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## Research Article

# Ascertaining the Growth Performance, Ileal Gut Morphology, Gastro-Intestinal Development and Viability of Broiler Chicken Fed L-Methionine (L-Met) Supplemented Diet

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## Abstract

**Objective:** The study was conducted to investigate the productivity of broiler fed diet supplemented with L-methionine.

**Materials and Methods:** Day-old broiler chicks (n = 216: Cobb500) were reared from day 1-33 in the battery cages. The chicks were distributed randomly into four treatments, i.e., D<sub>0</sub> (DL-Met), D<sub>1</sub> (0.20% L-Met), D<sub>2</sub> (0.25% L-Met) and D<sub>3</sub> (0.30% L-Met) in a CRD. Each treatment was replicated 6 times with 9 birds per replicate. Chicks were fed commercial starter diet *ad libitum* up to 2 weeks. After that, test diets were supplied throughout the trial period (15-33 day). All the formulated diets were iso-caloric and iso-nitrogenous. Data were collected for feed intake (FI), live weight (LW), feed conversion ratio (FCR) and livability. Visceral organs and ileal samples were collected on day 33 to assess the gut morphology and gastro-intestinal development of broiler. **Results:** The data revealed that FI (p<0.01) and LW (p<0.05) of broiler were influenced by treatment without affecting the FCR (p>0.05) up to 33 day. Birds fed D<sub>3</sub> diet had higher (p<0.038) LW (1996.50.0 g bird<sup>-1</sup>) at the expense of greater FI (3047.40 g bird<sup>-1</sup>) than that of other diets on day 33. The livability (%) of broilers was unaffected (p>0.05) between treatments. No significant (P>0.05) differences were found in the visceral organ weights (small intestine, proventriculus, gizzard, liver, heart, spleen and bursa) of broilers except for pancreas, which was found higher (p<0.029) in the birds fed D<sub>3</sub> diet. The data of gut morphology revealed that broiler fed L-Met diets (D<sub>2</sub>, D<sub>3</sub>) had increased (p<0.05) villi width, crypt depth and surface area compared to the birds fed D<sub>0</sub> and D<sub>1</sub> diets. **Conclusion:** Chick fed diets supplemented with L-Met had better growth response than that of chicks fed diets with DL- Met.

**Key words:** Growth, gastro-intestinal organs, intestinal morphology, survivability, amino acid, broiler chicken

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**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Amino acids (AA) are the building block of protein. The basic function of dietary protein is to supply adequate amounts of required AA for the animal diets. The protein requirement for poultry is basically the requirement of AA, because AA are the end product of protein hydrolysis<sup>1</sup>. Proper body growth and tissue development of poultry need both essential and non-essential AA through the dietary supplement. As it is reported that indispensable AA can not be synthesized by the birds to meet up their protein requirement. For this reason, the diet is always fortified with synthetic AA to attain optimum performance of birds for meat and egg production.

Amongst the essential AA, methionine (Met) is very important and is commercially available as synthetic form for the diet formulation of poultry. Plant protein sources are very insignificant source of Met, which is regarded as the first limiting amino acid for the avian species<sup>2,3</sup>. In addition, the evaluation of protein sources is rendered based upon the amounts of the availability of three limiting amino acids (lysine, methionine and tryptophan)<sup>4</sup>. In this regard, the methionine warrants the higher amount than others for the optimum productivity of the broiler chicken.

Methionine and cystine are the sulphur containing AA. It is observed that marginal concentrations of total sulfur AA (Met and Cys) in animal diets could lead to a great demand and production of supplemental Met compounds and it takes 40% of total feed grade AA used for animal production<sup>2</sup>. Either DL-Met (99% purity powders) or an aqueous solution of 2-hydroxy-4-(methylthio) butanoic acid is used as the most conventional sources of supplemental Met in animal feeds<sup>5</sup>. For the synthesis of protein, Met as a main limiting AA can act as methyl donor, anti-oxidant and the precursor of several bioactive compounds say glutathione and taurine<sup>6-8</sup>. Further, it could play an important role for the development and health status of animals<sup>9,10</sup>. Met tends to have a greater rate of first pass metabolism in the gut than some other essential AA<sup>9</sup>. The significant splanchnic Met metabolism indicates that the gastrointestinal tract could have a functional requirement for Met<sup>11</sup>.

