Energy dynamics, nitrogen balance, and performance in broilers fed high- and reduced-CP diets

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Primary Audience: Nutritionists, Broiler Producers

SUMMARY

There has been extensive research on feeding broilers low-CP (**LCP**) diets to reduce nitrogen (**N**) excretion in the environment. It is well established that broilers fed LCP diets exhibit improvements in N efficiency, but this is coupled with inferior growth performance and poor carcass quality. Therefore, 2 experiments were undertaken to explore energy and N balance and performance in birds fed LCP diets to determine bird responses to dietary energy content. Both experiments used isoenergetic grower–finisher diets formulated to reduced- or high-CP (**HCP**) level. Measurements of AME, net energy, and N balance were conducted in a calorimetry system (experiment 1), and bird performance was measured in a floor pen feeding study (experiment 2). In experiment 1, birds fed the LCP diet had a comparatively higher ratio of energy (AME and net energy) intake to N retained, higher N efficiency (N retained/N intake), and higher ratio of energy retained as fat to total energy retention. In experiment 2, the LCP-fed birds had a comparatively higher feed conversion ratio at day 14 to 35 and a higher relative fat pad weight on day 35. Abdominal fat pad was positively correlated with the energy (AME and net energy) intake–to–weight gain ratio, suggesting that energy in excess was deposited as fat. These results present more highly efficient use of N in broilers reared on LCP diets. However, these birds also consumed excess energy relative to N retained, which was deposited as body fat accretion, thereby increasing the feed conversion ratio.

Key words: high-CP diet, low-CP diet, net energy, AME, abdominal fat pad

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DESCRIPTION OF PROBLEM

Intensive animal production plays a notable role in environmental pollution in agriculture, largely because of low efficiency of dietary nutrient utilization, namely nitrogen (**N**) (Aletor et al., 2000). High-CP (**HCP**) diets provide sufficient N to allow for nonessential amino acids (**AA**) synthesis, but the quantity of N excreted by chickens fed this diet is high, thereby increasing N pollution in the environment, through ammonia emissions and nitrate pollution of fresh water (Morse, 1995; Aletor

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et al., 2000; Bregendahl et al., 2002). This is primarily induced through excretion of N from dietary AA, such as uric acid, when protein in excess is used as an energy source (Nieto et al., 1997; Sarmiento-Franco et al., 2000). In addition, increased use of soybean meal, which is rich in potassium, increases water intake, thus heightening excreta moisture, a contributing factor for transformation of excreta N into ammonia (Meda et al., 2011; Belloir et al., 2017). In the poultry industry, there has been extensive research conducted into decreasing the environmental impact of N excretion, such as feeding low-CP (**LCP**) diets supplemented with synthetic essential AA. However, a balance must be achieved between establishing these environmental improvements while still maintaining economic broiler production and good carcass characteristics for consumers (Aletor et al., 2000).

It is well established that broilers fed LCP diets exhibit improvements in N efficiency (Belloir et al., 2017; Alfonso-Avila et al., 2019). Although some researchers reported that LCP diets do not alter bird performance and meat quality (Belloir et al., 2017; Marayat et al., 2019), a number of experiments have demonstrated that reducing dietary CP can adversely affect bird performance, by lowering growth rate and increasing carcass fat content (Aletor et al., 2000; Bregendahl et al., 2002; Swennen et al., 2004). Potential reasons behind this inferior performance include a change in the AME:CP ratio (Kamran et al., 2008), suboptimal supplement of nonessential AA (MacLeod, 1990), and insufficient N pool for nonessential AA synthesis (Namroud et al., 2008). It has previously been demonstrated that an LCP diet with a high energy:CP ratio impairs growth performance, coupled with increased body fat accretion (MacLeod, 1997; Collin et al., 2003), whereas feeding an LCP diet with a low energy:CP ratio negatively affects bird growth performance without increasing carcass fat content (Kamran et al., 2008).

Although the effects of LCP diets on broiler growth performance and carcass characteristics have been documented previously, there is little evidence in the literature about how energy and N balance influences performance of birds fed diets containing different levels of CP content. The experiments described here were therefore conducted with the objective to investigate the effect of feeding diets with differing CP levels but similar net energy (**NE**) levels on energy dynamics, N balance, heat production (**HP**), and performance responses of broilers to understand the utilization of dietary energy by birds fed LCP diets. The study was conducted in closed-circuit calorimetry chambers (**Exp. 1**) to observe the magnitude of energy and N balance responses to LCP diet and in a floor pen feeding trial (**Exp. 2**) to assess bird performance.

