

EFFECT OF USING SYNTHETIC AND HERBAL METHIONINE IN BROILER BREEDER DIETS ON SOME BIOCHEMICAL PARAMETERS AND HUMORAL IMMUNE RESPONSE- FIELD TRIAL

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Received: 3 October 2020; **Accepted:** 20 October 2020

ABSTRACT

A study was conducted to compare the effect of substituting synthetic DL-methionine (DL-Met) with herbal methionine (H-Met) as a natural substitute on some biochemical parameters and the humoral immune response of broiler breeder hens aged (50-55 weeks). 900 females and 108 males broiler breeders were distributed randomly into two groups, each group consists of 3 replicates, in three commercial modern opened houses. The birds were fed throughout the experiment on the two iso-energy, iso-crude protein diets according to nutritional requirements table of Ross-308. The difference between two diets was in the type of methionine added. DL-Met was added to the diets of group A at the rate of 1Kg/ton, while H-Met was added to the diets of group B instead of DL-Met at the same level for female broiler breeders. The results of the experiment cleared that there were no differences in production efficiency between the group containing DL-Met and other herbal alternative H-Met. The biochemical blood parameters were in the range of physiological values, and there was no significant ($P>0.05$) difference between the values of the group containing DL-Met and H-Met except the serum glucose value of H-Met which was higher significantly ($P<0.05$). As well as the antibody titers of ND before vaccination were significantly lower ($P<0.05$) than the antibody titers for experimental groups after vaccination at the end of the experiment. There were no significant ($P>0.05$) differences between the antibody titers of the group received DL-Met and herbal substitute H-Met. Results of the current study concluded that synthetic methionine DL-Met can be substituted with herbal substitute H-Met at the same rate without any adverse effects on the blood biochemical parameters or the humoral immune response to vaccination against Newcastle disease, as well as no negative effect of using H-Met on the productivity of broiler breeder. It is suggested that the effects of partial substitution of DL-Met with H-Met on the immune response of the broiler breeders should be studied more broadly in the future.

Keywords: Methionine–herbal–Broiler Breeder–Blood Parameters–Humoral immunity–field.

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INTRODUCTION

There does appear to be a direct relationship between animal performance and a 'healthy' gu Methionine plays active roles in the bird's body as considered the one of the most important sulfur amino acids. It is consider as first limiting amino acid that the bird's body does not synthesis it (Fancher and Jensen, 1989), and as a donor of methyl group (Schutte *et al.*, 1997), and sulfur element in the body (Bunchasak, 2009), which helps body cells to minimize oxidative stress (Luo and Levine, 2009). It is an important component in the building of immune response and in the formation of humeral immune response specially the antibody (Kumar *et al.*, 2014) through direct effects (protein synthesis and breakdown) and indirect effects (derivatives of methionine) (Bunchasak, 2009) this amino acid is vital for birds to preserve its growth and productivity (Jankowski *et al.*, 2014), and this form of methionine is provided in large-scale by chemically synthesis (figge *et al.*, 2010).

By the end of the twentieth century, the decline of using animal protein source because of the hazard of pathogenic and toxic material contamination (Chadd *et al.*, 2002). Poultry diets depend entirely on plant ingredients, and corn has been adopted as the main component of the feed (North and Bell, 1990), and many researchers have succeeded in using plant-based diets completely in feeding poultry without affecting the growth or productivity of broiler chickens and broiler breeder. Not all plant ingredients used in poultry diets, especially corn, has a sufficient amount of methionine. So that, adding methionine from an external source to repair the deficiency in poultry diets was an inevitable procedure (North and Bell, 1990). DL-methionine (DL-Met) was the main and common source of industrial methionine added to chicken diets (figge *et al.*, 2010).

