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Abstracts of Short Presentations 2019

Recent Advances in Animal Nutrition – Australia 2019

Abstracts of Short Presentations

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Contents

Range usage or body weight has minor effect on the gastrointestinal passage rate of commercial free-range laying hen

M. Kolakshyapati, T.V. Nguyen, T.Z. Sibanda and I. Ruhnke

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Free-range flocks are composed of hens that prefer to spend the majority of their time in sheds, "stayers", and hens that prefer to spend the majority of their time on the range, "rangers". While it was previously shown that rangers and stayers differ significantly in egg production, the rationale and underlying mechanism for the different performance remains unknown (Ruhnke and Sibanda, 2018). Range usage may contribute to improved digestive organ development such as the gizzard due to the positive effects of coarse fibre and grit stones leading to an increased grinding activity, and subsequently a decreased passage rate resulting in an increased exposure time of nutrients to digestive enzymes and overall increased nutrient digestibility (Amerah et al. 2009; Hetland et al. 2004). On the other hand, body weight may influence passage rate, due to a larger digestive tract convolute and subsequently larger quantities of enzyme secretion, and a larger intestinal absorption surface.

The aim of this study was to evaluate the impact of range usage and body weight on the gastrointestinal passage rate of commercial free-range laying hens. Eighty-four Lohmann brown hens at 74 weeks of age were selected from a commercial free-range farm and classified as heavy (n = 42; 2.04 ± 0.02 kg) or light (n = 42; 1.71 ± 0.09 kg), and as rangers (n = 42; accessed the range on 84.1 % of the available days; 246 ± 1.55 days) or stayers (n = 42; accessed the range on 5.15 % of available days, 59.8 ± 9.56 days). Each individual hen was orally inoculated using a bolus of 0.15 g titanium dioxide (TiO_2) while having *ad libitum* access to mash feed and water. At 1, 2, 6, 8, 12, 24 and 36 h post inoculation (p.i.) hens were sacrificed and the crop, proventriculus, gizzard, duodenum, jejunum, ileum, cecum and coprodeum, including the digesta, were collected. The samples were analysed for TiO_2 content (Short et al. 1996) and the detected TiO_2 expressed as percentage of the total inoculated amount. A two-way factorial ANOVA was conducted to compare the main effects of range usage and body weight and their interactions taking three replicate hens into account. When interactions were detected, comparison of means was determined using Tukey's test. The level of significance was set at $P < 0.05$. Statistical analysis was performed using JMP Statistics software (v14 IBM SAS Institute Inc., Cary, NC).

There was no main effect of range usage on the passage rate of feed particles at any point of time. Heavy hens retained significantly more TiO_2 in the proventriculus and gizzard at 1 h p.i. compared to light hens. An interaction was observed between range usage and body weight at 1 h and 6 h p.i., where heavy stayers showed a significantly longer retention time of chyme in the proventriculus and gizzard at 1 h p.i., in comparison to light stayers ($P = 0.019$). On contrary, 6 h p.i., light stayers had significantly more TiO_2 in the crop compared to heavy stayers ($P = 0.037$).

In conclusion, body weight and range use had a minor impact on digesta passage rate of hens subject to this research.

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Short F, Gorton P, Wiseman J, Boorman K (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology, 59, 215-22

Black soldier fly larvae does not compromise broiler meat quality

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The demand for novel commercial protein sources in replacement of soybean and fishmeal in livestock feed has largely increased as a result of a costly soybean and fish meal-based diets. Black soldier fly larvae (BSFl) can partially or totally replace soybean and fish meal in broiler diets without compromising bird performance (Maurer et al., 2015; Vilela et al., 2019). However, the impact of BSFl on meat quality is just as relevant as bird performance, as the final product (chicken meat) has to be accepted by consumers. Thus, it was hypothesized that the composition of the BSFl would affect meat quality of broiler meat fed high inclusion rates of BSFl.

A total of 400 Ross - 308 broilers were housed in 40 cages allowing for 8 replicates of the 5 treatment groups. Birds were fed with 0, 2.5, 5, 7.5 and 10 % of BSFl during the starter period (2-10 days of age), and 0, 5, 10, 15 and 20% of BSFl during grower and finisher periods (11-42 weeks of age). On day 42, 2 birds per cage were randomly selected, humanely killed, and breast meat immediately removed and placed in plastic bags at 4 ℃ overnight. The next day, meat colour was assessed using a Minolta Chroma Meter CR 300 (Minolta Co., Ltd., Japan). The cut meat surface was exposed to the air for 35 minutes, three replicate measurements were taken placing the colorimeter across the fibre position on the bloomed surface of each breast. Colour parameters were set on the L^*, a^*, b^* system where L^* measures lightness, a^* relative redness and b^* relative yellowness. Measurements of pH and temperature were undertaken by a direct insertion of a pH probe (IJ44C probe, Ionode, Pty Ltd., Australia) in the muscle samples. The measurements were taken in duplicate using a digital pH meter with a combination electrode. A subsample was collected, vacuum packed and frozen at -80 °C for lipid oxidation analysis using TBARS (thiobarbituric acid reactive substance) qualification method (Zhang et al., 2019). Data was analysed as a completely randomised design which included levels of BSFl as fixed effect, using pH as covariate in colour analysis. IBM SPSS version 25 was used to conduct Univariate Analysis of Variance.

There was no significant effect of pH as a covariate on colour parameters ($P > 0.05$). Feed treatment had no effect on pH (5.74 ± 0.11), colour (L^* 59.0 ± 0.88; a^* 4.47 ± 0.91 and b^* 0.69 ± 0.88) and lipid oxidation (0.72 ± 0.07 mg MDA/kg) of breast chicken muscle ($P > 0.05$).

In conclusion, the inclusion of up to 10% BSFl fed until day 10 of age followed by 20% BSFl in broiler diets fed until 42 days of age had no detrimental effect on colour, pH and lipid oxidation of chicken breast.

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ViLela, J, Andronicos, N, Hilliar, M, Andrew, N, Swick, R, Ruhnke, I (2019) "Black Soldier Fly Larvae in Broiler Diets Did Not Affect Performance but Decreased Cellular Immune Parameters " 22nd European Symposium in Poultry Nutrition (ESPN 2019), Gadank – Poland.

Zhang, Y, Benjamin, W. B. H, Eric, N. P, Matthew, G. K, Kristy, L. B, Ashleigh, K. K, Damian, C, David L. H (2019) Understanding Beef Flavour and Overall Liking Traits Using Two Different Methods for Determination of Thiobarbituric Acid Reactive Substance (TBARS). Meat Science 49, 114-19.

Hydroxy methionine: beyond its methionine value for piglets' growth performance and health status

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Recent research has focussed on the importance of sulphur amino acids (SAA) and sulphur metabolites (i.e. taurine, glutathione (GSH)) under challenging conditions. Hydroxy-methionine (OH-Met) can act as an efficient precursor of these down-stream sulphur compounds. Therefore, the following research has focused on increasing the supply of methionine as OH-Met on piglet growth and health.