MATERIALS AND METHODS

Animal Management

Two experiments were conducted in this study: Exp. 1 was an energy and N balance study using closed-circuit calorimetry chambers in a climate-controlled room, and Exp. 2 was a floor pen feeding trial. Day-old as-hatched Ross 308 broiler chicks used in both experiments were obtained from a commercial hatchery (Baiada Poultry Pty Ltd., Tamworth, NSW, Australia) and were feather sexed on arrival. The Animal Ethics Committee of the University of New England approved the procedures used in this study (AEC18-001). Temperature and light program were undertaken in accordance with Ross 308 management guidelines (Aviagen, 2014a).

In Exp. 1, 40 birds were used to measure feed energy values in closed calorimetry chambers across 2 runs. From day 0 to 21, birds were reared in floor pens in a climate-controlled room. They were then acclimatized to the calorimetry chambers (pumps running with the chamber lids open) from day 21 to 25. The calorimetric run was performed from day 25 to 28 using 2 birds (1 male and 1 female) per chamber, replicated 5 times per treatment per run. During this period, total excreta were collected, BW of bird and feed intake (**FI**) recorded, and respiratory gas exchange (oxygen consumed and CO_2 exhaled) measured.

In Exp. 2, a floor pen feeding study was undertaken in a completely random block design in a 2 × 2 factorial arrangement of treatments using rooms as blocks. Factors included gender, females vs. males, and dietary

Table 1. Composition of the common starter and basal test diets.

Ingredients, g/kg as-fed	Common starter diet	Basal test grower–finisher diets	
		HCP diet	LCP diet
Corn	50.0	100	429
Wheat	526	402	50.0
Wheat pollard	10.0	62.2	170
Full-fat soy	50.0	50.0	149
Soybean meal	250	271	20.0
Canola meal cold pressed	50.0	50.0	110
Canola oil	25.5	35.0	27.9
Dicalcium phosphate, 18% P 21% Ca	15.0	12.4	12.0
Limestone	11.2	10.4	10.8
Sand	-	-	8.98
L-lysine HCl	-	-	2.40
Na bicarbonate	2.00	2.00	2.00
Salt	2.14	1.73	1.85
D,L-methionine	2.69	1.09	1.76
Choline Cl 70%	0.631	0.560	1.43
Trace mineral premix[1]	1.00	1.00	1.00
Vitamin premix[2]	0.700	0.700	0.700
L-threonine	-	-	0.55
L-isoleucine	-	-	0.260
Carbohydrase enzymes [3]	0.050	0.050	0.050
L-valine		-	0.030
Nutrient, g/kg unless otherwise indicated	Calculated nutrient	Analyzed nutrients	
AME, MJ/kg	12.8	14.5	14.6
CP	235	235	189
		Calculated nutrients	
Ether extract	53.5	63.6	75.0
Crude fiber	35.7	38.1	45.7
SID amino acids			
Lysine	12.3	11.19	9.90
Methionine	5.62	4.15	4.57
Methionine + cysteine	8.90	0.740	0.74
Threonine	7.80	7.35	6.40
Isoleucine	8.72	9.05	7.00
Valine	9.70	10.1	7.90
Calcium	8.80	8.00	8.00
Available phosphorus	4.40	4.00	4.00

Abbreviations: HCP, high CP; LCP, low CP; SID, standard ileal digestible.

[1]Formulated to supply 23 mg copper, 1.79 mg iodine, 57 mg iron, 171 mg manganese, 0.43 mg selenium, and 143 mg zinc per kg finished feed.

[2]Formulated to supply 5,040 mg retinol, 17.5 mg cholecalciferol, 105 mg tocopheryl acetate, 4 mg menadione, 4 mg thiamine, 11 mg riboflavin, 77 mg niacin, 18 mg pantothenate, 7 mg pyridoxine, 0.35 mg biotin, 3.0 mg folate, and 0.02 mg cyanocobalamin per kg of finished feed.

[3]Rovabio Advance (xylanase, β-glucanase, and arabinofuranosidase).

CP content, low vs. high. In total, 720 sexed birds were randomly allocated into 72 floor pens with 10 single-sexed birds per pen and 18 replicates per dietary treatment. Birds and feed were weighed on a weekly basis from day 14 to day 35, and 3 birds/pen were sampled on day 35. The sampled birds were weighed, electrically stunned followed by cervical dislocation, and then abdominal fat was collected and weighed. The overall mortality rate was less than 5% in Exp. 2.

Dietary Treatments

In both experiments, feed and drinking water were offered ad libitum, and the same diets were fed in both experiments. All diets were formulated to meet or exceed as-hatched Ross 308 nutrient

Table 2. Results from calorimetric trial using broiler chickens fed diets with LCP or HCP content from day 25 to 28.