The use of DL-Met. is influenced by two main factors, namely its price variation dependent on its fossil raw materials (Bhutyal *et al.*, 2019) as well as the human tendency to reduce the chemicals source used in feeding birds (Chattopadhyay *et al.*, 2006), and these two factors have encouraged researchers focus on finding natural alternatives for DL-Met. that means less costs and less negative impact on birds.

Makinde *et al.* (2017) found that, the herbal substitute for methionine, named (HM) like (Methiorep®), which it's adding as a substitute for industrial methionine DL-Met didn't has negative effects on the productivity performance of broiler chickens. Also Kalbande *et al.* (2009) emphasized that adding H-Met to the broiler diets had no effect on broiler performance compared with birds that consumed industrial methionine DL-Met. Subh *et al.* (2013) found the possibility of adding herbal methionine to broiler breeder diets without negatively affecting productivity, fertility, or hatchability and that also what agree with Bishnol (2008) in another trial.

This study was designed to complete the picture on the use of the H-Met in broiler breeder diets and the effect of substitution of DL-Met by H-Met on the bird's health, blood biochemical parameters and the humoral immune response to Newcastle disease vaccination titre.

MATERIALS AND METHODS

Place of experiment:

Broiler breeders flock have been reared in private poultry breeders farm contains 3 open side commercial houses, and when the flock completed 48 weeks old, 900 Females and 108 Males of this flock were selected and distributed randomly into two groups, each group consists of 3 replicates, were in a three commercial modern opened houses. The experimented birds were fed the same experimental diets during accustomed period

and the experiment began at the age of 50 weeks and continued until the age of 55 weeks.

From each group, 24 females were chosen randomly and numbered with numbers fixed on the wing and marking these birds with a suitable paint on the back for easy identification, at a rate of 8 female per replicate.

The diet was provided separately for the two sexes with a special hanging pan for males and another manual hanging pan suitable for the females. Females were isolated from the males diets at the appropriate height, and males were isolated from the female diets with a special net that prevents the males head from reaching the diet.

Vaccination of birds against Newcastle disease:

The experimental groups were vaccinated with live vaccination for ND - strain Lasota-produced by MSD company by fine spraying in the farm at the start of the experiment. Blood samples were taken at the start of the experiment before vaccination process and at the end of the experiment.

Experimental diets:

Females were feed on a production breeder diets, which iso-caloric and iso-nitrogenous according to the nutritional requirement tables of Ross 308 (Ross, 2016) and have 0.35% methionine. The composition of the used diets was shown in Table 1. Dry Matter %, crude protein % and crude fat % were analyzed in the laboratory of Animal Nutrition Dep., faculty of veterinary medicine, Hama University, Syria.

DL-Met was added to the diet of group A at the rate of 1Kg/ton, while diet of group B was formulated to contain 1Kg/ton of H-Met instead of DL-Met to cover the need of methionine in female diets. Daily fixed

amount of feed (158) g/day/ female was provided in the morning.

Males fed on a male breeder diets which is iso-caloric and iso-nitrogenous according to the nutritional requirement tables of Ross 308 (Ross, 2016) of 0.3% methionine formale diets. In group A, DL-Met was added at 0.8 kg/ton to meet the minimum needs of methionine, while in group B, the amount of DL-Met was replaced by 0.8 kg / ton of the studied H-Met to meet the requirement of methionine in male diets, and a daily fixed amount of feed (135) g/day/ male was provided in the morning.

H-Met is a commercial mixture of an Indian company offered in the local market at prices 10-15% lower than DL-Met, and these material was studied before by Subh *et al.* (2013). It consists of several medicinal plants, namely *Andrographis Paniculata* by 35%, *Ocimum Sanctum* plant by 25%, *Asparagus Racemosus* by 10%, and *Zee Mays* by 30%, and the mixture contains 8% crude protein and 4.54% methionine, and these ratios were carefully selected by the producing laboratories to ensure their synergistic participation as an alternative to methionine manufactured by simulating its effect on the body.