However, in poultry feed, DL-Met has long been used as a source of supplemental Met. Although D-Met is generally assumed as efficacious as L-Met for the growth of poultry due to the conversion to L-Met in the liver and kidneys<sup>12,13</sup>. D-Met is not utilized directly by the cells of the gastrointestinal tract until it is converted to L-Met in either the liver or kidneys. In fact, L-Met is the biologically functional form of Met readily

absorbed by the intestinal cells and therefore, L-Met can directly deliver beneficial effects to the gastrointestinal tract of chickens compared with D-Met<sup>14</sup>.

The introduction of L-Met in poultry diets is not much conventional as like as DL-Met available in the market. There is a plethora of research on dietary DL-Met AA supplementation in broiler chickens. Much data are not available regarding supplementation of L-Met in poultry. Recently, feed-grade supplemental L-Met became available from a fermentation process, which provides opportunities to use a naturally occurring form of Met in animal feed. Further, L-Met could be used as an immediate source of Met, which can play an important role for intestinal or splanchnic metabolism. Therefore, we could speculate that inclusion of L-Met in broiler diet could have a greater potential for the growth performance, redox status, gastro-intestinal development and glutathione level of broiler in compared to use of DL-Met<sup>15</sup>.

Feed grade L-Met is a new commercial product in the feed industry. Research focusing on L-Met in broiler chicken, could play a significant role to boost up the poultry industry of the country. For this why, the present study was undertaken so that the findings retrieved from this study, would be a guideline to determine the recommended level of L-Met for broiler feed formulation. Feed grade L-Met supplement in conventional diet could have a potential to improve the productivity of the broiler production. Considering the above, the current study was attempted to investigate the impact of various levels of L-Met (feed grade) on the growth response, livability, gastro-intestinal development and gut morphology of broiler chicken.

## MATERIALS AND METHODS

**Ethical approval:** The study was conducted following the guidelines of the research policy of Chattogram Veterinary and Animal Sciences University (CVASU) and approved by the Animal Ethics Committee of CVASU, Bangladesh [Approval no. CVASU/Dir(R&E). EC/2019/94(4)].

**Animal husbandry and experimental design:** A total of 216 (Cobb 500) day-old broiler chicks was procured from the local renowned hatchery weighing on an average of  $45.70 \pm 0.38$  g each. The chicks were weighed on receipt and then randomly assigned into four dietary treatment groups i.e. D<sub>0</sub> (DL-Met), D<sub>1</sub> (0.20% L-Met), D<sub>2</sub> (0.25% L-Met) and D<sub>3</sub> (0.30% L-Met) (D<sub>0</sub>, D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub>), where each treatment was replicated six times with nine birds per replicate in a completely randomized design

(CRD). Birds were raised in battery cages for the entire trial period (day 1-33). Cages were divided into 16 pens of equal size furnished with one feeder and one drinker. All the birds had a 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 birds were vaccinated against Ranikhet (New Castle Disease) and Gumboro disease, as per the schedule mentioned in Table 3, recommended by Department of Livestock Services (DLS), Chattogram, Bangladesh from where the vaccines (lyophilized) were collected.

**Diet:** 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 chemical composition of ready-made starter diet are 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 15-33 day. Four different test diets (D<sub>0</sub>, D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub>) were formulated as per the requirements of NRC<sup>16</sup>, shown in Table 2. All the diets were iso-caloric and iso-nitrogenous. Control diet (D<sub>0</sub>) was formulated with the all feedstuffs without L-Met, whereas D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> 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) are shown in the Table 2.

**Data and sample collection:** Mortality of bird was recorded as it occurred, while body weight and feed intake were recorded weekly for the calculation of body weight gain and feed conversion ratio (FCR). Livability was calculated from mortality of birds per replicate cage. Two birds per pen were selected randomly, weighed and killed humanely on day 33 to record the relative weights of gastro-intestinal organs (liver, pancreas, bursa of fabricius, heart, small intestine, proventriculus, gizzard, spleen) for assessing the gastro-

intestinal development of the birds. Tissue samples (2 or 3cm) were also collected from the ileum, by killing two birds from each replicate cage, for the measurement of intestinal morphology such as villi height, villi width, crypt depth and surface area on day 33. Feed samples were also collected prior supplying to the birds to determine the nutritive value of the feeds.