	Mean			*P*-value	
Parameter[1]	HCP diet	LCP diet	Pooled SEM	Run	Diet
Feed energy value, kJ/g DM					
GE	19.88	19.83	-	-	-
AME	14.49	14.59	0.1100	0.026	0.625
AMEn	13.63	13.81	0.1070	0.018	0.898
NE	10.90	11.01	0.0980	0.107	0.308
Energy utilization, %					
AME/GE	72.90	73.60	0.0056	0.026	0.518
NE/AME	75.24	76.07	0.0036	0.620	0.271
Growth performance					
FI, g as-is/d	129.0	130.0	3.350	0.001	0.837
WG, g/d	84.92	84.66	2.900	0.005	0.958
FCR (g:g as-is)	1.528	1.546	0.0190	0.275	0.643
AMEi/WG, kJ/g	19.88	20.86	0.2130	0.795	0.496
NEi/WG, kJ/g	14.96	15.36	0.1640	0.933	0.244
N balance, g/d					
N intake	4.852[a]	3.930[b]	0.1520	0.001	<0.001
N excreted	1.952[b]	1.280[a]	0.0877	0.004	<0.001
N retained	2.901	2.650	0.0827	0.001	0.055
N excreted/N intake	40.24[b]	32.65[a]	0.0100	0.627	<0.001
N retained/N intake, %	59.80[b]	63.40[a]	1.000	0.627	<0.001
Energy balance, kJ/kg $BW^{0.70}$/d					
HP	779.0	785.5	9.620	0.006	0.694
RE	549.5	613.9	25.70	0.003	0.124
AMEi	1,329	1,399	33.70	0.002	0.189
NEi	1,000	1,064	25.70	0.003	0.124
RE as fat/RE	36.93[b]	46.33[a]	1.740	0.002	<0.001
Respiratory quotient	0.9907	1.007	0.0055	0.055	0.125

[a,b] Means in a row not sharing a superscript differ significantly at $P < 0.05$ for treatment effect.

Abbreviations: AMEi, AME intake; AMEn, AME corrected with N retention; $BW^{0.70}$, metabolic BW; FCR, feed conversion ratio; FI, feed intake; HCP, high CP; HP, heat production; LCP, Low CP; N, nitrogen; NE, net energy; NEi, net energy intake; RE, energy retention; WG, weight gain.

[1]Each mean represents measurements from 10 respiratory chambers. All data were corrected for the variations between 2 calorimetric runs.

specifications (Aviagen, 2014b). The standard ileal digestible AA were calculated using AminoChic 2.0 software (Evonik, Essen, Germany). Birds were fed a common starter diet from day 0 to 14. Test grower–finisher diets were then introduced from day 14 to 28 in Exp. 1 (calorimetry study) and from day 14 to 35 in Exp. 2 (feeding study in floor pens). The common starter diet was formulated based on wheat and soybean meal, with 12.8 MJ/kg AME and 235 g/kg CP (calculated). The test isoenergetic grower–finisher diets were formulated to LCP and HCP concentration, at 189 and 235 g/kg CP (analyzed), respectively. In addition, the LCP diet contained 14.59 MJ/kg AME, 11.1 MJ/kg NE (analyzed), and 75.0 g/kg ether extract (calculated), whereas the HCP diet contained 14.49 MJ/kg AME, 10.9 MJ/kg NE (analyzed), and 63.6 g/kg ether extract (calculated, Table 1). The LCP diet was supplemented with crystalline essential AA to meet the minimal standards for Ross 308 broilers. All diets were supplemented with carbohydrase enzymes (Rovabio Advance) based on xylanase, β-glucanase, and arabinofuranosidase.

Chemical Analysis and Calculations

In Exp. 1, the CO_2 trapped in KOH solution was recovered using the $BaCl_2$ precipitation technique, as described previously (Annison and White, 1961; Swick et al., 2013; Wu et al., 2019). The ratio of the volume (in liter) of CO_2 produced by the chickens to the volume (in liter) of O_2 consumed was calculated to obtain the respiratory quotient. The volumes of CO_2 and O_2 were also used to calculate HP (in kcal) using the modified Brouwer (1965) equation as follows:

$$HP = 1.200 \times CO_2 + 3.866 \times O_2$$

Feed and excreta samples were analyzed for DM by placing the samples in an oven at 105°C until consistent weight was achieved, and for gross energy (GE) using an adiabatic bomb calorimeter (IKA Werke, C7000; GMBH and Co., Staufen, Germany). Feed AME (kcal/kg DM) was calculated using the following equation:

$$AME = [(\text{feed GE} \times FI) - (\text{excreta GE} \times \text{total excreta output})] / FI$$

Feed NE and NE intake (**NEi**) were calculated according to Noblet et al. (1994). In brief, heat increment was obtained by subtracting 450 kJ/kg $BW^{0.70}$ from total HP. The value 450 kJ/kg $BW^{0.70}$ is a fasting HP estimate by Noblet et al. (2015). Retained energy was calculated as the difference between AME intake (**AMEi**) and HP. Net energy intake was calculated as energy retention + fasting HP × metabolic $BW^{0.70}$. Feed NE was obtained by dividing NEi by FI.

