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RESEARCH ARTICLE

COMPARISON BETWEEN DIFFERENT DIETARY METHIONINE SOURCES ON PRODUCTIVE PERFORMANCE, HEMATOLOGICAL, BLOOD METABOLITES, AND HISTOLOGICAL PARAMETERS IN BROILER CHICKEN UNDER SEMI-ARID CONDITIONS.

Amal M Hassan[1], Nematallah Ali[2] and Ayman Ahmed[2].

1. Animal & Poultry Physiology Dept., Desert Research Centre, Egypt
2. Poultry. Prod. Dept., Fac. of Agric., Ain Shams Univ., Egypt.

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Abstract

This research was guided to investigate the effects of supplementation of DL-methionine (DLM) and liquid DL-methionine hydroxy analog free acid (MHA) in diets on productive performance, hematological, blood metabolites and histological parameters in broiler chickens under summer conditions in Sina. A total number of 180 one day old Hubbard broiler chicks, with initial body weight (46.9 ± 2.5 g), were divided into 6 equal treatment groups. The treatment were DL-methionine (DLM) at 0.25% level, acid liquid methionine (ALM) and methionine hydroxyl analogue Ca salt (MHA) at 0.29 and 0.30%, respectively with and without dietary L-Threonine (0.0 and 1.0 g/kg diet). Chicks were raised for 35 days in battery cages under semi – arid conditions (high ambient temperature and relative humidity) in open-side housing.

The obtained results indicate: The productive performance of DLM and MHA were not significantly different. Supplementation of DLM and MHA significantly improved final body weight, body weight gain, average daily gain, and feed conversion ratio when compared to the control group (P<0.01). The use of DLM enhanced the plasma methionine concentration (P<0.01) and increased the heterophil/lymphocyte ratio (P<0.05). While MHA elevated the plasma urea and uric acid concentration levels (P<0.05).

***Corresponding Author:-* Amal M Hassan.**

Introduction:-

Methionine is the first limiting essential amino acid in poultry nutrition. It plays main roles including protein synthesis, precursor of important intermediates in metabolic pathways and synthesis of polyamines **(Vazquez-Anon *et al*. 2006 and Fanatico, 2010).** The common sources of supplemental methionine used in broiler diets are methionine hydroxy analogue-free acid (DL-M), DL-methionine (AL-M) and methionine hydroxy analogue calcium salt (MHA). Several researchers **(Waldroup *et al*., 1981; Elkin and Hester, 1983 and Garlich, 1985)** found that AL-M or MHA has nearly equal biological activity to DL-M. On the other hand, **Garlich (1985)** found significant differences in weight gain and feed conversion among the three-methionine sources of broilers (AL-M, DL-Met and L-M). However, **(Baker and Boebel, 1980 Christensen *et al*., 1980; and Boebel and Baker, 1982)** reported that performance of birds fed diets containing MHA was not equal to those fed DL-M and had significantly less weight gain and poorer feed conversion. Threonine (third most limiting amino acid) deficiency may decrease the efficiency of lysine (second most limiting amino acid) utilization. That makes formulating broiler diets to be adequate in threonine relatively critical. Lysine and threonine have shown to interact in such a way that, at optimum dietary concentrations, to increase broiler body weight gain and breast fillet yields **(Baylan *et al*., 2006**

and Canogullari *et al.*, 2009). Additionally, **Barkley and Wallis (2001)** observed that increasing dietary threonine concentration from 5.7 to 7.2 g / kg improved growth and feed conversion ratio of broilers.

Broiler chicken are homeothermal and can live comfortably only in a relatively narrow thermal zone. The optimal ambient temperature for efficient production of broiler chicken is 20°C **(Aviagen 2009).** High ambient temperature or other unsuitable environment conditions can result in significant economic loss due to reduced feed consumption, growth rate, feed conversion, macrophage activity, and survivability of poultry **(Bunchasak, 2009 and Quinteiro-Filho *et al.* 2010).** In addition, **Virden and Kidd (2009)** reported that heat stress exposure might result in impaired digestibility of major nutrient and various essential amino acids. In view of that, **Swennen *et al.* (2011)** and **Willemsem *et al.* (2011)** reported that supplementation of MHA to the low-protein diet displayed a better antioxidant status of chicks under heat stress condition.

