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INVESTIGATIONS ON THE EFFECT OF VITAMIN SUPPLEMENTS ON BODY WEIGHT, HAEMATOLOGICAL AND BIOCHEMICAL INDICES IN QUAIL

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Abstract

The research was carried out on 40 Japanese quails (Coturnix japonica), they were divided into two groups of 20 quails each, control and experimental. Both groups were given the same forage regime, combined quail forage, growth phase. The experimental group additionally received a supplement of vitamin C (L-ascorbic acid) and vitamin E (DL-alpha tocopheryl acetate), both vitamins in a dose of 1000 mg/kg of combined feed. The duration of the experiment was 6 weeks. The investigations performed were: body weight, hemogram and some biochemical indices. These investigations were carried out at 21 days and the end of the experimental period, i.e. 42 days. At 21 days and 42 days, significant differences were observed in the individuals of the experimental group: increases in erythrocyte count, haemoglobin and haematocrit were observed. Biochemical investigations showed increases in total protein, alkaline phosphatase and calcemia. Decreases in AST, ALT, blood glucose and creatinine were observed in the experimental group. The body weight was statistically significantly higher in the group receiving the feed diet with vitamin C and E supplementation.

Key words*: biochemistry, body weight, haematology, quail, vitamin C, vitamin E.*

INTRODUCTION

Globally, the concern of growing healthier and more productive poultry is a desideratum that more and more breeders are trying to reach. This is particularly important because poultry meat, along with meat from other species, is a major source of protein worldwide (Petcu, 2013; Petcu, 2015). Thus, in the poultry industry for the best possible results in terms of productivity and reproducibility, many additives are added in animal feed (Marin et al., 2010; Marin et al., 2015; Bălăceanu, 2017; Abd El-Hack et al, 2022; Ghimpețeanu, 2022). In several specialized works the idea of using substances derived from natural sources as additives to replace the use of antibiotics is suggested in order to obtain safe food for consumers (Mitrea, 2003; Gonciarov, 2004; Petcu, 2007; Savu, 2013). The supplementation of the feed ration with different vitamin adjuvants is particularly important to support the metabolic processes of the organism (Ghiță et al., 2021).

Vitamin complexes, in particular those containing vitamin C and vitamin E, play a particularly important role as antioxidants, these two vitamins preventing lipid peroxidation. Vitamin E acts as an oxygen free radical scavenger. Vitamin C, also plays its role as an antioxidant, by reacting with peroxide radicals and at the same time works as an adjuvant for vitamin E (Surai, 2002; Kurutas, 2016; Predescu, 2018). There are numerous researches emphasizing the role and effects of vitamins C and E on the body homeostasis but also on the reproductive performance of animals (Pop et al., 2006; Răduță et al., 2017). Vitamin E is closely involved in the synthesis of several hormones involved in fertility. Deficiencies in vitamin E can materialize in malfunction of the reproductive system, but also in a poor response to the action of stressors (Mălăncuș et al., 2024). Also in the literature, numerous works are cited that emphasize the importance of vitamin C, as an adjuvant in egg production but also for hatching percentage. Several researchers have emphasized that supplementation of feed with vitamin E and vitamin C, together or separately, is a beneficial practice to eliminate the negative effects of stress, thus for better growth performance (Akinyemi et. al., 2003; Mălăncuș et al., 2022).

The aim of this study is to observe the effect of high doses of vitamin C and vitamin E on growth as well as haematological and biochemical parameters in quail.

