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Executive Summary

This Poultry CRC project intended to establish the principle reasons for the performance gap between free-range and conventionally reared broilers and layers and evaluate a range of nutritional interventions that would reduce the magnitude of the effect of this on production. Six studies (five broiler and one layer) were conducted within this project. In the first instance the project looked at explaining the contribution of two factors that are the core of free range production in Australia: absence of in-feed antibiotics and exposure to range from d 21 onwards. Alkane analysis was used to measure grass consumption and was estimated at 13.5-14.7% of total "as fed" intake by free range broilers which equates to 6.34-6.78 g of grass per bird per hour of range access.Taking into consideration grass consumption, total feed intake increased significantly by about 4.1% ($P < 0.05$) on an "as fed' basis and FCR which showed a 8-10 point increase ($P = 0.07$) for broilers on range.

Nutrient analysis of fresh grass (Kikuyu, *Pennisetum clandestinum)* showed high levels of potassium (K) (crude protein: 16.8 %, K: 2.7% dry matter (DM) basis). Buchanan et al. (2007) reported that, apparent metabolisable energy (AME) levels of pasture forages are very low for poultry (AME: 1.53 MJ/kg). Implications of grass consumption were studied at different levels of consumption, both for energy density and dietary electrolyte balance (DEB).

The effect of different levels of grass consumption was studied along with provision of two different diet densities in free range broilers. A significant improvement in weight gain, FCR and the apparent ileal digestibility of dry matter, energy and nitrogen was observed with 2-4 % grass inclusion, but, significantly poorer performance and digestibility thereafter. Provision of a diet with a higher energy density improved performance at low concentrations of grass only, resulting in a significant grass*energy interaction for most parameters.

Broilers fed diets with combinations of two different dietary electrolyte balance (DEB) levels and standard and high energy densities indicated that low DEB level and high energy density diets showed a significant effect on weight gain ($P < 0.001$) and improved FCR, dry matter digestibility and digestible dry matter content ($P < 0.01$). Lower levels of DEB were also seen to improve plasma levels of Na, Cl and Ca. High density diets however, increased blood pH by 0.24 and caused depletion of K from the optimal 5mmol/L, both indicative of respiratory alkalosis. Overall, a lower DEB and high density (HD) diet significantly improved performance and digestibility parameters in this trial, indicating it to be a good strategy to address the consequences of grass consumption on range especially in hot summer conditions .

Free range broilers were provided free choice feeding of whole wheat grains with an aim to determine if it will stimulate development of the digestive tract and improve the digestive capabilities of the bird while allowing it to better cope with heat stress. Despite the relatively low intake levels of whole grains in the diet throughout the trial, there was still a substantial dilution effect of the whole grains on the ileal digestible energy and protein. However, despite the overall lower feed intake, and lower digestibility , birds were able to maintain a comparable live weight compared to controls. The improved FCR of birds fed whole grains, may have occured due to either an improvement in the utilisation of nutrients or a decrease in the energy use for non-growth functions such as heat dissipation. However, free choice feeding may not have been the best option for birds to consume a sufficient amount of whole grain wheat to stimulate development of the digestive tract of the bird.

Inclusion of Zinc Bacitracin or mannan-rich fraction of carbohydrates (Actigen®; Alltech Australia, Melbourne) in the standard free range (control) diets improved growth rates in both range access and non-range access birds. Actigen® provided similar growth to an antibiotic

feed additive and superior weight gain to a control diet under very good health conditions. Actigen® provided effects on nutrient digestibility that were not as significant as the antibiotic feed additive but were superior to the control diet. Range access showed lower protein digestibility in birds as compared to those with no range access.

The laying hens study undertaken within this project aimed to characterise populations based on the duration and frequency of visits to the range and evaluate the differences in gut characteristics and digestibility of nutrients between the non-users versus range users.It was found that increasing range access was a promising strategy to improve gut characteristics and digestibility of free-range layers, but the type of benefit may depend on the nature of range usage by the birds.

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Introduction

For the first time, since introduction of cage and barn-laid egg production, the retail turnover of free-range eggs (47%) exceeded that of cage (39%), barn laid (11%) and organic eggs (3%) (AECL, 2013). Given that some of the major Australian retailers are phasing out caged-egg sales by 2018, there is little doubt that this trend is set to continue well into the future. Furthermore, the demand for free-range meat chicken has grown significantly to approximately 15% of the total market, with a value of approximately $840 million (ACMF, 2011). A major reason for this changing market demographic is increased demand by the public for poultry products produced in less intensive production systems. These production systems are perceived to be superior for bird welfare, product quality and food safety.

However, free-range production in Australia is associated with poorer bird performance, poorer feed conversion efficiency and higher mortality compared to conventional production systems (Durali et al. (2012). Free-range broiler production accounts for between 15-20% of poultry production in Australia. Analysis of data from a preliminary study on several commercial farms had revealed that there is a 10-12 points increase ($P<0.05$) in FCR in free-range broilers compared with their conventional counterparts at the same location (Durali et al. (2012). Moreover, free-range production was associated with higher ($P<0.05$) mortality than conventional systems (5-6% in free-range compared with 3-4% in conventional). A similar response was reported with a 20-30 points increase in FCR and 2-3% increase in mortality in layers grown in free-range system as compared to cage or barn system (Anderson, 2009; Nagle et al., 2006). Discussions with free-range farmers have also revealed other problems such as higher energy requirements, less hen house eggs, less hen day eggs, shorter production peaks, shorter flock longevity, less weight uniformity and higher hierarchical behaviour when compared to their conventional counterparts.

Putatively, antibiotic growth promoters (AGPs) improve FCR of market-age broilers by three per cent, equivalent to five or six points in FCR (Rosen, 1995; Rosen, 2004; Hruby and Remus, 2007). Diseases of poultry are a major cause of mortality and loss of production occurs in the absence of AGP.

Management issues related to production system have shown to play important roles in the poor performance of free-range birds. Nutritional deficiencies are particularly important and common due to the often poor and unbalanced diet of free-range birds (dilution of complete diets with grass etc.), which may have significant negative effects on growth and disease resistance. Thus, there is a need to improve the current free-range practices for more efficient and profitable production and an important prerequisite is to identify where the performance gap originates.

This project aimed to identify and describe the reasons for the reduced performance in a free-range production systems and look into strategies for improvement in performance. In the first instance the project looked at delineating the contribution of two factors that are the core of free range broiler production in Australia: absence of in-feed antibiotics and exposure to range from d 21 onwards. Diets intended for consumption by free-range broilers are not routinely formulated to accommodate the modifying effects of grass consumption on digestible nutrient intake. Buchanan et al. (2007) reported that, apparent metabolisable energy (AME) levels of pasture forages are very low for poultry (AME: 1.53 MJ/kg). Formulation of standard diet with incremental addition of grass at 2%, diluted apparent metabolisable energy (AME) by around 0.21MJ/kg for every 2% grass consumed. Furthermore, grass contains a high potassium concentration (*2.7% on DM basis*) and its consumption increases DEB by around 20mEq/kg for every 2% grass consumed. The implications of these changes in dietary nutrient supply were explored in subsequent trials.

Another nutritional strategy to improve the performance of free range broilers were ensued by investigating free choice feeding of whole grain to determine the effect on development of the digestive tract and digestive capabilities. The use of whole grain to mitigate the effects of heat stress in hot summer months was also investigated.

Commercially available product with mannan rich fraction of carbohydrate was used to see the effect on performance of free range vs conventionally produced birds while its efficacy as a substitute for AGP was also investigated.

Another component of the project included looking into the free-range layer production systems. Sub-populations of hens, those that use the free-range on a regular basis and those that rarely/never use the range were identified. Differences in growth rates, gut characteristics and digestibility were assesed with an aim to generate information in order to formulate diets for this multimodal population.

As demand for free-range poultry products grows rapidly and as free-range eggs and chicken meat become increasingly mainstream, the premium unit prices on these products will decline, exerting cost pressures up-stream. Therefore, It is likely that free-range production, in order to remain cost-effective and sustainable, will need to address the issues that contribute to the performance gap compared with more conventional intensive production systems.

Objectives

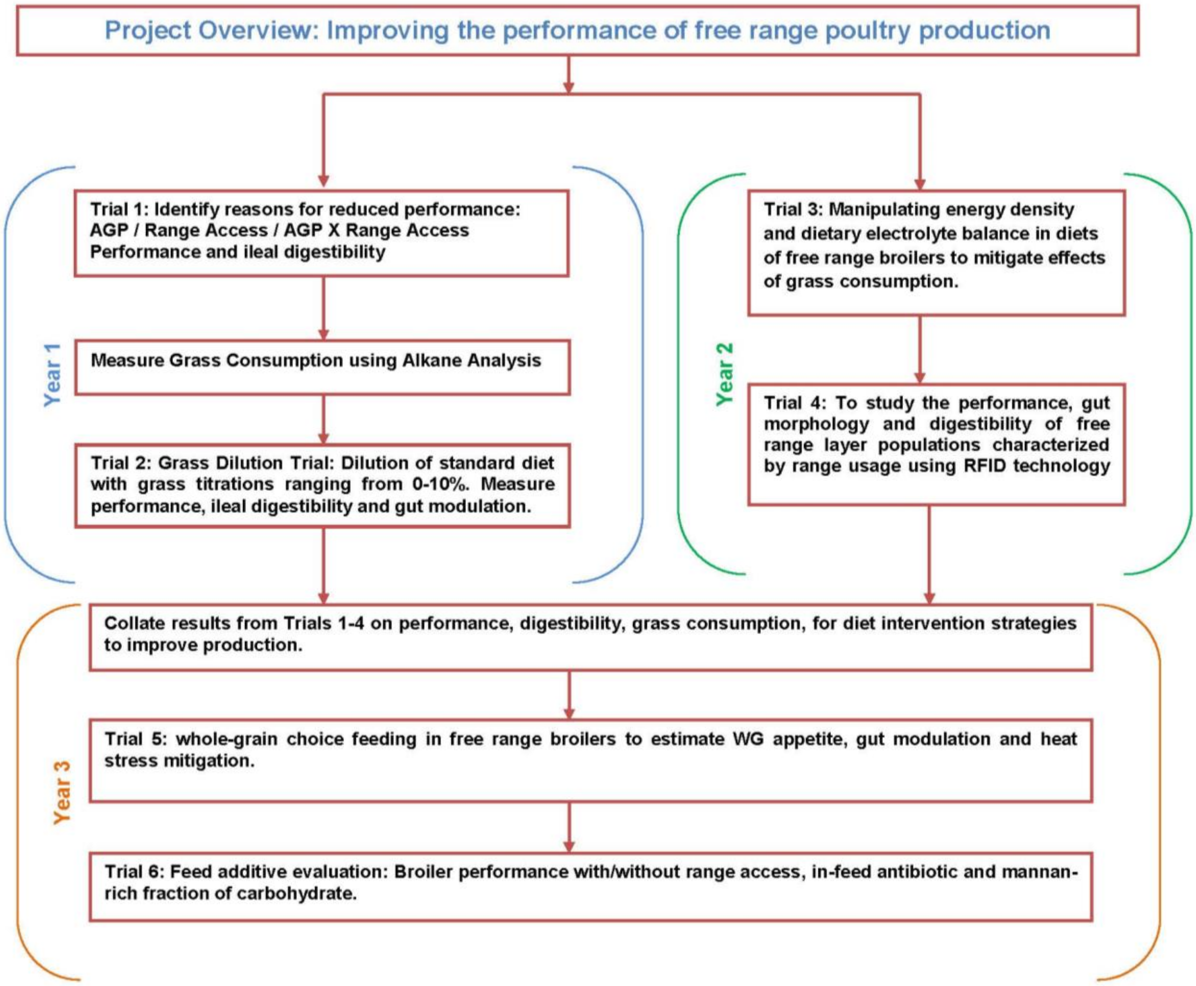
This project had two main objectives:

1. Establish the principle reasons for the performance gap between free-range and conventionally reared broilers and layers.

2. Evaluate a range of nutritional interventions that will reduce the magnitude of the performance gap.

Experimental outline

A total of six trials were conducted in this project. All except Trial 4 were conducted on broilers at the Poultry Research Unit of University of Sydney. Trial 4 was conducted on laying hens in collaboration with University of New England, Armidale at the free range facilities based in CSIRO, Chiswick.
The project was organised over three years as below:



Year 1. Describe, define and identify reasons for reduced performance in free-range broilers and layers.

Trial 1 (broiler)

A 2x2 factorial arrangement of treatments was used to study the effects of dietary AGP supplementation(diets with vs. without), production systems (free-range vs. conventional) and their interactions. Performance parameters (weight gain, feed intake, FCR and mortality) were measured.

Grass consumption was quantified in birds and its effect on performance, growth measures and ileal nutrient digestibility evaluated. The work on grass consumption was done in collaboration with the Dairy Research Foundation based at University of Sydney using alkane analysis methodology.

Trial 2 (broiler)

A 2 x 6 factorial arrangement was used where standard (12.8 MJ/kg) and high energy (13.20 MJ/kg) density broiler diets were systematically diluted with 2, 4, 6, 8 and 10% grass. The aim of the study was to investigate the effects of different levels of grass dilution and implications of using a high energy density diet to mitigate these effects. Performance parameters (weight gain, feed intake and FCR), gut characteristics and nutrient digestibility were measured.

Year 2. Determining effects of free-range production system

Trial 3 (broiler)

Grass contains a high concentrations of potassium and by formulating diets with incremental grass inclusion showed increase in DEB by around 20mEq/kg for every 2% grass consumed. A 2 x 2 factorial design using DEB (at 180 mEq/kg and 220 mEq/kg) and two density diets, standard (12.78 MJ/kg) and high density (13.2 MJ/kg) was used to study their effects and interactions. Performance parameters (weight gain, feed intake, FCR), nutrient digestibility, plasma electrolyte levels and thermal profiles were studied.

Trial 4 (layer)

In collaboration with UNE, Armidale and CSIRO Chiswick, RFID technology was used to characterize sub-populations of free range birds based on their range usage; i.e. birds that used the range 1. with less number of visits but longer duration per visit, 2. high number of visits but shorter durations per visit and 3. birds that did not use the range at all. Performance (individual weight, egg production and feed intake) and gut characteristics were studied for 12 birds in each of the contrasting sub-populations.

Year 3. Developing nutrition based strategies for improving free-range performance

Trial 5 (broiler)

Provision of whole grains in the diet has been found to improve performance of birds through physical and functional mechanisms as a result of improvements in the development of the digestive tract. Moreover,it was hypothesised that by providing a feed source that was lower in energy and protein and thus had lower heat increment than pellets, may allow birds to cope with heat stress in hot summer months. Birds were randomly allocated to one of two treatment groups; birds fed complete pelleted diet (**P**), and birds fed pellets and choice fed whole grain wheat (**WG**) and given access to range at 21 days of age. Performance parameters (bird weights, feed intake, whole grain intake, FCR), gut characteristics, nutrient digestibility and blood parameters were measured.

Trial 6 (broiler):

The earlier trial (Trial 1) conducted to decipher the impact of in-feed antibiotics and range access did not result in a conclusive inference. It was therefore considered appropriate to revisit that aspect of the project. In addition to that, the addition of mannan-rich fraction of carbohydrate was tested for ability to compensate for the absence of in-feed antibiotics in free range broiler production systems. A 2 x 2 x 2 factorial arrangement consisted of diets with or without AGP, and with or without mannan-rich fraction of carbohydrate for birds with or without access to range. Performance parameters (weight gain, feed intake, FCR), and nutrient digestibility was measured.

General Methodology

All broiler experiments conducted in this project were in accordance with the University of Sydney Animal Ethics Committee while the laying hens trial (Trial 4) was conducted in accordance with the CSIRO Chiswick Animal Ethics Committee, and the Australian code for the care and use of animals for scientific purposes (National Health and Medical Research Council 2004).

Methods commonly used in the project are described below:

Animals and Husbandry

For all broiler trials (except Trial 6, where mixed sex Ross 308 were used), day-old Cobb 500 male chicks were obtained from a commercial hatchery, weighed at one day old and randomly allocated to pens/cages. Feed and water was provided *ad lib* using appropriate feeders and drinkers (feed troughs and nipple lines for cages and gravity pan feeders and nipple lines in floor pens).

Broilers were kept at a temperature of 31 °C for days 1-4 and thereafter this was reduced by 0.5 °C/day to 24 °C. The lighting regime for the study consisted of 23L:1D for the first 4 days and then 18L:6D for the remainder of the experiment.

Diets

All experimental diets met the National Research Council (1994) requirements. An indigestible marker (acid insoluble ash; AIA) (Celite 281, Filchem Australia Pty Ltd, Castle Hill, NSW, Australia) was added to diets at a concentration of 20 g/kg.

Performance parameters

Body weights (BW) and feed intake(FI) were recorded on a weekly basis either on a pen or individual basis, depending on the trial design. Body weight gain (BWG), average feed intake (FI) and feed conversion ratio (FCR) were determined for each treatment group in all trials.

Sample collection

At the end of trial, birds to be sampled were euthanised with an intravenous injection of a diluted sodium pentobarbitone solution (Lethabarb, Virbac Australia Pty Ltd, Milperra, NSW, Australia). The contents of the lower ileum were collected and pooled for each pen/cage, immediately frozen and freeze-dried thereafter according to the methods of Ravindran *et al*. (2005). Diet samples were collected for all treatment groups at three different time points in the feed pelleting or mixing process. Grass samples were collected from different sites in the range area, pooled and the representative sample freeze-dried and ground for further analyses.

Chemical analyses

The acid insoluble ash (AIA component) of diets and ileal digesta samples were determined according to the method of Siriwan *et al*. (1993).

The GE of diets and excreta were determined using a Parr 1281 adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA) that was standardised with benzoic acid.

Nitrogen concentration of samples was determined by the Dumas method using a FP-428 N analyser (LECO Corporation, St Joseph, MI, USA) as described by Sweeney (1989).

The diets and digesta were also analysed for the minerals (P, Ca, Cu, K, Mg, Mn, Na, Sr, Fe and Zn). Samples were wet acid digested using nitric acid and hydrogen peroxide before the determination of mineral concentration by Inductively Coupled Plasma-Optical Emission Spectroscopy using a Perkin Elmer OPTIMA7300 (Perkin Elmer Inc., Waltham, MA, USA).

ADF was determined by the method of Goering & Van Soest (1970). NDF was determined by the method of Robertson & Van Soest (1981).

Calculation of coefficient of apparent Ileal digestibility (CAID) of nutrients

The CAID of DM, energy, N, amino acids and minerals were calculated as per Ravindran *et al*. (2005) as below:

Coefficient of apparent ileal nutrient digestibilities was calculated as follows:

$$\text{Nutrient digestibility coefficient} = \frac{(NT / AIA)_d - (NT / AIA)_i}{(NT / AIA)_d}$$

Where, $(NT / AIA)_d$ = ratio of nutrient to acid-insoluble ash in the diet,
and $(NT / AIA)_i$ = ratio of nutrient to acid-insoluble ash in ileal digesta.

Ileal digestible energy was calculated by the following formula.

IDE (MJ/kg DM) = Coefficient of Ileal digestible energy x GE of the diet

Statistical Analyses

All data were exported to JMP v9.0 (SAS Institute, Cary, NC, USA) and subjected to analysis of variance using the general linear models procedure. Means were considered significant at $P<0.05$ and were separated by LSMeans Differences Tukey HSD, where, levels not connected by same letter were significantly different.

Chapter 1. Effect of range access, antibiotic growth promoter, and grass (*P.clandestinum*) consumption on performance and digestibility of free range broilers

Introduction

Free range broiler production is growing rapidly in Australia. In 2006 free-range broiler production accounted for 4% of total broiler production and today it is around 15% (ACMF, 2011). Commercial free-range broiler production is associated with poorer growth rate, higher feed conversion and higher mortality compared with conventional broiler production. Weeks et al., (1994) demonstrated that free range broilers had significantly lighter body weight (4.08 ± 0.08kg) than conventionally reared broilers (4.49 ± 0.08kg) at ten weeks of age. This performance gap has been observed in a long term commercial comparison study as a 2-3% increase in mortality, 10-15 points increase in feed conversion ratio and a retardation in growth rate in that birds took an extra 2.5 days to reach a 2.45 kg target body weight (Durali et al.). This 'performance gap' may be as a result of the absence of in-feed antibiotics (AGP), poorer digestive health, gastrointestinal disease challenge, nutritional inadequacy due to unpredictable consumption of pasture and insects and increased activity.

