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Evaluation of Two Sources of Methionine and Antioxidants in Drinking Water upon Some Physiological and Biochemical Traits of ISA BROWN Laying Hens

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ABSTRACT

The experiment was conducted at the poultry farm of Animal Production Department-College of Agriculture-Kirkuk University during the period from 1/2/2020 to 24/4/2020 for 84 days (12 weeks) to evaluation of two sources of methionine and antioxidants in drinking water upon some physiological and biochemical traits of laying hens ISA BROWN. Used 200 layer aged 35 weeks were randomly assigned to 10 treatments with 5 replicates of 4 hens per replicate placed in a vertical batteries contain four floors and each floor contains three cages with dimensions (48×45×40 cm Length, width and height respectively) with one hen/cage. Fed and water was *ad libitum* and the treatments of this study were; T1: Basel diet meets of dietary requirement of methionine by adding liquid synthetic methionine, T2: Basel diet meets of dietary requirement of methionine by adding liquid herbal methionine, T3: Basel diet meets of dietary requirement of methionine by adding liquid synthetic methionine by 120 %, T4: Basel diet meets of dietary requirement of methionine by adding liquid herbal methionine by 120 %, T5: Basel diet meets of dietary requirement of methionine by adding liquid synthetic methionine by 110 %, T6: Basel diet meets of dietary requirement of methionine by adding liquid herbal methionine by 110 %, T7: Basel diet meets of dietary requirement of methionine by adding liquid synthetic methionine and adding herbal antioxidant Force®6, T8: Basel diet meets of dietary requirement of methionine by adding liquid synthetic methionine and adding synthetic antioxidant BHT, T9: Basel diet meets of dietary requirement of methionine by adding liquid herbal methionine and adding herbal antioxidant Force®6, T10: Basel diet meets of dietary requirement of methionine by adding liquid herbal methionine and adding synthetic antioxidant BHT, The results showed significantly differences for treatments adding liquid herbal and synthetic methionine by 110 % and 120 % in serum glucose, cholesterol , high density lipoprotein , GOT , GPT , MDA , GSH , percentage weight of overy and percentage weight of ovi duct. while no significantly differences ($P \leq 0.05$) in serum albumin, uric acid, percentage weight of abdominal fat and liver. Treatments addition herbal or synthetic antioxidant (Force®6 and BHT) with liquid herbal or synthetic methionine improved GOT, GPT, MDA and GSH compared with treatments no added of herbal or synthetic antioxidant (Force®6 and BHT) with liquid herbal or synthetic methionine.

Key words
methionine, antioxidants, physiological, biochemical, ISA BROWN

Introduction

Methionine is the first specific essential amino acid in poultry to obtain optimum production performance and high quality [1,2].With securing the positive functioning of vital activities in the body, including the action of methionine as an inhibitor of oxidation by considering methionine as a precursor or a generator of the compound Glutathione, especially the production of the enzyme (Glutathione Peroxidase) that breaks down peroxides and prevents the formation of free radicals that are damaged by living cells [3].the general trend towards the production of so-called organic poultry, alternatives to nutritional additives for naturally occurring poultry diets, which are parts of the plants of the plant kingdom called photobiotic [4,5], including the herbal essential amino acid Herbal methionine and in liquid form(Liquid Herbal methionine) showed that synthetic methionine is made from among the petroleum derivatives hydrogen cyanide, acrolein, methyl mercaptan.

Therefore, this study aimed to compare the efficiency of liquid herbal methionine in its action as an antioxidant agent compared to liquid synthetic methionine and natural (Force®6) and synthetic (BHT) antioxidants in their effecton some physiological characteristics related to criteria indicative of the comparison of oxidative stress through some criteria of blood biochemistry.

Materials and Methods

The experiment was conducted in the poultry field of the Department of Animal Production - College of Agriculture for the period from 1/2/2020 until 4/24/2020 for a period of 84 days (12 weeks) in addition to a 14-day introductory period before the actual experiment began to accustom chickens to the diets of the experiment with the

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aim of study of the use of two types of methionine in the diets of laying hens and its effect on productive performance, physiological and biochemical characteristics of blood, antioxidant properties, and some specific characteristics of laying hens eggs.

