# Possible role of available phosphorus in potentiating the use of low-protein diets for broiler chicken production

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**ABSTRACT** A total of 945 male Ross 308 broiler chicks were used in a growth study to explore the interaction between dietary crude protein concentration and available phosphorus. Nine experimental treatments were constructed factorially by offering low, medium, or standard protein concentrations without or with low, standard, or high available phosphorus. Diets were based on corn, wheat, and soybean meal and all nutrients other than protein/amino acids and available phosphorus were maintained at or above breeder guidelines. Additional synthetic amino acids were used in the diets with low protein concentration in attempt to maintain digestible amino acid supply. Diets were offered to 7 replicate pens of 15 chicks per pen from day 8 to 35. Growth performance was measured during the grower (day 8–24) and finisher (day 25–35) periods. On day 35 carcass composition was determined, blood was drawn for various biochemical measurements and the tibia was excised for mechanical and compositional analyses. Birds that received the low-protein diet had lower terminal body weight and higher feed conversion ratio compared with those that received diets with adequate crude protein content. However, addition of available phosphorus to the low-protein diet resulted in significant reductions in weight-corrected feed conversion that were not evident in the diet with adequate protein content. Bone architecture was only moderately influenced by dietary treatment but birds that ingested the diets containing low and medium protein concentrations had relatively heavier abdominal fat pad weight. Blood biochemistry, especially ammonia, uric acid, and phosphorus, was influenced by both dietary protein and available phosphorus and trends suggested that both axes are involved in protein accretion and catabolism. It can be concluded that performance losses associated with feeding low protein diets to broiler chickens may be partially restored by additional available phosphorus. The implications for use of exogenous enzymes such as protease and phytase and protein nutrition *per se* warrants further examination.

**Key words:** protein, amino acids, broiler, phosphorus, nutrition

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## INTRODUCTION

Before the availability of synthetic amino acids, broiler diets were formulated to contain up to 70% soybean meal (**SBM**) and 35% crude protein to meet the requirement for the first limiting amino acid, methionine (Pesti, 2009). After the introduction of synthetic methionine, and later, lysine and threonine, broiler diets can be formulated with SBM inclusion of between 25 and 30% and crude protein concentrations of 18 to 22% while still satisfying the birds' requirement for essential amino acids. Further reductions in dietary crude protein are desirable to promote economic and environmental sustainability of poultry production. However, the response of broiler chickens to radically low protein concentrations varies, even when augmented with an array of synthetic amino acids (Corzo et al., 2005, 2010; Belloir et al., 2017) and feeding diets with high protein and energy concentrations remains associated with maximum growth performance and somatotropic response (Saxena et al., 2020). Reasons for the variability in response of broilers to low-protein diets and supplemental synthetic amino acids are not clear but may be associated with alterations to dietary fiber or potassium (**K**) content with changing SBM inclusion, changes to the net energy density of the diet, a general requirement for nitrogen, nonessential amino acids or perhaps amino acids that are conditionally essential such as glycine and serine (Fancher and Jensen, 1989; Siegert et al., 2016; Chrystal et al., 2020).



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One overlooked axis in feeding low-protein diets to livestock is the supply of available phosphorus (**P**). Available P is defined as the bioavailability of P relative to an inorganic P source, for example, dicalcium phosphate and is nomenclature that is common for least-cost feed formulation systems. Available P is similar to but not synonymous with direct measures of P digestibility such as apparent ileal P digestibility or P retention, but for the purposes of the present study, the terms can be considered equivalent. Maize contains around 0.23% phytate P and 0.08% nonphytate P and this is similar for alternative cereal grains (Eeckhout and De Paepe, 1994; Weremko et al., 1997). Alternatively, SBM contains around 0.38% phytate P and 0.25% nonphytate P (Eeckhout and De Paepe, 1994; Weremko et al., 1997). Thus, both the total concentration of phytate P and total P and the ratio of phytate P to total P in cereals (75–80%) and in SBM (50–60%) are substantially different and these differences declare themselves in the bioavailability of P in these grains. For example, Weremko et al. (1997) reviewed 14 independent studies on the *per se* availability of P in maize (approximately 13%) and 10 independent studies on the same for SBM (approximately 30%) in pigs, concluding that there are significant differences between cereals and protein meals in terms of the bioavailability of P. In addition to changes in phytate and nonphytate P concentrations and ratios as diet composition is adjusted to generate lower crude protein content, it is relevant that animals requirements for P may be influenced by protein intake. Hammoud et al. (2017) observed that feeding a low-protein diet (10% vs. a control diet at 20%) to rats significantly retarded growth but that these deleterious effects could be largely offset by increasing dietary phosphorus content, both in growth rate and also in body composition. Similar effects have been reported more recently where addition of lysine and P to a low protein diet in rats generated significant synergistic effects on growth rate (Ragi et al., 2019). Putatively, if P supply is marginal then adenosine triphosphate (**ATP**) synthesis may be inadequate (Hettleman et al., 1983) to support protein synthesis and growth and this may restrict feed intake to prevent circulatory accumulation of amino acids that can result in toxicity. Protein synthesis requires substantial energy investment (0.67 kcal/1 g of protein; Shariatmadari and Forbes, 1993) and so inadequacy of P supply and restriction on ATP synthesis may have a profound effect on protein accretion. Thus, the objective of the experiment reported herein was to explore whether the performance of broiler chickens could be enhanced by supplementation of low-protein diets that were balanced in amino acid provision and potassium, with additional digestible P.

## MATERIALS AND METHODS

### Birds and Diets

The study procedures were reviewed and approved by the University of New England Animal Ethics Committee to ensure compliance with welfare and humane practices.

A total of 990 male broiler chickens (Ross 308) were obtained from a local hatchery (Aviagen, Goulburn, NSW, Australia). All chicks were offered a common starter diet formulated to meet or exceed Ross 308 nutrient specifications (Aviagen, 2014) with an apparent metabolizable energy content of 3,000 kcal/kg, 1.28% digestible lysine, 0.90% calcium (**Ca**), and 0.45% available P. On day 8, 945 healthy chicks were weighed and distributed to 63 floor pens, 15 chicks per pen, to achieve an equivalent pen weight (±50 g/pen). A total of 9 dietary treatments were generated by factorially arranging 3 concentrations of crude protein (21.5/19.5, 19.5/17.5, or 17.5/15.5%; grower/finisher, respectively) and 3 concentrations of available P (0.48/0.45%, 0.43/0.40%, or 0.38/0.35%; grower/finisher, respectively). Chicks were raised in a windowless and environmentally controlled house. The ambient temperature was initially set and maintained at 33 ± 1.0°C for the first 3 d on chick's arrival and then gradually decreased by 1.0°C every 2 d to reach 23.0°C and kept constant thereafter to the end of the trial. Lighting and ventilation program followed the recommendations set forth in the Ross 308 breed management manual (Aviagen, 2018). Feed and water were available throughout the experiment ad libitum.

Diets were based on corn, wheat, and SBM (Tables 1–4) and were formulated to be equivalent in all nutrients other than those that were the focus of the experiment. Digestible amino acids were added in increasing concentrations as dietary crude protein was reduced to ensure essential amino acid requirements were met, even at the lowest protein level. Dietary electrolyte balance and K provision was maintained as SBM was displaced by addition of K carbonate.

### Measurements

Body weight gain and feed consumption were measured and FCR calculated for the grower (day 8–24) and finisher (day 25–35) periods and over the entire experimental period (day 8–35). Mortality, on a pen basis, was used to correct FCR values. On day 35 body weight corrected FCR (**FCRc**) was also calculated and presented as there were treatment-associated differences in body weight. This correction was achieved by consider a 30 g difference in body weight was equivalent to 1 point in FCR. The primary reason for this additional calculation is to accommodate the fact that under commercial growing conditions birds are reared to a target weight and not a fixed age.

