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A retrospective analysis of *Salmonella* monitoring programs in Australian feed mills

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Animal feed and feed mills have long been regarded as possible vectors of *Salmonella* spp. transmission (Davies and Wray 1994; Parker 2008) with potential for subsequent consequences in human health and well-being (Sexton, 2016). As such, the provision of safe, commercially prepared stock feed for production animals is critical in ensuring the safety of food-animal products for human consumption. Since 2002, the Australian stock feed industry has applied FeedSafe®, a comprehensive quality assurance program based on the principles of hazard analysis and critical control points (HACCP) and good manufacturing practices (GMP). Microbiological monitoring forms part of the FeedSafe® quality assurance approach. Parker et al. (2008) observed a reduction in the incidence of *Salmonella* following the introduction of FeedSafe® microbiological monitoring principles. The current study sought to retrospectively analyse microbiological sampling data collected from 22 FeedSafe® accredited mills over a 13-year period and in particular, to determine the effectiveness of FeedSafe® in controlling the risk factors that contribute to *Salmonella* spp. contamination of commercially prepared stock feed.

The 22 feed mills surveyed were located in multiple states (Queensland, New South Wales, Victoria and South Australia). All were FeedSafe® accredited and had implemented an HACCP program for the control of *Salmonella* spp. During the study period (January 2003 to August 2016), 19,900 microbiological samples of raw materials, milling equipment and finished feed were collected. From this data the prevalence, distribution and diversity of *Salmonella* serotypes was determined across different mills and at different points along the stock feed production chain.

At the commencement of the study period, 7.24% of all samples collected (raw materials, environment and finished feed) were positive for *Salmonella*. By 2016 this rate had fallen to 3.34%. Of the 19,900 samples, 4.9% were positive for *Salmonella* and the highest incidence of positive results was obtained from raw materials and certain pieces of milling equipment. Raw materials, namely animal (16.6%) and vegetable (11.2%) protein meals demonstrated the highest incidence of *Salmonella* spp. Of the mills surveyed, the incidence of *Salmonella* in the milling equipment was 2.9%; cooler equipment had the highest risk (5.7%). Over 58 different *Salmonella* serovars were detected; however, those of public health interest in Australia (*S. enteritidis* and *S. typhimurium*) were rarely detected.

This retrospective study demonstrated the *Salmonella* spp. risk factors during the commercial preparation of stock feed. The study also highlighted that through implementation of, and strict adherence to, the principles of FeedSafe® and HACCP, it is possible to control these risks factors, providing assurance that the risk of animal feed or feed mills contributing to food-borne illness is low.

Davies RH, Wray C (1997) Distribution of *Salmonella* contamination in ten animal feedmills. Veterinary Microbiology 51, 159–169.

Parker AJ, Parkes RW, Overend D, Hepworth G (2008) Prevalence of *Salmonella* spp. in feed mills following the introduction of FeedSafe®. Proceedings of the 2008 Australasian Milling Conference, 163–166.

Sexton M (2016) *Salmonella* and *Campylobacter* in poultry in Australia. Australian Poultry Science Symposium 27, 116–124.

Intermittent lighting improves broiler efficiency during a necrotic enteritis challenge

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Intermittent lighting (IL) programs have been shown to increase feed retention in the crop and reduce the pH in the foregut compartments compared with *ad libitum* feeding (Svihus et al., 2013). Although IL is also known to improve feed conversion ratio (FCR) in broilers (Buyse et al., 1996), no research has been conducted to assess its effect during a disease challenge.

To test whether IL would improve the resilience of broilers to necrotic enteritis (NE), a 2 × 2 experiment was conducted. There were two factors: lighting schedule (continuous lighting (CL; 10–12 lux; 18 h of light (L) and 6 h of darkness (D)) vs intermittent lighting (IL; 1L:3D:1L:3D:1L:3D:1L:3D:2L:6D)) and sub-clinical NE challenge (challenge vs no challenge). A total of 390 Cobb 500 mixed sex, day-old chicks (initial weight, 35.8 ± 5.0 g) were raised until day 7 under 23 h of light (20 lux) and 1 h of darkness. On day 7, 12 birds were selected at random (day 7 weight, 154.8 ± 1.4 g) and allocated to four treatments in single floor pens (0.7 m × 0.7 m). A sub-clinical NE challenge was administered to half of the birds and consisted of oral inoculation with *Eimeria* spp. on day 9 followed by oral inoculation with *Clostridium perfringens* on day 14 as described by Wu et al. (2014). Birds and feeders were weighed on days 7, 14 and 21 to ascertain the effects of IL and NE on bird performance (Table 1). Data were analysed using

Table 1. Performance of broilers in the seven days following *Clostridium perfringens* type A inoculation (FI, feed intake; BWG, body weight gain; FCRc, mortality-corrected feed conversion ratio; BW, body weight).

Lighting program	Necrotic enteritis (NE) challenge	BW Day 14 (g/bird)	Days 14 to 21				BW Day 21 (g/bird)
			FI (g/bird)	BWG (g/bird)	FCRc	Livability (%)	
Continuous	No challenge	403.8	580.8	429.0	1.356[c]	99.71	832.7
Continuous	Challenge	370	548.8	259.0	2.117[a]	100.00	628.9
Intermittent	No challenge	381.5	547.1	413.0	1.327[c]	99.86	794.5
Intermittent	Challenge	344.1	421.6	257.6	1.647[b]	99.88	601.7
	Pooled SE	3.5	27.3	14.7	0.062	0.13	15.0
		Main effects					
Continuous		386.9[a]	564.8[a]	343.9	1.737	99.86	730.8[a]
Intermittent		362.8[b]	484.3[b]	335.3	1.487	99.90	698.1[b]
	No challenge	392.6[a]	564.0[a]	420.9[a]	1.342	99.79	813.6[a]
	Challenge	357.0[b]	485.2[b]	258.3[b]	1.882	99.94	615.3[b]
		P values					
Lighting program		0.000	0.007	0.566	0.000	0.945	0.039
	NE challenge	0.000	0.008	0.000	0.000	0.245	0.000
Lighting program × NE challenge		0.622	0.100	0.624	0.002	0.304	0.713

[ab]Means within a column with different superscripts differ significantly ($P < 0.05$).

ANOVA (SPSS Statistics, version 24) and means were compared using the Tukey multiple range test.

Effects of sub-clinical NE challenge were observed five days after *Eimeria* inoculation: body weight (BW) of challenged birds was 9% less ($P < 0.05$) than that of unchallenged birds on day 14. The difference was more obvious on day 21: BW of challenged birds was 24% less than that of unchallenged birds. Lighting affected BW ($P < 0.05$) on days 14 and 21: birds exposed to IL were 5% lighter than birds exposed to CL. Although livability was unaffected for all groups, BW gain of challenged animals in the period after *C. perfringens* inoculation was 40% less ($P < 0.05$) than that of the unchallenged birds. Intermittent lighting diminished the negative effect of NE on FCR as shown by the two-way interaction between IL and NE challenge ($P < 0.05$).