The AME and NE values of feed obtained from Exp. 1 were used to calculate AMEi and NEi in Exp. 2. The individual bird weight on day 14 and day 35 in Exp. 2 was recorded to calculate the CV as a measure of flock uniformity. The average CV per pen was used to determine the mean CV per treatment.

Statistical Analyses

Data from Exp. 1 were analyzed in a random block design (blocked by run) using one-way ANOVA, with run as a covariate, on Minitab GLM procedure (Minitab Inc., State College, PA). The experimental unit was a calorimetry chamber (n = 20). Data in Exp. 2 were analyzed as a 2 × 2 factorial in a completely randomized block design, with room as block, using 2-way ANOVA on the GLM procedure on Minitab. The experimental unit in Exp. 2 was a pen (n = 72). Pairwise comparisons between treatments with significant means was carried out using Tukey's mean separation test. Mean differences were considered significant at a probability level of $P < 0.05$ and tendency at $0.05 < P < 0.10$. Pearson correlation test was used to determine correlation matrix.

RESULTS AND DISCUSSION

The present study investigated the effect of LCP and HCP diets with similar NE content on energy and N utilization and performance in broiler chickens. The study applied feed energy parameters (feed AME and NE) obtained from Exp. 1 to calculate AMEi and NEi in Exp. 2. As the feeding trial (Exp. 2) was run over an extended period (from day 14 to 35), this allowed a more holistic comparison of energy and performance factors between the LCP and HCP diets. The important message derived from this study is that 2 different experiments with different focuses (calorimetric and feeding trials) were undertaken, yet the outcomes from both trials were consistent.

Feed Intake Responses to Dietary CP Content

As per the results from Exp. 1 and Exp. 2, birds reared on the LCP diet consumed a similar amount of feed as those fed the HCP diet. In addition, there was no difference in NE consumption between the 2 groups of birds. This means that birds were consuming feed to satisfy their NE needs, as opposed to protein requirements. Feed intake being dictated by dietary energy level has previously been reported by Ravindran, 2013 and also agrees with the study by MacLeod (1990), who found that the ability of birds to control energy intake takes priority over AA intake when fed the LCP diets. However, this is in contrast with the findings of

Parr and Summers (1991), who suggested that birds eat to satisfy their essential AA requirements rather than energy needs, as established when offering LCP diets with varying energy levels. To the best of our knowledge, this is the first study to observe that birds consume to meet NE requirements when fed different levels of CP.

Nitrogen Balance and HP

Feed energy values, energy utilization, energy balance, N balance, and bird performance in response to dietary CP contents in Exp. 1 are summarized in Table 2, and correlation matrix of data from Exp. 1 is illustrated in Table 3. The N balance analysis evidently showed that Ni was more ($P < 0.001$) pronounced in birds fed the HCP diet than those fed the LCP diet. However, N efficiency (N retained [**Nr**]/N intake [**Ni**]) was 5.7% higher in the LCP broilers than that in the HCP group ($P < 0.001$). Improved N efficiency in birds fed LCP diets was also reported by Belloir et al. (2017). The high N efficiency in the LCP-fed birds led to comparatively less N excreted and less N excreted/Ni ($P < 0.001$ each) because they consumed dietary CP lower than their protein requirements, which was 19% less Ni compared with the HCP-fed birds. In addition, the negative correlations observed between FCR and Ni, along with FCR and Nr (r = −0.446, $P < 0.05$ and r = −0.646, $P < 0.01$, respectively), coupled with a lack of correlation between FCR and N efficiency, suggest that there was reduced N excretion as a result of N sparing effect with a lower Ni (MacLeod, 1997). Moreover, AME:CP and NE:CP ratios were highly positively correlated with N efficiency and negatively correlated with Ni, with no correlation with Nr, suggesting that increased feed energy relative to CP impaired Ni, with a tendency for decreased Nr, thereby increasing N efficiency. These low Ni and Nr, which are required for muscle deposition, might explain why the growth rate of the LCP-fed birds lagged behind ($P < 0.01$) their HCP counterparts from day 14 to 21 and day 14 to 28 in Exp. 2.