Randomly distributed 200 laying hens of the ISA Brown at 35 weeks of age to 10 feeding treatments with 5 replicates / treatment, each replicator included 4 birds placed in four-battery cages, and each floor contained two cages with dimensions of 48 x 45 x 40 cm length, width andheight . The birds fed were *ad libitum*, and water was available continuously, with the system of nipples and daily lighting was 17 hours of lighting compared to 7 hours of darkness according to the company’s guide, for a period of 12 weeks. Experiment treatments were: T1: basic diet, T2: basic diet that meets the nutritional requirement of methionine by adding liquid herbal methionine. T3: basic diet that meets the nutritional requirement of methionine by adding liquid synthetic methionine by 120%, T4: basic diet that meets the nutritional requirement of methionine by adding liquid herbal methionine by 120%, T5: basic diet that meets the nutritional requirement of methionine by adding industrial methionineamounted to 110%, T6: basic diet that meets the nutritional requirement of methionine by adding the liquid herbal methionine by 110%, T7:basic diet that meets the nutritional requirement of methionine by adding liquid synthetic methionine with the addition of a natural antioxidant Force®6 (as per the company's recommendation), T8: basic diet that meets the nutritional requirement of methionine by adding liquid synthetic methionine with the addition of an industrial antioxidant (BHT) (as recommended by the company), T9: basic diet that meets the nutritional needs of methionine with the addition of liquid herbal methionine with the addition of an industrial antioxidant (BHT) T10: basic diet that meets the nutritional needs of methionine by adding liquid herbal methionine with the addition of a natural antioxidant Force®6.

The chickens were weighed, then the treatments were fixed, and fed to the diets of the experiment shown in Table (1) for a period of 14 days as a preparatory period before starting the actual experiment, in order to make the chickens adapt to the new feed.Some biochemical characteristics were calculated, glucose, cholesterol, total protein, globulin, uric acid, GPT , GOT, and the antioxidant characteristics MDA and GSH.The results were analyzed statistically using the SAS program, (2001) and the Duncan test, (1955) to test the significance between the parameters at the 5% probability level.

Table (1): The percentages of forage materials included in the formation of experiment coefficients feeds with the calculated chemical analysis.

Feed material%	Nutritional Transactions									
	1	2	3	4	5	6	7	8	9	10
Crushed wheat	20.23	20.23	20.23	20.23	20.23	20.23	20.23	20.23	20.23	20.23
Crushed yellow corn	43	43	43	43	43	43	43	43	43	43
Soybean cake (47%)	21	21	21	21	21	21	21	21	21	21
Sun flower oil	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Di calcium phosphate	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83
Limestone	9.63	9.63	9.63	9.63	9.63	9.63	9.63	9.63	9.63	9.63
Mixed Vitamins and Minerals [a]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60% concentration)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Table salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Liquid Synthetic Methionine [b]	0.15	___	0.18	___	0.165	___	0.15	0.15	___	___
Methionine Herbal Liquid [b]	___	0.15	___	0.18	___	0.165	___	___	0.15	0.15
Force®6)[b]	___	___	___	___	___	___	0.05	___	0.05	___
BHT)[b]	___	___	___	___	___	___	___	0.05	___	0.05
Total %	100	100	100	100	100	100	100	100	100	100
Calculated chemical composition (c)										
Representative energy (Kcal / kg)	2908	2908	2908	2908	2908	2908	2908	2908	2908	2908
Crude protein%	16.32	16.32	16.32	16.32	16.32	16.32	16.32	16.32	16.32	16.32
The ratio of energy to protein	178	178	178	178	178	178	178	178	178	178
Methionine%	0.40	0.40	0.43	0.43	0.41	0.41	0.40	0.40	0.40	0.40
Lysine%	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
Calcium %	4.09	4.09	4.09	4.09	4.09	4.09	4.09	4.09	4.09	4.09
Available phosphorous%	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32

(a) -Obtained from Kosar Company for Feed and Poultry Materials / Erbil
(b) –It was obtained from the commercial markets in Erbil Governorate
(c) - According to the chemical composition of the American National Research Council (NRC) (1994).

