were measured. Bone strength was tested with a texture analyser post-mortem at each time point. A one-way ANOVA with Duncan Post Hoc tests were then performed using SPSS (v25).

### Results

There was a statistically significant difference in bone strength between ages and diets (Table 1). The phytase only (0.35% digestible P) diet showed no significant difference to the low

digestible P (0.45% dig P) control on days 14 and 21. There was a significant difference between the high digestible P (0.45% digestible P) control and low digestible P (0.45% digestible P) control when compared to the phytase only diet (0.35% digestible P) on day 7 only. There was no significant difference in FCR & FI between all diets at all ages. On day 7 & day 21 there was significant difference in BWG between the high dig P control when compared to both the low dig P control and the phytase only P diets. On day 14 there showed a significant difference between all three diets. There was a significant interaction between age and diet ( $P < 0.001$ ) on body weight gain (BWG), bone strength at all time points. Feed conversion rate (FCR) on day 7 and feed intake (FI) on day 14.

### Conclusion

Inorganic mineral P supplement can be excluded from the diet of broilers safely without any compromise to bone strength of the birds from day 14. The lack of significant difference between the Low dig P control and the phytase only P diet suggests phytase is initially unable to supply adequate P. Investigation will be conducted to explore the interplay between the exogenous phytase added to the diet and the endogenous mechanisms developed by the birds to manage the low levels of bioavailable P.

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## Which beak is best: investigating the relationship between beak shape and pecking damage in commercial laying hens

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### Application

Understanding the amount of physical damage different beak shapes can cause could help guide the genetic selection of hens whose beak shapes are less apt to cause damage when they engage in severe feather pecking (SFP) behaviour.

### Introduction

SFP is a serious welfare concern within the egg industry. Outbreaks of SFP can result in cannibalism and mortality reaching 50% or more in extreme cases (Kjaer & Sørensen, 2002). To move away from beak treatment (an effective method of reducing SFP-related damage commonly performed at day-



old by infrared energy), it has been suggested that traits such as feather cover, liveability, and beak shape could be selected in non-beak treated laying hen breeding flocks. Significant variation in beak shape exists within and between breeding laying hen lines and there are distinct beak phenotypes within these lines that could be selected for (Struthers et al., 2021). This suggests that it may be possible to incorporate beak shape data into selection indices. However, first it is important to understand the relationship between beak shape and pecking-related damage. It is hypothesised that blunt beaks are less able to grasp and pull feathers and body tissue. Previous research has found that laying hens with blunter beaks (whether naturally or by infrared beak treatment) have better feather cover and less mortality, suggesting that there is a relationship between beak shape and the bird's ability to cause damage (Icken, Cavero, & Schmutz, 2017; Struthers, Classen, Gomis, Crowe, & Schwean-Lardner, 2019). However, the amount of physical damage that different beak shapes can cause has yet to be quantified. Therefore, the objective of this study was to determine if there are differences in pecking damage between hens with different beak shapes.

### Material and methods

This study was approved by the Animal Experiments Committee at Scotland's Rural College (SRUC). Twenty-four 33-week-old Lohmann Brown laying hens were selected from a commercial flock. Hens were sorted into two groups based on pre-determined beak criteria: 12 were classified as having 'sharp' beaks (i.e. minimum of 0.35 cm top beak overhang) and 12 were classified as having 'blunt' beaks (i.e. maximum of 0.15 cm overhang). Hens were transported to SRUC's poultry unit and housed in six floor pens (4 hens/pen). Hens were given a 1-week acclimatisation period to their home pens and a 1-week habituation period to the test pen. The test pen consisted of a 'chicken' model (foam block covered with feathered chicken skin) and a high-speed camera. Following habituation, each hen was placed in the test pen and recorded for 15 min twice a week for 3 weeks (6 tests/hen total). Pecking at the model was encouraged using crushed meal worms and red food colouring to imitate blood. Before and after each test session, the foam block was weighed to determine weight change (since pecking resulted in block material removal, as a proxy for tissue damage potential) and the number of feathers was counted to determine feather removal. The study was analysed as a one-way analysis

of variance in a completely randomised design with 12 replicates per group (bird as replicate unit). Data were analysed using the R stats package (v.4.1.0). Differences were considered significant when  $P \leq 0.05$ .

### Results

Beak shape did not affect the change in block weight over the three-week testing period (8.6 vs 9.3 g, for sharp and blunt beak groups, respectively;  $P = 0.59$ ). However, hens in the sharp beak group removed significantly more feathers from the models than hens in the blunt beak group (59 vs 31 feathers, respectively;  $P = 0.03$ ).

### Conclusion

The results of this study suggest that birds with a sharp beak shape (i.e. one where the top beak extends out over the bottom) are more capable of removing feathers. No difference in the change in block weight suggests that there are other factors beyond just the 'sharpness' of the beak that contribute to feather removal and/or the potential to cause tissue damage; however, it is important to note that the beak shapes in the present study were less 'extreme' than those observed in breeding flocks. This study contributes to knowledge of how best to prevent SFP-related damage and improve welfare in non-beak treated laying hens.

### Acknowledgment

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## Effects of xylo-oligosaccharide supplementation with different dose and degree of polymerisation on short-chain fatty acid caecal production

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### Application

Low levels (stimbiotic) of xylo-oligosaccharides (XOS) instead of high (prebiotic) may be used to improve the gut health of poultry.

### Introduction

Although XOS is known for its prebiotic effects and potential to improve gut health, there is a lack of information on the possible effect of different doses (stimbiotic vs prebiotic mode of action) and DP when included in broiler diets. Therefore,

the study was conducted to investigate the effect of XOS supplementation on caecal fermentation of broiler chickens through the measurement of short-chain fatty acids (SCFA).

### Material and methods

The experiment was conducted at the National Poultry Institute (UK) and approved by the research ethics committee of HAU (0333–202203-PGMPHD). A 35-day study was conducted on 360 male Ross 308 broiler chicks, reared in 36 pens, and fed *ad libitum*. Chickens were fed maize and

**Table 1.** The effect of XOS source and dose on SCFA caecal production (mmol/kg)

XOS source	XOS Dose	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Lactic acid	SCFAs	VFAs	BCFAs
XOS 2–9 DP		85.2	6.57	16.67	1.393	2.15	114.5	112.4	2.14
XOS 2–6 DP		75.9	5.98	15.10	1.348	2.27	103.4	101.2	2.31
	High	72.2 <sup>b</sup>	6.50	12.14 <sup>b</sup>	1.221 <sup>b</sup>	2.58	97.4 <sup>b</sup>	94.9 <sup>b</sup>	2.29
	Low	88.9 <sup>a</sup>	6.06	19.63 <sup>a</sup>	1.519 <sup>a</sup>	1.83	120.4 <sup>a</sup>	118.6 <sup>a</sup>	2.15
SEM		3.34	0.396	1.120	0.0679	0.265	4.51	4.47	0.222
XOS 2–9 DP	High	74.8	6.70	11.68	1.169	2.61	99.6	97.0	1.92
XOS 2–9 DP	Low	95.6	6.44	21.66	1.617	1.68	129.4	127.7	2.35
XOS 2–6 DP	High	69.7	6.29	12.61	1.274	2.55	95.2	92.8	2.66
XOS 2–6 DP	Low	82.1	5.68	17.59	1.422	1.98	111.5	109.5	1.95
SEM		4.73	0.560	1.584	0.0960	0.375	6.38	6.32	0.314
<b>Probabilities</b>									
XOS source		0.058	0.304	0.329	0.644	0.752	0.091	0.086	0.589
Dose		0.001	0.442	<.001	0.004	0.055	0.001	<.001	0.660
XOS source x Dose		0.382	0.758	0.125	0.129	0.631	0.299	0.278	0.079

<sup>a,b</sup>P < 0.05; SEM, standard errors of mean; DP, degree of polymerisation; BCFAs, branched-chain fatty acids.

soybean-based meal diets in two phases: starter (0–21d) in crumb form and finisher (22–35d) in pellet form. A basal experimental diet was mixed containing 500 FTU/kg of phytase and 16,000 BXU/kg of xylanase with calculated 12.91 MJ/kg AME and 229 g/kg CP content. XOS supplements were added, with degrees of polymerisation between 2 to 6 and between 2 to 9 at two dosages of 50 (Low) and 500 (High) g/T, producing 4 diets. On day 35, caecal digesta samples from one bird randomly selected from each pen were collected in Biofreezer® tubes for the measurement of SCFA as described previously González-Solé et al. (2022). The data were analysed in GenStat (20th edition) by ANOVA with 2 × 2 factorial design including two XOS sources and 2 doses. At P < 0.05, differences were reported as significant.

## Results

There were no XOS source x Dose interaction in any of the parameters measured (P > 0.05) (Table 1). XOS source did not influence SCFA either (P > 0.05). Although there were no treatment effects on animal performance in this trial, the low dose of XOS was more effective in stimulating acetic, butyric, valeric, SCFA and volatile fatty acid (VFA) concentrations in

the caeca than the higher dose. This suggests the stimbiotic mode of action may be distinct from the prebiotic mode of action, and the use of XOS levels in excess of the stimbiotic effect may eliminate this response altogether.

## Conclusion

The results of the study suggest that low levels of XOS, defined as a stimbiotic mode of action, may be more effective to influence the gut microbiota and their fermentation pattern compared to high doses of XOS, which may saturate their fermentation activity much faster.

## Acknowledgment

Richard James and Charlotte Arthur are acknowledged for their technical support.

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## Assessing the efficacy of bacteriophage therapy to reduce *Salmonella* colonisation in broiler chickens

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### Application

Bacteriophages can be used as an alternative to antibiotics against bacterial pathogens.

### Introduction

Non-typhoidal *Salmonella* species are responsible for 93.8 million infections annually of which 85% of human infections are foodborne. Within animal production high levels of antimicrobial resistance have been observed in bacterial species linked to human food poisoning and recently the European Food Safety Authority reported multi-drug resistant (MDR) *Salmonella* spp were recovered from broiler, pig, calf and Turkey carcasses at levels 53.6%, 43.3%, 23.1% and 19.1% respectively. The highest levels of MDR were found in broilers and from 984 *Salmonella* strains used in the analysis 51.2% of strains were resistant to three or more antimicrobials

commonly used to treat the infection (EFSA and ECDC, 2022). Worryingly these MDR bacterial strains have entered the human food chain, and are becoming increasingly difficult to treat. Alternatives to antibiotics are needed and bacteriophages (phages for short) could provide an effective alternative. Phages are viruses of bacteria and are naturally the most abundant organism in the world (Thanki, Hooton, Gigante, Atterbury, & Clokie, 2021). The aim of this study was to determine if a bacteriophage (phage) cocktail delivered in feed is able to reduce *Salmonella* colonisation in experimentally-challenged chickens and to determine optimal phage dose.

### Materials and methods

The experimental protocol was presented and accepted by Drayton Animal Health Welfare and the Ethical Review Body prior to commencement of study. The study was in

accordance with the Animals (Scientific Procedures) Act 1986 and was conducted at Drayton Animal Health Ltd (UK). In this study 672 Ross 308 male broilers were used, and the trial was conducted at Drayton Animal Health, UK. Chickens were divided into six treatment groups and each group included 112 chickens (7 chickens per pen and 16 replicates per treatment group). The treatment groups were T1 (control birds); T2 (birds fed the phage diet at dose  $10^6$  PFU/day); T3 (challenged birds); T4 (birds fed the phage diet at dose  $10^5$  PFU/day and challenged); T5 (birds fed the phage diet at dose  $10^6$  PFU/day and challenged); and T6 (birds fed the phage diet at dose  $10^7$  PFU/day and challenged). The phage cocktail was mixed with starter (0–13 days), grower (14–27) and finisher (28–42) diet and the diet was changed accordingly through the trial. Chickens were challenged via oral gavage on day 4 with *S. Typhimurium* at dose  $5 \times 10^6$  CFU/per bird. Pooled faecal samples from each pen were collected on days 6, 7, 8, 9, 10, 14, 28 and 42 (last day of the trial) to determine *Salmonella* and phage counts. One-way ANOVA was used for statistical analysis.