Interestingly, HP showed no significant ($P > 0.05$) difference between birds fed the LCP and HCP diets, which is in agreement with previous investigations (MacLeod, 1990; 1992; 1997; Collin et al., 2003). As HP had a high ($P < 0.001$) positive correlation with FI and weight gain (**WG**), with r = 0.820 and r = 0.801, respectively, and a negative correlation with FCR (r = −0.456, $P < 0.01$, Table 4), it implies that HP increased with increasing muscle accretion (Close, 1990; Olukosi et al., 2008). It was also demonstrated that HP increases with increasing protein accretion (MacLeod, 1997). The similar HP observed in the present study between birds fed the LCP and HCP diets, in spite of their different ($P = 0.055$) values for Nr, means that the LCP-fed birds retained less N that was used for muscle accretion. However, N retained by the HCP birds in excess to their requirement for muscle deposition was used for energy, as shown by reduced HP (Leeson and Summers, 2001). As the HCP diet contained lower energy (AME and NE) per CP compared with the LCP diet and birds fed the HCP diet showed lower energy intake per Ni and per Nr, actual energy intake from other sources may not meet the energy requirement, thus excessive protein may have to be used as energy but accretion.

Body Fat Accretion

There were no significant interactions between gender and diet treatments Exp. 2, thus only the mean values resulting from the main effects are presented in Table 4. Table 5 presented correlation between some of the performance results from Exp. 2. Abdominal fat pad weighed 29% more in the LCP-fed birds than the HCP-fed counterparts in Exp. 2 ($P < 0.001$). This was likely due to energy retained as fat–to–total energy retention ratio observed in Exp. 1, which was 20% higher in the LCP birds than that in the HCP birds. This increase in fat pad weight in broilers fed diets with low CP concentration is in agreement with the literature (Buyse et al., 1992; Collin et al., 2003; Swennen et al., 2004; Aftab et al., 2006). There are a number of possible explanations for the increased fat gain observed in the LCP-fed birds. First, the same level of FI for the LCP- and HCP-fed birds indicates that, by consuming a similar amount of feed, the excess energy intake relative to Nr led to increased body fat

Table 3. Correlation matrix for energy and N utilization criteria of birds fed diets containing different CP levels from day 25 to 28.

Parameter[1]	AME	NE	FI	WG	FCR	Ni	Nr	Nr/Ni	HP	RE	REf/RE	AME:CP	NE:CP	AME:Nr
NE	0.848***													
FI	0.433†	0.277												
WG	0.516*	0.390†	0.945***											
FCR	−0.512*	−0.509*	−0.502*	−0.751***										
Ni	0.256	0.051	0.689**	0.685**	−0.446*									
Nr	0.521*	0.337	0.886***	0.917***	−0.646**	0.885***								
Nr/Ni	0.432†	0.531*	0.117	0.181	−0.211	−0.546*	−0.096							
HP	0.617**	0.238	0.820***	0.801***	−0.456*	0.534*	0.757***	0.232						
RE	0.725***	0.678**	0.833***	0.867***	−0.628**	0.390†	0.742***	0.507*	0.772***					
REf/RE	0.627**	0.687**	0.620**	0.604**	−0.392†	0.011	0.362	0.650**	0.536*	0.845***				
AME:CP	0.372	0.448*	0.153	0.134	−0.039	−0.580**	−0.178	0.935***	0.245	0.473*	0.748***			
NE:CP	0.352	0.509*	0.114	0.110	−0.060	−0.602**	−0.203	0.935***	0.143	0.464*	0.753***	0.988***		
AME:Nr	0.252	0.282	0.183	0.068	0.163	−0.500*	−0.229	0.683**	0.230	0.359	0.746***	0.898***	0.871***	
NE:Nr	0.224	0.421†	0.107	0.028	0.104	−0.558*	−0.276	0.717***	0.031	0.358	0.767***	0.893***	0.917***	0.948***

Abbreviations: AME:CP (kJ/g:%), %, AME to CP ratio; AMEi:Ni (kJ/kg:g/kg), AME intake/$BW^{0.70}$ to Ni/$BW^{0.70}$; AMEi:Nr (kJ/kg:g/kg), AMEi/$BW^{0.70}$ to Nr/$BW^{0.70}$ ratio; $BW^{0.70}$, metabolic body weight; FCR (g:g as-is), feed conversion ratio; FI (g as is/b/d), feed intake; HP (kJ/kg $BW^{0.70}$/d), heat production per metabolic BW; N, nitrogen, Ni (g/d), N intake; Nr (g/d), N retained; Nr/Ni (%), N efficiency; NE (MJ/kg DM), net energy; NE:CP (kJ/g:%),%, NE to CP ratio; NEi:Ni (kJ/kg:g/kg), NEi/$BW^{0.70}$ to Ni/$BW^{0.70}$; NEi:Nr/$BW^{0.70}$ (kJ/kg:g/kg), NEi/$BW^{0.70}$ to Ni/$BW^{0.70}$; RE (kJ/kg $BW^{0.70}$/d), total energy retained; REf/RE (%), proportion of energy retained as fat per total energy retained; WG (g/d), weight gain.