The positive effects of antibiotic supplementation to diet has been well documented since their introduction in the 1950's (Moore et al., 1946; Couch and Reed, 1950; Whitehill et al., 1950; Elam et al., 1951a; Elam et al., 1951b). Most of the in-feed antibiotics have two common features; they are mostly active against Gram positive bacteria and their absorption from the gastro intestinal tract is limited Feighner and Dashkevicz (1987). The mechanisms of AGP action vary for different groups of antibiotics (Ferket, 2004). They may interfere with cellular metabolism of microorganism, bacterial cell wall building and maintenance or protein transformation at ribosomal (Ferket, 2004). As a result, antibiotics appear to limit proliferation of bacteria and thus permit the host to perform more closely to their genetic potential.

Free range broilers and layers consume unknown amounts of pasture with obscure nutritional value and this may influence performance. Moreover, parameters like FCR and feed intake may still be under-represented considering free range birds also have access to supplementary feed sources on range which are not systematically considered. The foraging activity and variable environmental conditions on range make it hard to apply the nutritional management guidelines recommended for intensive birds (Glatz et al., 2006). Although it has been established by observation studies that chickens eat grass while on range (Glatz et al., 2005; Miao et al., 2005), there have been minimal attempts to quantify the amount of grass consumed and its effect on performance and digestibility in birds.

One of the most suitable methods is to use n-alkanes (Dove and Mayes, 1991) to estimate diet composition and intake. If grass consumption can be quantified accurately then an important outcome would be to provide birds with feed that would compensate for the nutritional imbalances caused. This study attempts to identify effect of range access and unknown pasture consumption on bird performance fed with diet with and without in-feed antibiotic. The study also evaluates the use of alkanes as internal markers for estimating the intake of Kikuyu grass (*P. clandestinum*) in free range broilers. The impact of grass consumption on performance was also assessed.

Materials and Methods

A total of 1440 Cobb 500 male broiler chicks were allocated to one of four treatments with twelve replicates in a 2x2 full factorial arrangement, the factors being conventional or free-range production system and a diet with (AGP+) or without (AGP-) the in-feed antibiotic Zinc Bacitracin (source: Albac G 150 antibiotic feed premix, Pfizer Australia Pty Ltd) included at 50 g/tonne of feed.

Day old chicks received a numbered wing tag at placement and were randomly allocated to 48 pens (4.5 m^2 each with 30birds/pen and a density of 6 birds/m^2). with ad libitum access to feed and water in a tunnel ventilated shed. The pen floor was covered with 75mm pine wood chip. Broilers were kept at a temperature of 31°C from d 1-4 and thereafter this was reduced by 0.5°C/day to 24°C. Range temperatures varied from 7-15°C minimum and 20-25°C maximum during the duration of the trial.

While chicks were assigned to treatment diets on d1, free range access was available to birds only from d 21 onwards as required by Free-range Egg & Poultry Australia Ltd., (FREPA). FREPA are responsible for setting standards of production and quality for free-range poultry production in Australia and their standards require that when fully feathered, birds must have easy access to an area on which to range during daylight hours). Twenty four outdoor pens (with an area of 4.5 m^2 each) were setup on range (Figure1) with half of the pen covered with shade cloth that could be moved over the pen depending on where it was needed during range access.

Figure 1. Outdoor pens setup for allowing assignment of range access from d 21 onwards

Ten percent of birds in the free range treatment were chosen randomly and manually assigned to the range at 9 AM, from d 21-28 for 2 hours. The number was increased to 20% birds from d 28-35 and to 30% from d 35-42 for three hours (all chosen randomly) on range.

The number of birds chosen per pen and the duration of range access were representative of the preference by broilers on a commercial free range farm (pers. comm., Durali, 2012). The range had a homogenous growth of young Kikuyu grass (*P. clandestinum*) as the main herbage and was mowed to about 6 cm before assigning the birds and not irrigated during the trial. Body weights and feed intake were recorded at weekly intervals on a pen basis, during the 42-day trial, and FCR calculated (corrected for mortality).

Diets

Wheat-sorghum based starter, grower and finisher diets were formulated as shown in Table1. Zinc Bacitracin (source: Albac G 150 antibiotic feed premix, Pfizer Australia Pty Ltd) was added at 50g for every tonne of feed for the treatment diets. Feed was allocated to pens on the basis of 0.7kg starter feed, 1.2 kg grower feed and ad libitum finisher feed per bird.

Table 1. Ingredient composition (%, as fed) and calculated nutrients of starter,grower and finisher diets and grass

Ingredient Composition (% , as fed)	**Starter**	**Grower**	**Finisher**	**Grass (** *P. clandestinum)*
Whole Wheat12.5%12.8MJ	0	15	15	
Wheat12.5%12.8MJ	46.08	28.42	30.74	
Sorghum 10.5%	21.15	28.27	28.08	
Meat/bone meal	6.82	7.1	5.41	
Soybean meal 48	16.87	5.32	3.77	
Canola meal (solvent extracted)	4	10	10	
Sunflower oil	1.21	2	2.99	
Limestone	0.25	0.17	0.32	
Salt	0.03	0	0	
Sodium Bicarbonate	0.51	0.48	0.48	
Lysine HCl	0.37	0.48	0.48	
DL Methionine	0.3	0.28	0.27	
Threonine	0.11	0.19	0.17	
Choline chloride 60	0.07	0.06	0.06	
Xylanase	0.02	0.02	0.02	
Vitamin/mineral premix*	2.2	2.2	2.2	
Phytase 500FTU	0.01	0.01	0.01	
Calculated nutrients				Expressed on Dry Matter basis (except DM %)
DM %	89.34	89.46	89.46	17.00
Poult AME (MJ/kg)	12.5	12.9	13.2	1.53**
Crude protein %	22.3	19.5	18.0	16.80
Calcium %	0.9	0.9	0.8	0.42
Avail Phos %	0.47	0.47	0.39	0.28
Lys%	1.32	1.18	1.10	0.58
Met %	0.63	0.58	0.54	0.19
Me+Cys %	0.98	0.90	0.85	0.32
Na %	0.22	0.20	0.19	0.04
Cl %	0.21	0.21	0.20	
K%	0.71	0.56	0.52	2.90
DEB (mEq/kg)	214	210	210	

**Supplied per kg of diet: retinol, 3,600 µg; cholecalciferol, 125 µg; α-tocopherol, 50 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 5 mg; cobalamin, 25 µg; niacin, 50 mg; pantothenic acid, 18 mg; folic acid, 2 mg; biotin, 200 µg; Cu, 20 mg; Fe, 40 mg; Mn, 110 mg; Co, 250 µg; I, 1 mg; Mo, 2 mg; Zn, 90 mg; Se, 300 µg; ethoxyquin, 125 mg.*
**Assumed Poult AME (MJ/kg) values for *P. clandestinum based on Buchanan et al. (2007).*

Alkane Analysis

Litter samples which consisted of woodchip along with faeces were collected on d 42 from each pen using the "coning and quartering" technique (IUPAC 1997) to represent an even distribution of the representative excreta. Grass, diet pellets and clean wood chip samples were also collected on d 42, and were dried to a constant weight in a freeze drier and ground through a 0.5 mm screen Cyclone mill.

Alkane concentration in grass, diet pellets, woodchip and litter samples was determined using a modification of Mayes *et al.* (1986) methodology and gas chromatography. Duplicate samples of litter (1.2 g), grass (1.5 g), diet pellets (1.5 g) and woodchip (1.5 g) were placed in a screw-top McCartney bottle (20ml) and weighed prior to the addition of 0.2 g of internal standard (2.5 mg of C34 (Tetratriacontane) / g of Undecane) and 10 mL of 1.5M KOH. Bottle contents were mixed thoroughly and placed in a water bath (90°C) for 3 h with thorough mixing every 30 min. After cooling of samples, 8.0 mL of heptane and 5.0 mL of distilled water were added to the bottle contents and mixed thoroughly and placed again on the water bath until just boiling. The top organic phase was removed with a pastured pipette and transferred to a scintillation vial. The process was repeated with 5 mL of heptane. The contents were evaporated to dryness and re-dissolved in 2ml of Heptane, and applied to a column consisting of silica gel contained in disposable pipette tips stoppered with glass wool. The column was eluted with approximately 10 mL of heptane in five steps of 2ml each. The eluent was again evaporated to dryness and re-dissolved in 0.8 mL of heptane before injection into the capillary column in a gas chromatograph GC-2010 equipped with mass spectrometer GCMS-QP 2010 PLUS (Shimadzu, Australia) with an ultra-low bleed, fused silica capillary column (Rxi-1ms; Crossbond, 100% dimethyl polysiloxane; 30m × 0.25mm × 0.25um film thickness). Helium was used as a carrier gas at flow rate of 0.60 mL/min, while the column oven temperature was programmed from 200ºC held for 2 min, then increased from 200 to 330ºC at 10ºC/min and held for 15 minutes. The ion source and the interface temperature of the mass spectrometer were 250 and 275ºC, respectively. The inlet was operated at 280ºC in splitless injection with a split ratio of 1:20.

Calculations

The identity of even and odd chain alkanes (C25 to C33) was determined from their retention times relative to the known standard. The area under the peak for each alkane was determined using an integrator (Model 3393A, Hewlett Packard), and peak areas were converted to amounts of alkane by reference to the internal standard C34.

Recovery of each alkane was used to calculate the proportion of the ingested ingredient, which was recovered in excreta. Diet proportions were estimated using a non-negative least squares procedure in the software “EatWhat” (Dove and Moore, 1995). In this study, only five odd chained alkanes (C25, C27, C29, C31, and C33) that were found in traceable concentrations were used for diet proportion estimates. On initial estimation of diet components from a litter sample, no grass was detected, woodchip was found to be the biggest component followed by diet pellets. Even though woodchip was not abundant in its higher order alkane concentrations, the large portion of wood chip in the litter sample was thought to be causing a cumulative effect and masking the grass content in the diet composition. However, since the portion of woodchip in the litter sample would confound its use as a diet component it was necessary to eliminate it in the final analysis. It has been reported earlier that even though all intake components are not listed, it does not affect the estimation of other individual intake components (Ferreira et al., 2005). Correction to the estimate was performed by subtracting the contribution of woodchip thus allowing diet pellets and grass as the only two intake components.

Other corrections that had to be made to accurately predict the intake of grass was to correct the faecal concentrations to represent range access for all birds in a pen and correction for their relative recovery rate based on data from Hameleers et al. (1996) . Grass intake on an hourly basis was calculated in relation to total feed intake and calculated on an “as fed” basis.

Feed intake and FCR were calculated and then corrected by adding the calculated grass consumption for d 21-42. Digestibility of nutrients was evaluated using chemical analysis and calculations as listed in the general methodology section of the report. Performance and alkane data were analysed using JMP 9.0, 2010. ANOVA was conducted to evaluate the system and diet effects on performance as well as effect of individual alkanes on faecal recovery rates. In all cases, significance was set at $P < 0.05$.

Results and Discussion

Performance

Over the 42 d trial, a significant System x Diet interaction was seen for average body weight (BW) ($P < 0.05$) and body weight gain (BWG) ($P < 0.05$) with birds in conventional system with diet containing AGP showing higher values than birds in other treatment groups (Table2).

Table 2. Effect of different production systems (conventional / free range) and antibiotic growth promoter (AGP) status (+ or -) on performance of birds 42 days after hatch.

System	Diet	BW	FI	BWG	FCR
Conventional	AGP +	1762.5^{a}	3655.5	1716.2^{a}	2.13
Conventional	AGP **-**	1691.1^{b}	3618.1	1644.6^{b}	2.20
Free Range	AGP +	1576.8^{c}	3198.1	1530.2^{c}	2.09
Free Range	AGP **-**	1567.2^{c}	3225.0	1521.2^{c}	2.12
Pooled SEM		16.06	72.09	12.1	0.02
P-value					
System		***	***	***	**
Diet		**	NS	**	*
System x Diet		*	NS	*	NS

*BW=bodyweight (g); BWG=bodyweight gain (g); FI=feed intake (g); FCR=feed conversion ratio; Means in columns with no common superscript are significantly different. * (P<0.05),** (P<0.01), ***(P<0.001).*

Looking at the main effects of System and Diet, based on the LSD, birds in free range system consumed almost 6% less feed than birds on the conventional system ($P < 0.001$) resulting in lower FCR with the free range system compared with birds reared under the conventional system ($P < 0.01$). FCR in AGP^{+} diets was around 5 points lower as compared to AGP^{-} diet ($P < 0.05$) (Table 1). However, these results did not account for grass consumption on range and therfore both FI and FCR are under-represented. Grass consumtion was quantified and these values modified as shown in later sections in the chapter.

The performance of birds in this trial was sub-optimal, mainly due to error in production of the first batch of grower diet, fed until day 28 which contained an almost 50% lower level of Cl as compared to thelevel calculated for the formula. The reason for the low Cl was not found but maybe have been accidental omission of lysine hydrochloride from the batch. This led to lower final body weights and higher FCR on d42 as compared to Cobb 500 standard (*Cobb 500 male standard at 42 days is LW 2.953kg, FCR 1.691)*. However, the differences between treatments were apparent to draw conclusions on performance parameters in this study.

Digestibility

There was a significant System × Diet interaction for coefficient of apparent ileal digestibility (CAID) of dry matter (DM) ($P < 0.05$), energy ($P < 0.05$) and apparent metabolisable energy (AME), with birds on conventional system and free range diet (AGP-) showing significantly lower coefficients than all other treatment groups (Table 3). Birds on a free range system showed higher CAID for nitrogen(0.80) as compared to those on conventional system (0.76) ($P < 0.05$) as also with birds that ate AGP+ diet (0.80) as compared to AGP- diet (0.74)($P < 0.01$).

Table 3. Effect of different production systems (conventional / free range) and antibiotic growth promoter (AGP) status (+ or -) on digestibility of nutrients 42 days after hatch.

System	Diet	CAID (%)			IDE (MJ/kg DM)
		DM	Energy	Protein (N)	
Conventional	AGP +	0.70^a	0.73^a	0.79	13.34^a
Conventional	AGP -	0.63^b	0.67^b	0.75	12.06^b
Free Range	AGP +	0.69^a	0.73^a	0.80	13.33^a
Free Range	AGP -	0.69^a	0.72^a	0.78	13.10^a
Pooled SEM		0.013	0.013	0.010	0.239
P-value					
System		*	*	*	*
Diet		**	*	**	**
System x Diet		*	*	NS	*

*CAID (Coefficient of Apparent Ileal Digestibility); DM (Dry Matter); N (Nitrogen), IDE(Ileal digestible Energy); Means in columns with no common superscript are significantly different. * (P<0.05),** (P<0.01).*

Alkane profile of diet components

The GC/MS profile of alkane patterns for diet pellets, woodchip and grass revealed higher peaks for all alkanes in grass followed by diet pellets and with negligible peaks represented in woodchip samples (Figure2).

The alkane profiles of the diet components showed that the predominant alkanes recovered were the odd chain alkanes C25, C27, C29, C31 and C33 while alkanes with even-numbered chain lengths had little appreciable concentration in the intake components.

Alkanes C31 (27.77 mg/kg DM) and C33 (21.12 mg/kg DM) showed up in higher concentrations as compared to any other alkanes in grass (Table 4).

Table 4. Concentrations of n-alkanes in intake components as determined by converting area under the peak to amounts of alkane by reference to the internal standard C34.

Intake component	Chain Length of *n*-alkane (mg/kg DM)						
	C25	C27	C28	C29	C31	C32	C33
Grass	4.37	5.80	0.16	6.30	27.77	0.95	21.12
Wood chip	0.02	0.20	0.25	0.25	0.06	0.11	0.10
AGP^+ pellet	1.06	1.36	0.25	1.40	0.314	0.1	0.12
AGP^- pellet	1.01	1.28	0.24	1.26	0.318	0.09	0.13

Woodchip showed the lowest amount of alkanes ranging from 0.028 mg/kg DM of C25 to 0.256 mg/kg DM of C29 recovered from it, whereas the two diet pellets showed higher concentrations of C25 (1.013 and 1.069 mg/kg DM), C27 (1.283 and 1.360 mg/kg DM), and C29 (1.260 and 1.407 mg/kg DM) (Table 4).

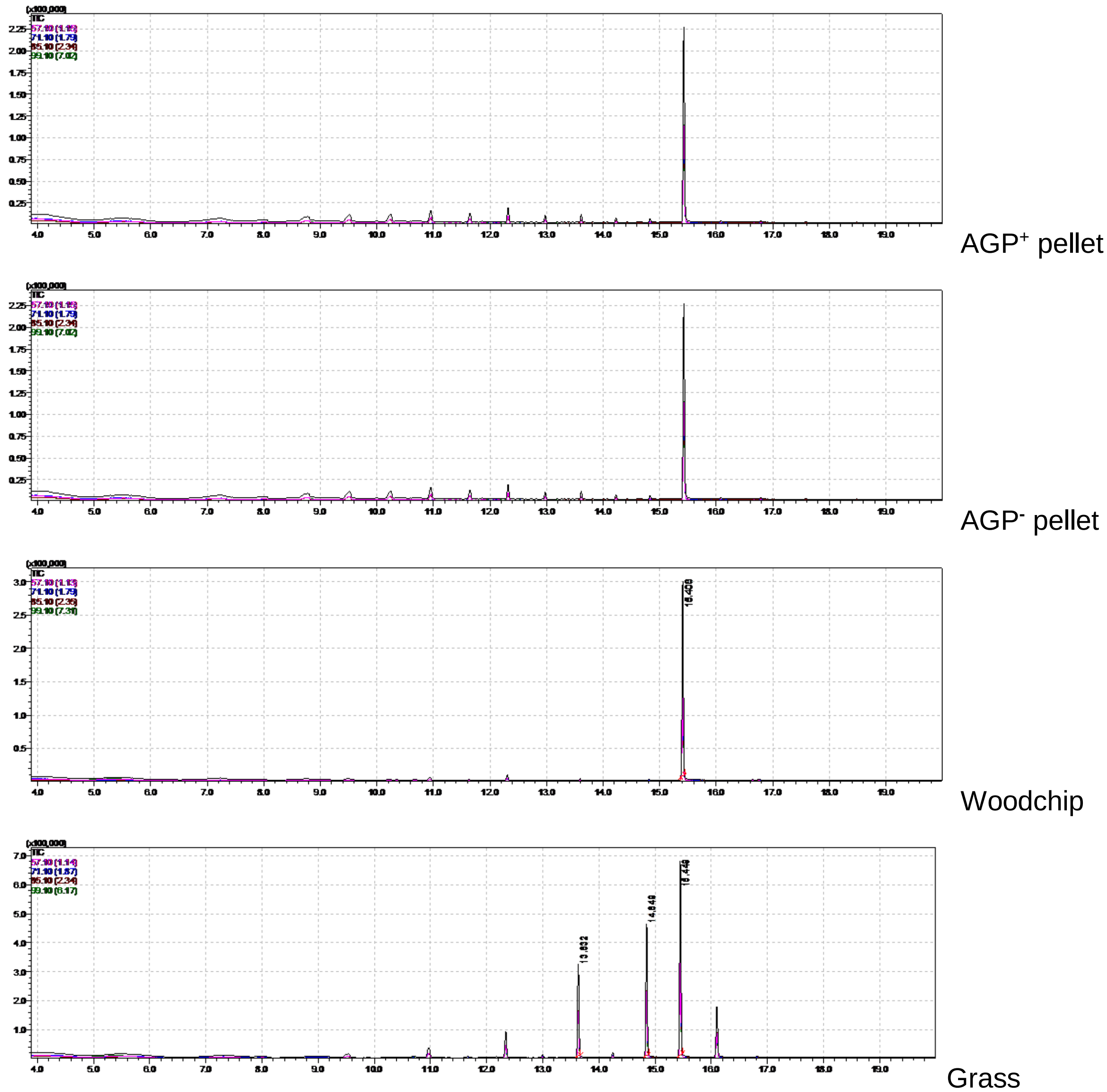


Figure 2. The GC/MS profile of alkane patterns for diet pellets, woodchip and grass used to estimate alkane concentrations of intake components. Higher peaks for all n-alkanes were seen in grass.

