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Original Research Article

Balanced nutrient density for broiler chickens using a range of digestible lysine-to-metabolizable energy ratios and nutrient density: Growth performance, nutrient utilisation and apparent metabolizable energy

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ABSTRACT

Currently, specific nutrient concentration, metabolizable energy (ME) and digestible amino acids are used as feed formulation criteria. A balanced nutrient density (BND) concept based on 2 criteria of nutrient density and balanced amino acids-to-ME ratio may offer more flexibility in optimisation of profit in formulation of diets compared with current formulation based on set values per unit of feed mass. A total of 672 one-d-old off-sex male Ross 308 broiler chickens were used across two 42-d performance trials in a 3 × 2 factorial arrangement of treatments with each diet replicated 8 times (14 birds per replicate). The experimental factors were 2 nutrient density levels (low [LD] and high [HD]) and 3 digestible lysine-to-ME ratios (DLYS:ME; low, medium, and high). Low density diets had ME of 2,876 and 3,023 kcal/kg for starter and finisher, respectively, while values for HD diets were 3,169 and 3,315 kcal/kg with proportionally higher non-nitrogenated nutrients. Separate digestibility and apparent metabolizable energy (AME) assays were conducted at d 21 and 42. Digestibility assays at d 7 were conducted on birds used for performance trials. Regardless of the diet density, birds fed low DLYS:ME had a lower ($P < 0.01$) feed intake (d 0 to 42) than medium and high DLYS:ME. Without interaction, birds fed low and medium DLYS:ME had a similar body weight gain being the heaviest while birds low DLYS:ME were the lightest. By an interaction ($P < 0.05$), the highest overall FCR value was observed for birds fed LD × low DLYS:ME and improved linearly when DLYS:ME increased to the highest level reaching a limit for birds fed HD × medium DLYS:ME. Calorie conversion linearly decreased ($P < 0.001$) with increments in DLYS:ME. Jejunal and ileal starch and protein digestibility were affected on d 21 and 42 but not on d 7 of age. Given the independence of response on BW and feed consumption, the use of BND as a flexible system in diet formulations has the potential to enable more accurate formulation for optimisation of growth performance of broiler chickens.

1. Introduction

Nutrient specifications and metabolizable energy (ME) have long been used for diet formulation and expressed as unit of feed mass. Changes in energy and amino acid concentrations should be considered proportionally together or a limit in lean tissue deposition may be reached to store or disperse energy when excess energy is fed. Also, when amino acids are independently increased, they may increasingly be used as energy sources (Gous et al., 2018; Richards and Proszkowiec-Weglarz, 2007). Therefore,

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the absolute intake of amino acids and their relative content to ME may be more important than concentration of amino acids for tissue synthesis and optimization of performance. In broilers, the positive effect of increasing amino acids-to-energy ratio on performance parameter is shown (Chen et al., 2019) but this concept is more known in pigs (Li et al., 2012). Although research has been done on balanced protein/amino acids relative to ME, most studies have considered ME as a fixed value without proportional changes in other essential nutrients (Sterling et al., 2006; Zhai et al., 2014), which may have biased the results by limiting tissue deposition in the most nutritionally concentrated diets. In other words, when ME is fixed, the energy level of the diet may determine the optimal amino acid level because a given supply of balanced protein may require proportional supply of non-nitrogenated energy substrates for growth. Such consideration may lead to a more flexible system for optimisation of profit in formulation of broiler diets.

In this study, we investigate the concept of balanced nutrient density (BND) defined as a nutrient specification system based on 2 criteria: 1) nutrient density, defined as the ME with an optimal supply of non-nitrogenated nutrients, and 2) balanced amino acids-to-energy ratio, defined as digestible lysine-to-ME ratio (DLYS:ME) with all limiting amino acids balanced relative to lysine. As a basic principle, these 2 criteria are required to be independent to be a reliable predictor of animal performance. Hence, it was hypothesised that there will be no interaction between nutrient density and amino acids-to-energy ratio for growth performance and feed efficiency of broiler chickens.

In parallel to maximisation of growth and feed efficiency, nutrient utilisation needs to be improved for a sustainable broiler production. Therefore, in line with consideration of macronutrients, nutrient digestion is considered in different ages since there is a known age effect on digestibility of nutrients in broiler chickens (Yang et al., 2020). Accordingly, nutrient digestibility of protein, starch and fat provide basic explanations for the expected performance differences associated with DLYS:ME and dietary density in different ages as there is a gap in the literature. As energy contributing nutrients, starch, fat and protein are considered together because their metabolism is critically important in performance of fast-growing animals, particularly poultry (Selle and Liu, 2019). Thus, the current project was designed to investigate the effect of DLYS:ME and nutrient density using the concept of BND on performance, nutrient utilisation at different ages and nitrogen-corrected metabolizable energy.

2. Materials and methods

The Animal Ethics Committee of the Primary industries and Regions South Australia approved all the experimental procedures.

2.1. Experimental design and diets

A 3 × 2 factorial arrangement of treatments was followed with factors being 2 nutrient density levels (low [LD] and high [HD]), 3 DLYS:ME (low, medium, and high). The nutrient density and DLYS:ME factors differed in the diet formulation of starter and finisher diets. The study comprised of a 2-phase feeding program, with a starter from hatch to d 21 of age (Table 1) and finisher from d 21 to 42 of age (Table 2). Nutrient density was defined as ME with an optimal supply of non-nitrogenated nutrients, and DLYS:ME was defined as DLYS:ME, with all limiting amino acids balanced relative to lysine. An optimal supply of non-nitrogenated nutrients relative to ME, and amino acids relative to lysine, were defined close to those recommended by the primary breeder (Ross, 2014). The ratios of DLYS to ME (% per megacalorie) were set as 0.355 (low), 0.396 (medium), and 0.437 (high) for starter. For finisher diets, DLYS:ME were 0.305 (low), 0.346 (medium) and 0.386 (high). The 2 chosen ME density values were considered extreme with respect to commercial practice. The medium DLYS:ME closely corresponded to the recommendations of Ross 308 (Ross, 2014), whereas the low and high DLYS:ME values were approximately 0.04% per megacalorie below or over the medium DLYS:ME.

