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Effect of zinc methionine supplementation on biochemical and hematological indices of growing rabbits

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Abstract

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This study was conducted to investigate the influence of different amounts of zinc methionine on the blood biochemistry, hematological parameters and productivity of growing rabbits. Studies have been conducted on 15 rabbits of the Termon white breed, which were randomly divided into three groups of 5 animals in each – one control and two experimental. Animals of the control group were fed with the commercially granulated feed, whereas animals in the experiment group G1 and G2 were fed with the same basal ration supplemented with 10 and 20 mg/kg Zinc-Methionine, respectively. The supplement was added from 50 days of animal age during 40 days. *Samples of blood from the marginal ear vein were taken for biochemical and hematologic researches* at ten days' intervals during experimental periods. *Body weight gain was determined by weighing. High Zn supplementation decreased ($P < 0.05$) mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), as compared to control group, while other hematological parameters of rabbits remained unaffected.* Results showed that serum albumin concentration were significantly increased on the 70th and on the 80th days of study ($P < 0.05$) in rabbits received zinc-methionine compared to control animals, while total protein content did not change. The creatinine concentration in the blood of rabbits of the both experimental groups was decreased ($P < 0.05$) on the 90th day of experiment, as compared to the control. Total cholesterol and triglycerides concentrations were decreased significantly during all period of feeding ZnMet-supplemented diet in both experimental groups. Serum ALT and AST constituent were not significantly affected by dietary treatments comparing to the control. However, serum ALP activity significantly ($P < 0.05$) increasedon the 80th day of experiment in rabbits fed Zn-Met at the both doses. Also, LDH content was significantly increased ($P < 0.05$) on the 80th and 90th day of the research in high dose-treated rabbits (G2 group), compared to the low dose-treated animals or control group ($P < 0.05$). Conclusively, the results revealed that fortification of diet with Zn-Met at level of 10 and 20 mg/kg could significantly enhance the nutrients digestibility of growing rabbits without harmful effect on blood constituents.

Keywords: rabbits; zinc methionine; blood, nutrition

Introduction

Trace elements are playing a vital role in basic physiology-biochemical processes, within the body of animals. They are involved in growth, production and reproduction. They also play an important role as catalysts in protein, lipid, carbohydrate and mineral metabolism, activate the functions of hormones, vitamins and enzymes.

According to the National Institutes of Health (NIH), zinc is crucial to many aspects of health. Zinc is one of the most common trace elements in the body and plays a substantial role in growth and development, acting as a signaling factor. Zinc has a catalytic, coactive, or structural role in a wide variety of enzymes that participates actively in protein synthesis and carbohydrate metabolism. This mineral is an essential component of carbonic anhydrase, glutamic dehydrogenase, alkaline phosphatase, pyridine nucleotide dehydrogenase, alcohol dehydrogenase, superoxide dismutase, pancreatic carboxypeptidase, and tryptophan desmolase, DNA-polymerase. Zinc plays an important role in polynucleotide transcription and thus in the genetic expression process. It`s also involved in the metabolism of other micronutrients (Salgueiro et al., 2000).

Recently, organic trace elements have been used often due to their potentially higher bioavailability (Ahmed et al., 2018). Numerous studies in cattle, rabbits and poultry have shown improvements in reproductive performance, immune system function and mineral status, when complexed organic trace minerals were used (Bomko et al., 2018; Dychok et al., 2018; Dzen et al., 2015). Bioavailability of organic Zn, Cu, Mn and Se relative to inorganic salts has been evaluated in many studies (Pavlata et al., 2012; Cao et al., 2000). Few studies have reported the organic Zn products, such as Zn-methionine (Met), Zn-yeast, can become alternative sources of the inorganic form of Zn (Nitrayova et al., 2012). Therefore, the main objective of the present study was to investigate the effect of dietary ZnMet supplementation on the some biochemical and hematological parameters in rabbits of the Termon white breed. They are fairly fertile, stable and not too fastidious to food.