Faecal concentrations of n-alkanes

The n-alkane faecal concentrations of litter samples from each treatment group showed a rise in C31 and C33 alkanes in birds on a free range system (and with access to grass) with either AGP+ (2.20 $\pm$ 0.11 mg/kg DM and 1.18$\pm$ 0.05 mg/kg DM) or AGP^{-}(2.14 $\pm$ 0.11 mg/kg and 1.06 DM$\pm$ 0.05 mg/kg DM) as compared to the conventional system (with no access to grass) with either AGP+ (0.51 $\pm$ 0.11 mg/kg DM and 0.24 $\pm$ 0.05 mg/kg DM) or AGP^{-} (0.55 $\pm$ 0.11 mg/kg DM and 0.29 $\pm$ 0.05 mg/kg DM)(Table 5).

Table 5. Faecal concentrations (mg/kg DM) of n-alkanes in different treatment groups

System	Diet	n-alkane (mg/kg DM)						
		C25	**C27**	**C28**	**C29**	**C31**	**C32**	**C33**
Conventional	AGP +	2.18	2.36	2.5	2.29	0.51^{b}	0.22^{b}	0.24^{b}
Conventional	AGP -	2.61	2.67	2.43	2.27	0.55^{b}	0.34^{a}	0.29^{b}
Free Range	AGP +	2.35	2.56	2.44	2.41	2.2^{a}	0.31^{ab}	1.18^{a}
Free Range	AGP -	2.5	2.51	2.17	2.4	2.14^{a}	0.3^{ab}	1.06^{a}
Pooled s.e.m.		0.18	0.12	0.24	0.13	0.11	0.04	0.05
P-value								
System		NS	NS	NS	NS	***	NS	***
Diet		NS	NS	NS	NS	NS	NS	NS
System x Diet		NS	NS	NS	NS	NS	NS	NS
Faecal recovery in chickens (Hameleers et al., 1996)		0.44	0.55	-	0.67	0.71	-	0.75

*Means in columns with no common superscript are significantly different. * (P<0.05),** (P<0.01), ***(P<0.001).*

On comparing the effects of the two production systems and diets individually, significantly high concentrations of C31 and C33 (P ≤ 0.001) were observed in the free range system as compared to others. No significant difference was seen in alkane recoveries between the two diet pellets (Figure 3).

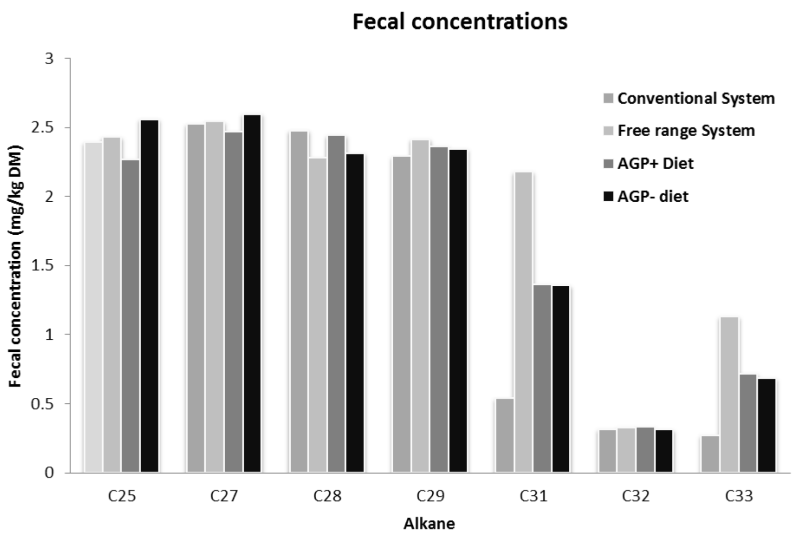


Figure 3. Faecal concentrations (mg/kg DM) for conventional and free range systems and AGP+ and AGP- diets. Significantly high concentrations of C31 and C33 (P ≤ 0.001) were observed in the free range system.

Composition of litter samples using “EATWHAT”

The composition of litter samples for the four treatment groups as determined by “Eat What” based on the alkane profiles showed that woodchip was the biggest component with up to 72% being accounted for in the litter sample and the other 28% representing the excreta composed of either of the two pellet diets and grass.

To obtain a true representation of only the faecal sample in the litter, the woodchip component was removed from the intake component list. Considering that the amount of alkanes contributed by woodchip was minimal, all the alkane quantities were considered to be derived from the faecal component in the litter sample. After correcting the concentration of individual alkanes in excreta, allowing for incomplete recovery based on published values by Hameleers *et al.* (1996) and number of birds per pen, the intake of pellets and grass was established in the faecal sample. Table 5 shows the proportion of ingested ingredients in all twenty four pens as recovered from excreta of birds that had access to grass and the two diet treatments.

No grass was detected in excreta of birds reared under the conventional production system. The mean grass consumption in the two treatments with free range access was 2.3-2.5% of the total feed intake on a DM basis with the rest accounted by the diet pellets (Table 6).

Table 6. Normalised best combination of grass and pellet diets (AGP^+ and AGP^-) ingested by birds in all 24 pens of free range production system.

	AGP^+ diet			AGP^- diet	
pen no.	pellet	grass	pen no.	pellet	grass
9	97.8723	2.12766	5	97.5	2.5
10	97.6636	2.33645	6	97.541	2.45902
11	97.7011	2.29885	12	97.5369	2.46305
14	97.351	2.64901	15	97.3856	2.61438
18	97.7612	2.23881	20	97.0238	2.97619
21	97.9167	2.08333	22	97.3154	2.68456
26	97.3856	2.61438	34	97.619	2.38095
27	97.8571	2.14286	35	97.2222	2.77778
39	98.2167	1.78326	36	97.619	2.38095
40	97.2973	2.7027	43	97.5155	2.48447
42	97.7901	2.20994	44	97.4522	2.54777
45	97.561	2.43902	48	97.1264	2.87356

Estimation of grass ingested by birds on range

The amount of grass consumed was evaluated using penwise feed intake data from d 21-42 to reflect the days birds had access to range. Birds spent an average of 267 hours/pen on range from d 21-42 for treatments that provided access to range. The mean grass consumption in the two treatments with free range access was 2.3% (AGP^+ Diet) and 2.5% (AGP^- Diet) of the total feed intake on a DM basis (2.9-3.2 g per bird day per pen). DM content of grass was measured to be 17%, thus grass consumption was estimated to be 13.5-14.7% of total “as fed” intake. Considering the number of hours the birds spent on the range per day (2.33 hours) and the average feed intake of bird per day, this equates to 6.34-6.78 g of grass per bird per hour of range access in this study.

Effect of grass consumption on performance parameters

Before accounting for grass consumption in the calculations, performance of birds on free range system appeared to be better than those raised on conventional system with a significant decrease seen in feed intake of birds (Table 1). Taking into consideration grass consumption, total feed intake increased significantly by about 4.1% ($P < 0.05$) on an “as fed’ basis (Figure 4) and FCR which showed a 8-10 point increase ($P = 0.07$) for birds on range (Figure 5).

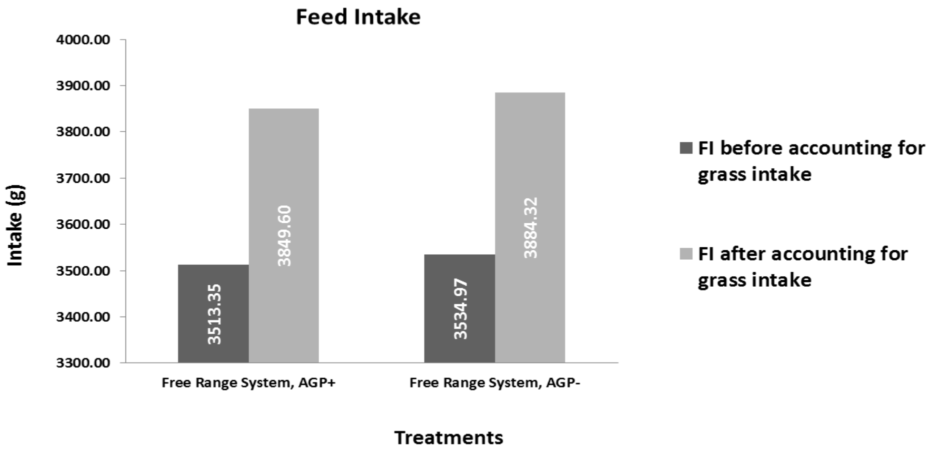


Figure 4. Effect of grass consumption on feed intake/bird/day (g)

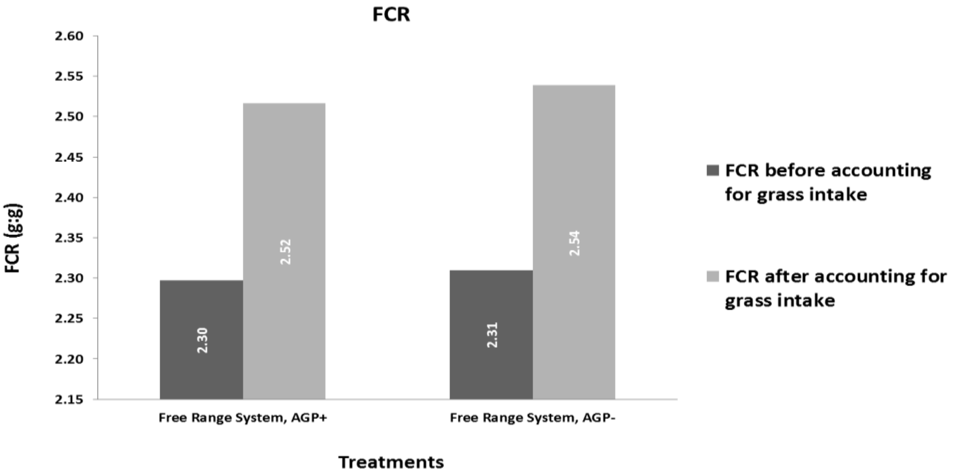


Figure 5. Effect of grass consumption on FCR (g:g)

Conclusion

Alkane analysis was used in this study to measure grass consumption. An estimated 13.5-14.7% of total “as fed” intake by free range broilers in this study was contributed to grass consumption. Considering the number of hours the birds spent on the range and the average feed intake from d21-42, this equates to 6.34-6.78 g of grass per bird per hour of range access.

Before taking grass consumption into account, average body weight and body weight gain in birds reared on conventional system was significantly higher as compared to birds reared on free range. However, feed intake was lower in free range birds thus leading to a lower FCR in comparison to conventionally reared birds. A significant increase in both feed intake and FCR on accounting for grass consumption gives an indication on the detrimental effects of grass consumption both for the bird and the production costs. Moreover, there was a significant difference in the amount of grass ingested by birds on AGP+ or AGP- diets with birds fed diets devoid of AGP consuming more.

Chapter 2. The effect of grass dilution on performance, gut characteristics and nutrient digestibility of broiler chicken

Introduction

In recent years, the contribution of grazing to the nutrition of the free range birds has been considered negligible. However, broilers on range appear to ingest grass (Figure 1), which offers very little nutrient value, except for some fibre and crude protein. In the earlier trial, grass consumption was estimated to be 13.5-14.7% of total “as fed” intake, which equates to 6.34-6.78 g of grass per bird per hour of range access.

Figure 1. Broilers eating grass on range access

This makes it difficult to determine if the birds have received adequate quantity of nutrients when they consume a portion of their diet as grass from the range. Diets intended for consumption by free-range birds are not routinely formulated to accommodate the modifying effects of grass consumption on digestible nutrient intake.

Using nutrient analysis of grass (chapter 1) and formulating diets with incremental inclusion of grass at 2%, it was found that due to a very low digestible energy density for broiler chickens, AME of standard diet (12.8 MJ/kg) and high density diet (13.20 MJ/kg), when calculted, was reduced by around 0.08-0.12 MJ/kg for every 2% dry matter grass consumed.

So far, the results of Trial 1 indicate that grass consumption is a critical measure in free range production, not only to estimate the amount of complete feed that is displaced but also to understand the potential impact it has on the performance gap between the free range and conventional production systems. This information may be used to formulate a free-range diet that considers the qualitative effects of grass consumption on total nutrient intake. The implications of changes in dietary nutrient supply were explored in this trial where both standard and high energy density broiler diets were systematically diluted with grass.

Objectives

- Investigate the effect of grass dilution in standard and high density diets.
- Measure indicators like weight gain, feed intake, gut characteristics, FCR, ileal DM, N, IDE, mineral and fibre digestibility.

Materials and Methods

A total of 768 Cobb 500 day-old male broilers were obtained from a commercial hatchery, weighed and 8 birds per pen were randomly allocated to 96 pens on wire. Birds were weighed individually on d 21, and a distribution curve created. Six birds each were reallocated to all 96 pens based on a Std.Dev. $\pm$ 2 for all bird group weights. Broilers were fed a standard starter diet *ad lib* followed by 12 treatments being introduced at d21, comprising a full factorial arrangement as shown in (Table1).

Table 1. Factorial arrangement of grower diets and treatments allocated at d 21

Grass g/kg	Std Diet (Ad lib control diet)	H D diet (Std + 0.42 MJ/kg AME)
0	Treatment 1	Treatment 7
20	Treatment 2	Treatment 8
40	Treatment 3	Treatment 9
60	Treatment 4	Treatment 10
80	Treatment 5	Treatment 11
100	Treatment 6	Treatment 12

The two basal grower diets were formulated with AME of 12.8 MJ/kg (Std. Diet) and 13.2 MJ/kg High Density diet (HD diet = Std. Diet + 0.42 MJ/kg AME) (Table 2).

Table 2. Ingredient composition (g/kg, as fed) of basal diets with two different energy densities

	Std. Diet	HD Diet (+0.42 MJ/kg AME)
Wheat - Feed	65.25%	63.03%
Meat/bone meal	6.00%	6.00%
Canola meal (solvent extracted)	10.00%	10.00%
Soybean meal 48	13.69%	14.14%
Sunflower oil	1.03%	2.81%
Salt	0.01%	0.02%
Sodium Bicarbonate	0.36%	0.36%
DL Methionine	0.36%	0.37%
Lysine HCl	0.53%	0.53%
Threonine	0.24%	0.24%
Limestone	0.24%	0.24%
Phytase	0.01%	0.01%
Choline chloride 60	0.06%	0.06%
Vitamin/mineral premix*	2.20%	2.20%
Xylanase	0.01%	0.01%

**Supplied per kg of diet: retinol, 3,600 μg; cholecalciferol, 125 μg; α-tocopherol, 50 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 5 mg; cobalamin, 25 μg; niacin, 50 mg; pantothenic acid, 18 mg; folic acid, 2 mg; biotin, 200 μg; Cu, 20 mg; Fe, 40 mg; Mn, 110 mg; Co, 250 μg; I, 1 mg; Mo, 2 mg; Zn, 90 mg; Se, 300 μg; ethoxyquin, 125 mg.*

The basal diets were diluted with six different levels (0, 20, 40, 60, 80 and 100 g/kg) of dried Kikuyu grass (*P. clandestinum).* Grass was harvested at day 48 of growth, air dried for 72 hrs, cut to 1cm length, weighed at the specified inclusion levels (g/kg) for different treatment diets, mixed and cold pelleted. Analysis of dried grass showed 83% DM and CP 16.8% DM; AME was assumed to be 1.53 MJ/kg DM based on values reported by Buchanan et al. (2007). Expected nutrient levels for the twelve treatment diets are presented in (Table 3).

Table 3 Expected nutrient composition of diets diluted with Incremental inclusions of 2% grass inclusion (GI)

	Std. Diet						HD Diet (+0.42 MJ/kg AME)					
Calculated nutrients	**0%GI**	**2%GI**	**4%GI**	**6%GI**	**8%GI**	**10%GI**	**0%GI**	**2%GI**	**4%GI**	**6%GI**	**8%GI**	**10%GI**
Poult AME (MJ/kg)	12.97	12.74	12.51	12.28	12.05	11.82	13.39	13.15	12.91	12.67	12.43	12.20
CP %	19.5	19.45	19.39	19.34	19.28	19.23	19.5	19.45	19.39	19.34	19.28	19.23
Calcium %	0.90	0.90	0.89	0.89	0.88	0.88	0.90	0.90	0.89	0.89	0.88	0.88
Phos %	0.78	0.77	0.76	0.75	0.75	0.74	0.77	0.77	0.76	0.75	0.74	0.73
Avail Phos %	0.55	0.55	0.55	0.54	0.54	0.54	0.55	0.55	0.54	0.54	0.54	0.53
Met %	0.65	0.64	0.63	0.62	0.62	0.61	0.65	0.64	0.63	0.63	0.62	0.61
Lys%	1.05	1.05	0.79	0.59	0.43	0.40	1.05	1.05	0.79	0.59	0.43	0.40
Cys %	0.34	0.34	0.33	0.33	0.33	0.32	0.34	0.34	0.33	0.33	0.32	0.32
Me+Cys %	1.00	0.99	0.98	0.96	0.95	0.94	1.00	0.99	0.98	0.96	0.95	0.94
Na %	0.18	0.18	0.19	0.19	0.19	0.19	0.18	0.18	0.19	0.19	0.19	0.19
Cl %	0.20	0.21	0.21	0.22	0.22	0.23	0.20	0.20	0.21	0.21	0.22	0.22
K %	0.66	0.70	0.75	0.79	0.83	0.87	0.66	0.71	0.75	0.79	0.83	0.87
DEB (mEq/kg)	191.7	201.9	212.0	222.1	232.3	242.4	192.0	204.6	217.3	230.0	242.6	255.3

Each treatment was replicated eight times with six chicks per replicate pen. Body weights and feed consumption was determined by pen at 0, 7, 14, 21 and 28 d of age, and BW gain (BWG), average feed intake (FI) and feed conversion ratio (FCR) were determined. On d28, the birds were euthanized with sodium pentobarbitone injection via the wing vein and the contents of the distal half of the ileum were collected (the ileum being defined as the portion of the small intestine from Meckels diverticulum to the ileo-caecal junction). Ileal contents were immediately frozen at -18ºC prior to being freeze-dried and ground to pass a 0.5mm screen. Gizzard Weight and Total Gut Mass (combined weight of duodenum, jejunum, ileum and caeca) was recorded for each bird.

Apparent ileal digestibility (AID) coefficients for protein (N) energy, fiber and minerals, were estimated using AIA marker and collecting ileal digesta on d 28 as described in 'General Methodology'. Samples were wet acid digested using nitric acid and hydrogen peroxide before the determination of mineral concentration by Inductively Coupled Plasma-Optical Emission Spectroscopy using a Perkin Elmer OPTIMA 7300 (Perkin Elmer Inc., Waltham, MA, USA). ADF was determined by the method of Goering & Van Soest (1970). NDF was determined by the method of Robertson & Van Soest (1981).

All data were exported to JMP v9.0 (SAS Institute, Cary, NC, USA) and subjected to standard least square analysis to determine the effect of grass dilution, diet densities and interactions between them. Means were compared using Tukey's HSD and were considered significantly different at $P<0.05$.

Result

Performance

All birds were fed the same commercial starter diet until d 21 and no significant differences were observed either in body weight gain (BWG), feed intake (FI) or feed conversion ratio (FCR) between birds on a weekly basis confirming their nutritional similarity. Mortality was low at 1.05% and not related to diet.

As shown in Table 4, significant differences in the final live body weight (BW) were observed on d 28 with an increase in GI. Final BW declined significantly at higher GI (*P*<0.001) and broilers fed STD diet were significantly lighter (*P*<0.01) compared to those fed the HD diet. Incremental dilution with grass resulted in a significant (*P*<0.001) decrease in FI. A significant difference was also observed between diet densities with HD diets showing lower FI than STD diets on average (*P*<0.001).