For each phase, 4 corner diets were formulated and prepared: 1) Low density × Low DLYS:ME, abbreviated as LDLA; 2) Low density × High DLYS:ME, abbreviated as LDHA; 3) High density × Low DLYS:ME, abbreviated as HDLA, and 4) High density × High DLYS:ME abbreviated as HDHA. From these 4 corner diets, 2 intermediate diets were then formulated: 5) Low density × Med DLYS:ME, by formulating 50% Low density × Low DLYS:ME and 50% Low density × High DLYS:ME, abbreviated as LDMA, and 6) High density × Med DLYS:ME, by formulating 50% High density × Low DLYS:ME and 50% High density × High DLYS:ME, abbreviated as HDMA. The cereals and protein sources were analysed for nutrient and amino acid composition using near infrared spectroscopy prior to feed formulation. Diets were based on corn and soybean meal. Canola oil was used to increase the ME of HD diets, while maintaining a minimum inclusion of oil in the LD diets. Similarly, wheat middlings were used to reduce the ME of LD diets, while maintaining a minimum inclusion in the HD diets. Soy protein concentrate was used to replace part of the protein from soybean meal to avoid potential confounding effects of allergenic soy proteins or other antinutritional factors. Calcium, available phosphorus, and all micronutrients included in the premix were increased proportionally to ME in the HD diets. Constant levels of sodium and chloride levels were maintained among diets. Titanium oxide (0.50%) was added to all diets as an indigestible marker. All diets were mixed and pelleted in a similar way.

The study comprised of 2 consecutive raised-pen trials to assess growth performance of broiler chickens including a digestibility assay conducted on d 7 of age. Two additional digestibility and apparent metabolizable energy (AME) assays were separately conducted in metabolism cages concurrent with the first run of growth studies on the same batch of birds at d 21 and 42 of age, described in following subsections.

2.2. Performance trials

Two consecutive performance trials were conducted using 1-d-old off-sex male Ross 308 broiler chicks. One-d-old birds, with an average initial body weight of 38.6 g, were obtained from Aviagen hatchery (Goulburn, NSW) and transferred to the poultry facilities at Roseworthy, SA. Each of the 6 dietary treatments was replicated eight times in 48 raised floor pens.

In each trial, upon arrival, birds were weighed and assigned to 24 pens with each pen accommodating 14 birds. The raised-pens were in 2 identically managed environmentally controlled rooms. Birds had a lighting schedule of 23 h light and 1 h dark in the first 5 d thereafter all birds were given 16 h of light and 8 h of dark. For the first 7 d of age, birds were kept on paper to avoid possible confounding effect of wood shavings on digestibility values. From d 8, each pen was provided with approximately 5 cm deep wood shavings as bedding material.

Throughout the experiment, birds had ad libitum access to feed and water through a feed hopper and a nipple drinker line. Room temperature was kept at 32 °C during the first 4 d of the study and gradually decreased to 23 °C by the end of the third week. Heating and cooling was automatically controlled via a digital heating and cooling controller. Feed was given as crumble in the first 7 d of age. From d 8 until d 42, feed was provided as pellet.

Table 1
Composition of experimental starter diets (as-is) fed to broilers from d 0 to 21 of age.

Item	LDLA	LDMA	LDHA	HDLA	HDMA	HDHA
Ingredients, %						
Corn	60.957	57.544	54.131	55.478	49.975	44.473
Wheat middlings	5.000	5.000	5.000	1.500	1.500	1.500
Soybean meal	26.179	27.730	29.280	29.556	32.554	35.551
Soy protein concentrate	2.000	3.500	5.000	2.000	4.000	6.000
Canola oil	1.036	1.307	1.578	6.067	6.590	7.113
Limestone	1.073	1.060	1.047	1.146	1.124	1.102
Dicalcium phosphate	1.914	1.899	1.883	2.198	2.175	2.152
Sodium chloride	0.149	0.140	0.132	0.135	0.145	0.154
Sodium bicarbonate	0.416	0.429	0.441	0.445	0.431	0.417
TiO_2	0.500	0.500	0.500	0.500	0.500	0.500
Vitamin and mineral premix[1]	0.200	0.200	0.200	0.220	0.220	0.220
Choline Cl (70%)	0.111	0.100	0.088	0.109	0.090	0.072
L-Lysine HCl (78.4%)	0.180	0.216	0.253	0.242	0.229	0.216
DL-Methionine	0.217	0.276	0.334	0.290	0.341	0.391
L-Threonine	0.069	0.101	0.133	0.115	0.127	0.139
Nutrients, %						
AME, kcal/kg	2,876	2,876	2,876	3,169	3,169	3,169
Crude protein	19.500	21.002	22.504	20.182	22.440	24.697
Crude protein (analysed)	19.791	21.821	23.153	20.731	22.91	24.932
Crude fat	3.235	3.645	4.055	8.070	8.771	9.472
Crude fat (analysed)	3.750	3.950	4.270	8.380	9.010	9.810
Crude fiber	2.554	2.623	2.692	2.358	2.477	2.596
Ash	5.866	6.012	6.157	6.169	6.401	6.633
DLYS:ME	0.355	0.396	0.437	0.355	0.396	0.437
Digestible Arg	1.156	1.262	1.367	1.206	1.371	1.536
Digestible Lys	1.021	1.139	1.257	1.125	1.255	1.385
Digestible Met	0.480	0.554	0.627	0.555	0.630	0.704
Digestible Cys	0.255	0.268	0.281	0.255	0.276	0.297
Digestible Met + Cys	0.758	0.846	0.933	0.835	0.932	1.028
Digestible Trp	0.213	0.229	0.244	0.222	0.248	0.273
Digestible Leu	1.504	1.567	1.630	1.526	1.630	1.734
Digestible Ile	0.758	0.823	0.888	0.796	0.898	1.000
Digestible Thr	0.686	0.766	0.845	0.756	0.844	0.931
Digestible Val	0.818	0.881	0.943	0.844	0.942	1.039
Starch	37.364	35.381	33.398	33.221	30.029	26.837
Calcium	0.915	0.915	0.915	1.006	1.006	1.006
Available P	0.457	0.457	0.457	0.502	0.502	0.502
Sodium	0.200	0.200	0.200	0.200	0.200	0.200
Chloride	0.200	0.200	0.200	0.200	0.200	0.200

LD = low nutrient density level; LA = low DLYS:ME; MA = medium DLYS:ME; HA = high DLYS:ME; HD = high nutrient density level; DLYS:ME = digestible lysine-to-metabolizable energy ratio.

