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Effects of digestible amino acid based formulation of low protein broiler diets supplemented with valine, isoleucine and arginine on performance and protein efficiency

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Abstract: The aim of the present study was to investigate the effect of digestible amino acid (DAA) based formulation strategy, and L-valine (L-Val), L-isoleucine (L-Ile) and L-arginine (L-Arg) supplementation to reduce the crude protein (CP) level of broiler diets on performance, carcass characteristics and protein efficiency ratio by comparing with the control diet formulated on total amino acid base. A total of 792 one-day-old Ross 308 broiler chicks were divided into 48 floor pens, with 24 pens containing 16 chicks and 24 pens containing 17 chicks. The experiment was organized in a completely randomized block design with four dietary treatments as follows; T1: formulated to meet minimum both total amino acid and DAA requirements without using L-Val, L-Ile, and L-Arg, T2: formulated to meet DAA requirements without using L-Val, L-Ile and L-Arg, T3: formulated to meet DAA requirements by using L-Val alone, T4: formulated to meet DAA requirements by using L-Val, L-Arg, and L-Ile. Each treatment had 12 replicates. Neither L-Val (T3) nor L-Val, L-Ile, and L-Arg supplemented (T4) low CP dietary treatments had any negative impact on growth performance, feed conversion ratio during experimental periods, as well as carcass parameters of broilers. Indeed, CP reduction by the addition of L-Val alone or L-Val, L-Ile, and L-Arg together even resulted in a significant improvement in protein efficiency ratio compared to T1 and T2 treatments. Supplementation of L-Val either alone or along with L-Ile and L-Arg to diets formulated based on DAA not only decreased dietary CP but also soybean meal inclusion. It can be concluded that maintained growth performance, and even improved protein utilization can be achieved due to the DAA based formulation strategy and supplementing broiler diets with L-Val alone or together with L-Ile and L-Arg.

Keywords: amino acids; carcass; chicken; protein intake

Poultry producers have been facing major challenges in recent years including drastic increases in feed costs and changes in available ingredients which are largely impacted by the increased use of grains for the growing human population, expected to reach almost 10 billion in 2050 from 7.8 billion, and for biofuel production. In the current year, the elevated price of soybean meal, the most available and common protein source of poultry diets, is very dramatic and makes the challenge even harder. At the same time, there is a growing environmental concern to reduce the excretions of nitrogen and carbon footprint from animal production despite the fact that the production of per kg of chicken meat produces 1.1 kg CO_2 equivalents, which is substantially less than that of pork (3.8 kg CO_2 equivalents) or beef (14.8 kg CO_2 equivalents) production (Fiola 2008). Given a conservative 250 g/kg dietary inclusion of soybean meal and a 2.25 : 1 conversion ratio of feed into carcass weight, 560 g soybean meal is needed to produce 1 kg chicken meat (Selle et al. 2020). Successful development of lower crude protein (CP) feed formulas can contribute to diminish the amount of soybean meal in broiler diets to alleviate the above-mentioned challenges. Broiler diets based on maize-soybean meal are deficient in methionine (Met), lysine (Lys) and threonine (Thr) to meet the requirements of modern broilers. That is why, as they are limiting, it became essential to supplement these critical amino acids (AA) in broiler diet to achieve the desired performance. Effects of supplementing these three limiting AAs in broiler diets are well documented, since feed grade crystalline Met, Lys and Thr have usually been used in chicken diets for years, starting from the first introduction of commercially available DL-Met in the late 1950s (Selle et al. 2020) and the accessibility of these essential amino acids (EAA) has already enabled an important decrease in dietary CP and soybean meal inclusion levels in poultry diets (Pesti 2009), although this evolution may not have been recognized. Some recent studies have focused on determining the fourth and fifth limiting AA for broilers (Berres et al. 2010). Valine (Val) has been proposed to be the fourth limiting AA after the sulphur containing AAs, Lys and Thr in vegetable diets based on maize and soybean meal (Golzar Adabi et al. 2019). However, either Val or isoleucine (Ile) may be the fourth limiting, or co-limiting AA in broiler diets according to the quality of protein sources such as soybean meal; whereas leucine levels are usually considered to be adequate (Selle et al. 2020).

Expanding commercial availability of L-Val, L-Ile and/or L-Arg as the fourth and fifth limiting amino acid (AA) thus seems to allow for some additional decrease in dietary CP and, in turn, higher reductions in soybean meal inclusion levels (Selle et al. 2020).

Many experiments and efforts have already been performed to reduce the dietary CP level of broiler diets since the early 2000s, and some of them failed to have satisfactory performance results compared to normal CP diets. The mechanisms behind the low growth performance of broilers have been mainly attributed to nonessential AA deficiency, and antagonism among branched-chain AAs (BCAA) (Golzar Adabi et al. 2019). The latter results from the altered AA profile in low CP broiler diets based on maize and soybean meal leading to an increased leucine (Leu) level, which potentially limits the utilization of Val and the performance of the birds (Golzar Adabi et al. 2019). Berres et al. (2010) reported that inadequate nonessential AAs may also limit growth performance when diets that are low in CP and L-Val are fed. The requirement for glycine (Gly) appears to be higher in diets that are low in CP than in broiler diets with higher levels of CP (Dean et al. 2006). However, restricted growth and feed conversion probably result from an inadequately exact recognition of EAA and nonessential amino acid (NEAA) demands, or ideal protein ratios. Ideal protein ratios or AA suggestions should be updated as the latest experimental data become accessible (Wu 2014) and this equally applies to the new and different context that the advent of reduced CP diets would create.

