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ORIGINAL ARTICLE

The impact of different levels of L-methionine (L-Met) on carcass yield traits, serum metabolites, tibial characters, and profitability of broilers fed conventional diet

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ABSTRACT

Objectives: The experiment was undertaken to investigate the performances of broilers with respect to meat yield traits, leg bone quality, blood metabolites, and economic profitability fed conventional diets supplemented with L-methionine (L-Met).

Materials and methods: Day-old broiler chicks (n = 144) of either sex were used to conduct the experiment from d1 to 33 days in a battery cage rearing system. Birds were distributed randomly into four dietary treatments, i.e., D_0 (DL-Met), D_1 (0.20% L-Met), D_2 (0.25% L-Met), and D_3 (0.30% L-Met) in a completely randomized design. Broiler chicks were fed complete starter ration for the first 2 weeks and then test diets were supplied *ad libitum* from d15 to 33 days. All the formulated rations had the same calorie and proteinous values. Similar housing, feeding, and rearing management were provided to the birds for all the experimental period. Data on carcass yield traits, such as dressing %, thigh, breast, back, drumstick, shank, neck, and wing weights, etc, were measured on the last day of the trial. Blood serum profile (total protein, glucose, albumin, uric acid, creatinine, and triglycerides), right tibial bone traits (bone weight, bone width), and mineral concentrations (Ca% and P%) were also assessed on the last day of the experiment. The economic profitability of broilers fed on the L-Met diet was also measured in this study.

Results: The results revealed that except for dressing % ($p < 0.05$) and back weight ($p < 0.01$); all other meat characteristics measured this study were found similar ($p > 0.05$) between treatments. The highest dressing % and back weight were observed in the D_3 group and the lowest being in D_0. Blood serum metabolites did not differ ($p > 0.05$) among treatments. Leg bone traits of broilers were found similar ($p > 0.05$) between treatments. Higher profit ($p < 0.01$) and lower production cost ($p < 0.05$) were observed in the birds fed the D_3 diet than other treatments.

Conclusion: It can be inferred that broilers might show improved dressed yield and profitable broiler production fed on L-Met supplemented diet (D_3).

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Introduction

Methionine (Met) is considered as the main limiting and essential amino acid (AA) of poultry. Indispensable AAs are very much important for normal growth and development of poultry [1]. Diets supplemented with an optimum amount of Met could ameliorate broiler performance, carcass yield characters, well-feathering, and reduction of heat stress of poultry [2–5]. Typically poultry diet is prepared mostly with plant-sourced feed ingredients for their optimal body growth and development, and this diet always lacks essential AA, i.e., methionine and lysine [6,7]. It is spotted that many synthetic AA, namely, methionine, lysine, threonine, and tryptophan are currently supplemented to vegetable protein diets to meet the AA requirements for commercial poultry production. It is common for all the animals that their digestive system can only utilize the L-amino acids for the accretion of protein that exists in both animal and plant sources feed materials. DL-amino acids must be converted to L-amino acid before biologically available in the animal body. The liver and kidney of chicken are the organs where the conversion

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of D-AA to L-AA occurs [8]. In monogastric animals, DL-Met is efficiently altered to L-Met in the liver and used for protein accumulation, including other metabolic activities [9–13].

However, in poultry feed, DL-Met is being used in broiler diet for many days as a synthetic source of Met, although this D-Met is considered as similar to L-Met in action for the growth and development of poultry [13], D-Met cannot be absorbed by the gastrointestinal tissues of readily without converting to L-Met in the liver or kidney. If Met can be used entirely as L-Met, it has more possibility to increase the utilization and assimilation of Met by intestinal cells of the animal. However, many previous investigators reported that the use of L-Met replacing DL-Met can increase the productivity of chickens [8,9,13]. The data on L-Met are very scarce as very few research works have been done so far. L-Met supplements in a conventional diet might be economical or could be used as an alternative to DL-Met for improving production and cutting the cost of the poultry and fish [14–17]. After all, it is academic research, so the scientific knowledge and the research findings in those specific fields of research study would be very useful for the higher studies in Poultry Science, biological Sciences, or other relevant research works of the agricultural sciences. Considering the above, the current study was undertaken to explore the impact of various levels of L-Met (feed grade) on the meat yield traits, blood metabolites, leg bone quality of broilers, and to measure the profitability of broilers fed test diets.