Materials and Methods

Experimental design and dietary treatments

The study was performed at the vivarium of the Institute of Animal Biology NAAS on 15 rabbits of the Termon white breed, which were randomly divided into three groups of 5 animals in each – one control and two experimental. Animals of the control group were fed with the commercially granulated feed (Table 1), whereas animals in the experiment group G1 and G2 were fed with the same basal ration supplemented with 10 and 20 mg/kg Zinc-Methionine (Zn-Met), respectively. The supplement was added from 50 days of animal age during 40 days. All manipulations with experimental animals were carried out in com-pliance with the rules of the European Convention for the Protection of Vertebrate Animals (Official Journal of the European Union L276/33, 2010). All rabbits were kept under the same conditions all over the experimental period (40 days). They were placed in stainless steel cages one animal per cage and continually provided with fresh water. The rabbits were acclimatized for one week before the commencement of the trials.

Sample collection and analysis of some biochemical parameters

The blood samples were collected precisely at ten days' intervals during the time of experiment. The blood samples were collected from marginal ear vein into vacuum blood collection tubes (Vacutainers), treated with sodium citrate and centrifuged for 20 min at 2000 x g to obtain plasma using a refrigerated centrifuge. The obtained plasma samples were stored at –30°C until analysis.

The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, albumin, total cholesterol, triglycerides (TG), creatinine, urea, total calcium and non-organic phosphorus were measured in rabbits' blood serum via spectrophotometry in the semiautomatic biochemical analyzer (Humalyzer 3000, GmbH, Germany), using commercial kits (Human GmbH, Germany).

Red blood cells (RBCs), hematocrit (HCT), haemoglobin (Hb), Mean cell volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Red cell distribution width (RDW) were examined using a Hema Screen 18 automatic hematology analyzer (Orphee Mythic 18, Switzerland).

Table 1. The component and chemical composition of granulated rabbit basal diet

Feed Ingredient	Content, %	Nutrient Composition	
Lucerne meal	30.0	Digestible energy (DE), kcal/kg	2150
Sunflower meal, extracted	19.0	Crude protein, %	14.5
Wheat bran	20.0	Crude fibre, %	14.5
Wheat grains	5.5	Crude fat, %	3.0
Maize	7.0	Lysine, %	0.73
Soy bean meal, extracted	6.0	Methionine, %	0.35
Barley grains	9.0	Methionine + cystine, %	0.4
Premix[a]	3.5	Calcium,%	0.7
Total	100	Na, %	0.15
		Available Phosphours, %	0.65

Table 2. Effect of dietary zinc-methionine supplementation on the blood biochemical parameters in rabbits (n=5, ±SEM)

Parameter	Control	Zinc-methionine	
		G1(10 mg/kg)	G2(20 mg/kg)
Total protein, g/L			
60	62.67±0.96	60.84±0.61	62.83±1.15
70	67.40±0.48	64.33±0.82	65.44±0.86
80	67.20±1.11	66.50±0.92	64.73±1.69
90	69.76±0.95	67.81±0.93	65.43±1.02
Albumin, g/L			
60	30.34±0.67	29.61±0.89	31.77±1.21
70	30.84±0.75	35.33±1.74	38.44±2.67*
80	36.30±1.39	41.96±2.12*	44.06±2.24*
90	34.47±1.52	36.53±1.05	33.76±2.17
Creatinine, μmol/L			
60	73.87±1.42	74.06±0.51	80.13±0.57
70	98.43±0.82	107.28±0.85	101.87±0.99
80	115.77±2.44	106.83±0.57	110.36±1.17
90	132.53±2.70	113.99±2.01*	118.90±0.50*#
Urea, mmol/L			
60	6.16±0.29	5.26±0.06*	5.70±0.06
70	5.94±0.08	5.20±0.06*	5.57±0.16
80	5.36±0.12	5.13±0.04	5.33±0.17
90	4.43±0.25	4.61±0.17	4.80±0.09

Note: * − compare to control group; # − compare to G1 group ($P < 0.05$).

Statistical analysis

The obtained data were analyzed using a program package Statistica 6.0 software (Stat Soft, Tulsa, USA). The significance of the difference between controls and treatments were evaluated using ANOVA (taking into account Bonferroni correction); differences were considered significant at $P < 0.05$.