On day 35, a total of 3 birds per pen were selected at random, electrically stunned and euthanized. Blood samples were individually collected in nonheparinized tubes from the jugular vein of 2 birds. Skinless breast meat, thigh + drumstick, and abdominal fat pad were removed, weighed, and calculated as a percentage of live body weight. Tibia samples were also collected for breaking strength test and mineral composition analysis. The digesta content of the ileum (portion of the small intestine from Meckel's diverticulum to approximately

**Table 1.** Experimental grower diets (%).

| | Standard CP | | | Medium CP | | | Low CP | | |
|---|---|---|---|---|---|---|---|---|---|
| Ingredients % | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP |
| Corn 3350–8.7% | 32.2 | 32.6 | 33.0 | 38.9 | 39.1 | 39.3 | 45.3 | 45.5 | 45.8 |
| Wheat 3,000–15% | 30.2 | 30.0 | 29.8 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| SBM 2380–47% | 28.2 | 28.2 | 28.2 | 20.7 | 20.6 | 20.6 | 12.8 | 12.8 | 12.8 |
| Canola oil | 5.07 | 4.98 | 4.90 | 4.63 | 4.55 | 4.48 | 4.31 | 4.23 | 4.16 |
| Dical Phos 18%P/23%Ca | 2.088 | 1.811 | 1.533 | 2.134 | 1.856 | 1.578 | 2.182 | 1.904 | 1.626 |
| Lime fine 38% | 0.819 | 0.988 | 1.156 | 0.831 | 0.999 | 1.168 | 0.842 | 1.011 | 1.179 |
| Dl-Methionine | 0.325 | 0.324 | 0.324 | 0.379 | 0.379 | 0.379 | 0.437 | 0.437 | 0.436 |
| L-Lysine HCL | 0.267 | 0.267 | 0.267 | 0.498 | 0.499 | 0.500 | 0.740 | 0.741 | 0.741 |
| Salt | 0.231 | 0.231 | 0.231 | 0.155 | 0.155 | 0.155 | 0.077 | 0.077 | 0.076 |
| Sodium bicarbonate | 0.220 | 0.221 | 0.221 | 0.336 | 0.336 | 0.337 | 0.457 | 0.457 | 0.458 |
| Vit/min premix | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 |
| L-Threonine | 0.136 | 0.136 | 0.135 | 0.239 | 0.239 | 0.240 | 0.348 | 0.348 | 0.348 |
| Choline chloride 75% | 0.057 | 0.057 | 0.057 | 0.082 | 0.082 | 0.082 | 0.109 | 0.109 | 0.109 |
| L-Valine | 0.009 | 0.009 | 0.009 | 0.133 | 0.133 | 0.133 | 0.263 | 0.263 | 0.263 |
| L-Histidine | 0 | 0 | 0 | 0.053 | 0.053 | 0.053 | 0.125 | 0.125 | 0.125 |
| L-Isoleucine | 0 | 0 | 0 | 0.107 | 0.107 | 0.108 | 0.244 | 0.244 | 0.244 |
| L-Phenylalanine | 0 | 0 | 0 | 0.126 | 0.126 | 0.126 | 0.268 | 0.268 | 0.268 |
| L-Glycine | 0 | 0 | 0 | 0.127 | 0.128 | 0.128 | 0.264 | 0.263 | 0.264 |
| Potassium carbonate | 0 | 0 | 0 | 0.199 | 0.199 | 0.199 | 0.422 | 0.422 | 0.422 |
| L-Arginine | 0 | 0 | 0 | 0.213 | 0.214 | 0.215 | 0.437 | 0.438 | 0.438 |
| L-Tryptophan | 0 | 0 | 0 | 0 | 0 | 0 | 0.024 | 0.024 | 0.024 |
| L-Leucine | 0 | 0 | 0 | 0 | 0 | 0 | 0.102 | 0.101 | 0.100 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Trace mineral concentrate supplied per kilogram of diet: 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; cereal-based carrier, 128 mg; mineral oil, 3.75 mg. Vitamin concentrate supplied 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.

Abbreviations: AvP, available phosphorous; CP, crude protein; Std, standard; Med, medium.

1 cm proximal to the ileocecal junction) were gently squeezed out and pooled per replicate pen, to determine digesta dry matter and water content.

## Chemical Analysis

The nitrogen (**N**) content of feed samples, in duplicate, were determined from a 0.25-g sample in a combustion analyzer (Leco model FP-2000 N analyzer, Leco Corp., St. Joseph, MI) using EDTA as a calibration standard, with crude protein being calculated by multiplying percentage N by a correction factor (6.25). All diets (in duplicate) were analyzed for total N, and mineral profile (Table 5).

The tibias were subjected to breaking strength test using an Instron instrument (Model 1011; Instron

**Table 2.** Calculated nutrient profile of the grower diets.

| | Standard CP | | | Medium CP | | | Low CP | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP |
| ME kcal/kg | 3,080 | 3,080 | 3,080 | 3,080 | 3,080 | 3,080 | 3,080 | 3,080 | 3,080 |
| Crude Protein % | 21.5 | 21.5 | 21.5 | 19.5 | 19.5 | 19.5 | 17.5 | 17.5 | 17.5 |
| Dig.Lys, % | 1.124 | 1.124 | 1.124 | 1.124 | 1.124 | 1.124 | 1.124 | 1.124 | 1.124 |
| Dig.Met, % | 0.588 | 0.588 | 0.588 | 0.611 | 0.610 | 0.610 | 0.635 | 0.634 | 0.634 |
| Dig.M + C, % | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 |
| Dig.Thr, % | 0.752 | 0.752 | 0.752 | 0.752 | 0.752 | 0.752 | 0.752 | 0.752 | 0.752 |
| Dig.Ile, % | 0.785 | 0.785 | 0.785 | 0.762 | 0.762 | 0.762 | 0.762 | 0.762 | 0.762 |
| Dig.Leu, % | 1.496 | 1.498 | 1.500 | 1.324 | 1.325 | 1.325 | 1.241 | 1.241 | 1.241 |
| Dig.Trp, % | 0.237 | 0.237 | 0.237 | 0.196 | 0.195 | 0.195 | 0.175 | 0.175 | 0.175 |
| Dig.Arg, % | 1.203 | 1.203 | 1.203 | 1.203 | 1.203 | 1.203 | 1.203 | 1.203 | 1.203 |
| Dig.Val, % | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 | 0.850 |
| Dig Gly, % | 0.797 | 0.797 | 0.797 | 0.797 | 0.797 | 0.797 | 0.797 | 0.797 | 0.797 |
| Dig Phe, % | 0.920 | 0.920 | 0.920 | 0.920 | 0.920 | 0.920 | 0.920 | 0.920 | 0.920 |
| Crude Fat % | 7.303 | 7.233 | 7.162 | 7.006 | 6.939 | 6.872 | 6.816 | 6.748 | 6.680 |
| Phytate P % | 0.261 | 0.261 | 0.262 | 0.242 | 0.242 | 0.242 | 0.221 | 0.221 | 0.222 |
| Ash % | 6.443 | 6.334 | 6.225 | 6.301 | 6.190 | 6.079 | 6.163 | 6.053 | 5.942 |
| Calcium % | 0.860 | 0.860 | 0.860 | 0.860 | 0.860 | 0.860 | 0.860 | 0.860 | 0.860 |
| Available P % | 0.480 | 0.430 | 0.380 | 0.480 | 0.430 | 0.380 | 0.480 | 0.430 | 0.380 |
| Total P % | 0.762 | 0.712 | 0.662 | 0.731 | 0.682 | 0.632 | 0.699 | 0.649 | 0.599 |
| Sodium % | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 |
| Chloride % | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 |
| Potassium % | 0.767 | 0.768 | 0.768 | 0.760 | 0.760 | 0.760 | 0.760 | 0.760 | 0.760 |
| DEB meq/kg | 207 | 207 | 207 | 205 | 205 | 205 | 205 | 205 | 205 |

Abbreviations: AvP, available phosphorous; CP, crude protein; DEB, dietary electrolyte balance; Dig, digestible; Std, standard; Med, medium.