Table 4. Influence of grass dilution to a wheat/soy-based diet on the performance of broiler chickens, d 21-28 post hatch

Grass Inclusion (GI) g/kg	Diet Density (DD)	df	BW d28 (g/bird)	BWG d21-28 (g/bird)	FI d21-28 (g/bird)	FCR d21-28 (g:g)
0	STD		1701	721[abcd]	1299	1.80[abc]
20	STD		1693	733[abc]	1254	1.71[bcd]
40	STD		1640	692[cdef]	1241	1.79[abc]
60	STD		1656	694[cdef]	1233	1.77[abc]
80	STD		1628	673[ef]	1229	1.82[ab]
100	STD		1625	661[ef]	1212	1.83[a]
0	HD		1712	746[ab]	1264	1.69[cd]
20	HD		1720	749[ab]	1239	1.65[d]
40	HD		1717	751[a]	1234	1.64[d]
60	HD		1675	703[bcde]	1227	1.74[abcd]
80	HD		1648	685[def]	1217	1.77[abc]
100	HD		1617	656[f]	1202	1.83[a]
Pooled SEM			14.23	10.01	6.40	0.025
P-value						
GI		5	***	***	***	***
DD		1	**	***	***	***
GI x DD		5	NS	*	NS	*

*GI=grass inclusion; DD=Diet density; BW=bodyweight; BWG=bodyweight gain; FI=feed intake; FCR=feed conversion ratio; Means in columns with no common superscript are significantly different * (P<0.05), ** (P<0.01), *** (P<0.001).*

A significant GI x DD interaction was seen for both BWG d21-28 and FCR d21-28. Significant improvement in performance indicators of BWG (*P*<0.05) and FCR (*P*<0.05) was seen with up to 20 g/kg GI in STD diet, and up to 40 g/kg grass GI in HD diet with a decline seen for both diets for higher inclusions of grass.

Gut Characteristics

A significant interaction of GI and DD was seen for NDF and ADF(% DM digesta) with an increase in levels seen with increase in grass inclusion for both STD and HD diets (*P<0.01*). A significant GI x DD interaction was also observed for effect on gizzard weights and relative gizzard weights (% BW) (*P<0.01*) (Table 5). There was no significant difference due to either GI or DD on the total gut mass (TGM) of birds, however, Relative Gut Mass (RGM, %BW) seemed to significantly decrease with increasing levels of GI (p< 0.05).

Table 5. Influence of grass dilution to a wheat/soy-based diet on ileal digesta composition and the gut morphology of broiler chickens, 28d post hatch

Grass Inclusion (GI) g/kg	Diet Density (DD)	df	NDF % DM digesta	ADF % DM digesta	BW d28 (g/b)	GW d28 (g/b)[1]	RGW d28 (% BW)	TGM d28 (g/b)[1]	RGM d28 (% BW)

0	STD		38.85^{l}	14.15^{l}	1701	23.13^{d}	1.36^{e}	80.48	4.74
20	STD		45.10^{j}	18.01^{j}	1693	26.80^{c}	1.60^{d}	79.81	4.81
40	STD		45.94^{h}	18.42^{h}	1640	28.80b^{c}	1.74^{bc}	76.92	4.67
60	STD		47.02^{f}	18.93^{f}	1656	28.73b^{c}	1.74^{bc}	76.41	4.63
80	STD		55.97^{d}	19.41^{d}	1628	31.88^{a}	1.97^{a}	77.92	4.79
100	STD		66.44^{b}	22.36^{b}	1625	31.78^{a}	1.94^{a}	77.29	4.74
0	HD		41.15^{k}	15.70^{k}	1712	21.53^{d}	1.27^{e}	79.19	4.72
20	HD		45.85^{i}	18.75^{i}	1720	27.91^{c}	1.64^{cd}	79.15	4.65
40	HD		50.07^{g}	18.83^{g}	1717	30.09^{ab}	1.78^{bc}	80.16	4.74
60	HD		50.27^{e}	19.61^{e}	1675	30.69^{a}	1.86^{ab}	78.44	4.79
80	HD		61.83^{c}	20.73^{c}	1648	31.80^{a}	1.93^{a}	78.50	4.75
100	HD		73.05^{a}	23.71^{a}	1617	32.17^{a}	1.99^{a}	78.22	4.85
Pooled SEM			9.02E-08	9.38E-09	14.23	0.5354	0.031	1.321	0.159
P-value									
GI		5	***	***	***	***	***	NS	*
DD		1	***	***	**	*	NS	NS	NS
GI x DD		5	***	***	NS	***	**	NS	NS

*NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber ; BW = Body Weight; GW = Gizzard Weight; RGW = Relative Gizzard Weight; TGM = Total Gut Mass; RGM = Relative Gut Mass; Means in columns with no common superscript are significantly different. * (P<0.05), ** (P<0.01), *** (P<0.001).*

Digestibility

The influence of GI and DD on CAID of DM, energy and protein (N) is presented in Table 6.

Table 6. Influence of grass dilution on the coefficients of apparent ileal digestibility (CAID) of dry matter (DM), energy and protein (N) and the ileal digestible energy content (IDE, MJ/kg) on d28.

Grass Inclusion (GI) g/kg	Diet Density (DD)	df	CAID			IDE
			DM	Energy	Protein (N)	(MJ/kg)
0	STD		0.68^{bc}	0.69^{bcde}	0.72	12.80^{bcd}
20	STD		0.68^{a}	0.74^{a}	0.77	13.51^{ab}
40	STD		0.70^{bc}	0.69^{bcde}	0.73	12.67^{cd}
60	STD		0.63^{bcd}	0.68^{cde}	0.71	12.34^{de}
80	STD		0.64^{fg}	0.61^{f}	0.66	11.12^{f}
100	STD		0.59^{efg}	0.61^{f}	0.64	11.14^{f}
0	HD		0.69^{ab}	0.69^{bcd}	0.75	12.63^{cd}
20	HD		0.68^{b}	0.70^{abc}	0.75	13.28^{abc}
40	HD		0.70^{ab}	0.72^{ab}	0.75	13.61^{a}
60	HD		0.63^{cde}	0.66^{de}	0.71	12.33^{de}
80	HD		0.63^{def}	0.65^{ef}	0.71	12.34^{de}
100	HD		0.59^{g}	0.61^{f}	0.66	11.67^{ef}
Pooled SEM			0.0078	0.008	0.012	0.159
P-value						
GI		5	***	***	***	***
DD		1	NS	NS	NS	***
GI x DD		5	***	***	NS	***

*Means in columns with no common superscript are significantly different. ***(P<0.001).*

A significant GI x DD interaction (*P*<0.001) was seen for ileal digestibility coefficients of DM and energy, and apparent ileal digestible energy content (IDE), with a trend of improvement for lower inclusions (up to 40 g/kg) and then a steady decline for higher GI for both STD and HD diets(Table 6). A significant effect of GI(*P*<0.001) was seen on nitrogen digestibility coefficient with an improvement at lower inclusions for up to 40 g/kg and a decline seen from >60g/kg inclusion for both STD and HD diet.

Mineral Digestibility

A significant interaction of GI x DD was seen for apparent ileal digestibility of Ca, P, Na and K with inclusion of grass resulting in significant decline (p<0.01) for STD and HD diets (Table 7).

Table 7. Influence of grass inclusion (GI) (g/kg) and diet density (DD) to a wheat/soy-based diet on the coefficients of apparent ileal digestibility of minerals for broiler chickens, 28d post hatch.

Grass Inclusion (GI) g/kg	Diet Density (DD)	df	CAID			
			Ca	P	Na	K
0	STD		0.32^{abc}	0.41^{abc}	0.56^{ab}	0.78^{ab}
20	STD		0.27^{abcd}	0.41^{abc}	0.52^{ab}	0.77^{ab}
40	STD		0.20^{cde}	0.33^{cd}	0.14^{ef}	0.76^{ab}
60	STD		0.22^{bcde}	0.39^{bcd}	0.24^{de}	0.77^{ab}
80	STD		0.10^{e}	0.28^{d}	-0.56^{h}	0.70^{c}
100	STD		0.17^{de}	0.51^{a}	-0.35^{a}	0.70^{c}
0	HD		0.28^{abcd}	0.40^{bc}	0.28^{de}	0.79^{a}
20	HD		0.34^{ab}	0.46^{ab}	0.34^{cd}	0.77^{ab}
40	HD		0.36^{a}	0.50^{ab}	0.44^{bc}	0.78^{a}
60	HD		0.27^{abcd}	0.43^{abc}	0.02^{f}	0.76^{ab}
80	HD		0.27^{abcd}	0.46^{ab}	-0.16^{g}	0.75^{b}
100	HD		0.22^{bcde}	0.43^{abc}	-0.82^{i}	0.70^{c}
Pooled SEM			0.0226	0.0199	0.0278	0.0057
P-value						
GI		5	***	**	***	***
DD		1	***	***	***	NS
GI x DD		5	**	***	***	***

*Means in columns with no common superscript are significantly different. * (P<0.05),** (P<0.01), ***(P<0.001).*

For HD diets, up to 4% GI showed a significant increase in digestibility of all minerals before declining with higher levels of GI.

Negative retention of Na was seen with higher levels of 8-10% GI indicating the balance that is maintained to account for constant dietary electrolyte balance (DEB).

Conclusion

Increased dietary grass concentration had a significant effect on most outcomes, with improved weight gain, FCR and the ileal digestibility of dry matter, energy and nitrogen up to around 20-40g/kg but significantly poorer performance and digestibility thereafter. Provision of a diet with a higher energy density improved performance at low concentrations of grass only resulting in a significant grass*energy interaction for most parameters. These results confirm previous observations that moderate grass intake may improve performance of broilers, perhaps mediated via functional fibre mechanisms. However, grass intake beyond 20-40g/kg results in impaired performance that appears to be independent of diet energy density but may be associated with deleterious changes to dietary electrolyte balance.

Chapter 3. Manipulating energy density and dietary electrolyte balance in diets of free range broilers

Introduction

Broiler chickens in free range conditions have range access from d21-42 as per Australian regulations and consume grass (*P. clandestinum)* that has high levels of potassium (K) and low energy density (DM: 83%; CP: 16.8 % DM ; K: 2.7% DM and AME assumed to be 1.53 MJ/kg (Buchanan et al. (2007)). This leads to changes in dietary electrolyte balance (DEB) and metabolisable energy (AME). By formulating diets with incremental inclusion of grass, calculated nutrient levels show increase in DEB by around 20mEq/kg for every 2% grass consumed (Figure 1).

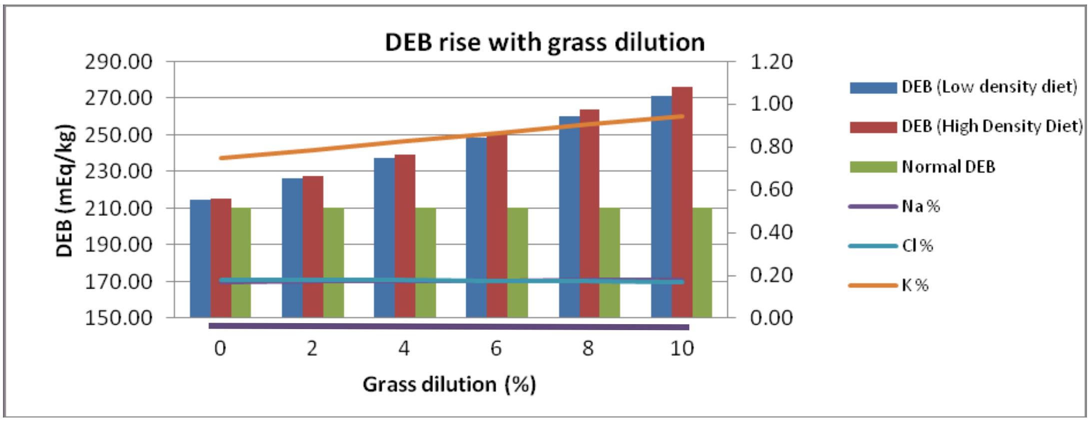


Figure 1. Rise in DEB due to increase in potassium with increasing level of grass dilution.

Potassium is readily absorbed from the upper small intestine and is excreted from the body primarily through the urine. The functions of potassium in the body include osmotic pressure regulation, maintenance of water and acid-base balance, nerve impulse conduction, muscle contraction and enzymatic reactions balance (Miller, 1995). The increase of dietary potassium level has also been associated, with an increase of water consumption and excretion, leading to depleted levels of K and higher excreta moisture. This leads to problems associated with wet litter, like increase of relative humidity, increased weight and volume of manure which increase their management, storage and removal costs, more fly development, increase of the rate of loss of ammonia into the environment, footpad dermatitis and breast burns and respiratory diseases (Francesch and Brufau, 2004).

The dietary electrolyte balance (DEB), also known as acid-base balance is critical especially in free range production where birds are exposed to high temperatures on range. Very high DEB can result in metabolic acidosis.

It is well known that nutrition and environment influenced the bird's acid-base balance. Therefore, the maintenance of this balance can be an important measure to improve the performance of broilers raised under high temperatures and to overcome the harmful effects of respiratory alkalosis resulting from heat stress.

High temperature, especially when coupled with high humidity, imposes severe stress on birds and leads to reduced performance. Some diets have been developed that reduce heat increment, for example fat feeding, and thus enabled improved growth performance at high ambient temperatures (Shannon and Brown, 1969; Dale and Fuller, 1980).

Objectives

To investigate the effect of two levels of DEB in two density diets and their interaction on the performance and digestibility of free range birds.

Measure indicators like weight gain (WG), feed intake(FI) , FCR, apparent ileal DM, N, and energy digestibility, IDE, plasma electrolyte levels and thermal profiles of shed and range areas as well as birds during the period of the trial.

Materials and Methods

All experimental procedures conducted in this study were in accordance with the University of Sydney Animal Ethics Committee and with the Australian code for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2004).

A total of 800 Cobb 500 day-old male broilers were obtained from a commercial hatchery, weighed at one day old and randomly allocated to pens. Fifty birds were placed in each of the 16 free range housing pens. Broilers were fed a formulated starter diet *ad lib* followed by 4 treatments being introduced at d 21, comprising a full factorial arrangement with two diet densities (DD): standard (STD) and high density (HD, +0.42 MJ/kg AME) and two different levels of dietary electrolyte balance (DEB) (180 mEq/kg and 220 mEq/kg). Four treatment diets were formulated as below (Table 1).

Table 1 Ingredient composition (g/kg, as fed) and estimated nutrient levels of grower diets with two different energy densities and two levels of dietary electrolyte balance (DEB) (mEq/kg)

Ingredient composition (%)	DEB 220_STD	DEB 220_HD	DEB 180_STD	DEB 180_HD
Wheat - Feed	66.96	65.00	67.21	65.02
Canola meal (solvent extracted)	5.00	4.52	5.00	4.93
Soybean meal 48	20.24	20.98	20.19	20.68
Canola oil	4.30	6.00	4.23	6.00
Salt	0.02	0.02	0.22	0.22
Sodium Bicarbonate	0.43	0.43	0.11	0.11
DL Methionine	0.22	0.22	0.21	0.21
Lysine HCl	0.24	0.24	0.24	0.24
Threonine	0.09	0.09	0.09	0.09
Limestone	0.68	0.67	0.68	0.67
Dicalcium Phos	1.26	1.28	1.26	1.28
Choline chloride 60	0.06	0.06	0.06	0.06
Vitamin/mineral premix*	0.50	0.50	0.50	0.50
Rovabio Excel	0.50	0.50	0.50	0.50
Nutrient profile				
CP%	19	19	19	19
Poult AME MJ/kg	12.8	13.2	12.8	13.2
Calcium%	0.80	0.80	0.80	0.80
Avail Phos%	0.40	0.40	0.40	0.40
Na%	0.16	0.16	0.15	0.15
Cl%	0.16	0.16	0.28	0.28
K%	0.76	0.76	0.76	0.76
DEB (mEq/kg)	220	220	180	180

**Supplied per kg of diet: retinol, 3,600 μg; cholecalciferol, 125 μg; α-tocopherol, 50 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 5 mg; cobalamin, 25 μg; niacin, 50 mg; pantothenic acid, 18 mg; folic acid, 2 mg; biotin, 200 μg; Cu, 20 mg; Fe, 40 mg; Mn, 110 mg; Co, 250 μg; I, 1 mg; Mo, 2 mg; Zn, 90 mg; Se, 300 μg; ethoxyquin, 125 mg.*

An indigestible marker (acid insoluble ash; AIA) (Celite 281, Filchem Australia Pty Ltd, Castle Hill, NSW, Australia) was added to diets at a concentration of 20 g/kg and the coefficient of apparent ileal digestibility (CAID) for dry matter, crude protein and energy were calculated as stated in 'General Methodology'. Each treatment was replicated four times with fifty chickens per replicate pen. Broilers were kept at a temperature of 31 °C for days 1-4 and thereafter this was reduced by 0.5°C/day to 24 °C by d 18. Birds were given access to range on d 21. The range had a homogenous growth of young Kikuyu grass(*P. clandestinum*) as the main herbage and was mowed to about 6 cm before assigning the birds and not irrigated during the trial.

Body weight gain (BWG), average feed intake (FI) and feed conversion ratio (FCR) were determined weekly on a pen basis for d1- 42. On d 42, five birds from each pen were euthanized and the contents of the distal half of the ileum were collected Ileal contents were immediately frozen at -18ºC prior to being freeze-dried and ground to pass a 0.5 mm screen.

Blood samples were collected for plasma electrolyte analyses and the blood pH was noted. FLIR T620 Thermal Imaging Camera (FLIR Systems AB, Germany) was used to take images of range and shed, birds on range and in the shed and were processed using FLIR ThermaCAM Researcher Pro 2.10 software (FLIR Systems AB, Germany). Weather data from the NSW meteorology website was also collected for the duration of the trial.

Data analysis

All data were exported to JMP v9.0 (SAS Institute, Cary, NC, USA) and subjected to least square analysis to determine the effect of diet densities, DEB and the interactions between them. Means were compared using Tukey's HSD and were considered significantly different at $P<0.05$.

Results

Performance

All birds were fed the same commercial starter diet until d 21 and no significant differences were observed either in body weight gain (BWG), feed intake (FI) or feed conversion ratio (FCR) between birds on a weekly basis confirming their nutritional similarity. Mortality was low at 1.45% and not related to diet.

Table 2. Performance of broiler chickens, 21- 42d, fed treatment diets

DEB	DD	BWG (g/bird) D21-42	FI (g/bird) D21-42	FCR(g:g) D21-D42
180	HD	2049.2	3729.9	1.82[b]
180	STD	1954.0	3655.9	1.87[ab]
220	HD	2023.5	3908.3	1.93[a]
220	STD	2009.9	3807.6	1.89[ab]
Pooled SEM		22.29	48.32	0.02
P-value				
DEB		NS	**	*
DD		*	NS	NS
DEB x DD		NS	NS	*

DEB=Dietary electrolyte balance; DD=Diet density; BWG=bodyweight gain; FI=feed intake; FCR=feed conversion ratio; Means in columns with no common superscript are significantly different (P<0.05); Significance level: NS, non significant, *, P ≤ 0.05, **, P≤ 0.01and ***, P≤ 0.001.

From d21-42 (Table 2), an interaction between DD and DEB was observed with improved FCR but only in birds that received HD diet at low DEB, with an opposite affect seen in the diet with high DEB. HD diets showed significantly better BWG for both high and low DEB diets (P<0.05). Low DEB diets performed significantly better than high DEB diets in both STD and HD diets with significantly lower FI (P<0.01) and FCR (P<0.05). Thus an improvement in performance of free-range broilers was observed by increasing dietary energy, but only if done in concert with a reduction in DEB to around 180 mEq/kg.

Digestibility

Increasing energy density improved coefficient of apparent ileal digestibility (CAID) of dry matter (DM) in the low DEB diet, having the opposite effect in the high DEB diet. Dietary interventions had no effect on CAID of energy, protein (N) or the digestible energy content (IDE) on d42 (Table 3).