## Results

In faecal samples collected on days 6, 7 and 8 *Salmonella* counts were not significantly different in the challenged treatment groups. On day 9 the average faecal *Salmonella*

counts from chickens in T6 were  $6.33 \times 10^4$  CFU/g, which was significantly lower than the counts from group T3 ( $8.40 \times 10^5$  CFU/g) ( $p < 0.01$ ). Further significant reductions in *Salmonella* counts were observed in phage treated groups T4, T5 and T6 ( $\sim 2.4 \times 10^2$  CFU/g) versus T3 ( $\sim 6 \times 10^4$  CFU/g) in faecal samples collected on day 28 ( $p < 0.01$ ). By day 42 no *Salmonella* was detected in the faecal samples collected from group T4, and in groups T5 and T6 *Salmonella* was only isolated from 3/16 and 2/16 pens respectively. In comparison *Salmonella* was isolated from 7/16 pens in T3.

## Conclusion

We showed delivering phages via feed was effective at reducing *Salmonella* colonisation in chickens and our study highlights phages offer a promising tool to target bacterial infections in poultry.

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## A microencapsulated blend of botanicals can support broiler chickens during an experimental model of coccidiosis

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## Application

Poultry producers can use AviPower\*2-500 in feed to improve broilers growth performance and gut morphology during coccidiosis.

## Introduction

Botanicals are increasingly used in poultry nutrition based on their numerous biological functions. Amongst them, the anticoccidial properties are of key interest to find new tools to control coccidiosis, still a major issue world-wide (Felici, Tugnoli, Piva, & Grilli, 2021). The aim of this study was to

test the anticoccidial activity of a microencapsulated blend of botanicals *in vivo* in broilers artificially infected with coccidia.

## Material and methods

The trial was conducted in the USA in a private research center authorized to run experimental research with animals. A total of 1,500 day-old male chicks (Cobb 500) were divided into floor pens (50 birds/pen) and assigned to 3 dietary groups (10 pens/group): negative control (NEG), basal diet, unchallenged; positive control (POS), basal diet, challenged;

**Table 1.** a) Growth performance, b) intestinal lesions and *Eimeria* shedding in faeces at d 28 during coccidiosis challenge

a.		NEG	POS	TRT	P-value	b.		POS	TRT	P-value
Phase: day 0–21	FI (kg/pen)	48.74 <sup>a</sup>	51.75 <sup>b</sup>	50.05 <sup>ab</sup>	0.001	Lesions				
	BWG (kg/bird)	0.667	0.718	0.688	0.21	<i>E. acervulina</i>	2.64 <sup>a</sup>	1.88 <sup>b</sup>	0.001	
	FCR	1.376	1.368	1.378	0.80	<i>E. maxima</i>	1.72 <sup>a</sup>	1.06 <sup>b</sup>	0.002	
Phase: day 21–28	FI (kg/pen)	33.32	32.81	32.17	0.18	<i>E. tenella</i>	2.38 <sup>a</sup>	1.38 <sup>b</sup>	0.003	
	BWG (kg/bird)	0.425 <sup>a</sup>	0.278 <sup>b</sup>	0.347 <sup>c</sup>	<0.0001	OPG				
	FCR	1.594 <sup>a</sup>	2.445 <sup>b</sup>	1.884 <sup>c</sup>	0.0001	<i>E. acervulina</i>	23,202	17,655	0.19	
Phase: day 21–42	FI (kg/pen)	116.40	124.32	124.88	0.06	<i>E. maxima</i>	3095	777	0.09	
	BWG (kg/bird)	1.424	1.407	1.497	0.12	<i>E. tenella</i>	15,249	10,994	0.62	
	FCR	1.843 <sup>a</sup>	2.015 <sup>b</sup>	1.849 <sup>a</sup>	<0.0001	Total <i>Eimeria</i>	41,547	29,426	0.17	
Overall: day 0–42	FI (kg/pen)	165.15 <sup>a</sup>	176.06 <sup>b</sup>	174.93 <sup>b</sup>	0.01					
	BWG (kg/bird)	2.091	2.125	2.185	0.15					
	FCR	1.677 <sup>a</sup>	1.770 <sup>b</sup>	1.684 <sup>a</sup>	<0.0001					

<sup>abc</sup>: Within a row different superscripts indicate significant differences ( $P < 0.05$ ).

FI = feed intake, BWG = body weight gain, FCR = feed conversion ratio, OPG = oocysts per gram of feces.

treated (TRT), basal diet + a microencapsulated blend of botanicals (AviPower\*2-500) at 250 g/MT, challenged. Basal diets were not-medicated, commercial type, formulated according to NRC (1994) for starter (d0-21), grower (d21-35) and finisher (d35-42) phases. The coccidiosis challenge consisted of an oral inoculation with multispecies *Eimeria* (*E. acervulina*, *E. maxima*, *E. tenella*) at day 21. The study lasted 42 days with intestinal lesions and oocysts per gram (OPG) of faeces determined on day 28 and growth performance recorded throughout the study. Data were analysed with ANOVA and differences considered significant at  $P < 0.05$ .

## Results

In the first week after *Eimeria* infection, the challenge had a negative impact on growth performance for POS compared to NEG, while TRT, despite the challenge, showed significantly better body weight gain and FCR than POS

(Table 1). From the challenge (d21) to d42 and for the overall period, FCR of TRT was comparable to NEG unchallenged group ( $P < 0.001$ ). Moreover, at d28 TRT showed reduced intestinal lesion scores for *E. acervulina*, *E. maxima*, and *E. tenella* ( $P < 0.05$ ) and a trend ( $P < 0.10$ ) towards lower fecal shedding of *E. maxima* oocysts compared to POS.

## Conclusion

To conclude, the microencapsulated blend of botanicals used in this study has the potential to contain loss of performance and intestinal lesions associated with coccidiosis challenge in broiler chickens.

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# Determining the impact of dietary phytate on the standardised ileal digestible calcium requirement of fast-growing male broilers from hatch to day 30 post-hatch

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## Application

Formulating broiler diets to meet digestible calcium requirements may improve growth performance, efficiency, and the digestibility of phosphorus and amino acids, thereby reducing excretion into the environment.

two levels of dietary phytate (standard (STD) and high) on the SID Ca requirement of broilers from hatch to day 30 post-hatch.

## Introduction

Broiler diets are currently formulated using digestible (dgP) and total calcium (Ca). Over- or under-supply of dietary Ca, high concentrations of very soluble Ca, or an imbalanced Ca to P ratio can have detrimental effects on broiler performance, gastrointestinal pH (Lee, Febery, Mottram, & Bedford, 2021), digestibility (Amerah, Plumstead, Barnard, & Kumar, 2014), and may exacerbate mortality associated with necrotic enteritis (Pavia, Walk, & McElroy, 2013). The standardised ileal digestible (SID) Ca requirements for fast-growing broilers have been determined by Walk, Wang, Wang, Sorbara, and Zhang (2022) and David, Abdollahi, Bedford, and Ravindran (2022) with differing results. One factor that may be influencing the estimated requirements is the use of high doses of phytase, to eliminate any potential Ca-phytate interactions, in the studies by Walk et al. (2022). The objective of this work was to determine the impact of

## Materials and methods

Ross 308 male broilers ( $n = 704$ ) were obtained and allocated to 64 battery cages with 11 birds/cage (d 0–16) and 5 birds/cage (d 16–30). There were 8 treatments and 8 replicate pens. Treatments were arranged as a  $2 \times 4$  factorial, with 2 levels of phytate P (STD, 0.25% or high, 0.32%) and 4 levels of SID Ca: 0.33, 0.42, 0.51, or 0.60% from d 0–16 or 0.29, 0.38, 0.47, or 0.56% from d 16–30. Diets were corn and soybean meal and fed in crumble and pellet form from d 0 to 16 and d 16 to 30, respectively. Rice bran was used to increase the phytate P content in the high phytate diet. Except Ca, all nutrients were equivalent and met requirements, including dgP at 0.46 and 0.41% from d 0–16 and d 16–30, respectively. Performance, tibia ash, and apparent ileal digestibility of Ca and P were measured on d 16 and 30. Blood samples were collected on d 30. All procedures complied with the European Union directive 2010/63/EU for animal experiments. Data were analysed using JMP. The model included phytate, SID Ca, and the interaction. Means were separated

**Table 1.** Estimation of the standardised ileal digestible (SID) calcium requirement of broilers fed standard or high phytate diets

Parameter	Day 0–16 of age		Day 16–30 of age	
	Response	Estimated SID Ca requirement, %	Response	Estimated SID Ca requirement, %
Feed conversion ratio, g:g	1.155	0.381	–	–
Tibia ash, %	50.13	0.482	–	–
Apparent ileal digested Ca, %	0.355	0.346	0.261	0.355
Apparent ileal digested P, %	0.425	< 0.330	0.371	< 0.290
Mean	–	0.385	–	0.323

using contrasts. The SID Ca requirement was estimated using quadratic models and 100% of the response. Significance was accepted at  $P < 0.05$ .

## Results

The analysed total Ca, P, and phytate P in the diets were within the expected values. There was no significant effect of phytate, SID Ca, or interaction on body weight gain or feed intake from d 0–16 or d 16–30. Feed conversion ratio (FCR) from d 0–16 was lowest (quadratic,  $P < 0.05$ ) in birds fed 0.33 or 0.42% SID Ca. Tibia ash percent was greatest (quadratic,  $P < 0.05$ ) in birds fed 0.51% SID Ca from d 0–16 and lower ( $P < 0.05$ ) in birds fed high phytate P from d 16–30. Apparent ileal digested Ca and P were greatest (quadratic,  $P < 0.05$ ) in birds fed the highest or lowest SID Ca, respectively, on d 16 and d 30. Total P (mg/dL) in the blood linearly ( $P < 0.05$ ) decreased as SID Ca in the diet increased. Due to the lack of significant phytate  $\times$  SID Ca interactions, the SID Ca requirement was estimated using the main effect of SID Ca (Table 1).

## Conclusion

There was no significant effect of dietary phytate on the estimated SID Ca requirement of broilers. The SID Ca requirement to optimise bone ash (d 0–16) was greater than that to optimize FCR or nutrient digestibility. Minimal effects of graded levels of dietary SID Ca were observed from d 16–30. This has been previously reported by Walk et al. (2022) and limited the ability to estimate the SID Ca requirement for broilers after d 16 post-hatch.

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# The relationship between ammonia emissions and the dry matter of broiler litter and hen manure

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## Application

Broiler litter or hen manure dry matter content may be a potential tool to assess the efficacy of on-farm ammonia mitigation strategies.