Materials and Methods

Ethical approval

The experiment was conducted at the Poultry Research Shed of Chattogram Veterinary and Animal Sciences University (CVASU), and all the experimental bird's care, handling, and management of this study were approved by the Animal Ethics Committees of CVASU, Bangladesh [Approval No. CVASU/Dir(R&E). EC/2019/94(4)].

Animals

Day-old broiler chicks (n = 144; Cobb 500) were procured from the local renowned breeder hatchery to conduct this experiment from d1 to 33 days. The chicks were weighed on receipt and then randomly assigned into four dietary treatment groups, i.e., D_0 (DL-Met)), D_1 (0.20% L-Met), D_2 (0.25% L-Met) and D_3 (0.30% L-Met), where each treatment was replicated four times with nine birds per replicate in a completely randomized design (CRD). Feeding trial with broiler chickens was undertaken at the Poultry Research Shed of CVASU from April to May 2018.

Diet

A ready-made broiler starter (crumble) diet was procured from the local market and used to feed the birds up to 2 weeks (day 1–14) as an adjustment period. The proximate composition and reporting values of the chemical composition of ready-made starter diet (RRP) were shown in Table 1. After that, finisher or test diets (mash) were prepared manually and provided the birds for the remaining trial period, i.e., from day 15 to 33. After purchasing the macro (maize, wheat, soybean meal, fish meal, palm oil, and limestone) and micro-feed ingredients four different test diets (D_0, D_1, D_2, and D_3) were formulated as per the requirements of National Research Council [18], as given below in Table 2. All the test diets were iso-energetic and iso-proteinous. The control diet (D_0) was formulated with all feedstuffs without L-Met, whereas D_1, D_2, and D_3 test diets were prepared with the supplementation of L-Met at the rate of 0.20%, 0.25%, and 0.30%, respectively. The composition and nutritive values (calculated and analyzed in the lab) of the formulated or test diets (finisher) were shown in Table 2.

Management

Broiler chicks were reared in battery cages for the entire trial period (day 1–33). Cages were divided into 16 pens of equal size furnished with feeder and drinker. Each pen was allotted for nine birds. After collection, chicks were first weighed and then distributed randomly into each pen. All the birds had free access to the diet, along with *ad libitum* fresh, clean drinking water during the entire trial period. The birds were exposed to a continuous lighting program. All the chicks were vaccinated to enhance the immunity against Newcastle (Ranikhet) and Gumboro diseases.

Data and sample collection

All expenditures, such as chick cost, feed ingredients, and others were recorded during the experimental period. Two birds per replicate cage were selected randomly and then blood samples were taken for serum analysis [triglyceride (TG),

Table 1. Nutrient composition of the starter diet.

Nutrient components (%)	Proximate values of RRP feed	Reporting values of RRP feed
ME (kcal/kg)	3,380.00	3,000.00
Moisture	9.52	11.0
DM	90.48	89.0
CP	20.13	22.0
CF	5.15	4.0
EE	7.91	–
Ash	6.13	–
Ca	2.55	–
P	0.46	–
Lys	–	1.3
Met	–	0.55

Table 2. Ingredient and nutrient composition of finisher diet.

