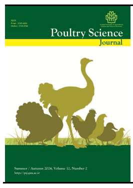

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Effects of Reduced Dietary Crude Protein and Amino Acids on the Performance, Carcass Characteristics and Intestinal Morphology of Broiler Chickens

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Abstract

A factorial experiment with a completely randomized design was carried out to examine the effects of reducing dietary crude protein (CP) and incorporating threonine, arginine, valine, isoleucine, and tryptophan synthetic amino acids supplements (SAA) on broiler chickens. The experiment involved 180 Ross 308 broilers divided into six treatments with six replicates each. The experimental treatments were as follows: 1- Standard diet, 2- Standard diet + SAA, 3- Diet with 2% reduced CP, 4- Diet with 2% reduced CP + SAA, 5- Diet with 4% reduced CP, and 6- Diet with 4% reduced CP + SAA. The 4% CP reduction diet decreased body weight gain in the starter and finisher periods ($P < 0.05$). The 4% lower CP also decreased feed intake in the grower, finisher and whole of the experiment ($P < 0.05$), and the same effect was observed for the 2% reduced CP diet only in the grower and entire experiment ($P < 0.05$). The same negative effects of lower dietary CP were also found for feed conversion ratio (FCR) ($P < 0.05$). The SAA supplements improved weight gain in the starter and the whole experimental period ($P < 0.05$). Feed intake did not show any change following SAA incorporation; however, SAA had a significant positive effect on FCR ($P < 0.05$). The only significant effect of experimental factors on small intestine morphology was a higher villus thickness in birds fed 2% less CP compared to the control ($P < 0.05$). The lower dietary CP led to increased serum cholesterol, triglyceride and HDL-c concentrations ($P < 0.05$). The serum total protein and albumen in the group fed 2% less CP was higher than the two other groups ($P < 0.05$). The only effect of SAA was an increased serum HDL level ($P < 0.05$). The results suggest that SAA supplementation could compensate for the negative effects of 2% but not the 4% lower dietary CP.

Introduction

Protein, along with energy, is the most important nutrient in a diet and protein-rich foods make up a significant portion of the cost of a broiler diet. Proteins play a critical role in the nutrition of broiler chickens, serving as essential components for growth, development, and overall health. The significance of protein in broiler chickens' diets lies in its function as the fundamental building blocks for muscle, feathers, enzymes, and hormones. Adequate protein intake is imperative to support optimal growth rates, feed efficiency, and immune function in broiler chickens. A deficiency in protein can result in stunted growth, decreased egg production, and compromised feather quality in broiler chickens. Understanding the importance of protein in broiler chickens' nutrition is crucial for ensuring their well-being and productivity (Leeson and Summers, 2009).

Certainly, finding a way to reduce broilers' dietary crude protein level without negatively impacting production efficiency can be an attractive idea. By introducing synthetic amino acids and increasing their

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availability and diversity, it is now possible to formulate diets that are perfectly tailored to the nutritional needs of poultry (Kidd *et al*., 2013). Chrystal *et al.* (2020) showed that reducing dietary crude protein from 210 to 165 g/kg reduced the diet soybean meal from 335 to 184 g/kg. This 45% reduction in soybean meal was accompanied by an increase in synthetic amino acids. This study indicates that reducing the crude protein in the diet not only helps maintain desirable performance but also emphasizes reducing dependence on soybean meal. Decreasing the protein content can lead to risks such as increased fat deposition and digestive imbalance.

Enhanced diet formulation utilizing the nutrients present in ingredients, lowering crude protein (CP) levels, and incorporating synthetic amino acids can lead to decreases in environmental pollution (Nahn, 2007). Low-quality bedding and the associated problems have become a welfare concern, but reducing crude protein in the diet can improve bedding quality and reduce foot pad lesions (Dunlop *et al.*, 2016). The current research was designed to investigate the effect of reducing 2 to 4% crude protein in the diet and using synthetic amino acids threonine, arginine, valine, isoleucine, and tryptophan on broiler chickens.

Materials and Methods

The authors affirm adherence to the journal's ethical guidelines and compliance with European Union norms for poultry welfare. In this study, 180 broiler chicks of Ross 308 strain were used. The experiment included six treatments and six replications, with five chicks each. Diets were formulated based on the specific nutrition requirements for Ross 308 broiler chickens. The total duration of the experiment was 42 days, including starter (days 1-10), grower (days 11-24) and finisher (days 25-42) phases. At the end of each feeding phase, the weight gain and feed consumption values were recorded, and the feed conversion ratio was calculated. Synthetic amino acid supplements (SAA) of threonine, arginine, valine, isoleucine, and tryptophan were used in this experiment. The experimental diets included: 1. Recommended crude protein without SAA, 2. 2% less crude protein without SAA, 3. 4% less crude protein without SAA, 4. Recommended crude protein with SAA, 5. 2% less crude protein with SAA and 6. 4% less crude protein with SAA.