Table 3. Influence of two density and two DEB diets on the coefficient of apparent ileal dry matter digestibility (CAID) of dry matter (DM), energy and protein (N) and the ileal digestible energy (IDE) content on d42.

DEB	DD	CAID			*IDE*(MJ/kg)
		DM	Energy	Protein (N)	
180	HD	0.61[a]	0.63	0.77	12.25
180	STD	0.57[b]	0.59	0.77	11.33
220	HD	0.55[b]	0.64	0.72	11.25
220	STD	0.54[b]	0.60	0.75	11.25
Pooled SEM		0.014	0.052	1.871	0.007
P-value					
DEB		NS	NS	NS	NS
DD		NS	NS	NS	NS
DEB x DD		***	NS	NS	NS

Means in columns with no common superscript are significantly different (P<0.05); Significance level: NS, non significant, *, P ≤ 0.05, **, P≤ 0.01and ***, P≤ 0.001.

Plasma mineral characteristics

There was an average 139g reduction of weight with the lower DEB as compared to higher DEB for both HD and STD diets, probably due to a reduction in FI. However, lowering of calcium and rise in phosphate levels were seen for higher DEB treatments. Heat stress was also suggested by lower levels of sodium and chloride in higher DEB treatments (Table 4).

Table 4 Influence of two density and two DEB diets on the blood plasma characteristics on d42

DEB	Diet Density (DD)	ABW (g)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Calcium (mmol/L)	Phosphate (mmol/L)
180	HD	2862	153.4	4.04[b]	108.8	3.10	1.67
180	STD	2930	153.5	4.31[ab]	108.6	3.13	1.67
220	HD	3050	152.7	4.24[ab]	107.8	3.04	1.71
220	STD	3019	152.2	4.37 [a]	107.3	2.96	1.86
Pooled SEM		72.15	0.488	0.079	0.399	0.051	0.061
P-value							
DEB		*	*	NS	**	*	*
DD		NS	NS	NS	NS	NS	NS
DEB x DD		NS	NS	*	NS	NS	NS

Means in columns with no common superscript are significantly different * (P<0.05),** (P<0.01), ***(P<0.001)

Litter moisture, bird temperature and blood pH

Although heat stress was prevalent regardless of treatment, litter moisture content was seen to be lower in pens with birds fed on HD diets as compared to standard (not statistically significant), irrespective of the DEB levels (Table 5).

Table 5. Litter moisture, Birds body surface temperatures and Blood pH levels for different treatments

DEB	Diet Density (DD)	Litter Moisture content %	Average Bird Temp in shed °C	Average bird temp on Range °C	Blood pH
180	HD	31.81	33.37	36.72	7.52[a]
180	STD	35.26	32.66	36.12	7.28[b]
220	HD	31.40	33.65	37.62	7.37[ab]
220	STD	32.31	32.70	36.52	7.35[b]
Pooled SEM		2.931	0.22	0.247	0.038
P-value					
DEB		*NS*	*NS*	*	*NS*
DD		*NS*	**	**	**
DEB x DD		*NS*	*NS*	*NS*	*

However, higher bird temperatures were recorded for birds fed on HD diets. Blood pH was significantly elevated for birds fed a STD diet and with low DEB (P<0.05).

Thermal profiles on range and in the shed for free range birds

Thermal images of birds out on the range and inside the shed taken by the FLIR Thermal Imaging Camera are shown in Figure 2.

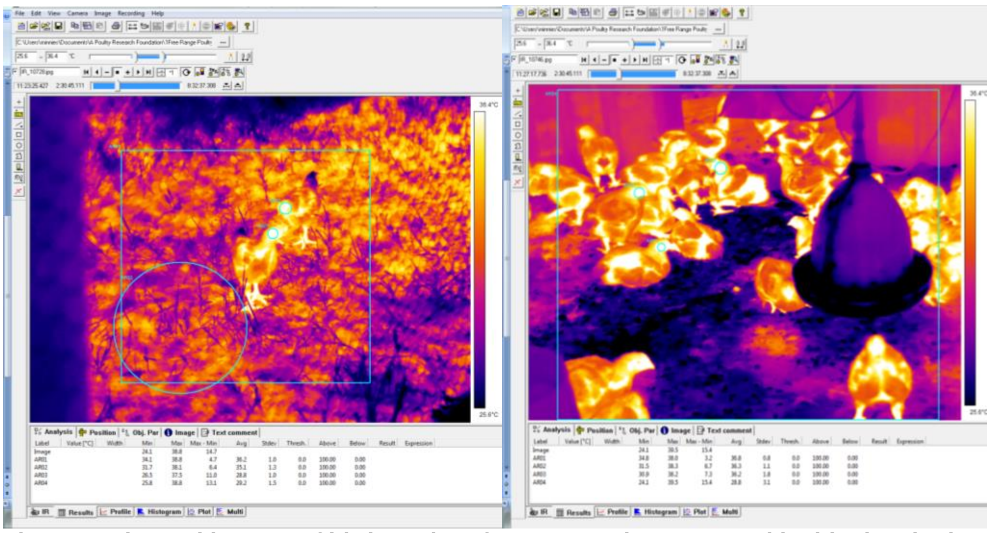


Figure 2. Thermal images of birds and surface out on the range and inside the shed

Thermal profiles of birds and surface inside the shed and out on range were recorded at two times of the day, 8.00 AM (just after opening the popholes in the morning) (Figure 3) and at 12.00 noon (Figure 4). The minimum temperature in the 18 d period was 13.4°C and maximum of 31.3 degrees. The shed temperature at 8.00 AM ranged between 24-26°C on most days, showing a maintained profile early in the mornings. The birds too showed ambient surface temperature averaging at 31°C inside the shed. The average range and bird temperatures outside did not show a big variation from the inside temperatures at this early hour showing an average of 24.2 and 31.3°C. The mean range temperature was highly correlated to the weather at 8 am in the morning.

Figure 3. Thermal profile at 8am for 18/21 days on free range

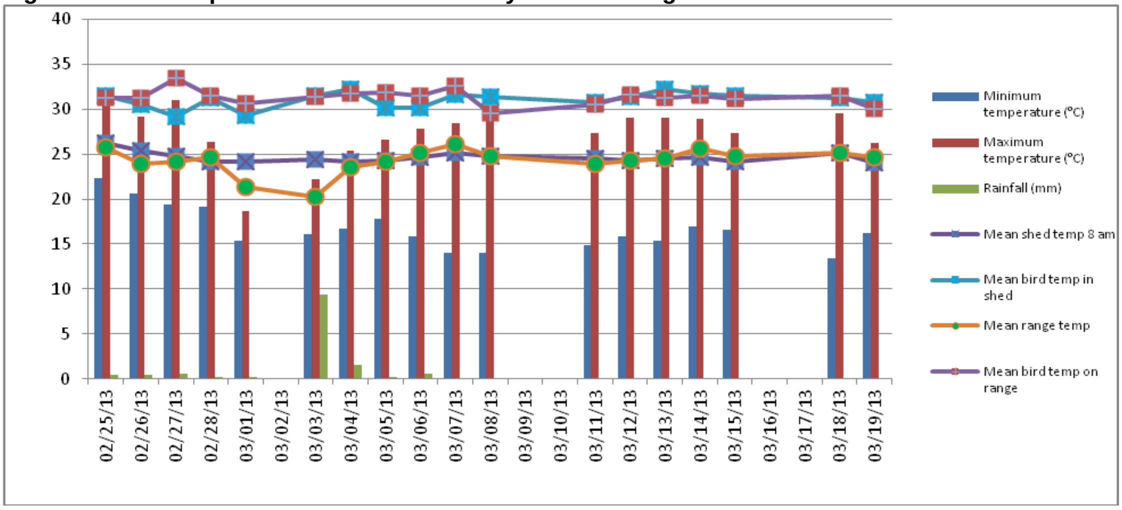
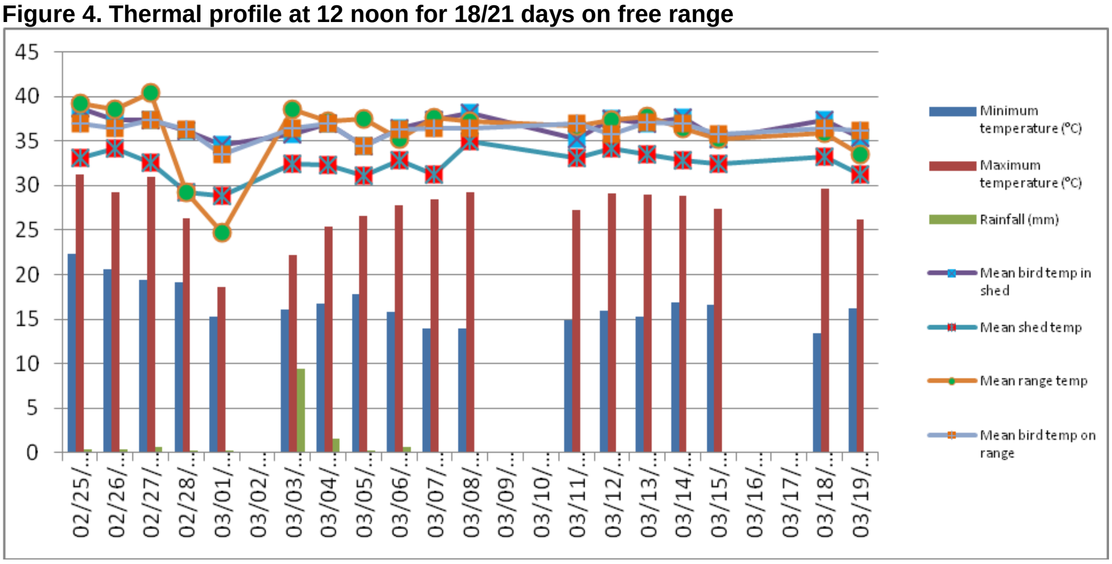


Figure 4. Thermal profile at 12 noon for 18/21 days on free range



At noon, however, the average shed temperature rose to 32.4°C, while the bird's surface temperature increased to 36.5°C, which was almost 5.5°C higher than the temperature at 8AM. Temperature profiles on range showed very high average temperature of range and birds on range with 36°C, and 36.3°C respectively. The noon range temperatures were almost 4°C higher than the shed temperature and almost 12°C higher than the 8AM temperature. Although the birds on range had a higher surface temperature by about 5°C at 12 noon than birds earlier in the day, they showed a slightly lower temperature (0.3°C) than the birds inside the shed at the same time.

Conclusion

Thermal profiles indicated surface temperatures in birds rose above ambient levels with a rise in day temperatures in all treatment groups, with the highest seen in birds inside the shed. Birds fed diets consisting of low DEB levels and high density showed a significant weight gain ($P < 0.001$) and an improved FCR ($P < 0.01$), dry matter digestibility and digestible dry matter content.

An effect of lower levels of DEB were seen with significantly lower feed intake ($P < 0.05$) for both low and high density diets and improved plasma levels of Na, Cl and Ca. High density diets increased blood pH by 0.24 and caused depletion of K from the optimal 5mmol/L, both indicative of respiratory alkalosis. However, the higher levels of sodium bicarbonate in DEB 220 might have helped allevitae stress as its inclusion is known to reduce respiratory alkalosis caused by panting/hyperventilation. Moreover, chloride levels in DEB 180 were higher perhaps increasing water intake and thus litter moisture. Thus lower DEB and HD diets significantly improved performance and digestibility parameters, indicating it to be a good strategy to address the consequences of grass consumption on range.

Chapter 4. The effect of range usage characterized using RFID technology on performance, gut characteristics and digestibility of free range layer populations

(In collaboration with CSIRO Chiswick and UNE Armidale)

Introduction

In free range systems, use of the range area is perceived to benefit hen welfare by lowering the density of birds indoors, increasing access to resources, allowing birds to perform natural behaviours and providing an enriched environment (Richards et al. 2011). It has been identified in many studies that a large proportion of hens use the range on a regular basis, while others rarely or never visit the range (Bubier 1998, Hegelund et al. 2005, Zeltner & Hirt, 2003). The challenge is how to feed an 'average' bird in this population.

Birds with access to the range eat pasture, seeds, soil and insects and exhibit greater incidences of running, walking, and wing flapping reflecting greater freedom of movement (Lomu et al. 2004). However, this results in a change of nutrient availability and utilization. The consumption of different materials on the range may facilitate gizzard development, potentially resulting in better nutrient utilisation (Svihus 2012). Moreover, there are differences in the way birds access the range. This study aims to characterise populations based on the duration and frequency of visits to the range and evaluate the differences in gut characteristics and digestibility of nutrients between the non-users versus range users.

Objectives

1. To use wireless technology (RFID tags) to monitor hens' movement between indoor and outdoor areas in a free-range egg production system and identify hens that preferentially "use" or "do not use" the outdoor space.

2. To assess differences in performance, gut characteristics and digestibility, in these sub-populations.

Materials and Methods

All experimental procedures conducted in this study were in accordance with the CSIRO Chiswick Animal Ethics Committee and with the Australian code for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2004).

A total of 200 laying hens (63 wk, ISA Brown) were used in this study. The birds were housed in an indoor barn with access to outdoor range at the CSIRO, Chiswick facility in

Armidale. Hens were fed a commercial layer pellet with 18% crude protein, 3.5% fat and 5.5% fibre (detailed composition of diet was not available from the supplier).

Figure 1. A collaborative trial setup between University of Sydney, University of New England and CSIRO Chiswick to study range access in free range layers.

Feed and water was provided *ad lib* in the barn. The range area was devoid of all vegetation during the 28 days of this trial. An indigestible marker (acid insoluble ash; AIA) (Celite 281, Filchem Australia Pty Ltd, Castle Hill, NSW, Australia) was added to diets at a concentration of 20 g/kg to calculate the apparent ileal digestibility (AID) coefficients for dry matter, crude protein and energy. Birds were weighed on day one (63 weeks of age) and day twenty eight (67 weeks of age) of trial. Body weight gain (BWG), average feed intake (FI) and daily egg production (DEP) was measured during the trial.

Employing RFID technology, individual hen movement between the indoor and outdoor area was recorded using RFID antennas and light-beam sensors fitted to ‘pop-holes’. Data were collected daily from 09:30 to16:00h for 28 contiguous days to identify variation in outdoor use. The system was able to individually identify the birds as they passed through the pop-holes and also record the date and time. On day 28, based on the RFID data, birds were classified into non-range (NR) users and range users. Range users were further classified according to duration and frequency of visits (low frequency, long visits (LL) and high frequency, short visit (HS)). Thirty-six birds were selected (12 birds each in three sub-populations), weighed and euthanized and the contents of the distal half of the ileum collected. Ileal contents were immediately frozen at -18ºC prior to being freeze-dried and ground to pass a 0.5 mm screen. Gizzard pH, gizzard weight and gut weight (duodenum, jejunum, Ileum and caeca) were also recorded for each bird.

Chemical analyses

The acid insoluble ash component of dried diets and ileal digesta samples were determined according to the method of Siriwan *et al*. (1993). The AID coefficient of DM, energy, N, and their digestible content were calculated as per Ravindran *et al*. (2005). The gross energy (GE) of diets and lyophilized digesta were determined using a Parr 1281 adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA) that was standardised with benzoic acid. Nitrogen concentration of samples was determined by the Dumas method using a FP-

428 nitrogen analyser (LECO® Corporation, St. Joseph, MI, USA) as described by Sweeny (1989).

Data analysis

All data were exported to JMP v9.0 (SAS Institute, Cary, NC, USA) and subjected to analysis of variance. Means were separated by Tukeys HSD and were considered significant at P<0.05.

Results

Performance of flock

An average of 149 eggs were produced per day (74.5% hen-day production) with feed conversion efficiency (FCE) of 2.17, and an average of three eggs per day being dirty, broken, or laid outside the nest box during the trial.

RFID Data and characterisation of sub-populations

RFID data from 28 days of trial was analysed to look into range access of free range layers. Figure 2 shows the distribution of birds that used the range for at least one day.

While 16% of the birds never went out on range, 84% accessed the range on at least one day. Sixty-three percent of birds went out on range every day. Birds visited the range on avearge 253 times and spent an average of 80 h on range. The maximum time spent on the range on a single visit was 0.58 h and the minimum was about 0.04 h.

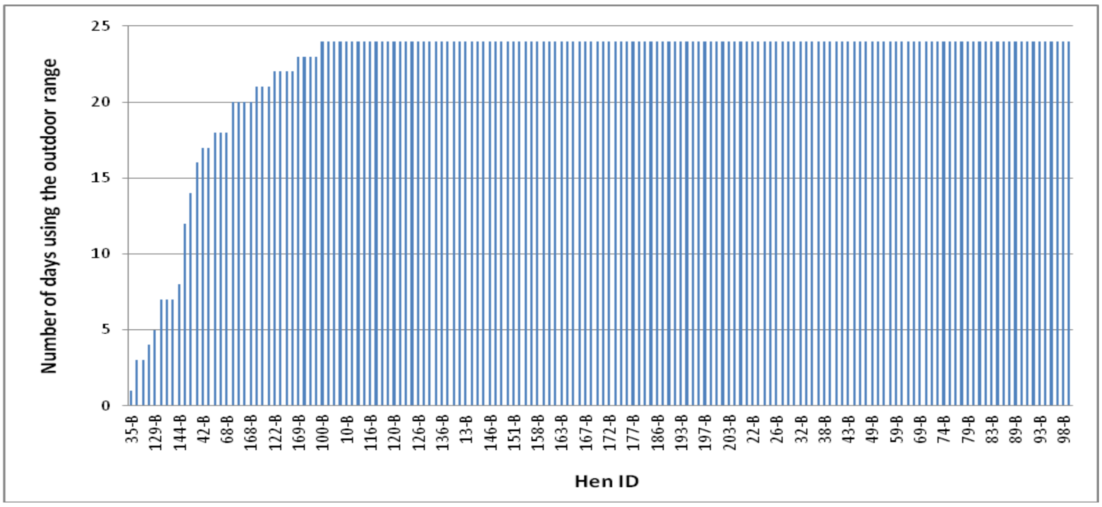


Figure 2. Number of days that individual hens were recorded as utilizing the range, using RFID technology. Data presented are for the hens that used the outdoor range in at least 1 day.

Birds that accessed the range on all days were further classified according to duration and frequency of visits as in Table 1.

Table 1. Characterisation of birds based on duration and number of visits to the range

Classification of range users	Average time outside (h)	Average duration per visit (h)	Average frequency of visits (n)	% of birds
Non-range users (NR)	0	0	0	16%
Daily range users				
Low frequency, Long visits (LL)	107	0.44	241	33%
High frequency, Short visits (HS)	90	0.18	491	30%

A total of 36 birds were selected from the three categories specified in Table 1 (12 per category). The twenty-four range users from LL and HS categories were characterised on the basis of frequency and duration of visits as shown in Figure 3.

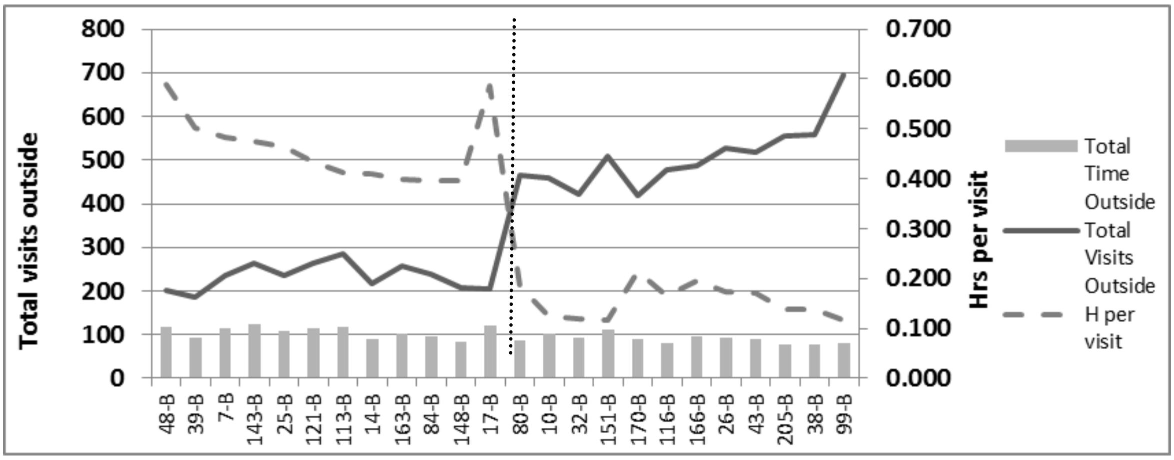


Figure 3- Selection of twenty four RFID-tagged hens from LL and HS categories

Performance and gut characteristics of subpopulations of birds

The average body weight gain was significantly higher ($P < 0.05$) by 48 g in LL and 16 g in HS as compared to NR birds after 28 days of trial (Table 2). Gizzard pH, although not significantly different, was lower for HS birds as compared to LL or NR.