[1] At 2 g/kg supplementation, vitamin and mineral concentrate supplied the following per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 μg; biotin, 200 μg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; mineral oil, 3.75 mg.

Birds were weighed weekly on d 0, 7, 14, 21, 35 and 42 and feed intake was recorded for each week. Feed conversion ratio was calculated by dividing the total body weight gain including weights of dead birds by total feed intake of each pen. The correction of feed intake and subsequently FCR for mortalities were made by correcting the number of birds per week of the experiment for any pens involving dead or culled birds.

2.3. Digestibility and AME assays

Concurrent with first run of the performance trials, digestibility (except d 7) and AME assays were conducted on the same batch of birds separately reared on floor. Therefore, 252 one-d-old off-sex male Ross 308 broilers were placed in an environmentally-controlled room and reared under similar management, temperature and lighting program as for the performance trials. All the birds were given a common commercial diet until d 15 when birds were transferred to metabolism group cages after being weighed. Each cage accommodated 4 birds in a total of 36 metabolism cages. Birds were assigned to 6 experimental diets each replicated 6 times for a 3-d adaption period followed by 3 d of total excreta collection on d 18, 19, and 20. Upon completion of AME procedure, 3 birds per cage were euthanized for jejunal and ileal digesta collection on d 21 of age. Using the remaining 108 male broilers, the exact same procedure was followed for finisher diets with an exception of placing 3 birds per cage due to higher body weight and growth rate. The adaption period and excreta collection were conducted from d 36 to 41 for AME followed by jejunal and ileal digesta collection from 2 birds per replicate on d 42. As previously indicated the digestibility assays for d 7 were performed on birds in rearing pens used in growth performance trials as the AME cages were not suitable for young birds. As such on d 7 of age, in each of the performance trial, 6 birds from each pen were euthanized for digesta collection obtaining 8 replicates per treatment.

At each age of d 7, 21 and 42, digesta was collected from the entire jejunum and ileum by gently squeezing the content into a container, placed on ice and subsequently frozen in −20 °C before being fully freeze-dried and ground to 0.1 mm. At the time of collection, digesta from birds within a cage/replicate were pooled.

2.4. Analysis and calculations

Titanium oxide was measured in all the diets and digesta samples according to the method described by Short et al. (1996). The

Table 2
Composition of experimental finisher diets (as-is) fed to broilers from d 22 to 42 of age.

Item	LDLA	LDMA	LDHA	HDLA	HDMA	HDHA
Ingredients, %						
Corn	65.979	60.608	55.236	58.835	53.195	47.555
Wheat middlings	5.000	5.000	5.000	1.000	1.000	1.000
Soybean meal	20.592	23.895	27.198	26.143	29.044	31.946
Soy protein concentrate	1.500	3.000	4.500	1.500	3.750	6.000
Canola oil	2.388	2.960	3.531	7.624	8.132	8.640
Limestone	0.956	0.934	0.913	0.998	0.976	0.953
Dicalcium phosphate	1.606	1.587	1.568	1.867	1.842	1.817
Sodium chloride	0.123	0.131	0.139	0.137	0.148	0.158
Sodium bicarbonate	0.463	0.451	0.439	0.453	0.437	0.422
TiO_2	0.500	0.500	0.500	0.500	0.500	0.500
Vitamin and mineral premix[1]	0.200	0.200	0.200	0.220	0.220	0.220
Choline Cl (70%)	0.134	0.116	0.099	0.124	0.105	0.086
L-Lysine HCl (78.4%)	0.241	0.233	0.226	0.224	0.207	0.191
DL-Methionine	0.235	0.289	0.343	0.284	0.341	0.398
L-Threonine	0.083	0.096	0.109	0.092	0.103	0.114
Nutrients, %						
AME, kcal/kg	3,023	3,023	3,023	3,315	3,315	3,315
Crude protein	17.090	19.182	21.273	18.442	20.806	23.170
Crude protein (analysed)	16.720	18.830	21.930	17.960	20.170	22.800
Crude fat	4.537	5.231	5.925	9.559	10.273	10.986
Crude fat (analysed)	5.010	5.930	6.490	9.430	10.410	10.750
Crude fiber	2.364	2.482	2.599	2.202	2.324	2.445
Ash	5.237	5.463	5.689	5.609	5.847	6.084
DLYS:ME	0.305	0.346	0.386	0.305	0.346	0.386
Digestible Arg	0.983	1.134	1.285	1.087	1.261	1.435
Digestible Lys	0.922	1.045	1.167	1.011	1.146	1.280
Digestible Met	0.471	0.547	0.622	0.531	0.613	0.695
Digestible Cys	0.230	0.249	0.268	0.238	0.260	0.281
Digestible Met + Cys	0.722	0.818	0.914	0.792	0.897	1.002
Digestible Trp	0.183	0.207	0.230	0.202	0.228	0.254
Digestible Leu	1.362	1.461	1.560	1.432	1.540	1.647
Digestible Ile	0.647	0.741	0.835	0.721	0.828	0.935
Digestible Thr	0.618	0.700	0.782	0.677	0.767	0.857
Digestible Val	0.714	0.803	0.891	0.772	0.875	0.978
Starch	40.248	37.136	34.023	35.014	31.741	28.467
Calcium	0.790	0.790	0.790	0.869	0.869	0.869
Available P	0.395	0.395	0.395	0.436	0.436	0.436
Sodium	0.200	0.200	0.200	0.200	0.200	0.200
Chloride	0.200	0.200	0.200	0.200	0.200	0.200

LD = low nutrient density; LA = low DLYS:ME; MA = medium DLYS:ME; HA = high DLYS:ME; HD = high nutrient density; DLYS:ME = digestible lysine-to-metabolizable energy ratio.
[1] At 2 g/kg supplementation, vitamin and mineral concentrate supplied the following per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 μg; biotin, 200 μg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; mineral oil, 3.75 mg.

total starch (996.11) was measured using Megazyme kit (K-TSTA-100A) following the methods of AOAC (2005). The nitrogen content was measured using a Leco TruSpec CNS analyser. Fat content was assayed using the method of Folch et al. (1957) for the diets and ileal samples. The gross energy (GE) content of experimental diets and excreta was determined using a Parr isoperibol bomb calorimeter (Parr Instrument Company, Moline, IL) with benzoic acid as the standard.

