# Effect of dietary supplement of Branched Chain Amino Acids on Production Performance and Some Physiological Traits of Aged Laying Hens

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## Abstract

The experiment was conducted in order to study the effect of adding a mixture of Branched Chain Amino Acids (BCAA) to the diet of laying hens on productive performance, egg quality traits and some physiological traits during the last stage of the productive life. 180 Lohmann Brown laying hens were bred at 58 weeks of age until at the age of 76 weeks, they were randomly distributed to 18 ground pens, the dimensions of each pen is 1.5 x 2 m, with 10 chickens per pen (as repeated), and after a period of two weeks of breeding, which was considered a preparatory stage for the beginning of the experiment, and at the age of 60 weeks, the hens were randomly distributed over six treatments. , and with three replicates (pen) for each treatment, the first treatment (T1) was fed a standard diet without addition (control treatment), the second treatment (T2), the third treatment (T3), the fourth treatment (T4), the fifth treatment (T5) and the sixth treatment (T6) was fed a standard diet supplemented with 0.5, 1, 1.5, 2, 2.5 g/kg mixture of Leucine, Isoleucine and Valine (mixing ratio 2:1:1), respectively, and the results showed a significant superiority ($P \leq 0.05$) for all treatments. Addition of BCAA in the percentage of egg production (HD%) compared with the control treatment, a significant superiority ($P \leq 0.05$) was obtained for the two treatments of adding BCAA T2 (0.5g/kg) and T3 (1g/kg) in egg weight, egg mass, feed conversion efficiency and body weight compared with the control treatment, and there was a significant superiority for the two treatments of adding BCAA T2 (0.5g/kg), T3 ( 1 g/kg) in the length of the villi and the depth of the crypts of the intestine compared with the control treatment, and a significant ($P \leq 0.05$) was observed for all BCAA addition treatments in the volumetric criterion of antibodies to Newcastle disease virus compared with the control treatment. There were no significant differences between the averages of the experimental treatments in the characteristics of each of blood sugar, uric acid, total protein, cholesterol, and the activity of AST and ALT transporter enzymes, and there was a significant improvement in the level of MDA for all BCAA addition treatments compared to the control treatment. It is concluded from this study that supplementing feed with a sufficient proportion of branched-chain amino acids is beneficial in improving the productive performance, quality traits of the eggs produced and the immune response of laying hens during the last stage of their productive life.

Key words: BCAA, laying hens, production, physiological traits.

## Introduction

The process of providing nutrients that closely correspond to the optimal requirements for each of the life stages of modern hybrids laying hens is one of the main strategies in overcoming the challenges facing this industry, to improve digestion and metabolism, and reduce the loss of nutrients with excrement, and its reflection on productive perseverance. Bird health and production costs (Jasim and Fadel, 2020؛ Macelline et al., 2021), so researchers and specialists turned to the use of balanced diets as well as food additives (Neveling, 2018؛ Ali et al., 2020; Dhiab and Al-Saadi, 2021). The essential amino acids are among the most important of these additions, including the addition of Branched Chain Amino Acids (BCAA), which includes Leucine, Isoleucine, and Valine, and they are distinct from the rest of the other essential amino acids in its chemical composition containing The cross-sectional chain of the carbon atom that makes it unique in its biochemical properties (Seager and Slabaugh, 2013), it enhances and regulates the energy source by increasing the response of cells to insulin and metabolism glucose and fats, and converting them to glucose in the liver before other amino acids by a process called gluconeogenesis when the animal is exposed to stress, thus preventing the catabolism of muscle proteins, as well as its role in vital building processes as the raw material for building proteins in the body (Simone et al., 2013; Shimomura et al., 2015), and it stimulates the immune response through its role in the formation of immune globulins, the maturation and differentiation of lymphocytes (Gu et al., 2019; Jasim and Mohammed, 2020). One study showed that the use of BCAA increases the expression of genes specialized in the production of antigens. Oxidation reduces the production of Reactive Oxygen Species (ROS), which leads to maintenance of skeletal muscle fiber size, enhanced physical endurance and increased life span in rats (DAntona et al., 2010).