Feed Ingredients	Finisher diet % (15–33 days)			
	Do	D_1	D_2	D_3
Corn/maize	50.00	50.00	50.64	50.00
Wheat	12.42	13.20	12.22	11.00
Soybean meal	23.40	23.00	23.46	23.80
Fish meal	5.61	5.20	5.00	5.00
Palm oil	5.18	5.12	5.15	5.70
DCP	0.70	0.70	0.70	0.71
Limestone	1.59	1.67	1.70	1.70
NaCl /Table salt	0.35	0.40	0.30	0.24
Choline chloride	0.04	0.03	0.036	0.034
Vitamin min premix	0.21	0.26	0.35	0.26
L-lysine	0.26	0.25	0.25	0.30
DL-methionine	0.21	0.00	0.00	0.00
L-methionine	0.00	0.20	0.25	0.30
Enzymes	0.036	0.036	0.036	0.036
Toxin Binder	0.25	0.25	0.25	0.25
Sand	0.00	0.00	0.00	0.92
Calculated nutrient components (%)				
ME (kcal/kg)	3165.00	3165.00	3165.00	3165.00
CP	20.00	20.00	20.00	20.00
Ca	1.31	1.31	1.31	1.31
P	0.68	0.68	0.68	0.68
CF	3.14	3.11	3.13	3.11
EE	3.33	3.32	3.32	3.28
Lysine	1.06	1.06	1.06	1.06
Met	0.47	0.47	0.47	0.47
Threonine	0.83	0.83	0.83	0.83
Proximate components (%)				
ME (Kcal/kg)	3511.00	3363.00	3549.00	3462.00
DM	88.50	88.73	88.62	88.85
CP	19.60	19.78	19.25	19.90
CF	4.20	5.33	3.50	4.34
EE	8.29	7.85	8.33	8.54
Ash	5.23	6.16	5.80	6.57
Ca	2.25	3.35	3.18	2.72
P	0.44	0.53	0.50	0.47
NFE	51.18	49.61	51.74	49.50

Control diet (Do) with DL-methionine and no L-methionine, whereas D_1, D_2 and D_3 diets are supplemented with 0.20%, 0.25%, and 0.30% L-methionine, respectively.

glucose, total protein (TP), albumin (Alb), uric acid, and creatinine] at day 33. These birds were then weighed and killed humanely to record the carcass yield traits (breast weight, thigh weight, wing weight, shank weight, drumstick weight, neck weight, and abdominal fat content). Right tibial samples were also collected to assess the leg bone traits (bone weight, length, width, head width, Ca% and P%). Formulated feed samples were also collected before providing the birds to determine the chemical composition of the test diets.

Sample processing and analyses

Feed sample

Samples of about 500 gm from each of the starter and finisher diets were taken and ground by a coffee grinder. After that, the samples were sent to the Poultry Research and Training Center lab for testing dry matter (DM%), moisture %, crude protein (CP%), crude fiber (CF%), ether extract (EE%), ash, calcium (Ca%), and phosphorus (P%) using standard laboratory procedure [19]. Metabolizable energy (ME) was estimated indirectly on the basis of true metabolizable energy (TME) contents of the feed samples, assuming that TME was 8% higher than the ME, as it is reported that TME is 5–10% higher than ME [20].

Evaluation of serum, carcass yield and leg bone parameters

On day 33, two birds per replicate were selected randomly for collecting blood samples for assessing blood serum profiles. Blood samples were collected from the wing veins into sterile vacutainers (4 ml) without anticoagulant. Collected blood samples were sent to the Department of Physiology, Biochemistry, and Pharmacology, CVASU, for measuring blood metabolites. Samples were kept undisturbed in a vertical position (using test tube rack) for 4 h into the refrigerator to allow the separation of serum. Then, the samples were centrifuged at 3,000 rpm for 10 min to separate the serum. Afterward, the serum samples from each tube (accumulated as supernatant) were collected carefully by using a micropipette and taken into the sterile Eppendorf tube (2 ml) followed by stored at −20°C till analysis. Glucose, TP, Alb, TG, creatinine, and uric acid were analyzed using standard kits (Randox Laboratories Ltd., UK) and automatic analyzer (Humalyzer 300, Merck®, Germany, semi-automated Benchtop chemistry photometer) according to the manufacturers' instructions.

