تأثير مصادر المثيونين المختلفة على الكفاءة الإنتاجية وحالة الأكسدة في بدارى التسمين

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(الإيداع:9 آيلول 2019 ، القبول 3 آذار 2020)

الملخص:

أجريت تجربة لدراسة تاثير مصادر المثيونين المختلفة على الكفاءة الإنتاجية وعلى حالة الأكسدة فى بدارى التسمين. ولما كان ذلك فقد استخدم عدد (510) من صيصان التسمين(سلالة روس) بعمر يوم واحد قسمت عشوائيا إلى ست مجموعات، وقد كان النظام الغذائى المتبع كالآتى: المجموعة الأولى (G1) غذيت بالعلائق الأساسية مع الدل مثيونين فى صورة بودرة ،المجموعة الثانية (G2) غذيت بنفس العليقة الأولى مع إضافة مركب BHT ، والمجموعة الثالثة (G3) غذيت بنفس العلائق مع استخدام MHA السائل، وتم تغذية المجموعة الرابعة (G4) بنفس العلائق مع مركب BHT، المجموعة الخامسة (G5) استخدم MHA–Ca فى صورة بودرة أماالمجموعة السادسة (G6) فقد أضيف إلى علائقها الأساسية شبيه المثيونين –MHA Ca مع BHT.

تم حساب التطورات في وزن الجسم، واستهلاك العلف ومن ثم حساب معدلات التحويل الغذائي في المراحل المختلفة من التجربة. جمعت عينات الدم بشكل فردي من خمسة طيور من كل تكرار في نهاية التجربة، وتم فصل مصل الدم لتحديد بعض مكونات مصل الدم، وجمعت العينات من الأنسجة الكبدية لتحديد مؤشرات الإجهاد التأكسدى، كما تم أيضا قياس مؤشرات نشاط الميتوكوندريا بالأنسجة الكبدية. وفي نهاية التجربة وعند عمر 42 يوما ذبحت الطيور ودرست مواصفات الذبيحة ومؤشرات بعض الأعضاء الداخلية.

هذا وقد أظهرت النتائج فروقا معنوية إيجابية وذات دلالة لمعدلات النمو وكفاءة استخدام الغذاء لصيصان المجموعات الأولى والثانية و الخامسة والسادسة، بينما كان هناك تاثير سلبي ومعنوي في المجموعات الثالثة و الرابعة. وعدم وجود تأثير معنوي على مكونات مصل والخمائر المدروسة. وأظهرت أيضا إرتفاع مستوى كلٍ من (GSH)، (CAT)، (GPx)، (المختزلGR)، وانخفاض مستوى إنزيم (MDA) في المجموعات الأولى والثانية والخامسة والسادسة، وتحسن واضح فى نشاط الميتوكوندريا بالخلايا الكبدية مع ارتفاع فى مستوى إنزيم المانوالهيد في المجموعات الثالثة والرابعة، وجد البحث فروقا معنوية ايجابية ذات دلالة فى أوزان عضلات الصدر للصيصان فى نفس المجموعات التى أظهرت نتائج إيجابية فى معدلات النمو ومؤشرات الإجهاد التاكسدى.

وخلصت نتائج هذه الدراسة إلى أنه من الممكن استخدام كل من (DL–Met) وشبيه المثيونين فى صورة ملح الكالسيوم MHA–Ca كمصدر للحامض الأمينى الأساسى والهام المثيونين فى علائق الدجاج اللاحم بما يغطى احتياجاتها لما لهما من تاثير فعال وإيجابى ليس فقط على معدلات النمو ومواصفات الذبيحة ولكن أيضا للحد من حالات الإجهاد التأكسدي التى قد تحدث في الدجاج وأيضا للجدوى الاقتصادية من استخدامها شريطة استخدامهم وحساب الكمية المستخدمة لتغطية الاحتياجات على أساس الكفاءة البيولوجية لكل منها.

الكلمات المفتاحية: المثيونين – دجاج اللحم – الإجهاد التأكسدي – مواصفات الذبيحة.

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Impact of different methionine sources on performance and oxidation status of broiler Chickens

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(Received: 9 September 2019, Accepted: 3 March 2020)

Abstract:

An experimental feeding trial was conducted to study the impact of different Methionine sources on performance and oxidation status of broiler chickens . A total of 510 One-day-old Ross (308) chicks were randomly assigned into six equal groups. (G1) was fed on basal diets with DL-M, (G2) was fed on the same diets with DL-M & BHT, (G3) was fed on the same diets with MHA-FAK, (G4) was fed on the same diets with MHA-FA (BE 65%) & BHT, (G5) was fed on the same diets with MHA- Ca, and (G6) was fed on the same diets with MHA-Ca & BHT. Results revealed significant ($P< 0.05$) positive effects of either DL-Met or MHA-Ca or in combination with BHT supplementation on most of the growth performance parameters, meanwhile the broiler chicks responded lesser to MHA-FA than DL-Met. None of serum parameters were affected except for the elevated levels of ALT of chicks in groups (G3 and G4). The highest (GSH), (CAT), (GPx) and (GR) and lowest significant MDA levels were also observed in the birds supplemented with DL-Met+ BHT (G2) and those supplemented with DL-Met alone (G1), also Mitochondrial Oxygen Consumption test as well as Mitochondrial function alteration in livers improve a situation that indicated the ability of both DL-Met or MHA-Ca to improve the oxidation status of the birds and confirmed the positive impact noticed in growth performance traits. The highest and significant (BMY%), were recorded in the birds supplemented with DL-Met+ BHT (G2) and those supplemented with DL-Met (G1).But the other carcass traits including organ indices seem to be not significantly altered . In addition groups received DLMet or MHA-Ca with or without fortification of BHT showed an increase in villus height and villus to crypt ratios. It could be concluded that the use of both D-Met and MHA-Ca with or without BHT supplementation had a better effect not only on growth performance, serum parameters carcass traits and gut morphology but also alleviated oxidative stress in broilers chickens. Knowing and understanding the relative biological value (RBV) of MHA-FA compared to DL-Met is an important precondition to cost-effective purchasing, feed formulation and broiler production.