Results and Discussion

The effect of using two types of methionine and two types of antioxidants in the diets of laying hens on blood biochemical characteristics:

High-density lipoprotein (HDL) glucose and cholesterol:

Table 2 shows the effect of using two types of methionine (synthetic and herbal) and two types of antioxidants (Force®6 and BHT) in laying hens' diets on the level of glucose (mg / 100 ml), cholesterol (mg / 100 ml) and high-density protein HDL mg/100 mL) in serum during the experiment.

It appears from the results of the statistical analysis that there are significant differences between treatments,T5 and T6 gave the highest blood glucose rate and significantly ($P \leq 0.05$) over all the experimental treatments.

Table (2): The effect of using two types of methionine and two types of antioxidants in the diets of laying hens on the level of glucose, cholesterol and high-density protein fats in the blood serum (mean ± S.E)

Treatments	Glucose (mg/100 ml)	Cholesterol (mg/100 ml)	HDL (mg/100 ml)
T1	1.17±34.86 d	3.68±60.42 abc	1.49±62.52 c
T2	0.85±40.40 bcd	2.18±70.00 a	1.79±68.94 bc
T3	3.78±47.08 b	9.33±66.41 ab	0.26±66.92 bc
T4	1.50±43.99 bc	3.78±55.12 bcd	0.17±68.01 bc
T5	1.64±55.17 a	3.51±46.67 cd	1.79±77.63 a
T6	3.02±58.20 a	1.05±49.00 cd	1.43±68.63 bc
T7	1.57±47.88 b	3.35±48.75 cd	1.79±73.91 ab
T8	1.52±38.87 cd	4.32±48.62 cd	1.70±73.13 ab
T9	2.68±37.49 cd	2.57±45.85 d	5.55±75.46 ab
T10	4.08±44.21 bc	4.29±42.06 d	4.12±72.04 ab
significantly	*	*	*

- NS – There were no significant differences within the column at the ($P \leq 0.05$) probability level.The first treatment is to add liquid synthetic methionine as needed, the second treatment is to add liquid herbal methionine as needed, the third treatment is to add liquid synthetic methionine by 120% more than the need, the fourth treatment to add liquid herbal methionine 120% more than needed. ‹The fifth treatment is an addition of 110% more synthetic methionine than the need, the sixth treatment is an addition of 110% more liquid synthetic methionine than is needed, the seventh treatment is an addition of liquid synthetic methionine as needed with Force®6 antioxidant, the eighth treatment is an addition of liquid synthetic methionine as needed.With BHT antioxidant, ninth treatment add liquid herbal methionine as needed with Force®6 antioxidant, tenth treatment add liquid herbal methionine as needed with BHT antioxidant.

for blood cholesterol, the table above showed that adding synthetic methionine and herbal methionine by 110% and 120% more than the nutritional requirement and adding antioxidants (Force®6 and BHT) improved the level of blood cholesterol, T2 gave the highest level of cholesterol in the blood, which did not differ significantly with T1 and T3,significant decrease in the level of blood cholesterol was recorded, especially the treatments that were added to

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the herbal methionine with the antioxidants Force®6 and BHT (T9 and T10).T5 recorded highest rate of HDL, and did not differ significantly with the T7, T8, T9 and T10, while T1 recorded lowest rate of HDL in the blood.

Total protein, albumin and globulin:
Table 3 showed the effect of using two types of methionine (synthetic and herbal) and two types of antioxidants (Force®6 and BHT) in laying hens' diets on the level of total protein (mg / 100 mL), albumin (mg / 100 mL) and globulin (mg / 100 mL) in blood serum,The difference between the total protein levels was significant (P≤0.05)different, T2, T3, T4, T5, T6 and T10 were highest for total protein, while T1 and T7 gave the lowest level for total protein inThe serum of the laying hens, T8 and T9 did not differ significantly compared with all the experimental treatments, while it was noticed that there were no significant differences (P≤0.05) between all the trial treatments regarding the level of albumin in the blood serum.