Although there has been a major shift from using TAA towards DAA in poultry feed formulation, some companies or nutritionists still calculate broiler formulas based on TAA requirements. DAA-based feed formulation allows diets to be calculated to closely meet animal requirements and reduce nitrogen excretion via higher protein utilization (Lu et al. 2020). Hence, the search to formulate lower CP diets successfully is still a complex problem that needs to be further investigated.

Based on all these statements, we theorized that supplementation of L-Val alone or together with L-Ile and L-Arg in broiler diets formulated on a DAA base to reduce CP, and soybean meal inclusion, and further to obtain better ideal AA ratios can favourably influence growth performance, protein utilization and carcass parameters in Ross 308 broiler chickens.

MATERIAL AND METHODS

Ethics

All experimental procedures were approved by Ankara University Animal Experiments Local Ethics Committee (2018-15-91).

Experimental design, animals and housing

A total of 792 one-day-old Ross 308 male broiler chicks were purchased from a commercial hatchery for an experiment. At the beginning of study (day 11) chicks were weighed and randomly allocated to 48 floor pens, littered with wood shavings and equipped with nipple drinkers and plastic hanging feeder, when 24 pens (1.4 × 0.9 m each) contained 16 chicks and 24 pens (1.5 × 0.9 m each) contained 17 chicks. Chicks were allowed *ad libitum* access to water and feed. Room temperature was maintained at 33 °C for the first three days and then gradually decreased to 23 °C by day 21 and then maintained at this level until the end of the experiment via automatic heating, cooling and ventilation systems. Continuous fluorescent lighting was applied during the experimental period. A commercial broiler starter diet was provided to all chicks until 11 days of age. The experimental diets were formulated to meet Aviagen (2014) recommendations for grower (11–24 days) and finisher (25–39 days) periods, and were fed in mash form. The experiment was arranged in a completely randomized block design with four dietary treatments as follows; T1: formulated to meet minimum both TAA and DAA requirements without using L-Val, L-Ile and L-Arg, T2: formulated to meet DAA requirements without using L-Val, L-Ile and L-Arg, T3: formulated to meet DAA requirements by using L-Val alone, T4: formulated to meet DAA requirements by using L-Val, L-Arg and L-Ile. Each treatment had 12 replicates. The main ingredients and experimental diets were analysed for proximate (AOAC 2005), amino acid (Liames and Fontaine 1994) and N-corrected apparent metabolizable energy content by near infrared reflectance spectroscopy (NIRS; Evonik Nutrition & Care GmbH, Hanau, Germany). The experimental basal diets were formulated to meet or exceed demands of chickens according to breeder guidelines except for the above-mentioned variations in crude protein and amino acids levels based on formulation strategy. Feed composition and analytical results are shown in Table 1. Pure crystalline L-Val, L-Ile and L-Arg in powder form were provided by Evonik Nutrition & Care GmbH, Hanau, Germany.

Measurements

Birds were weighed at day 11, 24 and day 39 for each replicate. FI were measured at the beginning of the grower period at day 11, 24 and at day 39 for each replicate. Feed conversion ratio (FCR) was calculated for days 11–24, 25–39 and 11–39 using feed intake and weight gain for each replicate on a pen base. Protein intake was calculated on a pen base by multiplying feed intake by the protein level of experimental diets. Protein efficiency ratio (PER) was also calculated as protein intake divided by body weight gain (g : g). Daily mortality was recorded for each pen and the weight of dead birds was used to correct FCR. At the end of experiment, two chickens per pen close to the average pen weight were selected for processing. Each bird was weighed, and leg-banded for identification. Feed was withdrawn 6 h before processing. Each bird was exsanguinated by cutting the jugular vein, allowed to bleed for approximately 2 min, scalded for 30 s, and defeathered in a rotary picker. Viscera and abdominal fat were removed. Then, weights of liver and abdominal fat were obtained. Thighs, drumsticks and breast as bone in and skin on were weighed. Carcass yield, abdominal fat, thighs + drumsticks and breast meat were weighed and calculated as a fraction of individual live body weight.

The ideal AA profiles were obtained as the digestible essential AA (DEAA) level of each treatment divided by the lysine level in the experimental diets (Table 2).

Statistical Analysis

The data for all response variables were analysed as a completely randomized block design with four dietary treatments and 12 replicate blocks by using General ANOVA/MANOVA procedure of the Statistica (1984; StatSoft, Tulsa, OK, USA). Floor pen was the experimental unit for all analyses. Furthermore, regression analyses were performed to compare dietary CP levels and PER. When significance was detected ($P < 0.05$), means were compared by Tukey HSD test. Mortality results were assessed by chi-square test.