In previous years, a number of studies were conducted on the addition of valine and isoleucine in laying hens' diets, which had returns in the economic feasibility of feeding and improving immunity and antioxidant capacity, while the use of Leucine was less interested by researchers (Dong et al., 2016; Wen et al., 2019; Jian et al., 2021), on the other hand, some studies indicate that these acids are more useful if added in combination than the single addition of each of them (Cynober, 2003).

Based on this information and not using branched-chain amino acids in a complete combination in laying hens diets, this study aimed to know the effect of adding them in combination and at several levels to the diet on productive performance, egg quality, intestinal biometrics, and biochemical blood traits of laying hens during the last stage of their productive life.

## Materials and Methods

### Birds and treatments experience

In this experiment, 180 Lohmann Brown laying hens at 58 weeks of age, obtained from a private poultry company, were randomly distributed to 18 ground buns (Pens) each with an area of 1.5 x 2 m (10 hens/pen), and after two weeks of breeding as a period for the adaptation of chickens, and at the age of 60 weeks, the birds were weighed individually at five o'clock in the afternoon and they were at an average weight of 1920 ± 4 g. Then the experiment's treatment were randomly distributed to the chicken pens, with three pens for each treatment (3 repeated/treatment).

1- First treatment (control) T1: standard diet without addition.

2- The second treatment T2: a standard diet plus 0.5g/kg (0.05%) BCAAs mixture at 0.250, 0.125, 0.1250g/kg of Leucine, Isoleucine and Valine, in order.
3- The third treatment T3: a standard diet added to it 1 g/kg (0.1%) mixture of BCAA at levels of 0.5, 0.25, 0.25 g/kg of Leucine, Isoleucine and Valine, in order.
4- Fourth treatment T4: a standard diet plus 1.5 g/kg (0.15%) of BCAA mixture at a level of 0.75, 0.375, 0.375 g/kg of Leucine, Isoleucine and Valine, in order.
5- Fifth treatment T5: Standard diet plus 2 g/kg (0.2%) BCAA mixture at a level of 1, 0.5, 0.5 g/kg of Leucine, Isoleucine and Valine, in order.
6- Sixth treatment T6: a standard diet supplemented with 2.5 g/kg (0.25%) BCAA mixture at a level of 1.25, 0.625, 0.625 g/kg of Leucine, Isoleucine and Valine, in order.

**The source of the branched-chain amino acids and the preparation of their mixture**

The Branched Chain Amino Acids (BCAAs: L-Leucine, L-Isoleucine, and L-Valine) that were used in this experiment are came from Bulk Supplements of American origin, and each of them was packed in sealed bags weighing one kilogram, and a mixture of them was prepared. 4 kg, from mixing 2: 1: 1 kg of Leucine, Isoleucine and Valine, in order, and the mixing process was done mechanically for five minutes with an electric mixer capacity of 5 kg from the German company Clartonic, and it was filled with a tightly closed bag until use.

**Mixing branched chain amino acids in the diet**

The feed additives for the experimental treatments were mixed from the mixture of branched-chain amino acids assigned to each treatment weekly, accompanied by the mixing of sunflower oil specified for the standard diet (to preserve the oil from oxidation and rancidity from the length of the fodder storage period), manually and gradually to ensure that it reached the stage of homogeneity, then it was packed in Plastic bags and marked to distinguish them.

**Chicken management**

The chickens were housed in ground area furnished with straw, each equipped with a hanging automatic plastic manhole and a hanging cylindrical plastic feeder and two nests for eggs, and fed on a standard productive ration in the form of crushed fodder (Mash) with a specific quantity of 115 g/bird/day, and from Table 1 it is shown the components of the ration used In the experiment and its chemical analysis, it was also provided by the environmental conditions and according to the recommendations of the management manual for Lohmann Company (Lohmann Tierzucht, 2019).

The vaccinations of birds against viral diseases were completed by the spray method. At the beginning of the experiment, they were immunized with the mixed vaccine against Newcastle disease, Clone 30 and infectious bronchitis, MA5, then it was followed 35 days later with the Lasota Newcastle disease vaccine, and this vaccination process was repeated every 35 days alternating between the two vaccines. ex.