**Sample processing and analyses**

**Feed sample:** Collected feed samples were processed by grinding with the help of coffee grinder machine thoroughly to analyze for dry matter (DM%), moisture%, crude protein (CP%), crude fiber (CF%), ether extract (EE%) and ash using standard laboratory procedure<sup>17</sup>. Dry matter was determined by oven dry method. Crude protein was recovered by Kjeldahl process. Ether Extract was quantified by Soxhlet apparatus. Ash was measured by igniting the pre-ashing sample on a Muffle furnace at a temperature of 600°C for four to 6 h. Additionally, calcium (Ca%) and phosphorus (P%) were determined by atomic absorption and spectrophotometry, respectively. Metabolizable energy (ME) was determined 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<sup>18</sup>.

**Tissue samples processing for morphometric measurement of broiler chicken:**

Collected tissue segments were flushed with 0.9% saline solution to remove the contents and put in the 10% neutral-buffered formalin. After that, the samples were dehydrated, cleaned and paraffin embedded. Duplicates tissue segments were processed by the paraffin sectioning at a 4 µm thickness, placed on a glass slides. The slides were stained with hematoxylin-eosin and covered with cover-slip. Histological indices were analyzed under a computer-aided light microscopic using image analyzer. Measurements of villus height and crypt depth were taken only from sections, where the plane of section ran vertically from the top of villus

Table 1: Nutrient composition of starter diet

Nutrient components (%)	Proximate values of RRP feed	Reporting values of RRP feed
ME (kcal kg <sup>-1</sup> )	3380.00	3000.00
Moisture	9.52	11.00
DM	90.48	89.00
CP	20.13	22.00
CF	5.15	4.00
EE	7.91	-
Ash	6.13	-
Ca	2.55	-
P	0.46	-
Lys	-	1.30
Met	-	0.55

Table 2: Ingredient and nutrient composition of finisher diet

Feed ingredients	Diets			
	D <sub>0</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
Yellow corn	50.000	50.000	50.640	50.000
Wheat (durum)	12.420	13.200	12.220	11.000
Soybean meal (45.50%)	23.400	23.000	23.460	23.800
Fish meal	5.610	5.200	5.000	5.000
Palm oil	5.180	5.120	5.150	5.700
Di-calcium phosphate	0.700	0.700	0.700	0.710
Limestone	1.590	1.670	1.700	1.700
Table salt	0.350	0.400	0.300	0.240
Choline chloride	0.040	0.030	0.036	0.034
Vitamin minerals premix <sup>1</sup>	0.210	0.260	0.350	0.260
L-lysine	0.260	0.250	0.250	0.300
DL-methionine	0.210	0.000	0.000	0.000
L-methionine	0.000	0.200	0.250	0.300
Enzymes (natuzyme)	0.036	0.036	0.036	0.036
Sand	0.000	0.000	0.000	0.920
<b>Calculated nutrient (%)</b>				
ME (kcal kg <sup>-1</sup> )	3165.000	3165.000	3165.000	3165.000
CP	20.000	20.000	20.000	20.000
Ca	1.310	1.310	1.310	1.310
P	0.680	0.680	0.680	0.680
CF	3.140	3.110	3.130	3.110
EE	3.330	3.320	3.320	3.280
Lysine	1.060	1.060	1.060	1.060
Methionine	0.470	0.470	0.470	0.470
Threonine	0.830	0.830	0.830	0.830
<b>Analytical value (%)</b>				
DM	88.500	88.730	88.620	88.850
CP	19.600	19.780	19.250	19.900
CF	4.200	5.330	4.500	4.340
EE	7.330	7.500	7.890	7.340
Ash	5.230	6.160	5.800	6.150
Ca	2.200	2.300	2.200	2.320
P	0.440	0.530	0.500	0.470

