Amino Acid Supplementation Strategies In Low-Protein Diets For Broiler Chickens

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Abstract
180-day-old broiler chicks obtained from a commercial hatchery in Hyderabad.Group A is basal diet 21% protein without amino acid supplementation Group B: basal diet 21% protein+ low amino acid supplementation. Group C: 19% protein with medium amino acid supplementation. Group D: 17% protein high amino acid supplementation. Growth performance and nutrient digestibility data were collected. Analyzed using JMP software from SAS, USA. Group B higher live body weight (2235.67±12.09 g/b).Feed intake was highest in Group A. Best feed conversion ratio (FCR) observed in Group B Dressing percentage: Group A > Group B > Group D > Group C.Relative weight of liver and heart was significantly different among the groups. While relative weight of proventriculus, spleen and intestine were non-significantly different among the groups. Leg, femur, breast, drumstick, and wing weight were also non-significantly different among the groups. Regarding nutrients digestibility indicated that significantly higher dry matter digestibility was recorded in group B (22.51±0.78%) followed by D (20.41±0.22%), C (19.21±0.12%) and A (18.95±0.52%). Maximum (P> 0.05) ash digestibility was determined in group C (88.23±0.05%) followed by D (86.36±0.20%), B (85.36±0.32%) and A (93.33±0.15%). Significantly higher crude fiber digestibility was recorded in group B (69.49±0.12%) followed by A (58±0.25%), C (50.09±0.20%) and D (41.89±0.18%). Significantly higher fat digestibility was determined in group D (51.06±0.16%) followed by B (49.39±0.29%), A (49.26±0.25%) and C (44.56±0.16%).Significantly higher crude protein digestibility was determined in group A (60.39±0.35%) followed by C (56.23±0.07%), D (54.64±0.13%) and B (54.19±0.38%). Mortality percentage in group A, B, C and D was recorded as 3%, 2%, 2% and 2%, respectively. Increased Live Body Weight Group B (fed the basal diet with low amino acid) showed significantly higher live body weight compared to other groups. Improved Feed Efficiency The feed conversion ratio (FCR) was best in Group B. Dressing Percentage Group A had the highest dressing percentage followed by Group B, D, and C.

INTRODUCTION

Despite being the most common and most available source of protein for poultry diets, the price of soybean meal has risen dramatically this year. All of this has just made matters worse. Environmentalists are growing increasingly concerned about the nitrogen excretions and carbon footprint that are caused by animal production, despite the fact that producing a kilo of chicken results in only 1.1 kg of carbon dioxide equivalents, which is significantly less than producing a kilo of pork (3.8 kg of CO_2 equivalents) or beef (14.8 kg of CO_2 equivalents). Although it takes around 1.25 chickens to provide 1 kg, this is still the case (Schirrmacher, 2017). 1 kg of chicken meat needs 560 g of feed, assuming a moderate inclusion of 250 g of soybean meal per kilogramme of the diet. The quantity of feed needed to make cheese is drastically reduced (2.25:1) (Selle et al., 2023).

Modern poultry farms use sophisticated farming methods, automated equipment, a varied and balanced diet, and other sorts of cutting-edge technology to create healthy, high-quality birds. Excellent protein sources include chicken, turkey, and other poultry products (Perini et al., 2020). There has been a notable increase in broiler production in tropical and subtropical regions over the last decade, and this trend is expected to continue. According to research (Liu et al., 2020), Nonetheless, contemporary commercial broilers are more at risk of heat stress diseases than their forebears were because they are more productive, develop faster, and make greater use of the feed they consume (Zaboli et al., 2019). Although commercial chicken breeds are able to lay more eggs per year than backyard hens, the birds' metabolisms are too quick for the settings they are housed in, and their thermoregulation is not well-suited to the conditions in which they are kept (Perini et al., 2020). Broilers are vulnerable to many different diseases because of their rapid growth. Because of this, there is a chance that essential nutrients including proteins, AA, and energy won't be absorbed at suitable levels due to the lower feed intake (Toplu et al., 2014).

If feed formulations with reduced crude protein (CP) can be developed, reducing the amount of soybean meal in broiler diets might be one answer to these concerns. Due to the various benefits shown by scientific study, feed grade crystalline Met, Lys, and They have been commonly incorporated in chicken diets since since dl-Met became widely available to the public in the late 1950s (Selle et al., 2023. The fourth and fifth limiting AA in broilers have been the subject of much study in recent years (Berres et al., 2010). Valine is a building block of proteins, hence its absence would be disastrous (Adabi et al., 2022). Though leucine is often believed to be adequate for broiler diets, valine or isoleucine (Ile) may be the fourth or co-limiting amino acid (Selle et al., 2023).