Besides, the absorption rate of DL-M by intestinal epithelial cells was lower than that of MHA in chicks exposed to heat stress **(Knight *et al.* 1994)**. It seems that MHA absorption is unaffected by heat stress, while Dl-M isomer seems to be reduced. In addition, **Dibner *et al.* (1992)** explained that the exact metabolic process that results in the bioavailability differences remains unclear. In addition, **Taghinejad-Roudbaneh *et al.*, (2013)** reported that threonine requirement under stress and non-hygienic conditions was 0.81% to support growth performance.

Therefore, the objectives of this study were to investigate the effects of three different sources of Methionine, being DL-M, AL-M and MHA and 2 levels of dietary L-Threonine (0.0 and 1.0 g/kg diet) under summer conditions on hematological profiles, blood biochemical; haematological parameters and productive performance in broiler chickens.

Materials and methods:-

A total number of 180 one day old Hubbard broiler chicks obtained from a commercial hatchery, with initial body weight (46.9 ± 2.5 g), were used in this experiment to compare three different sources of Methionine, being DL-methionine (DLM), acid liquid methionine (ALM) and methionine hydroxyl analogue Ca salt (MHA) and 2 levels of dietary L-Threonine (0.0 and 1.0 g/kg diet). The chicks were randomly distributed into six treatment groups consisting of three replicate pens of 10 chicks each. Experimental treatments consisted of a 3 x 2 factorial arrangement with 3 different sources of Methionine, DLM was used at 0.25 % level, while ALM and MHA were added at 0.29 and 0.30 %, respectively with and without L- Threonine (0.0 and 1.0) As presented in Table (1) experimental diets were formulated to meet the nutritional recommendations for broilers from 0-35 days of age for all nutrients according to **NRC (1994).**

The chicks were raised for a total of 35 days under high temperature conditions in metal batteries consisting of four cages. Ambient temperature and relative humidity ranged between 27°C and 35°C (31± 0.9°C) and 78 % to 86 % (79 ± 4 % RH), respectively during the day. Feed and water were provided *ad libitum*.

All chicks were individually weighed weekly during the experimental period. Feed intake were recorded weekly, while the average daily gain (ADG) and feed conversion ratio (FCR) were calculated weekly (1–35 days) for each treatment. At the end of the experiment, five chicks per treatment were weighed and punctured at the wing vein to obtain a blood sample for chemical analysis and hematological profiles. The sample transferring it into plastic vials containing EDTA as an anticoagulant automated hematology analyzers measured hematocrit, hemoglobin concentration and total red blood cell count in blood samples and differential counts of leukocyte. Then, the whole blood samples were centrifuged at 3,000-×g for 10 min at room temperature to separate plasma and stored at (−20°C) until biochemical analysis. Total protein (TP), albumin (AL), cholesterol and Triglycerides (Tg). Liver enzymes (alanine transaminase (ALT), aspartic transaminase (AST)), and Thyroid hormone (T3). While, globulin and albumin ratio (A/G ratio) was calculated. All samples were determined calorimetrically by using commercial kits (By Bio Systems S.A. Costa Brava 30, Barcelona (Spain, Barcelona)). Thyroid hormones (Tri-iodothreonine) was measured by ELISA method using IMMUNOSPEC kits supplied by (Immunospec Corporation, 7018 Owensmounth Ave. Suite 103 Canoga Park, CA 91303, USA).

At slaughtering time, three birds of each treatment were taken for histological studies. Small section from small intestine was taken, immediately fixed in 10 % buffered formalin solution for histological technique. All sections [4-5 µm thick] were stained with haemotoxylline and eosin [H & E] stains according to **(Bancroft *et al.*, 1994).** Sections were examined under light microscope and then photographed using computerized program.

Data were analyzed using two-way analysis of variance with methionine source (M), threonine supplementation (T) and their interaction (T*M) using the General Linear Model Procedure of **SAS** (**2002**) as following model: Yijk = μ + Ti + Mj + (TM) ij + eijk.

Where: Yijk = Trait measured, μ = Overall mean, Mi= Methionine source, Tj= Threonine, supplementation, (TM) ij = Interaction between methionine source and threonine, Eijk=Experimental error. When significant differences among means were found, means were separated using Duncan's multiple range tests (**Duncan**, **1955**).

Result and discussion:-

Average daily weight gain (ADG) feed intake (FI) and feed conversion ratio (FCR) of broiler as affected by deferent dietary methionine sources (DL-M; AL-M and M-HA) and the addition of threonine at the rate of 1g/kg are illustrated in table (2). The addition of threonine increased ADG significantly ($P<0.05$) regardless of different sources of methonunine (40.06 vs. 36.89 for with vs. without threonine, respectively). Daily feed intake values or feed convention were not affected by adding threonine.