Key words: Methionine, broilers, performance, oxidation stress, mitochondrial, carcass traits

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1- Introduction

The high rate of productivity of poultry results in relatively high nutrient needs. Methionine (Met.) is usually the first limiting amino acid in most broiler diets followed by lysine, threonine, and tryptophan or isoleucine. Met. is crucial to the production of meat, synthesis of enzymes and hormones. Hence, a deficiency of methionine is often a roadblock to achieve higher growth and better health performance. ***Esteve-Garcia & Austic (1987).*** Met. is a powerful antioxidant, and the sulphur it contains helps neutralize free radicals that are formed as a result of various metabolic process in the body. If not neutralized, free radicals interact with DNA and the proteins in healthy cell and damage tissues and organs. One of the important roles of sulphur containing amino acids Met. is the formation of glutathione which is the main antioxidant in the body. Met. products which are commercially available in the market in order to be used as Met. supplements includes DL-methionine as dry DL-Met (DLM; 99% pure), or Met-hydroxy analogue (MHA) as either liquid Met hydroxy analogue (MHA-FA) with (65%) biological efficiency, or as Met. hydroxy analogue calcium salt (MHA-Ca) containing 88% of active substance ***Thomas et al. (1991).*** All these sources allow for accurate balancing of the dietary amino acid profile, but they greatly differ regarding their chemical and physical properties. Many research activities in poultry have indicated that inferior digestion and transformation of MHA-FA to L-Met. so reduces their Met. value **(Koban and Koberstein 1984; Lemme, 2001; Drew et al. 2005). Xie et al., (2004). Hoehler et al.(2005)** stated that DL-Met and MHA-FA products greatly differ regarding their biological effectiveness because MHA-FA: (a) is not an amino acid in biochemical term and has to be converted into Met or cystine in metabolism (b) is not pure but contains 12% water and impurities; (c) is partially subjected to microbial degradation in the small intestine and hence not fully available for absorption; (d) is composed of mono-, di- and oligomers-, the latter being poorly absorbed. ***Robert et al. (2006)*** reported that there are biochemical and metabolic differences between liquid MHA-FA and DL-Met as MHA-FA is poorly utilized and in order to properly utilize liquid MHA-FA, it is essential to understand how it differs from DL-Met, and how these differences affect its biological role as a Met source.

2- Aim Of Work:

The objectives of the present study were to highlighting the impact of different Met sources on growth performance parameters, selected serum parameters, antioxidant biomarkers and carcass traits as well as the related gut histomorphological picture.

3- Material and methods

The current study was carried out at a private broiler farm located in Hehya town- Zagazig – Egypt and its protocol was approved by the Institutional Animal Care and Use Committee in Egypt.

3.1. Birds and husbandry

A total of 510 day-old- broiler chicks (ROSS 308) were obtained from the commercial hatchery of El-Wady Poultry company, they were divided into 6 experimental groups each of 5 replicates (17 bird/replicate).Chicks were reared in an open house system bedded by a layer of wood shaving with a constant lighting program employed during the whole experimental period (six weeks) and were provided with clean plenty drinking water. All birds were kept under standard hygienic conditions and were subjected to prophylactic vaccination and management program against viral and bacterial diseases during the whole experimental period.

3.2 Diets and Experimental Design

The birds were fed on basal diets which were formulated according to the requirement as recommended by the breed producer using Alex software linear programming. The experimental groups were as follow: the first group (G1) fed on basal diets supplemented with DL-Met (DLM; 99%), the second (G2) was fed the basal diets supplemented with DL-Met (DLM; 99%) + BHT, the third group (G3) was fed on basal diets supplemented with MHA-FA (65%). the fourth (G4) was fed on basal diets and supplemented MHA-FA (65%) +BHT, the fifth group (G5) was fed on the basal diets supplemented with MHA-Ca (88%) and the sixth group(G6) was fed on the basal diets supplemented with MHA-Ca (88%) +BHT. The experimental corn-soy based basal diets were formulated on the basis of the ideal protein concept (ileum digested A.A.), to meet the nutrient requirements of (ROSS 308) broiler chicks. The composition, calculated and chemical analysis of different diets according to ***AOAC (2005)*** are illustrated in table (1). All the experimental basal diets in different stages were analysed to determine their proximate chemical composition and their amino acids profile to insure mixing integrity of different methionine sources using amino acid auto analyser. Results of amino acids profile in different groups at different rearing stages were showed in tables (1).