At the end of each rearing period, the weight gain and feed consumption were determined, and the feed conversion ratio was calculated. These production parameters were also calculated for the entire rearing period. On day 42, two birds were selected from each replicate, blood samples were taken through the wing vein. The blood samples were collected in coagulation tubes and the serum was separated. This serum was kept at a temperature of – 20 ºC. Then, the blood samples were sent to the laboratory for measuring triglycerides, cholesterol, HDL-c, total protein, and albumin in the blood serum. The measurement of metabolites was done using enzymatic kits (Pars Azmoon Co, Iran) and a spectrophotometer (Alcyon 300, USA). Then, the selected birds were slaughtered to determine the weight of their internal organs. Since the length of the intestine is dependent on the body size of the bird, the ratio of small intestine length to live weight was calculated.

The duodenum section of the slathered chicken’s small intestine was examined for morphology. For this purpose, 2-centimeter-long segments were excised from the midsections of the duodenum. The intestines were processed and paraffin-embedded sections were prepared, stained, and then subjected to microscopic and histomorphometric analysis. Morphological features evaluated included the villus height and width, crypt depth and width, and villus height to crypt depth ratio. The specimens underwent two rounds of cleansing with saline phosphate solution (PBS) to eliminate any contents before being placed in plastic containers filled with 6-7 mL of 10% formalin. Thin tissue slides were then prepared using the paraffin wax technique. Morphological analyses were conducted following the protocol established by Iji *et al*. (2001).

In this factorial experiment, the effects of the level of crude protein in the diet and the use or non-use of synthetic amino acid supplements were examined. The data was recorded and analyzed in Excel and then analyzed using the SAS statistical software using the GLM method and 2×3 factorial analysis, with dietary CP levels and SAA supplementation as the main factors. Ultimately, to investigate significant differences between the means of the data, a Duncan's comparative test with a significance level of 5% was used.

Results

Table 2 shows the effect of experimental treatments on the average daily weight gain, feed intake and feed conversion ratio of broiler chickens. The average daily weight gain of broiler chickens fed a diet containing 4% less CP, in the starter and finisher phases was significantly lower than that of the control group ($P < 0.05$). A 2% reduction in CP in the diet did not lead to a significant difference in the daily weight gain of the broiler chickens compared to the control group. However, a significant dose-dependent reduction in weight gain was observed during the entire rearing period with the decrease in dietary CP ($P < 0.05$).

Table 1. Ingredient and nutrient composition of the diet (as-fed basis)