Barkley and Wallis (2001) reported that increasing threonine concentration of the diet improved broiler and layer hen performance. They also showed that increasing threonine concentration in broiler diets improved FCR.

The results of the present study are in harmony with those reported by several researches. **Barkley and Wallis, (2001)** reported that no significant differences between the methionine sources for FI, ADG and FC in broilers.; they concluded that LBW and ADG of broiler fed (M-HA) or (Al-M) diets were equal to those fed control (DL-M) diets. The void effect of different dietary methionine sources might be due to the insignificant differences in bone weight distribution **(El-Faham, *et al.*,2017)** in different carcass parts (e.g. breast, thigh, drumstick and total cuts). Also, there were insignificant differences in skin and subcutaneous fat percentages of carcass parts of chicken fed diets supplemented with (threonine 1.0 g/kg) or (AL-M) as a source of methionine The performances of chicks receiving low TSAA diet or amino acid imbalance diet are commonly depressed **(Bunchasak 2009** and **Rakangtong and Bunchasak 2011)**. In contrast, Chicks fed diets supplemented with DL-M and M-HA showed improved LBW and ADG compared to chicks fed a methionine-deficient diet **(Rachawadee and Chaiyapoom 2012).**

Several investigators have reported that the average relative bioavailability of M-HA compared with DL-M in various animal species ranged from 65 % to 95 %, and chicks fed an M-HA diet had lower growth performance than those fed a DL-M diet **(Vazquez-Anon *et al.* 2006 and Sauer *et al.* 2008).** In contrast, under heat stress conditions, some researchers have observed that chicks fed MHA showed better growth performance and feed efficiency and lower mortality **(Gonzales-Esquerra and Leeson 2006). Willemsen *et al.* (2011)** reported that M-HA supplementation partially prevented the growth-depressing effects of chronic heat exposure compared with DL-M supplementation. It can be inferred that M-HA is more efficient in alleviating high temperature-induced oxidative damage because of a more favorable reduced glutathione (GSH) / total GSH ratio. Under thermoneutral conditions, the mechanisms for intestinal absorption differ between M-HA and DL-M. **Knight *et al.* (1994)** found that the uptake of DL-M by intestinal epithelial cells was reduced in chicks exposed to short periods of heat stress. **Dibner *et al.* (1992)** reported that total epithelial uptake of 14C-DL-M is reduced by 34 % in the intestines of acute heat stress chicks. Therefore, these results may explain the impaired growth observed in DL-M fed chicks and the elevated growth observed in M-HA-fed chicks under heat stress conditions. In the present study, there was no significant difference in growth performance between supplementation of DL-M and M-HA. This indicates that at least the effects of DL-M and M-HA on productive performance are exactly equal under semi-arid condition in this experiment.

Hematological profiles:-

The effects of dietary methionine source on the hematological profiles are presented in Table 3. There were not significant differences between all treatments in hematological parameters, but numerically threonine supplementation had improved some parameters shush as Ht and increased MO and BA counts. Ht was improved and that is mean threonine decreased the stress on birds.

In contrast to our results, recent studies have revealed that broilers exposed to heat stress conditions experienced effects on the pathophysiology of white blood cells and an increase in the percentage of heterophil and H/L ratio by glucocorticoid influence **(Quinteiro-Filho *et al.* 2010).** Supplementation of DL-M and M-HA in the

diet to meet the TSAA requirement improved growth performance and metabolic rate, but physiological stress may be enhanced. Supplementation of DL-M in diet enhanced protein synthesis in chicks **(Richards *et al.* 2005),** while supplementation of M-HA in diet increased nitrogen catabolism and intermediates in metabolic pathway synthesis such as urea **(Martin-Venegas *et al.* 2006).** Accordingly, M-HA can be catabolized to urea and enhanced uric acid in plasma in this study. Both intermediates are well recognized as important biological antioxidants that reduce physiological stress, and they may also depress the H/L ratio in the blood of chicks reared under heat stress. This may explain the difference in hematological response between DL-M and M-HA. In conclusion, the productive performance of DL-M and M-HA is equivalent to an equimolar methionine basis under tropical conditions. DL-M and M-HA enhanced plasma methionine and cystine concentration, while M-HA improved urea and uric acid concentration in plasma. DLM reduced lymphocyte (percent) and increased the H/L ratio in blood, data not shown.