Performance studied variables:

The experiment lasted 5 weeks, from week 50 to 55. Average weekly production percentage of each group was calculated, and the average hatchability and fertility percentage of eggs were estimated for each experimental group by examining the eggs collected on the last day of the experiment.

The hatchability percentage was calculated as the number hatched chicks suitable for sale divided on the number of the total incubated egg x 100. The fertility percentage was calculated as the number of fertilized eggs divided on the total incubated eggs x100.

Measurement of blood parameter:

Blood samples were collected from the marked and numbered females in the early morning before feeding at the start and at the end of this experiment. Blood samples were collected from the wing vein of birds without anticoagulant, and the blood samples were left in the syringe diagonally at room temperature 25°C until the completion of the blood clotting process. A centrifugation process was carried out for 30 seconds at a low speed of 3000 revolutions / min for the serum samples collection. Blood cells remnants were concentrated at the bottom of the tube and clear serum was transported to Appendroph tube which a plastic tube with cap of 1.5 ml and these tubes were kept in bags in the freezer at 20° C until further analysis.

Three replicates for each test simultaneously were made for each sample to exclude the effect of repeated negative freezing on blood parameter values of the sample. The total serum protein, serum albumin, blood sugar, triglycerides, cholesterol, high-density lipoproteins (HDL) and Low-density lipoproteins (LDL) were determined in the serum by using diagnostic test kits (kits) produced by the Spanish company Bio Systems, and the absorbance of samples was measured by using a Spectronic Instrument, Model. 2001 / 4 in the laboratory of scientific research and graduate studies.

Measurement of antibody level for Newcastle disease:

The level of antibodies in birds against Newcastle disease was tested using the Hem agglutination- Inhibition Test simultaneously according to the method (Lu, 2007) by determining the highest serum dilution (standard) that causes complete Inhibition of blood cells agglutination.

In order to study the values of dilution, a statistical study was made with considering the titer of Inhibition of agglutination with an inverted logarithm of serum dilution in

the last hole in which the prevention of hem-agglutination occurs, as in the example:

Dilution 1/8 is flipped to become 8, and therefore $Lg(8) = 3$, and the square of the logarithm $(Lg(8))^2 = (3)^2 = 9$. The results of inhibition of blood agglutination were recorded for each sample and then statistically studied.

Statistical analysis:

The independent samples T-test was used to compare the averages of the variables studied for the experimental groups, test for the presence of significant differences between the means of these variables for the levels of significance 1% and 5% was made by the use the statistical program, the Statistical Package for Social Sciences (SPSS, 2008) to compare and analyze the statistical results.

RESULTS AND DISCUSSION

Table 2 shown the effect of each of DL- Met and H-Met as an alternative of DL- Met on the percentage of egg production and weight, and chart (1) showed the differences of egg production at the beginning and the end of the trial for each group. The egg productions % of our experiment was higher than Ross 308 Broiler Breeder standard 2016 by 3% and this may be attributed to the selection process which omitted the bad females before starting of the experiment. So that effects increased the beginning egg production percentage up of standard, but the egg weight was on the standard and that may be because of the selection process which chose the females on the target weight.

There were no significant differences between the average egg production or egg weight, and this indicated that H-Met has no negative effect on both the percentage of egg production and weight. The differences between the values of the control and

experimental group remained closed, which is an indication that, there was no negative effect on overall productivity when using the H-Met, and a statistical study was not conducted due to the small number of replicates. These results agreed with the findings of Subh *et al.* (2013) who reported that DL-Met can be effectively replaced by H-Met at the same level in female broiler breeder diets without effect on the egg production ratio and weight.

Table 3 showed the effect of DL-Met and H-Met on fertility and hatchability percentages. Hatchability % was higher than Ross 308 Broiler Breeder standard 2016 (Ross, 2016) by about 2.5 to 3%, and this can be understood because the good physical situation of males and females contributed in this experiment after primary selection. There were differences between groups (DL-Met and H-Met) in values of hatchability and fertility percentages at the end and beginning of the experiment. Addition of H-Met had no negative effect on fertility and hatchability percentages during the experimental period. These results also supported by Subh *et al.* (2013) who studied the same H-Met and its effects on broiler breeder productivity.