Table (3): The effect of using two types of methionine and two types of antioxidants in the diets of laying hens on the level of total protein, albumin and globulin in blood serum (mean ± S.E)

Treatments	Total protein (mg/100 ml)	Albumin (mg/100 ml)	Globulin (mg/100 ml)
T1	1.44±15.77 b	0.97±6.89	2.33±8.87 b
T2	0.20±23.45 a	0.92±5.32	1.04±18.13 a
T3	1.22±21.72 a	0.34±5.54	1.55±16.18 a
T4	0.94±20.83 a	0.80±6.52	1.69±14.30 ab
T5	0.45±21.64 a	1.09±5.27	0.71±16.36 a
T6	0.94±23.16 a	0.42±7.43	0.61±15.73 a
T7	0.52±15.12 b	1.03±6.96	1.55±8.16 b
T8	2.40±19.66 ab	0.85±6.87	3.17±12.79 ab
T9	2.69±19.29 ab	0.35±4.84	2.63±14.44 ab
T10	1.58±22.75 a	0.96±6.42	2.38±16.32 a
significantly	*	NS	*

- NS – There were no significant differences within the column at the (P≤0.05) probability level.The first treatment is to add liquid synthetic methionine as needed, the second treatment is to add liquid herbal methionine as needed, the third treatment is to add liquid synthetic methionine by 120% more than the need, the fourth treatment to add liquid herbal methionine 120% more than needed. ‹The fifth treatment is an addition of 110% more synthetic methionine than the need, the sixth treatment is an addition of 110% more liquid synthetic methionine than is needed, the seventh treatment is an addition of liquid synthetic methionine as needed with Force®6 antioxidant, the eighth treatment is an addition of liquid synthetic methionine as needed.With BHT antioxidant, ninth treatment add liquid herbal methionine as needed with Force®6 antioxidant, tenth treatment add liquid herbal methionine as needed with BHT antioxidant.

As for the level of globulin in blood, it is noticed in the above table that T2, T3, T5, T6 and T10 gave the highest significant level of globulin (P≤0.05), while T1 and T7 recorded the lowest level for blood serum.

Uric acid, GOT and GPT levels:
Table 4 shows the effect of using two types of methionine (synthetic and herbal) and two types of antioxidants (Force®6 and BHT) on the level of uric acid , GOT and GPT in laying hens.It is evident from the table that there were no significant differences between all treatments in the level of uric acid in the blood serum of bird, As for the effectiveness of the GOT enzyme, it is noted that there are significant differences (P≤0.05) between the experimental treatments, as well as the treatments for which the antioxidants of Fores®6 and BHT were showed a significant

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decrease (P≤0.05) in the level of the GOT enzyme compared with the treatment that was added.It has methionine as per the recommended nutritional requirement ،As for the level of activity of the GPT enzyme, it is noticed through the table that there are significant differences (P≤0.05) between the experimental treatments.

Table (4): The effect of using two types of methionine and two types of antioxidants in the diets of laying hens on the level of uric acid and the effectiveness of GOT and GPT enzymes in the blood serum (mean±S.E)

Treatments	Uric acid (mg/100 ml)	GOT (IU/ml)	GPT (IU/ml)
T1	1.05±7.45 a	0.45±4.15 a	0.23±9.59 a
T2	0.26±7.32 a	0.14±3.34 bc	0.60±9.46 a
T3	1.52±9.51 a	0.33±3.60 ab	0.75±6.48 c
T4	0.39±9.88 a	0.10±2.47 d	0.31±9.76 a
T5	2.02±8.47 a	0.21±3.49 abc	0.95±6.79 bc
T6	0.65±9.72 a	0.07±2.76 cd	0.48±8.20 abc
T7	0.06±10.35 a	0.18±3.09 bcd	0.28±8.00 abc
T8	1.48±8.05 a	0.26±2.90 bcd	0.41±8.24 ab
T9	1.22±8.11 a	0.18±3.14 bcd	0.21±7.16 bc
T10	1.62±7.63 a	0.13±2.84 bcd	0.50±8.45 ab
significantly	NS	*	*