3.3. Measurements, observations and statistical analysis

3.3.1 Growth Performance Parameters

Birds in different experimental groups were weighed at the initial time and then weekly until the termination of the experiment at 40 days of age. Body weight gain, feed intake were recorded and feed conversion ratios were calculated accordingly. Results showing the impact of different dietary treatments on growth performance traits are shown in table (2)

3.3.2 Serum Parameters

At the end of experiment, blood samples were collected after cervical dislocation of birds (3 birds/ replicate) and sera were separated, refrigerated and subsequently analyzed for the determination of serum total protein ***Gornal et al., (1949)*** Serum albumin ***Doumas et al., (1979)*** using commercial kits (Biodiagnostic).Serum globulin was calculated ***Coles, (1974).*** A / G ratio was calculated accordingly. Liver function Tests (**AST** (SGOT) and ALT (SGPT) were determined according to ***Reitman and Frankel,(1975)*** using commercial kits. Kidney function Tests (Uric acid ***Barham and Trinder, (1972).*** Serum creatinine ***Lasen, (1972)*** using commercial kits (Biodiagnostic).Results (table 3)

3.3.3 Ant-Oxidant biomarkers

Liver tissues were collected from all groups at end of experiment (2 samples / replicate) were taken for assessment of Glutathione reduced (GSH) ***Beutler et al. (1963).*** Glutathione Peroxidase (GPx) ***Paglia andValentine (1967).*** Glutathione reductase (GR) ***Goldberg and Spooner (1983)*** Catalase activity (CAT) ***Aebi (1984).*** and Lipid peroxidation "Malondialdehyde" (MDA) according to ***Kei, (1978).***Results of the examined ant-Oxidant biomarkers in different experimental groups are shown in table (4)

3.3.4. Mitochondrial Activities

Mitochondrial oxygen consumption measurement was done according to ***Hofhaus et al., (1996).*** Hydroxy-2'-deoxy-guanosine measurement. Competitive ELISA assays for 8-OHdG were performed according to ***Schmerold and Niedermüller, (2001)*** using 8-OHdG-EIA kit (OXFORD, USA). Results are shown in (table 5)

3.3.5. Carcass traits:

At 42 days of age, 3 birds from each replicate of were randomly chosen, left overnight in the waiting yard where only water was allowed. Each bird was weighted then hanged, slaughtered, scalded at 55-65 C^0, defeathered, eviscerated and washed with tap water. The carcass was then placed on a processing table where the carcass traits and organ indices were recorded according to ***El-Banna et al. (2008).***Results are shown in table(6).

3.3.5. Histomorphological examination

Specimens from duodenum at the end of experiment were taken (2 birds/ replicate) and fixed in 10 % formalin solution. These specimens were then dehydrated cleaned and embedded in paraffin wax blocks and were sectioned at 5 microns. Sections were stained by haematoxylin eosin method. Methods of histopathological techniques were adopted according to those of ***Carleton et al. (1967).*** Results are presented in (table 7)

3.3.6. Statistical analyses

The obtained data were calculated and statistically analysed according to **(Wayne, 1998)** using by SPSS software version 11 for Windows. The differences between groups were determined with variance analysis (one-way analysis of variance {ANOVA} using the probability level of 0.05 for the rejection of the null hypnosis. Significant differences among means were determined by the Student-Newman-kuel test. All data were recorded on an individual basis, except the feed consumption because of group feeding, thus no statistical analysis was performed for feed consumption and feed conversion ratio. Data were expressed as means SEM.

4- Results and Discussion

Impact of different Met sources on:

4.1.Growth Performance parameters

The overall cumulative growth performance Traits at the end of experimental period were illustrated in Tables (3a,3b,3c). Results revealed that the best growth performance was observed for the birds fed DL-Met and MHA-Ca since no significant (P< 0.05) differences in the Final body weight, cumulative weight gain of chicks in experimental groups fed on diets supplemented with DLMet. (G1) or supplemented with DL-Met+ BHT (G2) or supplemented with MHA-Ca (G5) and those supplemented with MHA-Ca +BHT (G6). Birds in G2 surpassing all groups and achieved the best weight gain. However Final body weight and the cumulative weight gains of chicks in groups (G3) and G4) were significantly reduced. Concerning the mean feed intake and mean feed conversion ratios no statistically significant differences were observed between the different dietary treatments using different methionine sources (except for G2), that is to say that the FCR seems to be not affected significantly by different Met. sources under the condition of our experiment. The significant (P< 0.05) positive effects of either DL-Met or MHA-Ca or in combination with BHT supplementation on most of the growth performance parameters irrespective of the source of supplemental methionine a situation that demonstrated a better efficiency of the broilers in these groups could be partially due to the fact that methionine stimulates growth by means of growth factors besides its influence on protein synthesis and breakdown. More or less similar observations were reported **by *Stubbs et al., (2002), Mandal et al.(2004) , Kimball and Jefferson (2006), Tesseraud et al.(2007) and Ana Paula et al. (2013*)**.Also may be due to the improvement in villus height and crypt/ villus ratio of duodenum as the small intestine, especially crypts and villi of the absorptive epithelium, plays a significant role in the final phase of nutrient digestion and assimilation ***Wang and Peng (2008).*** Since many research activities and studies have shown that one third of dietary intake