Control diet (D<sub>0</sub>) with DL-methionine and no L-methionine, whereas D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> diets are supplemented with 0.20, 0.25 and 0.30% L-methionine, respectively, <sup>1</sup>Provided per kg of diet (mg): Vitamin A (as all-trans retinol): 3.6 mg, Cholecalciferol: 0.09 mg, Vitamin E (as d- $\alpha$ -tocopherol): 44.7 mg, Vitamin K<sub>3</sub>: 2 mg, Thiamine: 2 mg, Riboflavin: 6 mg, Pyridoxine hydrochloride: 5 mg, Vitamin B<sub>12</sub>: 0.2 mg, Biotin: 0.1 mg, Niacin: 50 mg, D- calcium pantothenate: 12 mg, Folic acid: 2 mg, Mn: 80 mg, Fe: 60 mg, Cu: 8 mg, I: 1 mg, Co: 0.3 mg and Mo: 1 mg

Table 3: Vaccination schedule

Age (day)	Name and type of vaccines	Name of diseases	Route of administration
5	BCRDV <sup>a</sup> , Live	Newcastle disease	One drop in one eye
12	IBD <sup>b</sup> , Live	Gumboro	One drop in one eye
17	IBD <sup>b</sup> (Booster dose), Live	Gumboro	One drop in one eye
21	BCRDV <sup>a</sup> (Booster dose), Live	Newcastle disease	One drop in one eye

<sup>a</sup>Baby chick ranikhet disease vaccine (BCRDV), <sup>b</sup>Infectious bursal disease (IBD) or Gumboro vaccine

to the base of an adjacent crypt. The villi height (from the crypt mouth to the villi tip), crypt depth (from the base up to the region of transition between the crypt and villi), villus width (½ of the villus length) were measured. The villi surface area (the area of the villous per length unit per bird) and villi height: crypt depth ratio were also calculated.

**Statistical analyses:** All collected data were subjected to analyzing by one way Analysis of Variance (ANOVA) using Minitab software (Minitab, Minitab Version, 16, 2000). The

significance of differences between means was tested using the Duncan's multiple range tests (DMRT). Statistical significance was considered at  $p \leq 0.05$ .

## RESULTS

**Growth responses of broiler chicken:** The results showed that the feed intake (FI) and live weight (LW) of broiler chickens up to 21 days were not influenced ( $p = 0.87$ ;  $p = 0.57$ ) by dietary treatments except for 33 days (Table 4). From 1-33 days, the

Table 4: Feed intake (FI), live weight (LW) and feed conversion ratio (FCR) of broiler chickens fed L-Met diet (day 1-33)

	Age (day)	Treatments				Pooled SEM	p-values
		D <sub>0</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>		
FI (g bird <sup>-1</sup> )	1-14	670.92	652.40	650.60	661.60	10.443	0.870
	1-21	1382.00	1339.00	1367.10	1376.00	16.850	0.811
	1-33	2980.00 <sup>b</sup>	2965.50 <sup>b</sup>	2968.50 <sup>b</sup>	3047.40 <sup>a</sup>	6.873	0.010
LW (g bird <sup>-1</sup> )	1-14	548.67	537.55	534.77	531.36	6.873	0.827
	1-21	985.10	986.00	1000.00	1024.00	10.975	0.577
	1-33	1812.50 <sup>b</sup>	1864.50 <sup>b</sup>	1885.50 <sup>a</sup>	1996.50 <sup>a</sup>	19.700	0.038
FCR	1-14	1.34	1.33	1.33	1.37	0.019	0.819
	1-21	1.47	1.42	1.44	1.40	0.024	0.731
	1-33	1.69	1.63	1.62	1.56	0.018	0.133

Data indicate mean values of four birds per treatment from day 1-33 days, D<sub>0</sub> refers to control diet with no supplemental L-methionine, whereas D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> diets are supplemented with 0.20, 0.25 and 0.30% L-methionine, respectively, SEM-Pooled standard errors mean

Table 5: Relative weight of visceral organs (g bird<sup>-1</sup>) of broilers fed L-Met diets

	Treatments				Pooled SEM	p-value
	D <sub>0</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>		
<sup>1</sup> Small intestine	60.65	66.44	73.49	73.42	3.155	0.442
Proventriculus and gizzard	44.61	44.00	43.00	48.00	1.583	0.718
Liver	27.92	31.91	32.80	32.10	1.489	0.658
Heart	6.36	7.00	7.48	7.56	0.397	0.653
Spleen	1.66	1.61	1.98	1.98	0.183	0.820
Bursa fabricius	1.76	2.11	2.20	2.57	0.159	0.393
Pancreas	2.45 <sup>b</sup>	2.69 <sup>b</sup>	2.64 <sup>b</sup>	3.08 <sup>a</sup>	0.065	0.029