	Starter (1-10 d)						Grower (11-24 d)						Finisher (25-42 d)					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Corn	51.71	58.70	65.72	52.05	60.55	69.5	57.10	64.08	71.08	67.37	65.98	74.96	62.44	69.42	76.43	62.66	71.38	80.35
Soybean Meal	39.71	33.54	27.35	39.23	31.32	22.95	34.25	28.08	21.90	23.85	25.82	17.41	28.85	22.68	16.50	28.53	20.38	11.97
Soy oil	3.98	2.88	1.76	3.87	2.4	0.84	4.50	3.40	2.29	4.41	2.92	1.36	5.04	3.94	2.83	4.97	3.46	1.89
DCP	2.04	2.09	2.15	2.04	2.12	2.19	1.76	1.82	1.87	1.77	1.83	1.91	1.47	1.53	1.59	1.48	1.56	1.63
CaCo3	1.05	1.06	1.07	1.05	1.06	1.07	0.93	0.94	0.95	0.93	0.95	0.96	0.82	0.83	0.84	0.82	0.83	0.84
Min Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.24	0.22	0.22	0.24	0.22	0.21	0.25	0.22	0.22	0.25	0.22	0.21	0.26	0.22	0.21	0.25	0.21	0.21
Ca(HCO3)3	0.16	0.19	0.19	0.17	0.19	0.19	0.15	0.19	0.19	0.16	0.19	0.19	0.14	0.19	0.19	0.14	0.19	0.19
DL- Met	0.37	0.41	0.45	0.37	0.43	0.49	0.33	0.37	0.42	0.33	0.39	0.45	0.28	0.32	0.36	0.28	0.34	0.40
L-Lys	0.24	0.41	0.59	0.25	0.48	0.72	0.23	0.40	0.58	0.24	0.47	0.71	0.20	0.37	0.55	0.21	0.44	0.68
Threonine	0	0	0	0.16	0.25	0.35	0	0	0	0.13	0.23	0.33	0	0	0	0.10	0.20	0.30
Valine	0	0	0	0.05	0.17	0.29	0	0	0	0.04	0.16	0.29	0	0	0	0.03	0.15	0.28
Isoleucine	0	0	0	0.02	0.14	0.27	0	0	0	0.02	0.15	0.27	0	0	0	0.03	0.15	0.28
Arginine	0	0	0	0	0.17	0.39	0	0	0	0	0.18	0.41	0	0	0	0	0.20	0.42
Tryptophan	0	0	0	0	0	0.04	0	0	0	0	0.01	0.04	0	0	0	0	0.01	0.06
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ME (Kcal/Kg)	3000	3000	3000	3000	3000	3000	3100	3100	3100	3100	3100	3100	3200	3200	3200	3200	3200	3200
CP %	22	22	20	20	18	18	20	20	18	18	16	16	17.5	17.5	15.5	15.5	13.5	13.5
Ca %	1.05	1.05	1.05	1.05	1.05	1.05	1.05	0.9	0.9	0.9	0.9	0.9	0.85	0.85	0.85	0.85	0.85	0.85
Ava P%	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.45	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42	0.42	0.42
Lys%	1.25	1.25	1.25	1.25	1.25	1.25	1.12	1.12	1.12	1.12	1.12	1.12	0.94	0.94	0.94	0.94	0.94	0.94
Met + Cys%	0.93	0.93	0.93	0.93	0.93	0.93	0.85	0.85	0.85	0.85	0.85	0.85	0.73	0.73	0.73	0.73	0.73	0.73
Met%	0.65	0.65	0.68	0.67	0.71	0.69	0.59	0.59	0.62	0.61	0.65	0.64	0.49	0.50	0.53	0.52	0.55	0.53
Threonine%	0.69	0.84	0.84	0.62	0.84	0.55	0.63	0.75	0.75	0.56	0.75	0.48	0.55	0.63	0.63	0.47	0.63	0.40
Valine%	0.90	0.94	0.94	0.81	0.94	0.72	0.82	0.85	0.85	0.73	0.85	0.64	0.71	0.73	0.73	0.63	0.73	0.54
Isoleucine%	0.82	0.84	0.84	0.73	0.84	0.64	0.74	0.76	0.76	0.65	0.76	0.56	0.64	0.65	0.65	0.55	0.65	0.46
Arginine%	1.35	1.34	1.29	1.19	1.19	1.03	1.20	1.19	1.16	1.04	1.16	0.88	0.02	1.02	0.99	0.86	0.99	0.70
Tryptophan%	1.60	1.58	1.42	1.47	1.25	1.35	0.21	0.21	0.18	0.18	0.18	0.15	0.18	0.18	0.15	0.15	0.15	0.12

Experimental treatments included: 1.Ration containing the recommended level of CP and without threonine, arginine, valine, isoleucine, and tryptophan synthetic amino acids Supplementations (SAA), 2. Ration containing 2% less CP than the recommended level and without SAA, 3. Ration containing 4% less CP than the recommended level and without SAA, 4.Ration containing the recommended level of CP with SAA, 5. Ration containing 2% less CP than the recommended level and with SAA, 6. Ration containing 4% more CP than the recommended level with SAA. Vitamin and mineral premix provided the following per kilogram of diet: vitamin A (retinyl acetate): 9000 IU; vitamin D (cholecalciferol): 5500 IU; vitamin E (DL-α-tocopheryl acetate): 68 IU; Menadione: 9.0 mg; Pyridoxine: 7.0 mg; Riboflavin: 26.0 mg; Ca-pantothenate: 26.3 mg; Biotin: 0.41 mg; Thiamine: 3.66 mg; Niacin: 75 mg; Cobalamin: 0.03 mg; Folic acid: 3.70 mg; Fe: 82 mg; Mn: 60 mg; Zn: 115 mg; Cu: 15 mg; I: 0.85 mg and Se: 0.4 mg

Table 2. Effects of reduced dietary crude protein and amino acids on the weight gain, feed intake and feed conversion ratio of broiler chickens