[1]Probability values are indicated as: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and †$P < 0.10$ (tendency).

Table 4. Performance of broiler chickens fed LCP or HCP diets from day 14 to 35.

	Diet		Gender			*P*-value		
Parameter[1]	HCP	LCP	Males	Females	Pooled SEM	Interaction	Gender	Diet
From day 14 to 21								
WG, g	65.19[a]	62.82[b]	66.72[a]	61.29[b]	0.5530	0.570	<0.001	0.008
FCR (g:g as-is)	1.435[b]	1.520[a]	1.487	1.468	0.0091	0.200	0.211	<0.001
FI, g as is/d	93.45	95.39	99.01[a]	89.83[b]	0.7880	0.117	<0.001	0.080
AMEi/$BW^{0.70}$, kJ/kg	17,775[b]	18,132[a]	18,081	17,826	88.50	0.586	0.137	0.039
NEi/$BW^{0.70}$, kJ/kg	13,497	13,752	13,722	13,527	72.40	0.639	0.159	0.067
AMEi/WG, kJ/g	18.88[b]	19.93[a]	19.48	19.33	0.1100	0.446	0.423	<0.001
NEi/WG, kJ/g	14.33[b]	15.12[a]	14.78	14.67	0.0866	0.506	0.446	<0.001
From day 14 to 28								
WG, g	81.54[a]	78.38[b]	84.40[a]	75.53[b]	0.7140	0.070	<0.001	0.007
FCR (g:g as-is)	1.553[b]	1.603[a]	1.510[b]	1.647[a]	0.0126	0.200	<0.001	0.006
FI, g as-is/d	126.3	125.4	127.4	124.3	0.9830	0.362	0.090	0.581
AMEi/$BW^{0.70}$, kJ/kg	30,741	31,030	30,835	30,935	135.0	0.917	0.668	0.218
NEi/$BW^{0.70}$, kJ/kg	23,342	23,535	23,403	23,475	114.0	0.885	0.708	0.320
AMEi/WG, kJ/g	20.57	21.02	19.88[b]	21.70[a]	0.1760	0.710	<0.001	0.063
ANEi/WG, kJ/g	15.62	15.94	15.09[b]	16.47[a]	0.1390	0.692	<0.001	0.092
From day 14 to 35								
CV (day 14), %	7.274	7.209	7.779[a]	6.704[b]	0.2410	0.743	0.028	0.893
CV (day 35), %	7.750	8.191	8.775[a]	7.166[b]	0.3220	0.837	0.009	0.402
WG, g	91.16	88.85	95.76[a]	84.25[b]	0.8390	0.153	<0.001	0.120
FCR (g:g as-is)	1.586[b]	1.618[a]	1.550[b]	1.654[a]	0.0098	0.106	<0.001	0.027
FI, g as-is/d	144.1	143.5	148.3[a]	139.3[b]	1.020	0.909	<0.001	0.716
AMEi/$BW^{0.70}$, kJ/kg	41,245	41,336	41,271	41,310	157.0	0.117	0.895	0.753
NEi/$BW^{0.70}$, kJ/kg	31,317	31,352	31,322	31,347	133.0	0.135	0.916	0.885
AMEi/WG, kJ/g	20.99	21.21	20.42[b]	21.79[a]	0.1380	0.102	<0.001	0.260
NEi/WG, kJ/g	15.94	16.09	15.50[b]	16.53[a]	0.1100	0.111	<0.001	0.339
Fat pad (day 35), %	0.7598[b]	1.044[a]	0.8243[b]	0.9790[a]	0.0263	0.318	<0.001	<0.001

[a,b]Means in a row not sharing a superscript differ significantly at $P < 0.05$ for treatment effect.

Abbreviations: AMEi, AME intake; $BW^{0.70}$, metabolic BW; FCR, feed conversion ratio; HCP, high CP; LCP, low CP; NE, net energy; NEi, net energy intake; WG, weight gain.

[1]Main effect results are only presented in this table because there was no statistical significance ($P > 0.05$) interaction between gender and diet treatment. Each mean represents 18 pen measurements.

Table 5. Correlation matrix for bird performance at day 35.