- NS – There were no significant differences within the column at the (P≤0.05) probability level.The first treatment is to add liquid synthetic methionine as needed, the second treatment is to add liquid herbal methionine as needed, the third treatment is to add liquid synthetic methionine by 120% more than the need, the fourth treatment to add liquid herbal methionine 120% more than needed. ،The fifth treatment is an addition of 110% more synthetic methionine than the need, the sixth treatment is an addition of 110% more liquid synthetic methionine than is needed, the seventh treatment is an addition of liquid synthetic methionine as needed with Force®6 antioxidant, the eighth treatment is an addition of liquid synthetic methionine as needed.With BHT antioxidant, ninth treatment add liquid herbal methionine as needed with Force®6 antioxidant, tenth treatment add liquid herbal methionine as needed with BHT antioxidant.

The improvement in most of the biochemical characteristics, including the concentration of serum glucose, may be attributed to the fact that adding methionine has a positive role in reducing the secretion of the hormone corticosterone to the lowest level and this leads to a decrease in the occurrence of gluconeogenesis and makes there a balance in the glucose in the blood or stimulates the secretion of the hormone insulin from pancreatic beta cells,Consequently, increases the entry of glucose into the tissues and maintains its normal level in the blood [6,7]. [8] did not notice any significant effect on the concentrations of glucose, Control indicates that adding methionine to both sources and in all proportions did not raise the concentration of uric acid in the blood, meaning that the body did not make any effort to get rid of excess methionine through the process of removing the amine group Deamination and the formation of uric acid and total protein, it means that adding both sources of methionine and in all proportions did not lead to the occurrence of any negative factor that raises the concentration of uric acid in the blood serum of birds such as cases of hunger, gout, severe tissue destruction, kidney diseases and kidney calcification caused by high levels of calcium and Uric in serum. noted from Table 2 that the addition of methionine led to a significant improvement in the concentration of serum cholesterol, and this may be due to the fact that these percentages of methionine were suitable for conjugation of taurine, which is the average product from the metabolism of methionine with bile acids, which in turn worked on the formation of emulsifying agents and obtainingphysical breakdown of fat, reduction of surface tension, and increased surface area of lipid droplets for the

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action of the lipase enzyme [9].The lower concentrations of triglycerides and cholesterol may be attributed to the fact that these percentages of methionine were the best in the contribution of giving the methyl group CH3 and the formation of significant amounts of phosphatidylcholine (lecithin), which reducedthe concentrations of triglycerides and cholesterol in the blood serum of birds [10].Lecithin lowers cholesterol according to the emulsification mechanism [11].With regard to GOT and GPT enzymes, where these enzymes work to transfer the amino group from amino acids to ketone acids. Conversely, the absence of clear significant differences in GOT and GPT enzyme activity in white chicken serum when adding two sources of methionine and their concentrations may be attributed to the addition of Synthetic and herbal methionine did not have any negative effect on the activity of some internal organs such as the heart, liver, kidneys and muscles, as the increase of these two enzymes is linked to the health of these organs. Consistent with results [12,13] who observed that GOT and GPT enzyme activity were unaffected and were within the normal level when adding herbal methionine to either laying hens or broilers.The ability of antioxidants to lower blood glucose levels can be attributed to the inhibitory mechanism by which antioxidants act on the hormones of the adrenal cortex the glucocorticoids and reduce the level of corticosterone (CS) in the blood serum, the hormone responsible for Gluconeogenesis (proteins and fats) [14], thus reducing the level of glucose in the blood, which is the reason why the resulting improved productive performance through the effect it has in inhibiting the degradation of fats and proteins by the process of Gluconeogenesis [15,16]synthesizing glucose and obtaining energy from non-carbohydrate sources.As for the ability of antioxidants to reduce the level of cholesterol and triglycerides in the serum of laying hens, the addition of antioxidants may increase the activity of the thyroid gland and the higher its activity.By increasing the weight of the thyroid gland, this is indicated by [17]agreed also with [18,19] who indicated a significant decrease in the level of blood cholesterol.The significant decrease in the level of cholesterol may be due to the positive action of antioxidants in affecting the thyroid gland responsible for cholesterol metabolism and increasing the activity of its hormones, especially the hormones Thyronine Tri-iodine (T3) and Thyroxine (T4), which leads to lowering the concentration of cholesterol in the blood serum.Concerning the results of Table (3), the increase in the level of total protein in the serum resulting from the addition of antioxidants is consistent with what [20,21] who indicated that the addition of antioxidants caused a significant increase in the level of total protein concentration in broiler blood serum.As for the effect of antioxidants on the level of GOT and GPT enzymes, Table (4) the reason for the absence of significant differences in the level of GOT and GPT enzymes in the blood serum of birds in some treatments during the period of the study may be due to what [22] stated regarding the stability of the level of these two enzymes.In serum, it is an indication that the liver and kidney functions were not significantly affected. The level of both GOT and GPT is considered an indicator indicating the integrity of cells and the cell membrane [23] and that increased levels in the blood are an indication of leakage from cells or loss of the cell membrane of the permeability characteristic.This decrease in the effectiveness of GPT and GOT enzymes when treated with antioxidants is due to the effectiveness of antioxidants in sweeping the active oxygen species, which in effect reduce the risk of oxidative stress and lipid peroxidation represented by the generation of active oxygen varieties that work to destroy different body cells [24,25]. Or it may be attributed to the low level of MDA and the high level of GSH due to the presence of a highly significant positive correlation coefficient with the activity of the enzyme GOT, GPT gives an indication of the preservation of antioxidants on body proteins by reducing the production of the hormone corticosterone from the adrenal cortex, which works on the production of glucose from sources Non-carbohydrates [26].

