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Original Article

Effect of Varying Protein Levels and Amino Acids on Growth Performance, Gas Emission, Organ Weight, and Body Condition Score in Laying Chicks

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ABSTRACT

There is increasing interest among nutritionists in formulating reduced crude protein (CP) diets for poultry. Nearly a century of research has offered biochemical insights into the effects of reduced CP diets in broilers but practical implementation of such diets for laying hens remains limited. Therefore, this study aims to investigate the effect of varying crude protein (CP) and amino acids (AA) levels on growth performance, gas emission, organ weight, and body condition score in laying chicks. n=480, 1d old chicks were allocated into six treatment groups with 5 replicates of 16 birds/pen. The dietary treatments for Phase 1 (0-6 weeks) were as follows: CON - 18.5% CP with 100% digestible AA; TRT1 - 18.5% CP with 100% digestible AA; TRT2 - 18.5% CP with 105% digestible AA; TRT3 - 18.0% CP with 100% digestible AA; TRT4 - 18.0% CP with 100% digestible AA; and TRT5 - 18.0% CP with 105% digestible AA. In Phase 2 (6-16 weeks), the CP level was reduced by 2% in the CON, TRT4, and TRT5 diets, and by 2.5% in TRT1, TRT2, and TRT3 diets, while the AA levels remained consistent in all treatments. Our study demonstrates that slight variations in protein levels and AA have no impact on growth performance, gas emission, organ weight, and body condition score in laying chicks. In summary, we infer that poultry diet can be flexible and potentially reduce protein levels if ensuring sufficient AA, thus optimizing performance without negatively affecting the growth.

INTRODUCTION

Protein and amino acids (AA) play important roles in the formulation of poultry diets to maximize production, ensure bird welfare, and minimize environmental pollution. Accordingly, there is growing interest among nutritionists in feeding reduced protein diets to broiler chickens. Nearly a century of research attempts has been done to decrease the crude protein (CP) content in broiler diets. Though the ideal protein concept has been successful to a certain degree (Aispuro *et al.*, 2022), the information concerning the use of AA in low CP diets for laying hens remains scarce. Incorporating low-CP diets in poultry production offers several benefits, particularly in terms of environmental impact and animal welfare (Zhang *et al.*, 2021). Firstly, it reduces the nitrogen (N) intake of broilers while maintaining N retention. This strategy supports increased N retention and reduced N excretion (Belloir *et al.*, 2017). Secondly, excessive levels of CP in the diets of broiler chickens usually rely on the inclusion of soybean meal (SBM), often linked to land use change and therefore global warming potential (Castanheira & Freire, 2013). Research by Méda *et al.* (2019) demonstrated that broiler fed low CP diets showed decreased volatilization of nitrogen from litter into gaseous emissions, such as ammonia (NH_3) and nitrous oxide (N_2O).

Similarly, Kebreab *et al.* (2016) noted that reducing dietary CP reduced the global warming potential of chicken meat production. In 2019, Van Harn *et al.* demonstrated that broilers fed diets supplemented with 20.8 % to 17.8 % of CP in grower (11–28d) and 19.8 % to 16.8 % of CP in finisher (28–35d) had no negative effect on growth performance. Furthermore, Torki *et al.* (2015) reported that layers fed 16.5 to 12.0% low CP diet supplemented with the essential amino acids maintained acceptable production performance under a high ambient temperature. From this literature, we hypothesize that feeding low-CP diets to poultry is not only an effective way to decrease ammonia emissions but also serves to reduce feed costs. The modern livestock industry uses corn–soybean–based diets, and the emission of nitrogen in the excreta of chicks is an important issue in the poultry industry. Considering both the rising cost of feed proteins and their environmental implications, the current research aims to evaluate the effects of low-CP levels supplemented with amino acids in laying chicks' diets. Specifically, this study assesses the impact of reduced CP levels combined with amino acids on laying chicks' growth performance, gas emissions, organ weight, and body condition score during each developmental phase.

MATERIALS AND METHODS

Ethical approval

The research protocol (DK-1-2417) of the present study was approved by the Animal Care and Use Committee of Dankook University (South Korea).