An area of investigation of SAA role was primarily assessed within the gut itself. Gut blood flow rate is important for nutrient absorption and amino acid net portal appearance. Fang et al. (2009) demonstrated that OH-Met may increase portal blood flow and the net portal absorption of amino acids, due to the increased portal appearance of essential amino acids. The enhanced portal appearance of dietary AA might imply an additional nutritional significance of OH-Met in swine growth. Zhang et al. (2015) performed a study where they increased the SAA content by 25% above NRC (2012) recommendations using either D,L-methionine (DLM) or OH-Met during the lactating period in sows and found that milk fat and lactose concentrations were increased when sows were fed 125% SAA diets as OH-Met. As a result piglet body weights were also increased. This was confirmed by Xu et al. (2017a) who observed a 24% increase in piglet body weight in the OH-Met group. The better milk quality was found to be related to the acetate production that was increased in the presence of OH-Met but decreased with DLM consumption given that lactating sows can utilise acetate as a carbon source for fatty acid synthesis. The maternal supplementation of OH-Met, 25% above SAA recommendations led to a significant improvement of piglets' body weight at weaning. Li et al. (2014) showed that piglets supplemented with OH-Met were heavier than the control or those supplemented with DLM. Histological analyses revealed that with OH-Met supplementaton, villus height and villus height/crypt depth were significantly increased. In addition, the assessment of redox status through GSH balance at in the jejunum in weaned piglets showed that the ratio of reduced GSH over oxidized GSH was significantly lowered with OH-Met showing better redox status. During the post-weaning period, piglets fed OH-Met improved their ability to cope with weaning or inflammatory stress (Li et al., 2014; Xu et al., 2017b). In the study of Xu et al. (2017b), OH-Met and DLM (125% SAA) fed piglets showed the highest body weights at d 49 and d 63, following a LPS challenge, in comparison to the control diet (Xu et al., 2017b). These data confirm the need to increase SAA supply during and inflammation.

In conclusion, these studies collectively demonstrate that both maternal and piglet supplementation of OH-Met improve piglet performance and the ability to cope with weaning stresses.

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Zhang X, Li H, Liu G, Wan H, Mercier Y, Wu C, We X, Che L, Lin Y, Xu S, Tian G, Chen D, Wu D, Fang Z (2015) Differences in plasma metabolomics between sows fed DL-methionine and its hydroxy analogue reveal a strong association of milk composition and neonatal growth with maternal methionine nutrition. British Journal of Nutrition 113, 585–595.

Hydroxy-methionine supplementation improves meat quality

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Redox reactions are well known as modulators of metabolic pathways in response to the oxidized or reduced status of a given molecule (Toledano *et al.* 2007). Meat is prone to producing free radicals such as reactive oxygen or nitrogen species (i.e. ROS & NOS) that can attack the constituents of the muscle such as pigments, lipids and proteins. This paper summarises the effects of hydroxy-methionine (OH-Met) on meat quality.

According to Martin-Venegas et al. (2006), the conversion of OH-Met into L-Methionine facilitates greater transsulfuration into L-Cysteine when compared to direct supply of L-Methionine *in vitro*. As a precursor of glutathione and taurine, L-cysteine plays a key role in the antioxidant system, modulating the redox status. Berri et al. (2012) fed broilers with diets supplemented with DL-methionine (DLM), DL-OH-Met, and a mix of both DLM and OH-Met (50/50) from 0 to 42 days and found similar feed conversion ratio between methionine sources. These authors observed that the total or partial inclusion of OH-Met reduced the meat thiobarbituric acid reactive substances (TBARS) index after 10 days of +4°C storage, pointing to lower lipid peroxidation. Additionally, Lebret et al. (2018) observed that raising dietary total sulfur amino acids (TSAA) from 0.45% to 1.33% by use of OH-Met in pigs, during the last fourteen days before slaughtering, increased ($P<0.05$) the ultimate pH of muscles and reduced drip loss. Pigs fed the highest TSAA level displayed lower TBARS and higher glutathione levels ($P<0.05$), lightness and hue ($P<0.10$) in the longissimus muscle, compared to the control diet. An increase of the dietary TSAA above the requirements with OH-Met, resulted to an improved technological quality of ham.

OH-Met was also found to be similar to DLM in improving duck carcass traits (Zhao et al., 2018). However, the total antioxidant capacity and glutathione peroxidase in *Pectoralis major* muscle were increased ($P<0.05$) only by OH-Met compared to DL-Met. OH-Met promoted also a higher ($P<0.05$) concentration of reduced glutathione and higher total antioxidant capacity compared to DLM.

In conclusion, dietary OH-Met is more efficient in promoting antioxidant status that improve meat quality traits, compared to DLM.

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Bacillus subtilis 29784 reinforces the gut barrier and prevents pro-inflammatory response

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Stressors such as high stocking density, heat, and infectious diseases can affect the health of poultry through dysbiosis and inflammation. Maintaining intestinal barrier integrity and preventing "leaky gut" is critical to avoid disease development and reduce use of antibiotics (Tellez and Latorre, 2017). In a previous study (Rhayat et al., 2019) we showed that *Bacillus subtilis* 29784 (*BS29784*) used as probiotic blunts production of pro-inflammatory interleukin (IL)-8 (IL-8) and inducible nitric oxide synthase (iNOS). The objective of the present study, based on two complementary *in vitro* experiments, was to explore the mechanisms involved in the anti-inflammatory properties of *BS29784*.

In experiment 1 (exp1), vegetative cells of *BS29784* were applied for 16 h to the apical surface of a 14 day-differentiated Caco-2 cells monolayer established in a Transwell system. Caco-2 cells exposed or unexposed to bacterial cells were then stimulated with IL-1β or deoxynivalenol (DON) and nuclear and cytosolic fractions were extracted. Western blotting was used to measure the nuclear factor of κ light polypeptide gene enhancer in β cells inhibitor (IkB) protein level in the cytosol and nuclear translocation of NF (Nuclear Factor) κB as well as for expression of zona occludens-1 (ZO-1), occludin and claudin-1 tight junction proteins. In experiment 2 (exp2), Caco-2 cells were used to track the effect of *BS29784* on trans-epithelial electrical resistance (TEER) using automated measurements over 24-48 h in normal and inflammatory conditions containing inteferon-γ and tmor necrosis factor-α. The tight junction proteins ZO-1 and occludin were visualized by confocal microscopy.

IL-1β treatment on Caco-2 cells induced a 5-fold increase (P>0.001) in NFκB amount in the nuclear fraction, demonstrating activation of the signaling pathway by the pro-inflammatory stimulus. When cells were pre-incubated with *BS29784,* there was no increase in nuclear level of NFkB. Exposure to DON was associated with a significant decrease in the expression of ZO-1, occludin and claudin-1, whereas *BS29784* caused an increase (P<0.001) in expression of these proteins (exp1). Among the tested *Bacillus* strains, the effect on TEER varied from a negative response of 0.5-fold baseline to positive effects from 1.5 - 3-fold above the baseline. Some tested strains were even able to alleviate and restore the decrease in TEER caused by the inflammatory stress. *BS29784* was able to increase the levels of ZO-1 and occludin proteins by 20% (exp2).