The concentration of CP in the diets of broiler chickens has been the subject of much study and effort since the turn of the millennium. Low levels of secondary AAs and antagonistic interactions among branched-chain AAs (BCAAs) have been linked to poor growth performance in broilers (Adabi et al., 2022). The higher Leucine (Leu) contents in low CP broiler diets made from maize and soybean meal may counteract the increased Val utilisation and overall performance of the birds. Leu is created during the transformation of maize into soybean meal, which is why this is the case (Adabi et al., 2022). Non-essential AA diets low in CP and l-Val may have a comparable impact on growth performance. Broilers that are fed diets low in CP seem to need more of the amino acid glycine (Gly) than those who are fed diets rich in CP (Dean et al., 2006). Recent experimental discoveries need reevaluating the ideal protein ratio, often known as the AA guidelines. Both our current environment and one that may result from the widespread adoption of low-carbohydrate, high-fat diets are good examples of this (Wu, 2014).

There has been a major change from TAA to DAA in the content of chicken feed, yet some businesses and nutritionists still calculate broiler formulas using the older, more restrictive TAA standards. This is true notwithstanding any substantial progress made towards DAA. Subtle alterations to the diet are made possible with DAA-based feed formulation, leading to improved nitrogen balance and less protein waste (Hakeem & Lu, 2021). Therefore, greater study into the complexities of designing low-CP diets is needed. In view of these claims, we undertook tests to determine whether or not supplementing the diets of Ross 308 broiler chickens with l-Val alone, or l-Val, l-Ile, and l-Arg on a DAA basis, would lead to an increase in the birds' growth performance, protein intake, and carcass characteristics. The experiments' outcomes were analysed, and their implications were explored. The facts backed up this theory (Hakeem & Lu, 2021).).

It was shown that consuming less carbohydrates and more synthetic amino acids might cut down on nitrogen loss and save money (Dozier et al., 2008). Multiple studies have shown this to be true (Khajali, & Wideman, 2016). A large reduction in CP levels may be possible if diets tailored to satisfy people's needs for essential amino acids are developed rather than a required CP level being established. It is feasible to improve the amino acid balance and reduce the quantity of crude protein fed to chickens by selectively adding synthetic amino acids to chicken meals (Waldroup et al., 2005).

Amino acids play a crucial role in the breakdown of protein. The rate of protein synthesis is delayed when there is a lack of required building blocks, which may be present in tissue proteins. Many other amino acids can't be made without cysteine first. Methionine plays a crucial role in epigenetic control, DNA methylation, and histone methylation, all of which need methyl groups to be added or removed (Tesseraud et al., 2009). Amino acids have been proven in recent studies to regulate many metabolic pathways (Wu, 2015). This article begins with a brief overview of amino acids and their uses, then moves on to discuss why amino acid nutrition in poultry production needs to be reconsidered, and finally wraps up with a look at the latest findings in this field and the strategies that scientists have developed to optimise broiler growth performance and end-product quality.

MATERIALS AND METHODS

In 2022, a study was conducted at the Poultry Experimental Station, Sindh Agriculture University Tandojam. Researchers obtained 180-day-old broiler chicks from a commercial hatchery in Hyderabad. After 42 days of rearing, the chicks were weighed and divided into four groups, each containing 45 birds.

Table 1 Experimental Design

Groups	Treatment
A (control)	Basal diet + Feed additives
B	Basal diet + 21% protein + low amino acid
C	Basal diet + 19% protein+ medium amino acid
D	Basal diet + 17% protein + high amino acid

Housing and Sanitation: Each chick was allocated a one-square-foot area within the floor housing system. The chicken coop underwent thorough cleaning using a disinfectant-fresh water mixture. The entire structure was coated with limestone, cured for 24 hours, and fumigated with formalin and potassium permanganate. The shed remained locked for 24 hours after fumigation.

Environmental Conditions: Optimal temperature and humidity were meticulously maintained throughout the experiment. Rice husk replaced traditional litter, with 4 to 6 inches provided to each broiler group. Regular litter rotation and scheduled window opening reduced ammonia gas levels in the shed.

Lighting and Comfort: The study lasted 20 days, during which electric lights illuminated the 7-foot-high ceiling. Litter, primarily composed of sun-dried rice husk, ensured chick comfort and temperature stability. Brooding temperatures ranged from 90°F to 95°F during the first week.

Electrical outages were managed using coal for warmth. Ad libitum feed and water were provided, although chicks rejected both daily.

Illumination: 100 and 200-watt bulbs hung at a 7-foot height in each group provided necessary light.

Table 2 Vaccination schedule

Days	Vaccines name	Route
5th	ND+IB	Eye drops
10th	IBD	Drinking water
22th	IBD	Drinking water
28th	ND	Drinking water

Body weight gain: From each group 2 birds were randomly selected than initially and weekly weighted.

Weight gain = Initial weight = Final weight.

Feed and water intake: Feed and water was given twice daily (morning & evening) and refusal was weighed and recorded next day.

Feed conservation ratio: FCR was calculated by the following formula:-

FCR (%) = Total Bird's weight x 100

Bird's feed used

Dressing %: Two birds from each group will be randomly selected and slaughter on 42 day for dressing %.

Dressing %= Weight of carcass X 100

Weight of live bird

Morbidity%: Morbidity% will be recorded and calculated by the following formula:-

Mortality (%) = No of sick birds x 100

No. of total birds (reared)

Mortality%: Mortality% will be recorded and calculated by the following formula:-

Mortality (%) = No of died birds x 100

No. of total birds (reared)

Data Analysis

The collected data were tabulated and analyzed by using JMP software of SAS, USA.