Animals, Housing, Experimental design and diets

A total of 480, Hy-line brown laying chicks (1-d old) were kept in multiple type cages with an evaporative cooling house system with 60% relative humidity, and 16-h of fluorescent light cycle. A temperature regimen starting at 33°C was applied and was gradually reduced by 3°C each week until reaching 24°C by the end of week 16. This experiment was conducted for a period of 16 weeks (Phase 1, 0- 6 weeks and Phase 2, 6 -16 weeks) in a completely randomized design, and all chicks were assigned to one of 6 dietary treatments. The test diets were provided in two phases: Phase 1 (0-6 weeks) and Phase 2 (6-16 weeks). The dietary treatments for Phase 1 were as follows: CON - 18.5% CP with 100% digestible AA; TRT1 - 18.5% CP with 100% digestible AA; TRT2 - 18.5% CP with 105% digestible AA; TRT3 - 18.0% CP with 100% digestible AA; TRT4 - 18.0% CP with 100% digestible AA; and TRT5 - 18.0% CP with 105% digestible AA. In Phase 2, the CP level was reduced by 2% in the CON, TRT4, and TRT5 diets, and by 2.5% in TRT1, TRT2, and TRT3 diets, while the AA levels remained consistent in all treatments. The above-mentioned diets were prepared in the laboratory of feed science and technology according to NRC (1994) guidelines and were examined for protein and AA prior to the trial. The analysis of AA was performed with an amino acid analyzer. The ingredients and nutrient composition of the experimental diets for phases 1 and 2 are presented in Table 1 and Figure 1.

Table 1 – Composition of laying chick diets (as fed basis).

Items	Phase 1		Phase 2		
	CP 18.5% AA 100%	CP 18% AA 100%	CP 16.5% AA 100%	CP 16% AA 100%	CP 16% AA 105%
Ingredients (%)					
Corn	63.03	64.85	63.81	65.66	65.65
Soybean meal (CP 44%)	30.77	29.24	26.60	25.06	24.95
Soy oil	1.53	1.14	1.28	0.88	0.88
Mono-dicalcium phosphate	1.72	1.72	1.91	1.91	1.91
Limestone	1.41	1.42	1.41	1.42	1.42
Salt	0.25	0.25	0.25	0.25	0.25
Methionine (50%)	0.45	0.47	0.28	0.29	0.33
Threonine (99%)	0.10	0.12	0.01	0.03	0.06
Tryptophan (99%)	-	0.01	-	0.01	0.01
Lysine (78%)	0.24	0.28	0.01	0.05	0.10
Vitamin mix1	0.20	0.20	0.20	0.20	0.20
Mineral mix2	0.20	0.20	0.20	0.20	0.20
Choline (50%)	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Analysed value					
Crude Protein, %	18.41	18.03	16.59	15.97	16.04
Crude Fat, %	3.76	3.48	3.66	3.38	3.24
Crude Fiber, %	3.39	3.26	3.01	2.95	2.94
Crude Ash, %	4.23	4.10	7.68	7.76	7.79
Caluclated value					
ME, kcal/kg	2950	2950	2850	2850	2850
Crude Protein, %	18.50	18.00	16.50	16.00	16.00
Crude Fat, %	3.86	3.53	3.62	3.29	3.28
Crude Fiber, %	3.35	3.28	3.07	3.00	2.99
Crude Ash, %	4.11	4.05	7.78	7.72	7.71
Calcium, %	1.00	1.00	1.00	1.00	1.00
Available Phosphorus, %	0.44	0.44	0.48	0.48	0.48
SID Lysine, %	1.07	1.07	0.79	0.79	0.83
SID Methionine, %	0.49	0.49	0.38	0.38	0.40
SID Methionine+Cystine, %	0.84	0.84	0.68	0.68	0.71
SID Threonine, %	0.70	0.70	0.55	0.55	0.58
SID Tryptophan, %	0.20	0.20	0.18	0.18	0.18

[1] Provided per kg of diet: vitamin A, 8,000 IU; vitamin D3, 3,300 IU; vitamin E, 20 g; vitamin K3, 2.5 g; vitamin B1, 2.5 g; vitamin B2, 5.5 g; vitamin B6, 4 g; vitamin B12, 23 mg; biotin, 75 mg; folic acid, 0.9 g; niacin, 30 g; D-calcium pantothenate, 8 g.
[2] Provided per kg of diet: Fe, 40 g as ferrous sulfate; Cu, 8 g as copper sulfate; Mn, 90 g as manganese oxide; Zn, 80 g as zinc oxide; 1.2 g as potassium iodide; and Se, 0.22 g as sodium selenite.