RESULTS

CP levels of the experimental diets for grower and finisher periods were reduced depending on the presence of crystalline AA (Table 1). When compared to TAA based diet (T1), the L-Val, L-Ile

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Table 1. Ingredients and chemical composition of experimental diets (g/kg; as-fed basis)

Ingredients	Starter (days 0–10)	Grower (days 11–24)				Finisher (days 25–39)			
		T1	T2	T3	T4	T1	T2	T3	T4
Corn	529.80	533.34	536.46	544.72	588.10	606.17	611.95	612.99	630.39
Soybean meal	356.18	287.51	284.52	276.74	234.80	289.61	276.07	273.45	224.91
Sunflower meal	30.00	80.00	80.00	80.00	80.00	10.94	18.51	18.91	44.59
Sunflower oil	38.54	58.20	57.77	56.51	49.44	55.00	55.00	55.00	55.00
DCP19	22.86	20.03	20.03	20.04	20.11	18.56	17.57	18.50	18.33
Limestone ($CaCO_3$)	6.45	5.99	6.00	6.06	6.35	5.46	5.54	5.55	5.81
Lysine sulphate (54.6%)	3.69	3.58	3.74	4.10	6.03	2.43	2.98	3.08	4.91
DL-methionine	3.84	3.18	3.04	3.11	3.50	3.09	2.99	3.00	3.22
Salt (NaCl)	2.83	3.12	3.12	3.11	3.05	1.70	1.68	1.68	1.61
Vit-min premix[1]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
L-threonine	1.28	0.95	1.20	1.31	1.90	0.69	1.03	1.06	1.54
Sodium bicarbonate	1.21	0.87	0.88	0.89	1.01	3.07	3.38	3.44	4.52
Choline chloride (70%)	0.82	0.73	0.74	0.76	0.92	0.78	0.80	0.81	0.90
L-valine	–	–	–	0.14	0.87	–	–	0.03	0.59
L-isoleucine	–	–	–	–	0.75	–	–	–	0.62
L-arginine	–	–	–	–	0.67	–	–	–	0.56
Total	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00
Calculated and analysed nutrients[2]									
CP (%)	23.15 (23.44)	21.5(21.68)	21.4(21.67)	21.12 (21.31)	19.80 (19.64)	19.50 (20.61)	19.17 (19.46)	19.11 (18.44)	18.14 (17.88)
AMEn (kcal/kg)	3 000.00	3 100.00	3 100.00	3 100.00	3 100.00	3 200.00	3 200.00	3 200.00	3 200.00
Ca (%)	0.96	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79
Pa (%)	0.48	0.43	0.44	0.44	0.43	0.39	0.39	0.39	0.39
Total P (%)	0.67 (0.69)	0.64 (0.64)	0.63 (0.65)	0.63 (0.65)	0.63 (0.64)	0.56 (0.60)	0.56 (0.54)	0.56 (0.54)	0.56 (0.55)
Na (%)	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.20
Cl (%)	0.23	0.25	0.25	0.25	0.25	0.16	0.16	0.16	0.16
Lys (%)	1.44 (1.42)	1.29 (1.29)	1.29 (1.29)	1.29 (1.34)	1.28 (1.28)	1.16 (1.25)	1.16 (1.16)	1.16 (1.10)	1.15 (1.12)
Met (%)	0.72 (0.76)	0.65 (0.64)	0.63 (0.65)	0.64 (0.60)	0.65 (0.59)	0.60 (0.61)	0.58 (0.60)	0.58 (0.54)	0.60 (0.62)
Met + Cys (%)	1.08 (1.12)	0.99 (0.97)	0.97 (0.99)	0.97 (0.95)	0.96 (0.91)	0.91 (0.94)	0.89 (0.81)	0.89 (0.83)	0.89 (0.90)
Thr (%)	0.98 (0.98)	0.89 (0.87)	0.91 (0.49)	0.90 (0.91)	0.89 (0.86)	0.79 (0.82)	0.81 (0.81)	0.81 (0.76)	0.80 (0.79)

Table 1 to be continued

Ingredients	Starter (days 0–10)	Grower (days 11–24)				Finisher (days 25–39)			
		T1	T2	T3	T4	T1	T2	T3	T4
Trp (%)	0.28	0.26	0.26	0.26	0.23	0.23	0.23	0.23	0.21
Arg (%)	1.57 (1.49)	1.46 (1.39)	1.45 (1.40)	1.43 (1.45)	1.36 (1.31)	1.29 (1.33)	1.26 (1.21)	1.26 (1.19)	1.22 (1.13)
Ile (%)	0.99 (0.98)	0.91 (0.90)	0.90 (0.88)	0.89 (0.91)	0.87 (0.87)	0.83 (0.87)	0.81 (0.80)	0.80 (0.77)	0.80 (0.77)
Leu (%)	1.88 (1.83)	1.73 (1.67)	1.73 (1.64)	1.70 (1.74)	1.58 (1.58)	1.65 (1.68)	1.62 (1.56)	1.61 (1.50)	1.50 (1.38)
Val (%)	1.08 (1.04)	1.00 (0.98)	1.00 (0.97)	1.00 (1.02)	0.99 (0.97)	0.91 (0.95)	0.89 (0.88)	0.89 (0.85)	0.88 (0.84)
Gly (%)	0.95 (0.93)	0.91 (0.90)	0.90 (0.89)	0.89 (0.91)	0.82 (0.83)	0.79 (0.82)	0.78 (0.77)	0.77 (0.74)	0.73 (0.71)
Ser (%)	1.11 (1.08)	1.02 (0.99)	1.01 (0.98)	1.00 (1.02)	0.91 (0.92)	0.95 (0.98)	0.92 (0.90)	0.92 (0.87)	0.84 (0.80)
Glu A (%)	4.08 (3.97)	3.81 (3.69)	3.79 (3.63)	3.73 (3.80)	3.41 (3.43)	3.45 (3.55)	3.38 (3.29)	3.37 (3.16)	3.13 (2.919)
NEAA (%)[3]	49.37	49.61	49.37	49.41	48.70	49.70	49.50	49.50	48.17
Digestible Lys (%)	1.32	1.18	1.18	1.18	1.18	1.06	1.06	1.06	1.06
Digestible Met (%)	0.69	0.62	0.61	0.61	0.63	0.57	0.56	0.56	0.58
Digestible Met + Cys (%)	0.99	0.91	0.89	0.89	0.89	0.84	0.82	0.82	0.82
Digestible Thr (%)	0.86	0.77	0.79	0.79	0.79	0.69	0.71	0.71	0.71
Digestible Trp (%)	0.25	0.23	0.23	0.23	0.20	0.21	0.20	0.20	0.18
Digestible Arg (%)	1.45	1.36	1.35	1.32	1.26	1.19	1.17	1.16	1.13
Digestible Ile (%)	0.89	0.80	0.81	0.80	0.80	0.75	0.73	0.73	0.73
Digestible Leu (%)	1.69	1.56	1.55	1.53	1.43	1.49	1.46	1.46	1.36
Digestible Val (%)	0.96	0.90	0.89	0.89	0.89	0.82	0.80	0.80	0.80