Makinde *et al.* (2017) supported results of current study and declared that Methiorep® as H-Met can completely substituted DL-Met in broiler diets without adverse effect on growth performance, On other hand, Kalbande *et al.* (2009) and Kumari *et al.* (2012) found that substitution of DL-Met by H-Met in broiler diets had no significant effect on performance.

Determinants of biochemical blood parameters:

Results in Table 4 showed that the mean \pm standard deviation of the female blood parameter values at the beginning of the trail (50 weeks) and for each studied variables DL-Met and H-Met at 55 weeks old.

The females blood parameter values including glucose, cholesterol, total protein, albumin and globulin were in the range of Ritchie *et al.* (1994). These blood parameter values were closed to the blood parameter values of adult females industrial broiler breeders (Ross 308) according to findings of Abdi-Hachesoo *et al.* (2011) who compared them with the blood parameter values of adult female indigenous broiler breeders. The biochemical parameters estimated in this study were acceptable and the health of the broiler breeder females used in this experiment was normal and reflecting the field rearing conditions.

There were significant ($P < 0.05$) differences only with glucose, while the rest of studied parameters did not have significant differences. An increase in serum glucose with H-Met supplementation does not indicated a pathological condition because it is still in the normal range similar to findings of Abdi-Hachesoo *et al.* (2011). These results did not agreed with the findings of Makinde *et al.* (2017) who reported no difference in serum glucose (g/dl) when replacing DL-Met with 0, 25, 50, 75% and 75% of H-Met in broiler chicken diets. Bhutyal *et al.* (2019) not found any positive effects of H-Met supplementation on serum glucose. Positive effects of H-Met in this study and the differences with other studies may be attributed to the role of H-Met components that arise scientifically serum glucose and need for further physiological studies to explain these effects. An increase could not consider as an adverse effect of H-Met which was similar to the values of serum glucose reported by Ritchie *et al.* (1994).

These results are agreed with the findings of Halder and Roy (2007), Rekhateel *et al.* (2010) and Chinnadurai *et al.* (2018) who found that H-Met supplemented diets had no significant effect on serum biochemical parameters of broiler chickens. Similar results also were obtained by Bhutyal *et al.*

(2019) who declared no adverse effect of H-Met supplementation on blood biochemical parameters and the difference only in serum glucose.

In contrast, Igbasan *et al.* (2012) reported that layer hens fed DL-Met and H-Met supplemented diets had significant difference in total serum protein and albumin, and this may be due to the differences in the type of reared chicken and the experiment substrates in this trail.

Table 5 showed that no significant differences were observed in alanine transferase (ALT) and aspartate transferase, (AST) in blood serum between variables studied H-Met and DL-Met, but It was noticed that the group received the plant alternative was numerically better ($P > 0.05$). This might be indicated, at least, the absence of a negative effect of vegetable methionine on the hepatocytes which may increase releasing of these two enzymes in serum.

The liver enzymes (ALT and AST) values in females were higher than the range of their values detected by Abdi-Hachesoo *et al.* (2011) who reported ($7.9 + 1.91$) and ($119 + 13.32$) UI/L respectively of adult female industrial broiler breeders.

However, a moderate increased of ALT and AST in this study were observed in the two experimental groups and this may be due to stress which increased the enzyme activity plus that means there were no adverse effects of replacing DL-Met by H-Met. Chinnadurai *et al.* (2018) found no significant difference of AST and ALT levels when replaced DL-Met by H-Met. In Addition, Sheila *et al.* (2014) reported no significant differences between the dietary treatment on AST and ALT of broiler at day 42.