Results and Discussion

Results of the current study showed that total protein values were similar for all the animal groups, while the albumin value increased in animals receiving ZnMet-supplemented diet. As shown in Table 2, the concentration of albumin had higher values in the blood of rabbits which received Zinc-methionine at lower dose on the 80th day of study ($P < 0.05$) compared to control animals, while in the G2 experimental group this index was significantly increased on the 70th and on the 80th days of experiment ($P < 0.05$). This improvement might be related to Zn requirements for normal protein synthesis and metabolism.

Results of our study indicated that dietary Zn-Met added doses did not reveal any significant changes in urea content between groups. Its content was slightly decreased in the both experimental groups during the study period compared to the control group, in particular the significant changes were noted in G1 group on the begin of the study. The research revealed a tendency towards changes in the level of urea. Creatinine concentration in the blood of rabbits of the both experimental groups was decreased ($P < 0.05$) on the 90th day of experiment as compared to the control.

Dietary supplementation of Zn-methionine during growing rabbits was accompanied with changes in the activity of enzymes related to Zn metabolism (Figures 1 and 2).

Activity of alkaline phosphatase (ALP) did not change significantly on the 60th and 70th day in all groups, while on the 80th and 90th day of experiment the levels of enzymatic activity were significantly elevated in both experimental groups compared to the control group ($P < 0.05$).

On the beginning of Zn-Met supplementation, the activity of LDH was significantly decreased in blood of rabbits from both experimental groups, whereas on the 80th and 90th day of the research in high dose-treated rabbits (G2 group) LDH content was significantly increased ($P < 0.05$) as compared to control and G1 groups. The change in activity of ALP and LDH may attribute to the increased Zn availability to the rabbits as Zn is as cofactor for those enzymes.

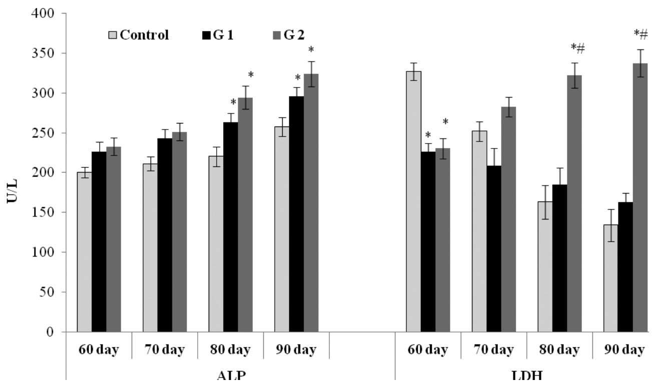

Fig. 1. Effect of feeding Zinc-methionine supplementation on the activity of activity of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) of control and experimental rabbits:
Control group (without Zn supplementation); G1group: 10 mg/kg Zn as ZnMet; G2 group: 20 mg/kg Zn as ZnMet. Values are expressed as mean ± SD; * − compare to control group; # − compare to G1 group, $P < 0.05$

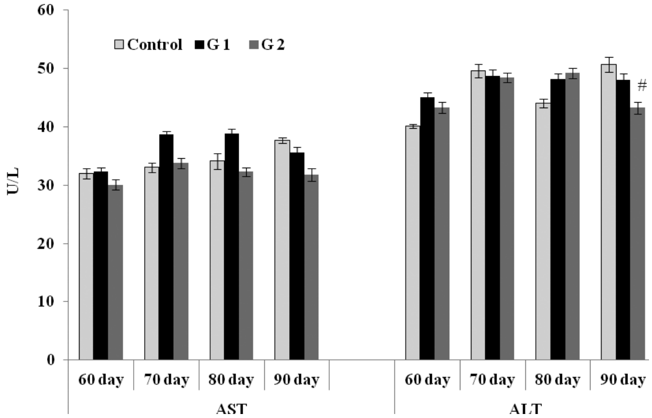

Fig. 2. Activity of the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of control and experimental rabbits:
Control group (without Zn supplementation); G1group: 10 mg/kg Zn as ZnMet; G2 group: 20 mg/kg Zn as ZnMet. Values are expressed as mean ± SD for 5 animals; # − compare to G1 group, $P < 0.05$

Serum AST activity was not significantly affected by dietary treatments comparing to the control. However, serum ALP content was elevated by supplementing the both doses of Zn-Met (10 and 20 mg/kg) and was significantly increased ($P < 0.05$) on the 90th day of experiment in G2 group as compared to the control (Figure 2).