Calculation of ileal and jejunal digestibility coefficients based on the concentration of indigestible marker was performed as reported by Barekatain et al. (2013):

$$\text{Apparent digestibility coefficient (ADC)} = \frac{(\text{NT/Ti})_{\text{diet}} - (\text{NT/Ti})_{\text{digesta}}}{(\text{NT/Ti})_{\text{digesta}}},$$

where NT meant nutrient, $(\text{NT/Ti})_{\text{diet}}$ was the ratio of NT to titanium (Ti) in diet and $(\text{NT/Ti})_{\text{digesta}}$ was the ratio of NT to Ti in digesta.

Ileal digestible intake of starch and protein (N) were calculated using a formula similar to Moss et al. (2018) but defined differently:

$$\text{Digestible nutrient intake (g/d per bird)} = \text{Daily feed intake} \times \text{Nutrient content}_{\text{diet}} \times \text{ADC}_{\text{ileum}}.$$

The feed intake values used for the ileal digestible nutrient intake calculation were the total feed intake (in cages) for 3 d recorded immediately before d 21 and 42 expressed on a daily basis. For values on d 7, the total feed intake of the first 7 d of age expressed on daily basis was used.

Dietary AME was calculated using the following formula:

$$\text{AME diet} = \frac{(\text{Feed intake} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{excreta}})}{(\text{Feed intake})}.$$

The AME values were corrected for N (AMEn kcal/kg) by correcting N retention to zero using the factor of 8.73 kcal/g N retained in the body (Hill and Anderson, 1958).

The following equation was used to calculate N retention:

$$\text{N retention}(\%) = \frac{(\text{Feed intake} \times \text{N}_{\text{diet}}) - (\text{Excreta output} \times \text{N}_{\text{excreta}})}{(\text{Feed intake} \times \text{N}_{\text{diet}})} \times 100.$$

2.5. Statistical analysis

General Linear Model of SAS (2003) was used to analyse all the data of the study. The results of both performance studies were combined and analysed together for the main effects of nutrient density, DLYS:ME and their interactions using 2-way ANOVA. The trial was considered as random effect. Each of the digestibility and AME assays was analysed as stand-alone for the same main effects and interactions. The data are presented as means with a pooled standard error of the mean. Each pen/cage or its representative sample was considered an experimental unit. When a significant effect was detected, means were separated using Least Square Differences test. The level of significance was specified as $P < 0.05$ and tendency was considered for $0.05 \leq P \leq 0.10$.

3. Results

3.1. Bird performance

The performance parameters for the 6-week study are shown in Table 3. In general, the performance of the birds (3,633 g BW at d 42) far exceeded the Ross 308 target standards (3,136 g BW at d 42) as specified by Aviagen (Ross, 2014). The mortality was less than 4% and not attributed to any of the experimental diets.

Feed consumption was only affected by DLYS:ME at any stage of the experiment with no effect of nutrient density or interaction. From d 0 to 7 of age, increasing DLYS:ME resulted in birds consuming more feed ($P < 0.001$). However, when assessed from d 0 to 21 ($P < 0.01$) and 0 to 42 ($P = 0.043$), birds fed diets containing medium and high levels of DLYS:ME consumed similar amount of feed but more than birds fed low DLYS:ME.

There was no interaction between DLYS:ME and nutrient density for BWG. During the first week of the experiment, birds fed HD diets gained more weight ($P < 0.01$) than LD group. BWG was also higher ($P < 0.01$) in birds fed high level of DLYS:ME than low and medium levels from d 0 to 7. As for d 0 to 21 ($P < 0.001$) and the entire study ($P < 0.001$), birds on HD diets had independently higher BWG than LD group. For the same periods of study, birds fed low DLYS:ME had lower BWG than other birds fed medium and high levels ($P < 0.001$).

In absence of an interaction, feeding birds with HD diets reduced ($P < 0.001$) FCR during the first week of study (Table 3). When assessed from d 0 to 21 ($P < 0.001$) and d 0 to 42 ($P < 0.001$), there was an interaction between nutrient density and DLYS:ME for FCR. From d 0 to 21, increasing DLYS:ME decreased FCR only in birds fed LD diets. However, when assessed for the entire study, the lowest FCR was observed in birds fed LDMA and HDHA.

3.2. Nutrient digestibility

3.2.1. Starch, nitrogen and fat digestibility

Starch and protein digestibility coefficients at d 7, 21 and 42 are shown in Table 4. At d 7, starch or nitrogen digestibility coefficients were not affected and there was no interaction between DLYS:ME and diet density in both jejunum and ileum. However, fat digestibility was higher ($P < 0.001$) in birds fed HD diets compared with birds fed LD diets (Table 6). There was no difference in fat digestibility related to DLYS:ME on d 7.

On d 21, starch digestibility was only affected in jejunum where HD independently decreased ($P < 0.001$) starch digestibility while birds fed low DLYS:ME tended ($P = 0.063$) to have the lowest values. Digestibility of starch was not affected in the ileum at d 21. On the same day, with an independent effect of DLYS:ME, jejunal nitrogen digestibility was highest ($P = 0.026$) in birds fed medium level of DLYS:ME compared with the other 2 levels. Nutrient density had no effect on jejunal nitrogen digestibility on d 21. Nutrient density and DLYS:ME interacted ($P < 0.001$) for ileal nitrogen digestibility on d 21. Accordingly, ileal nitrogen digestibility was the lowest in birds fed LDHA and the highest in birds fed HDMA and HDHA. As shown in Table 6, with no interaction, ileal fat

Table 3
Growth performance of broiler chickens fed experimental diets from d 0 to 42 of age.