**Table 3.** Experimental finisher diets (%).

| | Standard CP | | | Medium CP | | | Low CP | | |
|---|---|---|---|---|---|---|---|---|---|
| Ingredients % | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP |
| Corn 3350–8.7% | 35.3 | 35.5 | 35.8 | 41.7 | 42.0 | 42.2 | 48.2 | 48.4 | 48.6 |
| Wheat 3,000–15% | 32.5 | 32.5 | 32.5 | 32.5 | 32.5 | 32.5 | 32.5 | 32.5 | 32.5 |
| SBM 2380–47% | 22.2 | 22.2 | 22.1 | 14.6 | 14.6 | 14.5 | 6.58 | 6.53 | 6.49 |
| Canola oil | 5.56 | 5.48 | 5.40 | 5.17 | 5.09 | 5.01 | 4.91 | 4.83 | 4.75 |
| Dical Phos 18%P/23%Ca | 1.947 | 1.669 | 1.391 | 1.994 | 1.715 | 1.437 | 2.044 | 1.766 | 1.487 |
| Lime fine 38% | 0.776 | 0.944 | 1.113 | 0.787 | 0.956 | 1.124 | 0.798 | 0.967 | 1.136 |
| L-Lysine- HCl | 0.346 | 0.347 | 0.347 | 0.580 | 0.581 | 0.582 | 0.829 | 0.830 | 0.831 |
| Dl-Methionine | 0.329 | 0.329 | 0.328 | 0.385 | 0.384 | 0.384 | 0.445 | 0.444 | 0.444 |
| Sodium bicarbonate | 0.257 | 0.257 | 0.258 | 0.374 | 0.374 | 0.375 | 0.498 | 0.499 | 0.499 |
| Salt | 0.207 | 0.207 | 0.207 | 0.131 | 0.131 | 0.130 | 0.050 | 0.050 | 0.050 |
| Vit/min premix | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 |
| L-Threonine | 0.154 | 0.155 | 0.155 | 0.260 | 0.260 | 0.260 | 0.372 | 0.372 | 0.372 |
| L-Arginine | 0.074 | 0.074 | 0.075 | 0.290 | 0.291 | 0.291 | 0.520 | 0.521 | 0.521 |
| Choline chloride 75% | 0.062 | 0.062 | 0.062 | 0.088 | 0.088 | 0.088 | 0.116 | 0.116 | 0.116 |
| L-Valine | 0.050 | 0.050 | 0.050 | 0.176 | 0.176 | 0.176 | 0.311 | 0.311 | 0.311 |
| L-Isoleucine | 0.031 | 0.032 | 0.032 | 0.163 | 0.163 | 0.164 | 0.304 | 0.304 | 0.304 |
| L-Leucine | 0 | 0 | 0 | 0.016 | 0.015 | 0.014 | 0.208 | 0.207 | 0.206 |
| L-Histidine | 0 | 0 | 0 | 0.068 | 0.068 | 0.068 | 0.142 | 0.142 | 0.142 |
| L-Glycine | 0 | 0 | 0 | 0.130 | 0.131 | 0.131 | 0.270 | 0.270 | 0.271 |
| L-Phenylalanine | 0 | 0 | 0 | 0.137 | 0.137 | 0.137 | 0.283 | 0.283 | 0.283 |
| Potassium carbonate | 0 | 0 | 0 | 0.208 | 0.208 | 0.209 | 0.438 | 0.438 | 0.438 |
| L-Tryptophan | 0 | 0 | 0 | 0 | 0 | 0 | 0.038 | 0.038 | 0.038 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Trace mineral concentrate supplied per kilogram of diet: 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; cereal-based carrier, 128 mg; mineral oil, 3.75 mg. Vitamin concentrate supplied 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.

Abbreviations: AvP, available phosphorous; CP, crude protein; Std, standard; Med, medium.

Universal Testing Machine, Instron Corp., Canton, MA) with Automated Materials Test System software version 4.2. The samples were placed on vertical brackets set 40 mm apart and a 10 mm compression rob was positioned near the center of the bone. The instrument was equipped with a 50 kg load cell and a crosshead speed of 50 mm/min was used during the breaking strength determination. After the breaking strength test, the broken tibia samples were collected and dried for 24 h at 105°C in a drying oven (Qualtex Universal Series 2000; Watson Victor Ltd., Perth, Australia) and reweighed after cooling in a desiccator. The dried tibias were then ashed in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK) at 600°C for 6 h after starting at 300°C with a 1 h ramp up time. Moisture-free tibia ash was expressed as the percentage of tibia ash relative to dry tibia weight. The ash samples were further ground. The mineral content of the tibia ash

**Table 4.** Calculated nutrient profile of the finisher diets.

| | Standard CP | | | Medium CP | | | Low CP | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP | High AvP | Std AvP | Low AvP |
| ME kcal/kg | 3,150 | 3,150 | 3,150 | 3,150 | 3,150 | 3,150 | 3,150 | 3,150 | 3,150 |
| Crude protein % | 19.5 | 19.5 | 19.5 | 17.5 | 17.5 | 17.5 | 15.5 | 15.5 | 15.5 |
| Dig.Lys, % | 1.046 | 1.046 | 1.046 | 1.046 | 1.046 | 1.046 | 1.046 | 1.046 | 1.046 |
| Dig.Met, % | 0.568 | 0.568 | 0.568 | 0.591 | 0.591 | 0.591 | 0.616 | 0.616 | 0.616 |
| Dig.M + C, % | 0.816 | 0.816 | 0.816 | 0.816 | 0.816 | 0.816 | 0.816 | 0.816 | 0.816 |
| Dig.Thr, % | 0.690 | 0.690 | 0.690 | 0.690 | 0.690 | 0.690 | 0.690 | 0.690 | 0.690 |
| Dig.Ile, % | 0.720 | 0.720 | 0.720 | 0.720 | 0.720 | 0.720 | 0.720 | 0.720 | 0.720 |
| Dig.Leu, % | 1.360 | 1.361 | 1.362 | 1.200 | 1.200 | 1.200 | 1.200 | 1.200 | 1.200 |
| Dig.Trp, % | 0.207 | 0.207 | 0.207 | 0.165 | 0.165 | 0.165 | 0.157 | 0.157 | 0.157 |
| Dig.Arg, % | 1.115 | 1.115 | 1.115 | 1.115 | 1.115 | 1.115 | 1.115 | 1.115 | 1.115 |
| Dig.Val, % | 0.799 | 0.799 | 0.799 | 0.799 | 0.799 | 0.799 | 0.799 | 0.799 | 0.799 |
| Dig Gly, % | 0.699 | 0.700 | 0.700 | 0.699 | 0.699 | 0.699 | 0.699 | 0.699 | 0.699 |
| Dig Phe, % | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 |
| Crude fat % | 7.857 | 7.790 | 7.722 | 7.602 | 7.535 | 7.467 | 7.467 | 7.399 | 7.331 |
| Phytate phosphorous % | 0.248 | 0.248 | 0.249 | 0.228 | 0.229 | 0.229 | 0.207 | 0.207 | 0.207 |
| Ash % | 5.942 | 5.831 | 5.720 | 5.803 | 5.692 | 5.582 | 5.660 | 5.550 | 5.439 |
| Calcium % | 0.800 | 0.800 | 0.800 | 0.800 | 0.800 | 0.800 | 0.800 | 0.800 | 0.800 |
| Available P % | 0.450 | 0.400 | 0.350 | 0.450 | 0.400 | 0.350 | 0.450 | 0.400 | 0.350 |
| Total P % | 0.709 | 0.659 | 0.609 | 0.678 | 0.628 | 0.578 | 0.645 | 0.595 | 0.545 |
| Sodium % | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 | 0.180 |
| Chloride % | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 | 0.240 |
| Potassium % | 0.674 | 0.674 | 0.674 | 0.670 | 0.670 | 0.670 | 0.670 | 0.670 | 0.670 |
| DEB meq/kg | 183 | 183 | 183 | 182 | 182 | 182 | 182 | 182 | 182 |

Abbreviations: AvP, available phosphorous; CP, crude protein; DEB, dietary electrolyte balance; Dig, digestible; Std, standard; Med, medium.