## Introduction

Ammonia is an environmental pollutant and as agricultural production is a major contributor of ammonia, there is a global drive to reduce ammonia emissions from production systems. In poultry, excess nitrogen is excreted in the form of uric acid which is converted to urea and then volatilised as ammonia. The extent of this volatilisation process is influenced by many factors including litter/manure dry matter (DM), temperature, pH, type and depth of bedding, stocking density, feeding quality, drinker management and manure/litter handling practices (Hayes, Curran, & Dodd, 2006). Previous work has shown increasing litter and manure DM can reduce ammonia emissions (Miles, Rowe, & Cathcart, 2011). As measuring DM is simple and inexpensive, DM could potentially be used to assess the effect of mitigation strategies on-farm. However the relationship has not been quantified and therefore the effect of increasing DM on ammonia emissions cannot be predicted. Mitigation

strategies must currently be assessed by ammonia emission measurement, which is difficult, time-consuming and expensive. The aim of this work was to quantify the relationship between broiler litter and hen manure dry matter and ammonia emissions to develop a tool to assess the efficacy of mitigation strategies. The relationship with other gases was also determined.

## Materials and methods

Representative subsamples of poultry litter and hen manure were collected from a commercial broiler house and a laying hen colony house with a belt-drying system. Two incubation trials were set up using 11.7 L containers ( $n = 18$ ) each containing 300 g of litter (Trial one) or hen manure (Trial two). Broiler litter samples were wetted with deionised water to obtain the following range of DM: 50%, 55%, 60%, 65%, 70% and 75%, and hen manure samples were wetted to obtain the following range of DM: 45%, 50%, 55%, 60%, 65% and 70%. Thus, there were three container pseudo replicates per DM treatment created from each sample of litter/manure. Each container lid was modified to include two septa for gas collection. One septum on each was drilled

**Table 1.** The relationship between broiler litter and hen manure DM and gaseous emissions

	Broiler litter				Hen manure			
	Equation	Type	R <sup>2</sup>	Prob.	Equation	Type	R <sup>2</sup>	Prob.
Ammonia	$Y = -0.80x + 82.0$	Linear	0.67	<0.001	$Y = -0.167x + 14.99$	Linear	0.77	<0.001
Carbon dioxide	$Y = -121.7x + 9978$	Linear	0.84	<0.05	$Y = -14.59x + 218.9$	Linear	0.31	NS
Methane	$Y = -0.82x + 1539$	Linear	0.03	NS	$Y = -0.0009x + 2.24$	Linear	0.00	NS
Nitrous oxide	$Y = -0.296x + 368.4$	Linear	0.77	<0.001	$Y = 7.59x + 828.6$	Linear	0.22	NS

out to accommodate a 6 mm airline to allow measurement of ammonia using a Dräger X-am 5000, whilst the other septum was used to extract headspace air for gas chromatography analysis of carbon dioxide, methane and nitrous oxide. Incubation containers were left open between successive gas sampling and were stored in fume cupboards at 21°C ( $\pm 3^\circ\text{C}$ ). Linear regression analysis was conducted to test the significance of the relationship between gaseous emissions and litter/manure DM.

## Results

There was a strong ( $R^2 = 0.67$ ) and significant ( $P < 0.001$ ) inverse relationship between broiler litter DM and ammonia emission (Table 1). This relationship equates to a 9.5% reduction in ammonia emissions for every 5% increase in DM. There were also significant inverse relationships between broiler litter DM and carbon dioxide ( $R^2 = 0.84$ ,  $P < 0.05$ ) and nitrous oxide emissions ( $R^2 = 0.77$ ,  $P < 0.001$ ). There was a strong ( $R^2 = 0.77$ ) and significant ( $P < 0.001$ ) inverse relationship between hen manure DM and ammonia emissions which equates to a 11% reduction in ammonia emissions for every 5% increase in DM.

## Conclusion

The relationships between broiler litter and hen manure DM and ammonia emissions have been quantified and these relationships can potentially be used to assess the efficacy of mitigation strategies that are designed to increase DM in commercial poultry houses. The significant relationship between broiler litter DM, carbon dioxide and nitrous oxide are also of interest given that these are potent greenhouse gases. This study has enabled robust prediction equations to be derived to quantify the effect of increasing DM on ammonia emissions and will be useful in evaluating mitigation strategies.

## Acknowledgment

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# Production benefit of full matrix application for a novel consensus bacterial 6-phytase variant using a phased-dosing strategy in broilers

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## Application

Application of full matrix for phytase including calcium (Ca), sodium (Na), digestible phosphorus (P), digestible amino acids (AA) and metabolisable energy (ME) is an important strategy to reduce feed cost and improve production benefit.

## Introduction

Phytase breaks down phytate P in plant ingredients and is widely used by the poultry industry to increase availability of phosphorus in diets. Phytase, by reducing the anti-nutritional effects of phytate P, also improves the availability of non-P nutrients like Ca, AA, and energy

(AME) (Dersjant-Li, Awati, Schulzem, & Partridge, 2015). Improvements in nutrient availability may be used to decrease nutrient levels in feed formulations as 'matrix values' without negatively affecting performance. This study validated full matrix application, including Ca, P, AA and AME, for a novel consensus bacterial 6-phytase variant (PhyG) in broilers.

## Material and methods

Ross 308 as-hatch broilers were randomly assigned to 3 dietary treatments with 8 pens of 55 birds per treatment. Diets were pelleted and based on corn, wheat, SBM, sunflower meal and

**Table 1.** Effects of phytase on cumulative growth performance and on total feed cost per kilogram of bodyweight gain (1–32 days)

	ADFI g	ADG g	FCR	Livability, %	Total feed cost (€/kg BWG)	CO <sub>2</sub> eq/kg BWG
PC	87.37 <sup>a</sup>	62.10 <sup>a</sup>	1.413 <sup>a</sup>	89.9 <sup>a</sup>	0.529 <sup>a</sup>	2208 <sup>a</sup>
NC	67.53 <sup>b</sup>	47.24 <sup>b</sup>	1.448 <sup>b</sup>	86.5 <sup>b</sup>	0.497 <sup>b</sup>	1985 <sup>b</sup>
NC+PhyG	88.12 <sup>a</sup>	62.54 <sup>a</sup>	1.417 <sup>a</sup>	89.6 <sup>a</sup>	0.491 <sup>b</sup>	1943 <sup>c</sup>
Pooled SEM	0.547	0.436	0.0055	0.364	0.002	8.62
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

**Table 2.** Effects of phytase on tibia ash content (% DM) and ash weight on 10 and 32 d of age

Diets	10 d of age		32 d of age	
	Tibia ash, %	Ash weight, g	Tibia ash, %	Ash weight, g
PC	47.29 <sup>a</sup>	0.232 <sup>a</sup>	52.3 <sup>a</sup>	1.94 <sup>a</sup>
NC	38.24 <sup>b</sup>	0.149 <sup>b</sup>	44.8 <sup>b</sup>	1.19 <sup>b</sup>
NC+PhyG	47.20 <sup>a</sup>	0.237 <sup>a</sup>	51.9 <sup>a</sup>	1.99 <sup>a</sup>
Pooled SEM	0.533	0.006	0.338	0.019
P-value	<0.001	<0.001	<0.001	<0.001

<sup>abc</sup>: Means in the same row having different superscripts differ significantly ( $P < 0.05$ )

rapeseed meal, fed in 3 phases (starter, 1–10 d; grower, 10–21 d and finisher, 21–32 d). Treatments included 1) a nutritionally adequate positive control (PC); 2) PC reduced in Ca by 0.24–0.21% points (p), digestible P by 0.20–0.18% p, Na by 0.05% p, digestible AA by up to 0.04% p and ME up to 79 kcal/kg across the 3 phases (NC); 3) NC supplemented with PhyG at 2,000, 1,500, and 1,000 FTU/kg (NC+PhyG) in starter, grower and finisher phase, respectively. Data was analysed using JMP 16.1 and means separated by Tukey test ( $P \leq 0.05$  as significant). All animal care procedures were approved and conformed to the guidelines of the NLR ethics committee prior to the start of the research.

## Results

The nutrient and energy reduction in NC reduced ( $P < 0.05$ ) 1–32 d BWG and FCR vs PC (Table 1). The PhyG supplementation to NC maintained BWG and FCR vs PC ( $P > 0.05$ ). Similarly, NC reduced bone ash

contents at d 10 and 32, and NC+PhyG maintained bone ash (Table 2). The estimated overall feed cost (€/kg BWG) was reduced by 7.2% and carbon footprint, g CO<sub>2</sub> eq/kg BWG was 12% lower with NC+PhyG vs PC ( $P < 0.05$ ).

## Conclusion

In conclusion, supplementation of the novel consensus bacterial 6-phytase variant with a phased dosing strategy, with respective full matrix application, maintained broiler growth performance, bone ash, carcass characteristics and resulting production benefit and could contribute to sustainable broiler production.

## Reference

Dersjant-Li, Y., Awati, A., Schulzem, H., & Partridge, G. (2015). *Journal of the Science of Food and Agriculture*, 95(5), 878–896.

# Supplementation of a combination of lysolecithin, a synthetic emulsifier and monoglycerides, supports bird welfare and performance of broilers

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## Application

Utilisation of an additive containing lysolecithin, a synthetic emulsifier and monoglycerides significantly enhances bird welfare and performance.

## Introduction

Ongoing high feed costs and increased consumer concerns regarding animal welfare continue to challenge the broiler industry, increasing interest in solutions that can mitigate rising production costs and/or support welfare. LYSOFORTE® EXTEND (LEX) (Kemin), is a combination of lysolecithin, a synthetic emulsifier and monoglycerides available in both liquid and dry forms. The study investigated application of LEX in broiler diets containing two different energy levels to elucidate the effect on welfare and performance.

## Material and methods

1248 day-old Ross 308 broilers were assigned to one of six treatments for a 42-day study: a control basal diet with a normal energy content (NE); NE + 300 g/t LEX liquid; NE + 500 g/t LEX dry; a control basal diet with a low energy content (LE, -90 kcal/kg respect to NE), LE + 300 g/t LEX liquid and a LE + 500 g/t LEX dry. Each treatment consisted

of 13 pen replicates, each of 16 birds. Feed intake (FI) and weight were measured on days 0, 10, 21 and 42. On day 42 a litter sample was collected from each pen (5 sites within the pen) and two birds per pen were assessed for footpad lesions. Data was analyzed in the Fit Model platform of JMP 16, with means separation achieved using Tukey's HSD. Statistical significance was declared at  $p < 0.05$ . The study was conducted in accordance with Guidelines on Good Clinical Practice for Clinical Trials for Registration of Veterinary Medicinal Products (VICH), and met appropriate current quality standards indicated by Regulation (EC) No 429/2008 (European Commission, 2008) and EFSA's technical guidance document on the assessment of efficacy of feed additives (EFSA, 2018). Approval from Kemin's internal ethical review panel was also obtained.

## Results

Results showed a higher ( $p < 0.05$ ) cumulative bodyweight gain (BWG) with LEX supplementation (Table 1). However, there was no effect ( $p < 0.05$ ) of energy level or interaction between energy and supplementation. An increased ( $p < 0.05$ ) FI was observed for the LE diets, however the cumulative feed efficiency (FCR) of the LE + LEX treatments remained similar ( $p > 0.05$ ) to the NE control, which suggests

Table 1. Cumulative performance results (0–42 days)

Treatment	NE	LE	Control	LEX Dry	LEX Liquid	Pooled SE	P Value Energy	P Value LEX	P Value Energy*LEX
BWG (g/bird)	2814.37	2798.34	2719.95 <sup>b</sup>	2808.17 <sup>ab</sup>	2893.65 <sup>a</sup>	60.994	0.8021	0.0331	0.9242
FI/bird (g)	4545.02 <sup>b</sup>	4665.09 <sup>a</sup>	4531.21	4590.68	4696.97	67.771	0.0400	0.0737	0.8376
FCR	1.617 <sup>b</sup>	1.674 <sup>a</sup>	1.674 <sup>x</sup>	1.636 <sup>xy</sup>	1.626 <sup>y</sup>	0.0208	0.0027	0.0747	0.9560

LEX application allowed the birds to cover the energy gap. The cumulative FCR of the LE diets were increased ( $p < 0.05$ ) compared to the NE, however there was a tendency ( $0.05 < p < 0.1$ ) towards improved FCR when LEX was used. Dry matter percentage of the litter of birds fed LEX, was significantly higher (Mean 66.33%  $p < 0.0001$ ), compared to the control NE groups (50.63% and LE 49.44%). This reflected into lower ( $p < 0.05$ ) occurrence and severity of footpad lesions in birds supplemented with LEX compared to the two control treatments (data not shown).