of essential amino acids (EAA) are utilized in first pass metabolism by the intestine ***Stoll et al. (1998).*** Moreover, metabolism of essential amino acids by the mucosal cells is quantitatively greater than amino acids incorporation into mucosal protein ***Stoll et al. (1998).*** So it has been proposed that the metabolism and functions of amino acids may represent a functional requirement by the intestine ***Stoll et al. (1998); Riedijk et al. (2007).***These findings also comes in accordance with the observations of ***Meirelles et al. (2003)*** who reported that DLMet. was more effective in promoting a better live performance of the birds when compared to methionine hydroxyl analogues. ***Kanokkarn Poosuwan et al.(2015)*** reported that it could be implied that adding DL-LMA in drinking water can be used as methionine source and an acidifier to achieve maximal growth performance of broiler chicks via the improvement of gut morphology. In addition to the above-mentioned Modality of action the positive impact of DL Met. Alone or in combination with BHT on growth could be attributed to the well-established fact that DL Met. Plays a very important role as a powerful antioxidant increasing the antioxidant capacity and reducing oxidative stress. These come in agreement with the findings of ***Brosnan and Brosnan (2006).*** Who reported that Met. is an important methyl donor for most biological methylation reactions, Met is also a precursor for cysteine, which plays a key role in maintaining protein function and redox status. In addition, Met serves as a precursor of glutathione, taurine, and inorganic sulfur, which are major cellular antioxidant ***Brosnan and Brosnan (2006).*** Thus, the functional role of Met. in gastric intestinal tract, especially its antioxidative effects, may be the key requirement of the growth and development of the gastric-intestinal tract of a rapid growing animal and consequently impact the growth. Also ***Robert et al. (2006)*** Reported that there are biochemical and metabolic differences between liquid MHA-FA and DL-Met. as MHAFA is poorly utilized and in order to properly utilize liquid MHA-FA, it is essential to understand how it differs from DL-Met, and how these differences affect its biological role as a Met source. On the other hand the significant ($P< 0.05$) negative impact of MHA-FA only or in combination with BHT in comparison to the other Met. sources may be attributed to the difference in physical and chemical properties between these products, these observations comes in agreement with those reported by ***Lemme et al. (2007).*** In addition it has been suggested also that differences in the response of broilers to different Met. sources could be influenced by the contents of cysteine and choline chloride (according to the proportional relationship between Met. and cystine provided by ***NRC (1994).*** These findings comes in accordance with the findings of ***Thomas et al.(1991) and Collin et al. (2003)*** who reported that the reduced efficiency in converting feed into body weight is associated, among other factors, to unbalanced diets or to the deficiency of a specific nutrient. Moreover ***Maenz and***

Engele-Schaan (1996a) and Drew et al. (2003) suggested that there was an interaction between the intestinal microfora and liquid MHA-FA which disrupts and decreases the ability of liquid MHA-FA to serve as a Met source for the animal. Although both DLMet and liquid MHA-FA are absorbed across the intestinal membrane in a similar fashion via active transport. These results are also supported by those of ***van Weerden et al. (1992),*** who reported that the polymeric forms of liquid MHA-FA had a lower bio- efficacy than the commercial mixture of mono-, di-, and oligomers, which they determined had a bio- efficacy of 66% relative to DL-Met. ***Okuno et al. (1989), Saunderson (1991), Crespo and Esteve-Garcia, (2002); Mandal et al.(2004)*** pointed out also that the broiler chicks responded lesser to MHAFA than DL-Met this may be further attributed to the time required for adjustment of the birds to the taste to MHA-FA, MHA-FA absorption efficiency which might be less, lesser conversion efficiency of MHA-FA to DL-Met may be due to lesser development of transamination process or inhibition of MHA-FA to the development of gastrointestinal tract. However, this point requires further investigation to prove these suppositions. The similar phenomenon is also observed previous researchers In another study ***Liu et al.(2003)*** reported that supplementation of DL-Met or MHA-FA did not improve growth performance and feed efficiency for broilers during 0-3 weeks of age, the contradiction may be attributed to the content of methionine or sulfur-containing amino acid in basal diet.

4.2. *On serum biochemistry*

Results (table (3) indicated that the, total Protein, albumin, globulin,A/ G ratio, ALT, AST, urea and creatinine were not affected by any of the dietary treatments except for the elevated levels of ALT of chicks in groups (G3 and G4) that were supplemented with MHA-FA and with MHA-FA+ BHT respectively. A situation indicated that the liver of such group was influenced by the use of MHA-FA as Met. Supplement and this could be attributed to the adverse effects of MHAFA on the antioxidant mechanisms of the bird as a sequence of decreased levels of Glutathione (GSH) , and elevated levels MDA levels as well as to Mitochondrial function alteration in livers. Concerning the other serum parameters results revealed that none of these parameters was significantly affected by supplementation of different Met. sources a condition that indicated that, the liver and kidney functions are normal, and the Met supplementation at the used level of fortification have no hepatotoxic or nephrotoxic effects on young and growing broiler chicks . Most published studies concerning the response of broilers to dietary supplementation of Met have been mainly focused on their effects on growth performance parameters and carcass characteristics. We are not aware of any study that was carried on the effects of Met. on blood parameters of broilers to justify our findings.

On oxidation stress indicators

Data concerning the Impact of different Met sources on some selected oxidation stress indicators in liver tissue including Glutathione (GSH), Glutathione peroxidase (GPx), Glutathione reductase (GR), Catalase(CAT) and Malonedialdehyde (MDA) levels of chicks in different experimental groups at the end ofthe illustrated in Table (4) . Results revealed that the highest significant Glutathione (GSH), Glutathione peroxidase (GPx), Glutathione reductase (GR) and Catalase (CAT) and the lowest Malondialdehyde (MDA) levels were observed in the birds supplemented with DL-Met+ BHT (G2) and those supplemented with DL-Met alone(G1). Meanwhile birds in groups (G3) that were supplemented with MHA-FA showed the lowest levels among the whole experimental groups. However no significant differences were detected among other experimental groups (G4, G5 and G6) which were supplemented with MHA-FA+BHT, DL-met and MHA-Ca and MHA-Ca +BHT respectively. The positive and significant effects of DL-Met on these levels in liver tissue could be explained on the basis that Met. in this form DL-Met or MHA-Ca orin combination with BHT supplementation is an important methyl donor for most biological methylation reactions, Met. is also a precursor for cysteine, which plays a key role in maintaining protein function and redox status. In addition, Met serves as a precursor of glutathione and increases total GSH production, taurine, and inorganic sulfur, which is major cellular antioxidant.. In addition these positive effects could be attributed in a part to the fact that supplementation of either forms of Met improved villus development. The positive impact of these forms of met. on (GPx) levels could be due to their role in potentiation of the anti-oxidant status by increasing the (GPx) production which protect from oxidative damage as it has the ability to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. GPx-1 is a seleno-protein and one of a family of peroxidases that reductively inactivate peroxides using glutathione as a source of reducing equivalents. GPx-1, in particular, is a major intracellular antioxidant enzyme that is found in the cytoplasm and mitochondria. More or less similar findings were observed by Similar observations were reported by **Kanzok et al. (2001) Brosnan and Brosnan, (2006), Glenda Chidrawi et al(2008), Bhabak and Mugesh (2010) and Deponte (2013)**