These two experiments showed that *BS29784* exerts its immunomodulatory properties by inhibiting IkB degradation, thus preventing NFkB translocation and, by doing this, limiting the expression of pro-inflammatory cytokines, such as IL-8. In conclusion, tThe expression of tight-junction proteins, regardless of the type of stress, was shown to be consistently upregulated by *BS29784*.

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Biofortification of animal products with L-selenomethionine for better human and animal health in Australia

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Selenium (Se) is an essential micronutrient for human and animal health, with anti-oxidant, anti-viral and anti-cancer effects. Prevention of disease of clinical symptoms often requires a supra-nutritional Se intake. Unfortunately, in some regions of the world Se is declining in the food chain and new strategies are needed to increase its intake. The biofortification of animal products, in this respect, is an efficient way to increase both animal and human health. This review discusses the effectiveness of supplementing different sources of organic Se into feed on the deposition of Se in animal products (e.g. eggs, meat and milk). Organic Se sources that may be added to the feed can be in the form of selenized yeast (SY), L-selenomethionine (L-SeMet) and hydroxy-selenomethionine (OH-SeMet).

Eggs, as a cheap source of protein that are readily available in the market, present a valuable candidate for Se biofortification. Delezie et al. (2014) reported the high efficiency of a dust free preparation of L-SeMet in augmenting Se concentrations in eggs compared to SY and SS. After 8 weeks of supplementation of 0.3 ppm of Se in the form of L-SeMet, concentration in the whole egg was 473 µg Se/kg. The addition of SY and SS resulted in 375 µg Se/kg and 288 µg Se/kg, respectively. Based on the average egg weight (67g) this amounts to 32µg Se/egg for the addition of L-SeMet. This value represents 45.7% of the adequate intake (AI) for adults, 70 µg Se/day (EFSA 2014). L-SeMet is able to increase the Se concentration of animal products to the highest extend as it is the only Se compound that can be directly incorporated in proteins. Meat, as it is high in protein, is also a valuable candidate for biofortification. Van Beirendonck et al. 2016 showed the highest deposition of Se in broiler breast muscle when birds were provided with a dust free preparation of L-SeMet (0.2 mg Se/kg diet) and this compared to SY and SS. The data clearly proved that the Se deposition in muscle is linearly correlated with the added amount of Se in the form of L-SeMet to the diet. More recent data (Bruneel 2018,) shows the benefit of L-SeMet compared to OH-SeMet. The efficiency of OH-SeMet, to biofortify broiler breast muscle with Se, was seen to be only 80% compared to L-SeMet. The hydroxy form of selenomethionine cannot be used directly by the animal as it has to be first converted to L-SeMet before it can be used in protein production. Literature states that the relative utilisation of hydroxy-methionine, compared to L-methionine, for chicken and pigs is only 80% (EFSA 2012). Next to eggs and meat, milk is also an interesting way of providing extra Se to humans, especially infants. Vandaele et al. 2014 analysed the Se content in milk samples of dairy cows after supplementation of 0.3 mg Se/kg DM from SS, SY and a dust free preparation of L-SeMet. After 7 weeks of treatment, the Se concentration in the milk was the highest in the L-SeMet group (75 µg/kg).

From the above mentioned studies it can be concluded that adding L-SeMet to the animal diet is the most efficient way to increase the Se content of animal products (e.g. meat, milk and eggs). This practice provides an important opportunity to support human health.

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Body weight is associated with welfare, health and egg production status in commercial free-range laying hens

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Free-range housing systems are known for their increased biosecurity risk due to the increased hen exposure to various pathogens. However, use of indoor structures such as aviary systems may also be a potential risk for keel bone damage and may impact feather cover due to the increased exposure to other hens. Hen body weight is known to influence hen health, the immune system, and hen ovulation rate, but also the behaviour of an individual hen and may subsequently increase or decrease the likelihood of pathogen infection (Kilpinen et al., 2004). The aim of this study was to assess the welfare, health and egg production status of hens with different body weight in commercial free-range laying hens.

A total of 7708 Lohmann Brown hens at 74 weeks of age were obtained from 4 commercial flocks and evaluated for beak, comb and wattle appearance, feather cover, keel bone damage, the prevalence of gastrointestinal parasites, liver health, and egg follicle status. Hens were classified according their body weight as light (1.65 ± 0.002 kg; n=1913), medium (1.86±0.010 kg; n=3879) and heavy (2.08±0.002 kg; n=1916). All the scores from all the parameters were analysed using chi-squared test. These analysis were followed up by the non-parametric multiple comparison Wilcoxon test to determine the differences of the scores between body weight groups. Statistical analysis was performed using JMP Statistics software (v14 IBM SAS Institute Inc., Cary, NC).

The results demonstrated that light hens had the highest prevalence of gastrointestinal helminths, cestodes and spotty liver compared to the medium weight and heavy hens (P=0.001). Heavy hens had the highest proportion of fatty liver incidence, better beak condition score, and highest neck feather score compared to hens of other body weight. Furthermore, 55.8% of heavy hens had keel deformation compared to 48.9 and 50.7% of medium and lighter hens respectively (P=0.0001). In the contrary, lighter hens had a significantly higher feather score on the chest (3.02±0.018) compared to medium and heavier hens with scores of 2.96±0.013 and 2.87±0.018 respectively, indicating better feather cover. Heavier hens had significantly higher proportion of ovary follicles in full production (95.3%) compared to the lighter hens (90.0%).

In conclusion, body weight is significantly related to hen health and welfare in commercial free-range hens.

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Defining mineral requirements in an expanding Australian industry for black tiger prawns, *Penaeus monodon*

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In Australia, it is expected that the aquaculture industry will double its volume by 2025, and is partly contributed by the ongoing expansion of prawn production, primarily the culture of black tiger prawns, *Penaeus monodon* (*P. monodon*). This growth in Australian aquaculture will in turn require a large volume of high-quality compounded feed that is cost effective, sustainable and easily accessible. Presently, feeding aquaculture is expensive due to the high protein requirements and the industry reliance on unsustainable wild-caught seafood-derived meals. Attempts at replacing fishmeal in formulated diets has proven complicated as fishmeal provides a diverse source of nutrients including, but not exclusive to, digestible amino acids, nucleotides, fatty acids, vitamins, chemo-attractants, unknown growth promotors and minerals. Feed formulation in prawns is made more difficult by the lack of species-specific nutrient requirements. Nutrient requirements of black tiger prawns are far behind those of other crustaceans, despite *P.monodon* being the second most produced prawn species worldwide, after white-legged shrimp, *L. vannamei* (FAO, 2018). Research at the Bribie Island Research Centre, CSIRO is aimed at improving the sustainability and productivity of Australia's black tiger prawn industry by using two approaches: i) producing a domesticated gene pool and ii) developing a more sustainable feed; in which the second approach will be discussed in this abstract.