Table 2. Performance and gut characteristics of sub-populations of hens based on range usage.

Sub-population	BWG (wk 63-67)	Gizzard pH	Gizzard weight (g)	Gut weight (g)	RGW	RGuW
LL	79^{a}	4.84	40.92^{b}	72.85^{a}	$2.18\%^{ab}$	$3.87\%^{a}$
HS	47^{ab}	4.77	46.45^{a}	74.84^{a}	$2.41\%^{a}$	$3.89\%^{a}$
NR	31^{b}	4.93	41.00^{b}	67.01^{b}	$2.05\%^{b}$	$3.36\%^{b}$
Pooled SEM	0.011	0.15	1.35	1.73	0.088	0.12
P-value	*	NS	**	**	*	**

*BWG=Average body weight gain, RGW=Relative gizzard weight (% of body wt, RGuW=Relative gut weight (% of body wt. Means in columns with no common superscript are significantly different *(P<0.05); **(P<0.01)*

Gizzard weight was significantly higher ($P < 0.01$) for HS birds compared to the NR and LL, while for gut weight (duodenum, jejunum, Ileum and caeca), both LL and HS were significantly higher than NR ($P < 0.01$). HS birds also showed significantly higher RGW (relative gizzard weights as % of body weight) (P<0.05) and RGuW (relative gut weight as % of body weight) (P<0.01) as compared to NR (Table 2).

Influence of range use on digestibility

Significantly improved coefficient of apparent ileal digestibility (CAID) of dry matter (DM) (P<0.05) and energy (P<0.05) was seen for LL birds, followed by birds in HS and NR populations. Ileal digestible energy (IDE) was also significantly higher (P<0.05) in LL, followed by HS and NR. No effect was however evident for the digestibility of nitrogen (N) between the categories (Table 3).

Table 3 . Influence of range usage on the ileal digestibility of dry matter, energy and protein (N).

	CAID			IDE
Sub-population	DM	Energy	Protein (N)	(MJ/kg)
LL	0.74^{a}	0.75^{a}	84.96	11.37^{a}
HS	0.68^{ab}	0.71^{ab}	82.37	10.70^{ab}
NR	0.63^{b}	0.64^{b}	78.31	9.69^{b}
Pooled SEM	0.027	0.03	2.07	0.45
P-value	*	*	NS	*

CAID (coefficient of apparent ileal digestibility), DM (dry matter), IDE (ileal digestible energy)
*Means in columns with no common superscript are significantly different * (P<0.05).*

Conclusion

Use of range resulted in lower body weight and body weight gain as compared to hens with no range access. Increased frequency of visits on range was directly related to the increased gizzard and gut weights. However, hens with lower frequency but longer duration of visits showed a significantly improved DM and energy digestibility. In conclusion, increasing range access is a promising strategy to improve performance and gut characteristics of free-range layers. Digestibility of birds with range access also showed an improvement, more so in birds that visited the range less frequently but for longer durations of time.

Chapter 5. Whole grain choice feeding approach in free range broilers improves performance and digestibility and heat stress management by modification of blood plasma characteristics

Introduction

Conventional diets typically consist of highly processed ground grains which are assumed to improve digestibility of the diet by increasing surface area and substrate availability to enzymatic digestion (Amerah and Ravindran, 2008). However, fine processing of the grains reduces the need for the gizzard's grinding function and can result in a dilation of the proventriculus and under-development of the gizzard (Singh et al., 2014). Provision of whole grains in the diet has been found to improve performance of birds through physical and functional mechanisms as a result of improvements in the development of the digestive tract (Erener et al., 2003; Gabriel et al., 2008; Ravindran et al., 2006; Svihus, 2012; Wu et al., 2011). By providing feed of varying particle size, the gizzard is challenged to increased contraction and muscular grinding, resulting in a larger, heavier gizzard. Improvements in the development of the intestinal segments have been variable across previous studies (Amerah et al., 2008; Gabriel et al., 2003; Gabriel et al., 2008). Improved digestive function may allow for a finer grinding of particles, greater accessibility of nutrients, and therefore a better utilisation of nutrients. In addition, improvements in the digestive capability of the bird may have some heat mitigation benefits and may allow birds to perform better under heat stress through a reduction in digestive heat production.

A free choice feeding strategy involves the provision of various feed ingredients and allows the birds to freely select between the feeds according to their preferences. Free choice feeding of chickens has been widely studied, and birds have been proven to be able to preferentially select feeds to adequately meet their nutrient requirements (Gous, 2005). However, their ability to preferentially select feeds to manage heat loads is unknown.

Conventional climate control systems are largely ineffective in a free range setting. The presence of pop-holes allows heat to flow freely between the internal and external environment, and internal shed temperature is essentially dictated by external shed temperature. Being an open system, free range systems typically experience higher ambient temperatures and more pronounced temperature fluctuations than conventional systems (Lima and Naas, 2005). These temperature fluctuations can exceed the thermo neutral zone of the bird and cause heat stress.

High ambient temperatures can lead to heat stress, resulting in increased mortality, increase in feed conversion ratio (FCR) and substantially lengthen the time for a bird to reach market weight. Therefore, as conventional climate control is not feasible in a free range system, alternative heat mitigation strategies such as nutritional interventions must be employed to minimise heat stress in broiler chickens kept in free range housing.

Birds will typically reduce feed intake when heat stressed to prevent excessive heat production (May and Lott, 1992). By providing a feed source that is lower in energy and protein than pellets, and therefore has a lower heat increment; it is thought that birds will preferentially select whole grain wheat when heat stressed to reduce the amount of heat produced during digestion. Consequently, birds may be able to maintain feed intake during heat stress and this may reduce the impact of birds going off feed. Choice feeding of whole grains has been attempted in previous studies, and has been found to facilitate sufficient whole grain inclusion levels in the diet to stimulate positive digestive tract developments (Amerah et al., 2008).

The hypothesis of this study is that provision of whole wheat grains will stimulate development of the digestive tract and improve the digestive capabilities of the bird while allowing it to better cope with heat stress.

Objectives

1. To identify the voluntary appetite for wholegrains by birds

2. To investigate whether WG intake can mitigate heat stress

3. To investigate whether FR broilers fed WG have more 'robust' GI tracts (esp. gizzard) that may improve their resilience to enteric disease and/or grass consumption etc.

Materials and Methods

Experimental plan

A total of 800 Cobb 500 day-old male broilers were obtained from a commercial hatchery, weighed at one day old and randomly allocated to pens. Fifty birds were placed in each of the 16 pens. Broilers were fed the same formulated starter diet *ad lib* for first two weeks. On d 14, Birds were randomly allocated to one of two treatment groups; birds fed complete pelleted diet (**P**), and birds fed pellets and choice fed whole grain wheat (**WG**). Each treatment consisted of eight replicate pens of approximately 50 birds allocated randomly.

Birds were housed in a completely enclosed environmentally controlled shed from 0 to 13 d of age. The lighting specifications were 23:1 (light: dark) hours for the first four days followed by 18:6 (light: dark) hours for the rest of the trial. Initial room temperature was set at 32C and was gradually decreased by 0.5ºC increments per day until birds were moved to the free range shed. Automatic heat lamps were provided in each pen to provide heat.

On d 14, birds were redistributed to sixteen free range housing pens with each pen consisting of 50 birds each, except two pens per treatment consisting of 48 birds per pen. Popholes were kept shut for one week before birds were given access to range on d 21. The range had a homogenous growth of young Kikuyu grass (*P. clandestinum*) as the main herbage and was mowed to about 6 cm before assigning the birds and not irrigated during the trial.

All birds were provided with identical starter crumble ration from 0 to 13 days of age (calculated nutrient levels : DM 90.23%, CP 20.9%, GE 17.64 MJ/kg DM), and a grower diet (calculated nutrient levels : DM 90.10%, CP 18.82%, GE 17.26 MJ/kg DM) from 14 to 42 d (Table 1). Ingredient composition and calculated nutrient values of diets as well as analysis of starter and grower diets and whole wheat for dry matter, crude protein, and gross energy are presented in Table 1.

Table 1. Ingredient composition and calculated nutrient values (g/kg as fed) for starter and finisher feed and whole wheat and analysed values (%DM).

Ingredient composition (%)	Starter	Grower	Whole Wheat
Wheat - Feed	63.63	65.12	
Meat/bone meal	4.30	0.0	
Canola meal Solv Ext	4.00	4.03	
Soybean meal 48	22.37	20.96	
Canola oil	3.18	5.91	
Sodium Bicarbonate	0.37	0.54	
DL Methionine	0.26	0.22	
Lysine HCl	0.32	0.50	
Threonine	0.10	0.10	
Limestone	0.89	1.05	
Dicalcium phosphate	0.00	1.01	
Choline chloride 60	0.06	0.06	
Vitamin/mineral premix*	0.50	0.50	
Rovabio Excel (NSP enzymes)	0.01	0.01	
Nutrient values (%)			
Crude protein	22.60	19.00	10.5
Calcium	0.90	0.75	0.05
Phos	0.63	0.61	0.35
Avail Phos	0.39	0.35	0.18
Fat	5.09	7.37	1.85
Fibre	2.88	2.79	2.45
Met	0.58	0.50	0.168
Cys	0.37	0.35	0.244
Me+Cys	0.95	0.85	0.412
Lys	1.30	1.29	0.294
Na	0.16	0.18	0.04
Cl	0.18	0.18	0.07
K	0.80	0.75	0.39
DEB (mEq/kg)	225.00	220.00	97.403
Poult AME MJ/kg	12.66	13.18	12.83
Analysed values (%)			
DM	90.23	90.10	89.96
CP	20.90	18.82	10.33
GE (MJ/kg)	17.64	17.26	16.38

**Supplied per kg of diet: retinol, 3,600 µg; cholecalciferol, 125 µg; α-tocopherol, 50 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 5 mg; cobalamin, 25 µg; niacin, 50 mg; pantothenic acid, 18 mg; folic acid, 2 mg; biotin, 200 µg; Cu, 20 mg; Fe, 40 mg; Mn, 110 mg; Co, 250 µg; I, 1 mg; Mo, 2 mg; Zn, 90 mg; Se, 300 µg; ethoxyquin, 125 mg.*

In addition to the grower diets, WG birds were also provided whole wheat grains in a secondary feeder (DM 89.96%, CP 10.33%, GE 16.38MJ/kg DM). The ingredient compositions of diets provided to each treatment were identical. Diets were provided ad-libitum in two separate feeders in each pen. For the P treatment, both feeders contained formulated pellets. For the WG group, one feeder contained pellets with a second identical feeder containing whole grain wheat. Whole wheat feeders were weighed at 11am and 3pm daily to measure intake of whole grains during the hottest part of the day.

Bird weights and feed intake were recorded on d 0, 7, 14, 21, 35, and 42, and used to calculate feed consumption, FCR, and weight gain between these periods as well as between d 14-42. On d 21 and 42, six birds per pen were selected and euthanized for sampling. Bird weight, gizzard pH, gizzard weight with contents, gizzard weight without contents, pancreas weight, jejunum length, ileum length, and caeca length were recorded. The contents of the distal half of the ileum were collected and Ileal contents were immediately frozen at -18ºC prior to being freeze-dried and ground to pass a 0.5mm screen.

On d 42, blood samples were taken from the birds prior to euthanization. Blood samples were analysed using an i-STAT® Analyzer (Abbott Laboratories, Abbott Park, IL, USA) along with Chem8+ (Abbott Laboratories, Abbott Park, IL, USA) chips to measure Na-, K+, Cl-, anion gap, base exchange extra cellular fluid, glucose, HCO_3^-, hemoglobin, hematocrit, pCO2, TCO2 and pH.

Internal shed temperature was measured using two dataloggers (Model 8829, Bacto Laboratories Pty Ltd, Mt Pritchard, NSW, Australia) placed inside the shed which measured ambient temperature and relative humidity hourly. External shed temperature data was taken from the Bureau of Meteorology Camden Airport AWS (068192).

Each pen was photographed individually using FLIR T620 Thermal Imaging Camera (FLIR Systems AB, Germany) and thermal images were analysed using ThermaCAM Researcher Pro 2.9 (FLIR Systems AB, Germany). The mean surface temperature of the heads of three birds per pen was used to estimate average bird core temperature.

Chemical analyses

The acid insoluble ash component of dried diets and ileal digesta samples were determined according to the method of Siriwan *et al*. (1993). The AID coefficient of DM, energy, N, and their digestible content were calculated as per Ravindran *et al*. (2005). The gross energy (GE) of diets and lyophilized digesta were determined using a Parr 1281 adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA) that was standardised with benzoic acid. Nitrogen concentration of samples was determined by the Dumas method using a FP-428 nitrogen analyser (LECO® Corporation, St. Joseph, MI, USA) as described by Sweeny (1989).

Data analysis

All data were exported to JMP v9.0 (SAS Institute, Cary, NC, USA) and subjected to analysis of variance. Means were considered significant at P<0.05 and were separated by LSMeans Differences Tukey HSD, where, levels not commected by same letter were significantly different.

Results

Performance

Whole grain inclusion in the diet had no significant impact on the live weights of birds throughout the trial (Table 2). However, feed intake was significantly affected by the introduction of wholegrain on d 14, with significantly higher feed intake between d 14-21 (P<0.001), and a significantly lower intake between d 21-42 (P< 0.001).

FCR showed a similar trend with significant higher value reported at d 21 (P<0.05) , but with lowered feed intake, improved significantly in WG birds on D42. FCR on d 42 was poor for both P and WG birds as compared to Cobb standard, with P birds showing almost 8 points higher than WG birds.

Table 2. Performance of pellet only (P) and pellet and whole grain (WG) birds and calculated weekly live weight, feed intake, weight gain, and feed conversion ratio (FCR) between days 14-21and 21-42.

Treatment	BWG (g/bird)		FI (g/bird)		FCR(g:g)		
	D14-21	D21-42	D14-21	D21-42	D14	D21	D42
p	466.6	2068.0	889.1	4347	1.42	1.68	1.98
WG	472.3	2084.3	946.0	4109	1.43	1.74	1.90
Pooled SEM	0.006	0.002	0.001	0.004	0.011	0.017	0.022
p-Value	NS	NS	**	***	NS	*	*

*P<0.05, ** P<0.01, ***P<0.001, NS Not significant

Digestive tract development

Gizzard pH (GpH) was significantly low for birds fed on WG on D42 (P<0.05) (Table 3). Gizzard weight with contents, gizzard weight without contents, and pancreas weight were standardised relative to total live weight of the bird. The jejunum, ileum and caeca lengths were also standardised to total length of gastrointestinal tract. The relative gizzard weights (both with and without contents: RGW and RGW-C) and relative pancreas weight (RPW) were not affected by supplementation of whole grain in the diet. Similarly, choice feeding of whole grain wheat did not significantly alter the relative proportions of the relative jejunum, ileum, and caeca lengths (RJ, RI, RC) when compared to birds fed on P diets on both days.

Table 3. Live weight, gizzard pH, gizzard weight with and without contents relative to live weight, and intestinal segment relative to total intestinal length on d 21 and 42 for pellet only (P) and pellet and whole grain (WG) birds

Treatment	BW (g)		GpH		RGW-C(%)		RPW(%)		RJ(%)		RC(%)		RI(%)	
	D21	D42	D21	D42	D21	D42	D21	D42	D21	D42	D21	D42	D21	D42
p	926	3098	2.30	3.19	1.98	1.00	0.35	0.15	40.0	39.8	18.2	20.0	41.6	40.1
WG	907	3065	2.31	2.86	2.05	1.04	0.37	0.15	40.6	39.6	17.7	20.1	41.6	40.2
Pooled SEM	13.9	43.2	0.07	0.10	0.04	0.02	0.01	0.00	0.38	0.28	0.26	0.22	0.50	0.30
p-Value	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

BW=bird weight; GpH= gizzard pH; RGW-C= relative gizzard weight without contents; RPW=relative pancreas weight; RJ= relative jejunum length; RI= relative ileum length; RC=relative caeca lengths; Means within rows with same superscript differ significantly at P<0.05, NS not significant , *P<0.05, ***P<0.001

Temperature

The mean surface temperature the heads of WG birds did not significantly differ to P birds (36.6C and 36.44C respectively, P=0.491).The average temperature from d 14-42 was consistently higher than recommended at 23.8C and fluctuated greatly, ranging from 20.79C to 26.93C (Figure 2). Similarly, maximum and minimum temperature was highly variable in the free range shed with temperatures ranging from 15.9C to 35.8C.

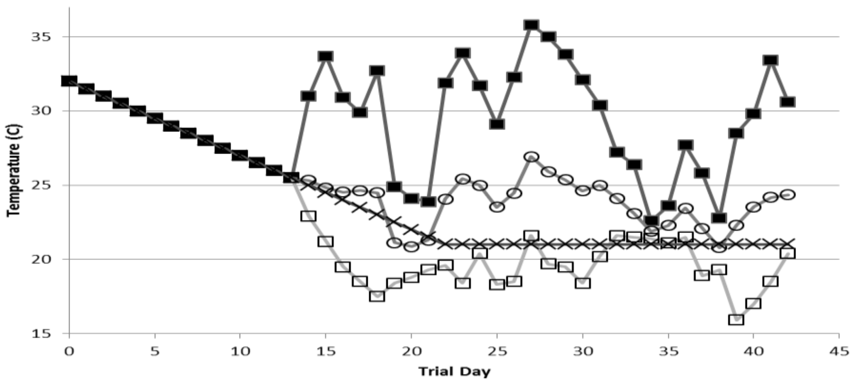


Figure 2. Internal shed temperature profile of both the deep litter shed (from d 0-13) and free range shed (from d 14-42) displaying internal ■ maximum temperature, □ minimum temperature, ○ average temperature, and x Cobb500 breeder recommendations

Whole grain intake

The proportion of whole grain wheat consumed relative to total feed intake was quite low (Table 4).

Table 4: Pellet and grain intake, total feed intake, proportions of whole grain wheat and pellets consumed relative to total feed intake, and the average weekly temperature for week 3, 4, 5 and 6 for birds in the WG treatment

Period	Feed intake (g/bird/week)	Pellet intake (g/bird/week)	Grain Intake (g/bird/week)	Pellet Intake (%)	Grain Intake (%)	Average Weekly Temperature (C)
Week 3	946c	883	63b^{c}	93.36^{ab}	6.64^{ab}	33.7^{c}
Week 4	1322b	1271	52^{c}	96.08^{a}	3.92^{b}	35.8^{a}
Week 5	1324b	1218	106^{ab}	92.01^{ab}	7.99^{ab}	35^{b}
Week 6	1463a	1333	130^{a}	91.00^{b}	9.00^{a}	33.4^{d}
Pooled SEM	32.3	38.5	13.0	1.06	0.106	0.001
p-Value	***	***	***	**	**	***

Means within rows bearing the same superscripts differ significantly at P<0.05, *P<0.05, ***P<0.001, NS Not significant

The proportion of whole grains significantly differed by Week (P<0.001) with a steady increase seen as birds got bigger and more familiar to whole grain provision. Whole grain intake varied per pen across weeks and ranged from ~1.8% to 14.19% of total feed intake. The proportion of grain consumed in week 6 was significantly higher than in week 5 (P<0.001), which in turn was significantly higher than weeks 3 and 4(P<0.001). Lowest consumption of whole grains occurred in week 4 when the average temperature was highest. Grain intake per bird recorded between 11am and 3pm (hottest time of the day) ranged from 0-10g/bird across the trial.