**Table 5.** Analyzed crude protein and mineral content of the experimental diets.

| | | Grower diets | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments[1] | | CP | Ca | P | K | Na | Cl | Mg | Cu | Fe | Mn | Zn |
| CP | AvP | % | % | % | % | % | % | % | mg/kg | mg/kg | mg/kg | mg/kg |
| Std | High | 21.7 | 1.16 | 0.815 | 0.96 | 0.187 | 0.273 | 0.212 | 21.8 | 182 | 159 | 127 |
| Std | Std | 21.6 | 1.18 | 0.779 | 0.96 | 0.194 | 0.265 | 0.210 | 22.2 | 162 | 148 | 130 |
| Std | Low | 21.8 | 1.17 | 0.743 | 0.94 | 0.190 | 0.276 | 0.211 | 19.0 | 212 | 145 | 122 |
| Med | High | 19.8 | 0.96 | 0.751 | 0.94 | 0.189 | 0.234 | 0.211 | 21.9 | 149 | 195 | 134 |
| Med | Std | 20.1 | 1.03 | 0.712 | 0.93 | 0.201 | 0.244 | 0.205 | 20.8 | 152 | 197 | 133 |
| Med | Low | 19.7 | 0.99 | 0.671 | 0.93 | 0.190 | 0.255 | 0.207 | 20.4 | 138 | 195 | 130 |
| Low | High | 17.8 | 0.95 | 0.699 | 0.88 | 0.192 | 0.221 | 0.178 | 18.7 | 139 | 179 | 125 |
| Low | Std | 18.2 | 1.07 | 0.658 | 0.88 | 0.205 | 0.235 | 0.189 | 19.5 | 142 | 195 | 137 |
| Low | Low | 18.0 | 0.96 | 0.606 | 0.84 | 0.182 | 0.213 | 0.173 | 21.6 | 134 | 181 | 129 |
| | | Finisher diets | | | | | | | | | | |
| Treatments | | CP | Ca | P | K | Na | Cl | Mg | Cu | Fe | Mn | Zn |
| CP | AvP | % | % | % | % | % | % | % | mg/kg | mg/kg | mg/kg | mg/kg |
| Std | High | 19.9 | 0.93 | 0.724 | 0.78 | 0.174 | 0.265 | 0.195 | 20.9 | 158 | 163 | 124 |
| Std | Std | 20.3 | 0.94 | 0.685 | 0.79 | 0.190 | 0.277 | 0.205 | 21.4 | 141 | 182 | 128 |
| Std | Low | 20.1 | 0.95 | 0.637 | 0.78 | 0.184 | 0.256 | 0.212 | 20.1 | 143 | 173 | 123 |
| Med | High | 17.4 | 0.93 | 0.705 | 0.77 | 0.189 | 0.241 | 0.198 | 19.2 | 169 | 184 | 131 |
| Med | Std | 17.7 | 0.91 | 0.648 | 0.78 | 0.191 | 0.222 | 0.198 | 20.7 | 164 | 188 | 132 |
| Med | Low | 17.8 | 0.95 | 0.602 | 0.75 | 0.189 | 0.234 | 0.190 | 21.4 | 164 | 187 | 130 |
| Low | High | 15.2 | 0.93 | 0.664 | 0.72 | 0.188 | 0.220 | 0.159 | 19.5 | 146 | 170 | 122 |
| Low | Std | 15.1 | 0.90 | 0.619 | 0.71 | 0.183 | 0.207 | 0.160 | 19.5 | 153 | 160 | 126 |
| Low | Low | 15.6 | 0.91 | 0.573 | 0.73 | 0.181 | 0.216 | 0.149 | 19.0 | 151 | 175 | 124 |

[1]AvP, available phosphorous; CP, crude protein; Std, standard; Med, medium.

and diets samples were determined using inductively coupled plasma–optical emission spectrometer (Agilent, Mulgrave, Victoria, Australia).

Blood samples were allowed to clot for 30 min at room temperature and then centrifuged at 3,000 × *g* for 10 min at 4°C (Sigma 4-15 laboratory centrifuge, Germany) to separate the serum. Individual serum samples were analyzed for ammonia, uric acid, total protein, high-density lipoprotein (**HDL**), low-density lipoprotein cholesterol and triglyceride, calcium, phosphorous,