MATERIALS AND METHODS

The experiment was carried out on the biobase of the Faculty of Veterinary Medicine in Bucharest in 2023. A number of 40-day-old Japanese quail (*Coturnix Japonica*) were used in the experiment. They were randomly divided into two groups, control and experimental, with 20 birds per group. Both control and experimental birds were kept in cages (0.70 m/0.50 m) with 10 birds in each cage. Microclimate conditions were the same for both groups. The temperature was maintained at 27ºC during the 6 experimental weeks. Water and feed were given *ad libitum*. The forage ration was purchased from the National Research and Development Institute for Animal Biology and Nutrition (IBNA) Balotești and consisted of combined feed for the quail, growth phase. The ingredients of the feed ration were: cereals, soybean meal, corn gluten, monocalcium phosphate, calcium carbonate, salt, amino acids and vitamin-mineral premix.
Nutritional values for the feed ration are: crude protein 22.50%, metabolizable energy (Kcal/kg) 3140, methionine 0.64%, methionine + cystine 0.98%, lysine 1.33%, calcium 0.96%, phosphorus 0.75%, choline 0.03%, salt 0.40%. Both groups were fed the same diet. The experimental group additionally received a supplement of vitamin C (L-ascorbic acid) and vitamin E (DL-alpha tocopheryl acetate), both vitamins at a dose of 1000 mg/kg of combined feed. Individuals were weighed twice, 3 weeks after the start of the experiment and at the end of the 6 weeks, using a Partner electronic balance. Blood samples were collected at 21 and 42 days, the experimental period, from each individual in both control and experimental groups. Blood was collected by subaxillary vein puncture from where 1 ml of blood/puncture was collected. From the blood samples the following determinations were performed: erythrocyte count, haemoglobin, haematocrit, then the derived erythrocyte constants (MCV MCH and MCHC) were calculated. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), calcium (Ca), glycemia (GLU) and creatinine (CREA) were performed as biochemical investigations. Hematological investigations were performed using an IDEXX ProCyte Dx apparatus, and a VetTest Chemestry Analyzer was used for biochemical investigations. All data obtained were tabulated and statistically interpreted using Student t test.

RESULTS AND DISCUSSIONS

Regarding body weight, both after 21 days and after 42 days, differences could be observed in the experimental group. Its mean body weight was 2.19% higher after 21 days and 5.26% higher after 42 days ($p<0.05$) than the values recorded in the control group (Figure 1 and Figure 2). This aspect shows a better weight gain in the group that received the feed with vitamin C and vitamin E supplementation.

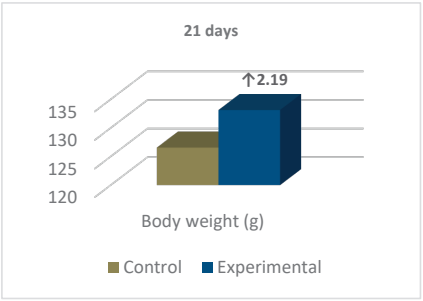

Figure 1. Mean values of body weight investigations in quails at 21 days of the experiment ($p<0.05$)

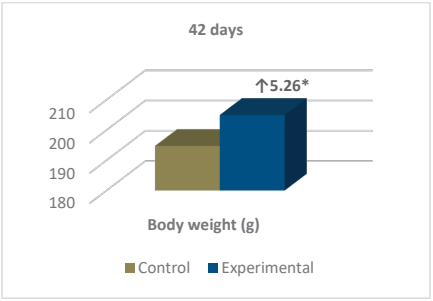

Figure 2. Mean values of body weight investigations in quails at 42 days of the experiment ($p<0.05$)

The two vitamins are particularly important as they respond to stress due to their antioxidant properties and vitamin C's ability to increase the utilisation of corticosteroids released during

stress phases (Phoprasit et al., 2014; Ghiță et al., 2015). Also, the requirements of animals for these two vitamins, especially under stress, may be much higher than the requirements normally fed through feed. It is well known that stress can lead to demineralization but also to the release of some vitamins from various tissues and their excretion (Haq et al., 2016). The interaction between vitamin C and vitamin E is of importance from both a pathophysiological and animal welfare point of view, thus of great importance for food safety. Various research in the field shows that under various temperature conditions, vitamin C and vitamin E added to the feed ration in quail promotes the well-being of the animals, leading to positive growth gain (Ali et al., 2012). Consecutive to the addition of vitamin C and vitamin E to the feed ration, as observed by other researchers, the body weight in the experimental group is higher. Providing an antioxidant barrier enables the body to effectively counteract the effects of oxidative stress and thus improve metabolism. An intake of vitamin C and vitamin E is necessary to obtain healthy individuals, either for use as breeders or to ensure an adequate carcass in the case of food birds. In several studies carried out over the years, the problem of loss of constitutive juices in the muscle of poultry has been reported. In the same studies, it has been hypothesized that this can be corrected by supplementing the feed with antioxidants at increased concentrations.