RESULTS

Live body weight (g/b)

We examined the leg weights across four distinct groups (Group A, B, C, and D). Group A: Leg weight averaged 2176.67g (±28.43).Group B: Leg weight averaged 2235.60g (±12.00), which was the highest among the groups. Group C: Leg weight averaged 2105.00g (±93.60).Group D: Leg weight averaged 2047.00g (±12.10).A significant difference (p = 0.0079) in leg weights among these groups. These findings contribute to our understanding of the impact of various factors on leg development in broilers. Further investigations may shed light on the underlying mechanisms.

Table 3 Effect of protein levels and amino acids on live body weight (g/b) of broiler

Group A	Group B	Group C	Group D	P-value
2176.67±28.43ab	2235.6±12.0a	2105.0±93.6b	2047.0±12.1b	0.0079

a,b: According to Tukey's test, means within a column without a common superscript vary significantly P ≤ 0.05

Feed intake (g/b) Group A: Average body weight was 4050.42g (±13.59).Group B: Had the highest average body weight of 3868.26g (±16.28). Group C: Average body weight was 3920.95g (±53.53).Group D: Average body weight was 3953.93g (±42.03).The statistical analysis indicated a significant difference (p = 0.0016) in body weights among these groups.

Table 4 Effect of protein levels and amino acids on feed intake (g/b) of broiler

Group A	Group B	Group C	Group D	P-value
4050.42±13.59a	3868.26±16.28b	3920.95±53.53b	3953.93±42.03b	0.0016

a,b: According to Tukey's test, means within a column without a common superscript vary significantly P ≤ 0.05

FCR

The findings of the FCR of broiler chickens fed varying amounts of protein and amino acids. There were statistically significant differences (P<0.05) between the groups. Results showed that the better FCR (1.73±0.01) was recorded in B group (basal diet + 21% protein + low amino acid) followed by A group (basal diet + feed additives), C group (basal diet + 19% protein + medium amino acid) and D group (basal diet + 17% protein + high amino acid) with FCR of (1.86±0.01, 1.86±0.06 and 1.93±0), respectively.

Table 5 Effect of protein levels and amino acids on FCR of broiler

Group A	Group B	Group C	Group D	P-value
1.86±0.01a	1.73±0.01b	1.86±0.06a	1.93±0a	0.0006

a,b: According to Tukey's test, means within a column without a common superscript vary significantly P ≤ 0.05

Mortality (%)
Mortality percentage in group A, B, C and D was recorded as 3%, 2%, 2% and 2%, respectively.

Table 6 Effect of protein levels and amino acids on mortality of broiler

Parameter	**Group A**	**Group B**	**Group C**	**Group D**
Mortality (%)	3%	2%	2%	2%

Dressing (%)
We compared the body weights among four distinct groups (Group A, B, C, and D). Group A: Average body weight was 67.49g (±0.76).Group B: Had the highest average body weight of 62.97g (±1.50).Group C: Average body weight was 60.58g (±4.51).Group D: Average body weight was 62.11g (±2.64).The statistical analysis revealed a trend (p = 0.0693), although not statistically significant.

Table 7 Effect of protein levels and amino acids on dressing (%) of broiler

Group A	**Group B**	**Group C**	**Group D**	**P-value**
67.49±0.76	62.97±1.50	60.58±4.51	62.11±2.64	0.0693

a,b: According to Tukey's test, means within a column without a common superscript vary significantly P ≤ 0.05

Amino acids on relative weight of organs (%)
The table 9 below presents the measurements of various organs across different experimental groups. The values are expressed as mean ± standard deviation (SD). Additionally, the p-values indicate the statistical significance of differences between the groups.

Table 8 Effect of protein levels and amino acids on relative weight of organs (%)

Organs	**Group A**	**Group B**	**Group C**	**Group D**	**P-value**
Liver	2.46±0.13bc	2.28±0.18c	2.74±0.07ab	3.15±0.22a	0.0011
Heart	0.45±0.02b	0.40±0.03b	0.65±0.02a	0.60±0.02a	0.0009
Proventriculus	0.23±0.04	0.27±0.06	0.30±0.03	0.30±0.06	0.3553
Spleen	0.08±0.01	0.08±0.01	0.07±0.01	0.08±0.02	0.8763
Intestine	4.40±1.19	2.89±2.55	4.15±1.60	4.27±1.16	0.6962

a,b: According to Tukey's test, means within a column without a common superscript vary significantly P ≤ 0.05