Grain intake had a strong negative correlation with maximum temperature and decreased as temperature increased (Figure 3).

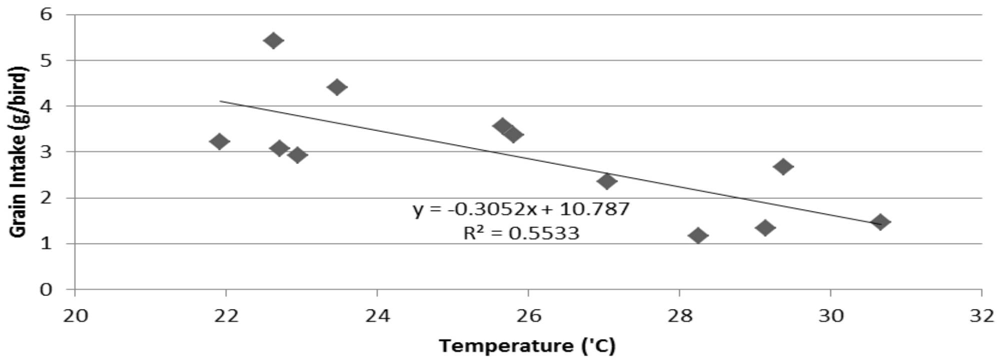


Figure 3: Correlation between whole grain intake (g/bird) between 11am and 3pm and temperature

Blood parameters

Blood samples collected on d 42 showed elevated levels for K, Cl, BEecf, pH, HCO_3^-, TCO_2, and Haematocrit in birds on both P and WG diets (Table 5) indicating the inability of birds to maintain homeostasis under stressful conditions and some level of heat stress for all birds in the shed. However, birds fed WG showed more normal values than birds fed just pellets for all blood parameters (standard ranges for chicken using the i-STAT as specified by Steinmetz et al, 2007).

Table 5. i-STAT blood parameters for pellet only (P) and pellet and whole grain (WG) birds measuring Na, K, Cl, anion gap, glucose, base exchange extra cellular fluid (BE ecf), pH, HCO3-, TCO2, pCO2, haemoglobin, and haematocrit. The standard range as specified for chickens by the i-STAT analyzer is also included (Steinmetz et al, 2007).

Item (mEq/L)	Range	P	WG	Pooled SEM	Significance
Na	138-146	145.4	144.8	0.33	NS
K	3-5	5.227	5.204	0.076	NS
Cl	96-106	112.2	112.7	0.50	NS
Anion Gap	10-20	10.95	10.85	0.41	NS
Glucose	13-14	13.69	13.18	0.11	**
BEecf	0 ± 3	5.104	3.729	0.50	NS
pH	7.35-7.45	7.501	7.513	0.012	NS
HCO_3^-	22-26	27.94	26.42	0.48	NS
TCO_2	23-27	28.88	27.31	0.50	*
pCO_2	25-45	35.41	32.73	1.11	NS
Haemoglobin (g/dL)	7-11	8.32	7.76	0.17	*
Haematocrit	38-51	24.4	22.8	0.52	*

*P<0.05, ***P<0.001, NS Not significant

Birds fed WG diet had significantly lower glucose (P<0.01), TCO_2 (P<0.05) and haemoglobin (P<0.05) levels than birds on diet P. Although not significantly different, whole grain inclusion resulted in the management of Na as compared to birds fed P diets by maintaining the allowed range for these parameters.This is indicative of a difficulty of birds to maintain homeostasis under stressful conditions and all birds suffered some level of heat stress.

Digestibility

Overall, coefficients of apparent ileal digestibility for DM, energy and nitrogen were very low (Table 6) and could be attributed to the heat stress condition of the birds. Apparent dry matter digestibility (P<0.05), digestibility of energy (P<0.001), protein (N) (P<0.01), and ileal digestible energy content (P<0.001) were all significantly lower in the WG treatment when compared to the P treatment.

Table 6. Apparent dry matter (DM) digestibility, dry matter digestibility coefficient, digestible energy content, digestible energy coefficient, digestible nitrogen content, and digestible nitrogen coefficient of the ileal contents of pellet only fed (P) and pellet and whole grain fed (WG) birds on d 42

Treatment	CAID			IDE (MJ/kg)
	DM	Energy	Protein (N)	
p	0.57	0.59	0.73	10.35
WG	0.54	0.44	0.71	7.14
Pooled SEM	0.008	0.018	0.61	0.35
p-Value	*	***	**	***

*P<0.05, **P<0.01, ***P<0.001, NS Not significant

Conclusion

Despite the relatively low intake of whole wheat throughout the trial, there was still a substantial dilution effect of the whole grains on the ileal digestible energy and protein in birds fed whole grains. However, despite the overall lower feed intake, and less digestible feed, WG treatment birds were still able to maintain a comparable live weight to birds in the P treatment. The improved FCR of birds fed whole grains, could be attributed to either an improvement in the utilisation of nutrients or a decrease in the energy use for non-growth functions such as heat dissipation.

Whole grain inclusion improved the bird's ability to cope with heat stress. All birds suffered from heat stress to some extent and struggled to maintain homeostasis; however birds were able to maintain body temperature despite high ambient temperatures. Whole grain inclusion did not decrease susceptibility to heat stress but did reduce energy spent on thermoregulation resulting in performance improvements. Free choice feeding may not have been the best option for birds to consume a sufficient amount of whole grain wheat to stimulate development of the digestive tract of the bird.

Chapter 6. Effect on broiler performance and digestibility with and without range access, in-feed antibiotic and mannan-rich fraction of carbohydrate

Introduction

Australian free range broiler production has two major differentiation points from conventional broiler production; range access and no in-feed antibiotics as growth promoters. Weeks et al. (1994) demonstrated that free range broilers had significantly lighter body weight (4.08 ± 0.08kg) than conventionally reared broilers (4.49 ± 0.08kg) at ten weeks of age. This performance gap has been observed in a long term commercial comparison study as a 2-3% increase in mortality, 10-15 points increase in feed conversion ratio. Free range birds required 2.5 days more to reach 2.45 kg target body weight (Durali et al., 2012). It also has been reported that there is a negative correlation between range usage and body weight gain of free range broilers. Durali et al. (2014) demonstrated that birds used range for more hours had lower body weight gain. This 'performance gap' is not totally understood but is thought to be as a result of variable pasture consumption, nutritional inadequacy and poorer digestive health. The earlier trial conducted to decipher the impact of in-feed antibiotics and range access did not result insignificant differences in performance. It was therefore considered appropriate to revisit that aspect of the project. In addition to that, we were also able to test if the addition of mannan-rich fraction of carbohydrate would be useful to compensate for the absence of in-feed antibiotics for free range broilers. The difference in performance of free range vs. conventional production system is either due to the absence of antibiotic growth promotants (AGP) in diet and/or access to range area which includes variable pasture consumption, nutritional inadequacy and poorer digestive health or the combination of these factors.

Objectives

1. Investigate the effect of range access and non-range access on bird performance and digestibility.

2. Investigate the effect of in-feed antibiotics vs mannan-rich fraction of carbohydrates (Actigen®; Alltech Australia, Melbourne)

3. Determine any interactions between the diets and range access on performance and digestibility of free range broilers.

Materials and Methods

A total of 1920 day-old Ross 308 chicks were obtained from a commercial hatchery. The birds were sexed then mixed and 32 males and 32 females were placed into each of 30 pens. Each pen was of 6.5 m^2 floor space and contained two tube feeders and one bell drinker. Brooding was maintained using gas-fired space heaters with shed ambient temperature starting at 32^oC and target temperature was decreased by 1^oC every second day until 21^oC was reached at d 21. Range access was provided from d 21 for all pens on the southern side of the shed. The range area for each pen is 9.75 m^2 or 1.5 times the pen size. The main herbage on range was a homogenous growth of young Kikuyu grass (*P. clandestinum*), which was mowed to about 6 cm before assigning the birds and not irrigated during the trial.

A standard diet with no Zn Bacitracin (but with an anticoccidial) was produced at the Poultry Research Foundation feed mill to which treatments were added as follows:

- Standard diet only, no range access
- Standard feed only with range access
- Standard diet plus Zn Bacitracin, no range access
- Standard diet plus Zn Bacitracin, with range access
- Standard diet plus Actigen, no range access
- Standard diet plus Actigen with range access

Zn Bacitracin (source: Albac G 150 antibiotic feed premix, Pfizer Australia Pty Ltd) was added at 50g/tonne that included it for the treatment diets.

Starter, grower and finisher diets were produced for each feed and labelled A (standard diet - control), B (standard diet including Zn Bacitracin (50g/tonne of feed)) and C (standard diet including Actigen® (400g/tonne)). Diets were fed at Starter 0.7 kg per bird, Grower 1.2 kg/ bird and Finisher thereafter to trial completion.

Table 1. Ingredient composition (%, as fed) and calculated nutrients of standard starter,grower and finisher diets .

Ingredient Composition (% , as fed)	Starter	Grower	Finisher
Whole Wheat12.5%12.8MJ	0	15	15
Wheat12.5%12.8MJ	46.08	28.42	30.74
Sorghum 10.5%	21.15	28.27	28.08
Meat/bone meal	6.82	7.1	5.41
Soybean meal 48	16.87	5.32	3.77
Canola meal (solvent extracted)	4	10	10
Sunflower oil	1.21	2	2.99
Limestone	0.25	0.17	0.32
Salt	0.03	0	0
Sodium Bicarbonate	0.51	0.48	0.48
Lysine HCl	0.37	0.48	0.48
DL Methionine	0.3	0.28	0.27
Threonine	0.11	0.19	0.17
Choline chloride 60	0.07	0.06	0.06
Xylanase	0.02	0.02	0.02
Vitamin/mineral premix*	2.2	2.2	2.2
Phytase 500FTU	0.01	0.01	0.01
Calculated nutrients			
Dry Matter %	89.34	89.46	89.46
Poult AME (MJ/kg)	12.5	12.9	13.2
Crude protein %	22.3	19.5	18.0
Calcium %	0.9	0.9	0.8
Avail Phos %	0.47	0.47	0.39
Lys%	1.32	1.18	1.10
Met %	0.63	0.58	0.54
Me+Cys %	0.98	0.90	0.85
Na %	0.22	0.20	0.19
Cl %	0.21	0.21	0.20
K%	0.71	0.56	0.52
DEB (mEq/kg)	214	210	210

**Supplied per kg of diet: retinol, 3,600 µg; cholecalciferol, 125 µg; α-tocopherol, 50 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 9 mg; pyridoxine, 5 mg; cobalamin, 25 µg; niacin, 50 mg; pantothenic acid, 18 mg; folic acid, 2 mg; biotin, 200 µg; Cu, 20 mg; Fe, 40 mg; Mn, 110 mg; Co, 250 µg; I, 1 mg; Mo, 2 mg; Zn, 90 mg; Se, 300 µg; ethoxyquin, 125 mg.*

Birds were weighed on a pen basis at placement and on d 7, 14, 21, 28, 34 and 41. Feed consumption was measured at each weighing occasion and FCR calculated. Any bird which died or was removed and euthanized was weighed and necropsied and the results were recorded.

Chemical analyses

The acid insoluble ash component of dried diets and ileal digesta samples were determined according to the method of Siriwan *et al*. (1993). The apparent ileal digestibility coefficient of DM, energy, protein (N) , and the ileal digestible energy content were calculated as per Ravindran *et al*. (2005). The gross energy (GE) of diets and lyophilized digesta and nitrogen concentration of samples was determined as specified in the 'General Methodology' section.

Data analysis

All data were exported to JMP v9.0 (SAS Institute, Cary, NC, USA) and subjected to analysis of variance. Means were considered significant at $P<0.05$ and were separated by LSMeans Differences Tukey HSD, where, levels not connected by same letter were significantly different.

Results

Performance

Starting weights for each group were a close average. Feeds with either Zn Bacitracin (B) or Actigen® (C) had significantly higher weights than the non-medicated control group (A) from d 0-14 and then from d 22-28. Although there was a slight, but significant difference in weight for birds of range vs non-range access group, it could not be attributed to the treatment since there was no range access before 21 days but was probably in reaction to the construction work outside the southern end of the shed. However the weights of the birds in either access group were not different, after range access was allowed on d 21(Table 1). The same effect was visible in weekly weight gains (Table 2).

Table 2. Average weight gain at ages by feed treatment and range access (after 21 days)

Feed	Range Access	Average Gain (gm) per period					
		Days 0-7	Days 8-14	Days 15-21	Days 22-28	Days 29-34	Days 35-41
A	**No**	122.7	277.3	456.7	545.2	599.2	775.3
	Yes	114.9	273.6	469.6	544.5	576.8	775.9
B	**No**	130.6	300.5	453.5	601.4	600.8	752.4
	Yes	126.2	297.7	490.1	568.9	563.8	800.7
C	**No**	131.2	290.5	465.7	607.2	569.0	771.2
	Yes	127.1	296.6	486.7	598.1	592.7	743.4
	P=	*0.61*	*0.97*	*0.75*	*0.53*	*0.25*	*0.28*
A		118.8 [b]	275.5 [b]	463.2	544.8 [b]	588.0	775.6
B		128.4 [a]	299.1 [a]	471.8	585.1 [b]	582.3	776.5
C		129.2 [a]	293.5 [a]	476.2	602.7 [a]	580.8	757.3
	P=	*<0.01*	*<0.001*	*0.71*	*0.002*	*0.91*	*0.66*
	No	128.2 [a]	289.4	458.7	584.6	589.7	766.3
	Yes	122.8 [b]	289.3	482.2	570.5	577.8	773.3
	P=	*<0.01*	*0.51*	*0.08*	*0.25*	*0.44*	*0.72*

A = Standard Diet-control; **B** = Standard diet+50ppm Zn Bacitracin; **C** = standard diet+Actigen®

[a, b, c] Means with different superscripts differ significantly (P<0.05)

In terms of weekly weight gain, birds on feeds B and C gained more weight than the controls (A) over the first 2 weeks and birds on feed C gained more than either of the other groups in the fourth week (22-28 days). Thereafter weekly gains in all groups were similar.

Feed intake per bird (Table3) was similar between groups up to 7 days. From 8 to 14 days the feed groups (B and C) consumed more feed per bird per day than the control feed group (A) but thereafter there was no significant difference in daily feed intake between any of the feed groups although group A continually had the lowest feed intake. Between days 15 and 21 the birds destined for range access consumed more feed per bird per day than those that would remain confined. This may have been a compensatory intake after the slower growth seen in the first week.

Table 3. Average weekly feed intake by feed treatment and range access (after 21 days)

Feed	Range Access	Feed intake (gm) per bird per day in period				
		days 0-7	days 8-14	days 15-21	days 22-34	days 35-41
A	**No**	19.9	50.2	88.4	148.1	201.7
	Yes	19.5	48.7	91.3	148.2	195.4
B	**No**	19.6	52.8	90.5	152.3	203.4
	Yes	20.1	51.8	95.4	150.2	212.6
C	**No**	20.1	52.4	89.4	157.1	203.4
	Yes	20.4	52.3	95.1	146.4	214.7
	P=	*0.13*	*0.42*	*0.65*	*0.29*	*0.44*
A		19.7	49.4 [b]	89.9	148.2	198.5
B		19.9	52.3 [a]	93.0	151.3	208.0
C		20.3	52.3 [a]	92.3	151.8	209.0
	P=	*0.06*	*<0.01*	*0.13*	*0.55*	*0.31*
	No	19.9	51.8	89.5 [b]	152.5	202.8
	Yes	20.0	50.9	94.0 [a]	148.3	207.5
	P=	*0.59*	*0.051*	*0.002*	*0.16*	*0.45*

A = Standard Diet-control; **B** = Standard diet+50ppm Zn Bacitracin; **C** = standard diet+Actigen®
[a, b, c] Means with different superscripts differ significantly (P<0.05)

FCR's differed at several points (Table 4). Over the first 7 days, FCR for the birds that would eventually have range access was poorer, again reflecting the slower growth rate in this section with no difference in feed intake (Tables 1 and 2) but FCR for the range access and no range access groups was not different thereafter. Feed additives (groups B and C) improved FCR over days 0-7. Extending this to day 14 however showed an FCR advantage to only the Zn Bacitracin additive group.

Table 4. Feed Conversion Ratios (FCR) by feed treatment and range access (after 21 days)

Feed	Range Access	FCR (cumulative) over periods					Corrected for mortality
		days 0-7	days 0-14	Days 0-21	Days 0-34	Days 0-41	Days 0-41
A	**No**	1.140	1.109	1.248	1.493	1.657	1.646
	Yes	1.236	1.111	1.250	1.510	1.674	1.648
B	**No**	1.054	1.073	1.237	1.477	1.661	1.651
	Yes	1.096	1.087	1.234	1.505	1.690	1.668
C	**No**	1.074	1.101	1.227	1.519	1.690	1.668
	Yes	1.111	1.098	1.240	1.446	1.676	1.651
	P=	0.36	0.50	0.89	0.06	0.43	0.47
A		1.188 [a]	1.110 [a]	1.249	1.502	1.666	1.647
B		1.075 [b]	1.080 [b]	1.235	1.491	1.676	1.660
C		1.093 [b]	1.099 [a]	1.234	1.482	1.683	1.660
	P=	<0.01	<0.01	0.64	0.70	0.61	0.58
	No	1.089 [b]	1.094	1.237	1.496	1.670	1.655
	Yes	1.148 [a]	1.099	1.241	1.487	1.680	1.656
	P=	<0.01	0.46	0.79	0.61	0.47	0.96

A = Standard Diet-control; **B** = Standard diet+50ppm Zn Bacitracin; **C** = standard diet+Actigen®
[a, b, c] Means with different superscripts differ significantly (P<0.05)

FCR over the whole trial did not differ significantly between any groups however even after correction for mortality.

Digestibility

Significant differences were seen in the digestibility of nutrients with the addition of additives, both Zn Bacitracin and Actigen®. However, there were no significant interactions noted between range access and the addition of additives on digestibility measures.

Both treatment groups (B and C), performed better than the control group (A) for apparent ileal digestibility of dry matter, energy and protein (N) as well as ileal digestible energy content. In all cases Actigen® was able to compensate for Zn Bacitracin by showing significant improvement as compared to control. However, this improvement in digestibility was not reflected in improvement of FCR. This could be attributed to significantly lower apparent ileal nitrogen digestibility for birds when given access to range (Table 5).

Table 5. Effect of range access and feed additives on the coefficient of apparent ileal digestibility (CAID)of dry matter, energy and protein (N), and Ileal digestible energy (IDE) on d42.

Feed	Range Access	CAID			*IDE*(MJ/kg DM)
		Dry Matter	Energy	Protein (N)	
A	**No**	0.61	0.60	0.66	11.34
	Yes	0.63	0.61	0.62	11.48
B	**No**	0.75	0.74	0.77	14.65
	Yes	0.76	0.74	0.74	14.79
C	**No**	0.66	0.66	0.70	13.09
	Yes	0.65	0.65	0.65	12.86
	***P*=**	0.55	0.55	0.54	0.39
A		0.60^{c}	0.60^{c}	0.64^{c}	11.42^{c}
B		0.74^{a}	0.74^{a}	0.76^{a}	14.71^{a}
C		0.65^{b}	0.65^{b}	0.67^{b}	12.96^{b}
	***P*=**	<0.001	<0.001	<0.001	<0.001
	No	0.66	0.67	0.71^{a}	13.15
	Yes	0.67	0.66	0.67^{b}	12.98
	***P*=**	0.97	0.97	<0.001	0.89

A = Standard Diet-control; **B** = Standard diet+ Zn Bacitracin; **C** = standard diet+Actigen®
[a, b, c] Means with different superscripts differ significantly (P<0.05)

Conclusion

Inclusion of Zn Bacitracin or Actigen® in the standard free range (control) diets improved growth rates in both range access and non-range access birds. Actigen® provided similar growth to an antibiotic feed additive and superior weight gain to a control diet.The improved growth rates afforded by Actigen® and Zn Bacitracin was achieved primarily from improved growth rate in the first 2 weeks of life. FCR was also improved by Zn Bacitracin and Actigen® but only over the first 2 weeks of life. Over the entire trial period FCR was not different between feeds nor between range access or full confinement. Allowing range access in this trial did not affect growth rate. Actigen® provided effects on nutrient digestibility that were not as significant as the antibiotic feed additive but were superior to the control diet. Range access showed lower protein digestibility in birds as compared to those with no range access. However, surprisingly these differences in ileal digestibility between treatments did not have any effect on growth rate, feed intake or FCR. Further studies on effect of materials that are ingested on range may be able to explain this effect.