The results of the haematological and biochemical investigations emphasize the beneficial effect of supplementation with vitamin C and vitamin E (Kabir et al., 2013; Lin J., 2014). Increased values of erythrocyte count, haemoglobin and haematocrit can be observed in the experimental group, both at 21 days and at the end of the experimental period.

After 21 days from the start of the experiment, some changes were observed concerning the haematological parameters: the mean erythrocyte count was 11.32% higher in the experimental group ($p<0.05$), haemoglobin and haematocrit had higher mean values than the control group, haemoglobin increasing by 6.25% and haematocrit by 3.3%.

The derived erythrocyte constants showed in the group of birds supplemented with vitamin C and vitamin E, some changes compared to the control group. The MCV was lower by 7.14% and the MCH by 4.07%. The mean erythrocyte haemoglobin concentration was higher in the experimental group, increasing by 2.71%

Similar results were also obtained at the end of the experimental period: the mean erythrocyte count was 19.65% higher in the experimental group ($p<0.05$), also haemoglobin and haematocrit had higher mean values, 7.57% and 6.45% respectively, compared to the mean values recorded in the control group ($p<0.05$).

The secondary erythrocyte constants MCV and MCH showed decreasing trends in the experimental group, 11.11% and 10.17%.

The mean erythrocyte haemoglobin concentration was higher in the control group, the mean of the determinations being 1.02% higher than in the vitamin-supplemented group (Table 1 and Table 2).

Table 1. Mean values of haematological investigations in quails at 21 days of the experiment

Parameter 21 days	Control Group	Experimental Group	Percentage (%)
E x 10^6 / µl	5.3	5.9	**↑11.32***
Hb g/dl	14.4	15.3	**↑6.25**
HTC %	30	31	**↑3.3**
MCV μ^3	56	52	**↓7.14**
MCH pg Hb/E	27	25.9	**↓4.07**
MCHC g Hb/dl E	48	49.3	**↑2.71**

*P<0.05 – significant differences

Table 2. Mean values of haematological investigations in quails at 42 days of the experiment

Parameter 42 days	Control Group	Experimental Group	Percentage (%)
E x 10^6 / µl	5.14	6.15	**↑19.65***
Hb g/dl	15.2	16.35	**↑7.57***
HTC %	31	33	**↑6.45***
MCV μ^3	60.3	53.6	**↓11.11**
MCH pg Hb/E	29.5	26.5	**↓10.17**
MCHC g Hb/dl E	49	49.5	**↓1.02**

*P<0.05 – significant differences

The increased values of erythrocyte count, haemoglobin and haematocrit are explained by the anti-oxidant effect of vitamin E and vitamin C (Figure 3 and Figure 4).