AMEn = apparent metabolizable energy, nitrogen-corrected; Arg = arginine; CP = crude protein; Cys = cysteine; Glu A = glutamic acid; Gly = glycine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; NEAA = nonessential amino acid; Pa = available phosphorus; Ser = serine; T1 = formulated to meet minimum both total amino acid and digestible amino acid (DAA) requirements without using L-Val, L-Ile, and L-Arg; T2 = formulated to meet DAA requirements without using L-Val, L-Ile and L-Arg; T3 = formulated to meet DAA requirements by using L-Val alone; T4 = formulated to meet DAA requirements by using L-Val, L-Arg, and L-Ile; Thr = threonine; Trp = tryptophan; Val = valine

[1] Premixes provided per kilogram of complete diet: vitamin A: 11 000 IU; vitamin D_3: 5 000 IU; vitamin E: 80 mg; vitamin K_3: 3 mg; thiamine: 2 mg; riboflavin: 6 mg; pyridoxine: 4 mg; pantothenic acid: 20 mg; niacin: 70 mg; folic acid: 1.750 mg; biotin: 200 mg; vitamin B_{12}: 16 mg; antioxidant: 125 mg; Cu: 16 mg; I: 2 mg, Se: 300 mg; Fe: 50 mg; Zn: 100 mg; Mn: 120 mg

[2] The parentheses indicate the analysed nutrient contents

[3] Sum of nonessential amino acids, % of total analysed amino acids

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Table 2. Ideal digestible amino acid (AA) profile of experimental diets (% of Lys), and digestible and ideal AA specifications for Ross 308 broilers

AA	Grower diets						Finisher diets					
	Ross digestible AA (%)	Ross ideal AA[1]	T1	T2	T3	T4	Ross digestible AA (%)	Ross ideal AA[1]	T1	T2	T3	T4
Lys	1.15	100	100	100	100	100	1.02	100	100	100	100	100
Met	0.47	41	53	52	52	53	0.43	42	54	53	53	55
Met + Cys	0.87	76	77	75	75	75	0.80	78	79	77	77	77
Thr	0.77	67	65	67	67	67	0.68	67	65	67	67	67
Trp	0.18	16	19	19	19	17	0.16	16	20	19	19	17
Arg	1.23	107	115	114	112	107	1.09	107	112	110	109	107
Ile	0.78	68	69	69	68	68	0.70	69	71	69	69	69
Leu	1.27	110	132	131	130	121	1.12	110	141	138	138	128
Val	0.87	76	76	75	76	76	0.78	76	77	75	76	76

Arg = arginine; Cys = cysteine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; T1 = formulated to meet minimum both total amino acid and digestible amino acid (DAA) requirements without using L-Val, L-Ile, and L-Arg; T2 = formulated to meet DAA requirements without using L-Val, L-Ile and L-Arg; T3 = formulated to meet DAA requirements by using L-Val alone; T4 = formulated to meet DAA requirements by using L-Val, L-Arg, and L-Ile; Thr = threonine; Trp = tryptophan; Val = valine

[1]Aviagen (2014)

and L-Arg supplementation (T4) significantly reduced the CP level from 21.5% to 19.8% and from 19.5% to 18.14% in grower and finisher period, respectively. The best ideal AA ratios were also obtained almost for all essential AAs in T4 as recommended by Aviagen (2014) (Table 2). The ideal AA profile of T1 diet exceeded recommended ratios of Trp, Ile, Arg and Leu.

Broiler performance results including body weight (BW), BWG, FI, FCR, mortality rate and protein intake, PER in grower (day 11–24), finisher (day 24–39) periods and whole trial (day 11–39) are given in Table 3.