The effect of using two types of methionine and two types of antioxidants in the diets of laying hens on the antioxidant properties:

Table 5 shows the effect of using two types of methionine (synthetic and herbal) and two types of antioxidants (Force®6 and BHT) in laying hens diets on the antioxidant status represented by the levels of MDA and glutathione (GSH) in blood serum.It is evident from the table that there are significant differences ($P \leq 0.05$) between the experimental parameters at the MDA and GSH level, T3, T4, T5, T7, T8 and T10 recorded lowest level of MDA, while T1 and T2 recorded highest level of MDA.As for the status of anti-oxidant GSH, it is evident from the table 5 that there are significantdifferences ($P \leq 0.05$) between the experimental parameters, represented in the high level of GSH for T4, T7 and T8, as this rise rose to the level of significance ($P \leq 0.05$) compared to the T2While it did not differ significantly with the rest of the experiment treatments.It is concluded from the results of the statistical analysis represented in the table 5 that adding methionine to the synthetic herbal by 110% and 120% higher than the recommended nutritional requirement and adding Force®6 and BHT antioxidants improved the antioxidant status by lowering the level of MDA and increasing the level of GSHIn serum.Methionine has a major role in raising the level of GSH and lowering the level of MDA. Methionine has shown a tendency to reduce the effects of oxidative stress. It is reported that when methionine is treated with the oxidizing agent, it changes into Methionine Sulfoxide. Thus, the surface methionine residues provide a high concentration of the reactants which are sufficient to beTraps for oxidizing agent,In addition, methionine may be reversibly reduced by the enzyme Methionine sulfoxidereductase, which leads to catalytically stimulating antioxidant action within the body[27].[28] indicated that treatment with antioxidants reduced the increase in lipid peroxidation in the testes and raised the level of testicular GSH in rats compared with alloxan-treated rats, and this protective effect against the induced oxidative effect is by maintaining the effectiveness of cellular antioxidants.

Table (5): The effect of using two types of methionine and two types of antioxidants in the diets of laying hens on the level of antioxidants in the blood serum (mean ± S.E)

Treatments	MDA µml/mol	GSH µml/mol
T1	0.01±1.39 ab	0.39±3.70 ab
T2	0.03±1.47 a	0.32±3.08 b
T3	0.03±1.24 c	0.60±4.06 ab
T4	0.06±1.23 c	0.29±4.59 a
T5	0.02±1.22 c	0.24±4.48 ab
T6	0.03±1.26 bc	0.10±4.18 ab
T7	0.05±1.16 c	0.58±4.65 a
T8	0.07±1.21 c	0.33±4.94 a
T9	0.06±1.25 bc	0.53±4.50 ab
T10	0.04±1.24 c	0.59±4.44 ab
significantly	*	*