On mitochondrial activity

Mitochondrial functions include production of energy, activation of programmed cell death, and a number of cell specific tasks, e.g., cell signalling, control of Ca2+ metabolism, and synthesis of a number of important biomolecules. As proper mitochondrial function is critical for normal performance and survival of cells, mitochondrial dysfunction often leads to pathological

conditions resulting in various diseases. Recently mitochondrial dysfunction has been linked to multiple organ failure (MOF) often leading to the death.

Data (table) revealed that the lowest levels of the oxidative damage marker 8- oxodG in mtDNA were observed in the birds of (G2) and those of (G1). Meanwhile birds in group (G3) showed the highest concentration of DNA 8-OHdG levels among the whole experimental groups followed by (G4) which were supplemented with MHA-FA. However no differences were detected in the levels of among groups (G5 and G6). On the other hand, data concerning the mitochondrial function alteration as indicated by the levels of mitochondrial oxygen consumption and respiratory control ratio in liver tissue revealed that the highest levels were observed in the birds of (G2) and (G1). Meanwhile (G3) showed the lowest concentration of levels among the whole experimental groups followed by (G4). However no significant ($P< 0.05$) differences were detected in the levels of among groups (G5 and G6). In this work it is shown for the first time that the different methionine sources that are differ in their bioavailability may affects mitochondrial ROS generation and oxidative damage to mitochondrial DNA and proteins. This strongly suggests that the decrease in methionine intake is the cause of these adverse effects but its mechanisms of action are not fully explained in the currently available literature and this point requires further investigation to reach higher level of accuracy. But it is a well-established fact that the mitochondrial function, energy state, and ion homeostasis are disrupted during oxidative damage **Shi et al., (2012)**. It is becoming increasingly evident that mitochondrial DNA, which is less protected by associated histones than nuclear DNA, is a major target for oxidative damage **Garner (1980)**. Oxidation stress also caused impairments in hepatic mitochondrial respiration rates and led to alterations in mitochondrial ATPase activity **Sajan et al. (1995)**. In a number of studies, the ability of antioxidants to defend against free radicals when administered prior to or concomitantly with oxidative stress was demonstrated. Butylated hydroxytoluene and butylated hydroxyanisole were found to inhibit ROS in rats **Williams et al. (1986)**. The intake of Met, beta- carotene, ascorbic acid, selenium, uric acid, and vitamin E reduced the incidence of oxidative damage in rats **Nyandieka et al. (1990)**. Hepatic lipid peroxidation and serum activity of transaminases were reduced by the pretreatment of rats with antioxidants, Met, selenium, and vitamin E **Shen et al. (1994)**.

On carcass traits

Results (table 6) revealed that the highest and significant (BMY%), were recorded in the birds of (G2) and (G1). Meanwhile no significant ($P< 0.05$) differences were detected among other experimental groups. It seems that most of the carcass traits were not significantly altered as

a result of the use of different Met sources with exception of few unexpected changes in some traits. This improvement in the BMY% in Groups (G1) or (G2) may be explained on the basis of the overall improvement in growth performance as consequence of proper nutrient utilization efficiency, a situation that confirm the data concerning the influence of different Met sources on growth, nutrient utilization and antioxidant status of the birds. More or less similar findings were observed by **Schutte and Pack (1995)** who stated that dietary supplements of DLMet increase breast meat yield and decrease abdominal fat in growing chickens, this phenomenon indicates that meat retention transferred from thigh or other parts to breast when methionine added to reach some level. In addition to the above-mentioned the positive impact of DL-Met with its higher bioavailability in comparison to other Met sources or without extra synthetic antioxidant supplementation could be attributed to the well- established fact that Sulfur containing amino acids were easy to be deposited in breast meat **Nitzan and Paroush (1981),** therefore, it is a sensitive parameter to estimate the Relative Biological Value (RBV) for different methionine sources. Also these findings supported those reported by **Zelenka et al. (2013), Sangali et al. (2014)** who concluded that the bioavailability of MHA-FA for carcass yield and breast meat yield was significantly ($P < 0.05$) lower than that of DLMet on a weight-to weight and on equimolar basis and the bioavailability of MHA-FA for carcass yield and breast meat yield was significantly ($P < 0.05$) lower than that of DLM on a weight-to-weight and on equimolar basis. On contrary **Mandal et al. (2004)** reported that supplementation of DL Met or methionine hydroxy analogue improved growth, efficiency of feed utilization, eviscerated yield and breast yield.