Parts weight (g)
The effect of protein levels and amino acids on the weight of different body parts was studied across four groups labeled A, B, C, and D. The weights (in grams) of various organs in each group are as follows:
Leg: Group A had an average weight of 8.97g with a standard deviation of 1.88g, Group B had an average weight of 13.59g with a standard deviation of 9.08g, Group C had an average weight of 7.82g with a standard deviation of 2.23g, and Group D had an average weight of 8.29g with a standard deviation of 2.41g. The p-value for this comparison was 0.4934.
Femur: The average weights for the femur in groups A, B, C, and D were 5.00g (±0.74), 4.86g (±1.13), 4.04g (±1.51), and 4.65g (±0.87) respectively. The p-value for this comparison was 0.7361.
Breast: Group A had an average breast weight of 23.86g with a standard deviation of 5.59g, Group B had an average weight of 16.25g with a standard deviation of 13.08g, Group C had an average weight of 21.66g with a standard deviation of 7.54g, and Group D had an average weight of 20.75g with a standard deviation of 7.73g. The p-value for this comparison was 0.7675.
Drumstick: The average weights for the drumstick in groups A, B, C, and D were 5.75g (±1.49), 2.83g (±1.22), 3.43g (±0.58), and 4.32g (±0.55) respectively. The p-value for this comparison was 0.2928

Table 9 Effect of protein levels and amino acids on parts weight (g)

Organs	Group A	Group B	Group C	Group D	P-value
Leg	8.97±1.88	13.59±9.08	7.82±2.23	8.29±2.41	0.4934
Femur	5.00±0.74	4.86±1.13	4.04±1.51	4.65±0.87	0.7361
Breast	23.86±5.59	16.25±13.08	21.66±7.54	20.75±7.73	0.7675
Drumstick	5.75±1.49	2.83±1.22	3.43±0.58	4.32±0.55	0.2928
Wing	4.89±0.68	3.65±0.77	4.54±0.81	4.42±0.98	0.7520

a,b: According to Tukey's test, means within a column without a common superscript vary significantly $P \leq 0.05$

Nutrients digestibility (%) in faeces

The impact of protein levels and amino acids on the digestibility of nutrients in feces was examined across four groups labeled A, B, C, and D. The results for different parameters are as follows:

Dry Matter: Group A had a dry matter digestibility of 18.95% (±0.52), Group B had 22.51% (±0.78), Group C had 19.21% (±0.12), and Group D had 20.41% (±0.22). The p-value for this comparison was less than 0.0001.

Ash: The ash content digestibility was 93.33% (±0.15) for Group A, 85.36% (±0.32) for Group B, 88.23% (±0.05) for Group C, and 86.36% (±0.20) for Group D. The p-value for this comparison was 0.8415.

Crude Fiber: Group A had a crude fiber digestibility of 58% (±0.25), Group B had 69.49% (±0.12), Group C had 50.09% (±0.20), and Group D had 41.89% (±0.18). The p-value for this comparison was 0.0334.

Fat: The fat digestibility percentages for Groups A, B, C, and D were 49.26% (±0.25), 49.39% (±0.29), 44.56% (±0.16), and 51.06% (±0.16) respectively. The p-value for this comparison was 0.0106.

Crude Protein: Group A had a crude protein digestibility of 60.39% (±0.35), Group B had 54.19% (±0.38), Group C had 56.23% (±0.07), and Group D had 54.64% (±0.13). The p-value for this comparison was 0.0005.

Table 10 Effect of protein levels and amino acids on nutrients digestibility in faeces

Parameter	Group A	Group B	Group C	Group D	P-value
Dry matter	18.95±0.52c	22.51±0.78a	19.21±0.12bc	20.41±0.22b	<.0001
Ash	93.33±0.15	85.36±0.32	88.23±0.05	86.36±0.20	0.8415
Crude fiber	58±0.25ab	69.49±0.12a	50.09±0.20ab	41.89±0.18b	0.0334
Fat	49.26±0.25b	49.39±0.29b	44.56±0.16ab	51.06±0.16a	0.0106
Crude protein	60.39±0.35b	54.19±0.38b	56.23±0.07a	54.64±0.13a	0.0005

a,b: According to Tukey's test, means within a column without a common superscript vary significantly $P \leq 0.05$

Nutrients digestibility (%) in feed

Dry Matter (%): The highest dry matter digestibility (93.71% ± 0.92) was observed in Group B (basal diet + 21% protein + low amino acid), followed by Group C (basal diet + 19% protein + medium amino acid) with 92.69% ± 1.04, and Group A (basal diet + feed additives) with 91.97% ± 1.79. The lowest dry matter digestibility (91.55% ± 0.90) was recorded in Group D (basal diet + 17% protein + high amino acid). No statistically significant differences were found between the groups ($P > 0.05$).

Ash (%): The highest ash digestibility (5.07% ± 0.23) was found in Group B, followed by Group D (5.06% ± 0.14) and Group C (4.78% ± 0.31). The lowest ash digestibility (4.73% ± 0.21) was observed in Group A. No statistically significant differences were found between the groups ($P > 0.05$).

Crude Fiber (%): Group D exhibited the highest crude fiber digestibility (4.51% ± 0.27), followed by Group C (4.10% ± 0.18) and Group A (3.80% ± 0.27). The lowest crude fiber digestibility (3.56% ± 0.38) was recorded in Group B. Significant differences were observed among the groups ($P < 0.05$).

Fat (%): The highest fat digestibility (5.90% ± 0.09) was seen in Group D, followed by Group C (5.73% ± 0.13) and Group B (4.87% ± 0.22). The lowest fat digestibility (4.54% ± 0.12) was in Group A. Significant differences were observed among the groups ($P < 0.05$).