This study suggests that IL may be used to minimize the negative effects of NE in broiler chickens.

Buyse J, Simons PCM, Boshouwers FMG, Decuypere E (1996) Effect of intermittent lighting, light intensity and source on the performance and welfare of broilers. World Poultry Science Journal 52, 121–130.

Svihus B, Lund VB, Borjgen B, Bedford MR, Bakken M (2013) Effect of intermittent feeding, structural components and phytase on performance and behaviour of broiler chickens. British Poultry Science 54, 222–230.

Wu SB, Stanley D, Rodgers N, Swick RA, Moore RJ (2014) Two necrotic enteritis predisposing factors, dietary fishmeal and *Eimeria* infection, induce large changes in the caecal microbiota of broiler chickens. Veterinary Microbiology 169, 188–197.

Dietary factors influencing performance of broiler chicks offered phytase-supplemented diets

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Multiple factors may influence responses to phytase inclusion in broiler diets; therefore, a Plackett and Burman (1946) factorial design was used to identify the influential factors (Table 1). Eleven variables were screened over 12 treatments (six replicate cages of six birds per treatment) at two levels. Broiler chicks were offered steam-pelleted diets based on maize or wheat and soybean meal with 1000 FTU/kg exogenous phytase from 7–28 days post-hatch and their growth performance met 2014 Ross 308 objectives. Outcomes are shown in Table 2 (significance was declared at $P < 0.10$). High levels of Ca ($P < 0.0001$), canola meal ($P < 0.0001$) and a xylanase and β-glucanase cocktail ($P < 0.10$) negatively influenced weight gain, whereas higher levels of digestible lysine ($P < 0.0001$), xylanase ($P < 0.003$), available P ($P < 0.04$) and wheat as the feed grain ($P < 0.06$) positively influenced weight gain. High levels of Ca ($P < 0.05$), canola meal ($P < 0.02$) and available P ($P < 0.1$) negatively influenced gain-to-feed ratio whereas high digestible lysine ($P < 0.0001$) positively influenced gain-to-feed ratio. Given the responses to digestible lysine, one possible implication is that phytase supplementation will be effective in low crude protein diets with less 'intact protein' and high crystalline amino acid contents. It is noteworthy that xylanase inclusion per se amplified phytase response in diets that were based on both maize and wheat. Contrary to expectations, phytate-P did not significantly influence performance.

Table 1. Factors of the experimental design matrix, their dietary levels and means by which each level was manipulated. All diets were formulated to an equal energy density of 12.97 MJ/kg.

Factor	Level 1	Level 2	Manipulated by
Canola meal (g/kg)	0	125	Partial substitution of soybean meal
Wheat (g/kg)	0	600	Total substitution of maize
Whole barley (g/kg)	0	200	Partial substitution of maize or wheat
Digestible lysine (%)	0.96	1.14	Partial substitution of soybean meal with synthetic amino acids
Phytate phosphorus (%)	0.26	0.33	Rice bran inclusion
Calcium (g/kg)	6	10	Limestone inclusion
Available phosphorus (g/kg)	3.0	4.5	Dicalcium phosphate inclusion
Sodium (g/kg)	1.6	2	Sodium chloride/sodium bicarbonate inclusion
Xylanase and β-glucanase (g/t)	0	100	Addition of a xylanase and β-glucanase enzyme cocktail
Xylanase (U/kg)	0	2000	Xylanase addition
Xylanase, amylase and protease (g/t)	0	100	Addition of a xylanase, amylase and protease enzyme cocktail

Table 2. Factors influencing weight gain and gain-to-feed ratios in birds offered diets supplemented with exogenous phytase.

Factor	Weight gain (g/bird)					Gain-to-feed (g/g)				
	–1 level	+1 level	Estimate[1]	t-Ratio	P-value	–1 level	+ 1 level	Estimate[1]	t-Ratio	P-value
Canola meal	1394	1252	–1.135	–8.87	< 0.0001	0.6888	0.6763	–0.000100	–2.45	0.0172
Wheat	1307	1339	0.053	1.98	0.0522	0.6847	0.6804	–0.000007	–0.86	0.3935
Whole barley	1322	1324	0.010	0.13	0.8988	0.6855	0.6796	–0.000029	–1.16	0.2519
Digestible lysine	1228	1419	100.495	11.94	< 0.0001	0.6494	0.7158	0.034950	13.06	< 0.0001
Phytate-P	1317	1330	17.943	0.79	0.4353	0.6848	0.6803	–0.006468	–0.89	0.3768
Calcium	1367	1280	–21.610	–5.41	< 0.0001	0.6916	0.6736	–0.004501	–3.54	0.0008
Available P	1306	1340	22.824	2.14	0.0364	0.6872	0.6779	–0.006214	–1.83	0.0717
Sodium	1321	1325	10.289	0.26	0.7978	0.6825	0.6826	0.000293	0.02	0.9817
XB	1337	1310	–0.273	–1.71	0.0929	0.6810	0.6841	0.000032	0.62	0.5367
Xylanase	1298	1349	0.026	3.21	0.0021	0.6823	0.6828	0.000001	0.10	0.9234
XAP	1320	1327	0.074	0.46	0.6455	0.6817	0.6835	0.000018	0.35	0.7244

[1]Estimate represents the magnitude of the effects

Plackett RL and Burman JP (1946) The design of optimum multifactorial experiments. Biometrika 33, 305–325.

Net energy prediction and energy efficiency of feed for broilers

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Chicken meat is the fastest growing protein source for humans in the world, and thus refinements in energy formulation techniques for broiler diets will gain importance as variability of feed sources and costs increase (Yu et al., 2006; Harri et al., 2009). Formulation of animal diets based on net energy (NE) has been implemented in ruminants and pigs (Noblet et al., 1994; Ferrell and Oltjen, 2008). However, it has not been realised for poultry feed formulation due to the lack of a compelling argument for using NE.

A closed-circuit respiratory calorimetric system and broilers aged 25–28 d were used to measure the metabolisable and NE values of 19 diets formulated with varying nutrient composition and to generate a prediction equation for NE. The diets encompassed the following ranges in nutrient composition (DM basis): apparent metabolisable energy corrected for N retention (AMEn), 11.66–15.02 MJ/kg; crude protein (CP), 18.5–29.9%; ether extract (EE), 1.6–8.9%; crude fibre (CF), 2.36–5.00%; lysine, 1.22–1.57%; methionine, 5.1–6.9%. The mean energy utilisation efficiencies of the diets are shown in Table 1.