**Table 6.** Performance parameters of birds in response to diets varying in crude protein and available phosphorous.

| Treatments [1] | | Body weight g/bird | | | Feed intake g/bird | | | FCR g/g | | | FCRc[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CP | AvP | Day 8 | Day 24 | Day 35 | Day 8–24 | Day 24–35 | Day 8–35 | Day 8–24 | Day 24–35 | Day 8–35 | Day 8–35 |
| Std | High | 244 | 1,270 | 2,562 | 1,370 | 1,981 | 3,350 | 1.334$^{b,c}$ | 1.534$^{b,c}$ | 1.445$^{b,c}$ | 1.445$^{c,d}$ |
| Std | Std | 246 | 1,280 | 2,555 | 1,357 | 1,957 | 3,315 | 1.312$^{c}$ | 1.535$^{b,c}$ | 1.435$^{b,c}$ | 1.438$^{c,d}$ |
| Std | Low | 243 | 1,260 | 2,529 | 1,327 | 1,933 | 3,261 | 1.304$^{c}$ | 1.524$^{b,c}$ | 1.426$^{c}$ | 1.437$^{c,d}$ |
| Med | High | 245 | 1,262 | 2,524 | 1,385 | 1,910 | 3,296 | 1.362$^{b}$ | 1.513$^{c}$ | 1.446$^{b,c}$ | 1.458$^{c,d}$ |
| Med | Std | 244 | 1,263 | 2,518 | 1,391 | 1,909 | 3,301 | 1.367$^{b}$ | 1.522$^{b,c}$ | 1.452$^{b}$ | 1.465$^{c}$ |
| Med | Low | 242 | 1,242 | 2,477 | 1,351 | 1,907 | 3,259 | 1.351$^{b}$ | 1.545$^{b}$ | 1.458$^{b}$ | 1.486$^{c}$ |
| Low | High | 242 | 1,225 | 2,395 | 1,381 | 1,883 | 3,264 | 1.396$^{a}$ | 1.600$^{a}$ | 1.506$^{a}$ | 1.552$^{b}$ |
| Low | Std | 242 | 1,221 | 2,375 | 1,372 | 1,847 | 3,220 | 1.402$^{a}$ | 1.597$^{a}$ | 1.512$^{a}$ | 1.571$^{b}$ |
| Low | Low | 242 | 1,177 | 2,274 | 1,328 | 1,772 | 3,100 | 1.419$^{a}$ | 1.614$^{a}$ | 1.524$^{a}$ | 1.620$^{a}$ |
| | SEM | 2.78 | 12.58 | 22.11 | 15.73 | 24.86 | 31.95 | 0.007 | 0.006 | 0.005 | 0.005 |
| Main effects | | | | | | | | | | | |
| CP | | | | | | | | | | | |
| Std | | 244 | 1,270$^{a}$ | 2,549$^{a}$ | 1,351 | 1,957$^{a}$ | 3,308$^{a}$ | 1.317$^{c}$ | 1.531$^{b}$ | 1.435$^{c}$ | 1.440$^{c}$ |
| Med | | 243 | 1,255$^{a}$ | 2,506$^{a}$ | 1,376 | 1,909$^{a}$ | 3,285$^{a}$ | 1.360$^{b}$ | 1.526$^{b}$ | 1.452$^{b}$ | 1.470$^{b}$ |
| Low | | 242 | 1,207$^{b}$ | 2,351$^{b}$ | 1,360 | 1,834$^{b}$ | 3,195$^{b}$ | 1.409$^{a}$ | 1.603$^{a}$ | 1.514$^{a}$ | 1.584$^{a}$ |
| AvP | | | | | | | | | | | |
| High | | 243 | 1,252$^{a}$ | 2,493$^{a}$ | 1,378$^{a}$ | 1,925$^{a}$ | 3,303$^{a}$ | 1.367 | 1.552 | 1.469 | 1.491 |
| Std | | 244 | 1,254$^{a}$ | 2,486$^{a}$ | 1,373$^{a}$ | 1,905$^{a,b}$ | 3,278$^{a}$ | 1.361 | 1.547 | 1.469 | 1.488 |
| Low | | 242 | 1,226$^{b}$ | 2,427$^{b}$ | 1,335$^{b}$ | 1,871$^{b}$ | 3,206$^{b}$ | 1.358 | 1.561 | 1.463 | 1.514 |
| Source of variation *(P-value)* | | | | | | | | | | | |
| CP level | | NS | <0.001 | <0.001 | NS | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| AvP level | | NS | <0.05 | <0.01 | <0.01 | <0.05 | <0.001 | NS | 0.067 | NS | NS |
| CP × AvP | | NS | NS | NS | NS | NS | NS | <0.05 | <0.01 | <0.01 | <0.001 |

[a–c]Values in a column with no common superscripts differ significantly ($P \leq 0.05$)—HSD test.
Mean values are based on 15 birds per replicate and 7 replicates per treatment.
[1]AvP, available phosphorous; CP, crude protein; Std, standard; Med, medium.
[2]FCR corrected to a body weight of 2,562 g (FCR adjusted by 1 point per 30 g of body weight difference).

**Table 7.** Carcass characteristics and ileal digesta DM of birds on day 35 in response to diets varying in crude protein and available phosphorous.

| Treatments [1] | | Carcass (g/100 g live weight) | | | Ileal digesta |
|---|---|---|---|---|---|
| CP | AvP | Breast | Thigh + drumstick | Fat pad | Water content (%) |
| Std | High | 17.7 | 18.7 | 0.95 | 61.6 |
| Std | Std | 17.5 | 18.6 | 0.92 | 62.1 |
| Std | Low | 17.9 | 18.3 | 0.91 | 61.3 |
| Med | High | 17.9 | 18.2 | 1.23 | 62.2 |
| Med | Std | 17.0 | 18.5 | 1.14 | 59.7 |
| Med | Low | 17.5 | 18.2 | 1.04 | 57.3 |
| Low | High | 17.9 | 18.2 | 1.30 | 57.0 |
| Low | Std | 17.6 | 18.3 | 1.28 | 57.3 |
| Low | Low | 17.2 | 18.0 | 1.40 | 55.8 |
| | SEM | 0.264 | 0.223 | 0.057 | 1.291 |
| Main effects | | | | | |
| CP | | | | | |
| Std | | 17.7 | 18.5 | 0.93[c] | 61.7[a] |
| Med | | 17.5 | 18.3 | 1.14[b] | 59.8[a] |
| Low | | 17.6 | 18.1 | 1.32[a] | 56.7[b] |
| AvP | | | | | |
| High | | 17.8 | 18.3 | 1.16 | 60.2 |
| Std | | 17.4 | 18.5 | 1.11 | 59.7 |
| Low | | 17.5 | 18.1 | 1.12 | 58.1 |
| Source of variation *(P-value)* | | | | | |
| CP level | | NS | 0.10 | <0.001 | <0.001 |
| AvP level | | 0.09 | NS | NS | NS |
| CP × AvP | | NS | NS | NS | NS |

[a–c]Values in a column with no common superscripts differ significantly ($P \leq 0.05$)—HSD test.

Mean values are based on 3 sacrificed birds per replicate and 7 replicates per treatment.

[1]AvP, available phosphorous; CP, crude protein; Std, standard; Med, medium.

alanine aminotransferase, and aspartate aminotransferase (**AST**) on Thermo Scientific Indiko and Konelab autoanalyzer, using a kit package specific to each test.

Ileal digesta samples were weighed and then oven dried at 95°C for 24 h to a constant weight.

### Statistical Analysis

Data were checked for normality and then subjected to statistical analysis using 2-way ANOVA of GLM procedure of SAS to assess the main effects of crude protein levels, available P, and their interaction. Each pen was considered an experimental unit and the values presented in the tables are means with pooled SEM. If a significant effect was detected, differences between treatments or main effects were separated by least square differences test. Significance was considered at $P < 0.05$ and $P < 0.1$ was indicated as a trend.

## RESULTS

Analyzed nutrient concentrations in the experimental diets are expressed in Table 5 and confirmed that diets had been correctly formulated and mixed. The desired crude protein, total phosphorus, and electrolyte levels were achieved and within acceptable ranges for sampling and analytical error.

The interactive effects of crude protein and available P on the performance of broiler chickens are presented in Table 6. Increasing crude protein and available P concentration resulted in an increase ($P < 0.01$) in body weight on day 24 and day 35 and for feed intake in both grower and finisher phases. There was no interaction ($P > 0.05$) between dietary crude protein and available P for body weight or feed intake. Over the entire trial, increasing available P generated a reduction in FCRc that was higher for birds that were fed the diets with low-protein concentration resulting in a significant protein * P interaction.

The effect of dietary protein and available P concentration on carcass composition and water content of the ileal digesta is presented in Table 7. There were no effects ($P > 0.05$) of available P concentration on any of the carcass parameters measured or on ileal digesta water content and no interaction ($P > 0.05$) between protein and P on these parameters. However, there was a tendency ($P = 0.09$) for breast yield to increase with increasing available P concentration. Increasing dietary crude protein concentration resulted in a significant increase in the water content of ileal digesta and a reduction ($P < 0.001$) in abdominal fat pad concentration.