On gut histo-morphological picture

Data (table7) revealed that the highest significant ($P < 0.05$) increases in villus height were observed in the birds of (G2) and (G1). Meanwhile birds in groups (G3) showed the lowest villus height among the whole experimental groups. However no significant differences were detected among other experimental groups. The significant positive impact DL-Met or MHA-Ca or in combination with BHT supplementation could be due to the major cellular antioxidant effects that mediated through its effects on different components of antioxidant defense mechanisms and in turn on the development of gastrointestinal tract also on the population of the intestinal micro flora, finally on the general health status of the birds and confirmed the beneficial effects noticed in growth performance, serum bio-markers, antioxidant indicators and carcass traits. These observations comes in accordance with those reported by **Thwaites and Anderson (2007)** who declared that the functional role of Met, especially its antioxidative effect, may be beneficial for the development of the gastric intestinal tract of a rapid growing

animal and its ability to exert a variety of biological functions in gastric intestinal tract level. **Kanokkarn Poosuwan et al. (2015)** reported that it could be implied that adding DL-LMA in drinking water can be used as Met source and an acidifier to achieve maximal growth performance of broiler chicks and its addition in drinking water significantly increased villous height, villous surface area and crypt depth in the duodenum and jejunum and achieve maximal growth performance of broiler chicks via the improvement of gut morphology. **Salary et al. (2015)** point out increasing levels of ALIMET inclusion in broiler chicken diets results in improvement in WG, serum antibodies titer, and intestinal beneficial bacteria in favor of harmful ones.

Table (1a): Physical composition of diets used in different stages of the experiment

Group / Ingredients	G1 & G2			G3 & G4			G5 & G6		
	Start.	Grow.	Finish.	Start.	Grow.	Finish.	Start.	Grow.	Finish.
Corn	532	624	664	530	623	662.2	531	624	665
SBM 48%Cp	320	250.5	210.6	319	250	210	322	252	211.5
C. Gluten (60%)	77.7	60	62	79.5	60.5	63.6	76.5	58.5	60
SB Oil	25	25	25	25	25	25	25	25	25
Premix*	2	2	2	2	2	2	2	2	2
MCP	17.4	15.5	14.3	17.4	15.5	14.3	17.4	15.5	14.3
CaCO3	16	13.5	13.1	16	13.5	13.1	15	12.5	12.3
Sod Bicarb	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
NaCL	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Lys- HCL	3.1	2.9	2.9	3.1	2.9	2.9	3.1	2.9	2.8
Threo	0.4	0.5	0.3	0.4	0.5	0.3	0.4	0.5	0.4
DL-Met**	2.2	1.9	1.6						
MHA-FA**				3.4	2.9	2.4			
MHA-Ca**							3.4	2.9	2.5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000

***broiler premix**: vitamin A 15.000 IU, vitamin D3 1.500 IU, vitamin E 20 mg, vitamin K3 5 mg, vitamin B13 mg, vitamin B2 6 mg, niacin 25 mg, vitamin B6 5 mg, vitamin B12 0.03 mg, folic acid 1 mg, D-biotin 0.05 mg, Ca-pantothenate 12 mg, carophyll-yellow 25 mg, and choline chloride 400 mg.

**Trace mineral premix (per kg of diet): Mn 80 mg, Fe 60 mg, Zn 60 mg, Cu 5 mg, Co 0.2

mg, I 1 mg, and Se 0.15 mg.

**DL-Methionine (Evonik supplier), Lysine HCL (Ajinomoto supplier), L-Threonine (Ajinomoto supplier) and BHT are supplied from a local company (Multi vita for animal nutrition). MHA-FA (Adisseo supplier) and MHA-Ca (Novus supplier) are been bought from a local company (Ronti Vita company).

NB, Premixes of T2, T4, T6 are enriched with BHT as anti-oxidant 150 g

Continued

Table (1b) :Chemical analysis and amino acids profile of diets used in different stages of the experiment

Group / Item	G1 & G2			G3 & G4			G5 & G6		
	Start.	Grow	Finish	Start.	Grow	Finish	Start.	Grow	Finish
Chem. analysis (calculated)									
ME Kcal/Kg	3025	3107	3155	3025	3025	3155	3025	3107	3155
Protein %	24.76	21.02	19.53	24.64	20.92	19.49	24.62	20.9	19.37
E.E	5.54	5.66	5.7	5.54	5.66	5.74	5.53	5.6	5.74
Calcium	1.05	0.8	0.85	1.05	0.9	0.85	1.05	0.9	0.85
Avail. Phos.	0.5	0.45	0.42	0.5	0.45	0.42	0.5	0.45	0.42
Cl	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Na	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
K	0.78	0.67	0.61	0.77	0.67	0.61	0.78	0.68	0.62
E.	204	178	162	203	177	162	204	178	163
*Electrolyte balance (mEq/Kg)									
Amino acids profile of diets used in in different stages of the experiment									
Lys.	1.38	1.17	1.06	1.38	1.17	1.06	1.38	1.17	1.06
Met.	0.62	0.54	0.49	0.62	0.54	0.49	0.62	0.54	0.49
Met+C.	1.02	0.88	0.82	1.02	0.88	0.82	1.02	0.88	0.82
Thr.	0.95	0.81	0.74	0.95	0.81	0.74	0.95	0.81	0.74
Trp.	0.26	0.22	0.2	0.26	0.22	0.2	0.26	0.22	0.2
Arg.	1.47	1.23	1.11	1.47	1.22	1.1	1.47	1.23	1.11
Ile.	1.03	0.86	0.79	1.03	0.86	0.79	1.03	0.86	0.78
Leu	2.45	2.1	2.02	2.46	2.11	2.03	2.44	2.1	2
Val.	1.13	0.96	0.89	1.13	0.96	0.89	1.13	0.96	0.89
SID Lys	1.26	1.07	0.97	1.26	1.07	0.97	1.26	1.07	0.97
SID Met	0.59	0.5	0.46	0.59	0.5	0.46	0.59	0.51	0.46
SID Cys	0.33	0.28	0.27	0.33	0.29	0.27	0.33	0.29	0.27
SID M+C	0.91	0.79	0.73	0.91	0.79	0.73	0.91	0.79	0.73
SID THR	0.8	0.69	0.63	0.8	0.69	0.63	0.8	0.69	0.63
SID Trp	0.23	0.18	0.17	0.22	0.19	0.17	0.23	0.19	0.17
SID Arg	1.36	1.13	1.02	1.35	1.13	1.02	1.36	1.13	1.02
SID Ile	0.46	0.77	0.71	0.46	0.77	0.71	0.92	0.77	0.7
SID Leu	0.72	1.91	1.84	2.23	1.92	1.85	2.21	1.91	1.83
SID Val	0.63	0.85	0.79	1	0.85	0.79	1	0.85	0.79
Met : Lys	0.18	0.47	0.47	0.46	0.47	0.47	0.47	0.47	0.74
M+C:Lys	0.72	0.74	0.75	0.72	0.74	0.75	0.72	0.74	0.75
Thr : Lys	0.63	0.64	0.65	0.63	0.64	0.65	0.63	0.64	0.65
Trp: Lys	0.18	0.17	0.17	0.18	0.17	0.17	0.18	0.17	0.17
Arg: Lys	1.08	1.06	1.05	1.07	1.06	1.05	1.08	1.06	1.05
Ile: Lys	0.73	0.72	0.73	0.73	0.72	0.73	0.73	0.72	0.73
Leu: Lys	1.76	1.79	1.9	1.77	1.78	1.91	1.76	1.78	1.88
Val: Lys	0.79	0.79	0.81	0.79	0.78	0.82	0.79	0.79	0.81