Each treatment has 5 replicates of 16 laying chicks per cage. All chicks were provided with *ad libitum* access to fresh water, and feed was introduced to each cage at 07:00 A.M and 16:00 P.M. The vaccination was provided at different time points and included in Figure 1. No mortality was found until the end of the 16th week.

AGE	Vaccine	Route of Administration
Day 1	Marek's (at hatchery)	S/C at neck
Week 2	IBD	Drinking Water
Week 4	Ma5+ ND clone 30	Coarse spray / eye drop
Week 6	Fowl pox	Wing Web
Week 8	Inactivated Salmonella	Intramuscular
Week 9	Ma5+ ND clone 30 (Booster)	Coarse spray / eye drop
Week 14	Inactivated Salmonella (Booster)	Intramuscular
Week 16	ND CLONE 30+ MA5	Spray eye drop/ drinking water

Figure 1 – Shows the vaccination schedule during the experimental period.

Sampling and laboratory analysis

Growth performance

Growth performance parameters such as body weight [(BW) weighed individually and averaged by pen] and feed intake [(FI) weighed on a pen basis] were calculated on phase basis [phase 1 (week 0-7) phase 2 (week 7-16), and overall experimental period], to determine BW gain (BWG) and feed conversion ratio (FCR). Simultaneously, all birds were inspected twice a day for general health and mortality rates.

Noxious Gas emission

At the end of phases 1 and 2, fresh excreta samples (approximately 300 g) were collected from 2 cages/ treatment around 17:00 (pm), pooled thoroughly, stored in an airtight container (2.6 L) with a slight hole on one side, fastened tightly with adhesive tape, and fermented at 25°C for 7 days. On the 8th day, a 100 ml sample was taken from the headspace (2cm) for air circulation, and the box was re-sealed. The sample container was manually shaken for about 30 seconds to determine the crust formation on the surface. Finally, NH3, H2S, Acetic acid, Propionic acid, Butyric acid, Iso butyric acid, Valeric acid, and Isovaleric acid were measured using a Multi-RAE Lite gas detection probe (model PGM-6208, RAE Systems, San Jose, CA, USA).

Organ Weight

At the end of phases 1 and 2, two birds/pen were picked randomly from each replication for carcass evaluation. The birds were slaughtered after starving for about 12 hours and weighed. Then the birds were de-feathered completely and eviscerated. The breast muscle, liver, spleen, abdominal fat, cloacal bursa, and gizzard were collected, weighed, and their percentages were determined in relation to live weight.

Body Lesion score

At the end of the trial, live birds were held individually with both legs in one hand and the head downwards. The palm of the other hand was used for palpating and grading the protuberance of the keel, the development of the breast muscles immediately alongside the ventral ridge of the keel, and the convexity or concavity of the breast muscle contour. Finally, the body condition score was assessed following the Gregory and Robins (1998) scoring system. In brief: score 0 corresponded to protruding keel bone and depressed contour to breast muscle; score 1 to prominent keel bone with poorly developed breast muscle; score 2 to a less prominent keel bone and moderate breast muscle development; and score 3 to plump breast muscle which provide a smooth contour with the keel.