The effect of available P and crude protein on tibia breaking strength and mineral content of the bone is presented in Table 8. Increasing dietary crude protein ($P < 0.001$) and available P ($P = 0.07$) independently increased bone breaking strength. Similarly, bone ash concentration was increased ($P < 0.05$) by elevating both available P and crude protein content in the feed. Tibia mineral composition was largely unaffected by

**Table 8.** Tibia breaking strength (N/mm$^2$) and mineral composition of birds on day 35 in response to diets varying in crude protein and available phosphorous.

| Treatments [1] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CP | AvP | Breaking strength | Ash % | Ca % | P % | K % | Cu mg/kg | Fe mg/kg | Mn mg/kg | Zn mg/kg |
| Std | High | 330 | 45.1 | 39.7 | 18.34 | 0.71 | 2.83 | 335 | 13.19 | 397 |
| Std | Std | 338 | 44.2 | 40.0 | 18.15 | 0.70 | 2.68 | 317 | 12.83 | 389 |
| Std | Low | 312 | 44.4 | 40.1 | 18.21 | 0.70 | 2.62 | 324 | 14.02 | 388 |
| Med | High | 293 | 44.9 | 40.3 | 18.53 | 0.73 | 2.64 | 355 | 12.79 | 395 |
| Med | Std | 296 | 44.7 | 39.8 | 18.07 | 0.69 | 2.77 | 321 | 13.53 | 393 |
| Med | Low | 305 | 44.2 | 39.5 | 17.88 | 0.68 | 2.83 | 290 | 13.46 | 394 |
| Low | High | 274 | 44.2 | 39.6 | 18.26 | 0.71 | 2.12 | 322 | 11.51 | 403 |
| Low | Std | 307 | 44.0 | 39.7 | 18.33 | 0.73 | 2.36 | 321 | 11.69 | 397 |
| Low | Low | 256 | 43.0 | 40.0 | 18.10 | 0.69 | 2.53 | 286 | 12.83 | 396 |
| | SEM | 11.95 | 0.363 | 0.394 | 0.186 | 0.021 | 0.199 | 14.88 | 0.551 | 8.348 |
| Main effects | | | | | | | | | | |
| CP | | | | | | | | | | |
| Std | | 327 | 44.6$^a$ | 40.0 | 18.23 | 0.71 | 2.71$^a$ | 325 | 13.35$^a$ | 391 |
| Med | | 298 | 44.6$^a$ | 39.9 | 18.16 | 0.70 | 2.75$^a$ | 322 | 13.26$^a$ | 394 |
| Low | | 279 | 43.7$^b$ | 39.7 | 18.23 | 0.71 | 2.34$^b$ | 310 | 12.01$^b$ | 399 |
| AvP | | | | | | | | | | |
| High | | 299 | 44.7$^a$ | 39.9 | 18.38 | 0.72 | 2.53 | 337$^a$ | 12.51 | 398 |
| Std | | 314 | 44.3$^a$ | 39.8 | 18.18 | 0.71 | 2.61 | 320$^a$ | 12.69 | 393 |
| Low | | 291 | 43.9$^b$ | 39.9 | 18.06 | 0.69 | 2.66 | 301$^b$ | 13.44 | 393 |
| Source of variation *(P-value)* | | | | | | | | | | |
| CP level | | <0.001 | <0.01 | NS | NS | NS | <0.05 | NS | <0.01 | NS |
| AvP level | | 0.07 | <0.05 | NS | NS | NS | NS | <0.05 | 0.09 | NS |
| CP × AvP | | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a–c]Values in a column with no common superscripts differ significantly ($P \leq 0.05$)—HSD test.
Mean values are based on 3 birds per replicate and 7 replicates per treatment.
[1]CP, crude protein; AvP, available phosphorous; Std, standard; Med, medium.

diet protein or P concentration although manganese (**Mn**) and copper (**Cu**) content were significantly increased with increasing dietary protein content whereas increasing available P generated an increase ($P < 0.05$) in tibia iron (**Fe**) content.

The interaction between dietary available P and crude protein concentrations on serum metabolites is presented in Table 9. Increasing dietary protein generated increases in plasma Ca and triglyceride concentration ($P < 0.01$). Increasing dietary available P content resulted in a reduction in plasma uric acid concentration only at a moderate crude protein level, resulting in a significant protein * P interaction. Increasing dietary protein content resulted in a reduction ($P < 0.01$) in plasma $NH_3$. Increasing dietary available P and protein content resulted in an increase ($P < 0.01$) in plasma P concentration. The increase in plasma P associated with increasing dietary available P concentration was more marked in low-protein diets resulting in a tendency for an interaction between available P and crude protein ($P = 0.05$). There was an inconsistent influence of diet available P and protein concentrations on plasma HDL where increases in available P reduced HDL in standard protein diets but the opposite occurred in moderate- and low-protein diets ($P < 0.01$). Plasma AST was independently increased by increases in dietary available P ($P < 0.05$) and protein ($P < 0.001$) although the effect of available P on AST was more apparent in diets with a low protein concentration resulting in a tendency for an interaction between treatments ($P = 0.06$). There was no effect ($P > 0.05$) of dietary treatment on plasma alanine aminotransferase, total protein, or low-density lipoprotein.

## DISCUSSION

There is a rich seam of scientific publication across multiple leading journals that report efforts to develop sustainable and effective strategies to feed low-protein diets to broiler chickens. However, responses are equivocal and the underlying cause of variance is not clear. Kidd and Tillman (2016) suggest that some of the variability in response of broilers to low-protein diets may be the animal models and experimental design used, genetics and age of the animals, dietary lysine concentration and ratio of other amino acids to lysine, nonessential amino acids (glycine and serine in particular) and the nitrogen content of the feed. Fancher and Jensen (1989) and Chrystal et al. (2020) examined the potential of added K or fiber (whole grain), respectively, but found no benefit in either case when low-protein diets were augmented. In addition, some concern has recently been expressed regarding the additivity of apparent ileal amino acid digestibility values in least-cost formulation and the potential confound associated with basal endogenous amino acid flow which may reduce experimental clarity (Kong and Adeola, 2013; Ravindran et al., 2017; Cowieson et al., 2019). Nonetheless, it is clear from the literature that broiler chicken performance is routinely compromised by feeding diets

**Table 9.** Serum concentration of biochemical metabolites of birds collected on day 35 in response to diets varying in crude protein and available phosphorous.

| Treatments[1] | | NH3 (μmol/L) | Uric acid (μmol/L) | ALT[2] (U/L) | AST[3] (U/L) | Total protein (g/L) | Calcium (mmol/L) | Phosphorus (mmol/L) | HDL (mmol/L) | LDL (mmol/L) | Triglyceride (mmol/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CP | AvP | | | | | | | | | | |
| Std | High | 652 | 394$^{a}$ | 4.864 | 446 | 28.10 | 1.714 | 2.191 | 2.684$^{d}$ | 0.593 | 0.854 |
| Std | Std | 569 | 363$^{a}$ | 4.660 | 382 | 27.78 | 1.767 | 2.030 | 2.698$^{d}$ | 0.588 | 0.762 |
| Std | Low | 803 | 404$^{a}$ | 5.008 | 464 | 27.48 | 1.653 | 2.092 | 2.792$^{c,d}$ | 0.596 | 0.778 |
| Med | High | 691 | 275$^{c}$ | 4.856 | 406 | 27.10 | 1.701 | 2.055 | 2.901$^{b,c,d}$ | 0.550 | 0.898 |
| Med | Std | 786 | 334$^{a,b,c}$ | 5.030 | 381 | 28.09 | 1.740 | 2.026 | 2.848$^{b,c,d}$ | 0.624 | 0.881 |
| Med | Low | 898 | 359$^{a,b}$ | 4.191 | 366 | 27.68 | 1.775 | 1.947 | 2.825$^{b,c,d}$ | 0.593 | 0.908 |
| Low | High | 860 | 270$^{c}$ | 4.886 | 393 | 27.39 | 1.788 | 2.008 | 3.050$^{b,c}$ | 0.526 | 1.059 |
| Low | Std | 826 | 290$^{c}$ | 4.908 | 350 | 28.38 | 1.862 | 2.066 | 3.383$^{a}$ | 0.615 | 1.143 |
| Low | Low | 774 | 299$^{c}$ | 4.875 | 317 | 27.87 | 1.929 | 1.814 | 3.096$^{b}$ | 0.606 | 1.022 |
| | SEM | 63.64 | 16.12 | 0.334 | 21.75 | 0.579 | 0.059 | 0.052 | 0.063 | 0.038 | 0.059 |
| Main effects | | | | | | | | | | | |
| CP | | | | | | | | | | | |
| Std | | 675$^{b}$ | 387$^{a}$ | 4.844 | 431$^{a}$ | 27.79 | 1.711$^{b}$ | 2.104$^{a}$ | 2.725$^{b}$ | 0.592 | 0.798$^{c}$ |
| Med | | 792$^{a}$ | 323$^{b}$ | 4.692 | 384$^{b}$ | 27.62 | 1.738$^{b}$ | 2.009$^{b}$ | 2.857$^{b}$ | 0.589 | 0.896$^{b}$ |
| Low | | 821$^{a}$ | 280$^{c}$ | 4.890 | 353$^{b}$ | 27.88 | 1.860$^{a}$ | 1.962$^{b}$ | 3.176$^{a}$ | 0.582 | 1.075$^{a}$ |
| AvP | | | | | | | | | | | |
| High | | 735 | 313$^{b}$ | 4.868 | 415$^{a}$ | 27.53 | 1.734 | 2.084$^{a}$ | 2.878 | 0.556 | 0.937 |
| Std | | 727 | 329$^{a,b}$ | 4.866 | 371$^{b}$ | 28.08 | 1.791 | 2.041$^{a}$ | 2.976 | 0.609 | 0.929 |
| Low | | 825 | 347$^{a}$ | 4.691 | 382$^{a,b}$ | 27.68 | 1.786 | 1.951$^{b}$ | 2.904 | 0.598 | 0.903 |
| Source of variation *(P-value)* | | | | | | | | | | | |
| CP level | | <0.05 | <0.001 | NS | <0.001 | NS | <0.01 | <0.01 | <0.001 | NS | <0.001 |
| AvP level | | NS | <0.05 | NS | <0.05 | NS | NS | <0.01 | NS | NS | NS |
| CP × AvP | | 0.07 | <0.05 | NS | 0.06 | NS | NS | 0.05 | <0.01 | NS | NS |