CP reduction from 21.5% to 19.8% in the grower period with supplementation of L-Val, L-Ile and L-Arg (T4) caused no significant difference in growth performance compared to both TAA and DAA based formula treatments without L-Val, L-Ile and L-Arg (T1), and did not cause any growth depression when FCR, FI, BWG and mortality results are considered ($P > 0.05$) (Table 3). There were not any significant differences between the other treatment groups either. Although T1 group had the highest protein content, growth and FCR of broilers fed T1 did not differ from the other treatments in the grower period (Table 3). A trend found in the grower period for the treatments continued through the finisher period as well. As seen in Table 3, reducing the protein level from 19.5% to 18.14% by supplementing Val, Ile and Arg AA did not result in any decrease in growth performance ($P > 0.05$). Although mortality and FCR results were so close and similar between all four treatments ($P > 0.05$), T4 birds that received the lowest protein in their diets consumed significantly more feeds than T2 and T3 ($P < 0.05$). In the complete trial period BWG, FCR and mortality results were not significantly different between the treatments except FI. During the whole experiment, feed intake increased in T4 chickens ($P < 0.05$). They consumed significantly more feeds than T2 and T3 (Table 3). T1 birds consumed more protein in both the grower and finisher period than the others and PER was also the highest in this group compared to the other treatments ($P < 0.05$) (Table 3). PER was linearly increased by decreasing the CP level in the present study ($P < 0.05$) (Figure 1).

Carcass yields, carcass parameters and some internal organ weights are shown in Table 4. Diets formulated by total and/or standardized ileal digestible amino acid based formulas added L-Val, L-Ile and L-Arg or without them to reduce the protein level did not have any significant effects on carcass parameters and internal organ weights ($P > 0.05$).

Table 3. Effects of L-valine, L-isoleucine and L-arginine supplementation on body weight, body weight gain, feed intake, protein intake, feed conversion and mortality rate

Items	T1	T2	T3	T4	*P*-value
Body weight (g)					
Day 10	243 ± 2.51	243 ± 2.53	243 ± 2.52	243 ± 2.45	0.987
Day 24	1 137 ± 8.91	1 136 ± 13.49	1 115 ± 11.80	1 132 ± 11.56	0.200
Day 39	2 547 ± 21.30	2 521 ± 38.24	2 499 ± 16.69	2 569 ± 27.84	0.277
Body weight gain (g)					
Grower period (days 11–24)	894 ± 7.67	893 ± 12.15	872. ± 10.09	889 ± 9.95	0.194
Finisher period (days 25–39)	1 410 ± 17.96	1 385 ± 32.90	1 384 ± 14.27	1 437 ± 20.95	0.274
Whole period (days 11–39)	2 304 ± 21.80	2 278 ± 37.16	2 256 ± 15.24	2 326 ± 26.43	0.181
Feed intake (g)					
Grower period (days 11–24)	1 217 ± 12.15	1 217 ± 13.55	1 206 ± 13.66	1 223 ± 16.50	0.808
Finisher period (days 25–39)	2 373 ± 34.48^{ab}	2 305 ± 42.68^{b}	2 305 ± 20.57^{b}	2 430 ± 24.78^{a}	0.013
Whole period (days 11–39)	3 583 ± 41.36^{ab}	3 520 ± 50.42^{b}	3 510 ± 21.56^{b}	3 647 ± 32.61^{a}	0.030
Feed conversion ratio					
Grower period (days 11–24)	1.36 ± 0.015	1.37 ± 0.016	1.39 ± 0.026	1.38 ± 0.008	0.698
Finisher period (days 25–39)	1.68 ± 0.022	1.67 ± 0.025	1.67 ± 0.013	1.70 ± 0.011	0.642
Whole period (days 11–39)	1.56 ± 0.015	1.55 ± 0.015	1.56 ± 0.008	1.57 ± 0.007	0.449
Mortality rate (%)					
Grower period (days 11–24)	1.0 ± 0.98	0.5 ± 0.49	1.0 ± 0.70	1.0 ± 0.70	0.944
Finisher period (days 25–39)	1.0 ± 0.70	0.0 ± 0.00	0.5 ± 0.52	0.5 ± 0.52	0.510
Whole period (days 11–39)	1.5 ± 0.80	0.5 ± 0.49	1.6 ± 0.81	1.6 ± 0.81	0.642
Protein intake (g)					
Grower period (days 11–24)	262 ± 2.61^{a}	261 ± 2.90^{a}	255 ± 2.89^{a}	235 ± 3.16^{b}	< 0.001
Finisher period (days 25–39)	463 ± 7.87^{a}	442 ± 8.18^{b}	441 ± 3.93^{b}	442 ± 4.50^{b}	0.050
Whole period (days 11–39)	724 ± 8.63^{a}	702 ± 10.06^{b}	695 ± 4.26^{bc}	676 ± 5.99^{c}	0.001

T1 = formulated to meet minimum both total amino acid and digestible amino acid (DAA) requirements without using L-Val, L-Ile, and L-Arg; T2 = formulated to meet DAA requirements without using L-Val, L-Ile and L-Arg; T3 = formulated to meet DAA requirements by using L-Val alone; T4 = formulated to meet DAA requirements by using L-Val, L-Arg, and L-Ile