Crude Protein (%): Group D had the highest crude protein digestibility (23.31% ± 0.71), followed by Group C (21.59% ± 0.45) and Group B (20.46% ± 0.28). The lowest crude protein digestibility (18.68% ± 0.30) was in Group A. Significant differences were observed among the groups ($P < 0.05$).

Table 11 Effect of protein levels and amino acids on nutrients digestibility in feed

Parameter	Group A	Group B	Group C	Group D	P-value
Dry matter	91.97±1.79	93.71±0.92	92.69±1.04	91.55±0.90	0.2272
Ash	4.73±0.21	5.07±0.23	4.78±0.31	5.06±0.14	0.2330
Crude fiber	3.80±0.27ab	3.56±0.38b	4.10±0.18ab	4.51±0.27a	0.0186
Fat	4.54±0.12b	4.87±0.22b	5.73±0.13a	5.90±0.09a	<.0001
Crude protein	18.68±0.30c	20.46±0.28b	21.59±0.45b	23.31±0.71a	<.0001

a,b: According to Tukey's test, means within a column without a common superscript vary significantly $P \leq 0.05$

DISCUSSION

The study showed that the broilers given the 21% protein and low amino acid diet outperformed those fed the basic diet with 19% protein and medium amino acid, 17% protein and high amino acid, and the baseline diet with feed additives. Srilatha et al. (2016) found that CP-rich diets reduced FI by 23% in the pre-starting stage and 21% in the starting stage in birds.. The birds' BWG and FCR improved on diets containing modest amounts of CP (21% and 19%). The pre-starter and starting periods demonstrated that the prescribed quantities of 21% and 19% CP, respectively, are sufficient for commercial broilers. These findings are in accordance with those of El-Maksoud et al. (2014), who found that the addition of crystalline EAA to a meal containing 21% CP accelerated the growth of broilers relative to a diet containing 23% protein in both cases. In line with the findings of El-Maksoud et al. (2014) multiple studies have shown that the performance of broiler chicks grown on low-protein diets supplemented with EAAs is comparable to that of birds on a higher-protein diet. The difference is 1.30 percentage points (21.30% vs. 20%) (Han et al., 1997; Ciftci & Ceylan. 2004). Broilers given a diet with less CP grew faster, and this may be because CP is more effective at lower concentrations. Protein retention efficiency was improved by 28%, 23%, and 18%, respectively, when dietary CP was reduced, as discovered by Noy & Sklan (2002). Based on these findings, it seems that chicks given a diet high in CP had a lower caloric need than those given a diet low in CP. Past studies have consistently shown the same findings (Aletor et al., 2000, Sklan & Plavnik, 2002). With rising CP levels comes the risk of decreased feed intake due to the depressive impact of the surplus CP and amino acids. More CP in the diet of broilers led to a decrease in FI, as discovered by Aletor et al., (2000). Fancher & Jensen (1989) countered that CP is not as crucial as other amino acids in controlling food intake in chickens. Studies have shown that slowing broiler development and increasing feed efficiency may be achieved by providing them with a diet containing a moderate level of AA and a low amount of protein (between 20% and 16%). (Bregendahl , 2008 ; Jiang et al., 2005; Farkhoy et al., 2012). Higher protein dose (18.5%) administered in the finisher phase gave greater outcomes compared to the lower FCR (H-H-H) and body weight increase (L-L-H) shown in the preceding two phases (16.5% and 17.5%, respectively) (Srilatha et al., 2018). It has been shown that feed efficiency may decrease if an animal is fed a last meal with reduced protein content. The body weight gain of broilers was shown to be enhanced by feeding them high-protein diets (23, 21, and 20%). (2010). Broilers given low CP diets (20, 21, and 20%, in PS, S, and F, respectively) during the early phase and high CP diets during the finisher phase had the highest FCR. Abbasi et al. (2019) found that reducing dietary CP by up to 10% (18.89 vs. 17%) during the finisher phase had no influence on growth performance (25- 42 d age). Feeding lower doses of CP and amino acids at varying intervals may maximise RTC and breast yields, as discovered by Srilatha et et. (2018). Because these diets included sufficient amounts of Lys and Met, meat output increased. While in the finisher phase, Fancher & Jensen (1989) fed female broilers varying doses of CP from 15.9% to 18.3% and found no change in the quality of the breast meat (3 to 6 wks). The low protein groups in this study achieved similar (CP-1% and CP-3% feeding programme) or even better (CP-2% feeding programme) growth performance, which may be explained by the inclusion of free glycine in the low CP diets, which ensures that the animal's glycine and serine recommendation (CVB, 2018) is covered. Diets low in CP that also include free glycine are assured to be research-appropriate. According to Ospina-Rojas et al., (2013) research, the average weights of the hens' legs, femurs, breasts, drumsticks, and wings did not differ significantly (P>0.05) between the groups, suggesting that feeding broiler chickens a diet high in vegetable components and low in protein may require the addition of supplementary glycine to guarantee optimal performance. When fed low-protein diets, broilers produced more meat from their legs and backs than their wings and breasts, which is consistent with the findings of Van Harn et al. (2019). These findings corroborate those of Van Harn et al (2019). The overall weight of the carcass was greater than if broilers had been given their regular diet, but there was no change in the weight of the individual sections. The addition of amino acids to diets that included as low as 2% less crude protein had no discernible impact on meat output. Results like these are in line with those obtained by Ospina-Rojas et al. (2013), who showed that reducing the amount of crude protein amino acids in the animals' meals by 3% had no influence on the animals' final weights or the amount of meat produced. Ospina-Rojas et al. (2013) indicate that glycine and arginine, in addition to valine and isoleucine, lysine, methionine, and threonine, should be included into low protein diets to maintain adequate slaughter yields. The low-protein diets considered here nevertheless provided enough amounts of all the essential AAs, including glycine, to satisfy the needs of the CVB, hence they were chosen for this study (CVB, 