Blood constituents:-

Table (4) showed the effect of different dietary treatment on some blood parameters. There were not significant different between treatments. That is mean not all dirtily methionine sources had effect on birds health and that is elucidated from results. Numerically, AL-M was the best one for blood measurement and improved them, decreased triglyceride and improved immunity by decreased A/G ratio. Therefore, methionine supplementation decreased the damage that resulted from increment of heat.

Rachawadee and Chaiyapoom (2012) reported that the concentrations of plasma urea and uric acid were elevated only in chicks fed the M-HA-supplemented diet. While, dietary methionine sources did not significantly influence the concentration of glutamic acid and total plasma protein. In this study, these factors were limited, except the dietary methionine source. Low plasma methionine and Cystine levels in the negative control group directly demonstrated the deficiency of TSAA in diet. When DL-M or M-HA in diet met the TSAA requirement, the level of TSAA in plasma was increased. This implied that DL-M or M-HA supplementation might improve dietary amino acid balance and increase amino acid bioavailability. However, the DL-M supplemented diet significantly increased plasma methionine to a higher level than that of the MHA supplemented diet. This might be explained by the difference in the metabolism pathway between DL-M and M-HA. Both DL-M and M-HA must be absorbed from the intestinal lumen of chicks and then converted to L-methionine, but the processes involved have different enzymatic mechanisms. L-methionine is necessary for many important functions: (1) protein synthesis, (2) transmethylation to form Sadenosyl methionine, (3) synthesis of polyamines, and (4) transsulfuration to form L-cysteine, which in turn is also a precursor amino acid for protein synthesis that can be incorporated into glutathione or catabolized to urea (**Song *et al.* 2001)** L-methionine converted from DL-M is mainly used in protein synthesis (**Richards *et al.* 2005).** In contrast, partialities of L-methionine converted from M-HA are a precursor of intermediates in metabolic pathways **(Martin-Venegas *et al.* 2006).** In the present study, the high plasma methionine concentration of the chick fed the DL-M-supplemented diet might support this hypothesis. The result of plasma urea concentration showed that chicks fed the M-HA supplemented diet have plasma urea concentration higher than control diet. A similar result was reported by **Martin-Venegas *et al.* (2006)** who showed that the urea content in chicken enterocytes was elevated by M-HA supplemented diet. They described that in the chicken intestine, L-cysteine from transsulfuration of M-HA might be diverted to urea synthesis. Therefore, M-HA is not only a potential methionine source, but it could also be more easily involved in.

Intermediates of metabolic pathways through the transsulfuration pathway. Accordingly, **Lobley *et al.* (2001)** reported in ruminants that the L-methionine derived from M-HA is utilized by the cells and only secreted into the blood stream in conditions of excess supply of methionine. This may explain why the plasma methionine level in chicks fed the M-HA supplemented diet was lower than those fed the DL-M supplemented diet. In poultry, plasma uric acid is the major end product of In poultry, plasma uric acid is the major end product of nitrogen catabolism; its concentration was directly reflected to dietary protein levels and it can be used to determine amino acid adequacy in diet **(Donsbough; 2008). Ribeiro *et al.* (2001)** reported that chicks fed M-HA had higher nitrogen retention than those fed DL-M in heat stress exposure. Our results showing that the plasma uric acid concentration was directly elevated by M-HA supplement at ion implies that M-HA satisfied TSAA in diet and increased nitrogen retention. Uric acid is also a potent antioxidant, and its concentration is inversely correlated with oxidative activity in poultry. Uric acid is a product of the degradation of adenine- and guanine-based purine compounds. The chicks use uric acid to scavenge hydroxyl, proxy, and superoxide radicals by an electron transfer before the oxidant can react with the targeted biological molecule and inhibits DNA damage and oxidation of lipids in cellular membranes **(Stinefelt *et al.* 2005). Swennen *et al.* (2011)** reported that M-HA supplement at ion partially prevented the growth-depressing effects of chronic heat exposure compared with DL-M supplementation, and chick fed supplementation

of M-HA to the low-protein diet has lipid peroxidation lower and hepatic concentrations of total and reduced glutathione higher than their DL-M counterparts do. This result displayed better anti-oxidant status of M-HA chick. Therefore, M-HA's effect of elevating plasma uric acid and urea .Therefore, MHA's effect of elevating plasma uric acid and urea levels may improve animal health status. Moreover, M-HA bears a hydroxyl group instead of an amino group; it is an organic acid until it is converted to L-methionine. Antimicrobial properties of M-HA resemble those of organic acids such as lactic acid. M-HA has a pKa of 3.8 **(Dibner and Buttin 2002).** Moreover, antibacterial effect of M-HA has already been demonstrated **(Mercier *et al.* 2005).** Thus, M-HA might be possible to promote chickens' health by better antioxidant status and modulate the bacterial content of the gut.