## Conclusion

Regardless of the energy content of the broiler diet, adding a combination of lysolecithin, a synthetic emulsifier and monoglycerides resulted in improved bird growth. In LE

diets, LEX ensured to maintain FCR similar as when feeding a NE control. The use of the LEX also improved litter quality and footpad health therefore improving animal welfare.

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# Effect of including oat hulls with varying particle size on amino acid digestibility of diets fed to broilers

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## Application

Adding oat hulls to lower protein of broiler diets improves the efficiency of amino acid digestion.

## Introduction

Low-protein diets can be beneficial for the environmental footprint by lowering the excretion of nutrients but impaired growth performance of poultry on low-protein diets without extra amino acids may be expected. Dietary insoluble fibres increase the digesta retention time in the upper gastrointestinal tract

improving nutrient utilisation. The study aimed to examine the effect of low-protein diets with oat hulls differing in particle size on amino acid digestibility in broilers on day 24 post-hatch.

## Materials and methods

The trial was approved by the NTU ethical review committee. A total of 336 Ross 308 male broiler chicks were given four dietary treatments; standard diet (PC), low protein diets (approx. 5%) either with celite (NC), oat hulls fine (OH400) or oat hulls coarse (OH850) as 12 pens per treatment, 7 chicks per pen, *ad libitum* from day-old to 35 days. The ileal digesta was pooled from two birds per pen cervically dislocated on day 24. Amino acid profile and coefficients of digestibility were determined by following Scholey, Marshall, and Cowan (2020). Data were analyzed by one-way ANOVA and means were compared using the LSD test.

## Results

Diet OH850 significantly increased aspartic acid and valine digestibility compared to PC, NC and OH400. Digestibility for some other amino acids (Table 1) was improved ( $P < 0.05$ ) on diet OH850 compared to NC and OH400 but similar ( $P > 0.05$ ) to PC. The treatments did not significantly affect ( $P > 0.05$ ) the digestibility of cystine, glutamine, glycine, alanine, histidine, lysine and arginine.

## Conclusion

The inclusion of coarse oat hulls in diets with protein lowered by around 5% improves amino acid digestibility in 24-day-old broilers.

## Reference

- Scholey, D. V., Marshall, A., & Cowan, A. A. (2020). *Poultry Science* 99 (5), 2566–2572.

**Table 1.** Effect of low protein diets with oat hulls differing in particle size on ileal amino acid digestibility on day 24

Parameter	Treatment <sup>a</sup>				SEM	P-value
	PC	NC	OH400	OH850		
D0-35 Body weight gain (g)	2101	1975	1981	2049	41.86	0.130
D0-35 Feed intake (g)	3218	3162	3198	3243	50.17	0.716
D0-35 Feed conversion ratio (g/g)	1.535 <sup>b</sup>	1.605 <sup>a</sup>	1.618 <sup>a</sup>	1.584 <sup>ab</sup>	0.021	0.040
Aspartic acid	0.798 <sup>b</sup>	0.780 <sup>b</sup>	0.791 <sup>b</sup>	0.824 <sup>a</sup>	0.009	0.007
Methionine	0.931 <sup>a</sup>	0.912 <sup>b</sup>	0.921 <sup>ab</sup>	0.932 <sup>a</sup>	0.004	0.002
Threonine	0.794 <sup>ab</sup>	0.766 <sup>c</sup>	0.771 <sup>bc</sup>	0.820 <sup>a</sup>	0.009	0.001
Serine	0.817 <sup>a</sup>	0.788 <sup>b</sup>	0.787 <sup>b</sup>	0.829 <sup>a</sup>	0.009	0.003
Valine	0.814 <sup>b</sup>	0.806 <sup>b</sup>	0.806 <sup>b</sup>	0.836 <sup>a</sup>	0.008	0.028
Isoleucine	0.822 <sup>ab</sup>	0.807 <sup>b</sup>	0.816 <sup>b</sup>	0.843 <sup>a</sup>	0.008	0.012
Leucine	0.839 <sup>ab</sup>	0.823 <sup>b</sup>	0.825 <sup>b</sup>	0.858 <sup>a</sup>	0.007	0.005
Tyrosine	0.863 <sup>a</sup>	0.795 <sup>b</sup>	0.826 <sup>b</sup>	0.874 <sup>a</sup>	0.013	<0.001
Phenylalanine	0.843 <sup>ab</sup>	0.827 <sup>b</sup>	0.833 <sup>b</sup>	0.861 <sup>a</sup>	0.007	0.010
Proline	0.856 <sup>a</sup>	0.832 <sup>b</sup>	0.833 <sup>b</sup>	0.873 <sup>a</sup>	0.007	0.001

<sup>a</sup>Starters diets (D0-10); PC: Commercial diet, CP:23.60%; NC: PC+5% celite, CP:22.41%; OH400: PC+5% Fine oat hulls of GMD: 400  $\mu$ m, CP:22.61%; OH850: PC+5% Coarse oat hulls of GMD: 850  $\mu$ m, CP:22.61%; Grower diets (D11-24); PC: Commercial diet, CP:21.74%; NC: PC+5% celite, CP:20.65%; OH400: PC+5% Fine oat hulls of GMD: 400  $\mu$ m, CP:20.85%; OH850: PC+5% Coarse oat hulls of GMD: 850  $\mu$ m, CP:20.85%.

<sup>ab</sup>Means within a row with different superscripts differ significantly ( $P \leq 0.05$ ). GMD: Geometric mean diameter.



## Effect of reduced protein diets diluted with the maize of differing particle size on nitrogen efficiency and litter characteristics in meat poultry

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### Application

Reduced-protein diets diluted with whole grain maize may be beneficial for the environment and litter characteristics in meat poultry production systems without affecting performance.

### Introduction

Low-density protein diets of broilers can be favourable for the environmental footprint by lowering the excretion of nutrients. Studies have shown impaired growth performance of broilers fed diets with reduced protein content (Lemme et al., 2019). The increased particle size of grains in the diet increases the digesta retention time in the upper gastrointestinal tract, improving nutrient utilization (Svihus, 2011). The study aimed to investigate the effect of low-protein diets diluted with the maize grain differing in particle sizes on nitrogen efficiency and litter characteristics.

### Materials and methods

The study was approved by NTU's ethical review committee. A total of 336 male broiler chicks (Ross 308) were given one of four dietary treatments; 'CON' (Commercial pellet diet – diet CP 22.50%), 'M350' (CON+20% maize of GMD 350  $\mu\text{m}$  – diet CP 19.90%), 'M2600' (CON+20% maize of GMD 2600  $\mu\text{m}$  – diet CP 19.90%) and 'WM' (CON+20% whole maize – diet CP 19.90%), with 7 pens per treatment (12 chicks per pen) fed *ad libitum* up to day 21. The maize of the defined format was added post-pellet to create the 3 test diets. Nitrogen retention, excretion and efficiency were calculated as described by Belloir et al. (2017) while litter nitrogen and moisture were determined by the standard methods of AOAC (1990). Data were analyzed by one-way ANOVA and means were separated using the LSD test at  $P \leq 0.05$ .

### Results

The whole maize low-protein diet significantly decreased N excretion compared to the standard diet without affecting N retention and efficiency and growth, feed intake and feed conversion ratio. Furthermore, it significantly decreased litter moisture (Table 1).

### Conclusion

In meat poultry production systems, reduced-protein diets with whole maize grain inclusion enhanced nitrogen utilisation and decreased litter moisture. Furthermore, growth and feed conversion ratio were maintained close to birds fed standard broiler diet. Therefore, whole maize grain may contribute to mitigating environmental concerns associated with poultry production, and concurrently improve rearing conditions but the solution may not be economically viable in the current market.

### Acknowledgment

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**Table 1.** Effect of standard and low protein diets with maize increasing in particle size on growth performance, nitrogen efficiency (D0-21) and litter characteristics (D20)

Parameters	Treatment				SEM	P-value
	CON	M350	M2600	WM		
Body weight gain (g)	964.1 <sup>a</sup>	625.8 <sup>c</sup>	727.8 <sup>b</sup>	919.3 <sup>a</sup>	22.06	<0.001
Feed intake (g)	1223 <sup>a</sup>	914.6 <sup>c</sup>	1054 <sup>bc</sup>	1165 <sup>ab</sup>	49.33	0.002
Feed conversion ratio (g/g)	1.273	1.471	1.462	1.262	0.072	0.087
N-retention (g/bird)	27.96 <sup>a</sup>	18.15 <sup>c</sup>	21.11 <sup>b</sup>	26.66 <sup>a</sup>	0.640	<0.001
N-excretion (g/bird)	16.08 <sup>a</sup>	10.97 <sup>b</sup>	12.45 <sup>ab</sup>	10.42 <sup>b</sup>	1.650	0.026
N-efficiency (%)	63.91 <sup>ab</sup>	62.91 <sup>b</sup>	63.60 <sup>b</sup>	72.59 <sup>a</sup>	3.022	0.036
D20 Litter N (%)	2.931	2.470	2.501	2.641	0.125	0.068
D20 Litter moisture (%)	15.40 <sup>a</sup>	8.174 <sup>b</sup>	8.374 <sup>b</sup>	9.335 <sup>b</sup>	1.566	0.013

<sup>abc</sup>: Means within a row with different superscripts differ significantly ( $P \leq 0.05$ ).

## Reused litter and faba beans impact on growth and caecal microbiome in slow growing broilers

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### Application

Reused litter improved bird performance concurs with beneficial shifts in caecal microbiome composition.

ANOVA for reused litter exposure, FB inclusion and interaction effects. SRUC's ethical review approved this study (POU AE 14–2021).

### Introduction

Previous studies have shown that exposure to reused litter improved growth performance in Ross 308 birds, independent on amino acid nutrition (Hussein, Khattak, Vervelde, Athanasiadou, & Houdijk, 2020). Here, we tested whether similar outcomes could be obtained in slower growing Hubbard JA787 birds fed rations with and without faba beans (FB). It was hypothesised that effects on growth performance would concur with modified caecal microbiome composition.

### Results

Mean BW did not differ between treatments at day 14 ( $368 \pm 2.4$  g) and day 28 ( $1192 \pm 7.4$  g). The greater final BW, Firmicutes abundance and F:BAP ratio for reused litter exposure tended to be more pronounced in the absence of FB (Table 1). Reused litter exposure birds had a better finisher FCR, which was increased for FB fed birds. Reused litter exposure reduced Bacteroidota abundance, which tended to be increased by FB, whilst reused litter and FB treatments interacted for Proteobacteria abundance.