Regression analysis showed that NE was positively related to AME ($P < 0.001$) and EE ($P < 0.01$) with a coefficient of 0.042, but was negatively correlated with CP ($P < 0.001$) with a coefficient of –0.037 when the intercept was included in the analysis. The R^2 for the model was 0.95. In addition, the heat increment of feed was positively related to EE and CP, but negatively related to CF. The NE prediction parameters were validated using a set of diets with balanced and imbalanced nutrient composition (Figure 1). The predicted values of the balanced diets were close to the measured values, but those of the imbalanced diets deviated from the

Table 1. Energy utilisation efficiencies of experimental diets fed to broilers.

Energy parameter	Mean	n	Measurement Range	
			Min	Max
Energy content (MJ/kg DM)				
GE	19.26	19	18.52	19.93
AME	13.99	135	11.84	16.34
AMEn	13.20	135	11.15	15.46
NE	10.43	135	8.55	12.87
[a]HIf	3.58	135	2.82	4.40
Energy utilization (%)				
AME/GE[b]	72.6	135	62.8	82.0
AMEn/GE	68.5	135	59.1	77.6
NE/AME	74.5	135	62.8	82.0
NE/AMEn	78.9	135	71.2	83.2

[a]Heat increment of feed . [b]Gross energy.

measured values, indicating the prediction is valid only with balanced diets.

It is concluded that these data indicate a positive effect of EE and a negative effect of CP on energy utilization in broilers. The NE, NE/ME and HI values of the diets were accurately predicted from dietary chemical composition using the prediction equation developed in this study. These results suggest that an NE system may be advantageous for broiler feed formulation.

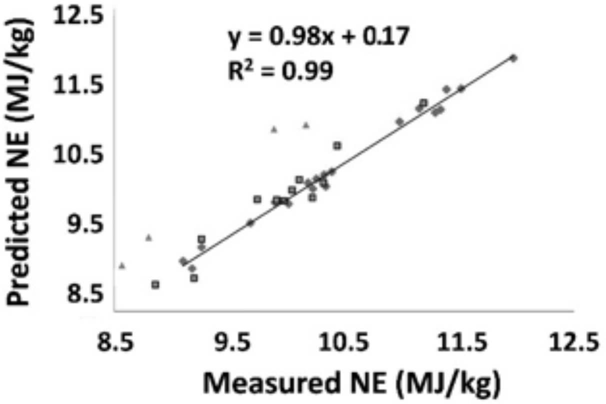

Figure 1. Predicted vs measured NE values. ◆ Diets used for prediction equations; ■ validation diets with balanced nutrient composition; ▲ diets with imbalanced nutrient composition.

Ferrell C, Oltjen J (2008) Net energy systems for beef cattle – concepts, application, and future models. Journal of Animal Science 86, 2779–2794.

Harri A, Nalley L, Hudson D (2009) The relationship between oil, exchange rates, and commodity prices. Journal of Agricultural and Applied Economics 41, 501–510.

Noblet J, Fortune H, Shi H, Dubois S (1994) Prediction of net energy value of feeds for growing pigs. Journal of Animal Science 72, 344–354.

Yu TH, Bessler DA, Fuller S (2006) Cointegration and causality analysis of world vegetable oil and crude oil prices. In: Proceedings of The American Agricultural Economics Association Annual Meeting, Long Beach, California.

Effect of dietary chelated minerals and antioxidant on broiler welfare and carcase integrity

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The integrity of a chicken carcase, the manner in which the bird is raised and the quality of the protein presented are important for market acceptance and loyalty of consumers to chicken meat. In addition to carcase integrity and issues such as footpad lesions and lameness that impact on welfare considerations (RSPCA approved farming scheme standards, 2013), new syndromes of carcase deformities have emerged in fast-growing birds. Kuttapan et al. (2016) discussed two emerging myopathies of great concern to poultry producers and processors, viz., 'white striping', a marked striation of fibrous tissue parallel to breast muscle fibres in the ventral plane of the pectoralis major muscle, and 'woody breast', in which muscle tissue is replaced with collagen because of insufficient vascularisation during growth. Both syndromes result in breast meat that is below the quality standard expected by the consumer. In a practical demonstration, Tijare et al. (2016) showed that both myopathies decreased water-holding capacity, resulting in increased cooking loss and reduced uptake of marinade by processed broiler meat.

Manangi et al. (2016) found that the inclusion of a synthetic antioxidant in broiler diets reduced oxidative stress markers and the incidence of woody breast. In this study we fed birds a bioavailable source of trace minerals associated with superoxide dismutase, viz., zinc, copper and manganese, to maximise the antioxidant activity of this enzyme. A highly bioavailable source of selenium was added to maximise the activity of the antioxidant enzyme, glutathione peroxidase. Ethoxyquin (125 ppm) was added to attenuate potential lipid oxidation and to promote the integrity of fat-soluble vitamins in the diet.

A total of 234 day-old Arbor Acres Plus male broilers were assigned to three replicated (n = 6) dietary treatments with 13 birds per replicate. All diets satisfied the nutritional requirements described in the breed strain's manual. Corn and soybean meal formed the basis of the diet. Dietary energy and crude protein concentrations were 3000 kcal and 23%, respectively, for Phase 1 (Days 1–10), 3100 kcal and 21.5%, respectively, for Phase 2 (Days 10–24) and 3200 kcal and 19.5%, respectively, for Phase 3 (Days 24–35).

Treatment 1 (T1) (Table 1) contained inorganic sources of trace minerals and served as the control; treatment 2 (T2) contained organic (chelated with 2-hydroxy-4-methylthio butanoic acid, HMTBA) sources of trace minerals and inorganic Se, and treatment 3 (T3) contained organic sources of trace minerals as in T2, plus an antioxidant (ethoxyquin) and Se in the form of selenium yeast.

Birds in all groups were exposed to mild stress by increasing house temperature to 30°C from Days 21 to 35. All birds were assessed for performance and four birds that were closest to the mean of each pen were assessed for carcase characteristics. At the end of the study, various welfare and carcase parameters were scored and analysed using the Chi square test (α = 0.05) and the t-test.

Table 1. Experimental treatments.

Treatment	Zn (ppm)	Cu (ppm)	Mn (ppm)	Se (ppm)	Antioxidant (ppm)
T1	110	6	120	0.3	–
T2	50 HMTBA	10 HMTBA	60 HMTBA	0.3	–
T3	50 HMTBA	10 HMTBA	60 HMTBA	0.3 yeast	125 ppm

The incidence of tibial-head lesions was greater ($P < 0.05$) in T1 (91.7%) than in T2 or T3 (45.8% and 41.6%, respectively). Treatments T1 and T2 resulted in a higher incidence ($P < 0.01$) of footpad lesions (67%) than T3 (62.5%). Treatments T2 and T3 had greater bone breaking-strength (367 N/m^2 and 399 N/m^2, respectively) than T1 (331 N/m^2), although only T3 was significantly greater ($P = 0.035$). Birds in groups T2 and T3 had a reduced incidence of white striping in breast meat (95.8% and 87.5%, respectively) compared with birds in the T1 control group (100%); differences between each of the three treatment groups were significant ($P < 0.05$). There was a reduction in the incidence of woody breast in the T2 and T3 groups (79.2% and 62.5%, respectively) compared with the T1 control group (91.7%); differences between each of the three treatment groups were significant ($P < 0.05$).