RESULTS AND DISCUSSION

To optimize production costs, hens' diets must contain adequate quantities of nutrients, particularly protein. It is generally known that providing high dietary protein to animals causes a low efficiency of protein utilization and high heat production, while limiting protein consumption and adding synthetic dl-methionine and l-lysine can improve the protein utilization and production performance of the hens (Bunchasak & Silapasorn, 2005). Thus, this study intends to examine the effect of varying protein levels and AA in the diets of laying chicks. The present study demonstrates that varying protein levels and AA in the diets of laying chicks did not significantly affect their growth performance during both Phase 1 and Phase 2 (Table 2). Particularly during phase 1, no differences in growth performance were observed between the control group (CON) and TRT1 chicks, both fed 18.5% crude protein (CP) with 100% AA, as well as between TRT2 chicks fed 18.5% CP with 105% AA. This suggests that increasing AA levels in conjunction with the fixed protein level did not impact the chicks' growth. Similarly, no performance differences were observed between the TRT3 and TRT4 chicks, which were fed 5% reduced CP with similar AA levels, and the TRT5 chicks that received the same reduced CP but with a 5% increase in AA. These results were aligned with Cabel & Waldroup (1990), who found similar results in broilers fed diets containing 23% and 19%

CP. However, Pesti & Fletcher (1984) observed improved FCR with high CP level. During phase 2, the CON and TRT1 chicks fed 16.5% CP with 100% AA also showed no difference in growth compared to the TRT2 chicks fed 16.0% CP with 105% AA. Moreover, no growth performance differences were observed between TRT3 and TRT4 chicks fed 16.0% CP with 100% AA, and TRT5 chicks fed 16.0% CP with 105% AA.

Table 2 – The effect of varying protein levels and amino acids on growth performance in layer chicks[1].

Items	CON	TRT1	TRT2	TRT3	TRT4	TRT5	SEM[2]	*p*-value
Phase 1								
Week 0-2								
BW, g	121	121	122	119	118	118	3	0.706
FI, g	219	219	221	218	216	217	5	0.966
Week 2-4								
BW, g	261	263	263	258	257	257	5	0.636
FI, g	369	372	372	365	363	362	8	0.604
Week 4-6								
BW, g	464	468	469	460	458	456	8	0.676
FI, g	548	555	553	542	543	539	14	0.942
Overall (wk 0-6)								
BW, g	464	468	469	460	458	456	8	0.676
BWG, g	430	435	435	426	425	422	8	0.702
FI, g	1136	1146	1147	1125	1122	1118	24	0.873
FCR	2.640	2.636	2.635	2.640	2.642	2.647	0.017	0.661
Phase 2								
Week 6-8								
BW, g	683	684	689	671	666	670	11	0.914
FI, g	628	625	636	622	621	627	17	0.997
Week 8-10								
BW, g	915	911	925	900	892	905	15	0.383
FI, g	735	728	745	730	721	735	22	0.540
Week 10-12								
BW, g	1107	1098	1123	1091	1078	1101	19	0.860
FI, g	821	808	836	815	799	826	27	0.915
Week 12-14								
BW, g	1272	1258	1297	1259	1237	1274	24	0.661
FI, g	959	943	982	953	930	969	33	0.763
Week 14-16								
BW, g	1410	1388	1447	1390	1365	1414	30	0.601
FI, g	1026	1004	1059	1017	988	1045	38	0.816
Overall (wk 6-16)								
BW, g	1410	1388	1447	1390	1365	1414	30	0.601
BWG, g	946	920	979	930	907	958	23	0.490
FI, g	4168	4107	4258	4136	4059	4202	121	0.854
FCR	4.407	4.462	4.350	4.449	4.477	4.382	0.065	0.907
Mortality	2.50	2.50	2.50	1.25	1.25	2.50	-	

[1]Abbreviation: Phase 1: CON - 18.5% CP + 100% digestible AA; TRT1 - 18.5% CP +100% digestible AA; TRT2 - 18.5% CP + 105% digestible AA; TRT3 - 18.0% CP + 100% digestible AA; TRT4 - 18.0% CP + 100% digestible AA; and TRT5 - 18.0% CP +105% digestible AA. Phase 2: the CP level was reduced by 2% in the CON, TRT4, and TRT5 diets, and by 2.5% in TRT1, TRT2, and TRT3 diets, while the AA levels remained consistent in all treatments.
[2]Standard error of means.

This result is in line with Humphrey and Klasing (2004), who reported that poultry fed protein- and methionine-deficient diets had no impact in their feed intake. We supposed that slight reductions in protein levels could be compensated by increased AA with no significant impacts on the growth performance of chicks. Protein and AA requirements are generally considered to decrease with increasing age (Cabel & Waldroup. 1990). We supposed that different properties in AA might intact proteins to decline growth rate in laying chicks.