### Material and methods

Day-old Hubbard JA 787 male broilers were placed in 32 pens (12 birds per pen), with 2 kg reused litter applied to half of the pens at days 0 and 14 of age. This reused litter treatment was factorially combined with 0 or 15% FB as grower/finisher feeding treatments ( $n = 8$  pens for each litter-feeding combination). Diets were wheat-SBM based, with FB exchanged against soya bean meal on a digestible lysine basis, and pure amino acids and small wheat and oil variations to maintain the same digestible amino acid and energy levels. Birds were fed a common starter crumb (day 0 to 14), grower pellet (day 14 to 28) and finisher pellet (day 28 to 42). Bird body weight (BW) and mortality corrected feed conversion ratio (FCR) were determined weekly but here reported fortnightly. Caecal content was collected from one randomly selected bird per pen at day 42 for bacterial phyla relative abundance assessment via 16S V4 rRNA gene sequencing, with Firmicutes divided over Bacteroidota, Actinobacteriota and Proteobacteria combined (F:BAP ratio) explored as a novel bioindicator. Data were analysed via a  $2 \times 2$  factorial

### Conclusion

This data supports the view that whilst birds may perform at slightly greater FCR in the presence of 15% FB, exposure to reused litter can improve performance, with concurring shifts in caecal microbial composition. The ratio of Firmicutes over the sum of Bacteroidota, Actinobacteria and Proteobacteria might be considered a novel bioindicator for performance.

### Acknowledgment

We thank Hubbard SAS for the provision of the birds, Legumes Translated (EU 817634) and Moy Park for financial support, and Allermuir staff for bird maintenance and technical support. SRUC receives support from Scottish Government (RESAS).

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**Table 1.** Body weight (BW), feed conversion ratio (FCR) and caecal phyla relative abundance and its ratio

Reused litter exposure:		No		Yes		s.e.d	P-values		
Faba beans (%):		0	15	0	15		Litter	FB	Litter × FB
BW (g)	Day 42	2431	2458	2531	2468	36	0.046	0.499	0.091
FCR	Day 0–14	1.260		1.277		0.025	0.500	-	-
FCR	Day 14–28	1.555	1.530	1.530	1.553	0.028	0.963	0.952	0.230
	Day 28–42	1.691	1.741	1.649	1.722	0.016	0.014	<.001	0.322
	Day 0–42	1.583	1.604	1.564	1.601	0.012	0.213	0.002	0.347
Firmicutes (F, %)		86.88	87.46	95.13	87.46	4.54	0.080	0.130	0.080
Bacteroidota (B, %)		8.13	10.18	2.19	7.39	4.34	0.054	0.106	0.473
Actinobacteriota (A, %)		1.82	0.72	1.03	1.41	0.92	0.912	0.444	0.118
Proteobacteria (P, %)		3.17	1.52	1.65	3.58	1.60	0.741	0.865	0.034
F:BAP ratio		10.5	11.9	23.3	11.4	4.98	0.096	0.154	0.074

## Bacterial 6-phytase supplementation can help improve bone mineralisation, phosphorus digestibility and growth performance of broilers fed corn-soyabean meal-based diet

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### Application

A holistic approach to fast degrade feed phytate and release additional phosphorus (P) from the indigestible feed phytate is required to substitute high-cost P sources and improve bird growth.

### Introduction

Development of new exogenous phytases demands a continued need to evaluate their efficacy. A novel biosynthetic bacterial 6-phytase, expressed in *Trichoderma reesei* (Rovabio PhyPlus) was characterized as having high affinity for myo-inositol hexakisphosphate (IP6) and other myo-inositol phosphate (IP) esters, and with a high intrinsic thermostability. Here, its impact on growth performance, tibia ash and tibia P content and pre-caecal digestibility of P (pcdP) in broilers was established.

### Material and methods

A total of 1296 male day-old Ross 308 broilers were allocated to one of three dietary treatments (T1, T2 and T3). T1 was a negative control (NC) diet, having similarly 0.15% points lower calcium and available P levels than T3, T2 was the NC diet plus Rovabio PhyPlus (Adisseo France SAS) at 500 FTU/kg diet and T3 was a positive control (PC) diet formulated to meet the broiler's requirement as recommended by Rhodimet Nutrition Guide (RNG, 2013) for amino acids and NRC (1994) for available P and Ca. All basal diets were corn-soyabean meal based. Each treatment was replicated 24 times with 18 birds/pen in a randomised complete block design. Birds were fed crumbs during the starter (d1-21) and pellets during the grower phases (d22-35). Both feed and water were available *ad libitum*. Average daily weight gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were determined for periods d0-21, d22-35 and d0-35. Tibia bones (at d21 and 35) and ileal digesta (d21) were collected from 5 birds/pen of 16 randomly selected pens/treatment. Birds were humanely killed to collect samples. Tibia bones were analysed for dry matter (DM), ash and P content and ileal digesta for pcdP. The data were analysed using Analysis of Variance (GenStat 19) with

a  $P < 0.05$  level of significance. This study was approved by Food Standard Scotland, and SRUC's Animal Welfare and Ethical Review body.

### Results

Table 1 shows that birds fed NC diet supplemented with Rovabio PhyPlus were significantly heavier and resulted in better FCR compared to both unsupplemented groups fed NC or PC diet. ADFI was similar between treatment group ( $P > 0.05$ ). The overall mortality was low (2.7%). Supplementing NC diet with Rovabio PhyPlus increased ( $P < 0.001$ ) the tibia ash and P contents compared to NC diet and bring it at par with PC diet. Tibia ash data also showed treatment and day interaction and ash content was reduced with age ( $P < 0.05$ ). In terms of pcdP, NC + Rovabio PhyPlus diet outperformed compared to both NC and PC diets.

### Conclusion

This study demonstrates that supplementing NC diets with Rovabio PhyPlus was efficacious in improving the growth performance of broilers from post-hatch to slaughter age. Furthermore, the data suggest that 500 FTU Rovabio PhyPlus/kg feed has the ability to restore performance, bone mineralisation, and P content of birds fed NC diet through the improvement of P digestibility and bring it at par or above what PC diets can offer.

### Acknowledgment

We thank Adisseo France S.A.S for financial support and Allermuir staff and students for bird maintenance and technical support. SRUC receives support from the Scottish Government (RESAS).

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**Table 1.** Effect of Rovabio PhyPlus on growth performance, tibia ash and P (phosphorous) contents, and P digestibility in broilers

Treatment	Growth performance				Tibia analysis				Digestibility
	ABW d35 g/bird	ADG d0-35 g/bird/d	ADFI d0-35 g/bird/d	FCR d0-35	Ash d21%	P d21%	Ash d35%	P d35%	pcdP d21%
NC	2445 <sup>a</sup>	65.23 <sup>a</sup>	95.02	1.458 <sup>b</sup>	47.02 <sup>a</sup>	8.30 <sup>a</sup>	41.34 <sup>a</sup>	7.11 <sup>a</sup>	51.7 <sup>b</sup>
NC + Rovabio PhyPlus	2559 <sup>c</sup>	68.28 <sup>b</sup>	95.48	1.399 <sup>a</sup>	49.34 <sup>b</sup>	8.78 <sup>b</sup>	43.63 <sup>b</sup>	7.61 <sup>b</sup>	60.4 <sup>c</sup>
PC	2506 <sup>b</sup>	66.28 <sup>a</sup>	95.85	1.446 <sup>b</sup>	49.53 <sup>b</sup>	8.78 <sup>b</sup>	42.65 <sup>b</sup>	7.49 <sup>b</sup>	47.9 <sup>a</sup>
s.e.d	0.019	0.814	1.521	0.018	0.292	0.079	0.487	0.105	1.21
P-value	<.001	0.002	0.861	0.005	<.001	<.001	<.001	<.001	<.001

ABW = Average body weight; ADG = average daily weight gain; ADFI = average daily feed intake; FCR = feed conversion ratio; pcdP = pre-caecal digestibility of P; s.e.d = Standard Error of Difference; P-value = Significantly different if  $P \leq 0.05$ .

## Nutritional sensitivity of the poultry gut microbiome

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The microbiome of animals is a main driver of animal performance and disease resistance. While coccidiosis is the most important intestinal pathology in chickens, also bacterial pathogens such as *Clostridium perfringens* are a major cause of performance losses. Apart from diseases caused by pathogens, also intestinal entities of unknown etiology are a major concern. One of these, the so-called dysbiosis, is a shift in microbial composition away from the normal condition, and associates with intestinal villus shortening, inflammation and thus digestive disturbances and performance losses. The most evident form of dysbiosis is caused by overgrowth of *Clostridium perfringens* in the small intestinal tract, resulting in necrotic enteritis. Mostly, however, intestinal microbial imbalances are less severe, can originate in the small but also distal intestinal tract (ceca). Reasons for unfavorable shifts in microbiota composition can have a dietary origin (e.g. excess of poorly digestible components) but can also be the consequence of infections. It has become clear that specific microbial taxa are regarded as beneficial, the most important ones being the butyrate-producing anaerobic bacteria. Butyrate is indeed regarded as a powerful anti-inflammatory and gut health promoting compound. On the other hand, specific taxa of the *Enterobacteriaceae* family are regarded as harmful. These are facultative anaerobic LPS-containing species that are promoting gut inflammation. Many dietary factors have been shown to support gut health by steering microbial composition. Obviously, probiotic strategies are one of them, as these are bacteria that can be added to the feed.

Bacilli are widely used as they are able to form spores and are heat-stable and have a variety of described mechanisms of action, depending on the strain (eg. production of antimicrobial molecules, beneficial metabolites, amongst others). Mixtures of strains are also in use, and competitive exclusion cultures are formulations that mimic fecal transplants so enabling rapid installation of a mature microbiota. Prebiotics are non-digestible saccharides that can be used as substrates by specific microbial populations and thus in this way promote colonization of beneficial microbes, that can use these substrates to produce fermentation acids and other beneficial molecules. Examples are xylo-oligosaccharides. Some of the prebiotic substances, such as glucans, have immunomodulatory effects. Also direct administration of short-chain fatty acids, including butyrates, have been shown to cause microbial shifts in addition to direct host effects. Because broilers have a very limited life span, it is evident that early establishment of a microbiota is crucial to avoid pathogen colonization. Also the feed composition and form is a factor that can affect the microbiota composition. A second important period of life is around 3 weeks of age, and supportive feed additives could then be needed to prevent dysbiotic shifts that affect performance, and cause wet litter and associated pathologies (e.g. pododermatitis, and translocation of bacteria and chondronecrosis with osteomyelitis). The lecture will focus on the beneficial and harmful microbiota shifts that are associated with animal performance and disease resistance, and that seem to have a universal pattern.

## An *in vivo* bone phenotype for use in genetic selection and welfare monitoring in laying hens

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### Application

Bone quality in laying hens is a welfare issue but no practical method has been available for genetic selection. We describe a method that can be used in a breeding programme and in nutritional trials.

### Introduction

When hens lay eggs there are major physiological changes to supply calcium for the formation of an egg shell. The changes include medullary bone formation as a depot of calcium for shell formation. This is related to structural bone quality that is particularly manifested in keel bone fractures and deviations, especially in aviary systems. Bone quality is a moderately heritable trait with around 40% of the variation attributable to genetics (Dunn et al., 2021). However, due to the absence of a practical method to measure bone quality in living hens that

would subsequently allow breeding there has been no possibility of progress. The data presented demonstrates a method to measure bone quality in living hens is possible using digital x-ray.