After collecting blood samples birds were then weighed and slaughtered humanely to collect different meat parts on the same day. After slaughtering, the birds were bled properly followed by skinning. Afterward, different meat yield parameters, such as carcass weight, dressed weight, weights of different meat cuts (neck, thigh, wings, breast, back, drumstick, shank and abdominal fat) were recorded.

Right tibia bone samples were also collected on the last day of the trial period to assess the leg bone quality of broiler chicken. Collected bones were processed by boiling 10 min in deionized water to facilitate the removal of the attached soft tissues and defatted. After that, bone length and head width were taken by using digital calipers (Insize, Japan) and the weight was recorded by digital balance. After that, the bone samples were assessed for bone mineral concentration, particularly Ca and P. At first, the samples were dried properly and then ground and later put the sample on a Muffle Furnace at a temperature of 600°C for 4 h to make ash. After taking bone ash weight, the samples later analyzed for Ca and P by atomic absorption and spectrophotometry.

Statistical analyses

Minitab statistical software was used to analyze all collected data of this study (Minitab Version 16, 2000). The data were subjected to one-way analysis of variance for CRD and tested for significance between the dietary treatment means by using Duncan's Multiple Range Test (DMRT), and statistical significance was considered at $p \leq 0.05$.

Results

Meat yield parameters of broiler chickens

Results of meat yield parameters shown in Table 3 demonstrate that only the dressing percentage ($p < 0.05$) and back weight ($p < 0.01$) were significantly affected by the dietary treatments. The highest dressing (%) and back weight were found in the broilers fed the D_3 diet and the lowest dressing (%) and back weight were on the D_0 diet. Broilers fed on L-meth supplemented diets accumulated similar fat content in their carcasses. Other meat characteristics, such as the weights of thigh, breast, drumstick, shank, neck, and wing, were not affected ($p > 0.05$) by dietary treatments.

Serum metabolites of broiler chickens fed L-Met diets

The results of blood metabolites (TP, glucose, Alb, uric acid, creatinine, and TG, etc.) of broiler chickens are shown in Table 4. The data indicate that the diets of different treatments have similar effects on serum metabolites ($p > 0.05$) of birds.

Leg bone traits and mineral concentration (Ca and P) of broiler chickens

The bone characteristics of broilers did not differ ($p > 0.05$) between treatments (Table 5). However, birds on the D_3 diet had the highest bone length (85.71 mm), while the birds of the D_0 diet group being the lowest bone length (79.91 mm), but the differences were tended to be significant ($p < 0.091$) between treatment. The bone mineral (Ca% and P%) contents of broiler were found to be unaffected ($p > 0.05$) by dietary treatments.

Table 3. Meat yield traits (gm/bird) of broilers fed L-Met diets.

	Treatments				Pooled SEM	*p*-values
	D_0	D_1	D_2	D_3		
Dressing (%)	58.00^b	63.00^a	64.02^a	66.03^a	0.734	**0.05**
Back weight	134.24^b	190.00^a	196.00^a	207.00^a	6.008	**0.01**
Thigh weight	114.00	124.00	129.00	128.00	2.198	0.109
Breast weight	418.0	461.00	467.00	471.00	12.275	0.422
Drumstick weight	96.36	103.04	106.04	111.00	2.438	0.259
Shank weight	49.00	53.00	55.00	56.00	1.592	0.424
Neck weight	33.48	31.00	32.49	37.00	1.732	0.694
Wing weight	77.29	76.33	77.08	80.00	1.986	0.505
Abdominal fat weight	17.00	16.47	19.00	19.11	1.377	0.855

Each value indicates the mean of four replicates consisting of four birds per treatment at 33 days; SEM: Standard Error Mean.

Table 4. Blood metabolites (mg/dl) of broilers fed L-Met diets.

	Treatments				Pooled SEM	*p*-values
	D_0	D_1	D_2	D_3		
Total protein	25.28	26.08	27.08	28.28	0.628	0.441
Glucose	221.18	266.13	262.23	274.93	9.82	0.270
Albumin	19.18	20.18	19.56	25.92	1.278	0.255
Uric acid	4.50	5.38	5.18	5.25	0.307	0.169
Creatinine	0.50	0.48	0.50	0.55	0.017	0.504
Triglycerides	81.25	95.08	94.00	106.43	3.252	0.108

Each value indicates the mean of four replicates consisting of four birds per treatment at 33 days.