- NS – There were no significant differences within the column at the ($P \leq 0.05$) probability level.The first treatment is to add liquid synthetic methionine as needed, the second treatment is to add liquid herbal methionine as needed, the third treatment is to add liquid synthetic methionine by 120% more than the need, the fourth treatment to add liquid herbal methionine 120% more than needed. ‹The fifth treatment is an addition of 110% more synthetic methionine than the need, the sixth treatment is an addition of 110% more liquid synthetic methionine than is needed, the seventh treatment is an addition of liquid synthetic methionine as needed with Force®6 antioxidant, the eighth treatment is an addition of liquid synthetic methionine as needed.With BHT antioxidant, ninth treatment add liquid herbal methionine as needed with Force®6 antioxidant, tenth treatment add liquid herbal methionine as needed with BHT antioxidant.

References

[1] Ahmed, M.E., and Abbas T.E. (2015). The effect of feeding herbal methionine versus dlmethionine supplemented diets on broiler Performance and carcasscharacteristics. *International Conference on Agricultural Ecological and Medical Sciences,10-11.*

[2] Manju , G. U ; B. S. V. Reddy ; Gideon Gloridoss ; T. M. Prabhu ; K. S. Giridhar ; and N. Suma . (2015). Effect of supplementation of lysine producing microbes vis-a-vis source and level of dietary protein on performance and egg quality characteristics of post-peak layers. *Veterinary World, EISSN: 2231-0916.*

[3] Thomas Willke. (2014). Methionine production—a critical review. *ApplMicrobiolBiotechnol. 98:9893–9914.*

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 17930- 17938
Received 25 April 2021; Accepted 08 May 2021.

[4] Kalbande, V.H., K. Ravikanth, S. Maini and D.S. Rekhe, (2009). Methionine supplementation options in poultry. *Int. J. Poult. Sci., 8: 588-591.*

[5] Hasan Al-Hadeedy, I. Y., Al-Nuaimi, M. I., & Ali, A. R. (2019). Effect of Adding Local Minerals Clay (Meqdadia) in the Female Japanese quail Diets upon Production Performance, Egg Quality and Carcass Traits. *Indian Journal of Public Health Research & Development*, *10*(8).

[6] Sturkie, P. (2000). Avian Physiology. *5th Ed. New York, Heidelberg, Barlin, Springer Verlag.*

[7] AL-Dalawi, R. H. H., & AL-Hadeedy, I. Y. H. (2019). Influence of different levels of dietary supplementation phytase enzyme upon growth performance, carcass traits and some biochemical parameters of local japanese quail. *Plant Archives*, *19*(2), 2287-2293.

[8] Makinde, O.J., A.J. Ajibade, S.K. Omotugba, M.S. Tamburawa, E.A. Ibe, E. Opoola and O.S. Zaccheaus. (2017). Herbal methionine (methiorep®) improves growth performance of broiler chickens without affecting carcass characteristics and blood indices. *Tropical and Subtropical Agroecosystems, 20: 131 – 139.*

[9] Tufft, L. S., and L. S. Jensen (1992). Influence of dietary taurine on performance and fat retention in broilers and turkey poults fed varying levels of fat. *Poultry Science, 71:880–885.*

[10] Navder, K.P., E. Baraona. and C. Lieber. (1997). Polyenyphosphatidylcholine decreases alcoholic hyperlipemia without affecting the alcoholinduced rise of HDL cholesterol. Life Sciences; 61:1907.

[11] Rousset .X., B. Vaisman, M. Amar, A. A. Sethi, and A. T. Remaley.(2009). Lecithin: cholesterol acyltransferase: from biochemistry to role in cardiovascular disease. *Current Opinion in Endocrinology, Diabetes and Obesity, 16(2): 163–171.*

[12] Rekhatel, D.H., S.Maini, V.P. Panthak, S.V. Ther, N. Datir, D.K. Kale and, R.P. Hardas.(2010). Haemobiological profile study in commercial broilers fed on diet supplemented with herbal methionine (methiorep®) and DL-methionine. *Indian Journal of Agricultural Research, 44: 197-203.*