Species-specific information on mineral requirements is paramount to formulate cost-effective diets. However, mineral requirements of *P. monodon* are not well known compared with other cultured prawn species where there are known requirements for only three minerals (NRC, 2011). At CSIRO, we employed a novel experimental method, the Plackett Burman (PB) screening design to determine the essentiality of twelve macro and trace minerals in black tiger prawns for the first time. The inorganic minerals investigated were boron (B), cobalt (Co), copper (Cu), magnesium (Mg), manganese (Mn), potassium (K), selenium (Se), sodium (Na), strontium (Sr), zinc (Zn) and calcium (Ca) and phosphorous (P) were considered in tandem at a 1:1 ratio, by PB design, to assess the response of dietary mineral supplementation in purified diets. Culture performance of juvenile prawns were influenced by dietary minerals where Ca, P, Mg, B, Mn, Se and Zn, produced the greatest positive effects on weight gain, feed conversion ratio (FCR) and nutrient retention. B had not been assessed in prawns previously and so a requirement for B was a highly novel outcome. Carcass mineral retention indicated the effect of mineral chemical form on diet stability and bioavailability, highlighting the need for further consideration in prawn feed formulations. This study demonstrated the necessity for several macro and trace minerals in prawn diets and the need to refine requirements for black tiger prawns.

Based on results of the PB screening of minerals, a follow-on experiment was conducted to identify the effect of mineral chemical form on prawn growth, nutrient utilisation and leaching of diets for black tiger prawns. Minerals of importance as determined by the PB study were assessed in high plant (34% soybean meal), low fishmeal (6% fishmeal)-based diets as an inorganic or an amino acid-chelate which included Ca as calcium phosphate or PIDOLin® PCa (13.5% Ca), Mg as magnesium oxide or PIDOLin® PMg (8.6% Mg), Mn as manganese sulphate or Availa®Mn 80 (8% Mn), Se as selenium selenite or Selisseo®Se (2% Se) and Zn as zinc sulfate or Availa®Zn 120 (12% Zn). Preliminary results from this trial found inclusions of macro-minerals as chelates reduced weight gains by 25% (158% vs. 212% initial body weight; $P < 0.05$) which were most likely associated with poor diet stability. Diet stability was correlated with prawn performance as shown in Figure 1. Conversely, chelated mineral inclusions obtained higher total tract digestibility of Ca, Mg and compared to all other treatments assessed. This preliminary finding

suggests that a balance between diet stability and mineral solubility needs to be obtained to achieve growth benefits from dietary minerals. Understanding the relationship between mineral delivery in feed and prawn mineral utilisation will assist with the production of sustainable aquafeeds and reducing reliance on wild-caught marine ingredients.

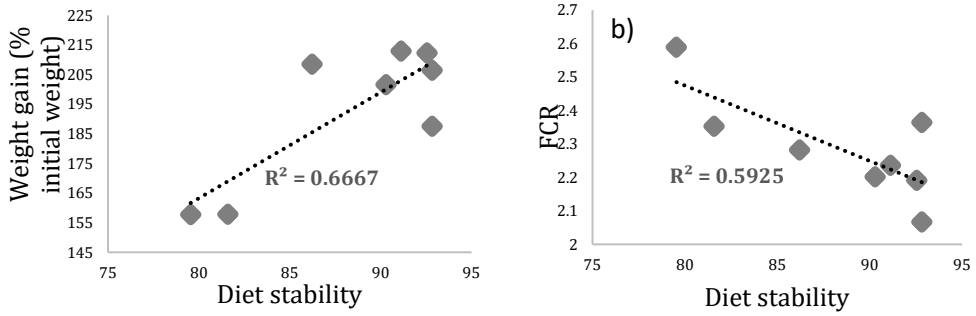

Figure 1. Linear relationship between diet stability (%dry matter after 5 h submerged) and a) weight gain (% initial weight; r = 0.817, p = 0.0134) and b) FCR (feed:gain, r = -0.770, p = 0.0255).

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Dietary protein source and level affect litter quality in meat chickens

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Soybean meal (SBM) is the most commonly used protein source in meat chicken diets and contains 460-480 g/kg crude protein (CP) (Ravindran et al., 2014). Canola meal (CM) contains approximately 340-370 g/kg CP and used as an alternative protein source to SBM for meat chickens at levels up to 150-200 g/kg. Meat and bone meal (MBM) is an animal by-product that is also used as a protein source in meat chicken diets at levels up to 120 g/kg. Soybean meal contains indigestible oligosaccharides and higher levels of potassium compared to CM and MBM. Canola meal contains higher levels of crude fibre, sulfur and magnesium compared to SBM and MBM. Meat and bone meal varies widely in nutritional composition and contains a lower level of digestible protein and amino acids than SBM but higher levels of calcium and phosphorus than SBM and CM. These nutrients and anti-nutrients have been shown to affect litter quality in meat chickens (Vieira and Lima, 2005; Eichner et al., 2007). Thus, dietary protein source may have significant effects on litter quality. Similarly, reduced protein diets use less protein meals and may contribute to higher litter quality.

Two experiments were conducted using Ross 308 male birds to investigate the effect of dietary protein source and level on water to feed intake ratio (WI:FI), litter moisture, litter pH, and odour emissions. In both the experiments, diets were based on wheat-SBM and they contained the same levels of metabolizable energy and digestible amino acids but different CP source (Exp. 1) and level (Exp. 2). In Exp. 1, birds were allocated to three treatments with five replications. The basal diet contained only SBM (230-290 g/kg) as a protein source. The other two diets used CM (170-190 g/kg) and MBM (70-110g/kg) at the expense of SBM. The diets were fed until d 32 and litter samples were collected on d 32. Water and feed intakes were measured from d 10 to d 32. Odorants were measured on d 24. The results showed that SBM and CM groups had higher water intake ($P < 0.01$) than the MBM group but WI:FI was highest in the CM group during the whole rearing period ($P < 0.05$). There were no significant differences in pH and moisture content of litter between SBM and CM groups but these were higher than that of the MBM group ($P < 0.05$). On d 24, emission of methyl mercaptan was the highest in SBM group and the lowest in MBM group ($P = 0.01$).

In Exp. 2, birds were assigned to two treatments with 12 birds per pen and six replicate pens per treatment. Two diets were formulated to contain the same ratio of SBM, CM and MBM but with different CP contents as follows: starter phase (260 g/kg vs 210 g/kg), grower phase (240 g/kg vs 195 g/kg) and finisher phase (230 g/kg vs 184 g/kg). The low CP diet was supplemented with crystalline amino acids including L-valine, L-isoleucine, L-arginine, L-lysine, D,L-methionine and L-threonine. Litter moisture, litter pH and odorants were measured at d 35. Reduction in dietary CP lowered the moisture content in litter by six percentage points ($P < 0.01$). The low CP diet also tended to lower litter pH ($P = 0.05$). Litter headspace concentrations of dimethyl amine, trimethyl amine, ammonia, hydrogen sulfide, phenol and benzene were lower in the low CP treatment ($P < 0.05$). The correlation between litter moisture and odorants showed that methyl mercaptan ($r = 0.453$, $P < 0.01$), hydrogen sulfide ($r = 0.482$, $P < 0.01$), dimethyl sulfide ($r = 0.621$, $P < 0.01$), trimethyl amine ($r = 0.526$, $P < 0.01$), phenol ($r = 0.409$, $P < 0.05$), indole ($r = 0.503$, $P < 0.01$) and skatole ($r = 0.344$, $P < 0.05$) had significant positive correlations with litter moisture. These results suggest that both dietary protein source and protein level may affect litter quality in meat chickens and odorous metabolites are correlated with litter moisture.