### Material and methods

Initial studies were carried out on cadavers to optimise positioning, exposure and methodology for analysis. Hy-line brown hens were used throughout. Temporary restraint of hens was achieved with a VSP Miami Vise. Image capture used a Cuattro Slate 6 DR X-ray system and an x-ray generator setting of 65kV/5mAs. A 100-pixel wide line was drawn across the tibiotarsus on the x-ray image and the plot profile pixel density (Tibiotarsus AUC) was calculated using the image analysis software Image J. Post-mortem measurements of radiographic density of tibia and keel bone and breaking strength of the tibia were made for

validation. A reproducibility study was performed using 60 hens at 30 weeks of age in four pens. To demonstrate the ability to identify bone density changes three diets were fed at 26 weeks of age, measured Ca P % were; Control 3.28%, 0.46%, 7:1; LowCa, 1.89%, 0.48%, 4:1; LowCaP 1.39%, 0.31%, 4.5:1. Tibiotarsus AUC were measured before; 3 weeks after and 5 weeks after treatment. Comparison of traits was made using Pearson correlation, attribution of variance was made using a Gage analysis and repeated measures ANOVA was used to compare dietary treatment. Experiments were approved by the Roslin Institute animal welfare and ethical review body.

## Results

Measurement took ~ 45 seconds per hen from holding crate back to holding crate. Tibiotarsus AUC measurements at 30 weeks of age and post-mortem tibiotarsus radiographic density were significantly correlated (0.76;  $p = 0.0001$ ) in the repeatability experiment. The vast majority of the variance (92%) was attributable to bird but only 8% from the repeated measurement of the tibiotarsus AUC. The correlation of tibiotarsus AUC measurements and post-mortem tibiotarsus radiographic density were significantly correlated (0.69;  $p = 0.0001$ ) in the experiment altering Ca:P levels and ratios. The control diet hens tibiotarsus AUC increased within 3 weeks  $5547 \pm 1226$  whilst the treatment groups reduced, LowCa,  $-574 \pm 1410$ ; LowCaP  $-5496 \pm 1182$  ( $p < 0.001$ ).

## Conclusion

The method was quick and easy to perform. The phenotypic correlations between the method on the live hen and dissected bone density estimates suggest the tibiotarsus AUC is a good proxy measurement. The ability to measure changes when the diet was altered was superior to post-mortem data in part because the bone quality could be assessed before and during dietary intervention. The small contribution of repeatability to variance meant that individual animals were easily separated on their bone quality. This favours genetic parameter estimation. The method should allow genetic progress and may reduce the number of animals required to test dietary interventions.

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## Laying hens display resilience to sleep disruption

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## Application

Mild disturbances such as noise, wind and light need not be considered immediate welfare concerns by poultry farmers.

## Introduction

Sleep is a behaviour that is found across the animal kingdom from jellyfish to humans, however, despite this the true function of sleep remains a mystery and sleep in birds is even more poorly understood than in mammals, though there are similarities. The quality and quantity of sleep is sensitive to physical and emotional stressors, which can result in a poor night's sleep. These characteristics may allow for the use of sleep as an indicator of stress and welfare in (commercially-housed) animals. Therefore, this study aimed to measure baseline sleep behaviours (slow-wave sleep, SWS; and rapid-eye-movement sleep, REM) and wakefulness in laying hens and then apply disturbances to observe the subsequent effects on sleep and wakefulness.

## Materials and methods

Experimental use of animals was approved by the SRUC Animal Experiments Committee. Twelve individually-housed H&N Brown Nick laying hens (56–77 weeks of age) were used

across four batches. Electroencephalograph (EEG) devices were surgically implanted under general anaesthesia to collect brain wave data to identify sleep states. Behavioural data was collected using infrared cameras. Following a week of surgical recovery, hens were exposed to disturbed, undisturbed and recovery nights to establish a baseline for sleep data and to observe the effects of mild disturbances on sleep. Disturbances were: noise (90 dB), wind, and light (20 lux) which were applied in a pre-determined order for 5 minutes every 30 minutes between 21:00–03:00 (lights off period between 19:00–05:00). Sleep states were scored with Slipanalysis (4 sec epochs/min) using both electrophysiological and behavioural data from which the proportion of time spent in each sleep state was calculated. Data on proportions of sleep behaviour in each 2-hour time interval per bird were analysed following angular transformation (to normalise data) using linear mixed models in R, after filtering out data rows that had >0.25 proportion of artefact (electronic noise) or missing data.

## Results

Birds spent ~30% of observations between 19:00–21:00 awake which increased to ~40% between 21:00–23:00, decreasing to ~35% by 03:00–05:00. SWS constituted ~60% of observations between 19:00–21:00 but steadily decreased

to ~45% by 03:00–05:00, whilst REM increased from ~10% of observations at 19:00–21:00 to 20% by 03:00–05:00. Overall, time interval in the day was significant ( $P < 0.001$ ) for all three states (wakefulness, SWS and REM), while disturbance treatments were not ( $P \geq 0.05$ ). The interaction between time interval and disturbance treatments was significant ( $P < 0.05$ ) for both wakefulness and SWS, but not for REM sleep ( $P > 0.05$ ).

### Conclusion

As observed in other avian species, sleep in laying hens showed expected signs of rhythmicity with SWS being higher during the early night and steadily being replaced by REM as night progressed. It is clear that birds were affected by disturbances

during the hours in which they were applied, with the exception of REM sleep which remained relatively constant during this time. Ultimately, hens seem to show resilience towards mild disturbances and appear to be able to return to sleep easily. Daytime sleep in these birds will be analysed to look for carry over effects of disturbance.

### Acknowledgment

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## Heritability and genetic correlation of keel bone morphometric phenotypes and injury grading in laying hens

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### Application

Improvement in the bone health of laying hens by genetic selection to lower incidence of bone injuries and improve animal welfare.

### Introduction

The chicken keel bone, part of the sternum, has the highest incidence of fracture (Freire, Wilkins, Short, & Nicol, 2003; Wilkins, Brown, Zimmerman, Leeb, & Nicols, 2004). Due to its anatomical location, the keel bone in the laying hen is subject to pressure when the hen is perching which could result in shape deformities (Kappeli, Gebhardt-Henrich, Frohlich, Pfulg, & Stoffel, 2011). Current keel phenotypes measured including radiodensity have low heritability and are not correlated with overall bone strength, suggesting that they would not be suitable for selection to reduce keel bone injury. The current methods of measurements are also time-consuming and carried out post-mortem. Therefore, there is no reliable keel phenotype that is associated with lower keel fracture incidence. One of the issues is that there are many types of injury that can occur in the keel bone including fractures and deviations. The aim of this study is to analyse morphological phenotypes of the keel bone and measure their heritability, genetic correlation, and correlation with keel injury.

### Materials and methods

Bone samples were collected from White Leghorn hens at 53 weeks of age ( $n = 956$ ). Radiographs of the keel bones were taken and novel phenotypes of the keel were measured. These include the depth of the keel at two points (cranial and middle), the localised radiodensity at two points (cranial and caudal), the area, the perimeter and the length of each keel bone. Heritability of these phenotypes were estimated using ASReml-W and genetic correlations estimated with other bone quality phenotypes such as strength, stiffness and density measurements of tibia and humerus. To quantify keel bone injuries, factors including proportion of bone affected, location of fracture/

callus, type of fracture, direction of fracture, healing state, deformity and deviation were each given a score between 1 and 4 based on severity. The scores were then categorised into grades 1 to 6 (least to most severe injuries). Analysis of variance was performed using Genstat. Geometric morphometry analysis of the keel bones was performed using landmark coordinates of each keel bone, using Geomorph package on R. This study was approved by the Animal Welfare and Ethical Review Body at The Roslin Institute, UK.

### Results

Of the novel keel phenotypes measured, cranial keel depth had a heritability of 0.3 and had significant genetic correlations with other bone phenotypes including tibia density ( $0.39 \pm 0.15$ ) and humerus density ( $0.60 \pm 0.20$ ). Cranial localised keel density had a significant genetic correlation with tibia density ( $0.93 \pm 0.24$ ), tibia stiffness ( $0.66 \pm 0.27$ ) and humerus density ( $0.68 \pm 0.30$ ). Keel overall density was higher ( $P = 0.05$ ) in the birds with keel grade 6 ( $0.69 \pm 0.04$  mm Al) than in the birds with keel grade 1 ( $0.62 \pm 0.01$  mm Al). Humerus breaking strength was higher ( $P = 0.004$ ) in hens with keel grade 1 ( $183.7 \pm 2.9$  N) than in hens with keel grade 6 ( $156.1 \pm 13.1$  N). Tibia breaking strength was also higher ( $P < 0.001$ ) in hens with keel grade 1 ( $215.6 \pm 3.2$  N) than in hens with keel grade 6 ( $170.7 \pm 13.0$  N).

### Conclusion

Novel keel phenotypes had moderate heritability and genetic correlation with other bone quality phenotypes suggesting that these phenotypes have similar genetic determinants. Some of these novel traits could be used as a proxy for bone quality measurement and potentially could be measured in living birds to aid selection. Morphometric traits may also be manipulated to improve keel quality and reduce injuries. The hens with higher overall bone strength had fewer and less severe keel injuries. Therefore, improving general bone health will improve keel quality and improve welfare.

## Acknowledgment

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## Practical application of sexing embryos before hatch

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Globally the development of applicable, reliable, and cost-efficient procedures to sex embryos could lead to the avoidance of the killing of male layer hybrids in the future. In basic research, the first task is to find a 'source of information' that provides reliable information about the sex of the embryo as early as possible. A biologically reliable and comprehensible identification of sex-specific distinguishing features (levels of male or female sex hormones, different DNA of the sex chromosomes, sex-typical biomarkers, or spectral differences) provides starting points for further developments.

During the research process, it is important to check various aspects for later practical application: Are the individual steps required suitable for automation? Can a damage to the embryo and/or negative impact on hatchability be ruled out? Can the information and measurement data obtained be easily interpreted and are they reproducible? Is the respective methodology socially acceptable? Above all, automation, and verification in extensive hatchery trials over the entire incubation period are the most important steps in this phase.

Four methods for *in ovo* sexing have successfully finished the development phase and reached practical application in Europe. To date, the highest amount of '*in ovo* chicks' – i.e. female layer chicks without killing the brother – were produced with the hyperspectral analysis system 'CHEGGY' (Agri Advanced Technologies GmbH [AAT], 2021), followed by endocrinological sexing 'Seleggt' (Seleggt, 2022), DNA analysis 'PlantEgg' (PLANTegg, 2021) and mass spectrometry In Ovo. All technologies are used in the second third of incubation.

### Hyperspectral analysis

In brown laying hybrids with sex-specific feather colour, sex differences in pigmentation levels are already visible during embryonic development between day 11 and day 14 of incubation. The major advantage of this method is that – unlike in other spectroscopy methods – the analysis can be performed non-invasively through the intact eggshell. Thus, cross contamination and negative influences on the development of the embryos, the hatching rate and later performance and health of the laying hens are not to be expected. At day 13 less than 5% sexing errors can be achieved.

### Endocrinology

Sex-specific hormonal concentration differences in samples from allantoic fluid are not significant apparent until day 9, and were more pronounced on day 10, allowing precise sex

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identification. Using advanced sampling techniques of allantoic fluid, an accuracy of 95–98% can be achieved. Since 2019, a fully automated process (3600 hatching eggs/hour) is available that carries out all process steps (Seleggt, 2022).

### DNA analysis

The PLANTegg process is using PCR (polymerase chain reaction) method to determine the differences between the two genders. After amplification of the DNA an optical signal is visible indicating the female DNA. Meanwhile the PLANTegg process is used commercially in hatcheries with a sexing precision of more than 98%. The sampling technology is identical with the procedure used by Seleggt (2022).

### Mass spectrometry

According to In Ovo, the concentration of glucose, choline and valine in the allantoic fluid is higher in females than in male eggs and could thus serve as biomarkers (Bruins & Stutterheim, 2014). In Ovo identifies biomarkers which can be detected by mass spectrometry in a fast and high throughput device. Their Alpha prototype realized 1800 eggs/hour with 95% accuracy. The sampling machine for allantoic fluid is using a syringe which penetrates the eggshell and collects the liquid for further processing.