Treatment	Body Weight Gain (g/bird/d)				Feed Intake (g/bird/d)				Feed Conversion Ratio			
	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total Period (1-42 d)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total Period (1-42 d)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total Period (1-42 d)
Amino Acids												
Supplement -	14.12^{b}	28.37	78.63	44.51^{b}	21.00	42.17	94.74	80.19	1.50_{a}	1.48_{a}	1.95_{a}	1.84^{a}
Supplement +	15.32^{a}	29.33	84.45	48.49^{a}	20.95	38.61	94.92	81.35	1.37_{b}	1.32_{b}	1.67_{b}	1.68^{b}
SEM	0.34	1.03	2.10	0.90	3.12	1.74	1.03	0.67	0.03	0.04	0.04	0.03
P-value	0.01	0.51	0.06	0.005	0.31	0.15	0.90	0.37	0.01	0.02	0.003	0.006
Crude Protein												
Recommended	15.47^{a}	33.48^{a}	90.00^{a}	52.50^{a}	21.20	48.72^{a}	100.02^{a}	87.30^{a}	1.37_{b}	1.45	1.58_{b}	1.66^{b}
2% less	15.03^{a}	29.92^{b}	89.50^{a}	48.87^{b}	21.05	40.38^{b}	97.88^{a}	82.55^{b}	1.40_{b}	1.36	1.84^{a}	1.71^{b}
4% less	13.66^{b}	24.18^{c}	65.12^{b}	38.12^{c}	20.68	33.59^{c}	86.59^{b}	72.47^{c}	1.55_{a}	1.39	2.00^{a}	1.91^{a}
SEM	0.41	1.26	2.57	1.10	0.38	2.13	1.26	0.82	0.04	0.06	0.06	0.04
P-value	0.01	0.001<	0.001<	0.001<	0.61	0.001<	0.001<	0.001<	0.01	0.53	0.001	0.001
AA × CP												
SEM	0.58	1.79	3.64	1.56	0.54	3.02	1.79	1.17	0.06	0.08	0.08	0.06
P-value	0.85	0.84	0.14	0.23	0.02	0.70	0.43	0.90	0.22	0.39	0.09	0.07

Different superscripts within a column indicate a significant difference ($P < 0.05$).

The use of synthetic amino acid supplements in all rearing phases resulted in an improvement in weight gain, however the statistically significant differences were observed only in the starter phase and overall experimental period ($P < 0.05$). No significant interaction was observed between the levels of CP and the consumption of synthetic amino acids on the daily weight gain.

Reducing the CP content in the diet led to a decrease in feed intake. The observed difference in the grower period for both 2% and 4% CP reduction was statistically significant in comparison to the control group ($P < 0.05$). In the finisher period, only the group fed a diet containing 4% less CP showed a significant decrease in feed intake compared to the control group ($P < 0.05$). Overall, a dose-dependent decrease in feed intake was observed throughout the experimental period with a reduction in dietary CP levels ($P < 0.05$). The consumption of synthetic amino acid supplements did not have any effect on feed intake. The interaction between the level of dietary CP and the consumption of synthetic amino acids only had a significant effect on feed intake during the starter period ($P < 0.05$). The only significant interaction related to feed intake in the starter period was observed in birds fed diets with lower protein intake, where supplementation of amino acids led to a decrease in feed intake ($P < 0.05$) (Figure 1).

A significant increase in feed conversion ratio was observed when the CP level in the diet was reduced by 4% compared to the control group ($P < 0.05$). The use of synthetic amino acid supplements in all the rearing phases resulted in a significant decrease in feed conversion ratio ($P < 0.05$). There was no significant interaction between the level of diet's CP and the consumption of synthetic amino acids regarding the feed conversion ratio.

Table 3 shows the effects of experimental treatments on carcass traits and internal organ weights. Reducing the CP content of the diet by 4% resulted in a decrease in carcass percentage, but the difference observed was only statistically significant compared to the group consuming a diet with 2% less CP ($P < 0.05$). Reducing the CP content of the diet resulted in a dose-dependent increase in relative liver weight ($P < 0.05$).