**Table 1. Ingredients and chemical composition of the ration used in the experiment.**

| The components of the diet | The ratio % |
|---|---|

| | |
|---|---|
| yellow corn | 40 |
| wheat | 27.5 |
| Soybean gain * | 20 |
| Premix ** | 2.5 |
| Limestone | 8 |
| Di-Calcium Phosphate | 1 |
| cooking oil | 1 |
| Total | 100 |
| Calculated chemical composition *** | |
| Crude protein% | 15.51 |
| Energy represented kcal / kg | 2745 |
| Leucine % | 1.32 |
| Isoleucine % | 0.63 |
| Valine % | 0.73 |
| Methionine% | 0.38 |
| Methionine and cysteine% | 0.65 |
| Lysine% | 0.76 |
| Calcium% | 3.82 |
| Available phosphorus% | 0.46 |

*Soybean meal of Argentine origin contains 44% of crude protein and 2230 kcal/kg as representative energy. ** LAYMIX premix produced by VB IFAW of Dutch origin contains 6% crude protein, lysine 1.50%, methionine 5.90%, methionine & cysteine 5.90%, calcium 25.12%, available phosphorous 10.20%, representative energy 433 kcal / kg. *** According to the chemical composition of the diet according to the analyzes of the feed materials contained in the reports of the US National Research Council (NRC, 1994).

**Studied traits**

**Productive qualities**

**Egg Production**

Eggs were collected twice a day at twelve noon and at five o'clock in the afternoon for the duration of the experiment. The percentage of egg production for each period was calculated on the basis of Hen Day production (%H.D), according to the equation:

$$\%H.D. = \frac{\text{Number of eggs produced during the period (28 days)}}{\text{Number of chickens at the end of the period x length of time in days (28 days)}} \times 100$$

**Egg Weight**

The weights of the eggs produced at the end of each week were collectively recorded for each replicate using a digital scale that reads to the nearest two decimal places, and then extracted the average egg weight (g).

**Egg Mass**

The mass of eggs produced per hen (g / hen / day) was calculated according to the formula:

**The mass of eggs produced (g egg / chicken /28 days) = the percentage of egg production% during the period × the average egg weight during the period.**

**Feed Conversion Ratio**

The feed conversion efficiency was calculated by calculating the amount of feed needed to produce one gram of eggs, according to the equation:

$$Feed\ conversion\ rate = \frac{daily\ feed\ consumption\ rate\ (\mathbf{hen\ /\ g})}{daily\ produced\ egg\ mass\ rate\ (\mathbf{hen\ /\ g})}$$

**Body weight**

The birds were weighed for each replicate collectively at the end of the experiment period (at the age of 76 weeks) at five in the afternoon (after the end of the ovulation period and the end of the consumption of the amount of feed provided), a digital scale was used and then the average weight of the bird was extracted within one replicate by applying the following equation:

$$\textbf{Birds average weight (g)} = \frac{total\ weight\ of\ birds\ in\ repeted\ (g)}{total\ number\ of\ birds\ in\ the\ repeted}$$

**Physiological traits**

**Slaughter of birds**

Three birds from each treatment were randomly slaughtered (in the morning and before providing the daily diet) at the age of 76 weeks (end of the experiment), in order to take histological measurements of the small intestine and collect blood samples.

**Villi Length & Crypts Depth**

The length of the villi and the depth of the crypts were measured after cutting 2 cm from the jejunum part of the small intestine of each bird (which is the area between the vestigial scar of the junction of the yolk sac and the duodenum), and then the process of preparing histological slides was carried out for it according to the method of Bancroft and Gamble (2008), The prepared slides were read using an optical microscope at a magnification of 40x, and the measurements were recorded for both the length of the villi and the depth of the crypts using the ocular micrometer after it was calibrated with the precise scale of the stage micrometer, noting that the length of the villi extends from the top of the villus up to It is related to the burrow, and the depth of the burrow is the distance from the base of the villus to the end of the burrow (Gao et al., 2008).

**Blood sample collection**

Blood samples were collected directly when slaughtering birds from the jugular vein, using blood collection tubes that do not contain anticoagulant, and placed in the refrigerator horizontally after closing for 24 hours, then placed in a centrifuge at 3000 rpm for 15 minutes to separate the serum that was frozen immediately until the required tests are performed.