$^{a–c}$Values in a column with no common superscripts differ significantly ($P \leq 0.05$)—HSD test.
Mean values are based on 3 birds per replicate and 7 replicates per treatment.
[1]CP, crude protein; AvP, available phosphorous; Std, standard; Med, medium.
[2]ALT, alanine aminotransferase.
[3]AST, aspartate aminotransferase.

with low-protein concentration, regardless of efforts made to supplement the diet with synthetic amino acids (Corzo et al., 2010) and this is in agreement with the results presented herein (Table 6). Indeed, reducing dietary crude protein from 21.5 to 17.5% in the grower diet and from 19.5 to 15.5% in the finisher diet at standard concentrations of available P resulted in a reduction in day 35 body weight of around 6.6% and a substantial increase in FCR. These deleterious outcomes on bird performance were despite a concerted effort to maintain the density of sulfur amino acids, lysine, threonine, arginine, valine, isoleucine, leucine, histidine, glycine, phenylalanine, and tryptophan by synthetic supplementation. Furthermore, the increase in relative fat pad weight with declining crude protein concentration in the feed is indicative of a failure of the low-protein diet to fully support protein accretion and efficient growth (Moran et al., 1992).

The importance of available P in broiler nutrition is well documented and has been the subject of multiple reviews involving critical adjacent factors such as vitamin D, bird age, phytase use, and Ca concentration (Selle and Ravindran, 2007; Li et al., 2016, 2017; Rodehutscord et al., 2017). However, there are very few reports on the interaction between available P and dietary crude protein concentration and, as far as the authors are aware, no published work in poultry. It is somewhat surprising that the potential for an interaction between dietary P status and crude protein has not received more attention given the fact that diets with low protein content typically contain a much lower concentration of phytate P and total P from organic sources (Eeckhout and De Paepe, 1994; Weremko et al., 1997). Furthermore, protein synthesis requires appreciable quantities of P for manufacture of ATP to meet the substantial energy demands of this process (Shariatmadari and Forbes, 1993). In the present experiment, the low protein, standard available P, grower (Table 2) and finisher (Table 4) diets contained approximately 0.6% less total P and 0.4% less phytate P than the standard protein, standard available P diets. Although available P was maintained as an experimental factor at the desired concentration by manipulation of dicalcium phosphate, it may be that the delivery of P to the animals on paper was different from reality due to these underlying changes in the origin of dietary P. Putatively, nonphytate P from different origins may have different availability for poultry and it may be that nonphytate P from SBM is more readily available than the same in corn. Some evidence that this may be the case is presented by Weremko et al. (1997) but further work on this topic is warranted given that formulating diets with low protein content has profound implications for organic P supply. Nevertheless, the results generated herein, particularly for FCR and FCRc, demonstrate that improvements in performance mediated by

additional available P are particularly marked in diets with marginal protein concentrations (Table 6).

Beneficial effects of additional available P in diets with marginal protein supply for rats has been presented by Hammoud et al. (2017) and Ragi et al. (2019) who noted that available P improved growth rate and had a range of post-prandial effects associated with elevated protein accretion. Ragi et al. (2019) noted that the addition of available P to a low-protein diet for rats resulted in a decrease in plasma urea nitrogen, HDL cholesterol and triglycerides. In the results presented herein (Table 9), there was no effect of available P on triglycerides although there was a reduction, congruent with Ragi et al. (2019), of ammonia, uric acid, and HDL associated with increased available P. It is possible therefore that increased available P reduced protein catabolism, perhaps by providing adequate P for ATP synthesis and protein accretion.

Although protein and P were the focal factors involved in the work presented herein, it is relevant that protein intake has been found to have a marked calciuretic effect (Margen et al., 1974; Zemel, 1988). The mechanisms involved in this process are complex but involve the effect of protein on renal function whereby increased protein intake results in a trade-off with reabsorption of Ca, increasing urinary losses (Linkswiler et al., 1981). Thus, axiomatically, a low-protein diet may be associated with a hypocalciuretic effect, increased Ca retention, and an increased requirement for available P to avoid Ca:P imbalance, hormonal flux, and bone mobilization. It is also relevant that P has been shown to exert a hypocalciuretic effect, partially offsetting the hypercalciuretic effect of dietary protein (Hegsted et al., 1981). It is therefore plausible that the interaction between dietary protein concentration and available P are both direct (via ATP synthesis and reduced protein catabolism) and indirect via hypocalciuretic effects and improved Ca and P balance. Although not significant in the present work, at least for Ca, the effect of available P concentration on plasma Ca and P (Table 9) were notably different, especially in the low-protein diet where increasing available P generated an increase in plasma P and a reduction in plasma Ca.

The effect of dietary available P on bone composition and mechanical characteristics (Table 8) are logical and in-line with previous observations on the effect of P on bone architecture (Onyango et al., 2003). The effect of protein or amino acid intake on bone strength and mineral composition in broilers is not as well explored compared with P or Ca. However, Patterson et al. (1986) found that the mechanical properties and strength of the tibia of broilers and turkeys could be enhanced with increased dietary protein concentrations although this may have been confounded with increased body weight and larger bones. By contrast, Skinner et al. (1991) and Yalcin et al. (1998) did not find consistent increases in bone strength with increasing dietary protein although these effects depend on additional diet parameters such as Ca and also on bird behavior and body weight. The lack of interaction between protein and P in the present experiment on bone characteristics (Table 8) suggests that protein and P may exert independent effects on this axis and the interactive effects observed on FCR are associated with mechanisms unrelated to bone mineral density or breaking strength.