Table 3. Effects of reduced dietary crude protein and amino acids on carcass traits of broiler chickens (% of live weight)

Treatment	Carcass	Heart	Liver	Abdominal Fat	Bursa of Fabricius	Spleen	Pancreas	Small intestine length ratio
Amino Acids								
Supplement -	60.72	0.59	2.55	1.72	0.17	0.10	0.22[a]	9.14[a]
Supplement +	59.72	0.58	2.44	1.61	0.19	0.09	0.19[b]	8.34[b]
SEM	0.54	0.02	0.06	0.10	0.009	0.008	0.008	0.21
P-value	0.23	0.71	0.27	0.47	0.36	0.27	0.01	0.01
Crude Protein								
Recommended	60.31[ab]	0.56	2.22[c]	1.59	0.19	0.10	0.20	8.10[b]
2% less	61.59[a]	0.57	2.48[b]	1.61	0.17	0.09	0.21	8.22[b]
4% less	58.87[b]	0.63	2.79[a]	1.80	0.18	0.10	0.22	9.90[a]
SEM	0.67	0.02	0.08	0.12	0.01	0.009	0.009	0.25
P-value	0.02	0.18	0.002	0.43	0.45	0.67	0.51	0.001<
AA × CP								
SEM	0.94	0.03	0.12	0.17	0.01	0.01	0.01	0.23
P-value	0.09	0.16	0.94	0.56	0.21	0.91	0.25	0.56

Different superscripts within a column indicate a significant difference ($P < 0.05$).

The ratio of small intestine length to body weight also increased in the group consuming a diet with 4% less CP compared to the other two groups ($P < 0.05$). The only significant effects resulting from the consumption of synthetic amino acid supplements were a decrease in relative pancreas weight and relative small intestine length ($P < 0.05$). No significant reciprocal effects were observed on carcass traits. The experimental treatments had just a significant effect on the morphological characteristics of the small intestine, as shown in Table 4. The villus Width in the group with 2% less CP was higher compared to the other two groups ($P < 0.05$). A significant interaction effect was observed for the traits of villus length, crypt depth, and muscle layer thickness ($P < 0.05$). The interaction plots for duodenum morphological traits are presented in figure 1. Significant interactions were observed on morphological traits of the duodenum section. With a reduction in dietary protein level, amino acid supplementation resulted in a decrease in villi height($P < 0.05$). With a decrease in dietary protein level, amino acid supplementation initially led to an increase and then a decrease in crypt depth, and in diets with standard protein and diets with a 2% reduction in crude protein, amino acid supplementation caused a decrease in villi thickness, but in diets with a 4% reduction in crude protein, the opposite trend was observed ($P < 0.05$). The reduction in dietary protein and amino acid supplementation resulted in a linear decrease in muscular layer thickness of the duodenum, but in the group without supplementation, the lowest dietary protein level led to an increase in thickness ($P < 0.05$).

Table 4. Effects of reduced dietary crude protein and amino acids on small intestine morphology of broiler chickens (μm)

Treatment	Villus Height	Villus Thickness	Crypt Depth	Crypt Thickness	Muscular layer Thickness
Amino Acids					
Supplement -	1145.8	163.2	156.5	12.4	231.7
Supplement +	1135.3	141.8	182.6	17.7	198.2
SEM	65.19	12.4	10.49	1.86	13.4
P-value	0.91	0.23	0.09	0.05	0.09
Crude Protein					
Recommended	1087.3	116.4[b]	182.7	11.3	235.5
2% less	1103.2	198.1[a]	153.3	16.1	187.4
4% less	1231.1	143.8[b]	172.8	17.8	221.9
SEM	79.84	15.22	12.85	2.28	16.41
P-value	0.39	0.003	0.28	0.13	0.12
AA × CP					
SEM	54.85	19.45	13.91	3.38	7.64
P-value	0.001	0.06	0.003	0.89	0.001

Different superscripts within a column indicate a significant difference ($P < 0.05$).

The effect of experimental treatments on blood parameters of broiler chickens is shown in Table 5. The lower dietary CP led to increased serum cholesterol, triglyceride and HDL-c concentrations. The serum total protein and albumen in group fed 2% less CP was higher than two other groups. The only effect of SAA was an increased serum HDL level. The plot illustrates the significant interactive effects of the measured traits shown in Figure 1. In the significant interactions observed in blood metabolites, the use of amino acid supplementation resulted in a decrease in total protein, albumin, and HDL levels in diets with standard protein level, but this trend was reversed in diets with reduced protein levels ($P < 0.05$).