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Effect of ethyl-cellulose rumen-protected methionine on dairy cow performance during transition phase

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The transition phase is the most critical period in the production cycle of dairy cows and is characterized by a challenged immune system. Preventing metabolic issues in this sensitive phase is a key to maximize peak performance. At the onset of lactation, dairy cows go into negative energy and protein balance. Applying the right feeding strategy to increase the dry matter intake in this close-up period and to support the optimum immune functioning will ensure a good start into lactation and improve the health status of cows. Methionine is considered as a first limiting amino acid in the majority of high producing dairy cows. Methionine is not only an essential amino acid, but it is also responsible for maintaining several immune functions. Therefore, the present study was conducted to determine the effect of ethyl-cellulose rumen-protected methionine (ERPM, Mepron®) on productive performance and health status of high producing dairy cows during transition phase.

The study was conducted at the Dairy Research Farm, University of Illinois, USA. Sixty multiparous Holstein cows were used in a randomized complete block design with 30 cows per treatment. Treatments included; 1. Control, cows were fed basal diets (close-up, fresh and high producing) without ERPM supplementation, and 2. Test, control diets with ERPM supplementation at 0.09% in close-up diet and 0.10% in postpartum (fresh and high producing) diets (Batistel et al., 2017). Close-up, fresh and high producing diets were fed from 28 days to calving, 1-30 days in milk and 31-60 days in milk, respectively.

Results showed that cows in test group (fed diets with ERMP supplementation) had greater dry matter intake (DMI) compared to cows in control group. The ERPM supplementation significantly increased ($P<0.05$) DMI of cows during close-up period by 1.2 kg/day, fresh period by 1.6 kg/day and high producing period by 1.5 kg/day. During fresh period, average milk yield (4.1 kg/day), milk protein yield (0.20 kg/day), milk fat yield (0.17 kg/day) and milk lactose yield (0.25 kg/day) in test group were significantly increased ($P<0.05$) compared to control group. During high producing period, the ERPM supplementation increased ($P<0.05$) milk yield by 4.4, milk protein yield by 0.17, milk fat yield by 0.19 and milk lactose yield by 0.30 kg/day/cow. The non-esterified fatty acids and γ-glutamyl transferase levels in blood were reduced by 25 and 37%, respectively in cows fed test diets compared to cows fed control diets. The reduction in fatty acids and γ-glutamyl transferase levels in the blood indicated better liver function and health status of dairy cows.

Based on results of this study, it can be concluded that feeding ERPM during transition phase of dairy cows improves dry matter intake and health status. The feeding of EPRM from 28 days prepartum improves productive performance not only in the immediate postpartum period but also through peak of lactation.

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Effect of pasture levels in a mixed dairy diet on enteric methane production from an in vitro digestibility system

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Climate change and effective measures to reduce global warming have gained significant attention over the last 30 years. In the latest international agreement, the Paris Agreement from 2016, individual countries set their own targets for reducing greenhouse gas (GHG) emissions. For example Australia announced a target of 26-28% reduction below 2005 by 2030 whereas the target set by New Zealand is a 30% reduction below 2005 by 2030 (Power, 2017). In an ambitious move the NZ government has recently introduced a bill which aims to reduce all GHG to net zero by 2050 in an effort to reach the global goal of 1.5°C global warming above pre-industrial levels as set by the IPCC (MacLauchlan, 2019). It is well known that Agriculture is a large contributor to Greenhouse gas (GHG) emissions although the actual contribution from agriculture does vary significantly between countries. In Australia agriculture contributes to around 15% of total GHG emissions on the other hand the contribution from agriculture in New Zealand is almost 50%. Intensive livestock production and methane emissions from cattle and small ruminants is estimated to be approximately 66% of all contributions from agriculture. A recent life cycle environmental impact study for dairy farms in in New Zealand concluded that brought in supplementary feed has a major negative impact on environmental impacts such as climate change or ozone depletion potential (Chobtang et al., 2017) which would lead to the hypothesis that feeding a high proportion of homegrown pastures could be a possible strategy to reduce overall GHG emissions from dairy farming. The impact of this strategy on enteric methane production needs to be assessed in view of the economic implications of changing farm feed systems both to individual farms and at an industry level.

The current study tested the effect of feeding increasing levels of pasture on enteric methane production using an *in vitro* fermentation model (IFM). Four mixed rations (55%, 70%, 85% and 100% pasture - plus equal amounts of maize silage (MS) and Palm Kernel Expeller (PKE)) were fermented in a closed *in vitro* system for 48 hours. Microbial biomass (MBM), volatile fatty acid production (VFA) and methane production were determined as indicators of ruminal digestion. Partitioning factor (PF) was used to determine efficiency of fermentation where PF = True dry matter digestibility (TDMD) * (1000/Total gas volume).

Overall MBM was independent of the % of pasture, however total VFA production was significantly higher in treatments with lower pasture (Table 1). In contrast methane emissions per unit of dry matter intake significantly increased as pasture content in the ration increased and the efficiency of the fermentation increases linearly as pasture content decreases. These results would indicate that ruminal digestion of diets with a higher proportion of added feed in the form of MS and PKM results in increased amounts of usable products, mainly VFA and lower waste in form of methane.

Enteric emissions are said to account for an estimated 40% of methane emissions from a dairy farm. Looking at enteric methane production in isolation these results show that the suggested strategy of increasing levels of pasture in place bough in feed as a means of reducing GHG is not a viable solution *per se*. It also has to be noted that the current study did not look at milk yield nor any input factures. Previous life cycle assessments reported that high yielding pastures in particular mixed pastures with clover have an overall beneficial effect on the Global Warming Potential (Basset-Mens et al., 2005). When devising strategy to

limit the potential liability of the dairy industry to the ETS, it is important to consider farm productivity, life cycle analysis of all the dietary components as well as enteric methane emission.

Table 1. Microbial biomass (MBM), methane (CH_4) and partitioning factor (PF) of *in vitro* testing of four mixed rations.

Treatment *	MBM (mg/gDM)	VFA mM	CH_4 (ml/gDM)	PF
T1: 55% pasture	138.9	36.27 ^{a}	45.14 ^{a}	6.03 ^{a}
T 2:70% pasture	139.1	30.66 ^{ab}	51.71 ^{a}	5.78 ^{ab}
T3: 85% pasture	144.4	28.65 ^{b}	55.22 ^{a}	5.43 ^{b}
T 4: 100% pasture	141.9	27.46 ^{b}	77.00 ^{b}	5.30 ^{b}
SEM	1.407	1.093	3.062	0.089
P-value	NS	0.01	0.001	0.01

*pasture plus ½ maize silage, ½ palm kernel expeller
^{a,b} values within column differ by $P<0.05$.