$^{a–c}$Means with different superscripts differ significantly

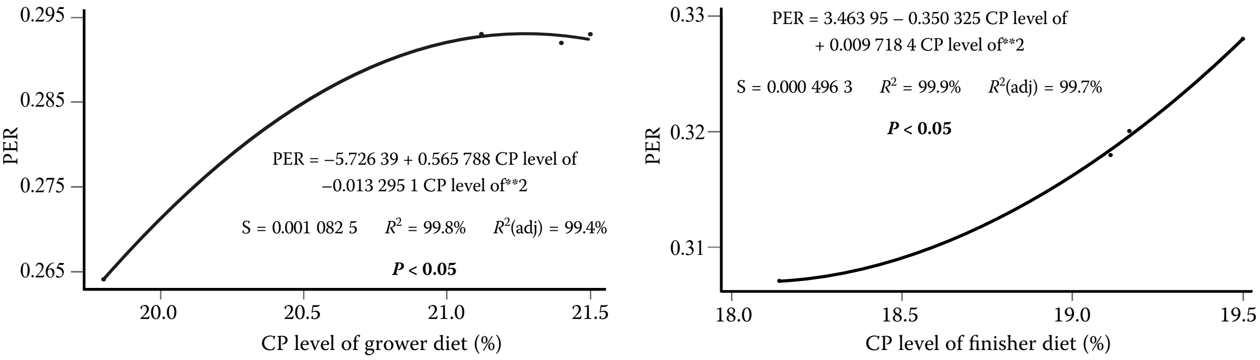

Figure 1. Relationship between the dietary crude protein level and protein efficiency ratio of broilers in the grower and finisher period

CP = crude protein; PER = protein efficiency ratio

Table 4. Effects of L-valine, L-isoleucine and L-arginine supplementation on carcass parameters (as percentage of live body weight)

Treatments	Carcass yield	Breast meat yield	Leg meat yield	Abdominal fat	Liver weight	Pancreas weight
T1	76 ± 0.40	28 ± 0.41	30 ± 0.35	1.17 ± 0.108	1.93 ± 0.054	0.23 ± 0.009
T2	76 ± 0.33	29 ± 0.30	29 ± 0.30	1.17 ± 0.091	1.91 ± 0.052	0.23 ± 0.016
T3	76 ± 0.20	28 ± 0.29	30 ± 0.31	1.19 ± 0.101	1.87 ± 0.036	0.23 ± 0.006
T4	76 ± 0.32	28 ± 0.34	30 ± 0.30	1.30 ± 0.099	1.92 ± 0.048	0.23 ± 0.005
P-value	0.682	0.298	0.316	0.786	0.796	0.991

T1 = formulated to meet minimum both total amino acid and digestible amino acid (DAA) requirements without using L-Val, L-Ile, and L-Arg; T2 = formulated to meet DAA requirements without using L-Val, L-Ile and L-Arg; T3 = formulated to meet DAA requirements by using L-Val alone; T4 = formulated to meet DAA requirements by using L-Val, L-Arg, and L-Ile

DISCUSSION

As seen in Table 1, the absence of L-Val, L-Ile and L-Arg significantly increased the inclusion of soybean meal in both the grower and finisher diet from 234.80 g/kg to 287.51 g/kg and from 224.91 g/kg to 289.61 g/kg, respectively. Meeting the Val and Ile requirements caused the higher CP level, and even the elevated Trp, Leu and Arg to Lys ratio, than recommended for Ross 308 broilers by Aviagen (2014). Davis (2009) mentioned that the production of soybeans in the Amazon rainforests is the leading cause of deforestation. This is a serious environmental problem, and an almost 6% reduction in soybean meal use achieved in the present experiment would substantially contribute to save the environment.

DAA based formulation strategy significantly reduced the CP level of grower and finisher diet by 7.90% and 6.96%, respectively, without compromising growth performance, FCR and carcass characteristics of broilers in this experiment when L-Val, L-Ile and L-Arg were supplied (Table 3 and 4). Similarly, Holsheimer and Janssen (1991) did not notice any performance drop when feeding chickens a reduced CP diet (170 g/kg) supplemented with L-Val (8.5 g/kg) from 21 to 49 days. Allameh and Toghyani (2019) observed that dietary L-Val supplementation could alleviate the adverse effect of feeding low CP diets to broilers due to the helpful effect on protein accumulation, and the intestinal morphology of broilers. A 3% reduction in dietary CP decreased body weight and feed conversion during 21–42 day of age in a study of Ospina-Rojas et al. (2014), but the supplementation of Val + Ile + Arg + Gly was able to improve these parameters. The study of Van Harn et al. (2019) also demonstrated successfully that CP content of grower and finisher diets can be reduced without adverse effects on growth performance of broilers. However, in the study conducted by Namroud et al. (2008) feeding broiler chickens diets containing a high proportion of crystalline amino acids, but being low in amino acids from natural protein sources, caused retarded growth as soon as dietary CP was < 19%. Dean et al. (2006) affirmed that even with supplementation of EAAs, a very high reduction of dietary protein may lead to a portion of the essential AAs being diverted to synthesis of NEAAs because of the lack of nonspecific nitrogen that would otherwise be used in this process. As seen in Table 1, compared to TAA based T1 diet, the NEAA/EAA ratio decreased in T4 diet from 49.61% to 48.70% and from 49.70% to 48.17% in the grower and finisher period, respectively. But it seems that there is not any NEAA deficiency in this study as 45/55 is a recommended value for the NEAA/EAA ratio (Bedford and Summers 1985).

Although there is no recommendation for Gly and Ser by Aviagen (2014) for Ross 308 broilers, the level of Gly + Ser of T4 diet is 1.73% (Table 1), and higher than 1.25% of NRC (1994) and very close to 1.9%, suggested level by Schutte et al. (1997). Unfortunately, there is a widespread belief that whenever CP concentrations are lowered, performance is negatively affected. But Burnham (2004) speculated that this belief was addressed from researchers who lowered CP concentrations beyond the practical formulation and then did not compensate sufficient amounts of limiting amino acids other than Met and Lys.