Each value indicates the mean of four replicates consisting of 4 birds per treatment at 33 days, <sup>1</sup>Organ (small intestine was weighed with contents), SEM: Standard Error Mean

birds of D<sub>3</sub> diet group consumed the highest feed ( $p = 0.01$ ) compared to other groups. Live weight was also significantly ( $p = 0.038$ ) greater for the broilers fed D<sub>3</sub> diet than the birds fed other diets up to 33 day. The feed conversion ratio (FCR) of broilers was unaffected ( $p = 0.133$ ) by dietary treatment from day 1-33 (Table 4). Birds on D<sub>3</sub> diet group had a comparatively lower FCR value (1.56) than the other diet group, although the FCR value between treatment was statistically identical or not affected ( $p > 0.05$ ) throughout the trial period.

**Livability:** The livability (%) of broiler was identical ( $p = 0.94$ ) between treatment as shown in Fig. 1. The data show that birds on D<sub>1</sub> group had the highest survivability (97.22%) and being similar (94.44%) in remaining dietary groups. The result of livability indicates that supplemental diets had no significant ( $p = 0.94$ ) influence on the viability of broiler chick. From the data it is also obvious that mortality (%) of broiler chicken was not significantly influenced ( $p = 0.94$ ) by the dietary treatment. Though numerically the mortality was lower (2.78%) for the birds fed D<sub>1</sub> diet than those of birds (5.56%) fed D<sub>0</sub>, D<sub>2</sub> and D<sub>3</sub> diets, respectively but it had no significant difference ( $p = 0.94$ ) between treatment.

**Gastro-intestinal development:** The relative weight of visceral organs of broiler chickens fed on the supplemented

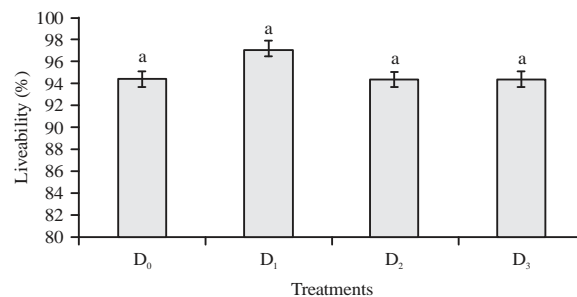


Fig. 1: Livability (%) of broilers fed L-Meth diets on day 33

Bar with similar letter has no significant differences ( $p > 0.05$ ) between treatments

diet (L-Met) at 33 days is shown in Table 5. The data show that except for pancreas, the relative weights of the remaining organs (small intestine, proventriculus, gizzard, liver, heart, spleen and bursa) of birds were found to be identical between treatment. The relative weight of pancreas was increased significantly ( $p = 0.029$ ) between treatment.

**Measurement of intestinal morphology of broiler chicken**

**fed L-Met diet:** The data show that most of the ileal characters i.e. villi width, crypt depth and surface area etc., were significantly ( $p < 0.05$ ) improved by supplemental diets, except for villi height ( $p = 0.265$ ) in this study (Table 6). The increasing

Table 6: Morphological measurement of ileal tissue of broilers fed L- Met diet

	Treatments				Pooled SEM	p-value
	D <sub>0</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>		
Villi height (cm)	0.560	0.450	0.570	0.560	0.0240	0.265
Villi width (cm)	0.065 <sup>b</sup>	0.068 <sup>b</sup>	0.100 <sup>a</sup>	0.090 <sup>a</sup>	0.0051	0.044
Crypt depth (cm)	0.290 <sup>b</sup>	0.250 <sup>b</sup>	0.390 <sup>a</sup>	0.380 <sup>a</sup>	0.0160	0.022
Surface area (µm <sup>2</sup> )	0.037 <sup>b</sup>	0.030 <sup>b</sup>	0.057 <sup>a</sup>	0.051 <sup>a</sup>	0.0030	0.040

Each value indicates the mean of four replicates consisting of 4 birds per treatment at 30 days, SEM: Standard error mean

trend was observed in the ileal tissue of villi width, crypt depth and surface area of broiler chickens by increased level of L-Met diets fed the bird. Broiler fed L-Met diets (D<sub>2</sub>, D<sub>3</sub>) had increased villi width (p = 0.044), crypt depth (p = 0.022) and surface area (p = 0.04) compared to the birds fed D<sub>0</sub> and D<sub>1</sub> diets.