This study shows that dietary addition of chelated organic minerals, an antioxidant and organic Se can minimise the incidence of carcase defects such as white striping and woody breast. Moreover, replacement of inorganic minerals with chelated minerals and addition of antioxidants could significantly reduce the occurrence of lameness in birds due to improved footpad health, bone strength and tibial-head integrity.

Kuttapan VA, Hargis BM, Owens C (2016) White striping and woody breast myopathies in the modern poultry industry: a review. Poultry Science 95, 2724–2733.

Manangi M, Chen J, Foran C, Vasquez Anon M, Walter K, Cerrate S, Corzo A, Halley J, Fancher B (2017) Synthetic antioxidant improves oxidative stability of breast meat and reduces incidence of Wooden breast myopathy in broilers fed diets containing oxidized fat. In: Proceedings of the International Poultry Scientific Forum, Atlanta, Georgia p. 49, abstract T167.

RSPCA Approved Farming Scheme Standards – Meat Chickens (2013). Accessed at https://rspcaapproved.org.au/wp-content/uploads/2017/02/RSPCAMeatChickenStandards_May2013.pdf

Tijare VV, Yang FL, Kuttapan VA, Alvarado CZ, Coon CN, Owens CM (2016) Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poultry Science 95, 2167–2173.

Enteric methane reduction in sheep consuming low quality chaff supplemented with nitrate or cysteamine hydrochloride

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Nitrate (NO_3) is an effective dietary additive for reducing enteric methane (CH_4) emissions from ruminants (Hristov et al., 2013; Lee and Beauchemin, 2014). Furthermore, NO_3 can replace urea as a non-protein nitrogen (NPN) source for microbial growth (Leng, 2008) and improve productivity in ruminants consuming protein-deficient diets (Nguyen et al., 2016). The possibility of nitrite (NO_2) toxicity is a limitation to widespread adoption of NO_3 supplementation. Like NO_3, cysteamine hydrochloride (CSH) has potential to reduce enteric CH_4 emissions and improve animal performance (Barnett and Hegarty, 2014; Sun et al., 2017). The aim of this study was to evaluate NO_3 and CSH as feed additives for reducing enteric emissions and improving productivity in sheep fed low quality chaff.

A complete randomized study using 24 Merino sheep (27.5 ± 0.77 kg live weight) was conducted over 35 days. Sheep were assigned to one of four dietary treatments, viz., a wheaten chaff mixture (600 g wheaten chaff and 200 g wheat grain) fed alone (control, CON) or with 2% NO_3 (provided as 3.14% calcium NO_3), urea (iso-nitrogenous with the NO_3 diet) or CSH (80 mg CSH/kg live weight). A diet adaptation period was included to prevent NO_2 toxicity in sheep fed NO_3. Rumen fluid samples were collected using oesophageal intubation 3.5 h after morning feeding on day 35 and used for analysis of concentrations of volatile fatty acids (VFA) and rumen ammonia (NH_3-N) and for enumeration of protozoa. Animals were offered 800 g of diet and fed once daily. Feed offered and refused was weighed daily to estimate dry matter intake (DMI). Open circuit respiration chambers (n = 6) were used to estimate CH_4 production (DMP) over a 22 h period. The results are shown in Table 1.

Table 1. Performance, rumen fermentation and methane production of sheep fed low quality chaff without (CON) or with nitrate (NO_3), urea or cysteamine (CSH) supplementation.

Parameter		CON	NO_3	Urea	CSH	SEM	P value
Initial live weight	(kg)	25.8	25.3	25	26.1	0.32	0.23
Final live weight	(kg)	25.2[a]	26[b]	23.9[a]	25.3[a]	0.34	< 0.05
ADG[1]	(g/d)	-23[a]	18[b]	–28[a]	–21[a]	8.09	< 0.10
DMI	(g DM/d)	430[a]	508[b]	450[ab]	492[ab]	18	0.10
Rumen NH_3-N	(mg/L)	37[b]	136[a]	137[a]	64[b]	0.85	< 0.01
Rumen pH		6.7[a]	6.9[b]	6.7[a]	6.7[a]	0.04	0.05
Total VFA	(mM/L)	58.1	50.7	60.2	59.1	2.45	0.45
Acetate (Ac)	(molar %)	70.7[ab]	75[a]	68.4[b]	70.8[ab]	1.04	< 0.05
Propionate (Pr)	(molar %)	22.1	20.8	24.7	22.7	0.92	0.57
Butyrate	(molar %)	7.2[a]	4.2[b]	6.9[a]	6.4[a]	0.42	< 0.10
Ac:Pr		3.3	3.7	3	3.1	0.16	0.44
Total protozoa	(10^5/mL)	11.8[a]	6.1[b]	9[ab]	5.5[b]	0.91	< 0.05
DMP	(g CH_4/d)	10.3[a]	7.7[b]	8.5[a]	10.9[a]	0.64	< 0.10
MY	(g CH_4/kg DMI)	20.6[a]	14.8[b]	19.3[a]	21.2[a]	0.06	< 0.01

[1]Average daily gain. Row means without common superscripts differ significantly.

Live weights of sheep were generally stable throughout the experiment but NO_3 supplementation tended to improve DMI ($P = 0.10$) and average daily gain ($P = 0.08$). Both NO_3 and urea supplementation increased rumen NH_3-N concentration ($P < 0.01$), confirming the roles of these two additives as NPN sources for microbial growth. The rumen protozoa population was reduced ($P < 0.05$) by NO_3 and CSH compared with the CON diet. No differences between diets were observed for rumen VFA concentration but NO_3 increased acetate percentage ($P < 0.05$) and tended ($P = 0.05$) to reduce butyrate percentage compared with urea. There was a tendency ($P = 0.08$) for NO_3 to reduce enteric DMP and CH_4 yield (MY; g CH_4/kg DMI) was significantly reduced ($P < 0.01$) by NO_3. Methane production of CSH-supplemented sheep did not differ from that of the CON sheep.

These findings confirm the role of NO_3 as a feed additive for reducing ruminant enteric emissions and rumen protozoa populations and for improving the productivity of sheep fed low quality diets.