In our study, as mentioned above, the NEAA ratio and level of Gly + Ser were not reduced to the level below recommendation and this may be one of possible reasons for similar or better results of low

CP diet compared to high CP T1 diet. However, there are also some more explanations of the results which need to be considered and discussed.

DAA based formulation strategy combined with L-Val, L-Ile and L-Arg supplementation did not only decrease the CP content, but also obtained the ideal AA profile (Baker and Han 1994) recommended for Ross 308 broilers (Aviagen 2014) by optimizing Arg, Thr, Trp, Ile and Leu ratios to Lys, which is usually in excess in common broiler diets (Vieira and Angel 2012). One of the most pronounced benefits of the diets having an ideal protein profile is to reduce the amount of absorbed AA being in relative excess to Lys, thus avoiding excess oxidation, decreasing metabolic burden, and improving AA balance (Vieira and Angel 2012; Chrystal et al. 2020; Selle et al. 2020).

Additions of imbalanced amino acid mixtures to reduced CP diets were investigated by Hill and Olsen (1963). They concluded that the resultant depressions in weight gain stemmed from the deamination of relatively large quantities of amino acids. In addition, Chrystal et al. (2020) showed that the overall amino acid digestibility increased by 14.1% in the jejunum and more modestly by 3.41% in the ileum following a reduction in dietary CP from 206 g/kg to 162 g/kg and might be attributed to the increased inclusions of unbound amino acids and their notional 100% digestibility and proximal sites of absorption.

Feeding lower protein levels significantly increased the FI of T4 birds in the whole period due to an increase in the grower phase. Although the main factor affecting feed consumption in poultry is related to the dietary energy level, broilers can adjust their feed intake to meet the requirement of CP/AA in diets containing adequate levels of energy. These results are in agreement with Aletor et al. (2000), who reported that wider energy and protein ratios in reduced CP diets increased dietary energy intake, which then might have deposited as abdominal fat after meeting the energy requirement. In our study the decreasing CP level also tended to increase the abdominal fat deposition in T3 and T4 birds (Table 4), although such increases were not found significantly different ($P > 0.05$).

The significant improvement of PER in T4 birds compared to T1 also supported the above-mentioned benefits of low CP broiler diets having an ideal AA profile. Cheng et al. (1997) found that PER was linearly improved by decreasing the CP level in poultry diet. Better protein utilization and almost the same performance results of reduced CP diet compared to the control having a regular protein level can also be attributed to the increasing unbound AA level in T4 feed. This is because their digestive dynamics is fundamentally different; unbound amino acids do not undergo digestion and are directly available for absorption in the upper small intestine and appear in the portal circulation more rapidly than protein-bound amino acids (Wu 2009). Notionally, unbound amino acids are totally 'digestible', and it has been concluded that the digestibility and bioavailability of crystalline lysine HCl in poultry is 100% (Lemme et al. 2019). As seen in Table 1, not only the level of unbound L-Val, L-Ile, L-Arg, but also the DL-Met, L-Lys and L-Thr inclusion level were also increased in both the grower and finisher T4 diet compared to other treatments. This may contribute to the better utilization of protein in T4 feeds (Figure 1) which have the best PER ($P < 0.05$), because of higher digestibilities of these six unbound AAs up to 100%. These results are in line with Lemme et al. (2019), who showed that reducing the CP level from 20.4% to 18.7% in broiler diets significantly improved protein utilization.

There was no effect of the dietary treatments on yields of the carcass and its part (breast, leg) or abdominal fat, liver and pancreas percentage of 39-day-old broilers ($P > 0.05$) (Table 4). Dozier et al. (2012) demonstrated similar results, when feeding broilers diets supplemented with Val in the finisher period. However, Sterling et al. (2002) indicated that very low crude protein diets may increase abdominal fat deposition in broiler and Pekin duck carcasses, respectively. These results may be attributed to the imbalance of other EAAs in low protein diets in their study.

Currently, the main drawback of T4 diet is the availability of L-Arg and L-Ile in the markets. Although they are expensive, about almost three times higher in price compared to DL-Met, increasing cost of protein sources and the concern about environmental pollution will push the broiler industry to use these amino acids much sooner than expected now. The results of the present research are a good example of declining the CP level, and reducing the cost of broiler diets whilst maintaining growth performance, and contributing to a more sustainable environment by preventing excessive use of protein sources as well.

CONCLUSION

The results of the present study showed that the formulation of broiler diet based on digestible AA's by using 4th and/or 5th EAA available as feed grade amino acid enables to reduce the CP level and soybean meal inclusion without compromising growth performance and feed utilization of modern broilers, additionally also resulting in a more sustainable environment.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Aletor VA, Hamid II, Niess E, Pfeffer E. Low protein amino acids supplemented diets in broiler chickens: Effects on performance, carcass characteristics, whole body composition and efficiencies of nutrient utilization. J Sci Food Agric. 2000 Apr;80(5):547-54.

Allameh S, Toghyani M. Effect of dietary valine supplementation to low protein diets on performance, intestinal morphology and immune responses in broiler chickens. Livest Sci. 2019 Nov 1;229:137-44.

AOAC – Association of Official Analytic Chemists. Official method of Analysis. 18th ed. Washington DC: Association of Official Analytical Chemists; 2005.

Aviagen T. Ross 308 broiler: Nutrition specifications (all plant-protein based feeds). Huntsville (AL): Aviagen Group; 2014. 10 p.

Baker DH, Han Y. Ideal amino acid profile for broiler chicks during the first three weeks posthatching. Poult Sci. 1994 Sep 1;73(9):1441-7.