**Humeral immunity**

The immune response was measured by measuring the titer of antibodies against Newcastle virus in the blood serum by using an enzyme-linked Immunosorbent Asay called ELISA Test, using a ready-made assay kit. From the American company SYNBIOTICS, according to AL-Mayah (2009).

**Biochemistry of the blood**

Using the Japanese-origin APPLE device to measure the biochemical characteristics of blood, after preparing it with the necessary samples and solutions from the ready-made kit for each trait. The enzyme activity of each of Alanine Transaminase (ALT) and Aspartate Transaminase (AST) was measured according to the method of Reitman and Frankel (1957). The concentration of glucose, uric acid, total protein and cholesterol were measured according to method of Wotton (1964); Arliss and Entvistle (1981); Henry et al. (1974); Richmond (1973) respectively, and ready-made measuring instruments from the company SPINRECT, of Spanish origin, were used to measure these characteristics. As for malondialdehyde (MDA), it was measured using Thiobarbituric acid (TBA) reaction method, based on the method of Esterbauer and Cheesman (1990).

**Statistical analysis**

Statistical analysis was carried out using the Complete Randomize Design (CRD) to analyze the data for each period, and the Randomized Complete Block Design (RCBD) in the analysis of the data of the general average (to remove the effect of the duration), and to test the significance of the differences between the averages, the Tukey test, at a significance level of 0.05, and the ready-made statistical analysis program SAS (2001) was used to analyze the data.

**Results and discussion**

**Productive traits**

It is noted from Table 2 the effect of the addition of BCAAs on the egg production rate (HD%) of aged laying hens during the experimental period for the period of age 60-76 weeks. As it is noticed in the results of the percentage of egg production that there is a significant superiority ($P \leq 0.05$) for all the addition treatments of BCAA T2, T3, T4, T5 and T6 compared to control treatment without addition (T1), and there were no significant differences between the addition treatments in the percentage of egg production, and in egg weight, the significant superiority was in the addition treatments T2, T3 compared to the control treatment. There were no significant differences between the addition treatments in the weight of eggs during the experiment period, and the significant superiority of the addition treatments in the percentage of egg production and egg weight during the duration of the experiment was reflected on the mass of the produced eggs. And there were no significant differences in the mass of eggs produced between the rest of the addition treatments T4, T5, and T6 and the control treatment. There was also a significant improvement in the efficiency of food conversion for the two addition treatments T2, T3 compared to the control treatment, and there was no significant difference between the rest of the T4, T5 and T6 addition treatments in conversion efficiency Diet compared to treatment control.

**Table 2. Effect of feed addition of BCAAs on the productive performance of aged laying hens during the experimental period of 60-76 weeks of age (mean ± standard error).**

| treatments | Egg production (%H.D) | egg weight (g/egg) | egg mass (g/chicken/day) | Feed Conversion Ratio (g feed/g eggs) | body weight (g/bird) |
|---|---|---|---|---|---|
| **T1** | 73.10±1.11b | 63.11±0.32b | 46.13±0.53b | 2.49±0.02b | 1967±2c |
| **T2** | 79.05±0.53a | 65.43±1.29a | 51.75±1.33a | 2.22±0.05a | 2006±9b |
| **T3** | 79.14±0.73a | 65.42±1.35a | 51.81±1.50a | 2.22±0.06a | 2080±7a |
| **T4** | 76.85±0.51a | 63.60±0.80ab | 48.89±0.94ab | 2.35±0.04ab | 1975±11bc |
| **T5** | 77.09±0.63a | 63.78±0.73ab | 49.19±0.96ab | 2.34±0.04ab | 1991±6bc |
| **T6** | 77.28±0.67a | 63.53±0.80ab | 49.12±1.05ab | 2.34±0.05ab | 1973±5bc |
| **Significance level** | ** | * | * | * | * |

T1: Standard diet without supplement (control). T2, T3, T4, T5, T6 Standard diet plus 0.5, 1, 1.5, 2, 2.5 g/kg mixture of Leucine, Isoleucine and Valine (mixing ratio 2:1:1), respectively. The different letters within the same column indicate that there are significant differences between the means at the probability level of $P \leq 0.05$ according to the Tukey test. ** There are highly significant effects at the probability level of $P \leq 0.01$ in the analysis of variance table. * There are significant effects at the probability level of $P \leq 0.05$ in the analysis of variance table. N.S. There are no significant differences between the means at the probability level of $P \leq 0.05$ according to Tukey's test