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Response of broilers challenged with sub-clinical necrotic enteritis to dietary inclusion of crimped kernel maize silage

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After the ban of in-feed antibiotics, strong concerns regarding an increased emergence of necrotic enteritis induced by *Clostridium perfringens* Type A have been raised in Europe. Various dietary strategies, including the use of probiotics and prebiotics as dietary supplements, have been implemented in attempts to prevent necrotic enteritis, with variable rates of success. Use of a fermented grain rather than fermented compound feed may avoid the negative effects of the latter (poor litter quality and degradation of supplemental synthetic amino acids) and benefit gastrointestinal health (Ranjitkar et al., 2016). Crimped kernel maize silage (CKMS) is characterized by high concentrations of acetic and lactic acid, low pH and high numbers of lactic acid bacteria. In a production trial with broilers fed diets with or without CKMS, there was no difference in performance or mortality between treatments, but CKMS supplementation improved litter quality and footpad health (Ranjitkar et al., 2016). However, scientific literature regarding the influence of CKMS on the course of necrotic enteritis is lacking. The objective of this experiment was to study the effects of adding various levels of CKMS (0, 15% or 30%) on necrotic enteritis lesions, microbiota counts and organic acid production in a subclinical necrotic enteritis disease model.

A total of 375 day-old male broilers (Ross 308) were allocated to one of three dietary treatments: a maize-based diet (Control diet) or diets supplemented with 15% (CKMS15) or 30% (CKMS30) crimped ensiled kernel maize from Days 0 to 28. Birds were challenged with *C. perfringens* and subsequently showed signs of necrotic enteritis. The disease model involved a tenfold overdose of an attenuated live vaccine against coccidiosis given orally on Day 17, followed by oral inoculation with *C. perfringens* Type A (S48, 10^8–10^9 bacteria per bird) twice daily on Days 18, 19 and 20. Intestinal lesions were scored on Days 22, 23, 25 and 28. Ileal and caecal digesta samples were collected for quantification of bacteria and organic acids. Counting of selected bacterial groups (lactose-negative enterobacteria, lactic acid bacteria, coliforms and *C. perfringens*) was done using the microbial plate-count method and for *C. perfringens* type A, using quantitative PCR as well.

Necrotic enteritis lesion scores in the small intestine peaked on Days 23 and 25 and decreased on Day 28 ($P < 0.01$). However, there was no effect of dietary treatments on lesion score ($P > 0.05$). No effect of age on microbiota count was observed, but the CKMS30 treatment reduced the number of coliforms in ileal contents ($P = 0.01$). Dietary treatments did not affect organic acid concentrations in the ileum and caeca, but there was an effect of age; butyric acid concentration was higher on Days 22, 23 and 25 compared with Day 28 ($P = 0.04$). Acetic and propionic acid concentrations in caeca were highest on Days 22 and 28 and lowest on Days 23 and 25.

In conclusion, the inclusion of CKMS in broiler diets had no effect on the course of necrotic enteritis but had potential benefits in terms of inhibition of potentially harmful microorganisms.

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Laying hens exposed to *Ascaridia galli*-contaminated free-range: impact on performance and egg quality

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The prevalence of *Ascaridia galli* infection has increased in free-range laying hens in Europe (Thapa et al., 2015). This is a cause of concern in Australia, where the poultry industry is rapidly moving towards free-range egg production and the impact of infection on nutrient absorption and performance is unknown. It is important to monitor the performance and egg quality of laying hens infected with *A. galli* to develop effective control measures in the future. Two studies were conducted to investigate performance and egg quality of laying hens either artificially or naturally infected with *A. galli*.

Experiments were conducted each using 200 Lohmann Brown laying hens allocated to one of four treatments with five replicates of 10 hens per pen. In Experiment 1, hens allocated to Treatment 1 (Control) were routinely dewormed using levamisole (4 mL per bird). Hens allocated to Treatments 2 (low infection group), 3 (medium infection group) or 4 (high infection group) were orally inoculated with 250, 1000 or 2500 embryonated *A. galli* eggs, respectively. Hens ranged and shed *A. galli* eggs for the duration of the trial (20 weeks). Experiment 2 was conducted four months later using the ranges previously used in Experiment 1. A new batch of 16-week-old hens was assigned to one of the following groups: Group 1 (negative control) ranged on a previously uninfected area (Control), Groups 2 and 3 ranged on pastures previously used by the low and medium infection groups, respectively, and Group 4, which served as a positive control, was artificially infected with 1000 *A. galli* eggs and ranged on previously used high infection group pens. Egg production and egg weight were measured daily. Feed intake and body weight were measured at 25 and 30 weeks of age and feed conversion ratio was calculated. Infection intensity was measured by counting worms in the intestine and analysing coprodeum content for the presence of *A. galli*. Six hen eggs per pen were collected at the end of the experiment at 30 weeks of age and measured to assess internal and external egg quality. Data were analysed using JMP statistical software version 8 (SAS Institute Inc., Cary, NC). Two-way ANOVA was performed to assess treatment group differences with age fitted as a linear covariate. A P-value < 0.05 was considered significant.

In Experiment 2, the medium infection group had higher worm counts in the intestine (43.92) compared with the low infection group (23.82) but was similar to the positive control group (34.36) (overall treatment effect, P < 0.01), whereas the negative control group had no worms. Excreta egg counts for *A. galli* followed the same pattern. The medium infection group had higher egg counts (3 437) compared with the low infection group (1 820) but was similar to the positive control (2 918), whereas the negative control had no eggs in the excreta (P < 0.01). *A. galli* infection acquired from the contaminated ranges did not decrease egg production, egg mass, feed intake, feed conversion ratio or onset of egg laying. Similarly, egg quality parameters (egg weight, shell reflectivity, shell weight, shell thickness, shell percentage, breaking shell strength, deformation, albumen height, Haugh unit and yolk score) were not influenced by *A. galli* infection (P > 0.05). This suggests that in ranging laying hens, *A. galli* infection at the levels observed in our trial did not affect performance or egg quality from the point of lay until 40 weeks of age. We also measured the liver lipid content of the birds. Heavily infected hens had consistently lower liver lipid content (7%) compared with uninfected hens (10%) (P < 0.01), which may indicate that the birds were using energy reserves to cope with the infection.

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Sugarcane bagasse upregulates the expression of pancreatic amylase and chymotrypsin of broilers fed corn-based diets

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The physical structure of feed and the use of dietary fibre in poultry diets have attracted a great deal of interest in recent years as a means to improve digestive efficiency and enhance gut health of birds. It has been speculated that larger ingredient particles and a moderate level of fibre improve gizzard function and enhance the release of cholecystokinin (Svihus et al., 2004), which acts through the vagus nerve (Li and Owyang, 1993) to stimulate pancreatic enzyme secretion. This study was conducted to investigate the effect of sugarcane bagasse (SB) and corn particle size on gizzard development and mRNA expression of genes encoding cholecystokinin (CCK) and the cholecystokinin receptor (CCK1R) in the intestine and pancreatic enzymes in broilers. A total of 336 day-old male Ross 308 chicks were randomly allocated to four treatments with six replicate pens, each housing 14 birds. A 2 × 2 factorial arrangement of treatments was applied with the following factors: corn particle size (coarse (3576 µm) or fine (1113 µm) geometric mean diameter) and level of SB (0 or 2%).