Parameter[1]	FCR	WG	FI	AMEi/WG	NEi/WG	Fat pad	AMEi/$BW^{0.7}$
WG	−0.641 ***						
FI	0.029	0.747 ***					
AMEi/WG	0.977 ***	−0.574 ***	0.092				
NEi/WG	0.952 ***	−0.537 ***	0.119	0.995 ***			
Fat pad	0.363 **	−0.463 ***	−0.282 *	0.280 *	0.263 *		
AMEi/$BW^{0.70}$	0.548 ***	0.083	0.582 ***	0.625 ***	0.655 ***	0.032	
NEi/$BW^{0.70}$	0.525 ***	0.090	0.570 ***	0.625 ***	0.669 ***	0.021	0.990 ***

Abbreviations: AMEi/$BW^{0.70}$ (kJ/kg), AMEi per $BW^{0.70}$; AMEi/WG (kJ/kg), AME intake per WG; $BW^{0.70}$, metabolic body weight; fat pad (%), abdominal fat pad weight; FCR (g:g as-is), feed conversion ratio; FI (g as is/b/d), feed intake; NEi/$BW^{0.70}$ (kJ/kg), NEi per $BW^{0.70}$; NEi/WG (kJ/kg), net energy intake per WG; WG (g/d), weight gain.

[1]Probability values are indicated as *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

accretion. This is evidenced by significant ($P < 0.001$) increases in AME:CP, NE:CP, AMEi:Ni, NEi:Ni, AMEi:Nr, and NEi:Nr ratios in birds reared on the LCP diet compared with the HCP-fed counterparts (Table 6). This indicates that the LCP diet contained more energy required to use CP for growth and consequently deposited as fat, as has been previously reported (Collin et al., 2003). This is also demonstrated by significant ($P < 0.001$) positive correlations observed between energy retained as fat:total energy retention vs. AME:CP, NE:CP, AME:Nr, and NEi:Nr, with r = 0.748, r = 0.753, r = 0.746, and r = 0.767, respectively. Second, an increase in adipose fat accretion is also evidenced by a significant ($P < 0.001$) increase in AME and NE consumption per WG observed at day 14 to 21 in birds fed the LCP diet, coupled with a positive, albeit weak correlation observed between abdominal fat pad weight and FCR, AMEi/WG, and NEi/WG at day 14 to 35 (r = 0.363, $P < 0.01$; r = 0.280, $P < 0.05$ and r = 0.263, $P < 0.05$, respectively) and negative correlations between abdominal fat pad weight and WG and FI (r = −0.463, $P < 0.001$ and r = 0.282, $P < 0.05$, respectively). These correlations also denote the fact that the LCP birds consume excessive energy to deposit fat and suppress FI and growth, with subsequent FCR increase. Increased energy intake per WG in

Table 6. Effect of CP levels on energy to N characteristics from day 25 to 28.

	Mean			*P*-value	
Parameter	HCP	LCP	SEM	Run	Diet
Feed energy:CP (kJ/g:%), %					
AME:CP	61.62[b]	77.23[a]	1.860	0.364	<0.001
NE:CP	46.36[b]	58.75[a]	1.490	0.079	<0.001
Energy intake:Ni (kJ/kg:g/kg)					
AMEi:Ni	346.4[b]	432.5[a]	10.30	0.364	<0.001
NEi:Ni	260.6[b]	329.0[a]	8.240	0.079	<0.001
Energy intake:Nr (kJ/kg:g/kg)					
AMEi:Nr	579.6[b]	642.5[a]	8.070	0.027	<0.001
NEi:Nr	436.0[b]	488.7[a]	6.740	0.111	<0.001

[a, b]Means within a column with different superscripts differ significantly ($P < 0.05$, Tukey Pairwise Comparison test).
Abbreviations: AME:CP, apparent metabolizable energy–to–crude protein ratio; AMEi:Ni, AME intake/$BW^{0.70}$ to N intake/$BW^{0.70}$; AMEi:Nr, AMEi/$BW^{0.70}$ to N retention/$BW^{0.70}$; $BW^{0.70}$, metabolic body weight; HCP, high CP; LCP, low CP; NE:CP, net energy-to-CP ratio; NEi:Ni, NE intake/$BW^{0.70}$ to Ni/$BW^{0.70}$; NEi:Nr, NEi/$BW^{0.70}$ to Nr/$BW^{0.70}$.

birds fed LCP diets has been previously reported (Blaxter, 1989; Bregendahl et al., 2002). Finally, the LCP birds could have consumed more energy from carbohydrates and fat than protein, which are more readily metabolized into fat compared with protein (MacLeod, 1997).