Item	Feed intake, g/bird			BWG, g/bird			FCR, g feed/g BWG		
	d 0 to 7	d 0 to 21	d 0 to 42	d 0 to 7	d 0 to 21	d 0 to 42	d 0 to 7	d 0 to 21	d 0 to 42
Main effect									
Density									
LD	155	1,254	5,045	139^{b}	1,008^{b}	3,470^{b}	1.117^{a}	1.257	1.458
HD	157	1,274	4,976	150^{a}	1,066^{a}	3,671^{a}	1.048^{b}	1.196	1.356
DLYS:ME									
LA	149^{c}	1,231^{b}	4,918^{b}	138^{b}	987^{b}	3,375^{b}	1.088	1.251	1.462
MA	155^{b}	1,274^{a}	5,047^{a}	142^{b}	1,046^{a}	3,633^{a}	1.097	1.220	1.397
HA	162^{a}	1,302^{a}	5,067^{a}	153^{a}	1,078^{a}	3,704^{a}	1.064	1.209	1.363
Treatments									
LDLA	149	1,228	4,920	133	936	3,221	1.121	1.313^{a}	1.530^{a}
LDMA	153	1,286	5,122	136	1,026	3,521	1.131	1.255^{b}	1.456^{b}
LDHA	162	1,279	5,094	148	1,062	3,667	1.101	1.204^{c}	1.390^{c}
HDLA	151	1,233	4,917	143	1,038	3,529	1.055	1.189^{c}	1.394^{bc}
HDMA	158	1,263	5,013	149	1,066	3,745	1.065	1.186^{c}	1.338^{d}
HDHA	161	1,326	5,001	158	1,094	3,741	1.027	1.215^{bc}	1.337^{d}
SEM	1.1	7.4	25.4	1.7	7.1	22.0	0.0093	0.0063	0.0065
Source of variation									
Density	0.335	0.519	0.185	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
DLYS:ME	<0.001	<0.01	0.043	<0.01	<0.001	<0.001	0.324	0.030	<0.001
Density × DLYS:ME	0.502	0.165	0.660	0.896	0.100	0.102	0.977	<0.001	0.031

LD = low nutrient density; HD = high nutrient density; DLYS:ME = digestible lysine-to-metabolizable energy ratio; LA = low DLYS:ME; MA = medium DLYS:ME; HA = high DLYS:ME.
[a-c] Means within the same column and same effect not sharing a superscript letter differ significantly at the P level shown ($n = 48$).

Table 4
Starch and protein (N) digestibility coefficients of broiler chickens fed experimental diets at 3 different ages.

Item	Day 7				Day 21				Day 42			
	Starch		Nitrogen		Starch		Nitrogen		Starch		Nitrogen	
	Jejunum	ileum	Jejunum	ileum	Jejunum	ileum	Jejunum	ileum	Jejunum	ileum	Jejunum	ileum
Main effects												
Density												
LD	0.610	0.937	0.542	0.792	0.678^{a}	0.930	0.608	0.785^{b}	0.734	0.917	0.588	0.823
HD	0.632	0.933	0.558	0.787	0.597^{b}	0.919	0.615	0.817^{a}	0.727	0.926	0.612	0.836
DLYS:ME												
LA	0.637	0.939	0.560	0.793	0.617^{b}	0.928	0.595^{b}	0.794^{b}	0.719	0.927^{a}	0.564^{b}	0.809^{b}
MA	0.636	0.935	0.537	0.787	0.666^{a}	0.926	0.636^{a}	0.796^{b}	0.754	0.930^{a}	0.603^{ab}	0.827^{b}
HA	0.591	0.931	0.548	0.788	0.637^{ab}	0.920	0.602^{b}	0.813^{a}	0.718	0.907^{b}	0.633^{a}	0.853^{a}
Treatments												
LDLA	0.642	0.945	0.551	0.800	0.634	0.933	0.611	0.800^{bc}	0.698^{b}	0.911^{b}	0.576^{b}	0.806
LDMA	0.610	0.934	0.540	0.786	0.714	0.933	0.624	0.786^{cd}	0.789^{a}	0.944^{a}	0.609^{b}	0.826
LDHA	0.579	0.934	0.537	0.791	0.689	0.926	0.589	0.770^{d}	0.713^{b}	0.896^{b}	0.579^{b}	0.838
HDLA	0.632	0.933	0.570	0.787	0.601	0.925	0.579	0.789^{cd}	0.741^{ab}	0.944^{b}	0.552^{b}	0.814
HDMA	0.662	0.936	0.535	0.788	0.607	0.920	0.650	0.841^{a}	0.719^{b}	0.916^{a}	0.597^{b}	0.828
HDHA	0.604	0.929	0.559	0.785	0.585	0.914	0.616	0.823^{ab}	0.723^{b}	0.919^{a}	0.688^{a}	0.868
SEM	0.011	0.002	0.015	0.006	0.0072	0.0037	0.0063	0.0033	0.0092	0.0034	0.0089	0.0043
Source of variation												
Density	0.317	0.310	0.683	0.636	<0.001	0.156	0.583	<0.001	0.736	0.173	0.181	0.127
DLYS:ME	0.170	0.377	0.824	0.879	0.063	0.614	0.026	0.048	0.216	0.017	0.012	0.0013
Density × DLYS:ME	0.518	0.478	0.915	0.861	0.072	0.955	0.107	<0.001	0.049	0.002	0.008	0.358

LD = low nutrient density; HD = high nutrient density; DLYS:ME = digestible lysine-to-metabolizable energy ratio; LA = low DLYS:ME; MA = medium DLYS:ME; HA = high DLYS:ME.
[a-d] Means within same column and same effect not sharing a superscript letter differ significantly at the P level shown ($n = 96$).

digestibility on d 21 decreased in birds fed LD diets ($P < 0.01$) and low DLYS:ME ($P = 0.037$).

On d 42, there was an interaction between DLYS:ME and nutrient density for both jejunal ($P = 0.049$) and ileal ($P < 0.01$) starch digestibility (Table 4). In jejunum, starch digestibility was the highest in birds fed LDMA compared with all the other birds except HDLA. For ileum, birds fed LDMA and HDLA had the highest starch digestibility on d 42. DLYS:ME and nutrient density interacted ($P < 0.01$) for jejunal nitrogen digestibility with HDHA increased digestibility compared with all the other treatments. While there was no effect of nutrient density, ileal digestibility of nitrogen at d 42 was independently increased ($P < 0.01$) by feeding high level of DLYS:ME. Fat digestibility was only increased ($P < 0.001$) by feeding HD diets at d 42.

3.2.2. Ileal digestible starch and nitrogen intake

The daily digestible intake of starch and nitrogen in ileum of birds at d 7, 21 and 42 are shown in Table 5. On d 7, there was a significant interaction ($P < 0.01$) between the 2 main experimental factors where HDHA and LDLA resulted in the lowest and highest ileal starch digestible intake, respectively. Both increasing nutrient density ($P < 0.01$) and DLYS:ME ($P < 0.001$) distinctively increased ileal nitrogen digestible intake on d 7.