### Research projects

#### Spectroscopic methods

Spectroscopic methods offer contactless examination, but also low costs for consumables. The examinations can be performed as early as 3 to 5 days of incubation. The spectroscopic examinations are non-invasive in the sense that no tissue must be collected. However, a hole in the eggshell is necessary to allow access to interior of the egg. The actual measurement to obtain the sex information takes only a few seconds, allowing sorting of eggs in near real time.

#### Raman- and fluorescence spectroscopy

In a Raman spectroscopic measurement, light of a defined wavelength is irradiated onto the object under investigation and the spectrum of the scattered light is analysed. Sex-specific information can be obtained from the developing embryonic vascular system. To identify the sex on the opened egg, a hole of about 12 mm diameter must first be made in the shell and the 'lid' removed while the inner

shell membrane remains intact. The latest generation of the method, which was primarily developed at the Technical University (TU) Dresden, Germany, uses two laser beams of different wavelengths. The scattered light from the haemoglobin is used to identify the sex in a combination of Raman and fluorescence spectroscopy. While Raman spectra provide molecular structural features, fluorescence primarily provides information on the concentration of haemoglobin.

#### **Time-resolved laser-induced fluorescence spectroscopy**

The method is based on the measurement of laser-induced fluorescence, which has an extremely high detection sensitivity and precision. In combination with the detection of the time-dependent behaviour of the fluorescence light and algorithms adapted and optimized for sex determination, the sex of an embryo can be detected reliably even before the 6th day of incubation. An opening in the eggshell of <2 mm diameter, with the membrane kept intact, minimizes the risk of contamination (Dörksen, 2022).

#### **Magnetic resonance imaging**

As another example of an imaging process is the magnetic resonance imaging (MRI). ORBEM combines Artificial Intelligence and deep-tech imaging technology to distinguish the gonadal development of male and female embryos between 12 and 13 days of incubation (ORBEM, 2022). The 'Genus-Focus', a fully automated end to end system (Vencomatic Group, 2022), is a non-invasive method which is applicable to all layer chicken genetics. Two installations with a capacity of 6,000 and 12,000 eggs per hour are now fully operational at two French hatcheries.

#### **Outlook**

Efforts in Germany to only allow *in ovo* procedures before the 7th day of incubation are fuelling research but will ultimately result in rearing the male chicks as the only method if proven technologies in the first trimester of incubation are not available for commercial use. Actual *in ovo* techniques in practice have little possibilities to detect the sex of an embryo at an earlier stage – be it of technical or biological reasons: sex specific hormones or feather colour are not developed before day 8 or day 10 respectively, and for liquid-based methods it is essential to get enough and securely a specific amount of allantoic fluid at a very early stage of development. The further development of the described spectroscopic techniques as well as other approaches are needed for solutions that reliably perform as early as possible during incubation.

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## **Practicalities of sexing embryos and rearing males in practice**

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Layer breeders produce fertile eggs that, if incubated, hatch out as approximately 50% male and 50% female. Female layer chicks are reared for egg production, but typically male layer chicks are culled at the hatchery at day old and used as a food source (birds of prey, reptiles, etc). This results in the annual culling of approximately 330 million chicks in the EU (Vinci, 2022). The ethics of culling millions of freely-living animals has come under scrutiny, and as a result, Germany and France have banned the culling of day-old male layer chicks and are calling for similar EU-wide legislation. Technological advances have resulted in several methods of identifying the sex of the embryo in the shell ('in-ovo sexing') in the second trimester of incubation, which means that male embryos can be culled during an early stage of development. This not only is more efficient in terms of energy (duration of time in the incubators) and staff time (sorting of hatched chicks) but is also more ethically acceptable. However, using in-

ovo sexing may increase the dead-in-shell % of the female embryos due to the introduction of pathogens, and it increases the cost of the resulting pullets where the technology has been implemented. Furthermore, concerns over the age at which embryos can feel pain are fueling requirements for early ( $\leq 7$  days) in-ovo sexing in Germany. Alternatively, male layers can be reared and processed for meat production. However, to reach a suitable weight for slaughter, male layers have to be reared longer than a typical broiler strain (e.g. 15 compared to <6 weeks) but are lighter than broilers (1500 versus 2400 g) (Van Horne, 2022). As a result, the onset of aggression can result in fighting, causing welfare issues such as fear and wounding. Layer males have poorer feed conversion compared to broilers, and combined with the longer grow-out period, this makes layer male rearing inefficient, with an estimated CO<sub>2</sub> equivalent per kg carcass weight of 9.6 in male layers versus 2.96 in broilers (Bessei, 2021). Furthermore, layer male carcasses produce



less meat overall and less breast meat specifically than a broiler, making them unsuitable for the European market. As a result, male layers reared in Europe are used for processed foods or male layer chicks are exported to Poland for rearing and slaughter, where the meat is exported to Africa (Blanco, 2022). This latter solution presents further ethical issues around rearing standards.

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## Exploring effect of housing system on skeletal development in laying hens throughout lay

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### Application

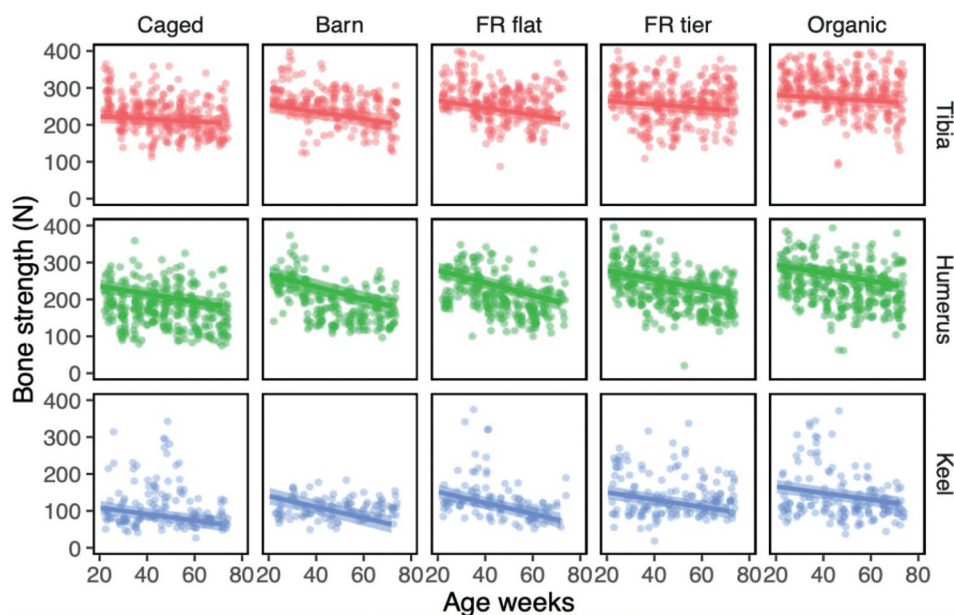
Age-related change in laying hens' bone strength appears to alter with housing system suggesting efforts to improve bone integrity should be undertaken using a bespoke approach for each housing system.

### Introduction

Caged housing systems limit movement, increasing risk of osteoporosis and significantly reducing tibia and humerus breaking strength compared to aviary systems. Conversely, loose housing systems provide increased opportunity for flight, potentially resulting in humerus fractures (Casey-Trott, Korver, Guerin, Sandilands, & Torrey et al., 2017). This study investigated how differing UK housing systems affect tibia, keel and humerus strength of laying hens up to 72 weeks of age.

### Materials and methods

After ethical approval, the study was conducted using 14 UK egg farms housing three bird strains (Lohmann Brown Classics, Bovan Browns and Hy-line Browns) from 18 to 72 weeks. Housing systems were free-range multi-tier (FR-Tier), caged (C), free-range flat deck (FR-Flat), organic flat deck (O) and barn (B), which included both flat deck and multi-tier. All farms followed the Welfare of Farmed Animals (England) Regulations 2007 and fed hens to meet all nutritional requirements during lay. Where possible, six hens were euthanised and collected from each farm every 6 weeks from 18 to 72 weeks of age resulting in 781 birds from a possible 840. From each bird, keel, humerus and tibia bones were excised and all soft tissue removed before measuring force (Newtons -N) required to break bones at their mid-point bone as using a texture analyser with a 3-point bent attachment. A Gaussian LMM in R version 4.1.0 and RStudio



**Figure 1.** Mean fitted bone strength of UK laying hens (solid line) and 95% confidence intervals (shaded area) over age (weeks) modelled with a Gaussian LMM, with farm and individual bird fitted as random terms in the model. Data is split by housing system and bone type.

version 1.1.463 was used as detailed in Kemp (2021) to model bone strength over laying period with farm fitted as a random term with tibia from caged housing system set as the baseline coefficient.

## Results

Figure 1 shows mean fitted bone strength with 95% confidence intervals for keel, tibia and humerus bones over time by housing system. As a main effect, age did not significantly effect tibia strength though, (as a trend:  $p = 0.072$ ; estimate =  $-5.75$ ), as age increased, tibia strength decreased. FR-Flat bone strength was significantly greater than C tibia strength ( $p = 0.020$ ; estimate =  $27.37$ ). FR-Tier and O tibia strength were significantly stronger than C ( $p < 0.001$ ; estimate =  $38.30$  and  $p < 0.001$ ; estimate =  $56.78$  respectively). FR-Tier keel bone was significantly weaker than C ( $p < 0.001$ ; estimate =  $-127.17$ ). Across all bone types, B and FR-Flat bone strength both declined significantly faster than C ( $p = 0.024$ ; estimate =  $-10.64$  and  $p = 0.025$ ; estimate =  $-10.92$  respectively). Across all systems, humerus and keel bone strength both declined significantly faster than tibia bone strength ( $p < 0.001$ ; estimate =  $-12.04$  and  $p < 0.001$ ; estimate =  $-8.74$  respectively).

## Conclusion

While limited to a small number of farms and failing to consider bird strain as a factor, this study suggests use of caged housing systems is generally associated with weaker bones, with one interesting exception: tibia strength of barn-housed hens does not differ to caged. Rate of deterioration in bone strength over time is greater in barn and free range flack deck than other systems, and humerus and keel bone strength declined faster than tibia for all systems, indicating multiple bones are required to assess impact of housing system as effect on keel, humerus and tibia strength differ.

## Acknowledgment

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## Using a live vaccine as a model for respiratory disease in hens: inducing clinical symptoms

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### Application

A vaccine model for disease stimulates measurable clinical symptoms without actually inducing illness.

### Introduction

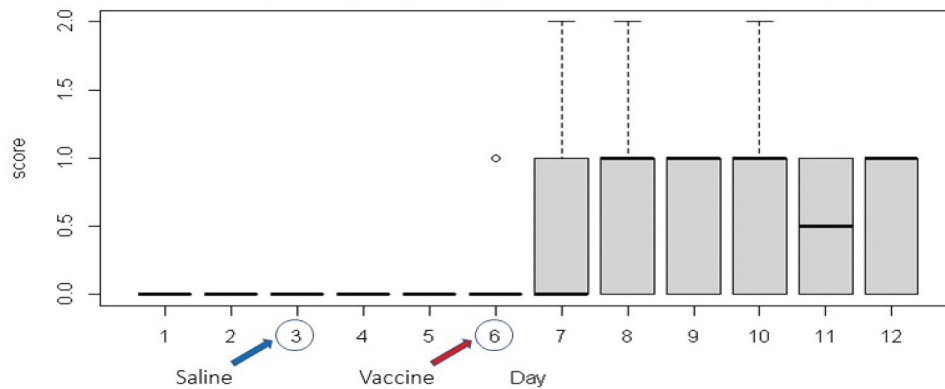
The health of animals is an important factor influencing the level of animal welfare, and sick or diseased animals are considered to have poor welfare (Broom, 2006). Monitoring changes in physiology and occurrence of sickness symptoms in laying hens could improve welfare and health management. As part of a project to determine whether wearable technologies that indicate activity and location could be used to identify sick birds, we used a live vaccine as a model to stimulate disease-type symptoms.