Table 6 shown the means of antibody titers against the causative agent of Newcastle

disease before the start of the experiment (before vaccination) and at the end of the trial for each of the experimental groups received H-Met and DL-Met supplemented diets.

There were significant ($P < 0.05$) difference between the mean of antibody titers at the start and at the end of the experiment and this indicated that the effects of the vaccination process which stimulate both mucosal and humeral immune responses (Rauw *et al.*, 2009).

At end of the trial, the mean value of antibody titer of DL-Met supplemented group was $8.22 + 2.25$ and was higher than H-Met supplemented group ($7.83 + 2.16$) but there were no significant ($P > 0.05$) differences were observed between the two experimental groups. Shewita *et al.* (2018) found no significant differences in HI titer after vaccination when DL-Met was replaced by a gradual percentage of H-Met in broiler diets along all the stages of the trial, but the best effect was observed in the mean values of antibody titer with mixing DL-Met and H-Met(1:1) to provide methionine requirement of broiler. Similar results reported by Kumari *et al.* (2012) who found that the mixing of H-Met and DL -Met at 50% to 50% in broiler chicken diet to cover methionine requirement increased scientifically ($P < 0.05$) the mean of HI titer against NDV.

The current study was conducted at the field conditions in private broiler breeder farm (two experiment groups). According to obtained results, we cannot detect the effect of DL-Met and H-Met on the humoral immunity of broiler breeders. There were no significant differences in production efficiency nor on the blood biochemical parameters. These results came in accordance with those reported by Makinde *et al.* (2017). Methionine requirements for immune responses were higher than those for performance (Hosseini *et al.*, 2012). The

importance of studying the partial substitution of DL-Met with H-Met in different proportions in broiler breeder diets was supported by Kumari *et al.* (2012); Shewita *et al.* (2018) and Chinnadurai *et al.* (2018).

We should take into account that, the products tested in the various researches

differ in composition, but in general, the H-Met is one or/a mixture of various herbs and supposed to be effective in its optimum activity for proper protein accretion and other important functions in chicks, so can reach better growth performance as well as humoral immunity of birds (Rahmani and Speer, 2005).

Table 1: Physical and Chemical Composition of experimental broiler breeder diets.

Component	DL-Met		H-Met	
	Female	Male	Female	Male
Maize	66.35	61.45	66.35	61.45
Wheat Bran	0	10	0	10
Barley	2	10	2	10
Soybean meal 44%	21.7	14	21.7	14
Monocalcium Phosphate	1.7	1.9	1.7	1.9
Calcium carbonate	7.5	1.2	7.5	1.2
Salt	0.3	0.3	0.3	0.3
Vitamin premix.	0.1	0.1	0.1	0.1
Mineral premix.	0.1	0.1	0.1	0.1
DL-Met.	0.1	0.08	0	0
H-Met	0	0	0.1	0.08
Choline Cl	0.15	0.15	0.15	0.15

Calculated chemical analysis:

AME (Kcal/Kg)	2790	2790	2756	2756
Crude protein %	15.19	13.31	15.19	13.31
Calcium %	3.03	1.04	3.03	1.04
Lys (dig) %	1.23	1.08	1.23	1.08
Met (dig) %	0.34	0.32	0	0
Met + Cys (dig) %	0.61	0.5	0.61	0.5