Results of economic efficiency (EE) and relative economic efficiency (REE) estimated for experimental diets are based on recent local market prices for feed ingredients and selling price of live broilers. Chicks fed diets supplemented with (Ali-Met + threonine) had the best economic and relative efficiency values being 78.54 and 145.55% respectively. While using (Ali-Met) or (Met-HA-Ca) or different source of methionine + threonine increased net return, EE and REE of broiler chicks compared with those fed diets supplemented with (DL-Met) and the corresponding increment values in REE were 2.67, 30.80, 44.28, 45.55 and 29.99, respectively **El Faham et al.,2017).**

Histological examination:-

The effects of different dietary treatment on son the histology of small intestine are presented in Fig. (1), the histological examination showed that the birds that reared under the different dietary treatment were more improved intestine villi than those of bird's control. This improvement was occurred through increasing the length of villi. In addition, all layers of intestine section have improved by of different dietary treatment especially mucosa layer that contained crypts of Lieberkühn. It appears from the previous observations that different dietary treatment caused an improvement in the histological appearance of the small intestine as observed by increasing the number and size of crypts, and the morphological changes in villi height and diameter. The increased number and size of crypts was known to enhance nutrients digestibility via their pH regulation in the digestive tract. It is well known that the crypts of lieberkühn secrete fluids containing different vital substances essential for the internal microenvironment of the small intestine segments

Conclusion:-

In could be conclude that under tropical conditions, there was no significant difference between DLM and MHA supplementation on productive performance. Plasma methionine concentration was increased by DLM supplementation, while plasma urea and uric acid concentration were significantly increased by MHA supplementation.

Table 1:- Feed ingredients and chemical analyses of experimental diets:

Ingredients	**Dietary Treatments - Starter (0-14 days)**					
	Without Threonine			**With Threonine 1 g/ Kg**		
	1	**2**	**3**	**4**	**5**	**6**
Yellow Corn	56.04	56.00	56.00	55.94	55.90	55.90
Soy 44%	28.82	28.82	28.81	28.82	28.82	28.81
Corn Gluten 60%	8.97	8.97	8.97	8.97	8.97	8.97
Vegetable Oil	1.50	1.50	1.50	1.50	1.50	1.50
Ca Carbonate	1.59	1.59	1.59	1.59	1.59	1.59
MCP	1.84	1.84	1.84	1.84	1.84	1.84
Lysine HCl	0.39	0.39	0.39	0.39	0.39	0.39
DL-Met	**0.25**	**-**	**-**	**0.25**	**-**	**-**
Ali-Met	**-**	**0.29**	**-**	**-**	**0.29**	**-**
Met-HA-Ca	**-**	**-**	**0.30**	**-**	**-**	**0.30**
L-Threonine	**-**	**-**	**-**	**0.10**	**0.10**	**0.10**
Salt (NaCl) + Premix	0.60	0.60	0.60	0.60	0.60	0.60
Total	**100**	**100**	**100**	**100**	**100**	**100**
Chemical Analysis (Calculated)						
Crude Protein %	23.01	23.00	23.00	23.01	23.00	23.00
ME Kcal/ Kg diet	3002	3000	3000	3002	3000	3000