Ammonia is a primary noxious gas produced in poultry houses, and it is typically influenced by factors such as dietary protein levels, the balance of amino acids, and overall nitrogen metabolism in birds (Wang *et al.*, 2021). Odor emissions can be controlled by proper management and dietary intervention (Park & Kim. 2020). Previously, Nahm (2007) demonstrated that reducing CP levels in diets lowered ammonia gas, VFA, and other odors in poultry manure. Similarly, Park & Kim. (2019) reported that hydrogen sulfide was decreased with low CP diets. However, in this study, there were no noticeable changes in laying chicks' gas emission performance by reducing protein and/or increasing AA in their diet (Table 3).

Table 3 – The effect of varying protein and amino acids levels on gas emission in layer chicks[1].

Items	CON	TRT1	TRT2	TRT3	TRT4	TRT5	SEM[2]	*p*-value
Week 6								
NH_3, ppm	14.50	14.38	14.50	13.75	13.88	13.75	0.82	0.945
H_2S, ppm	2.15	2.13	2.18	2.00	2.03	2.03	0.39	0.995
Acetic Acid, mmol/L	9.99	10.00	9.96	9.74	9.88	9.60	0.53	0.382
Propionic Acid, mmol/L	0.70	0.71	0.72	0.66	0.66	0.65	0.04	0.167
Butyric Acid, mmol/L	0.46	0.45	0.48	0.41	0.39	0.43	0.03	0.573
Isobutyric Acid, mmol/L	9.21	9.28	9.25	9.13	9.08	9.10	1.12	0.997
Valeric Acid, mmol/L	0.20	0.21	0.21	0.17	0.19	0.18	0.01	0.486
Isovaleric Acid, mmol/L	0.63	0.66	0.65	0.59	0.60	0.56	0.08	0.781
Week 16								
NH_3, ppm	21.63	20.38	19.75	21.38	20.25	19.50	1.12	0.674
H_2S, ppm	3.90	3.78	3.65	3.83	3.70	3.63	0.52	0.847
Acetic Acid, mmol/L	14.83	14.64	15.15	14.70	14.49	15.06	0.72	0.208
Propionic Acid, mmol/L	0.95	0.88	1.03	0.93	0.87	0.99	0.10	0.938
Butyric Acid, mmol/L	0.62	0.56	0.64	0.59	0.53	0.60	0.06	0.642
Isobutyric Acid, mmol/L	12.10	12.01	12.52	11.89	11.71	12.36	0.80	0.741
Valeric Acid, mmol/L	0.33	0.29	0.37	0.29	0.27	0.36	0.04	0.625
Isovaleric Acid, mmol/L	0.85	0.72	0.88	0.79	0.70	0.80	0.06	0.385

[1]Abbreviation: Phase 1: CON - 18.5% CP + 100% digestible AA; TRT1 - 18.5% CP +100% digestible AA; TRT2 - 18.5% CP + 105% digestible AA; TRT3 - 18.0% CP + 100% digestible AA; TRT4 - 18.0% CP + 100% digestible AA; and TRT5 - 18.0% CP +105% digestible AA. Phase 2: the CP level was reduced by 2% in the CON, TRT4, and TRT5 diets, and by 2.5% in TRT1, TRT2, and TRT3 diets, while the AA levels remained consistent in all treatments.
[2]Standard error of means.

This suggests that the changes in protein content and AA supplementation did not have a substantial impact on the birds' nitrogen metabolism in the short term, which is consistent with some previous studies indicating that small adjustments in protein and AA levels may not always lead to changes in gas emissions (Roberts *et al.*, 2007). At this stage, this result is unclear and requires further research.