It is noted from Table 2 the results of body weight at the age of 76 weeks, as the T3 addition treatment was superior to the rest of the addition treatments and the control treatment, and the T2 addition treatment recorded a significant superiority in body weight compared to the control treatment, while the rest of the addition treatments did not register a significant difference compared to the control treatment. The control treatment, and there were no significant differences in body weight between the addition treatments T2, T4, T5, and T6.

The results of the productive characteristics of this study show that the addition of BCAA's in the diet of elderly laying hens had a significant effect in improving these traits, and this improvement may be due to the synergistic effect that combines the positive properties of Leucine, Isoleucine and Valine acids in improving the health status of birds. And the histological characteristics of the alimentary canal, as noted from the results of this study, by stimulating the immunity of birds (Fig. 3) and increasing the length of the villi and the depth of the crypts of the small intestine (Fig. 1, 2), which leads to an increase in the efficiency of the intestine in the processes of digestion and absorption of nutrients, which is reflected in the improvement of health Birds and their productive performance (Jian et al., 2021), as the gut health of domestic birds is the key to their general health and the basis for achieving their target production rates (Huang et al., 2004).

The improvement in the productive performance of birds may be due to the role of BCAA in reducing the oxidative stress to which the bird's body cells are exposed as a result of the vital processes of maintenance and daily production, and this is what is observed in the results of this study, as the MDA (one of the indicators of oxidative stress) decreased in the group of birds fed with BCAA supplementation (Table 3), and this is consistent with what was confirmed by Iwasa et al. (2013) about the importance of BCAA in reducing free roots that cause oxidative stress to body cells.

**Physiological traits**

**Villi Length & Crypts Depth**

It is noticed from Figure 1 that there is a significant difference (P≤0.05) between the average lengths of the experimental treatments at the age of 76 weeks, as the two addition treatments T2, T3 recorded a significant improvement compared to the control treatment, and there was no significant difference in the length between the addition treatments.

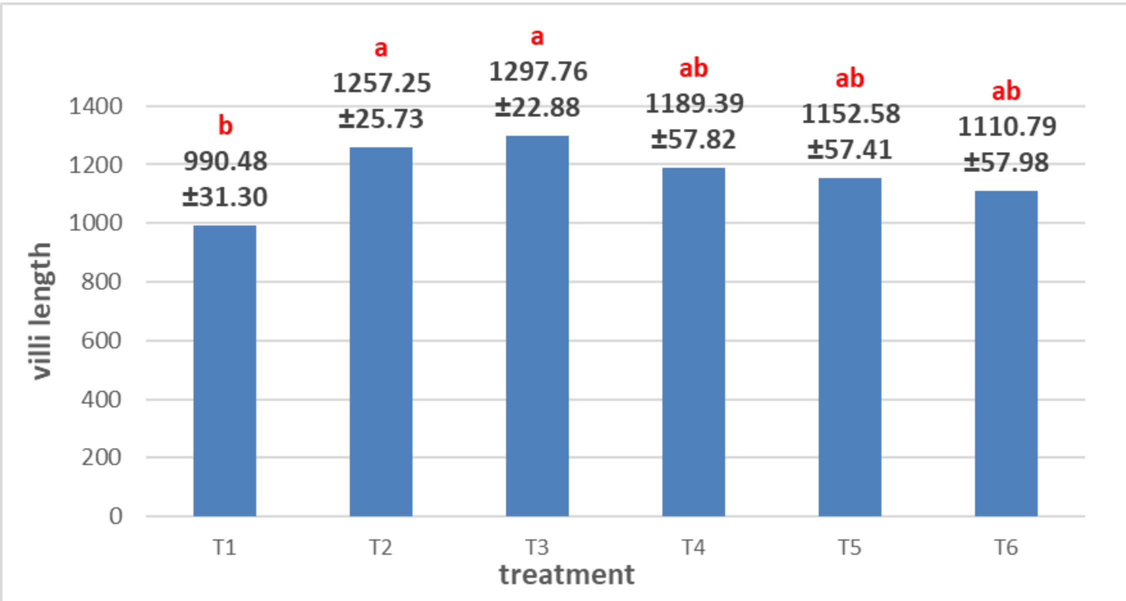


**Figure 1. Effect of feed supplementation of BCAAs on villi length (micrometers) of aged laying hens at the end of the experiment at 76 weeks of age (mean ± standard error).**