Calcium %	1.00	1.00	1.00	1.00	1.00	1.00
Available Phosphorus %	0.50	0.50	0.50	0.50	0.50	0.50
Lysine %	1.40	1.40	1.40	1.40	1.40	1.40
Methionine & Cysteine %	1.05	1.06	1.06	1.05	1.06	1.06
Threonine %	0.84	0.84	0.84	0.94	0.94	0.94
	Dietary Treatments - Grower (15 - 35 days)					
Yellow Corn	59.93	59.90	59.90	59.83	59.80	59.80
Soy 44%	26.38	26.37	26.36	26.38	26.37	26.36
Corn Gluten 60%	6.94	6.94	6.94	6.94	6.94	6.94
Vegetable Oil	2.50	2.50	2.50	2.50	2.50	2.50
Ca Carbonate	1.45	1.45	1.45	1.45	1.45	1.45
MCP	1.63	1.63	1.63	1.63	1.63	1.63
Lysine HCl	0.32	0.32	0.32	0.32	0.32	0.32
DL-Met	**0.25**	-	-	**0.25**	-	-
Ali-Met	-	**0.29**	-	-	**0.29**	-
Met-HA-Ca	-	-	**0.30**	-	-	**0.30**
L-Threonine	-	-	-	**0.10**	**0.10**	**0.10**
Salt (NaCl) + Premix	0.60	0.60	0.60	0.60	0.60	0.60
Total	**100**	**100**	**100**	**100**	**100**	**100**
Chemical Analysis (Calculated)						
Crude Protein %	21.00	21.00	20.99	21.00	21.00	20.99
ME Kcal/ Kg diet	3101	3100	3099	3101	3100	3045
Calcium %	0.90	0.90	0.90	0.90	0.90	0.90
Available Phosphorus %	0.45	0.45	0.45	0.45	0.45	0.45
Lysine %	1.26	1.26	1.26	1.26	1.26	1.26
Methionine & Cystein %	0.98	0.99	0.99	0.98	0.99	0.99
Threonine %	0.77	0.77	0.77	0.87	0.87	0.87

DL-Met: DL methionine, Ali-Met: Acid liquid methionine, Met-HA Ca: methionine hydroxy-analogue calcium salt, MCP: mono-calcium phosphate, AP: available phosphorus. Each 3 Kg of premix contains: Vitamins: A: 12000000 IU; Vit. D3 2000000 IU; E: 10000 mg; K3: 2000 mg; B1:1000 mg; B2: 5000 mg; B6:1500 mg; B12: 10 mg; Biotin: 50 mg; Colin chloride: 250000 mg; Pantothenic acid: 10000 mg; Nicotinic acid: 30000 mg; Folic acid: 1000 mg; Minerals: Mn: 60000 mg; Zn: 50000 mg; Fe: 30000 mg; Cu: 10000 mg; I: 1000 mg; Se: 100 mg and Co: 100 mg.

Table 2:- Effect of dietary treatments on bird performance.

Items	**Thréonine 1 g/ Kg (T)**	**Dietary Methionine Source (M)**			**Over all**
		DL-Met	Ali-Met	Met-HA Ca	
LBW (at 5 weeks)	Without	1268.63±48.38	1262.25±35.06	1485.29±90.20	1338.72[b]
	With	1412.71±40.85	1531.63±73.24	1402.17±48.58	1448.83[a]
	Overall	1340.67	1396.94	1443.73	
DWG (0-5 weeks)	Without	34.89±1.37	34.72±0.99	41.06±3.07	36.89[b]
	With	39.02±1.18	42.43±2.09	38.74±1.39	40.06[a]
	Overall	36.96	38.58	39.90	
DFI (0-5 weeks)	Without	65.50±4.21	58.29±4.81	69.62±3.73	64.47
	With	62.36±6.16	68.78±6.54	64.43±2.79	65.19
	Overall	63.94	63.54	67.03	
FCR (0-5 weeks)	Without	1.90±0.18	1.69±0.17	1.71±0.08	1.77
	With	1.60±0.17	1.61±0.07	1.66±0.04	1.63
	Overall	1.75	1.65	1.67	
Probability					
Trait	**T**		**M**	**T*M**	
LBW (at 5 weeks)	0.05		NS	NS	
BWG (0-5 weeks)	0.05		NS	NS	

DFI (0-5 weeks)	NS	NS	NS
FCR (0-5 weeks)	NS	NS	NS

(LBW) live body weight - (DWG) daily weight gain - (DFI) daily feed intake - (FCR) feed conversion ratio –

Means within the same row or column with different superscripts are significantly different. NS = Non Significant

Table 3:- Effect of dietary treatments on blood hematology parameters.