On d 21, ileal digestible starch intake was linearly decreased by increasing nutrient density ($P < 0.001$) and DLYS:ME ($P < 0.001$). For ileal digestible nitrogen intake, there was an interaction ($P < 0.001$) between nutrient density and DLYS:ME with birds fed increasing levels of DLYS:ME led to a linear increase in digestible nitrogen intake at HD diet.

On d 42, with a significant interaction ($P < 0.01$), at each levels of nutrient density, maximisation of DLYS:ME decreased the ileal digestible starch intake with birds fed HDMA and HDHA produced the lowest digestible starch intake values. On the same day, in absence of an interaction, feeding HD diets ($P < 0.001$) and medium and high levels of DLYS:ME ($P < 0.0001$) elevated the ileal nitrogen digestible intake.

As shown in Fig. 1, the regression analysis found a quadratic relationship ($P < 0.01$) between digestible starch intake to digestible protein intake ratios in the ileum at d 42 with FCR when assessed from d 0 to 42.

3.3. Nitrogen retention, energy and nitrogen intake and their conversion to body weight

As shown in Table 6, on d 21, with no interaction, birds fed HD diets retained more ($P < 0.001$) N than birds fed LD diets. At the same day, birds fed medium level of DLYS:ME had the highest ($P < 0.01$) N retention. On d 42, only highest level of DLYS:ME independently increased ($P < 0.01$) the N retention.

Calorie intake calculated for d 0 to 42 was only influenced by nutrient density where HD increased ($P < 0.001$) the values. Calorie conversion to BW was the highest ($P < 0.001$) in birds fed low DLYS:ME. Nitrogen intake was also independently increased by HD ($P < 0.0001$) and increasing level of DLYS:ME ($P < 0.001$). For N conversion, there was interaction ($P < 0.01$) between nutrient density and DLYS:ME. The highest conversion of N to BW was observed in birds fed HDHA and LDHA while the lowest conversion was seen in birds fed HDLA.

3.4. AMEn

As shown in Table 6, there was an interaction between DLYS:ME and nutrient density for AMEn values for starter ($P = 0.026$) and finisher diets ($P < 0.001$). For starter diets, HDLA and HDHA had higher AMEn values compared with other treatments while there was no difference between LD diets. For finisher diets, the lowest level of DLYS:ME increased the AMEn within each nutrient density.

4. Discussion

The analysis of protein and fat showed that diets were close to the formulated values. The experimental diets in general had profound effects on performance of broiler chickens in particular BWG consistent with large differences in AME and DLYS:ME. The unaffected overall feed consumption as a response to the diet density is in agreement with the recent observations concluding energy *per se*

Table 5
Starch and protein (N) digestible intake in the ileum of broiler chickens fed experimental diets at 3 different ages (g/d per bird).

Item	Day 7		Day 21		Day 42	
	Starch	Nitrogen	Starch	Nitrogen	Starch	Nitrogen
Main effects						
Density						
LD	7.78	3.98^{b}	35.9^{a}	18.1	81.4	30.4^{b}
HD	7.08	4.20^{a}	29.4^{b}	18.3	67.3	32.3^{a}
DLYS:ME						
LA	7.73	3.61^{c}	35.3^{a}	16.7	81.1	26.3^{c}
MA	7.45	4.12^{b}	33.0^{b}	18.6	76.6	30.9^{b}
HA	7.09	4.52^{a}	29.8^{c}	19.4	65.4	36.7^{a}
Treatments						
LDLA	8.02^{a}	3.60	38.1	17.3^{c}	90.3^{a}	25.8
LDMA	7.57^{bc}	3.97	36.2	18.5^{b}	86.6^{a}	30.3
LDHA	7.75^{ab}	4.36	33.7	18.6^{b}	67.3^{bc}	35.1
HDLA	7.46^{bc}	3.64	32.5	16.0^{d}	72.0^{b}	26.9
HDMA	7.34^{c}	4.27	29.9	18.8^{b}	66.5^{c}	31.7
HDHA	6.44^{d}	4.70	26.0	20.2^{a}	63.4^{c}	38.3
SEM	0.058	0.040	0.23	0.13	0.65	0.32
Source of variation						
Density	<0.0001	0.008	<0.001	0.446	<0.001	0.007
DLYS:ME	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Density × DLYS:ME	0.002	0.268	0.199	<0.001	<0.001	0.395

LD = low nutrient density; HD = high nutrient density; DLYS:ME = digestible lysine-to-metabolizable energy ratio; LA = low DLYS:ME; MA = medium DLYS:ME; HA = high DLYS:ME.
[a-d] Means within same column and same effect not sharing a superscript letter differ significantly at the P level shown ($n = 96$).

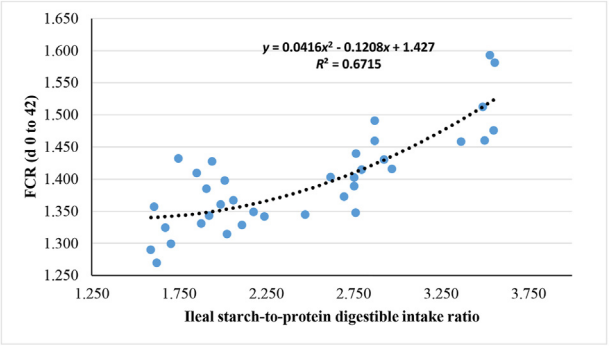

Fig. 1. The quadratic regression between digestible starch-to-digestible protein intake ratios in the ileum at d 42 with FCR in broiler chickens from d 0 to 42 ($P < 0.01$).

may not be the primary driver of feed intake in broilers (Classen, 2017). Instead, in this experiment, feed intake followed a pattern by which a limit was reached in most cases when DLYS:ME was increased to the medium level and therefore further increasing the DLYS:ME had no effect on feed consumption. Given a similar BWG of birds fed medium and high levels of DLYS:ME, a similar feed consumption is anticipated due to limitation in physical intake ability of birds. A basic hypothesis of this study was that energy density and DLYS:ME demonstrate independent effects on performance parameters. Throughout the experiment, the effects on feed consumption and BWG appeared to be mostly independent for DLYS:ME but this was not the case for FCR. Independent response for feed intake and BWG was in line with the hypothesis of the experiment. This indicates that both feed intake and BWG could be robust criteria for response to DLYS:ME and density presenting an opportunity for prediction of feed intake being used for optimisation of growth and profitability (Gous, 2007).