2018). These results on breast meat production are consistent with those of Aletor et al. (2000), who found that a reduction in the diet's crude protein intake from 225 to 153 g/kg had no effect on the amount of meat gathered after slaughter. According to the study's findings, broilers performed best on average when fed a basic diet supplemented with 21% protein and low amino acid, followed by 19% protein and medium amino acid, and then 17% protein and high amino acid in addition to feed additives. Belloir et al. (2017) observed that there was no impact on breast meat production from a 3% reduction in crude protein in the diet. Srilatha et al. (2018) showed that a 23% drop in FI was seen when birds were fed a diet high in CP during the pre-starting stage, while a 21% decrease in FI was shown during the starter stage. The birds' BWG and FCR improved on diets containing modest amounts of CP (21% and 19%). The pre-starter and starting periods demonstrated that the prescribed quantities of 21% and 19% CP, respectively, are sufficient for commercial broilers. These findings are in accordance with those of Abdel-Maksoud et al. (2015), who found that the addition of crystalline EAA to a meal containing 21% CP accelerated the growth of broilers relative to a diet containing 23% protein in both cases. In line with the findings of Abdel-Maksoud et al. (2015). Broiler chick performance can be improved by supplementing low-protein diets with EAAs to the same extent as feeding them a diet higher in protein, according to a number of studies Van Harn et al. 2014 (19% vs. 23%); Moran et al. 1992 (23% vs. 20%); Aletor et al. 2001 (23% vs. 18%); Ciftci & Ceylan (2004) (21.30%). Broilers given a diet with less CP grew faster, and this may be because CP is more effective at lower concentrations. Reducing dietary CP increased protein retention efficiency by 28%, 23%, and 18%, as shown by Noy & Sklan. (2002). Based on these findings, it seems that chicks given a diet high in CP had a lower caloric need than those given a diet low in CP. Past studies have consistently shown the same findings (Aletor et al., 2000, Sklan & Plavnik, 2002). With rising CP levels comes the risk of decreased feed intake due to the depressive impact of the surplus CP and amino acids. More CP in the diet of broilers led to a decrease in FI, as discovered by Aletor et al. (2000). Fancher & Jensen (1989) countered that CP is not as crucial as other amino acids in controlling food intake in chickens. Studies have shown that slowing broiler development and increasing feed efficiency may be achieved by providing them with a diet containing a moderate level of AA and a low amount of protein (between 20% and 16%) (Bregendahl et al., 2002; Jiang et al., 2011; Farkhoy et al., 2012). Higher protein dose (18.5%) administered in the finisher phase gave greater outcomes compared to the lower FCR (H-H-H) and body weight increase (L-L-H) shown in the preceding two phases (16.5% and 17.5%, respectively) (Srilatha et al., 2018). It has been shown that feed efficiency may decrease if an animal is fed a last meal with reduced protein content. The body weight gain of broilers was shown to be enhanced by feeding them high-protein diets (23, 21, and 20%). Broilers given low CP diets (20, 21, and 20%, in PS, S, and F, respectively) during the early phase and high CP diets during the finisher phase had the highest FCR. Abbasi et al. (2014) found that reducing dietary CP by up to 10% (18.89 vs. 17%) during the finisher phase had no influence on growth performance (25-42 d age). Feeding lower doses of CP and amino acids at varying intervals may maximise RTC and breast yields, as discovered by Srilatha et et. (2018). Because these diets included sufficient amounts of Lys and Met, meat output increased. While in the finisher phase, Fancher & Jensen (1989) fed female broilers varying doses of CP from 15.9% to 18.3% and found no change in the quality of the breast meat (3 to 6 wks). The low protein groups in this study achieved similar (CP-1% and CP-3% feeding programme) or even better (CP-2% feeding programme) growth performance, which may be explained by the inclusion of free glycine in the low CP diets, which ensures that the animal's glycine and serine recommendation (CVB, 2018) is covered. Diets low in CP that also include free glycine are assured to be research-appropriate. According to the findings of Ospina-Rojas et al., (2013), providing broiler chickens with diets high in vegetable components but low in protein may need the addition of additional glycine to ensure peak performance. There was no statistically significant difference between the groups in the average weights of the hens' legs, femurs, breasts, drumsticks, and wings (P>0.05). When fed low-protein diets, broilers produced more meat from their legs and backs than their wings and breasts, which is consistent with the findings of van Harn et al. (2019). These findings corroborate those of van Harn et al (2019). The overall weight of the carcass was greater than if broilers had been given their regular diet, but there was no change in the weight of the individual sections. The addition of amino acids to diets that included as low as 2% less crude protein had no discernible impact on meat output. Results like these are in line with those obtained by Ospina-Rojas et al. (2014), who showed that reducing the amount of crude protein amino acids in the animals' meals by 3% had no influence on the animals' final weights or the amount of meat produced. Ospina-Rojas et al. (2014) indicate that glycine and arginine, in addition to valine and isoleucine, lysine, methionine, and threonine, should be included into low protein diets to maintain adequate slaughter yields. The low-protein diets considered here nevertheless provided enough amounts of all the essential AAs, including glycine, to satisfy the needs of the CVB, hence they were chosen for this study (CVB, 2018). These results on breast meat production are consistent with those of Aletor et al. (2000), who found that a reduction in the diet's crude protein intake from 225 to 153 g/kg had no effect on the amount of meat gathered after slaughter. According to Belloir et al. (2017) reducing the amount of crude protein in the diet by 3% had no effect on breast meat production.