It has been reported that dietary CP concentration higher than normal requirements can promote leaner birds (Buyse et al., 1992; MacLeod, 1997; Collin et al., 2003). Therefore, low fat accretion in the HCP chickens observed in the present study demonstrated more efficient use of consumed energy for protein metabolism, assimilation, and maintenance, as also shown by Pirgozliev and Rose (1999). The low fat gain observed in the HCP-fed birds, coupled with high Ni (observed in Exp. 1) agree with the findings of Leeson et al. (1996), who reported that birds deposit less carcass fat when there is either a decrease of energy intake or an increase in protein intake. As an increase in abdominal fat content is regarded as economic loss in the slaughterhouse (Zerehdaran et al., 2004), reducing the energy:CP can lower abdominal fat weight in the LCP-fed birds, although the overall growth performance may still be affected (Kamran et al., 2008).

The LCP diet also adversely ($P < 0.05$) affected FCR during the entire experimental period (Table 4). A significant ($P < 0.001$) positive correlation between FCR and AMEi/WG, NEi/WG, AMEi/$BW^{0.70}$, and NEi/$BW^{0.70}$ (r = 0.977, r = 0.952, r = 0.548, and r = 0.525, respectively) at day 14 to 35 (Table 5) indicates that poor feed efficiency resulted in an increase in energy intake but still low Ni (observed in Exp. 1), thus resulting in less growth. Poorer FCR in birds fed diets containing LCP concentration has been recorded in previous studies (Nawaz et al., 2006; Cardinal et al., 2019).

Influence of Gender and Treatments on Experimental Results

The results from the feeding study (Exp. 2) clearly indicated that males had better ($P < 0.001$) growth performance (FI, WG, and FCR) than females from day 14 to 35, as expected. However, the BW was more uniform in the females (lower CV) than in the males on day 14 ($P < 0.05$) and day 35 ($P < 0.01$), whereby on day 14 and 35, female BW was 14 and 18%, respectively, more uniform than the males. This is in accordance with previous findings (Howlider and Rose, 1992; Dozier et al., 2001; Awad et al., 2017). Body weight uniformity was not affected by CP level ($P > 0.05$). The feeding study results did not indicate any interaction between gender and dietary treatments for any of the measured parameters. This indicates that males and females responded equally to changes in feed CP content.

In this study, AA in all diets were balanced in accordance with AMINOChick 2.0 (Evonik Nutrition and Care GmbH, Hanau, Germany), but some AA in the HCP diet were slightly higher than the AA requirements, owing to the raw ingredients used. For instance, lysine was approximately 11.5% higher in the HCP diet than that in the LCP diet. Sterling et al. (2003) stated that increasing dietary lysine and CP results in increased FI and WG, as well as reduced FCR. However, this improvement in growth performance could have been due to not only an increase in lysine but also in dietary CP. In fact, high lysine content in a diet does not necessarily improve performance when the ratio of lysine:CP is higher than the optimal point. Therefore, the authors concluded that broiler AA requirements have a constant proportion to dietary CP levels (Sterling et al., 2003). In addition, MacLeod (1997) observed an AMEi decrease in a low-lysine diet and described that a depression of energy retention arising from a diet deficient in a single AA is caused by reduced FI. Therefore, better growth performance in the HCP birds from the present study was not due to high lysine content, as the dietary lysine expressed per dietary CP was higher in the LCP diet than the HCP diet (52 vs. 48 g lysine/kg CP) but was likely owing to the provision of sufficient N pool for nonessential AA synthesis (Namroud et al., 2008). In addition, as the FI and NEi were similar between the 2 diet treatments in the present study, it is likely that both diets were not deficient in AA. Moreover, although the CP concentration in the LCP diet (18.9% CP) was slightly lower than the recommended Ross 308 nutrient specification (19.5%) and the HCP diet (23.5% CP), birds fed the LCP and HCP diets had better growth performance (high WG, high FI, and low FCR) than the Ross 308 standards. Therefore, the reduced performance seen in the LCP-fed birds compared with HCP fed birds does not necessarily denoted essential AA deficiency.

CONCLUSIONS AND APPLICATIONS

1. Results from the present study showed that birds fed the LCP diet presented higher N efficiency, which means that they excreted less N than birds fed the HCP diet.
2. The LCP diet adversely affected bird FCR and carcass composition compared with the HCP diet, in spite of similar NE intake and HP.
3. This study provided clear indication to the poultry industry that dietary CP level can be reduced from the normal guideline recommendations, but slightly compromised feed efficiency and increased fad pad may be the consequence compared with a diet with higher CP.
4. Energy-to-CP ratios can be lowered to reduce body fat accretion in birds fed LCP diets, although the overall growth performance may still be compromised.
5. Further research on optimal broiler feed CP concentration is warranted to promote lean meat production in an economically efficient way, with less environmental impacts.

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DISCLOSURES

The authors declare that the present manuscript has no conflict of interest related to its publication.

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