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Tibial mineralization in broilers as influenced by two levels of dietary calcium and phytase during subclinical necrotic enteritis

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Bone development is an important welfare and production concern in the broiler industry. It is affected by a number of factors including genetics and nutrition (González-Cerón, Rekaya, & Aggrey, 2015). Improved genetics resulting in higher growth and body weight places additional demands on nutrition to maintain skeletal structure. Calcium is one of the most limiting minerals in skeletal development. The presence of phytic acid in many ingredients is a major issue as calcium is easily chelated and forms phytate-mineral complexes that reduce its availability. Furthermore, high levels of calcium reduce the ability of exogenous phytase to hydrolyse phytate. Decreased calcium uptake may result in higher levels in the hindgut and may favour the proliferation of *Clostridium perfringens*. Therefore, it is hypothesized that high dietary calcium would lower phytase activity thus decreasing bone development during challenge with necrotic enteritis (NE).

A total of 768-day-old- Ross 308 male chicks were randomly allocated to eight treatments with six replicate pens, each housing 16 birds. A 2 × 2 × 2 factorial arrangement of treatments was applied and the factors were: dietary calcium (0.6 or 1.0%), phytase (500 or 1500 FTU/kg; Quantum BlueTM, (AB Vista, Malborough, UK) and NE challenge (no or yes). Half of the birds (384) were challenged with *Eimeria spp.* (Eimeria Pty Ltd) on d 9, and *C. perfringens* strain EHE-NE18 (CSIRO) on d 14 and 15 to induce NE. Tibiae were excised from the right legs of two birds per pen on d 16 and 29 for determination of ash, breaking strength and bone minerals. Tibiae breaking strength was measured using a universal texture analyzer with a 50 N load cell and 3 point fixture bed at a test speed of 10 points of data per second. The remnants of the bones were dried at 100 ℃ for 24 h, weighed, and ashed at 600 ℃ overnight for determination of ash. Approximately 1 g of the ash was homogenized and microwave-digested with nitric acid in inductively coupled plasma emission spectroscopy (ICP-OES).

On d 16, birds given challenge and high Ca had higher tibia breaking strength ($P<0.01$) compared to those in the non-challenged and high Ca group. Main effects for bone parameters on d 16 - non-challenge vs challenged, tibia Mn (20.9 vs 31.8 mg/kg, $P<0.001$); low vs high Ca - tibia ash (46.2 vs 49.9%, $P<0.001$), tibia Ca (37. 8 vs 38.8%, $P< .05$); low vs high phytase – tibia breaking strength (91.6 vs 103 N, $P < 0.001$), tibia Mn (25.2 vs 27.5 mg/kg, $P < 0.05$) and tibia Zn (450 vs 478 mg/kg, $P < 0.01$). There was a phytase by Ca interaction for Mn on d 29, where low dietary Ca increased ($P < 0.01$) Mn concentration in high phytase diets. Main effects for bone parameters on d 29 - non-challenge vs challenged - tibia breaking strength (377 vs 317 N, $P < 0.001$); low vs high Ca – tibia Mg (1.00 vs 0.88%, $P < 0.001$) and Zn (474 vs 448 mg/kg, $P < 0.05$); low vs high phytase – tibia Zn (440 vs 481 mg/kg, $P < 0.001$).

It was concluded that high dietary Ca and phytase were beneficial in bone mineralization while NE had a minimal effect on bone development. Therefore, nutritional manipulation may improve bone mineralization in chickens during NE.

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Efficacy of feed additives on immunity and gut integrity in broiler chickens challenged with *Eimeria*

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This study aimed to examine the efficacy of feed additives on serum IgA and gut integrity in broilers challenged with *Eimeria* spp (*E. acervulina*, *E. maxima* and *E. brunetti*). Additives were: A) synergistic blend of medium chain fatty acids (MCFA), slow release C12 fatty acid, target release butyrates, organic acids (OA) and a phenolic compound; B) synergistic blend of partly buffered OA with MCFA; C) synergistic blend of partly buffered OA with a high concentration of MCFA.

A total of 1170 male Ross 308 chicks (breeder flocks) were assigned to 65 pens each stocked with 18 birds. The treatments included: T1 - unchallenged group, without additives; T2 - challenged group, without additives; T3 - challenged group plus additive A at 1.5, 1.5 g/kg feed; T4 - challenged group plus additive B at 2.5, 2.0 g/kg feed; T5 - challenged group plus additive C at 2.0, 1.5 g/kg feed in starter and grower phases, respectively. Diets were wheat and soybean meal based supplemented with xylanase and phytase. Birds except unchallenged group were challenged with field strains *Eimeria* spp oocysts consisting of *E. acervulina* (5000), *E. maxima* (5000) and *E. brunetti* (2500) at d 9. Serum IgA and fluorescein isothiocyanate dextran (FITC-d) as leaky gut marker were measured on d 13. A higher concentration of serum FITC-d was observed in T2 compared to A and C additives groups ($P<0.05$). Birds treated with C had higher concentration of IgA compared to unchallenged and challenged group.

These findings showed that additives A and C enhanced gut integrity and additive C boosted immune responses of birds during the challenge period.

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Biopolymer composites for slow release of toxinsto manage *Pimelea* poisoning in cattle

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Cattle grazing the pastures of inland Australia can be poisoned by ingestion of certain native *Pimelea* plant species, particularly *Pimelea trichostachya* and *Pimelea simplex* (Fletcher et al., 2009). The *Pimelea* toxin, simplexin, causes restriction of the pulmonary venules, with resultant heart impacts and characteristic fluid accumulation (oedema) of the jaw and brisket regions. In certain years, heavy livestock losses can occur. Currently, there is no effective vaccine or antidote for *Pimelea* poisoning; the only management strategy beef producers have is to reduce contact between toxic plants and susceptible stock, to avoid potentially devastating poisoning events. Nevertheless, previous research has demonstrated that prolonged low dose feeding diminishes the effect of poisoning in animals. It was postulated that the animal exposed to prolonged low doses developed a mechanism for detoxifying simplexin, possibly through adaptation of the rumen microbial environment (Fletcher et al., 2014).

The present study investigates the use of a biopolymer/toxin composite to foster toxin-degrading microbe populations. The objectives are to manufacture biopolymer composites based on biodegradable polyhydroxyalkanoates (PHAs) and/or polycaprolactone (PCL), as toxin slow-release systems for the rumen that would have broad utility across a range of plant toxins and other beneficial rumen compounds. The performance of these biopolymer/toxin composites were investigated in an *in vitro* laboratory trial.

Preliminary results of different biopolymers/composites containing *Pimelea* material in an *in vitro* simulated rumen environment for up to 30 days demonstrated differing rates of weight loss dependent on the biopolymer type. The scanning electron microscopy imaging revealed that in the case of PHA biopolymer composites, numerous holes were observed on the surface (Figure 1), suggesting that they were good source of energy for the microbial environment.

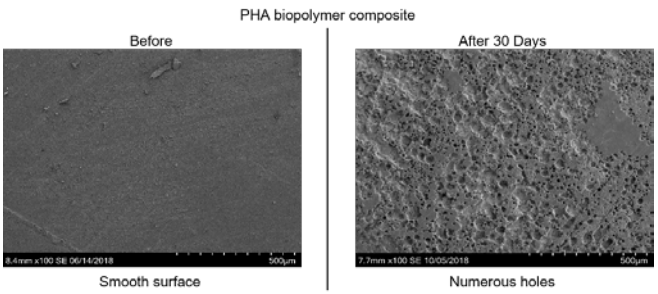

Figure 1. Microscopic imaging of the surface of PHA biopolymer before and after 30 days in an *in vitro* fermenter.