Total cholesterol and TAG concentrations were decreased significantly during all period of feeding Zn-Met-supplemented diet in both experimental groups (Figure 3). The experimental groups from 60th to 90th day, which were given the organic zinc form Zn-Met at dose 10 mg and 20 mg, showed a significant ($P < 0.05$) decrease of triglycerides in their blood serum compared to control group. The total cholesterol concentration was significantly changed in the blood serum of rabbits both groups during the experimental period compared with that of the control group.

In the research we did not find large differences among experimental groups in Ca, P content between control and experimental groups of rabbits those fed with Zn-Met supplemented diets (Figure 4). Also, the ratio between calcium

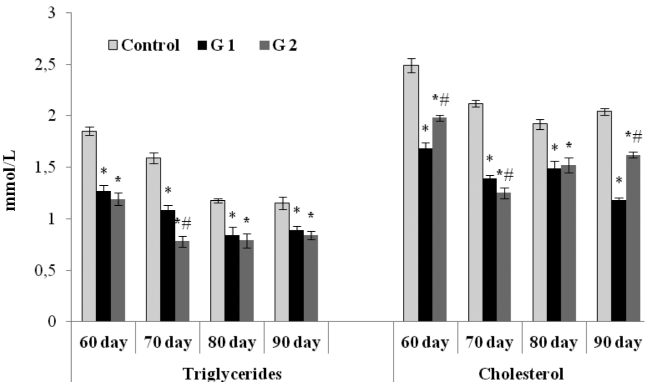

Fig. 3. Indices of lipid metabolism in rabbits fed diets supplemented with Zinc-methionine:
Control group (without Zn supplementation); G1group: 10 mg/kg Zn as ZnMet; G2 group: 20 mg/kg Zn as ZnMet. Values are expressed as mean ± SD for 5 animals; * − compare to control group; # − compare to G1 group, $P < 0.05$

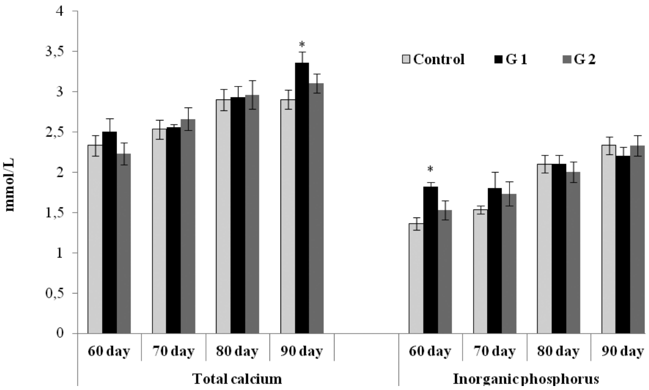

Fig. 4. Content of calcium and phosphorus in blood of rabbits fed diets supplemented with Zinc-methionine:
Control group (without Zn supplementation); G1group: 10 mg/kg Zn as ZnMet; G2 group: 20 mg/kg Zn as ZnMet. Values are expressed as mean ±SD; * − compare to control group, $P < 0.05$

and phosphorus in blood of rabbits control and experimental groups was accompanied by tendential changes during experimental period, ranging within the 1.24–1.71.

The morphological composition of the blood of rabbits of all groups during the fattening were showed in Table 3.

The number of erythrocytes of the blood of animals of all groups during experiment did not change, however the average haemoglobin content was significantly increased in the blood of animals from the G1 group after the use of lower concentration of Zn-Met at 80th and 90th day of treatment as compared with rabbits in the control group. The hematocrit level was significantly decreased ($P < 0.05$) at the 80th day in animals both experimental groups received Zn-Met as compared to control rabbits.