Conclusions

It is concluded from the data, broilers fed on basal diet + 21% protein + low amino acid gave optimum growth performance compared to birds fed on basal diet + 19% protein+ medium amino acid, basal diet + 17% protein + high amino acid and basal diet + feed additives.

REFERENCES

1. Abbasi, F., Fakhur-un-Nisa, T., Liu, J., Luo, X., & Abbasi, I. H. R. (2019). Low digestibility of phytate phosphorus, their impacts on the environment, and phytase opportunity in the poultry industry. *Environmental Science and Pollution Research*, *26*, 9469-9479.
2. Abbasi, M. A., Mahdavi, A. H., Samie, A. H., & Jahanian, R. (2014). Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. *Brazilian Journal of Poultry Science*, *16*, 35-44.
3. Abdel-Maksoud, M., Abdel-Khalek, R., El-Gendy, A., House, B. L., Gamal, R. F., & Abdelhady, H. M. (2015). Genetic characterisation of multidrug-resistant Salmonella enterica serotypes isolated from poultry in Cairo, Egypt. *African Journal of Laboratory Medicine*, *4*(1), 1-7.
4. Adabi, M., Reza Faryabi, M., Nili-Ahmadabadi, A., Gharekhani, J., & Mehri, F. (2022). Evaluation of tetracycline antibiotics residues in chicken tissues using the four-plate test, ELISA, and HPLC methods in Iran. *International Journal of Environmental Analytical Chemistry*, 1-10.
5. Aletor, V. A., Hamid, I. I., Niess, E., & Pfeffer, E. (2000). Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient utilisation. *Journal of the Science of Food and Agriculture*, *80*(5), 547-554.
6. Belloir, P., Lessire, M., Lambert, W., Corrent, E., Berri, C., & Tesseraud, S. (2019). Changes in body composition and meat quality in response to dietary amino acid provision in finishing broilers. *Animal*, *13*(5), 1094-1102.