It is important to clarify that the effect of nutrient density as defined in this study cannot be directly compared to studies where the response of broilers to ME changes has been assessed at fixed levels of minerals and other essential nutrients. For instance, Liu et al. (2019) found that BWG was not influenced by energy density but was affected by amino acid density and starch to lipid ratio. Marked and independent improvement in BWG, in the current study, in response to an increase in nutrient density of the diets may therefore be associated with proportional increase of other essential nutrients in addition to extreme changes in ME content. Nonetheless, consistent with results from Liu et al. (2019), both density and amino acid ratios improved feed efficiency during the first 3 weeks of age and an overall period. Specifically, both nutrient density and DLYS:ME had to be increased in order to achieve the best overall FCR (1.33) but a limit again seemed to be reached for HDMA. An interaction between digestible Lys and dietary energy for improved FCR has also been recently observed (Hirai et al., 2020). In contrast, Sharma et al. (2018) found that increasing digestible Lys and energy independently affected growth performance parameter. As previously mentioned, it is possible that limitations on essential nutrients may bias performance responses solely based on ME in these studies. It is therefore prudent to associate the interaction observed in the current study to the changes in micronutrients and essential nutrients in 2 different density diets. Hence, it can be proposed that the utilisation of nutrient density as criteria for diet formulation would allow providing adequate nutrients at different ME concentrations. Nevertheless, distinct improvement of performance parameters in particular BWG and FCR as a result of increasing DLYS:ME supports the assumption that the requirements of amino acids increase faster compared with energy for modern broilers and that fast-growing broilers require higher amino acids to energy ratios (Gous, 2010).

In the current study, increasing the density of the diet increased nitrogen retention and growth performance to 42 d, even at the high level of performance exhibited by these birds. This result is in accordance with conclusions made by Gous et al. (2018) that broilers exhibit an energy-dependent phase when high-protein feeds are offered and that an increase in energy is required to process the high protein present in the diets. It may also be deduced that independent increase in ME alone may not increase growth while all the other essential nutrients including minerals are sufficiently available for tissue accretion. As an example, this explanation is supported by results obtained by Sharma et al. (2018) when increasing ME only improved growth performance when broilers were fed low P diets and not high P.

Table 6
AMEn, nitrogen retention obtained in AME and digestibility assays and subsequent fat digestibility coefficients, calculated energy and nitrogen conversion for performance trials.

Item	Fat digestibility			AMEn, kcal/kg		N retention, %		Calorie intake, Mcal/bird	Calorie conversion, kcal/g BWG	N intake, g/bird	N conversion, g/kg BWG
	d 7	d 21	d 42	Grower	Finisher	d 21	d 42	d 0 to 42	d 0 to 42	d 0 to 42	d 0 to 42
Main effects											
Density											
LD	0.815[b]	0.819[b]	0.909[b]	2,954	3,054	68.7[b]	60.8	15.15[b]	4.38	167.2[b]	48.1
HD	0.883[a]	0.877[a]	0.946[a]	3,129	3,299	70.9[a]	62.4	15.99[a]	4.36	178.6[a]	48.5
DLYS:ME											
LA	0.841	0.815[b]	0.921	3,056	3,250	69.3[b]	61.2[b]	15.5	4.60[a]	150.9[c]	44.8
MA	0.854	0.860[a]	0.929	3,001	3,151	70.7[a]	60.1[b]	15.6	4.29[b]	177.1[b]	48.7
HA	0.851	0.868[a]	0.932	3,068	3,128	69.5[b]	63.7[a]	15.6	4.22[b]	190.6[a]	51.5
Treatments											
LDLA	0.801	0.804	0.895	2,953[c]	3,111[d]	68.3	59.5	14.92	4.64	146.8	45.7[d]
LDMA	0.835	0.828	0.911	2,951[c]	3,005[e]	69.1	58.7	15.25	4.34	168.5	47.9[c]
LDHA	0.810	0.828	0.921	2,958[c]	3,046[e]	68.9	64.5	15.29	4.18	186.2	50.8[ab]
HDLA	0.882	0.827	0.947	3,159[a]	3,390[a]	70.4	62.8	16.10	4.56	155.2	44.0[e]
HDMA	0.874	0.894	0.947	3,050[b]	3,298[b]	72.4	61.4	15.91	4.25	185.7	49.6[b]
HDHA	0.893	0.909	0.945	3,180[a]	3,210[c]	70.1	62.9	15.98	4.27	195.1	52.2[a]
SEM	0.0062	0.0087	0.0025	6.7	9.5	0.178	0.435	0.080	0.020	0.906	0.221
Source of variation											
Density	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	0.093	<0.001	0.575	<0.001	0.336
DLYS:ME	0.662	0.037	0.176	0.015	<0.001	<0.01	0.006	0.806	<0.001	<0.001	<0.001
Density × DLYS:ME	0.278	0.388	0.090	0.026	<0.001	0.067	0.058	0.328	0.115	0.092	0.006

LD = low nutrient density; HD = high nutrient density; DLYS:ME = digestible lysine-to-metabolizable energy ratio; LA = low DLYS:ME; MA = medium DLYS:ME; HA = high DLYS:ME.
[a-e] Means within the same column and same effect not sharing a superscript letter differ significantly at the *P* level shown ($n = 96$).

The AMEn assays found the measured values in agreement with formulated energy values at least between the LD and HD diets for all diets. The starter LD diets were however slightly higher than formulated values that could be due to underestimation of AME values for grains in young birds. Noteworthy, birds fed low DLYS:ME diets resulted in a higher AMEn particularly for finisher diets compared with other DLYS:ME. This could be related to a lower protein content of those diets. Similar energy sparing effect was observed recently by Chrystal et al. (2020) in broilers fed a diet with a low protein content. These authors attributed this phenomenon to the fact that less intact protein in the diet may result in less uric acid synthesis for N excretion. This could also be related to the generally higher starch digestibility in LD diets. It is important to note that such differences in AME of diets between different ratios of the DLYS:ME need to be taken into consideration when interpreting the results of the current study as a basic hypothesis was to assume similar energy at each level of tested DLYS:ME. Nevertheless, the calorie intake was not affected by DLYS:ME whereas expected differences were observed for energy density of diets (Table 6). In opposite, there was a clear increase in the total nitrogen intake with increments in DLYS:ME. Increments on feed intake and growth of broilers in response to increments of dietary balance protein have been widely demonstrated (Sterling et al., 2006). Feed intake of modern broilers appears to be refractory to amino acid intake, and they have a high capacity for utilisation of excess amino acids as energy source without significant changes in metabolic efficiency (MacLeod, 1991).