## DISCUSSION

**Growth responses of broiler chicken fed L-Met diet:** From the result it is obvious that live weight of broiler was increased by the increased level of the supplemental L-Met offered to the bird. The higher feed intake of broiler might be a result of increased body weight of broiler chicken fed L-Met diet, as is observed in this current study. It is noteworthy that feed intake was more in the dietary group of D<sub>3</sub> in spite of the mortality rate was numerically higher than D<sub>1</sub>. It can be speculated that the reason for the increased feed intake of broiler on L-Met supplemented diets could be due to amino acid balance<sup>19</sup>. Further, the increased absorption of L-Met diet might increase the protein synthesis and thereby the increased growth of the broiler chicken. It is reported that the L-Met is directly absorbed by the intestinal tissue of broiler compared to the DL-Met<sup>15</sup>, once after absorption by the intestinal tissues of the birds, L-Met is directly incorporated into body protein. Besides, the L-Met molecules derived from D-methionine or methionine hydroxy analogue can then be used by the animals to build up body tissues or accretion of protein. It is supported by the Xue *et al.*<sup>20</sup> who reported that L-Met could be incorporated directly into body proteins but the D-methionine must first be converted into keto-methionine and then into the L-methionine before being incorporated into body protein.

However, our present findings agree with the report of previous many researchers<sup>15,21-23</sup>. Park *et al.*<sup>23</sup> reported that young turkeys fed with L-Met had 131% better live weight than that of DL-Met. In this study, the greater feed consumption of broiler fed a diet supplemented with L-Met than DL-Met are speculated probably due to the difference in live weight of the birds. One of the objectives of the current

study was to compare the growth response of young broiler chicks fed diets supplemented with either L-Met or DL-Met, confirming that L-Met is better utilized for intestinal development and, consequently, growth by young broiler chick compared with DL-Met. The difference in the functionalities and roles for intestinal development of young broiler chick are speculated as the reasons for the difference in growth performance. This finding largely agrees with the findings of previous investigators<sup>24,25</sup>, who indicated that chicks fed with L-Met grow better than those fed with DL-Met. Bhargava *et al.*<sup>26</sup> also showed the improved growth by using L-Met associated with increased antibody production. It is clear from the study that feed intake and body weight of broiler chicken increased with the increasing level of L-Met in diets. The increased body weight might be due to the prompt absorption and conversion of L-Met into protein<sup>14,15</sup>. Once absorbed, L-methionine is directly incorporated into body protein while D-methionine undergoes a two-step process to be converted into L-methionine and absorbed. After that, they can be used by the animal to build up protein<sup>21</sup>.

**Livability and L-Met diet:** The livability (%) of broilers was unaffected between treatments, as is observed from the current study. It can be assumed that L-Met diet had no detrimental effect on the growth, development and the viability of broiler chicken. Further, it can be surmised that L-Met feedstuff in the broiler diet can be used undoubtedly, as it had no detrimental impact on the growth and survivability of the broiler chicken. Our result agrees with the report of previous investigator<sup>27</sup>, who observed no difference in the viability or mortality of broiler when fed L-Met diet.