SID : standardized ileal dige

Table (2) :Cumulative growth performance parameters of broilers in different experimental groups along the whole experimental period (Mean ±SE)

Group / Parameter	(G1) DLMet	(G2) DLMet + BHT	(G3) MHA–FA	(G4) MHA–FA + BHT	(G5) MHA– Ca	(G6) MHA–Ca +
Av. Initial	47.83 ±0.81	47.65 ±0.82	47.81 ±0.103	48.63 ±0.74	48.33 ±0.96	48.66 ±0.88
Av. Final weight (g)	1743.93^{a} ±25.57	1761.77 ^{a} ±78.95	1546.35 ^{c} ±19.90	1678.01 ^{b} ±48.70	1744.91 ±63.22^{ab}	1734.98 ^{ab} ±65.28
Av. Weight gain(g)	1696.10^{a} ±25.76	1714.12^{a} ±78.77^{a}	1498.54^{c} ±19.75	1629.83^{c} ±47.91c	1696.58^{ab} ±63.44	1686.32 ^{ab} ±66.07
Av. Feed intake(g)	2875.13 ^{ab} ±59.13	2942.44 ^{a} ±35.85	2859.67 ^{b} ±75.40	2831.35 ^{b} ±46.34	2865.11^{ab} ±61.98	2876.31 ^{ab} ±85.17
Av. Feed conversion	1.70 ^{b} ±0.03	1.72 ^{b} ±0.07	1.91 ^{a} ±0.06	1.74 ^{b} ±0.03	1.69 ^{b} ±0.04	1.71 ^{b} ±0.03

a,b,.... Means within the same row, with different superscripts, are significantly different (P< 0.05).

Table (3): Selected Serum Parameters in Different Experimental Groups at the End of the experimental period (mean

Group / Parameter	(G1) DL Met	(G2) DL Met + BHT	(G3) MHA-FA	(G4) MHA-FA + BHT	(G5) MHA-Ca	(G6) MHA-Ca + BHT
Total Protein g/dl	5.95 ±0.45	5.99 ±0.34	6.12 ±0.57	5.93 ± 0.48	5.88 ±0.62	6.35 ± 0.71
Albumin g/dl	3.34 ±0.18	3.81 ±0.29	3.88 ±0.3	3.85 ± 0.25	4.11 ±0.24	4.00 ± 0.28
Globulin g/dl	2.36 ±0.21	1.93 ±0.17	1.99 ±0.18	1.83 ± 0.15	1.97 ±0.19	2.10 ± 0.18
A/G ratio	1.35 ±0.14	1.97 ±0.19	1.94 ±0.23	2.12 ± 0.25	2.09 ±0.74	1.89 ± 0.19
ALT (U/L)	7.60 ±3.35	6.80± 2.19	9.40 ±4.80	9.32 ± 3.35	8.40 ± 2.83	7.80 ± 2.19
AST U/L	19.86 ±1.14	19.75 ±1.12	19.24 ±0.95	19.72 ± 1.27	20.08 ±1.36	19.8 ± 1.25
Urea mg/dl	10.23 ±0.81	10.18 ±0.76	10.10 ±0.83	10.16 ± 0.75	9.95 ±1.11	9.42 ± 0.68
Creatinine mg/dl	1.43 ±0.09	1.44 ±0.11	1.77 ±0.5	1.12 ± 0.05	1.31 ±0.23	1.03 ± 0.07

Means with different letters (a, b, c, d) within the same row are significantly different at P value ≤ 0.05.