T1: Standard diet without supplement (control). T2, T3, T4, T5, T6 standard diet supplemented with 0.5, 1, 1.5, 2, 2.5 g/kg mixture of Leucine, Isoleucine and Valine (mixing ratio 2:1:1), respectively. The different letters above the columns indicate that there are significant differences between the means at the probability level of P≤0.05 according to the Tukey test.

It is noticed from Figure 2 that there is a significant difference between the depth of crypts for the experimental treatments at the age of 76 weeks, as the two addition treatments T2 and T3 recorded the highest values compared to the control treatment, and no significant difference appeared in the depth of crypts between the addition treatments.

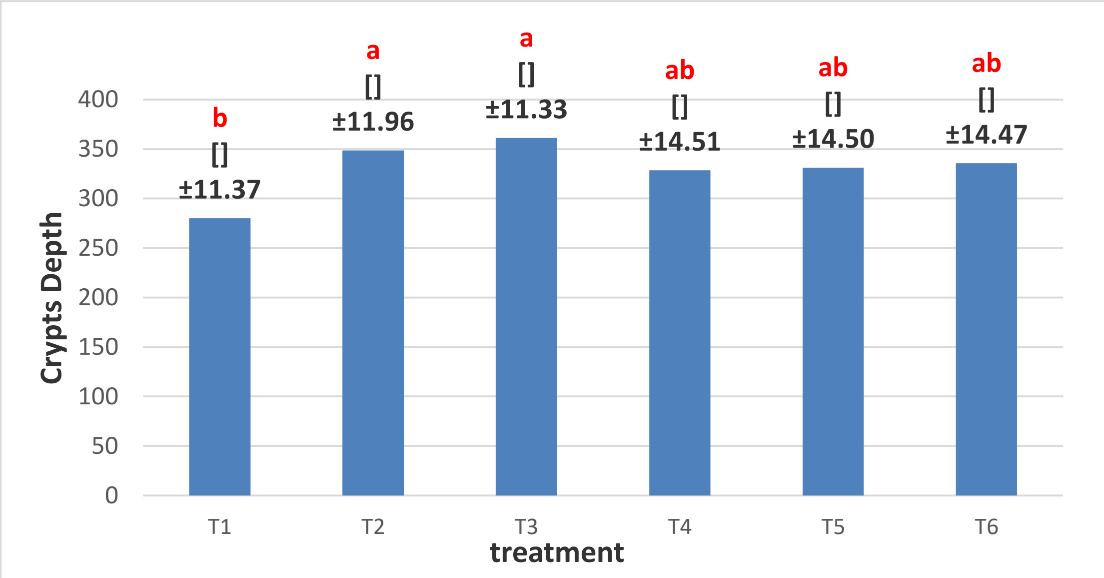


**Figure 2. Effect of feed supplementation of BCAAs in burrow depths (micrometers) for aged laying hens at the end of the experiment at 76 weeks of age (mean ± standard error).**

T1: Standard diet without supplement (control). T2, T3, T4, T5, T6 standard diet supplemented with 0.5, 1, 1.5, 2, 2.5 g/kg mixture of Leucine, Isoleucine and Valine (mixing ratio 2:1:1), respectively. The different letters above the columns indicate that there are significant differences between the means at the probability level of $P \leq 0.05$ according to the Tukey test.