Organ weight in poultry is an important indicator of overall health and metabolic function (Wang *et al.*, 2021), and it can be influenced by various factors such as diet composition, nutrient intake, and AA requirements (Plavnik & Hurwitz. 1983). Protein and amino acids play a key role in tissue growth and repair, and any alterations in dietary protein levels or AA balance can affect organ development. However, in this study, the lack of significant differences in organ weight across treatments implies that the protein and AA levels tested were within the range that adequately supported organ growth and function in the laying chicks (Table 4). Previously, Nastain *et al.* (2021) reported that broilers fed slight variations in the protein and AA in their diets showed no difference in their organ weight. Similarly, Badawi *et al.* (2009) found no considerable difference in dressing percentage and visceral organ weight in broilers fed diets containing reduced CP. However, Kobayashi *et al.* (27) reported that broilers fed low CP diets have little difference in their breast muscle, with no substantial difference in the AA diet. The inconsistencies among findings could be due to the adjustments made in this study, such as the reduction in protein or increase in AA. In fact, many studies have demonstrated that the essential amino acids provided in sufficient quantities along with lowering CP does not negatively affect organ weight or overall health (Shao *et al.*, 2018). As long as the AA profile of the diet is balanced and sufficient to meet the birds' metabolic needs, small changes in protein levels may not result in noticeable differences in organ weight. However, the exact cause for the lack of this outcome is currently unknown, and thus further research is needed. Previously, Gregory & Robins (1998) addressed that birds with the lowest condition score were skinny and were distinguished by a low breast muscle: bone ratio and a low breast muscle: leg muscle ratio. However, in this study, since the dietary treatments did not result in significant differences in body lesion scores (Table 5), it is likely that the protein and AA levels used could support the chicks' health and well-being. To our knowledge no study analyzing the effect of protein and AA diet in the body score of laying chicks exists, and thus sufficient comparisons could not be made.

Table 4 – The effect of varying protein levels and amino acids on organ weight in layer chicks[1].

Items	CON	TRT1	TRT2	TRT3	TRT4	TRT5	SEM[2]	*p*-value
Week 16								
Relative organ weight, %								
Breast muscle	20.90	20.67	21.15	20.73	20.38	21.04	0.25	0.594
Liver	1.70	1.64	1.74	1.66	1.59	1.71	0.06	0.211
Spleen	0.13	0.11	0.13	0.12	0.11	0.13	0.03	0.651
Abdominal fat	0.99	1.08	1.07	1.01	1.10	1.07	0.13	0.963
Cloacal bursa	0.08	0.08	0.09	0.08	0.07	0.09	0.02	0.970
Gizzard	1.51	1.45	1.54	1.47	1.39	1.52	0.11	0.915

[1]Abbreviation: CON - 16.5% CP + 100% digestible AA; TRT1 – 16 % CP +100% digestible AA; TRT2 – 16 % CP + 105% digestible AA; TRT3 – 16 % CP + 100% digestible AA; TRT4 - 16.0% CP + 100% digestible AA; and TRT5 - 16.0% CP +105% digestible AA.
[2]Standard error of means.

Table 5 – The effect of varying protein levels and amino acids on body score in layer chicks[1].

Items	CON	TRT1	TRT2	TRT3	TRT4	TRT5	SEM[2]	*p*-value
Body score[3]	4.00	4.50	4.25	4.00	4.75	4.50	0.34	0.698

[1]Abbreviation: CON - 16.5% CP + 100% digestible AA; TRT1 – 16 % CP +100% digestible AA; TRT2 – 16 % CP + 105% digestible AA; TRT3 – 16 % CP + 100% digestible AA; TRT4 - 16.0% CP + 100% digestible AA; and TRT5 - 16.0% CP +105% digestible AA.
[2]Standard error of means.

CONCLUSION

This study demonstrates that slight variations in protein levels and amino acids do not significantly affect the growth performance of laying chicks, provided that the AA balance is maintained. Based on these findings, we propose that poultry nutrition strategies can be flexible, allowing for reduced protein levels while ensuring sufficient amino acid supplementation, without negatively affecting growth.

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AUTHOR CONTRIBUTIONS

Conceptualization, SV and LCB ; methodology, SV and IHK ; software, SV and LCB ; validation, IHK; formal analysis, SV and LCB; investigation, IHK; writing—

original draft preparation, SV; writing—review and editing, IHK; supervision, IHK. Authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data sets will be available from the correspondence upon reasonable request.

CONFLICT OF INTEREST

No potential conflict of interest was filed in this article.

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