Table 1 (see next page) shows the ingredient and nutrient composition of the experimental diets. The relative gizzard weight was measured using three birds per pen on Day 24. mRNA expression of genes encoding CCK and CCK1R and pancreatic enzymes was measured using one bird per pen.

A significant particle size × SB interaction was observed for relative gizzard weight ($P < 0.05$), in which SB increased relative gizzard weight only in birds fed the finely ground corn diet. Whereas corn particle size did not affect the expression of pancreatic amylase (AMY2A), chymotrypsin (CELA1) or lipase (PNLIP; $P > 0.05$), addition of 2% SB upregulated pancreatic AMY2A and CELA1 expression ($P < 0.05$). The expression of both CCK and its receptor (CCK1R) in the duodenum, jejunum, ileum and pancreas was not affected by particle size or SB addition ($P > 0.05$). This suggests that CCK is not responsible for stimulation of pancreatic secretion by dietary fibre. These findings suggest that inclusion of coarsely ground corn and 2% SB in broiler diets is beneficial for gizzard development and thus digestion and performance through upregulation of digestive enzymes.

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Table 1. Composition and nutrient content of the basal diet (%)[1]

Ingredients	Starter	Grower
Corn	60.6	62.3
Soybean meal	32.6	29.3
Meat and bone meal	3.00	3.60
Canola oil	0.644	1.91
Limestone	0.970	0.814
Dicalcium phosphate	0.607	0.269
Phytase[2]	0.01	0.010
Salt	0.154	0.161
Na bicarbonate	0.219	0.200
Vitamin premix[3]	0.200	0.200
Choline	0.111	0.103
L-lysine HCl 784	0.305	0.226
D, L-methionine	0.392	0.336
L-threonine	0.204	0.148
TiO_2	60.6	0.500
Nutrients		
ME (kcal/kg)	3000	3100
Crude protein	22.2	21.0
Crude fat	2.85	4.14
Crude fibre	2.07	2.01
SID arginine	1.37	1.27
SID lysine	1.28	1.15
SID methionine	0.684	0.616
SID methionine + cysteine	0.950	0.870
SID tryptophan	0.244	0.226
SID isoleucine	0.860	0.807
SID threonine	0.860	0.770
SID valine	0.992	0.939
Calcium	0.960	0.870
Available phosphorus	0.480	0.435
Sodium	0.160	0.160
Chloride	0.250	0.242
Choline	0.170	0.160

[1] The composition of diets was diluted when 2% SB was added to the complete feed.
[2]Phyzyme XP5000G (100 g/t) Dupont.
[3]Vitamin-mineral concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamine, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg; SID = standard ileal digestible.

Curcumin xanthorrhiza for treatment of ulcers in horses

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Curcumin is the bioactive component of turmeric, which has been shown to have multi-species anti-inflammatory, antimicrobial, antioxidant and neuroprotective effects (Gupta et al., 2013). Turmeric may also have a role in treating ulcers (Kim et al., 2005). There are several varieties of turmeric. *Curcuma xanthorrhiza* has unusual qualities attributed to the xanthorrhizol oil. Horses can suffer from ulceration of the squamous and glandular regions of the stomach. The usual treatment consists of medicated compounds such omeprazole. This study was conducted to evaluate the effect of supplementing the diet offered to horses with *C. xanthorrhiza*. Gastric ulceration was induced by confinement and dietary modification.

Ten horses were used in a randomised, controlled and blinded, two-period crossover study. There was a washout period of two months before the second period. The horses were confined to stables and offered a hay diet twice daily. The experimental period lasted for 16 days. Each animal received hay plus pre-meal feed daily for the first nine days before commencing a seven-day intermittent feed deprivation protocol designed to induce ulcers (Murray and Eichorn, 1996; Husted et al., 2009). All horses were given 50 g/day of powdered coconut oil. Horses in the treatment group were given 20 g/day of *C. xanthorrhiza*. Pre-treatment gastroscopy (Day 0) was performed on each horse to determine a baseline gastric-ulcer grading score using the scoring system currently recommended by the European College of Equine Internal Medicine Consensus Statement (Sykes et al., 2015). Lesions were graded separately and recorded as both an overall grade and the sum of grades for squamous and glandular mucosa as previously described (Birkmann et al., 2014). Ulcer scores were determined by gastroscopy on Days 0, 9 and 16.

Squamous ulcer scores increased in the control horses during confinement and feed restriction, but not in turmeric-treated horses (Figure 1). These results suggest that oral supplementation with turmeric (*C. xanthorrhiza*) may be effective in reducing the severity of squamous ulceration in horses.

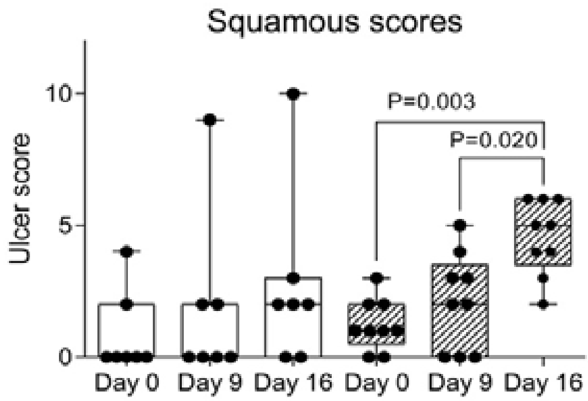

Figure 1. Gastric squamous ulcer scores following administration of turmeric (20 g once daily in feed, white boxes) or placebo (hatched boxes).

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Effect of grain type on performance, gut permeability and digesta viscosity of broilers during a necrotic enteritis challenge

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Necrotic enteritis (NE) is a major concern for the poultry industry worldwide as it causes reduced production and increased mortality, resulting in an estimated annual economic loss of US$6 billion (Wade and Keyburn, 2015). It is widely accepted that feed composition strongly influences the gut environment, consequently affecting the prevalence and severity of necrotic enteritis (Branton et al., 1997). Cereal grains such as wheat, barley and rye contain high levels of non-starch polysaccharides (NSPs) that have a direct negative impact on nutrient utilization. Insoluble NSPs can act as a physical barrier to enzymes. Soluble NSPs increase digesta viscosity, which may affect intestinal barrier function. Compromised barrier function results in translocation of luminal pathogens and a non-specific inflammatory response (Tellez et al., 2014).