As DLYS:ME increased the calorie conversion decreased while the opposite was observed for N conversion. These results are in agreement with a study by Zeng et al. (2015) although in regard to CP and ME. It may be possible that in low DLYS:ME, the excess energy available to the birds may possibly have been deposited more into fat tissues rather than muscle deposition compared with higher levels of DLYS:ME. Improved production of lean meat is associated with additional dietary lysine (Fouad and El-Senousey, 2014), which may further explain the changes in calorie conversion. With fat being a more efficient energy source than protein (Close, 1990), a higher calorie conversion in low DLYS:ME may also be explained. The improved N conversion for both HD and high DLYS:ME were expected consequences of dietary N content composition and digestibility as well as N retention. As expected, the total N retention was greater for HD compared to LD diets, which reflected a difference in balanced protein between both groups of diets. This difference in N retention was also accompanied by a difference in the apparent ileal digestibility of N particularly for finisher diets.

In general, the digestibility of nutrients in both jejunum and ileum was studied to explain some of the differences in performance of the birds as well as confirmation of expected differences between the formulated experimental diets. In most cases, the measured values of digestibility were reflective of the wide range of ingredients used and somewhat expected differences in digestibility of nutrients in those ingredients rather than directly related to the performance of the birds. A clear age effect was evident when the effects of treatments were only observed in d 21 and 42 and not d 7 of age. Independent analysis of age effect from Table 4 (analysis not shown) revealed that ileal starch digestibility slightly decreased with age while the other nutrients had relatively higher digestibility at d 21 and 42 compared with d 7. This may reflect the adaptability and development of gastrointestinal tract and possible effect of increasing feed intake on digestibility of nutrients in particular starch (Cowieson et al., 2019). The consistently lower starch digestibility in jejunum compared to ileum is in agreement with the results of Stefanello et al. (2015). It should be noted that the differences observed for a slight decline in starch digestibility in older birds as well as significant differences in N digestibility between different ratios of DLYS:ME in both jejunal and ileum present opportunities for optimisation of a BND system using exogenous enzyme such as amylase and protease in future studies.

By complex interactions, a higher starch digestibility was observed in LD diets and to a lesser extent in low DLYS:ME which

may simply be the result of higher starch content of those diets and a generally high starch digestibility of corn (Moran, 1982). The complexity and dynamic of starch and protein and their consideration at the same time can be related to a possible competition of these nutrients for uptake in enterocytes (Selle and Liu, 2019). Benefits of slowly digestible starch is demonstrated in poultry (Weurding et al., 2003). The calculation of digestible nutrient intakes per day for ileum was attempted to indicate if they could explain some of the differences in performance of birds. Defined in different terms, similar to the results by Selle et al. (2013), our quadratic regression analysis (Fig. 1) showed that the FCR of birds was negatively correlated with ileal digestible starch intake to digestible protein intake ratio indicating that as the rate of starch digestion decreases, proportionate to that of protein, the feed efficiency improves. However, it is not fully clear whether the observed correlation and the calculated ratios are cause or effect of starch content in the diet, feed intake and other unknown factors. Given the wide range of expected differences in starch and protein content of the diets in the present study as a result of extreme differences in AME and DLYS-to-ME ratios and therefore corresponding digestible nutrient intake, the interpretation of nature of such relationship may be difficult.

Fat digestibility across 3 different ages was mainly affected by the density of the diets with an expected higher digestibility in HD diets with higher concentration of supplemented oil.

Utilising the BND concept based on 2 criteria of DLYS:ME and nutrient density as defined by ME proportionate to other non-nitrogenated nutrients, the data demonstrated that both criteria of nutrient density and DLYS:ME can exhibit independent responses on overall BWG which is an advantage for optimization of performance and profitability. The independent feed intake response of broilers to DLYS:ME also highlights the robustness of this criteria to influence performance of broiler chickens. However, various interactions existed for FCR, AME, N conversion, jejunal and ileal starch and nitrogen digestibility between nutrient density and DLYS:ME. There are known limitations of ME system in both overestimation and underestimation of energy value of ingredients for broiler chickens (Barekatain et al., 2014). These limitations may have contributed into these interactions. Some unavoidable differences in fibre and fat contents of the diets may have also led to some biases related to physiological response to different feed ingredients. A move towards an NE system may have benefits in the valuation of diets (Barekatain et al., 2014) and profitability in integrated operations, and may allow greater flexibility to proactively manipulate energy densities of diets to maximise economic returns.

It was evident from the results of this study that modern broilers require proportionally higher amino acids compared with ME as shown by significant interaction between DLYS:ME and nutrient density. Both nutrient density and DLYS:ME triggered independent responses on BWG given the wide range of nutrient tested in this study. The use of BND in commercial feed formulation may allow reaching the profit maximisation objective of the broiler production system more accurately compared to the current per mass of feed nutrient specification system, by taking full advantage of ingredient price movements with less density constraints in the formulation. However, separate economic analysis along with detailed carcass yield analysis is required to prove such assumptions. Additionally, in future studies, use of exogenous enzymes may provide further optimisation of a BND system as there were differential impacts of both DLYS:ME and diet density on digestibility and utilisation of nutrients including starch and protein in different ages. Nonetheless, the application of such system requires new developments in diet formulation and the modelling of poultry enterprise profitability.

Author contributions

Reza Barekatain: Conceptualization, Investigation, Formal analysis, Data curation, Resources, Project administration, Writing – original draft. **Luis F. Romero:** Conceptualization, Methodology, Writing – original draft. **José Otávio B. Sorbara:** Conceptualization, Funding acquisition, Writing – review & editing. **Aaron J. Cowieson:** Conceptualization, Methodology, Writing – review & editing.

Conflict of interest

We, as the authors of the manuscript, declare that any affiliation with the funder of the study does not interfere with our adherence to all policies regarding sharing data and materials published in *Animal Nutrition*.

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