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Effect of organic acids and chromium supplementation on amelioration of heat stress in growing pigs

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Heat stress (HS) compromises pig health and welfare due to resultant respiratory alkalosis and oxidative stress (Cottrell et al., 2015). The elevation of respiration rate (RR) during heat exposure resulted in a decrease in blood CO_2 concentrations leading to respiratory alkalosis (Liu et al., 2018). In addition, the redistribution of blood flow away from visceral tissues triggers oxidative stress in the gastrointestinal tract (GIT) with a resultant reduced barrier function and increased permeability (Cottrell et al., 2015). Recently, there has been an increased focus on the use of nutritional supplements as cost effective strategies for the amelioration of HS. Chromium, a trace mineral, has been suggested as an additive to ameliorate HS due to increase insulin sensitivity and reduced respiration rate (RR) and rectal temperature (RR) in HS pigs (Liu et al., 2017). Butyric acid, a short chain fatty acid, which is synthesized via microbial fermentation (Bedford and Gong, 2018) had a beneficial for growth of epithelial cells and maintained mucosal integrity (Fang et al., 2014). Another organic acid, benzoic acid, lowered urinary pH and after absorbed into blood stream and positively influence on GIT development (Chen et al., 2017). Therefore, this study investigated the effect of chromium and a combination of these organic acids on ameliorating HS in growing pigs.

A total of 48 female Large White × Landrace grower pigs (32.7 ± 0.27 kg) were allotted in a 2 × 3 factorial experimental design with 2 different temperatures (TN vs HS) and 3 diets. Pigs were acclimated to one of the 3 diets: control (CON) (standard grower diet), chromium (Cr, + 400 ppb Chromium picolinate), Organic acids (OA) (+ 1.5 g/kg butyric acid (ButiPERL) and 5 g/kg benzoic acid) for 14 days under TN condition (20°C). After dietary acclimation, pigs were then given a climate challenge (CC) of either TN (constant 20°C) or cyclic heat-stress conditions (HS; 8 h 35°C, 16h 28°C/d) for 3 days. During heat exposure, RR and rectal and skin temperatures (RT and ST) were observed every 2 hours. Pigs were euthanised on day 3 of the CC with terminal blood samples obtained for blood gas analysis and quantification of stress and inflammation markers. Tissue samples were collected to measure transepithelial electrical resistance (TER) and macromolecule (fluorescein isothiocyanate–dextran; molecular mass 4 kDa, FD4) permeability with Ussing chambers. All data was analysed using an ANOVA with Duncan's post-hoc tests using Genstat v18.

Heat stress increased RR, RT and ST compared to TN. However, no effects organic acids or chromium were observed on these physiological measures under HS condition. The OA diet lowered urinary pH overall (5.95 and 6.07 vs 5.37 respectively, $P<0.001$), indicating that benzoic acid was absorbed and metabolised. Additionally, HS pigs had lower urinary pH compared to TN (6.04 vs 5.55, $P=0.003$). HS decreased $_pCO_2$ (49.8 vs 43.8 mmHg, $P<0.001$), total CO_2 concentration (34.4 vs 30.3 mmol/L, $P<0.001$) compared to TN, which resulted in a large reduction in bicarbonate under HS (32.9 vs 29.0 mmol/L, $P<0.001$). The OA tended to reduce CO_2 ($P=0.068$) and bicarbonate ($P=0.064$) concentrations, which would support the onset of respiratory alkalosis. HS pigs had a lower base excess than pigs housed in TN (7.39 vs 4.11 mmol/L, $P<0.001$) while the OA diet lowered base excess under both TN and HS conditions ($P=0.065$). HS reduced TER in th eileum (115 vs 99.9 Ω/cm^2, $P=0.026$) and colon (124 vs 87.4 Ω/cm^2, $P=0.005$) but no dietary effects were observed. There was an interaction between diet and temperature in the jejunum such that the TER was reduced with the OA diet in TN pigs only and chromium reduced the TER

in HS pigs only. Regarding the stress and inflammation markers, only a significant reduction in thyroid hormone (T_4) under TN (43.3 vs 29.6 nmol) was observed. There were no changes in other stress or inflammatory markers.

The efficacy of organic acids and chromium on physiological responses and protection of intestinal integrity in GIT were not observed as expected. However, the lower urinary pH in urine indicated that organic acids may have a role in buffering blood pH and further work should be done to understand if these acids ameliorate respiratory alkalosis.

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Antioxidant supplementation improves production performance in Holstein Friesian dairy cattle during summer

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Heat stress (HS) is one of the greatest challenges compromising dairy cattle production during hot summer months (St-Pierre et al., 2003). The present study investigated the supplementation of antioxidants (Oxi-Care; vitamin and mineral premix containing Vitamin E, Vitamin D_3, 25-hydroxycholecalciferol, Beta Carotene, Selenium, and Betaine) as a nutritional strategy to reduce the impacts of HS on dairy cow production during summer months. The study was conducted at The University of Melbourne Robotic Dairy at Dookie Campus (Northeast Victoria) during January-March 2019 and was approved by The University of Melbourne Animal Ethics Committee (AEC # 1814699.1). Ambient temperature and relative humidity data for the duration of the study were collected from the automatic weather station located at the Dookie dairy farm to calculate Temperature Humidity Index (THI). Eighty-eight (88) lactating Holstein Friesian cows were selected for feeding trial were divided into 2 groups of 44 cows each (blocked by milk yield, body weights, lactation numbers, and stage of lactation) and allocated to receive standard pellets (CON) or antioxidant pellets (OXI-CARE) at the robotic milking station for 60 days including 5 days transition period to new diets. The cows were managed under their normal routine husbandry system, milking, and allowed to graze pasture as a single group under the robotic milking system. The experimental pellets were formulated by adding OXI-CARE to standard pellets (16% crude protein) to deliver extra vitamin and antioxidants (OXI-CARE @ 15 g/cow/day). Pellets were offered (8kg/day) during milking by programming the automatic feeders to allocate pellets from either control or antioxidant silo as per the cow collar numbers. Cows voluntarily entered the dairy to be milked by the Lely Astronaut automatic milking system and individual cow daily production data were collected automatically by robots via RFID ear tags (Table 1).

	CON	OXI-CARE	SED	P Value
Daily milk production (kg)	26.01[a]	26.93[b]	0.27	0.039
Rumination time (mins)	468.0[a]	538.4[b]	13.8	0.024
Fat %	4.004[a]	4.245[b]	0.02	< 0.001
Protein %	3.006	3.043	0.01	0.169
Fat-protein ratio	1.331[a]	1.387[b]	0.01	< 0.001
Somatic cell count	226.6	137.3	69.6	0.709
Milking frequency (24hrs)	2.02[a]	2.43[b]	0.02	0.004

[ab]Means within rows with common supercrips are not significantly different (P>0.05)

Table 1. Mean production performance of Holstein Friesian dairy cows (n=88) grazing pastures during summer (THI=68-84) and offered standard pellets (CON) or supplemented with additional antioxidant pellets (OXI-CARE) at the Robotic dairy.