### Materials and methods

A total of 20 previously unvaccinated hens (Hy-Line Brown, 25–35 weeks old) were used in 5 replicated experiments (4 hens/replicate, 12 days each). Hens were moved to a room (approximately 180 m<sup>2</sup>) on day 1, which contained food, water, nest boxes and perches. On day 3, saline control was administered to all birds by nasal and ocular drop. On day 6, birds were given ILT (Infectious Laryngotracheitis) vaccine using the same method. Clinical symptoms were recorded daily: unusual breathing, nasal and ocular discharge, conjunctivitis, and depression (assessed using a scoring system where 0 = no symptoms, 3 = severe symptoms), cloacal temperature and respiratory rate (counts/min). Data were analysed in RStudio using linear and generalised linear mixed models. The study was approved by SRUC's Animal Experiments Committee.

**Table 1.** Mean clinical scores (range: 0–3) and respiratory rate (counts/min) in hens subjected to a live vaccine, which were significantly affected by day (all  $P < 0.001$ ). Mean values are from raw data, with standard deviation (SD) range shown

	Clinical Scores				Respiratory rate
	Abnormal breathing	Ocular discharge	Conjunctivitis	Depression	
Mean score	0.29	0.47	0.65	0.08	18.0
SD range	0.00–0.76	0.00–1.20	0.00–1.39	0.00–0.93	0.93–4.53



**Figure 1.** Mean scores (n=20 hens) of abnormal breathing, scored on a scale of 0-3, with standard deviation shown. Arrows show the day saline control and vaccination via ocular and nasal drop occurred.

## Results

There were no significant differences over the 12 days in mean cloacal temperature (range: 41.3–41.5°C) or mean nasal discharge scores (range: 0.0–0.1) ( $P > 0.05$ ). Mean scores of abnormal breathing, ocular discharge, conjunctivitis and depression, and respiratory rate were significantly different over days (all  $P < 0.001$ ) (Table 1). Mean scores of abnormal breathing were higher on days 7–12 than previous days (Figure 1).

## Conclusion

Using a live vaccine for a respiratory disease was successful at stimulating measurable changes in clinical symptoms in hens. This method could be used in future to test if wearable activity monitors can detect changes indicative of disease symptoms in hens.

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## Dietary black pepper and supplementary xylanase improve blood lipids profile in broilers

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## Application

Feeding black pepper or xylanase to broiler chickens may respectively elevate high-density lipoprotein or reduce the low-density lipoproteins in blood, thus improving their health and wellbeing.

## Introduction

Abnormalities in lipid metabolism are associated with a high-risk profile for cardio vascular diseases and obesity (Meriga et al., 2017), thus lipid balance is important for maintaining health and wellbeing. Dietary intake of piperonal, a constituent of black pepper (BP; *Piper nigrum L.*), displayed elevated plasma levels of high-density lipoprotein (HDL) and decreased plasma levels of total cholesterol, triglyceride (TG), and low-density lipoproteins (LDL) cholesterol in rats (Meriga et al., 2017). Xylanases (XYL) are commonly used in wheat-based formulations to help improve their feeding value (Bedford, 2000). However, information on interaction between BP and XYL is lacking. Therefore, the current experiment was designed to determine if dietary BP and XYL, alone or in a combination,

have an impact on blood lipid fractions. Blood total protein (TP) and minerals such as Ca, P, Fe and MG were also measured.

## Material and methods

The experiment was conducted at the National Institute of Poultry Husbandry and approved by the Harper Adams University Research Ethics Committee. A wheat-soy-based basal feed containing 650 g/kg wheat and 220 g/kg soybean meal as main ingredients (12.67 MJ/kg AME and 206 g CP; relatively low to industry recommendations) was mixed. The basal feed was then split into four batches as one of them stayed as it is; second batch was supplemented with *Aspergillus oryzae* commercial preparation of endo-1,4-beta XYL at 200 FXU/kg (Ronozyme WX, DSM, Switzerland); third batch was supplemented with 10 g/kg diet of milled BP (determined 4.2% piperine); the fourth batch was supplemented with both, XYL and BP, as in the previous two batches. Enzyme and BP in each diet were added on the top of the basal feed. Sixty-four male Ross 308 chicks were used in the study. At 7 d age (study start) the birds were allocated to 32 pens, 2 birds per pen, following randomisation.

**Table 1.** Effect of dietary black pepper (BP) and xylanase (XYL) on blood serum chemical composition

Treatment	HDL (mmol/L)	LDL (mmol/L)	TG (mmol/L)	TP (g/L)	Ca (mmol/L)	P (mmol/L)	Fe (umol/L)	Mg (mmol/L)
No BP	2.9	0.55	1.39	22.6	3.3	2.10	12.4	1.17
Yes BP	3.1	0.61	1.37	24.0	3.0	1.88	12.9	1.01
No XYL	3.0	0.66	1.37	23.3	3.2	2.14	12.9	1.12
Yes XYL	3.0	0.51	1.39	23.3	3.1	1.84	12.4	1.14
SEM	0.07	0.037	0.141	0.67	0.07	0.1145	0.85	0.029
Probabilities								
BP	0.017	0.278	0.916	0.174	0.022	0.176	0.703	0.083
XYL	0.918	0.010	0.916	0.953	0.947	0.073	0.644	0.639
BP x XYL	0.857	0.103	0.566	0.343	0.979	0.429	0.216	0.507

Standard rearing conditions for broilers were used (Aviagen Ltd., Edinburgh, UK). At the end of the study, at 21 d age, one bird per pen was electrically stunned and blood from jugular vein was obtained in heparin coated tubes. Blood lipid profiles including HDL, LDL and TG, and TP, Ca, P, Fe and MG were measured (Jóźwik et al., 2012). Blood data were analysed by ANOVA following 2 × 2 factorial arrangement with main factors being BP and XYL.

## Results

Feeding BP led to increased HDL ( $P < 0.05$ ), reduced Ca ( $P < 0.05$ ) and tended ( $P = 0.83$ ) to reduce Mg in blood. Feeding XYL reduced ( $P < 0.05$ ) blood LDL level. There was no BP by XYL interaction observed. No differences were found for the rest of the studied variables ( $P > 0.05$ ) (Table 1).

## Conclusion

These data showed that dietary BP may increase HDL although dietary XYL reduces LDL in blood of young broilers. Thus, suggesting that simultaneous supplementation of both ingredients may bring more health benefits to the birds. Research on meat lipids of broilers fed BP and XYL is warranted.

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## The effect of xylanase on growth performance and AMEn in broiler chickens when 7.5% of maize-based diet is replaced with cereal brans with different non-starch polysaccharide composition

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## Application

Supplementary xylanase can improve nutrient utilization and feed efficiency in a maize-based diet, but its efficacy may be influenced by the fibre source, inclusion level and their soluble/insoluble non-starch polysaccharide ratio.

interaction between exogenous xylanase (XYL) and different sources of dietary fibres on growth performance, including feed intake (FI), weight gain (WG) and feed conversion ratio (FCR), and dietary N-corrected apparent metabolisable energy (AMEn).

## Introduction

Nowadays the interest in fibre has increased due to their possible beneficial effect on the gut health, although understanding of their impact on the gastrointestinal tract (GIT) development and the performance of broilers is poor (Mateos, Jiménez-Moreno, Serrano, & Lázaro, 2012). Fibre is the sum of the soluble and insoluble non-starch polysaccharides (sNSP, iNSP respectively) fractions and lignin in grains. Excessive amounts of high molecular weight sNSP in broiler diets can cause a negative effect on performance by increasing digesta viscosity, but it is also a substrate for enzymatic hydrolysis, which becomes a subsequent source of energy for GIT microbes via fermentation, the end products of this process being in the form of short-chain fatty acids (Aftab & Bedford, 2018). The aim of the study was to investigate the potential

## Material and methods

A total of 336 day-old male Ross 308 broiler chickens were used in the experiment which was approved by the Harper Adams University Research Ethics Committee (1162–202122-PGMPHD). On arrival, birds were individually weighed and assigned to 48 raised floor pens with seven birds in each pen. Birds were fed 8 diets in total, as one was maize-based diet as control (C); second C + 75 g/kg wheat feed (WB); third C + 75 g/kg rice bran (RIB); fourth C + 75 g/kg rye bran (RYB), each supplemented with or without xylanase (XYL) (Econase XT, AB Vista, Malborough, UK) at 16000BXU/kg. All diets were formulated to be isocaloric and isonitrogenous. The fibres were chosen based on the amount of sNSP, with RYB high, WB medium and RIB low on soluble components. Diets were fed as mash from d 0–7 and as pellets (cold pelleted) from d 8–28. On day 28, all birds and feed were weighed to calculate FI, WG and FCR.

**Table 1.** The effect of added fibres and xylanase and their interaction on performance and AMEn

Fibre	Xylanase	Feed intake (g/b/d)	Weight gain (g/b/d)	Feed Conversion Ratio	AMEn
Control (C)	-	62.9	47.2	1.459 <sup>b</sup>	12.14 <sup>a</sup>
	+	62.9	48.4	1.413 <sup>a</sup>	12.66 <sup>c</sup>
Wheat bran (WB)	-	61.6	45.2	1.485 <sup>c</sup>	12.33 <sup>ab</sup>
	+	61.5	45.9	1.460 <sup>b</sup>	12.33 <sup>ab</sup>
Rye bran (RYB)	-	59.0	42.0	1.523 <sup>d</sup>	12.57 <sup>bc</sup>
	+	59.3	40.7	1.562 <sup>e</sup>	12.62 <sup>c</sup>
Rice bran (RIB)	-	61.3	44.1	1.516 <sup>d</sup>	12.93 <sup>d</sup>
	+	60.3	43.3	1.510 <sup>d</sup>	13.04 <sup>d</sup>
SEM		1.21	0.93	0.0078	0.093
<b>P-value of interaction</b>		0.956	0.482	<0.001	0.033

<sup>a,b</sup>Different superscripts in a column show a significant difference ( $P < 0.05$ ).

During the last three days of the study, excreta was collected and analysed for gross energy and nitrogen to calculate dietary AMEn. Data were analysed by ANOVA.

## Results

The main effects of fibres were significant on FI (g/bird/day: C = 62.9, WB = 61.6, RYB = 59.1, RIB = 60.8; SEM = 0.86;  $P = 0.032$ ) and WG (g/bird/day: C = 47.8, WB = 45.5, RYB = 41.3, RIB = 43.7; SEM = 0.66;  $P < 0.001$ ), but not significant ( $P > 0.05$ ) for XYL (data not shown) (Table 1). Dietary XYL reduced FCR for C and WB, increased it for RYB and did not change it for RIB diets, respectively ( $P < 0.001$ ). XYL increased AMEn in the control diet only ( $P = 0.033$ ).

## Conclusion

All dietary fibre sources are not the same and a more detailed description of NSP fragments is likely needed if structure/function relationships are to be identified. In addition, the source of fibre will most likely influence the efficacy of xylanase.

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