Items	**Thréonine 1 g/ Kg (T)**	**Dietary Methionine Source (M)**			**Overall**
		DL-Met	Ali-Met	Met-HA	
RBC	Without	4.53±0.85	3.36±0.20	3.62±0.27	3.84
	With	3.79±0.12	3.70±0.02	3.61±0.07	3.70
	Overall	4.16	3.47	3.61	
HB	Without	8.86±1.67	6.56±0.39	7.07±0.53	7.50
	With	7.40±0.23	4.81±2.41	7.04±0.13	6.56
	Overall	8.13	5.81	7.06	
Ht	Without	20.88±3.95	15.47±0.92	16.67±1.25	17.67
	With	17.44±0.56	11.35±5.67	16.61±0.32	15.47
	Overall	19.16	13.70	16.64	
MO	Without	48.54±13.51	47.18±31.68	56.19±3.81	50.79
	With	54.36±5.63	49.29±1.32	55.36±3.02	53.24
	Overall	51.04	48.45	55.72	
BA	Without	17.89±0.98	19.49±1.18	26.78±5.16	31.80
	With	24.53±5.39	34.39±8.38	37.13±9.59	21.70
	Overall	21.69	25.87	31.95	

Probability			
Trait	**T**	**M**	**T*M**
RBC	NS	NS	NS
HB	NS	NS	NS
Ht	NS	NS	NS
MO	NS	NS	NS
BA	NS	NS	NS

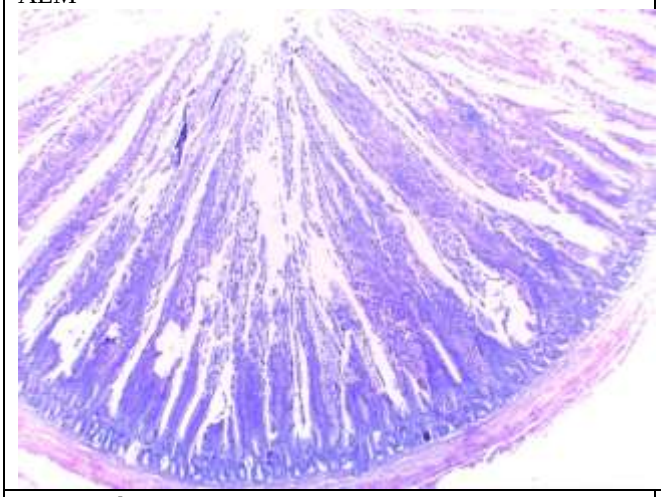
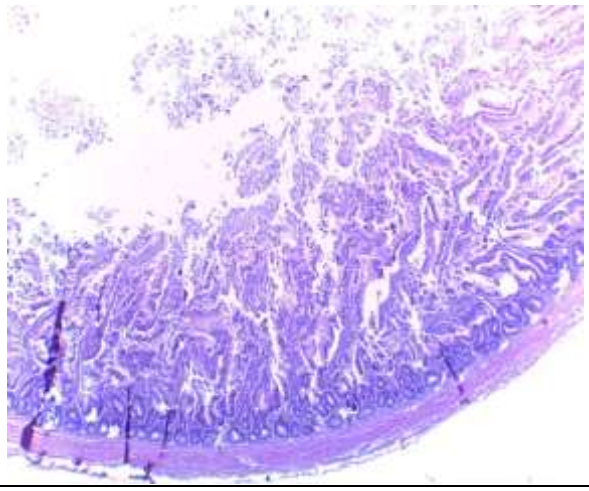
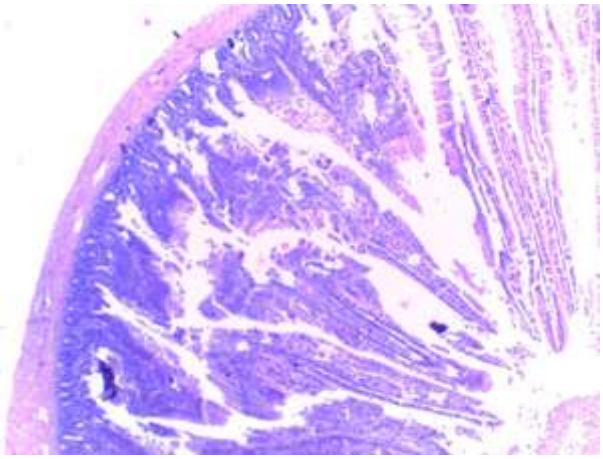
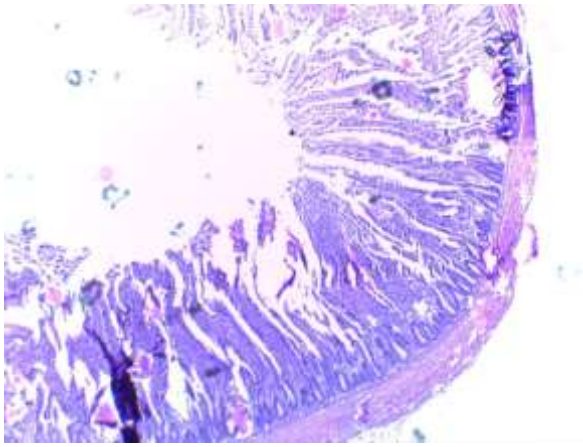
Means within the same row or column with different superscripts are significantly different. NS = Non Significant

Table 4:- Effect of dietary treatments on blood plasma parameters.