A total of 468 one-day-old male Ross 308 chicks were assigned to 36 floor pens in a 2 × 3 factorial arrangement of treatments from Days 0 to 35. Factors were (a) the presence or absence of an NE challenge and (b) grain type (wheat, barley or rye). According to the protocol of Keerqin et al. (2017), birds were challenged with *Eimeria* (5000 oocysts) on Day 9 followed by inoculation with *Clostridium perfringens* (10^8 CFU) on Days 14 and 15. Birds and feed were weighed on Days 0, 10, 24 and 35. On Day 16 two birds per pen were gavaged with fluorescein isothiocyanate–dextran and serum samples were obtained for evaluation of gut permeability. Jejunum content viscosity was also measured (Brookfield LVDV-III ultra-rheometer).

Table 1. Effect of grain type and necrotic enteritis (NE) challenge on growth performance, jejunum viscosity and gut permeability of birds from Days 0 to 35 (standard deviations are reported in parentheses; FITC-d[2], fluorescein isothiocyanate–dextran).

Factor		Performance			Jejunum digesta viscosity (mPa-s)	Serum FITC-d[2] (mg/mL)
		Weight gain	Feed intake	Feed conversion ratio		
Grain type	Wheat	1849 (108)^a	3658 (212)^a	1.980 (0.065)^b	3.76 (1.26)^b	0.79 (0.16)^b
	Barley	1849 (85)^a	3526 (180)^{ab}	1.909 (0.097)^b	3.45 (0.77)^b	0.84 (0.24)^b
	Rye	1632 (85)^b	3492 (183)^b	2.142 (0.110)^a	8.59 (3.05)^a	1.02 (0.29)^a
NE challenge	No	1851 (123)^a	3668 (179)^a	1.988 (0.122)	6.29 (3.60)^a	0.76 (0.19)^b
	Yes	1703 (111)^b	3450 (168)^b	2.033 (0.143)	4.25 (2.35)^b	0.99 (0.25)^a
P value	Grain type	0.001	0.045	0.001	0.001	0.001
	Challenge	0.001	0.001	0.172	0.002	0.001
	Grain type × challenge	0.680	0.174	0.502	0.165	0.218

Column means within a category sharing the same superscripts are not significantly different (P > 0.05).

The NE challenge reduced feed intake and weight gain from Days 0–35, irrespective of dietary treatment ($P < 0.001$), but feed conversion ratio (FCR) was not affected by the challenge ($P > 0.05$). Birds fed the rye-based diet had higher FCR ($P < 0.001$) than those fed other grains, probably because of increased viscosity of the jejunal contents ($P < 0.001$). Gut permeability was greater ($P < 0.001$) in birds fed the rye diet compared with those fed other grains. Digesta viscosity was decreased ($P < 0.002$) and gut permeability was increased ($P < 0.001$) by the NE challenge.

These results show that rye diets can influence digesta viscosity and intestinal permeability, which can have a direct impact on bird performance.

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Improving the nutritive value of cottonseed meal in diets for broiler chickens using composite microbial enzymes

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A 4 ×2 factorial study investigated the effects of four levels of cottonseed meal with or without a xylanase and β-glucanase blend on the nutritive value of wheat/sorghum/soybean meal-based diets for broiler chickens. Eight isocaloric and isonitrogenous diets were formulated using the least-cost method to meet the nutrient specifications of Ross 308 male broilers. Cottonseed meal was included in the starter diet at a level of 0, 4, 5 and 6%, in the grower diet at a level of 0, 8, 10 and 12% and in the finisher diet at a level of 0, 12, 15 and 18%. The diets were fed with or without 100 mg/kg of a xylanase and β-glucanase supplement (Danisco Animal Nutrition, Marlborough, UK). The metabolisable energy and lysine contents of the control diets were 12.1–12.3 MJ/kg and 11.9–12.1 g/kg, respectively. Each treatment was randomly assigned to six replicates of 10 birds each.

Data analysis showed a cottonseed meal × enzyme interaction ($P < 0.05$) with regard to feed intake and weight gain in the starter phase. The enzyme supplement improved ($P < 0.05$) feed conversion ratio in the grower and finisher phases and increased weight gain in growing and finishing birds. Cottonseed meal reduced ($P < 0.05$) the combined relative weight of the gizzard and proventriculus in starter chicks, but these organs were bigger ($P < 0.05$) in the grower phase. Cottonseed meal decreased ($P < 0.05$) the relative weight of the small intestine in starter and grower birds. Cottonseed meal increased the relative weight of thighs ($P < 0.05$) at 35 days, and that of breast at 35 days was increased ($P < 0.01$) by enzyme addition. Starch digestibility was improved ($P < 0.01$) by the enzyme supplement and was decreased ($P < 0.01$) by cottonseed meal. Enzyme supplementation improved ($P < 0.05$) the digestibility of gross energy and protein.

These results show that the nutritive value of cottonseed meal-containing diets can be improved by enzyme supplementation and that cottonseed meal can substitute for soybean meal without compromising broiler performance.

Feed enzymes and nutrient digestion in horses fed oats

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Performance horses require starch-rich diets to meet their energy requirements. Incomplete digestion of carbohydrates in the equine small intestine can increase fermentation in the hindgut, predisposing the horse to digestive disorders such as colic, acidosis and laminitis. Addition of feed enzymes may increase digestion in the small intestine and reduce the incidence of digestive disorders. Six Standardbred geldings (each acting as its own control) were initially fed three diets without enzymes and were then fed diets containing a multi-enzyme product (Natuzyme®; Bioproton Pty Ltd, Australia) at a level of 20 g/day for 72 days. Diets 1, 2 and 3 contained 0.5, 3 and 6 kg of oats per 500 kg body weight, respectively. Copra meal was included in Diet 1. Calcium carbonate and a protein and mineral balancer pellet were added to all diets. Each dietary period (23 days) consisted of 10 days of adaptation (no added enzyme), three days of total faecal collection and seven days of the diet plus enzyme followed by another three days of total faecal collection. Glycaemic responses were determined on the final day of each collection period. Blood samples were collected for glucose analysis prior to the morning feed, then at 30-minute intervals for four hours and hourly for four hours thereafter. Digestibility of dry matter, organic matter, nitrogen, starch, neutral detergent fibre and acid detergent fibre was determined. Dry matter digestibility was similar for the three diets but there was a reduction ($P < 0.05$) in digestibility with the inclusion of feed enzymes, except for Diet 3, resulting in a diet × enzyme interaction ($P < 0.05$). Similar trends were observed for nitrogen digestibility but were not statistically significant. Minimal starch was present in all faecal samples. Faecal pH decreased as the concentration of oats in the diet increased ($P < 0.001$), but the addition of enzymes had no effect. There were increases in glycaemic responses ($P < 0.001$) as the concentration of oats in the diet increased; enzymes did not modify the responses. This suggests that horses are able to digest high concentrations of oats without enzyme supplementation. The changes observed in faecal pH are attributed to greater fermentation of either structural or storage carbohydrates in the hindgut induced by an increase in the level of oats in the diet. Thus, it is not unexpected that there would be a diet × enzyme interaction in relation to dry matter digestibility because of changes in microbial activity. In the oats-based diets, the enzymes used did not increase dietary digestibility. These enzyme combinations were developed for pig and poultry diets, which consist primarily of wheat and sorghum. Future equine studies should examine enzyme combinations that are specifically designed for the diet that is fed.