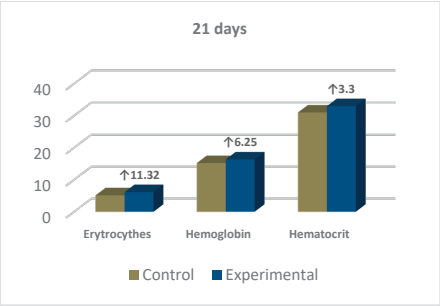

Figure 3. Eritrocytes, haemoglobin and hematocrit at 21 days of the experiment

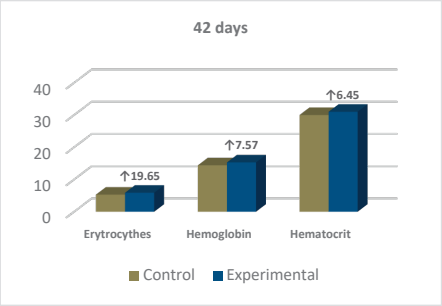

Figure 4. Eritrocytes, haemoglobin and hematocrit at 42 days of the experiment

The effect is manifested by an increase in haemato-forming activity in the haematogenous bone marrow (Răduță et al., 2017; Ghiță et al., 2022). The quantitative increase in the number of erythrocytes and haemoglobin ensures better functioning of tissues and the onset of oxygenation deficiency at the tissue level is avoided so that the skeletal muscle and the central nervous system have a correct oxygen supply. The addition of vitamin C and vitamin E to food will lead to better tissue oxygenation through increased erythrocyte counts and a prolonged lifespan. All these are explained by protecting cell membranes from the oxidative activity of various stressors (Yehmed et al., 2023). Providing better oxygenation at the tissue level also has beneficial effects for the commercial aspect of different avian products. In poultry reared for meat production, the increased oxygen supply will lead to a faster but also more harmonious body development of the carcass. The use of natural supplements instead of antibiotics in the feed ration is desirable, thus reducing the risk of antibiotic resistance, both for livestock and humans (Surai, 2002; Răduță et al., 2017).

In terms of secondary erythrocyte constants, a decrease in the MCV and in the MCH can be observed.

These decreases can be explained by a smaller volume of young erythrocytes released into the blood circulation as a result of enhanced haematopoiesis. A smaller erythrocyte volume will automatically lead to a lower mean erythrocyte haemoglobin (Figure 5 and Figure 6).

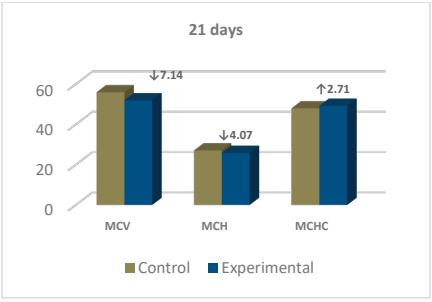

Figure 5. Mean values of haematological of indirect constants in quails at 21 days of the experiment

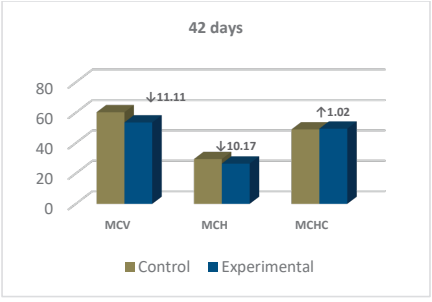

Figure 6. Mean values of haematological of indirect constants in quails at 42 days of the experiment

From the data presented in Table 3, it can be seen that 21 days after the start of the experiment, the average AST determinations in the experimental group were lower by 11.96%, and ALT showed lower values by 14.18%. Alkaline phosphatase increased by 12.24%, total protein by 11.63%, and calcium by 16.67%. Serum glucose decreased in the experimental group by 18.9% and creatinine by 5.41%. Forty-two days after the start of the experiment, the experimental group had a 12.6% lower mean AST and 35.74% lower ALT. Serum total protein increased by 22.83% (Table 4).

Table 3. Mean values of biochemical investigations in quails at 21 days of the experiment

Parameter 21 days	Control Group	Experimental Group	Percentage (%)
AST (U/L)	149,28	131,43	↓**11.96***
ALT (U/L)	32.12	28.13	↓**14,18***
ALP (U/L)	129	147	↑**12.24***
TP (g/dl)	9.03	10,8	↑**11.63***
Ca (mg/dl)	13.2	15.4	↑**16.67**
GLU (mg/dl)	212.7	172.3	↓**18.9***
CREA (mg/dl)	1.85	1.75	↓**5.41**

*P<0.05 – significant differences

Table 4. Mean values of biochemical investigations in quails at 42 days of the experiment

Parameter 42 days	Control Group	Experimental Group	Percentage (%)
AST (U/L)	151.32	132.25	↓**12.6***
ALT (U/L)	36.54	23.48	↓**35.74***
ALP (U/L)	138.3	157	↑**11.91***
TP (g/dl)	9.20	11.3	↑**22.83***
Ca (mg/dl)	14.3	16.2	↑**13.29**
GLU (mg/dl)	225.5	184.9	↓**18***
CREA (mg/dl)	1.98	1.22	↓**38.38**

*P<0.05 – significant differences

Mean serum glucose determinations in the experimental group were 18% lower than in the control group. Alkaline phosphatase (ALP) increased by 11.91%. There was also a 38.38% decrease in creatinine in the experimental group (Figures 7-10).