A reliable decrease of average concentration of hemoglobin in erythrocytes ($P < 0.05$) was observed in the G1 group during research and at the beginning and the end of treatment period in the G2 experimental group ($P < 0.05$).

Table 3. Hematological parameters of rabbits fed diets supplemented with Zinc-methionine (n=5, Mean±SD)

Parameter	Control	Zinc-methionine	
		G 1 (10 mg/kg)	G 2 (20 mg/kg)
RBC, ×10^{12}/L			
60 day	4.73±0.39	4.89±0.16	4.20±0.47
70	5.08±0.28	5.10±0.17	5.22±0.39
80	5.12±0.45	5.26±0.15	5.37±0.60
90	5.53±0.54	5.56±0.25	5.44±0.58
Hb, g/L			
60	100.0±7.9	100.5±4.5	89.75±9.38
70	110.75±5.35	113.5±3.79	105.25±8.43
80	107.33±4.40	141.0±12.76*	107.0±12.0
90	114.0±2.0	152.0±16.0*	107.5±43.5
HCT, L/L			
60	0.44±0.02	0.46±0.01	0.37±0.03
70	0.41±0.03	0.42±0.01	0.43±0.03
80	0.57±0.04	0.45±0.02*	0.44±0.05*
90	0.54±0.05	0.48±0.01	0.36±0.04*
MCV, fl			
60	87.75±0.92	88.47±0.63	87.2±1.17
70	88.3±1.23	89.36±1.65	84.10±1.03*
80	87.16±0.75	89.22±0.35	83.15±0.74*
90	86.05±1.95	87.25±2.05	81.15±1.25*
MCH, pg			
60	26.82±0.22	26.55±0.26	25.80±0.23
70	26.17±0.34	25.80±0.17	25.15±0.22
80	26.26±0.29	26.03±0.23	24.95±0.05**
90	26.40±0.39	25.50±0.60	22.20±0.90*
MCHC, g/L			
60	348.75±1.03	343.75±1.37*	338.75±1.88*
70	339.75±2.86	330.33±4.91	339.25±1.60
80	344.0±3.00	336.0±1.73*	339.5±2.50
90	349.0±1.00	335.0±1.00*	329.5±2.50*
RDW, %			
60	10.40±0.21	9.65±0.23*	9.72±0.11*
70	10.47±0.41	9.9±0.43	9.95±0.23
80	9.73±0.28	10.2±0.09	10.10±0.09
90	9.15±0.04	11.0±0.46*	10.95±1.05

Note: C: Control group (without Zn supplementation); G1: Base diet supplemented with 10 mg/kg; G2: Base diet supplemented with 20 mg/kg; * − compare to control group; # − compare to G1 group ($P < 0.05$).

Table 4. Effect of Zn-Met addition to rabbit`s diet on growth parameters (n=5, Mean±SD)

Parameter	Control	Zinc-methionine	
		G 1(10 mg/kg)	G 2 (20 mg/kg)
Live body weight, 40th day, g	1440±35	1566±22	1590±18
Live body weight, 90th day, g	2995±23	3234±31	3066±24
Daily weight gain, g	38.85±1.29	41.7±2.12	36.9±1.03
%, to Control	100	107.3	94.9

The growth performance parameters of growing rabbits fed diet supplemented with Zn-Met are shown in Table 4.

Live body weight was insignificantly higher in the rabbits fed diet supplemented with different Zn-Met levels as compared with those fed the control diet. Moreover, daily weight gain was ($P < 0.05$) improved by 7.3% in the rabbits fed diet supplemented with 10 mg Zn-Met/kg, while the total increment in the body weight of rabbits of the G1 experimental group and control were approximately similar during all experimental period.