[13] Igbasan, F.A., Ibrahim, A.M., Osho, B.I. (2012). Comparative efficacy of herbal and synthetic methionine on performance of some haematological and biochemical parameters in domestic laying hens. *African Journal of Biotechnology. 11(46):10617-10625.*

[14] Attia, K. H. M., Tawfeek, F. A., Mady, M. S., and Assar, M. H. (2015). Effect of dietary chromium, selenium and vitamin C on productive performance and some blood parameters of local strain Dokki-4 under Egyptian summer conditions. *Egypt. Poult. Sci, 35, 311-329.*

[15] Bashir, S., I. Peter, R. L.O., Aka, A. M., Umaru, A. J. Is'haq, M. M. Oyiza, H. M. Ibrahim, B Garba and Bello, M. B. (2013). Effect of water restriction and ascorbic acid supplementation on hematology and serum biochemistry of Japanese quails in sokoto, *Nigeria. Saidu. Ijavms, vol. 8(2): 34-40.*

[16] Sahin, N., M. Onderci, K. Sahin, M.F. Gursu and Smith, M.O. (2014). Ascorbic acid and melatonin reduce heat-induced performance inhibition and oxidative stress in Japanese quails. *British Poultry Science Volume 45, Number 1. 116–122.*

[17] Sturkie, P. D. (1986). Heart: contraction, conduction, and electrocardiography. *In Avian physiology (pp. 167-190). Springer New York.*

[18] Saleem, Q.R., Sahni, Y. P., and Singh, S. K. (2011). Effect of Emblicaofficinalis on Serum Lipid Profile in Birds. *Journal of animal and veterinary advances, 10(21), 2792-2796.*

[19] Kumari. K.S. P., S. N. Tiwari, M.J. Saxena, K. Ravikanth and S. Maini. (2012). Studies on comparative efficacy of herbal amino acid (Methiorep) supplement with synthetic dl-methionine on broiler growth performance and carcass quality traits. *International Journal of Scientific and Research Publications, 2(8): 1-5.*

[20] Ferit, M. G., Onderci, M., Gulcu, F., and Sahin, K. (2004). Effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress in vivo. *Nutrition Research, 24(2), 157-164.*

[21] Rindhe, S., U. S. Shrinivas, K., Kakasaheb, Kotagiri, R.A., and Reothia, A. (2015). Effect of supplementation of herbal antistress product and synthetic vitamin C on performance of broiler birds under physiological heat Stress. *World J Pharm Sci 2016; 4(1): 54-59.*

[22] Ismail, I. B., Al-Busadah, K. A., and El-Bahr, S. M. (2013). Oxidative stress biomarkers and biochemical profile in broilers chicken fed zinc bacitracin and ascorbic acid under hot climate. *Am J BiochemMolBiol, 3, 202-214.*

[23] Olayinka, E. T., Ore, A., Ola, O. S., and Adeyemo, O. A. (2014). Protective effect of quercetin on melphalan-induced oxidative stress and impaired renal and hepatic functions in rat. *Chemotherapy research and practice, 2014.*

[24] Zhang, J., Jiang, S., and Watson, R. R. (2001). Antioxidant supplementation prevents oxidation and inflammatory responses induced by sidestream cigarette smoke in old mice. *Environmental health perspectives, 109(10), 1007.*

[25] Ball, B. A., Medina, V., Gravance, C. G., and Baumber, J. (2001). Effect of antioxidants on preservation of motility, viability and acrosomal integrity of equine spermatozoa during storage at 5 C. *Theriogenology, 56(4), 577-589.*

[26] Siegel, H. S. (1985). Immunological responses as indicators of stress. *World's Poultry Science Journal, 41(1), 36-44.*

[27] Levine , R Land Mosoni, L and Boriett, Bs and stadman , ER. (1996). Methionine residues as endogenous antioxidants inproteins . *Proc. Nat. Acad, Sci. 93 : 15036 – 15040* .

[28] EL-Missiry, M.A. (1999). Enhanced testicular antioxidant system by ascorbic acid in alloxan diabetic rats . *Comp. Biochem. Physiol., part C. 124 : 233 – 237* .