Managing plasma phosphorous concentrations in beef heifers with slow-release 25-hydroxy-vitamin D supplementation

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We evaluated the efficacy of a rumen bolus containing 25-hydroxy-vitamin D (25OHD) and/or monensin on blood P and Ca concentrations and production parameters of Brahman cross heifers grazing unimproved native pastures. Eighty-four heifers, initial live weight (mean ± sem) 184 ± 2.0 kg, were allocated to one of four groups: (1) placebo (control), (2) monensin (120 mg/d), (3) Hy-D® (6 mg/d) or (4) monensin plus Hy-D®. Live weight, hip height and body condition scores were recorded and faecal and jugular blood samples were collected over 7 months.

Monensin had a significant effect ($P < 0.05$) on daily gain for the first 25 days. Interactions between 25OHD and monensin and between time and monensin were also significant ($P < 0.05$). Mean plasma concentrations of 25OHD in the control and monensin groups ranged from 33.5 ± 5.01 to 42.6 ± 6.987 ng/mL. Plasma Ca concentrations decreased, whereas mean plasma P concentrations reached 6.6 mg/dL after 25 days and plateaued between 8.5 and 9.0 mg/dL for up to 109 days.

This study demonstrated that sustained and elevated plasma concentrations of both 25OHD and P, compared with control animals, could be achieved. High plasma concentrations of 25OHD can be maintained using a slow-release bolus. The target plasma 25OHD concentration for increasing P absorption in beef cattle is between 200 and 300 ng/mL.

Responses of broiler chickens to optimum levels of carbohydrases and a superdose level of phytase in maize-based diets

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This study was carried out to investigate the responses of broiler chickens to novel microbial enzymes in maize/soybean-based diets. A total of 648 male and female Ross 308 broiler chickens were randomly assigned to a 2 (0 or 100 mg/kg xylanase (optimum level)) × 2 (0 or 100 mg/kg β-glucanase (optimum level)) × 3 (0, 100 mg/kg phytase (optimum level) or 300 mg/kg phytase (superdose level)) full factorial study in a complete randomized design. The diets were formulated to meet the breeder specifications for Ross 308 broiler chickens. The enzyme supplements were supplied by AB Vista, UK. Each of the 12 treatments was replicated six times, with nine birds per replicate. The diets were fed *ad libitum* for 35 days in three phases: starter (Days 1–10), grower (Days 11–24) and finisher (Days 25–35). The birds were raised in cages in climate-controlled rooms.

Data on feed intake and body weight were collected on Days 10, 25 and 35 and feed conversion ratio was calculated and corrected for mortality. On Day 35, two birds per pen were randomly selected, slaughtered by cervical dislocation and processed for assessment of meat yield. A general linear model procedure was used to analyse the data (Minitab, version 17).

Feed intake between hatch and Day 24 ($P < 0.001$) and Day 35 ($P < 0.01$) was greater in the superdose phytase group than in the optimum phytase and phytase-free groups. Xylanase supplementation also increased ($P < 0.05$) feed intake between hatch and Day 24. Superdosing with phytase resulted in greater ($P < 0.001$) body weight gain in all three feeding phases. Xylanase and β-glucanase supplementation also improved body weight gain, but only up to the end of the grower phase. Superdose phytase level affected feed conversion ratio in a pattern similar to that observed for body weight gain but the other enzymes had no effect on feed conversion ratio. The absolute weights of the breast, thighs and drumsticks were greatest ($P < 0.001$) in birds fed the superdose level of phytase but only the relative weight of the breast was improved ($P < 0.01$) by this level of phytase. There were no interactions between the three enzymes or between levels of supplementation.

This study showed that these enzyme supplements improved the performance of broiler chickens fed maize-based diets, with the greatest improvement in the phytase superdose groups.

Validation of predicted net energy ingredient values for laying hens

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Feed represents 65–75% of the cost of egg production. Accurate estimates of the nutrient content of ingredients are essential for diet formulation. The apparent metabolisable energy system, corrected for zero-nitrogen balance (AMEn), is the most common diet formulation system for poultry. During digestion some energy is lost as heat; this is called the heat increment (HI). Net energy (NE) = metabolisable energy (ME) – HI (Armsby and Fries, 1915). The NE system is more accurate than the ME system as it estimates the amount of feedstuff energy available for egg production. It is possible to estimate HI *in vivo* using calorimetry. Equations to predict the ME (Sibbald et al., 1963) and NE (Nehring et al., 1969) contents of ingredients can be generated from energy balance data obtained during feeding of complete diets.

Sixteen diets differing in nutrient composition were fed to laying hens in calorimeter chambers to generate a prediction equation for estimating net energy contents of the raw materials used. Two further diets were formulated using the equation and birds were placed in chambers to measure energy balance and to validate the equation. The diets were: low NE:AMEn (Diet 1) and high NE:AMEn (Diet 2). Diet 1 contained 195 g/kg crude protein (CP) and 31 g/kg ether extract (EE) and Diet 2 contained 137 g/kg CP and 65 g/kg EE. Each diet was fed to 24 birds in eight chambers containing three birds each for three days after a four-day acclimatisation period. The experimental design was completely randomised. Feed intake, body weight, egg production, AMEn, oxygen consumption, carbon dioxide generation, total heat production, HI and NE were determined assuming a value of 370 kJ/kg$^{0.75}$ for fasting heat production (Wu et al., 2016). Results are shown in Table 1. Predicted and measured energy

Table 1. Energy partitioning in birds fed high and low NE:AMEn diets.

	Diet		
	Low NE:AMEn	High NE:AMEn	P > F
Respiratory quotient	0.982	0.986	0.754
Heat production (kJ/kg$^{0.75}$)	573.4	550.7	0.04
HI (kJ/kg$^{0.75}$)	347.5	307.4	0.018
NE (MJ/kg feed)	7.64	8.71	< 0.001
NE:AMEn	0.732	0.777	0.005

Table 2. Predicted vs measured NE values (as is) in birds fed high and low NE:AMEn diets.

	Diet	Predicted	Measured
NE	Low NE:AMEn	7.89	7.64
	High NE:AMEn	8.95	8.71
NE:AMEn	Low NE:AMEn	0.755	0.732
	High NEA:MEn	0.798	0.777

balances were compared (Table 2). Our findings show that high and low NE and NE:AMEn contents of layer diets can be estimated using the prediction equation.

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