Cost-benefit analyses

The data on cost-benefit analyses of broiler was presented in Table 6. Higher ($p < 0.05$) total production cost was found in the birds fed non-supplemented or basal diets (D_0). The total cost of production (Tk/kg live broiler) was ($p < 0.05$) less for the broilers fed L-Met supplemented diets (D_2 and D_3). Higher ($p < 0.01$) profit margin was obtained for L-Met supplemented dietary group. It might be due to the increased body weight gain and reduced production cost per treatment group. On the other hand, lower profit ($p < 0.01$) (Tk/Kg live broiler) was counted for the birds fed diets without L-Met diets.

Discussion

Among other meat yield parameters, dressing % and back weight of birds fed different level L-Met diets were significantly improved than the birds had basal or control diet. For dressing %, our result could be correlated with the report of previous investigators [15,21]. Increased level of back weight seen in the birds fed a higher level of L-Met that supports the results found by Wang et al. [22] that might be due to higher body weight gain. Therefore, the elevated level of L-Met can result in the desired level of dressing %, and thus the meat yield of the broiler. Furthermore, the increased dressing yield % and back weight of broilers might be an outcome of increased body weight of broilers fed L-Met diet. The increased body weight of broilers on the L-Met diet might give rise to better dressing yield and other associated organs of the body. The findings can be partly correlated with the findings of several researchers [22,23], who found the increased dressed yield of broilers when the bird was fed the higher amount of Met (100% and 140%) than those of lower recommended level (80%). However, these current findings of our study could claim to supply the Cobb 500 strain with a greater amount of L-Met concentrated diets, if we wish to attain a higher amount of dressing percentage of broiler chickens.

In this study, it is clear that the level of different blood metabolites in serum did not vary significantly and remain constant within the normal range. These findings are partially supported by Rath et al. [24], where author noticed that blood metabolites (e.g., TP, Alb, and globulin) were normal entire the trial period, regardless the sources of AA added in the diets, whereas the finding of plasma glucose, TP, and Alb did by Zeid et al. [25], contradicts with our study. Besides, the present findings could be partly correlated with the previous report of Halder and Roy [26], who observed that the total blood plasma did not differ between treatment due to herbal Met and DL-Met supplementation in the broiler diets. It is clear from the result that total plasma protein, glucose, Alb, uric acid, creatinine,

Table 5. Bone (right tibia) characteristics of broiler chickens fed L-Met diets.

	Treatments				Pooled SEM	*p*-values
	D_0	D_1	D_2	D_3		
Bone weight (gm/bird/tibia)	4.91	5.73	5.57	6.06	0.191	0.244
Bone length (mm)	79.91	85.12	84.53	85.71	0.799	**0.091**
Bone width (mm)	6.71	6.63	7.26	7.00	0.123	0.327
Bone head width (mm)	13.95	14.36	13.60	14.33	0.232	0.456
Ca %	7.48	7.56	7.33	7.76	0.112	0.621
P %	3.10	3.20	4.32	4.25	0.179	0.131

Each value indicates the mean of four replicates consisting of 4 birds per treatment at 33 days.

Table 6. Cost of production and profit of broilers fed L- Met diets on 33 days.

	Dietary treatment				Pooled SEM	*p*-values
	D_0	D_1	D_2	D_3		
Live weight (gm/bird)	$1,812.50^b$	$11,864.50^b$	$1,885.50^a$	$1,996.50^a$	19.700	0.038
Survivability (%)	94.44	97.22	94.44	94.44	1.923	0.94
Feed cost (Tk/kg live bird)	60.67^a	58.64^b	57.91^b	56.43^c	0.088	0.05
Market price (Tk/kg live weight)	135.00	135.00	135.00	135.00	–	–
Total production cost (Tk/kg live broiler)	119.17^a	114.26^b	113.93^b	109.46^c	0.562	0.05
Profit (Tk/kg live broiler)	15.83^c	20.74^b	21.07^b	25.54^a	0.286	0.01
Cost: Benefit Ratio	7.53	5.51	5.41	4.29	–	–

Values bearing different superscript in a row differ significantly.