The improvement in the length of the villi and the depth of the crypts in the small intestine was due to nutritional addition of a mixture of branched-chain amino acids. This imporvment may be due to the ability of BCAA to stimulate the formation of epithelial cells lining the intestines, restoring the intestinal tissue and stimulating the secretion of mucus as raw materials that enter into the composition of the glycoproteins that make up this mucus, which Forms a layer to protect the surface of the intestine from exposure to the action of digestive enzymes and from invasion by pathogens (Holecek et al., 2006; Ren et al., 2015; Jian et al., 2021), as BCAAs are essential substrates for protein building, and their regulation of protein and energy metabolism as well as their direct absorption by enterocytes to be a source of energy and growth for these cells, stimulating and activating them, enhancing the absorption of nutrients (Shimomura and Kitaura, 2018; Adabi et al., 2019).

**Humeral immunity**

It is noticed from Figure 3 that there is a significant difference because of adding the supplementation treatments of BCAAs in the volumetric standard of antibodies to Newcastle disease virus at 76 weeks of age compared with the control treatment.

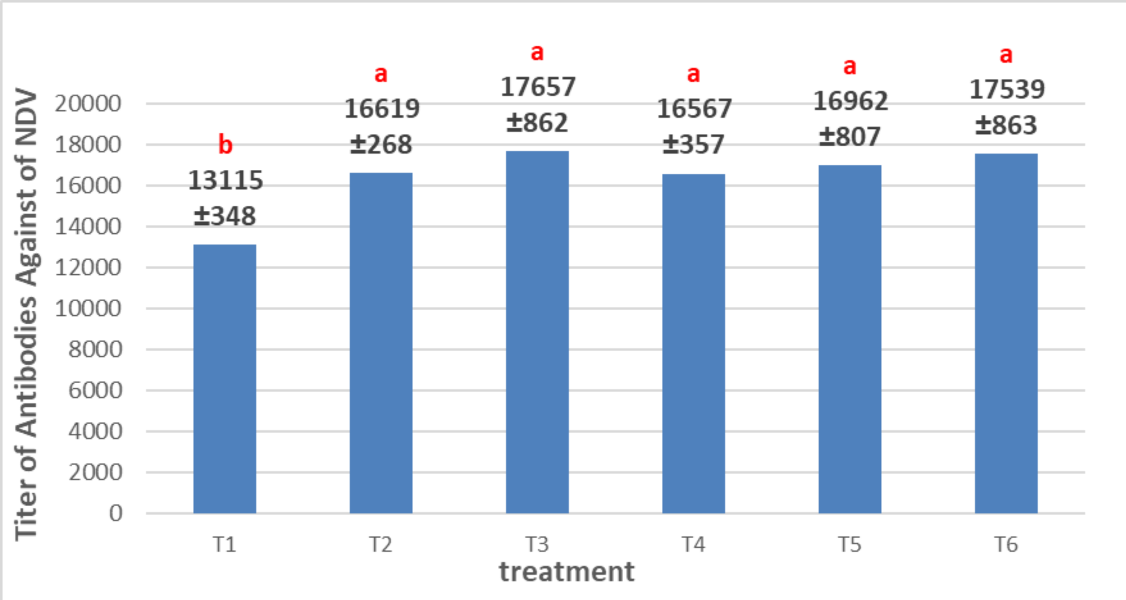


**Figure 3. Effect of feed addition of branched-chain amino acids on the antibody titer of NDV for aged laying hens at the end of the experiment at 76 weeks of age (mean ± standard error).**

T1: Standard diet without supplement (control). T2, T3, T4, T5, T6 standard diet supplemented with 0.5, 1, 1.5, 2, 2.5 g/kg mixture of Leucine, Isoleucine and Valine (mixing ratio 2:1:1), respectively. The different letters above the columns indicate that there are significant differences between the means at the probability level of $P \leq 0.05$ according to the Tukey test.

The improvement in the immune response through a significant increase in the antibody titer of Newcastle virus to the elderly laying hens fed from the addition of the mixture of branched-chain amino acids to the feed, may be due to the role of BCAA in the maturation and differentiation of immune cells and the production of cytokines and antibodies, as well as the regulation of pathways Basic Metabolism of the Immune Response to Pathogens (Simone et al., 2013; Bhanja et al., 2014; Gu et al., 2019), (Zhang et al. 2017) confirms that BCAAs are precursors to the formation of immune cells and an energy source for these cells.