General Discussion

Free-range broilers and layers are less efficient converters of feed into saleable meat and eggs and generally have higher mortality than conventionally-reared poultry. In broilers, the performance gap has been quantified as a 10-12 point increase in FCR and a 2-3% increase in mortality in free-range compared with conventionally-reared birds (Durali et al., 2012) (this does not consider the intake of DM from the range by some birds). This Poultry CRC project aimed to establish the principle reasons for the performance gap between free-range and conventionally reared broilers and layers and evaluate a range of nutritional interventions that would reduce the magnitude of the effect of this on production.

In the first instance the project looked at delineating the contribution of two factors that are the core of free range production in Australia: absence of in-feed antibiotics and exposure to range from d 21 onwards. This involved a factorial study to assess the magnitude of response to AGP and range challenge and interaction between the two (Chapter 1). During the experiment birds were observed to consume grass during their time on range. N-alkanes were successfully used to estimate the amount of grass consumed by free range broilers on range. An advantage of using naturally occurring n-alkanes is that it eliminates the need for using artificial dosing with external markers. Moreover, this methodology not only allows an estimate of the diet composition, but also partitions the total intake into its component plant species which is useful for future mixed pasture studies (Almeida et al., 2012; Antell and Ciszuk, 2006; Horsted et al., 2007; Milby, 1961; Ponte et al., 2008b)

In Chapter 1, average body weight and body weight gain in birds reared on conventional system was significantly higher as compared to birds reared on free range. However, feed intake was lower in free range birds thus leading to a lower FCR in comparison to conventionally reared birds. However, taking into consideration the grass consumption, the total feed intake increased significantly by about 4.07% ($P < 0.01$) and FCR which showed a 9-11 point increase for birds on range.The higher weight seen for conventional birds was in agreement with a number of studies that have earlier reported results for weight gain when compared between the two production systems (Lazzari et al., 2007; Milosevic et al., 2005; Pavlovski et al., 2009; Ward et al., 2001). Milosevic et al. (2005) also reported a lower FCR for free range birds (2.35) as compared to chicken coop birds (2.46) which is a similar trend to this study. The lower body weight in free range birds may be attributed to lower intramuscular and abdominal fat due to use of range area (Mikulski et al., 2011). The significant depression in body weight gain of free range birds could also be attributed to consumption of grass and other materials (rocks, soil, seeds etc.) on range that may result in dilution of whole diet and lead to nutritional deficiencies. Moreover, lower feed intake and FCR in free range birds are a result of the non-inclusion of diet components ingested on range, especially grass and other pastures in these calculations.

For free-range broilers, alkane concentrations in the digesta was measured and compared with alkane profiles of the pasture in order to estimate total DM intake from the range area. An estimated 13.5-14.7% of total “as fed” intake by free range broilers in this study was contributed to grass consumption. Considering the number of hours the birds spent on the range and the average feed intake from d21-42, this equates to 6.34-6.78 g of grass per bird per hour of range access.

Diets intended for consumption by free-range birds are not routinely formulated to accommodate the modifying effects of grass consumption on digestible nutrient intake. For example, grass has a very low metabolisable energy for broiler chickens, diluting AME by around 0.21 MJ/kg for every 2% grass consumed. Furthermore, grass contains a high K concentration and its consumption increases DEB by around 20mEq/kg for every 2% grass consumed. The implications of these changes in dietary nutrient supply were explored in subsequent trials (Chapter 2 and 3) where both standard and high energy density broiler diets were systematically diluted with grass (0-10% inclusion) and two different levels of DEB respectively. The outcomes of this work shed light on possible nutritional contributions to the relatively poor performance of free-range broilers in Australia. Inadvertent changes in either dietary energy density or in DEB may be of importance, especially during summer months where DEB balance becomes critical to control metabolic alkalosis.

Chapter 2 explained the significant negative effect of incremental levels of grass inclusion which was translated into a decline in feed intake and lower body weight at day 28. The low BWG and FCR of chicken fed a diet containing 40 g/kg grass or more could be attributed to reduced supply of energy and nutrients caused by low FI. Feed formulated to meet the nutrient requirement of chicken by increasing its density by +0.42 MJ/kg energy showed only a slight improvement for low levels of GI, but showed a similar trend of declining performance to the STD diet at higher GI levels. Lower FI could also be related to poor digestibility coefficients for DM, energy and N and also for ileal digestible energy content. Although a number of studies looking into poultry feeding and nutrition have traditionally showed deleterious effects of diets with fibre on performance and digestibility (Janssen and Carré (1985), Rougière and Carré, 2010, Mateos et al., 2012), some recent studies have suggested that inclusion of moderate amounts of fibre source in the diet improves digestive organ development and acid and enzyme secretions leading to improvement of nutrient digestibility (González-Alvarado et al., 2007; Jimenez-Moreno et al., 2009; Mateos et al., 2012; Sklan et al., 2003; Svihus, 2011). This phenomenon was observed with up to 40 g/kg GI in the present study.

Chapter 3 outlines the effect of modifying DEB values in response to increased K levels due to grass consumption. This trial was conducted in hot summer months and body surface temperatures in birds rose with a rise in day temperatures in all treatment groups, with the highest seen in birds inside the shed. Birds fed diets consisting of low DEB levels and high density showed a significant weight gain ($P < 0.001$) and an improved FCR ($P < 0.01$),while dry matter digestibility also improved. An effect of lower levels of DEB were seen with significantly lower feed intake ($P < 0.05$) for both low and high density diets and improved digestible nitrogen content and plasma levels of Na, Cl and Ca . High density diets increased blood pH by 0.24 and caused depletion of K from the optimal 5mmol/L, both indicative of respiratory alkalosis. Thus, lower DEB and HD diets significantly improved performance and digestibility parameters, indicating it be a good strategy to address the consequences of grass consumption on range. The importance of DEB in heat stress conditions was also reiterated by Ahmad and Sarwar (2006) who reviewed the effects of DEB in heat stressed broilers and concluded that DEB affects the bird's performance. High DEB levels caused by imbalance of electrolytes were known to cause metabolic alkalosis (Mushtaq and Pasha, 2013; Abdallah et al., 2011; Ahmad and Sarwar, 2006). Thus feed formulations with appropriate DEB levels and diet densities need to be designed for birds exposed to range access.

Whole grain consumption improved bird performance (Chapter 5). However, free choice feeding may not have been the best option for birds to consume a sufficient amount of whole grain wheat to stimulate development of the digestive tract of the bird. Poor consumption rates are likely due to the method of presentation of WG in the diet. High intake rates in previous studies were obtained using mixed feeding, sequential feeding, and restricted feeding strategies (Erener et al, 2003; Ravindran et al, 2006; Svihus et al, 2004). In contrast, although successful, a free choice feeding strategy results in inconsistent and highly variable whole grain intake rates. Free choice feeding in the Erener et al (2003) study resulted in very low whole grain intake between days 7-14 at 0%, which increased to 32% of the total feed consumption at the end of the trial. However, overall grain consumption accounted for only 18% from the point of introduction to the end of the trial. Poor consumption rates of whole grains may also be due to the concentration of nutrients in the pellets, which are more capable of successfully meeting the bird's nutrient requirements. This may have prevented high grain intake as the birds requirements were satisfied with the pellets. It may also be that the structural fiber need of the birds was satisfied by grass inclusion.

In this study, gizzard pH of WG birds was significantly lower than P birds on day 42 but not on day 21 and is consistent with the results of previous whole grain feeding studies (Gabriel et al, 2003, Engberg et al 2004). This decrease in pH is likely due to an increase in HCl secretion from the proventriculus into the gizzard to solubilise nutrients and improve digestion. It is likely that the lack of development of the digestive tract experienced in this trial may be due to the relatively low whole wheat inclusion levels in the diet. In the Gabriel et al (2008) study, physical improvements were observed when whole wheat was included at a rate of 200g/kg-400g/kg. It has been recommended that a minimum of 20% grain inclusion with particles larger than 1.5-2.0mm in size are adequate to stimulate development of the gizzard (Svihus, 2011).

The dissimilarity between the breeder recommendations for Cobb 500 birds of housing ambient temperature at 21^{0}C from day 21 and the actual average temperature in the free range shed, highlight the suboptimal growth conditions on free range systems. Fluctuations in maximum and minimum temperature as the trial progressed highlight inability of free range systems to maintain internal shed temperature. Whereas conventional systems may be completely enclosed, opening up pop-holes in free range systems can result in a disruption of the internal shed temperature and heat flows freely between the internal and external environment. Clearly, internal and external maximum temperatures were very similar and frequently exceeded the thermoneutral zone of the bird. Temperatures above the thermoneutral zone of the bird result heat stress, and an increased investment of energy into heat dissipation.

Physiological responses of the bird to heat stress were able to be measured by i-STAT blood analysis. Many of the blood parameters were higher than normal ranges and indicated the inability of the bird to maintain homeostasis under stressful conditions. As birds lack sweat glands, the main form of heat loss occurs through panting to increase evaporative cooling. Hyperventilation may have resulted result in a depletion of CO_2 in the blood, thereby causing an increase in blood pH or respiratory alkalosis. Evidently, blood pH was raised and was slightly higher than the normal range. The high HCO_3^- levels and only slight increase in blood pH suggest the effects of respiratory alkalosis may have been compensated for by endogenous release of HCO_3^- which buffers changes in blood pH. The increase in Cl^- levels is likely due to heat stress induced respiratory alkalosis resulting in an increased requirement of Cl^- and HCO_3^- to normalise blood pH (Ruiz Lopez and Austic, 1993).

Actigen (Alltech, Inc., Nicholasville, Kentucky, USA) is a second-generation, yeast cell wall product considered to be a "growth permitter" through its roles in immune modulation and improved intestinal health. Inclusion of Zn Bacitracin or Actigen in the standard free range (control) diet improved growth rates in both range access and non-range access birds (Chapter 6). Actigen provided similar growth to an antibiotic feed additive and superior weight gain to a non-medicated control ration under very good health conditions.The improved growth rates afforded by Actigen and Zn Bacitracin was achieved primarily from improved growth rate in the first 2 weeks of life.FCR was also improved by Zn Bacitracin and Actigen but only over the first 2 weeks of life. Over the entire trial period FCR was not different between feeds nor between range access or full confinement. Allowing range access in this trial did not affect growth rate. Actigen provided effects on nutrient digestibility that were not as significant as the antibiotic feed additive but were superior to a non-medicated control diet. Birds with range access showed lower nitrogen digestibility as compared to those with no range access.

A multi-modal distribution of free range layers resulted, with a proportion of the flock that use the range routinely and a proportion that use the range rarely, if at all (Chapter 4). Functionality of the digestive tract in birds is pivotal for optimal performance, and access to the outdoor range appears to influence digestive function. Use of the range resulted in higher body weight gain compared to birds with no range access, probably due to increased gut modulation and nutrient utilisation. Increased frequency of visits on the range was directly related to increased gizzard and gut weights.

Forage and grit stones ingested on range are structural components which strongly stimulate gizzard development (Steenfeldt et al. 2007, Hetland et al. 2003). Thus, it is logical to assume that birds in a free-range system will benefit from a more developed gizzard, which will potentially improve nutrient utilization and gut health. It is also possible that access to an outdoor area will increase retention time in the crop, and thus potentially improve efficacy of the digestion process (Svihus, 2012).

Birds with lower frequency but longer duration of visits (LL) showed a significantly improved DM digestibility and ADE. However, the digestibility values for birds that had access to the range did not account for the ingestion of grass, soil, grit, insects and other components of the range. These might have a different AIA value to the 20 g/kg added in the complete diet and may therefore have some implications in the digestibility estimates reported in this study. Steenfeldt et al. (2007) found high rates of fiber digestibility in layer hens fed large amounts of roughage. The above-mentioned strong adaptive capacity of the caeca may also result in significant increases in fermentative capacity of layer hens kept for long periods with access to forage.

Finally, this project successfully demonstrated the significance of range access, specifically grass intake in free range broilers and its implications and the challenges the need for diet adjustments to balance the overall nutrient intake. The challenge of less than ambient climatic conditions for birds with range access has also been demonstrated and nutritional strategies tested to mitigate those effects. The use of modern technology to monitor range access lead to better understanding of bird behaviour and difference in performance that can be used for further interventions. Feed additives to compensate the lack of inclusion of in-feed antibiotics was also explored within this project.

Implications

The performance gap between free-range and conventional production systems is costly to both the producer and the environment. In the Australian broiler and layer industry alone, a 1 pt improvement in FCR is worth an estimated $6.5m per annum and saves the industry almost 20,000 tonnes of feed. The production gap as identified in Trial 1 due to grass consumption, accounted for about 9-11 points in FCR. Interventions using higher energy diets in trial 2 were able to compensate for upto 4% grass consumption by improving FCR as compared to higher levels of grass which led to an increase of FCR by 12-14 points. By modifying the DEB and increasing the diet density to accommodate the increase in K concentrations due to grass intake and heat stress conditions during hot summer months, FCR was reduced by almost 11 points. Whole grain choice feeding also helped improve FCR by 8 points.

Recommendations

- Access to range has been identified as a major factor causing the production difference between free range and conventional systems. Especially grass consumption on range which leads to modifying effect on net nutrient intake leading to sub-optimal performance. Diets intended for consumption by free-range birds should be formulated to accommodate for the modifying effects of grass consumption on digestible nutrient intake.

- Further research into energy modulations and DEB levels needs to be undertaken in different seasons to determine best practice diets for birds that are subjected to variable conditions on range.

- Moderate levels of grass inclusion showed improvement in performance of free range birds. Research is needed to find the best nutrition pasture or combinations for free range broilers and layers and their effects studied on various performance parameters.

- Effect of whole grain feeding on free range birds needs to be studied further especially in relation to its effect on heat stress mitigation.

- RFID technology along with more advanced GPS technologies can be used to better understand the movement of birds both inside the shed and out on the range, both for broilers and layers. This information can lead to informed decisions on nutrition, welfare, disease and biosecurity practices.

- It was found that increasing range access is a promising strategy to improve performance and gut characteristics of free-range layers. Range enrichment with pasture and artificial structures needs to studied in order to promote range access for layers.

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Plain English Compendium Summary

Sub-Project Title:	**Improving the performance of free range poultry production**
Poultry CRC Sub-Project No.:	Cowieson 2.1.7
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Sub-Project Overview	This Poultry CRC project intended to establish the principle reasons for the performance gap between free-range and conventionally reared broilers and layers and evaluate a range of nutritional interventions that would reduce the magnitude of this effect on production.
Background	Free-range broilers and layers do not convert feed into saleable meat and eggs as efficiently and generally have higher mortality than conventionally-reared poultry.
Research	In the first instance the project looked at delineating the contribution of two factors that are the core of free range production in Australia: absence of in-feed antibiotics and exposure to range. Range access was found to be a major factor for the gap with grass consumption accounting for feed intake increased significantly by about 4.07% ($P < 0.01$) and feed conversion ratio (FCR) which showed a 9-11 point increase for birds on range. Grass has a very low digestible energy density and contains high potassium (K) concentration. The implications of these changes in dietary nutrient supply and interventions were explored by supplying both standard and high energy density broiler diets with different grass inclusions in one study and with two different levels of dietary electrolyte balance (DEB) in another. It was determined that these inadvertent changes in either dietary energy density or in DEB may be of importance, especially during summer months where DEB balance becomes critical to control metabolic alkalosis. Free choice feeding whole grain to free range broilers was investigated and improved the bird's ability to cope with heat stress. However, it may not have been the best option for birds to consume a sufficient amount of whole grain wheat to stimulate development of the digestive tract. Inclusion of Actigen in the standard free range (control) ration improved growth rates in both range access and non-range access birds. Actigen provided similar growth to an antibiotic feed additive and superior weight gain to a non-medicated control ration under very good health conditions. Radio frequency identification technology (RFID) was used to characterise a free range layer population based on their range usage. A multi-modal distribution of free range layers resulted, with a proportion of the flock that use the range routinely (subclassified based on durations and number of visits) and a proportion that use the range rarely. Increasing range access was determined to be a promising strategy to improve performance and gut characteristics of free-range layers. Digestibility of birds with range access also showed an improvement, more so in birds that visited the range less frequently but for longer durations of time.
Implications	The performance gap between free-range and conventional production systems is costly to both the producer and the environment. A 1-pt improvement in FCR is worth an estimated $6.5m per annum and saves the industry almost 20,000 tonnes of feed. The production gap as identified due to grass consumption, accounted for about 9-11 points in FCR. Interventions using higher energy diets were able to compensate for upto 4% grass consumption, while modifying the DEB and increasing the diet density to accommodate the increase in K concentrations due to grass intake and heat stress conditions during hot summer months, reduced FCR by almost 11 points. Whole grain choice feeding also helped improve FCR by 8 points.

Publications	***Journal Papers*** **Singh, M**, Durali T and Cowieson AJ (2015). Use of n-alkanes for determination of Kikuyu grass (*P. clandestinum*) intake in free-range broilers. *Animal Production Science*: DOI:10.1071/AN14778 **Singh M**, Cowieson AJ (2013) Range use and pasture consumption in free-range poultry production. *Animal Production Science* **53**: 1202-1208. **(Four other papers under preparation)** ***Invited Presentations*** **Singh, M** (2014) Role of pasture in the nutrition of free range birds. 2014 SA Poultry Industry Day organized by the South Australian Sub Branch of the WPSA., PIRSA-SARDI, University of Adelaide – Roseworthy Campus. October 2nd 2014. **Singh, M** (2013) Nutrition and welfare of free range poultry production, Poultry CRC Ideas Exchange, 25th September, 2013, Gold Coast, Australia. **Singh, M** (2013) A review on use of range and pasture consumption in free range poultry production, Recent Advances in Animal Nutrition (RAAN), 25th October, 2013, Armidale, Australia. **Singh, M** (2013) The Use of Range and Pasture Consumption in Free-Range Poultry Production, 20th November, 2013, AECL Forum, Perth, Australia. ***Conference Proceedings*** **M. Singh,** C.E. Hernandez, C. Lee, G. Hinch, A.J. Cowieson (2016) Wanderers versus stay at home: who has the better guts? In: 27th Annual Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 14-17th February 2016 pp.78-81. **Singh M**, Cowieson AJ (2014) Manipulating energy density and dietary electrolyte balance in diets of free range broilers. In: The XIVth European Poultry Conference (EPC), Stavanger, Norway, 23 – 27, June, 2014. pp.438 Durali T, Groves P, Cowieson AJ, **Singh M** (2014) Evaluating range usage of commercial free range broilers and its effect on bird performance using radio frequency identification (RFID) technology. In: 25th Annual Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 16-19 February 2014. pp. 150-153. **Singh M**, Durali T, Cowieson AJ (2013) Effect of grass dilution on performance and digestibility of free range chickens. In: The 19th European Symposium on Poultry Nutrition, Potsdam, Germany, 26-29 August, 2013. **Singh M**, Durali T, Walker T, Cowieson AJ (2013) Are we turning chickens into cows: how much grass do free range broilers eat? In: 24th Annual Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 17-20 February 2013' pp. 146-149. Durali T, **Singh M**, Groves P, Cowieson AJ (2013) Comparison of free-range and conventional broiler performance and digestibility. In: 24th Annual Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 17-20 February 2013. pp. 150-153. **Singh M**, Durali T, Walker T, Cowieson AJ (2013) Use of Alkanes in Determination of Grass Intake for Free Range Chickens. In: The 2013 International Poultry Scientific Forum (IPSF), Atlanta, Georgia, USA, 29-31 January 2013.