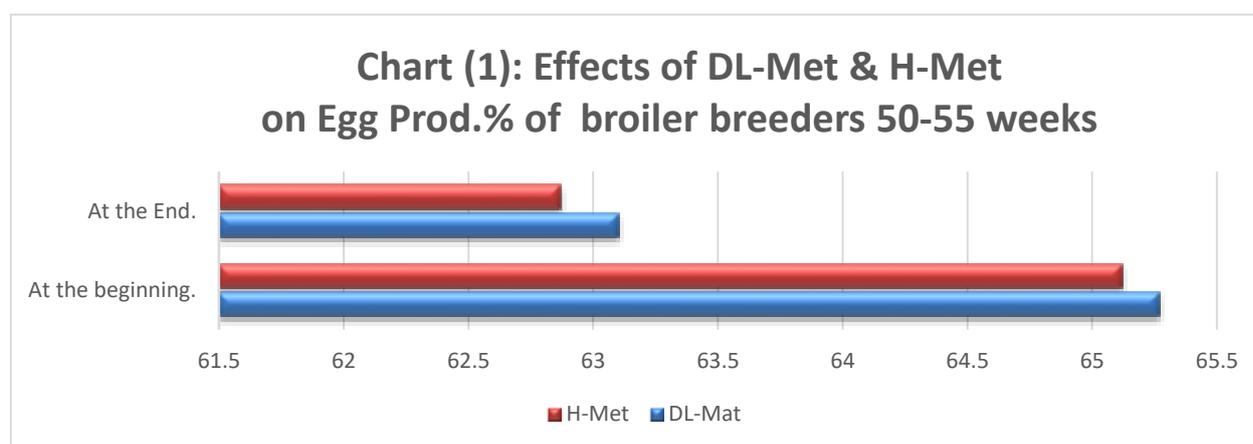
Laboratory chemical analysis:

Dry matter (DM, %)	89.6	89.8	89.5	89.7
Crude protein %	15.32	12.9	15.29	12.11
Crude fat %	4.2	4.8	4.3	4.9

Minerals and vitamins premixes were provided from local source to prevent the requirements recommended by the company producing the trading hybrid.

Table 2: Effect of DL-Met & H-Met on the Egg Prod. % and Egg Weight of Broiler Breeders (50-55 wk).

Treatment		DL-Met%	H-Met%	Difference %
Prod. %	At the beginning.	65.27	65.12	0.15
	At the End.	63.1	62.87	0.23
Egg Weight g.	At the beginning.	67.95	67.49	0.46
	At the End.	69.26	69.75	0.51

**Table 3:** Effect of DL-Met & H-Met on the Fertility and Hatchability of broiler breeders (50-50 wks).

Treatment		DL-Met%	H-Met%	Diff. %
Hatchability %	At the beginning.	84.1	83.63	0.47
	At the End.	82.53	82.2	0.33
Fertility %	At the beginning.	92.3	91.1	1.2
	At the End.	91.53	90.3	1.23

Table 4: Effect of DL-Met & H-Met on Biochemical Blood Parameters of broiler breeders (50-55 wks).

Parameters	beginning of trial	DL-Met	H-Met
Glucose (mg/dl)	^a 250.85 ±12.849	^a 250.96 ±13.322	^b 259.6 ±11.352
Triglycerides (mg/dl)	66.17 ±5.571	66.97 ±2.665	67.92 ±1.072
Cholesterol (mg/dl)	170.75 ±4.689	170.48 ±4.399	173.61 ±4.678
HDL (mg/dl)	63.54 ±1.609	63.58 ±1.849	64.69 ±1.445
LDL (mg/dl)	93.54 ±3.505	93.43 ±3.272	94.18 ±3.49
Total protein (mg/dl)	5.21 ±0.134	5.34 ±0.136	5.08 ±0.135
Albumin (mg/dl)	2.72 ±0.04	2.73 ±0.042	2.66 ±0.082
Globulin (mg/dl)	2.49 ±0.098	2.51 ±0.102	2.42 ±0.086

* Small later a, b, and c means there are significant differences in the same line.

Table 5: Effect of DL-Met & H-Met on Liver Function Parameters of Broiler Breeders (50-55 wks).

Parameters	beginning of trial	DL-Met	H-Met
ALT(U/L)	16.34 ±0.586	16.21 ± 0.522	16.62 ± 0.544
AST(U/L)	182.72 ±6.402	183.13 ± 6.375	185.44 ± 6.511

*There are no significant differences ($P > 0.05$) between experimental groups.