TG, etc. were in the normal range and not influenced by the sources of AA supplemented with the diets in this study.

The welfare and economic issues of growing poultry enterprises incur leg bone quality. Many leg abnormalities, such as lameness, rickets, tibial dyschondroplasia, angular bone deformities, leg weakness, perosis, muscular dystrophy, and footpad dermatitis, determine leg health and leg bone quality of poultry. These leg disorders of broiler chickens are directly related to various metabolic diseases in rapidly growing meat-type chickens, which incur greater production loss and pose a negative impact on the birds' welfare [27–29]. However, it is obvious in this study that, birds on the D_3 diet had the highest bone length, while the birds in the D_0 diet being the lowest bone length, but the differences were marginally significant between treatments.

It is obvious that broilers fed diet without supplementation had lower body weight and attained lower profit. The lower profit margin might be occurred due to attaining reduced body weight and higher production cost. Net costs differed among the treatment groups as well. However, the dissimilarity of return or profit margin could be likely due to differences in feed consumption, feed price (per kg) and viability in different dietary treatments. Halder and Roy [26] reported that net profit was significantly highest in the broilers fed herbal supplemented AA compared to DL-Met.

Besides, findings of the present study might partly agree with the previous study of Zhai et al. [23] who showed that the increasing amount of supplemented Met in broiler diet reduced the ratio of feed cost: the value of cut-up parts. However, the criteria by which the cost and benefit and performance of birds are evaluated as well as the feed costs and market prices could influence feed cost or production cost with respect to its economic returns [30–32]. Because the prices of feed, meat and another production cost of poultry regularly alter; hence, it is very important to re-consider the relationship between ingredient costs and subsequently another necessary item of poultry production cost to increase profit margin.

Feed undoubtedly incurs higher costs than any other production cost required for running a poultry business. Increasing feed cost and optimum production or performance of poultry are the burning issues to every nutritionist nowadays, which warrant re-evaluating the present feed formulation policy for the broiler chicken. In this particular type of study, the ultimate feed cost of individual bird consumed per treatment group should consider minutely, as both diet price per kg and feed consumption of each feed phase [31] are very important. Because today's broiler industries are booming with an aim to sell their finished products in the market in different forms (e.g live bird, dressed carcass, different meat cuts, deboned or fillet meat and so on), to adjust farm's profitability through mitigating feed cost of individual bird.

There were several limitations of this study, such as insufficient funds, using either sexed birds, formulation of ration without considering digestible AA, less number of birds and replication numbers, lack of feed pelleting facilities, lack of feed AA analytical facility in our lab and so on. However, AA profiling of test diets, which seems crucial for this study could not be done due to financial constraints.

Conclusion

It is obvious from the results that carcass meat yield and increased profitability of production Cobb 500 broilers were significantly increased without affecting other parameters measured herein. It can be inferred that L-Met has the potential to be used as an alternative feed ingredient to poultry for profitable broiler production with a good dressing yield under farming conditions. Future research studies could be done by rectifying the aforementioned limitations to elucidate the present findings, and it might be helpful to establish the feasibility of L-Met over the conventional DL-Met supplement in broiler diets.

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Conflict of interests

The authors solemnly declare that there is no contradiction, conflicting or clash of interests relating to our activities of the study by an individual or an institution.

Authors' contribution

Nasima Akter was involved in the conduction of the study, collection of data, analyses of the samples, and preparation of the manuscript. Mohammad Abul Hossain designed the study, analyzed, and interpreted the data. Md. Saiful Islam and Sharmin Zaman took part in data collection and sample analyses. Ishrath Jahan helps in the final preparation and critical checking of this manuscript.

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