**Development of gastro-intestinal organ:** The main objective of our study was to compare the growth response of broiler chickens fed diets supplemented with either L-Met or DL-Met, confirming that L-Met is better utilized, absorbed and assimilated for gastro-intestinal development and, consequently, growth responses by broiler chickens compared with DL-Met. Nitsan *et al.*<sup>28</sup> and Iji *et al.*<sup>29</sup> stated that gastro-intestinal organs along with the digestive activities develop

most rapidly within the first 7-10 days of life. However, it is still unclear to us how the nature of the diets influence this development, that has not been adequately studied. In this study, broiler chickens fed L-Met diet (D<sub>3</sub>) showed better growth of pancreas (weight) than the control diet, which might cause a positive effect on the birds' growth. The increased growth and development of the digestive organs, for example, pancreas and liver could accelerate the secretion of digestive juices or enzymes that might gear up the digestive activities of the birds. It is reported that enzyme supplementation to diets could enhance the nutrient digestibility of bird and thus growth performance of the birds<sup>30</sup>. The relative weight of the pancreas was found to be significant between treatments. It seems that the increased pancreas weight of broiler chicken fed L-Met diet (D<sub>3</sub>) might induce more secretion of various pancreatic juices or enzymes. The secreted enzymes and pancreatic juices might enhance the digestibility of feed, which could be a result of better growth responses of the birds in this study<sup>31</sup>.

**Gut morphology of broiler chickens fed L-Met diet:** It is obvious from the data that birds provided L-Met diet caused a significant improvement in villus width (VW), crypt depth (CD) and surface area (SA) as compared to those having diet supplemented with DL-Met. The findings agree with the report of Shen *et al.*<sup>15</sup>, who revealed that chicks fed a diet supplemented with 0.285% L-Met had greater villus width and villus height: crypt depth ratio compared with chicks fed a diet supplemented with 0.285% DL-Met. However, it could be assumed that the reason for this improvement of gut physiology might be due to acidifying effect of L-Met as reported by previous investigators<sup>32,33</sup>.

The multi-functional role of L-Met (e.g methyl donor, anti-oxidant, precursors of cystine, glutathione, taurine and inorganic sulphur etc.) might be responsible for the improved growth of VW, CD and SA of ileal epithelial tissues of broiler chickens fed L-Met supplemented diet in this study. Besides, the increased growth of VW, CD and SA might be an indication for the greater absorption, assimilation and utilization of nutrients by the birds fed L-Met diets resulting in heavier body growth responses, as is observed in this study. As we know that ingested nutrients are absorbed by the intestinal wall of the villi. This study demonstrated that intestinal epithelial tissue was proliferated by the supplementation of L-Met in broiler diet, as the methyl group donor character of L-Met could help to grow and multiply the intestinal epithelial tissues; and the antioxidant activity of L-Met could reduce the intestinal oxidative damage<sup>34</sup>. Studies reported that a number of functions say protein accretion, tissue signaling,

antioxidative effect and immune response etc., are required for the growth and development of the gastro-intestinal tracts of AA metabolism<sup>11,35</sup>. It is known that one-third of the dietary intake of indispensable AA is removed in first pass metabolism by the intestine. Metabolism of indispensable AA by mucosal tissues is comparatively higher than that of AA inclusion into mucosal protein<sup>9</sup>. The findings indicate that the beneficial role of L-Met on the growth and development of the gastro-intestinal tracts might be attributed to its anti-oxidative function<sup>15</sup>.

## CONCLUSION

The results obtained in this study revealed that, feed consumption and live weight of broiler fed L-Met supplemented diet were improved without affecting FCR. Viability and most of the visceral organ weights were unaffected except for pancreas. Most of the ileal tissues say VW, CD and SA of the broilers fed L-Met diet were improved except for villi height. It is clear from the results that growth performances and gut morphology of the Cobb 500 broilers were greatly benefited from being fed increased level of L-Met density diets. Overall, L-Met favourably affected the intestinal structure and tissue growth that could account for the boosted growth performance of the broiler in this study.

## SIGNIFICANCE STATEMENT

This study discovered the efficacy of L-Met as an alternative feed ingredient for poultry that could be beneficial for the diet formulation of poultry including poultry integrators, poultry farmers, researchers, academicians and poultry industry across the globe. Though DL-methionine is commonly used for the diet formulation of poultry as synthetic form, L-Met is not so much conventional or available in the market as a new feedstuff. So it can be used as an alternative feed resource for the diet formulation of poultry, as it is absorbed more efficiently by the birds than that of DL-methionine. This study would help the researcher to uncover the critical areas of feed supplements that many researchers were not able to explore. Thus, the findings of this research study could draw the attention of the promising researchers, for assessing the effects of L-Met on broiler chicken to elucidate the current data.

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