Table 5. Effects of reduced dietary crude protein and amino acids on serum parameters of broiler chickens

Treatment	Total Protein (mg/dL)	Albumen (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-c (mg/dL)
Amino Acids					
Supplement -	3.05	1.45	168.28	159.83	50.22[b]
Supplement +	3.45	1.67	179.72	161.83	60.11[a]
SEM	0.17	0.11	11.45	14.22	2.69
P-value	0.11	0.20	0.48	0.92	0.01
Crude Protein					
Recommended	2.67[b]	1.23[b]	124.17[b]	93.16[b]	48.42[b]
2% less	3.94[a]	2.09[a]	209.50[a]	205.42[a]	56.92[ab]
4% less	3.15[b]	1.37[b]	188.33[a]	183.92[a]	60.16[a]
SEM	0.21	0.14	14.02	17.41	3.30
P-value	0.001	0.004	0.005	0.002	0.04
AA × CP					
SEM	0.31	0.20	19.83	24.63	4.67
P-value	0.003	0.006	0.12	0.31	0.002

[a,b] Different superscripts within a column indicate a significant difference ($P < 0.05$).

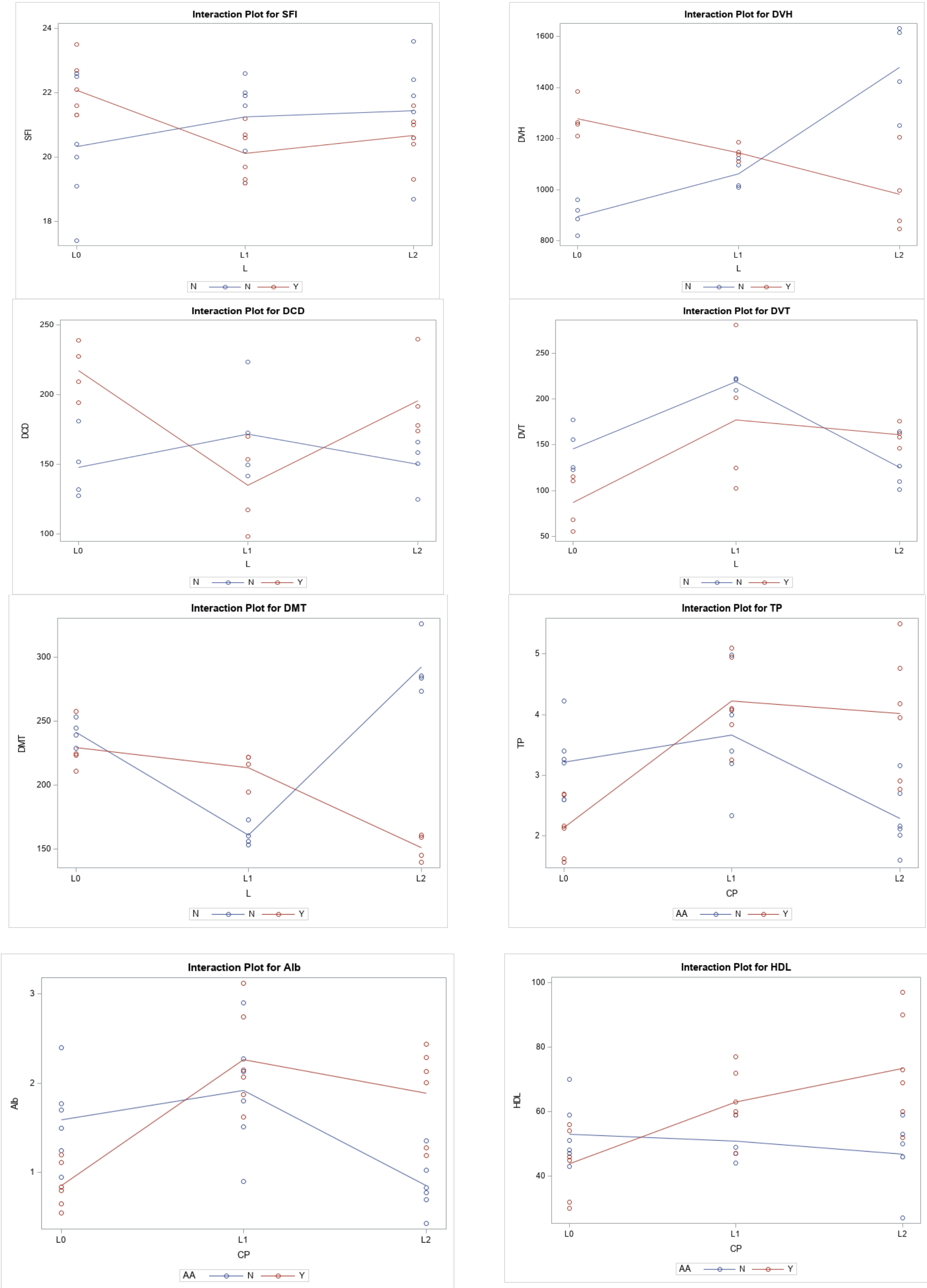

Figure 1. Interaction plots for dietary crude protein levels and synthetic amino acid supplementation. L0: Recommended CP, L1: 2% less dietary CP, L2: 4% less dietary CP, N: No synthetic amino acid supplementation, Y: synthetic amino acid supplementation, SFI: Starter Feed Intake, DVH: Duodenum Villus Height, DVT: Duodenum Villus Thickness, DCD: Duodenum Crypt Depth, DMT: Duodenum Muscular Layer Thickness. TP: Total Protein, Alb: Albumen, HDL: High Density Lipoprotein.

Discussion

Reduction of crude protein and the use of synthetic amino acid supplements in broiler diets have been proposed as an effective solution for reducing the cost of broiler feed and also addressing environmental issues by decreasing nitrogen and ammonia output from farms (Corzo *et al*., 2009; Van Harn *et al*., 2019). In the present study, a 4% reduction in dietary CP levels resulted in decreased performance indicators such as weight gain, feed intake, and increased feed conversion ratio; however, the use of synthetic amino acids improved performance. In line with the findings of this experiment, Neto *et al.* (2000) found that feeding broiler chickens with 17% CP compared to 24% for 21 days led to a significant decrease in body weight gain, as well as an increase in feed intake and abdominal fat weight.

It has been reported that the use of synthetic amino acid supplements can reduce the CP content of starter diets for broiler chickens to 19-20% without any adverse effects on performance (Awad *et al*., 2014). Kobayashi *et al.* (2012) also reported similar findings, showing that weight gain in birds consuming low CP diets supplemented with synthetic amino acids did not significantly differ from birds fed a standard diet. Ospina-Rojas *et al.* (2014) also reported that a 3% reduction in CP in diets containing synthetic amino acids had no adverse effects on the growth performance of broiler chickens at day 42. Saleh *et al*. (2021) provided Cobb 500 broiler chickens with low-protein diets while maintaining consistent metabolizable energy levels and amino acid concentrations does not have a negative impact on growth performance, carcass characteristics, blood parameters, and liver function. However, supplementing amino acids in inadequate protein diets can result in decreased growth efficiency, especially in high-temperature environments.