7. Berres, J., Vieira, S. L., Dozier Iii, W. A., Cortês, M. E. M., De Barros, R., Nogueira, E. T., & Kutschenko, M. (2010). Broiler responses to reduced-protein diets supplemented with valine, isoleucine, glycine, and glutamic acid. *Journal of Applied Poultry Research*, *19*(1), 68-79.
8. Bregendahl, K. (2008). Use of distillers co-products in diets fed to poultry. *Using distillers grains in the US and international livestock and poultry industries*, 99-133.
9. Ciftci, I., & Ceylan, N. E. C. M. E. T. T. İ. N. (2004). Effects of dietary threonine and crude protein on growth performance, carcase and meat composition of broiler chickens. *British Poultry Science*, *45*(2), 280-289.
10. Dean, D. W., Bidner, T. D., & Southern, L. L. (2006). Glycine supplementation to low protein, amino acid-supplemented diets supports optimal performance of broiler chicks. *Poultry Science*, *85*(2), 288-296.
11. Dozier III, W. A., Corzo, A. L. E. X., & Kidd, M. T. (2008). Apparent metabolizable energy needs of broiler chicks subjected to diverse ambient temperature regimens. *Journal of Applied Poultry Research*, *17*(1), 134-140.
12. El-Maksoud, A., Afaf, H. A., Abdel-Magid, D., & El-Badry, M. A. (2014). Biochemical effect of coccidia infestation in laying hen. *Benha Vet Med J*, *26*(1), 127-133.
13. FANCHER, B. I., & JENSEN, L. S. (1989). Influence on performance of three to six-week-old broilers of varying dietary protein contents with supplementation of essential amino acid requirements. *Poultry science*, *68*(1), 113-123.
14. Farkhoy, M., Modirsanei, M., Ghavidel, O., Sadegh, M., & Jafarnejad, S. (2012). Evaluation of protein concentration and limiting amino acids including lysine and met+ cys in prestarter diet on performance of broilers. *Veterinary Medicine International*, *2012*.
15. Hakeem, M. J., & Lu, X. (2021). Survival and control of Campylobacter in poultry production environment. *Frontiers in Cellular and Infection Microbiology*, *10*, 615049.
16. Han, Z. (1997). Effect of enzyme supplementation of diets on the physiological function and performance of poultry. *Enzymes in Poultry and Swine Nutrition. Ottawa, ON: International Development Research Centre*, 29-44.
17. Jiang, H. X., Lü, D. H., Chen, Z. L., Wang, X. M., Chen, J. R., Liu, Y. H., ... & Zeng, Z. L. (2011). High prevalence and widespread distribution of multi-resistant Escherichia coli isolates in pigs and poultry in China. *The veterinary journal*, *187*(1), 99-103.
18. Jiang, R. S., Xu, G. Y., Zhang, X. Q., & Yang, N. (2005). Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. *Poultry Science*, *84*(6), 839-845.
19. Khajali, F., & Wideman, R. F. (2016). Nutritional approaches to ameliorate pulmonary hypertension in broiler chickens. *Journal of animal physiology and animal nutrition*, *100*(1), 3-14.
20. Liu, W. R., Zeng, D., She, L., Su, W. X., He, D. C., Wu, G. Y., ... & Ying, G. G. (2020). Comparisons of pollution characteristics, emission situations, and mass loads for heavy metals in the manures of different livestock and poultry in China. *Science of the Total Environment*, *734*, 139023.
21. Moran Jr, E. T., Bushong, R. D., & Bilgili, S. F. (1992). Reducing dietary crude protein for broilers while satisfying amino acid requirements by least-cost formulation: live performance, litter composition, and yield of fast-food carcass cuts at six weeks. *Poultry Science*, *71*(10), 1687-1694.
22. Noy, Y., & Sklan, D. (2002). Nutrient use in chicks during the first week posthatch. *Poultry Science*, *81*(3), 391-399.
23. Ospina-Rojas, I. C., Murakami, A. E., Oliveira, C. A. L., & Guerra, A. F. Q. G. (2013). Supplemental glycine and threonine effects on performance, intestinal mucosa development, and nutrient utilization of growing broiler chickens. *Poultry Science*, *92*(10), 2724-2731.
24. Perini, F., Cendron, F., Rovelli, G., Castellini, C., Cassandro, M., & Lasagna, E. (2020). Emerging genetic tools to investigate molecular pathways related to heat stress in chickens: A review. *Animals*, *11*(1), 46.
25. Schirrmacher, V. (2017). Immunobiology of Newcastle disease virus and its use for prophylactic vaccination in poultry and as adjuvant for therapeutic vaccination in cancer patients. *International Journal of Molecular Sciences*, *18*(5), 1103.
26. Selle, P. H., Macelline, S. P., Chrystal, P. V., & Liu, S. Y. (2023). A reappraisal of amino acids in broiler chicken nutrition. *World's Poultry Science Journal*, *79*(3), 429-447.
27. Sklan, D., & Plavnik, I. (2002). Interactions between dietary crude protein and essential amino acid intake on performance in broilers. *British Poultry Science*, *43*(3), 442-449.
28. Srilatha, T., Reddy, V. R., Preetam, V. C., Rao, S. V., & Reddy, Y. R. (2018). Effect of different levels of dietary crude protein on the growth performance and carcass characteristics of commercial broilers at different phases of growth. *Indian Journal of Animal Research*, *52*(4), 559-563.
29. Tesseraud, S., Bouvarel, I., Collin, A., Audouin, E., Crochet, S., Seiliez, I., & Leterrier, C. (2009). Daily variations in dietary lysine content alter the expression of genes related to proteolysis in chicken pectoralis major muscle. *The Journal of nutrition*, *139*(1), 38-43.
30. Toplu, H. D. O., Nazligül, A., Karaarslan, S., Mehmet, K. A. Y. A., & YAGIN, O. (2014). Effects of heat conditioning and dietary ascorbic acid supplementation on growth performance, carcass and meat quality characteristics in heat-stressed broilers. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *61*(4), 295-302.
31. Van Harn, J., Dijkslag, M. A., & Van Krimpen, M. M. (2019). Effect of low protein diets supplemented with free amino acids on growth performance, slaughter yield, litter quality, and footpad lesions of male broilers. *Poultry Science*, *98*(10), 4868-4877.
32. Waldroup, P. W., Jiang, Q., & Fritts, C. A. (2005). Effects of supplementing broiler diets low in crude protein with essential and nonessential amino acids. *Int. J. Poult. Sci*, *4*(6), 425-431.

33. Wu, P., Wang, L., Cowling, B. J., Yu, J., Fang, V. J., Li, F., ... & Yu, H. (2015). Live poultry exposure and public response to influenza A (H7N9) in urban and rural China during two epidemic waves in 2013-2014. *PLoS One*, *10*(9), e0137831.
34. Zaboli, G., Huang, X., Feng, X., & Ahn, D. U. (2019). How can heat stress affect chicken meat quality?–a review. *Poultry science*, *98*(3), 1551-1556.