**Biochemical traits of blood**

It is noted from Table 3 the effect of the feed addition of branched-chain amino acids on the activity of enzymes transporting the amino group (ALT), (AST) and the level of malondidehyde (MDA) for aged laying hens at the age of 76 weeks, as it is noted that there are no significant differences between all the experimental treatments in the activity of enzymes (ALT), (AST) after 16 weeks (at the age of 76 weeks) of feeding chickens from the diet supplemented with BCAA, As for the level of MDA, a significant improvement was observed in the two addition treatments T2, T3, which recorded the lowest values, followed by the two addition treatments T4, T5 compared to the control treatment that recorded the highest values, and there were no significant differences between the addition treatments T4, T5, and T6.

**Table 3. Effect of feed addition of BCAAs on the activity of amino group transfer enzymes (ALT, AST) and MDA level in aged laying hens at the end of the experiment at the age of 76 weeks (mean ± standard error).**

| treatments | ALT (unit/liter) | AST (unit/liter) | MDA (nmol/ml) |
|---|---|---|---|
| **T1** | 15.24±0.23 | 282.30±29.77 | 9.01±0.32c |
| **T2** | 13.59±0.43 | 259.56±23.12 | 5.67±0.21a |
| **T3** | 13.91±0.34 | 300.34±24.16 | 5.40±0.29a |
| **T4** | 14.25±0.40 | 281.07±37.76 | 7.34±0.22b |
| **T5** | 14.61±0.34 | 263.87±50.40 | 7.71±0.22b |
| **T6** | 15.00±0.39 | 320.55±29.76 | 8.03±0.22bc |
| **Significance level** | N.S | N.S | ** |

T1: Standard diet without supplement (control). T2, T3, T4, T5, T6 Standard diet plus 0.5, 1, 1.5, 2, 2.5 g/kg mixture of Leucine, Isoleucine and Valine (mixing ratio 2:1:1), respectively. N.S There are no significant differences between the means at the probability level of $P \leq 0.05$ according to Tukey's test. ** There are highly significant effects at the probability level of $P \leq 0.01$ in the analysis of variance table.

The decrease in the level of MDA in the blood of old laying hens fed a mixture of branched chain amino acids is one of the indications for the decrease in oxidative stress to which these birds are exposed as a result of the vital activities of perpetuation, production and aging, and this may be due to the role of BCAA In reducing the production of free roots by the mitochondria of cells to counteract the effect of oxidative stress on birds (Iwasa et al., 2013; Li et al., 2016).

It is noted from Table 4 the effect of the feed addition of branched-chain amino acids on the biochemical traits of the aged laying hens at the end of the experiment at the age of 76 weeks, as it is noted that there were no significant differences between the mean levels of glucose, uric acid, total protein, cholesterol for all experiment treatments at the end of the period. Experience (at the age of 76 weeks).

**Table 4. Effect of supplementation with BCAAs on the biochemical characteristics of blood for aged laying hens at the end of the experiment at 76 weeks of age (mean ± standard error).**

| treatments | Glucose (mg/100ml) | Uric acid (mg/100ml) | Total Protein (g/L) | Cholesterol (mg/100ml) |
|---|---|---|---|---|
| **T1** | 199.26±5.58 | 5.91±0.17 | 40.12±0.11 | 253.89±2.55 |
| **T2** | 199.80±4.50 | 5.85±0.17 | 39.58±0.69 | 251.71±3.26 |
| **T3** | 200.88±5.22 | 5.80±0.15 | 35.88±1.28 | 251.21±2.61 |
| **T4** | 202.86±6.48 | 5.95±0.18 | 37.37±1.60 | 253.85±2.61 |

| | | | | |
|---|---|---|---|---|
| **T5** | 205.20±5.04 | 5.87±0.17 | 36.66±1.07 | 250.92±1.47 |
| **T6** | 201.78±5.22 | 5.77±0.18 | 38.59±0.58 | 256.27±1.11 |
| **Significance level** | N.S | N.S | N.S | N.S |

T1: Standard diet without supplement (control). T2, T3, T4, T5, T6 Standard diet plus 0.5, 1, 1.5, 2, 2.5 g/kg mixture of Leucine, Isoleucine and Valine (mixing ratio 2:1:1), respectively. N.S There are no significant differences between the means at the probability level of $P \leq 0.05$ according to Tukey's test.

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