During summer grazing (Table 1), OXI-CARE supplementation significantly ($P<0.05$) improved the daily milk production of cows as compared to the cows offered standard pellets. Similarly, there was significant effect of OXI-CARE supplementation on milk fat% and milk fat-protein ratio such that cows on supplemented pellets produced milk with higher fat% and fat-protein ratio. However, there was no effect of OXI-CARE supplementation on milk protein % and SSC. The OXI-CARE supplementation also increased the cow milking frequency as well as the rumination time.

These findings indicate that extra supplementation of mineral and vitamin antioxidants during summer helps to improve the production performance of dairy cattle plausibly by improving the antioxidant capacity and overall oxidative status of the cows which is known to be compromised by HS (Bernabucci et al., 2002).

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Maximising and effective measurement of energy from poultry feeds

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The most important economic factor in poultry production is feed cost in which energy sources represent a major part. Maximisation of feed utilisation and energy estimation are therefore the major targets of a nutritionist. Feed digestion and nutrient absorption are key elements for feed utilisation. These can be boosted by exogenous enzymatic activities, such as carbohydrase enzymes, through counteracting the effect of anti-nutrition factors (ANF) and reducing nutrient flow in the hind gut thereby ensuring a balanced gut microbial community. The latter can also be improved by functional feed, such as probiotic use. Different methods are used to measure dietary energy for poultry. Of these methods, the metabolisable energy (ME) system is commonly used to express energy concentration in diets, because it is easy to use and may provide repetitive values. However, ME value is not completely available to the bird to meet its energy requirements as part of it is lost through heat increment (HI) of feeding. The net energy (NE) system was then suggested to provide energy values closer to the true requirements of the bird. However, its use has been limited by the variability of its values due to environmental, dietary and animal factors. Studies performed so far suggest that the NE system may be employed to measure poultry feeds, because the main variation factors can be eliminated by using modern broiler breeds coupled with modern husbandry management and, more importantly, accurate systems to measure NE in chickens. The different method used to measure NE for poultry diets (comparable slaughter technique, direct calorimetry, indirect calorimetry and NE prediction equations) have been reviewed and will be presented.

Age-related changes in apparent digestibility of non-starch polysaccharides in broiler chickens fed maize- or wheat-based diet

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Poultry diets contain a substantial amount of carbohydrates, primarily in the form of non-starch polysaccharides (Choct, 1997). As chickens do not have endogenous enzymes to hydrolyse these carbohydrates, their digestion occurs via bacterial fermentation which may change with age (Montagne et al., 2003). However, the effect of age on NSP has yet to be determined.

The present study evaluated the effect of age on apparent total tract digestibility of soluble and insoluble non-starch polysaccharides in broiler chickens offered a wheat-based or maize-based diet. Each treatment was tested with 12 replicate groups of 10 birds grown to 35 days of age. All experimental diets contained 0.5 % of titanium dioxide as an indigestible marker. Excreta from each pen was collected and pooled on day 12, 24 and 35. Birds offered the wheat-based diet had greater ($P < 0.05$) apparent total tract digestibility of insoluble NSP compared to those offered the maize-based diet on day 12 and 24. Furthermore, birds fed the wheat-based diet had higher ($P < 0.05$) apparent total tract soluble NSP digestibility compared to those fed the maize-based diet on day 24 and 35. There were no significant differences between the two diets on the apparent total tract digestibility of insoluble NSP on day 35 and soluble NSP on day 12. A significant linear decrease with age from day 12 to 35 was observed on the apparent total tract digestibility of insoluble NSP in birds offered the wheat-based ($P < 0.05$; adjusted $r^2 = 0.61$) and the maize-based diets ($P < 0.05$; adjusted $r^2 = 0.25$). In contrast, there was a linear ($P < 0.01$; adjusted $r^2 = 0.30$) increase in the apparent total tract soluble NSP digestibility with age from day 12 to 35 in birds fed the wheat-based diet. No linear effects on soluble NSP digestibility with age were detected in birds offered the maize-based diet.

In conclusion, the digestibility of NSP differs depending on the diet type, with the digestibility of both insoluble and soluble NSP being higher in birds fed wheat-based diets compared to maize-based diet. Insoluble NSP digestibility was linearly decreased with age regardless of diet type, but the soluble NSP digestibility in the wheat-based diet showed a linear increase with age.

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Ileal morphology and jejunal gene expression of broilers fed meat and bone meal, phytase and antibiotics during necrotic enteritis challenge

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Poultry diets contain a substantial amount of carbohydrates, primarily in the form of non-starch polysaccharides (Choct, 1997). As chickens do not have endogenous enzymes to hydrolyse these carbohydrates, their digestion occurs via bacterial fermentation which may change with age (Montagne et al., 2003). However, the effect of age on NSP has yet to be determined.

A total of 672 one-day-old male Ross 308 chicks were weighed and randomly assigned to 8 dietary treatments with 6 pens/treatment and 14 birds per pen. A 2 x 2 x 2 factorial arrangement of treatments was employed with a completely randomized design. Factors were: MBM (0 g/kg or 60g/kg in starter (S) 50g/kg in grower and finisher (G/F); AB (0 or 100 mg/kg zinc bacitracin in S/G and 50 mg/kg in F plus 60 mg//kg salinomycin; and phytase (500 or 1500 FTU/kg; Quantum Blue™, AB Vista Feed Ingredients, UK). On d 28, jejunal and ileal tissues were collected from 2 birds per pen for the determination of morphology and gene expression respectively. Ileal weight was measured from 2 birds per pen on d 28. The fixed ileal tissues were processed and embedded in paraffin wax for histological analysis. Expression of mRNA for genes was measured on following the gene expression assay described by Cowieson et al. (2018).

Muscularis layer was thicker with low vs high phytase (208.4 vs 242.3 μm, $P > 0.05$) and crypt depth was greater with none vs those fed diets with AB added (275.8 vs 246.2 μm, $P > 0.05$). Ileal weight was greater in birds not fed AB compared to those consuming diets with AB. The expression of calbindin was higher in birds not fed AB compared to their counterparts administrered with AB (1.67 vs 1.11, $P > 0.05$). However, mucin 2 expression was greater in birds fed diets with AB compared to those not fed AB (1.28 vs 1.02, $P > 0.05$). A phytase by AB interaction was observed for villi length ($P < 0.05$). Villi height was increased in birds fed high phytase with no-antibiotics as compared to the high phytase and antibiotics group. A phytase by AB interaction was detected for villi surface area ($P < 0.05$). Villus surface area decreased in birds fed low phytase without AB compared to their counterparts on low phytase with AB. A 3-way interaction was detected for apical width ($P < 0.05$). The MBM, low phytase and AB group recorded the highest apical width compared to MBM, low and no-AB group which recorded the lowest.

In conclusion, this study suggests that AB and high phytase improved mucosa health and would be beneficial to birds fed diets containing MBM.

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