Items	**Thréonine 1 g/ Kg (T)**	**Dietary Methionine Source (M)**			**Overall**
		DL-Met	Ali-Met	Met-HA	
Total protein mg/dl	Without	3.38±0.67	3.41±0.31	3.28±0.99	3.36
	With	3.62±0.91	4.46±0.88	3.05±0.86	3.71
	Overall	3.50	3.94	3.17	
Albumin mg/dl	Without	1.34±0.22	1.57±0.33	1.47±0.30	1.46
	With	1.52±0.07	1.55±0.11	1.35±0.21	1.48
	Overall	1.43	1.56	1.41	
Globulin mg/dl	Without	2.04±0.54	1.84±0.54	1.81±0.84	1.89
	With	2.09±0.97	2.92±0.94	1.70±1.04	2.24
	Overall	2.07	2.38	1.75	
Albumin / Globulin ratio	Without	0.86±0.35	1.19±0.41	1.89±1.01	1.32
	With	2.02±1.20	1.19±0.76	4.22±2.89	2.48
	Overall	1.44	1.19	3.06	
Cholesterol mg/dl	Without	142.43±6.85	148.70±3.87	126.37±18.91	139.17
	With	152.14±20.61	152.22±9.22	121.71±7.08	142.03
	Overall	147.29	150.46	124.04	
Triglycerides mg/dl	Without	166.71±19.45	187.99±25.70	137.57±15.10	164.10

	With	212.14±73.10	154.42±17.68	247.57±73.90	204.71
	Overall	189.43	171.21	192.57	
Probability					
Trait	**T**	**M**	**T*M**		
Total protein	NS	NS	NS		
Albumin	NS	NS	NS		
Globulin	NS	NS	NS		
A / G ratio	NS	NS	NS		
Cholesterol	NS	NS	NS		
Triglycerides	NS	NS	NS		

Means within the same row or column with different superscripts are significantly different. NS = Non Significant

ALM 	ALM +TH 
DLM without 	DL + TH 
HA	HA + TH

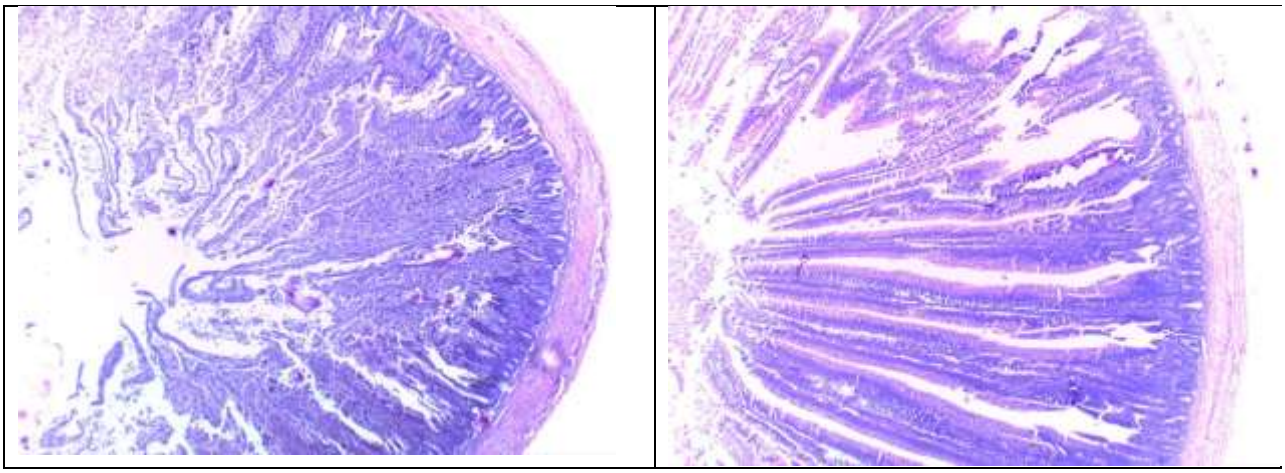


Fig. 1:- the histological structure (at 40x) of the small intestine from broilers fed different dietary methionine sources.

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