Table 6: Means of Newcastle disease (ND) antibody titers estimated with Hem agglutination Inhibition Test.

Parameters	beginning of trial	DL-Met	H-Met
HI Ab Titer	6.89 ±2.13 ^a	8.22 ±2.25 ^b	7.83 ±2.16 ^b

Means with different letters in the same row differ significantly at ($p < 0.05$).

CONCLUSION

According to the obtained results, it may be concluded that H-Met can completely use instead of DL-Met in broiler breeder diets without adverse effects on the production parameter and blood biochemical parameters, as well as without clear effects on antibody titer levels for Newcastle disease. It is suggested that the effects of partial substitution of DL-Met by H-Met on the immune response of the broiler breeders must be studied more broadly in the future.

ACKNOWLEDGEMENTS

I especially thank High Administration, Al-Ashour Poultry, for its support.

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أثر استخدام الميثيونين الصناعي والنباتي في أعلاف أمات دجاج اللحم على بعض القياسات الكيميائية والبيولوجية والاستجابة المناعية الخلطية – تجربة حقلية

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أجريت دراسة لمقارنة تأثير استبدال الميثيونين الصناعي DL-Met بالميثيونين النباتي H-Met كبديل طبيعي له على بعض القياسات الكيميائية للدم والاستجابة المناعية الخلطية عند فرخات أمات دجاج اللحم بعمر 50-55 أسبوع.

تم توزيع 900 أنثى و 108 ذكر من قطيع تجاري لأمات دجاج اللحم بشكل عشوائي إلى مجموعتين، كل مجموعة تتكون من 3 مكررات، في ثلاث بيوت تجارية حديثة مفتوحة. تم تغذية الطيور في التجربة بأعلاف متساوية بالطاقة والبروتين، وتختلف فيما بينها في نوع الميثيونين المضاف إليها. حيث تمت إضافة DL-Met إلى الخلطات العلفية الأولى لتلبية احتياجات الشركة المنتجة، وفي الخلطات العلفية الثانية تم استبدال DL-Met بالبديل النباتي المدروس H-Met بنفس النسبة.

أكدت نتائج التجربة عدم وجود فروق في كفاءة الإنتاج بين المجموعات المحتوية على DL-Met وعلى بديله النباتي (H-Mat)، وأشارت هذه الدراسة إلى أن القياسات الكيميائية للدم كانت في نطاق القيم الطبيعية، ولم يكن هناك فرق معنوي ($P > 0.05$) بين قيم المجموعة التي تحتوي على DL-Met و H-Met الخاص بها باستثناء قيمة سكر الدم (H-Met) والتي كانت أعلى معنوياً ($P < 0.05$). كذلك كانت معايير الأجسام المضادة لمرض النيوكاسل قبل التحصين أقل معنوياً ($P < 0.05$) من معايير الأجسام المضادة للمجموعات التجريبية بعد التحصين في نهاية التجربة، بينما لم تكن هناك فروق معنوية ($P > 0.05$) بين معيار الأجسام المضادة للمجموعات التي تناولت DL-Met بالبديل النباتي H-Met.

خلصت الدراسة إلى أنه يمكن استبدال الميثيونين الاصطناعي DL-Met بالبديل النباتي H-Met بنفس النسبة وبدون أي آثار سلبية على القياسات الكيميائية للدم أو على الاستجابة المناعية الخلطية للتحصين ضد مرض النيوكاسل لأمات دجاج اللحم، فضلاً عن عدم وجود تأثير سلبي لاستخدام الميثيونين النباتي على إنتاجية أمات دجاج اللحم، ويقترح أخيراً، دراسة تأثير الاستبدال الجزئي لـ DL-Met بـ H-Met على الاستجابة المناعية لتربية أمات دجاج اللحم بشكل أوسع في المستقبل.

الكلمات المفتاحية: ميثيونين – نباتي – أمات دجاج اللحم - محددات الدم - مناعة خلطية - الحقل.