Bedford MR, Summers JD. Influence of the ratio of essential to nonessential amino acids on performance and carcase composition of the broiler chick. Br Poult Sci. 1985 Oct 1; 26(4):483-91.

Berres J, Vieira SL, Kidd MT, Taschetto D, Freitas DM, Barros R, Nogueira ET. Supplementing L-valine and L-isoleucine in low protein corn and soybean meal all vegetable diets for broilers. J Appl Poult Res. 2010 Dec 1; 19(4):373-9.

Burnham DJ. Dietary strategies to lower nitrogen load in poultry. Proceedings of the 41st Eastern and Nutrition Conference; 2004; Montreal (QC). [Montreal (QC)]: ENC; 2004. p. 151-70.

Cheng TK, Hamre ML, Coon CN. Responses of broilers to dietary protein levels and amino acid supplementation to low protein diets at various environmental temperatures. J Appl Poult Res. 1997 Mar 1;6(1):18-33.

Chrystal PV, Moss AF, Yin D, Khoddami A, Naranjo VD, Selle PH, Liu SY. Glycine equivalent and threonine inclusions in reduced crude protein, maize based diets impact on growth performance, fat deposition starch protein digestive dynamics and amino acid metabolism in broiler chickens. Anim Feed Sci Technol. 2020 Mar 1;261: 14 p.

Davis RJ. The mean bean: The biological, economic, and social consequences of soybean production within the Brazilian Amazon [honours thesis]. [Denver (CO)]: Regis University; 2009 May. 74 p.

Dean DW, Bidner TD, Southern LL. Glycine supplementation to low protein, amino acid supplemented diets supports optimal performance of broiler chicks. Poult Sci. 2006 Feb 1;85(2):288-96.

Dozier WA 3rd, Tillman PB, Usry J. Interactive effects of digestible valine and isoleucine to lysine ratios provided to male broilers from 4 to 6 weeks of age. J Appl Poult Res. 2012 Dec 1;21(4):838-48.

Fiola N. Meeting the demand: An estimation of potential future greenhouse gas emissions from meat production. Ecol Econ. 2008 Oct 15;67(3):412-9.

Golzar Adabi S, Ceylan N, Ciftci I, Ceylan A. Response of growing chicks to supplementation of low protein diets with leucine, valine and glycine glutamic acid. S Afr J Anim Sci. 2019 Jun;49(6):1047-62.

Hill DC, Olsen EM. Effect of the addition of imbalanced amino acid mixtures to a low protein diet, on weight gains and plasma amino acids of chicks. J Nutr. 1963 Mar 1; 79(3):296-302.

Holsheimer JP, Janssen WMMA. Limiting amino acids in low protein maize-soyabean meal diets fed to broiler chicks from 3 to 7 weeks of age. Br Poult Sci. 1991 Mar 1; 32(1):151-8.

Lemme A, Hiller P, Klahsen M, Taube V, Stegemann J, Simon I. Reduction of dietary protein in broiler diets not only reduces N emissions but is also accompanied by several further benefits. J Appl Poult Res. 2019 Dec 1; 28(4):867-80.

Liames CR, Fontaine J. Determination of amino acids in feeds: Collaborative study. J AOAC Int. 1994 Nov 1; 77(6):1362-402.

Lu P, Wang J, Wu S, Gao J, Dong Y, Zhang H, Qi G. Standardized ileal digestible amino acid and metabolizable energy content of wheat from different origins and the effect of exogenous xylanase on their determination in broilers. Poult Sci. 2020 Feb 1;99(2):992-1000.

Namroud NF, Shivazad M, Zaghari M. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult Sci. 2008 Nov 1;87 (11):2250-8.

NRC – National Research Council. Nutrient requirements of poultry. 9th rev. ed. Washington, D.C.: The National Academy Press; 1994. 176 p.

Ospina-Rojas IC, Murakami AE, Duarte CRA, Eyng C, Oliveira AL, Janeiro V. Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. Br Poult Sci. 2014 Nov 2;55(6):766-73.

Pesti GM. Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. J Appl Poult Res. 2009 Oct 1;18(3):477-86.

Schutte JB, Smink W, Pack M. Requirement of young broiler chicks for glycine + serine. Arch Geflugelkd. 1997 Jan; 61(1):43-7.

Selle PH, de Paula Dorigam JC, Lemme A, Chrystal PV, Liu SY. Synthetic and crystalline amino acids: Alternatives to soybean meal in chicken meat production. Animals. 2020 Apr;10(4): 20 p.

Sterling KG, Costa EF, Henry MH, Pesti GM, Bakalli RI. Responses of broiler chickens to cottonseed- and soybean meal-based diets at several protein levels. Poult Sci. 2002 Feb 1;81(2):217-26.

Van Harn J, Dijkslag M, Van Krimpen M. Effect of low protein diets supplemented with free amino acids on growth performance, slaughter yield, litter quality, and footpad lesions of male broilers. Poult Sci. 2019 Oct 1;98(10):4868-77.

Vieira SL, Angel CR. Optimizing broiler performance using different amino acid density diets: What are the limits? J Appl Poult Res. 2012 Mar;21(1):149-55.

Wu G. Amino acids: Metabolism, functions, and nutrition. Amino Acids. 2009 May 1;37(1):1-17.

Wu G. Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. J Anim Sci Biotechnol. 2014 Dec;5(1): 12 p.

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