## CONCLUSIONS

It can be concluded that offering broiler chickens low-protein diets supplemented with an array of synthetic amino acids was not fully effective in promoting maximal growth and both body weight and FCR were compromised relative to a higher-protein diet. However, growth rate and FCR were promoted by addition of available P to the low-protein diet and this strategy was effective in restoring some, but not all, of the performance losses associated with the diet with the lowest protein concentration. Available phosphorus may reduce protein catabolism and promote protein accretion by provision of P for ATP synthesis. Furthermore, addition of P may offset the hypocalciuretic effect of low protein intake, restoring Ca and P balance. It is recommended that the interactive effects of dietary Ca, P, phytate, and vitamin D on protein nutrition and digestible amino acid supply be systematically explored to optimize sustainability of broiler production.

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## DISCLOSURES

The authors declare no conflict of interest.

## REFERENCES

Aviagen. 2014. Ross broiler 308 nutrient specifications. Pages 1–9 in Aviagen, Newbridge, UK.

Aviagen. 2018. Broiler management Handbook. Pages 1–147 in Aviagen, Newbridge, UK.

Belloir, P., B. Meda, W. Lambert, E. Corrent, H. Juin, M. Lessire, and S. Tesseraud. 2017. Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilization. Animal 11:1881–1889.

Chrystal, P. V., A. F. Moss, A. Khoddami, V. D. Naranjo, P. H. Selle, and S. Y. Liu. 2020. Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. Poult. Sci. 99:505–516.

Corzo, A., C. A. Fritts, M. T. Kidd, and B. J. Kerr. 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. Anim. Feed Sci. Technol. 118:319–327.

Corzo, A., M. Schilling, R. Loar, L. Mejia, L. Barbosa, and M. Kidd. 2010. Responses of Cobb x Cobb 500 broilers to dietary amino acid density regimens. J. Appl. Poult. Res. 19:227–236.

Cowieson, A. J., J. O. Sorbara, G. Pappenberger, M. R. Abdollahi, F. F. Roos, and V. Ravindran. 2019. Additivity of apparent and standardized ileal amino acid digestibility of corn and soybean meal in broiler diets. Poult. Sci. 98:3722–3728.

Eeckhout, W., and M. De Paepe. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47:19–29.

Fancher, B. I., and L. S. Jensen. 1989. Male broiler performance during the starting and growing periods as affected by dietary protein, essential amino acids and potassium levels. Poult. Sci. 68:1385–1395.

Hammoud, R. U., M. N. Jabbour, A. N. Tawil, H. Ghattas, and O. A. Obeid. 2017. Phosphorus supplementation mitigated food intake and growth of rats fed a low protein diet. Curr. Dev. Nutr. 1:1–8.

Hegsted, M., S. A. Schuette, M. B. Zemel, and H. M. Linkswiler. 1981. Urinary calcium and calcium balance in young men as affected by level of protein and phosphorus intake. J. Nutr. 111:553–562.

Hettleman, B. D., R. L. Sabina, M. K. Drezner, E. W. Holmes, and J. L. Swain. 1983. Defective adenosine triphosphate synthesis. An explanation for skeletal muscle dysfunction in phosphate-deficient mice. J. Clin. Invest 72:582–589.

Kidd, M. T., and P. B. Tillman. 2016. Key principals concerning amino acid responses in broilers. Anim. Feed Sci. Technol. 221:314–322.

Kong, C., and O. Adeola. 2013. Additivity of amino acid digestibility in corn and soybean meal for broiler chickens and White Pekin ducks. Poult. Sci. 92:2381–2388.

Linkswiler, H. M., M. B. Zemel, M. Hegsted, and S. Schuette. 1981. Protein-induced hypercalciuria. Fed. Proc. 40:2429–2433.

Li, X., D. Zhang, and W. L. Bryden. 2017. Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess? Anim. Prod. Sci. 57:2304–2310.

Li, X., D. Zhang, T. Y. Yand, and W. L. Bryden. 2016. Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. Agric 6:1–15.

Margen, S., J.-Y. Chu, N. A. Kaufmann, and D. H. Calloway. 1974. Studies in calcium metabolism. 1. The calciuretic effect of dietary protein. Am. J. Clin. Nutr. 27:584–589.

Moran, E. T., J. R. Bushong, and S. F. Bilgili. 1992. Reducing dietary crude protein for broilers while satisfying amino acid requirements by least-cost formulation: live performance, litter composition, and yield of fast-food carcass cuts at six weeks. Poult. Sci. 71:1687–1694.

Onyango, E. M., P. Y. Hester, R. Stroshine, and O. Adeola. 2003. Bone densiometry as an indicator of percentage tibia ash in broiler chickes fed varying dietary calcium and phosphorus levels. Poult. Sci. 82:1787–1791.

Patterson, P. H., M. E. Cook, T. D. Crenshaw, and M. L. Sunde. 1986. Mechanical properties of the tibiotarsus of broilers and poults loaded with artificial weight and fed various dietary protein levels. Poult. Sci. 65:1357–1364.

Pesti, G. M. 2009. Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. J. Appl. Poult. Res. 18:477–486.

Ragi, M.-E., C. El Mallah, I. Toufeili, and O. Obeid. 2019. Concomitant lysine and phosphorus addition to a wheat gluten diet highly amplified growth measures of rats. Nutr. 63:69–74.

Ravindran, V., O. Adeola, M. Rodehutscord, H. Kluth, J. D. van der Klis, E. van Eerden, and A. Helmbrecht. 2017. Determination of ileal digestibility of amino acids in raw materials for broiler chickens – results of collaborative studies and assay recommendations. Anim. Feed Sci. Technol. 225:62–72.

Rodehutscord, M., O. Adeloa, R. Angel, P. Bikker, E. Delezie, W. A. Dozier, M. Umar-Faruk, M. Francesh, C. Kwakernaak, A. Narcy, C. M. Nyachoti, O. A. Olukosi, A. Preynat, B. Renouf, A. Saiz del Barrio, K. Scedle, W. Siegert, S. Steenfelt, M. M. van Krimpen, S. M. Waititu, and M. Witzig. 2017. Results of an international phosphorus digestibility ring test with broiler chickens. Poult. Sci. 96:1679–1687.

Saxena, R., V. Tripathi, N. A. Mir, K. Dev, J. Begum, R. Agarwal, and A. Goel. 2020. Dynamics of gene expression of hormones involved in the growth of broiler chickens in response to the dietary protein and energy changes. Gen. Comp. Endocrinol. 288. In press.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 134:1–41.

Shariatmadari, F., and J. Forbes. 1993. Growth and food intake responses to diets of different protein contents and a choice between diets containing two concentrations of protein in broiler and layer strains of chicken. Br. Poult. Sci. 34:959–970.

Siegert, W., K. J. Wild, M. Schollenberger, A. Helmbrecht, and M. Rodehutscord. 2016. Effect of glycine supplementation in low protein diets with amino acids from soy protein isolate or free amino acids on broiler growth and nitrogen utilization. Br. Poult. Sci. 57:424–434.

Skinner, J. T., J. N. Beasley, and P. W. Waldroup. 1991. Effects of dietary amino acid levels on bone development in broiler chickens. Poult. Sci. 70:941–946.

Weremko, D., H. Fandrejewski, T. Zebrowska, K. Han, J. H. Kim, and W. T. Cho. 1997. Bioavailability of phosphorus in feed of plant origin for pigs. Asian-Aust. J. Anim. Sci. 10:551–566.

Yalcin, S., P. Settar, and O. Dicle. 1998. Influence of dietary protein and sex on walking ability and bone parameters of broilers. Br. Poult. Sci. 39:251–256.

Zemel, M. B. 1988. Calcium utilization: effect of varying level and source of dietary protein. Am. J. Clin. Nutr. 48:880–883.