Similar results to the above investigations were also published by Samantha Sigolo and colleagues in 2019. In that paper, the beneficial effect of supplementing quail feed with vitamin C and vitamin E is emphasized. In birds haematological and biochemical investigations are very important indicators for their well-being. Any change in the homeostasis of the organism will be reflected in the physiological response of the birds (Scholtz et al., 2009; Saracilă et al., 2023). In line with the results obtained, our determinations have shown that feed supplementation with vitamin C and vitamin E has beneficial effects on protein, renal and liver metabolism (Cotelle et al., 2003).

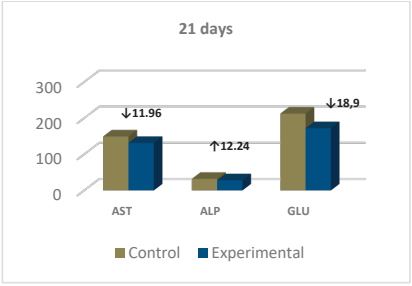

Figure 7. Mean values of AST, ALP and GLU investigations in quails at 21 days of the experiment

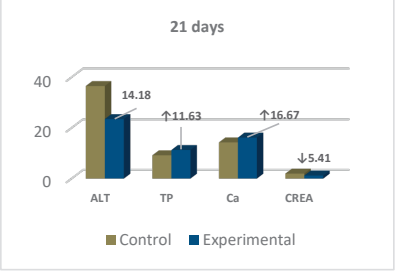

Figure 8. Mean values of ALT, TP, Ca and CREA investigations in quails at 21 days of the experiment

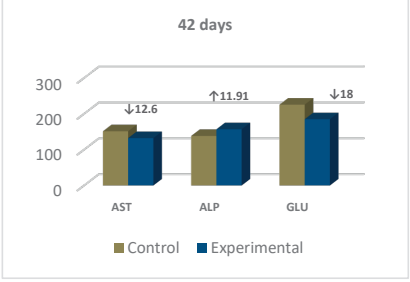

Figure 9. Mean values of AST, ALP and GLU investigations in quails at 42 days of the experiment

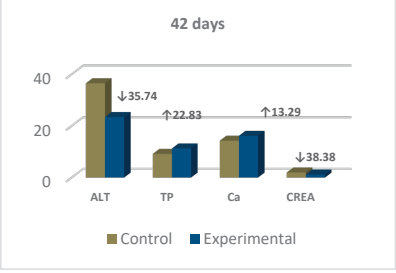

Figure 10. Mean values of ALT, TP, Ca and CREA investigations in quails at 42 days of the experiment

The low mean AST and ALT values, but increased ALP suggest an improvement in liver metabolism by reducing the permeability of cell

membranes so that the enzymes no longer leave the cytosol and are found in a lower percentage in the blood. The increase in alkaline phosphatase, most likely muscle and skeletal shows an enhancement of metabolism, a greater increase in the experimental group, an increase that may correlate with data found in body weight investigations (Goma et al., 2024).

The decrease in serum glucose concentration is explained by a decrease in its availability, as a consequence of the utilization of glucose more adequately in cellular metabolisms.

The increase of serum proteins is another aspect that suggests the improvement of metabolism, they being in higher concentration in the blood are more readily available for utilization by various systems in the body.

The property of muscle proteins to attract water and hold it at the cellular level is particularly important for meat quality (Petcu, 2015). The correlation between meat quality and muscle antioxidant resistance is well known. Differences in antioxidant defence systems in several animal species have been reported in several literature papers, with different muscle groups showing calpain damage, and protein lysis, these abnormalities being influenced by proteolysis (Cotelle et al., 2003; Haq et al., 2016).

CONCLUSIONS

As a result of supplementation with vitamin C and vitamin E in the experimental group, the body weight was 5.26% higher than in the control group. The supplementation of vitamin C and vitamin E in quail feed leads to an improvement in haematological parameters: erythrocyte count, haemoglobin, and haematocrit, the increases being statistically significant. The mean erythrocyte volume and mean erythrocyte haemoglobin show a decreasing trend, due to the smaller volume of young erythrocytes. Results of biochemical investigations showed decreases in AST, ALT, ALP and glucose in the experimental group. Mean increases in total protein and calcemia were recorded in the group fed the vitamin C and vitamin E supplements.

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