Qiu *et al.* (2023) reported that lowering the amount of CP in the diet of broiler chickens can lead to reduced growth performance and immune function while also causing an increase in abdominal fat accumulation and the apparent digestibility of protein and amino acids in the ileum. Adding protease to the diets can improve the growth performance of broilers by enhancing amino acid digestibility, but it does not have a significant impact on carcass characteristics or overall health. Benahmed *et al*. (2023) found that, the crude protein levels in the grower and finisher diets of broiler chickens can be decreased by up to 3.0% without negative impacts on performance or meat quality, as long as the birds' amino acid needs are properly fulfilled. It is essential to understand that if the minimum requirements of amino acids are met to maintain the growth and muscle of broiler chickens, the amount of CP in the diet can be reduced (Firman and Boling, 1998).

It appears that even without supplementation of amino acids, a slight reduction in dietary protein does not have a significant effect on performance. In an experiment, broiler chickens fed a diet with 1% less CP, constant ME, and equal levels of essential amino acids did not show any negative impact on growth performance, liver function, or carcass parameters (Salah, 2016). The increase in relative liver weight due to a decrease in dietary CP level may be due to increased lipogenesis in hepatocytes. Rosebrough *et al.* (1996) suggested that dietary protein intake per se is a potent regulator of de novo lipid metabolism in birds. The switch from a high to a low-protein diet increased lipogenesis and produced a response similar to that caused by meal feeding (Rosebrough *et al.*, 1996). In vitro lipogenesis and malic enzyme activity were inversely related to dietary protein levels (12-30%) and to acute changes from 12 to 30% (Rosebrough *et al.*, 2002).

The results showed that a decrease in dietary protein level and no SAA supplementation can lead to an increase in the length of the small intestine in broiler chickens. This is because lower protein levels in the diet may result in slower digestion and absorption of nutrients, leading to a compensatory increase in the length of the small intestine to enhance nutrient absorption (Montagne *et al*., 2003, Kar *et al*., 2017). It is possible that the use of synthetic amino acid supplements could potentially affect the size of the pancreas in broiler chickens. Synthetic amino acid supplements are often added to poultry diets to optimize protein levels and improve growth performance. If the synthetic amino acids are properly balanced and utilized by the birds, it is unlikely to have a negative impact on pancreatic size. However, excessive supplementation of certain amino acids could potentially disrupt the balance and function of the pancreas, leading to changes in size or function, as shown in this study.