Table (4): Selected oxidation stress indicators in liver tissue (n5/group) of different experimental groups at the end of the experimental period (mean±SD)

Parameter \ Group	(G1) DL Met	(G2) DL Met + BHT	(G3) MHA- FA	(G4) MHA-FA + BHT	(G5) MHA- Ca	(G6) MHA- Ca + BHT
Glutathione (mmol/g tissue)	2.93 ^{a} ±0.53	3.12 ^{a} ±0.46 ^{a}	1.22 ^{c} ±0.53	1.98 ^{b} ±0.54	1.89 ^{b} ±0.34	2.22 ^{b} ±0.34
GPx (U/g tissue)	822.88 ^{ab} ± 89.04	869.57 ^{a} ±78.00	717.83 ^{c} ±51.19	782.03 ^{bc} ±17.67	801.48 ^{ab} ±26.28	817.04 ^{ab} ±34.39
Glutathione reductase (U/L)	759.19 ^{ab} ±45.26	784.91^{a} ±85.43	689.66 ^{c} ±14.79	710.96 ^{bc} ±46.73	723.02 ±14.93 ^{bc}	741.91 ^{abc} ±18.82
CAT (U/g tissue)	0.85 ^{a} ±.05	0.90 ^{a} ±0.04	0.42 ^{d} ±0.09	0.51^{c} ±0.06	0.63 ^{b} ±0.07	0.70 ^{b} ±0.05
MDA nmol/g tissue)	10.56 ^{cd} ±3.02	6.08 ^{d} ±1.87	19.20 ^{a} ±4.04	16.86 ^{ab} ±4.39	15.44 ^{ab} ±4.53	13.46 ^{bc} ±2.30

Means with different letters (a, b, c, d) within the same row are significantly different at P value ≤ 0.05.

Table (5): Mitochondrial DNA 8-OHdG content and Mitochondrial function alteration in liver tissues of different groups.

Group	DLM (G1)	DLM + BHT (G2)	MHA- FA (G3)	MHA-FA + BHT (G4)	MHA- Ca	MHA-Ca + BHT (G6)
Mitochondrial DNA 8-OHdG content	21.^{22b} ± 1.88239	11.84^{a} ±1.25004	39.28^{d} ±2.23168	31.16^{c} ±2.25269	29.68^{c} ±2.07085	27.34^{b} ±1.20025
Mitochondrial function alteration	3.18^{a} ±0.14967	3.56^{a} ±0.22935	1.88^{b} ±0.11136	2.2^{b} ±0.15811	2.66^{b} ±0.39319	3^{a} ± 0.2429

a,b,c…. Means within the same row, with different superscripts, are significantly different (P< 0.05)

Table (6): Carcass traits% in different experimental groups at the end of experimental period.

Parameter \ Group	(G1) DL Met	(G2) DL Met + BHT	(G3) MHA-FA	(G4) MHA-FA + BHT	(G5) MHA-Ca	(G6) MHA-Ca + BHT
Live weight	2236.00^{a} ± 140.64	2324.00^{a} ±117.60	2282.00^{a} ±129.31	2018.80^{b} ±123.65	2327.60^{a} ±121.57	2230.40^{a} ±113.99
Carcass %	77.37^{ab} ±2.20	76.53^{b} ±1.61	78.79^{ab} ±0.83	76.78^{b} ±2.99	76.72^{b} ±1.94	79.52^{a} ±2.37
Breast with bone%	32.20^{a} ±2.16	30.43^{ab} ±2.15	30.73^{ab} ±3.16	28.90^{b} ±2.15	29.66^{ab} ±3.56	28.96^{ab} ±1.01
Liver %	3.60 ±0.57	3.97 ±0.52	3.38 ±0.36 a	3.64 ±0.49 a	3.51 ±0.51 a	3.75 ±0.40 a
Thigh %	15.10 ±1.13 a	15.48 ±1.46 a	15.22 ±1.83	16.18 ±0.67	14.82 ±0.87	15.99 ±0.49
Drum stick %	11.11^{ab} ±0.76	11.94^{a} ±0.65	12.12^{a} ±2.08	12.49^{a} ±1.48	11.81^{a} ±0.40	10.01^{b} ±0.73
Wing %	7.28^{bc} ±0.36	7.19^{bc} ±0.29	6.96^{c} ±0.24	8.14^{a} ±0.62	7.61^{ab} ±0.51	7.66^{ab} ±0.40
Abdominal fat%	2.13 ±0.47	2.55 ±0.27	2.52 ±0.57	2.42 ±0.39	2.38 ±0.28	2.29 ±0.60
Heart %	0.51^{b} ±0.05	0.54^{ab} ±0.05	0.49^{b} ±0.07	0.59^{a} ±0.10	0.54^{ab} ±0.03	0.57^{ab} ±0.03
Gizzard %	1.98 ±0.56	1.82 ±0.33	1.87 ±0.23	2.12 ±0.33	2.34 ±0.30	2.23 ±0.69
Proventriculus %	0.46 ±0.02	0.43 ±0.10	0.47 ±0.10	0.51 ±0.07	0.43 ±0.06	0.50 ±0.07
Neck %	12.97 ±0.72	12.13 ±1.44	12.01 ±2.07	11.34 ±2.46	12.66 ±2.29	12.46 ±3.77

Means with different letters (a, b) within the same row are significantly different at p value ≤ 0.05.

Table (7) :selected Villi height, crept depth, and Villus: crept ratio in different experimental groups at the end of the experimental period.

Parameter \ Group	(G1) DL Met	(G2) DL Met + BHT	(G3) MHA- FA	(G4) MHA- FA + BHT	(G5) MHA- Ca	(G6) MHA- Ca + BHT
Villus height (µm)	937.41^{ab} ±64.9	976.17^{a} ±83.5	791.89^{b} ±66.4	798.68^{b} ±73.6	847.54^{ab} ±77.3	881.82^{ab} ±54.5
Crypt depth (µm)	212.69 ±22.5	216.52 ±22.8	179.33 ±17.2	187.89 ±19.3	204.5 ±18.43	209.11 ±22.5
Villus: crept ratio	4.41 ±0.31	4.51 ±0.65	4.42 ±0.75	4.25 ±0.49	4.14 ±0.66	4.22 ±0.58

a,b,c…. Means within the same row, with different superscripts, are significantly different (P< 0.05)

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