Previous studies have shown that the negative impact of low-protein diets on the morphology of the intestine may be due to a decrease in the levels of non-essential amino acids such as glycine, glutamine, and proline necessary for the growth of the gastrointestinal epithelium and the production of digestive and mucin secretions (Law *et al*., 2018). Recently, Macelline *et al.* (2020) observed that broiler chickens fed a low-protein diet supplemented with essential amino acids did not show any adverse effects under sanitary conditions for 14 days post-hatch, while leading to a reduction in nitrogen excretion during the experiment.

Furthermore, they also observed that feeding low-protein diets supplemented with a synthetic amino acid leads to the upregulation of tight junction genes (ZO1 and claudin 1) that are essential for maintaining intestinal health. Additionally, a significant increase in liver weight in broiler chickens fed low-protein diets compared to those fed higher-protein diets was observed (Swennen *et al*., 2007), which may be associated with the increase in the energy-to-protein ratio in the low-protein diet. Our findings align with Kobayashi *et al*. (2012), who stated that in low-protein diets, carcass yield was lower compared to the control group, regardless of supplementation with essential amino acids.

Increasing the energy-to-protein ratio effectively improves body lipogenesis (Rosebrough, 1996). This is supported by the increase in blood plasma cholesterol concentration in broiler chickens fed low-protein diets in current study. The inverse relationship between visceral fat and crude protein level in the current experiment was observable, although the differences were not statistically significant, and with a larger sample size, they could potentially become significant.

In accordance with our results, Saleh *et al*. (2021) reported that abdominal fat in birds fed a low-protein diet compared to a standard diet showed no significant difference. Additionally, Badawi *et al*. (2019) studied the effect of feeding broiler chickens with a reduction of 2, 4, and 6% in CP from the standard diet and did not observe any significant changes in the relative weight of visceral organs. The increase in energy availability and the promotion of body lipogenesis in broiler chickens fed low-protein diets ultimately leads to an increase in abdominal fat content (Saleh *et al*., 2021). Contrary to the mentioned report, it has been observed that the abdominal fat weight increases by 104% when broiler chickens are fed a diet containing 17% crude protein compared to 24% (Van Harn *et al*., 2019). Increasing the energy-to-protein ratio is one of the key elements for increasing abdominal fat with a low-protein diet (El-Moneim *et al*., 2019).

The results of the present study demonstrated that high-protein diets lead to an increase in carcass performance, enhancing the high genetic potential of modern strains of broiler chickens for breast meat yield. This is of particular importance as the market for processed products and convenience food is expanding globally.

In the present study, a 4% reduction in CP level in the diet resulted in a decrease in the height of villi in the jejunum compared to the 22% crude protein level. This finding is consistent with the report by Abbasi *et al*. (2014), which showed that reducing CP in the diet led to a significant decrease in villus height and crypt depth in broiler chickens. Abbasi *et al*. (2014) also reported that reducing dietary CP had a similar effect on the villus height to crypt depth ratio. However, this result contradicted the findings of Sritiawthai *et al*. (2013), who found that ducks receiving 18% CP had greater villus height in the duodenum and jejunum compared to higher levels of CP, thus this result was also contradictory to our findings. Gu and Li (2004) reported that increasing dietary CP leads to an increase in the number of goblet cells in the distal jejunum of piglets, which is correlated with the growth of villus height and size of epithelial cells. It is believed that high dietary CP aids in the digestion and absorption capacity of the small intestine mucosa compared to low dietary CP. Wu (1998) also mentioned that the small intestine may utilize 30 to 50 percent of essential amino acids. Additionally, some amino acids may not be readily available for extra-intestinal tissue. Schaart *et al*. (2005) found that the small intestine mucosa utilizes dietary threonine for protein synthesis. Contrary to expectations, the use of synthetic amino acid supplements did not result in improvements in intestinal morphology parameters in broiler chickens.

The results of the present study showed that a 4% reduction in dietary crude protein level led to a decrease in performance traits of broiler chickens, and the use of synthetic amino acid supplements was not able to compensate for it. However, reducing the level of dietary crude protein up to 2% can be compensated for by adding synthetic amino acid supplements. Therefore, the use of synthetic amino acids and reducing the dietary protein level by up to 2% can be suggested as a method to reduce the consumption of protein ingredients in the diet, such as soybean meal.

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