Zinc is one of the most common and essential trace element and plays a substantial role in many metabolic processes and physiological functions of animals (McDowell, 2003; Roozbeh et al., 2009; Foster et al., 2009). Zinc has a catalytic, coactive, or structural role in more than 300 enzymes, 2000 transcription factors and cell-signaling proteins, hence, participates in many enzymatic and metabolic functions in the body. Therefore, Zinc deficiency adversely affects clinical, biochemical, and immunologic functions. In recent years, organic Zinc sources have been used progressively due to their potentially higher Zn bioavailability. Zinc-methionine is devoid of free divalent cations for chelation in the intestinal lumen with physic acid, which facilitate enhanced absorption of Zn (Ahmed et al., 2018). Therefore, Zn-Met could be used at lower quantities as compared to inorganic Zn (Sunder et al., 2013).

Our research showed that supplemention of the Zn-Met diet decreased serum TAG and total cholesterol. Change of triglycerides and total cholesterol levels in blood may be due to the zinc's role in enzyme action, which an integral part of several metalloenzymes that are severed in lipid digestion and absorption (Hazim et al., 2011). Some current researches have demonstrated that Zinc supplementation significantly reduces total cholesterol, LDL-c and triglycerides and elevates HDL-c levels (Ranasinghe et al., 2015). Zinc regulates the lipid synthesis via affecting the expression of genes encoding enzymes contributing to liver lipid homeostasis (Hambidge & Krebs, 2001; Qiong Huang et al., 2019).

Our results are in agreement with Wang et al. (2011), who reported that serum cholesterol concentration decreased upon supplemented with organic complexes of Zn. Noha&Tag-El Din (2019) also reported that dietary nano-zinc supplementation non significantly reduced blood serum triglycerides while increased HDL in broiler chicks. These results might be related to anti-atherogenic effect of zinc. Few studies have reported that Zinc deficiency caused increased plasma cholesterol and triglycerides levels in mice with zinc-deficient diets (Reiterer et al., 2005). Also, Ahmed et al. (2018) reported that zinc-methionine supplementation (25, 50 and 100 mg/kg) improved growth performance, nutrient digestibility and reduced plasma total cholesterol, meat lipid peroxidation in broilers subjected to heat stress. The reduce in plasma cholesterol and triglycerides due to Zn was explained by the fact that Zn is involved in lipid metabolism (Midilli et al., 2014).

Results of the current study showed that serum creatinine concentrations decreased by feeding Zn-Met supplemented diet, while urea content, activity of ALT and AST were not significantly changed. Serum level of urea and creatinine have been used as indicators of renal injury. So, these results show that Zn-Met had positive effect on kidney and liver function of treated rabbits. El-Moghazy et al. (2019) showed similar effects of dietary supplementation with Zn-Met (50, 100 and 150 mg/kg diet) on serum concentrations of creatinine, ALT and AST of growing rabbits. The present data are in harmony with Noha & Tag-El Din (2019), who reported that activities of liver enzymes ALT and AST in blood plasma were no significantly influenced by dietary 60 mg Nano-Zn supplementation to New Zealand rabbits diet. Similar results were obtained by Korniichuk (2020), who indicated that the total proteins, albumins and globulins, urea and creatinine increased; AST, ALT, LF enzymes do not change, and Ca level increased, while P decreased of rabbits aged from 36 to 119 days.

Zinc is an essential component in ALP and lactate dehydrogenase, as cofactor catalytic, regulatory or structural functions at the active site of these enzymes. Serum activity of these enzymes are required parameter of evaluating Zn uptake status, hepatic function and nutrient digestion (Sun et al., 2014). The current study found that adding of Zn-Met at 10, 20 mg/kg in the rabbits' diet increased serum activity of ALP, which was consistent with Zhong Cheng Wang

(Zhong et al., 2019), who reported that supplementation of zinc pectin oligosaccharides chelate in broilers` diet resulted in increased ALP activity in serum, contribute to the uptake and biological availability of Zn.

In this study, the activity of LDH, a zinc-related enzyme was affected by the level of Zn supplemental. The enzyme activity of LDH was much higher in rabbits fed diet with 20 mg/kg Zn-Met than that of the Control and 10 mg/kg Zn-Meth. Similar to our research, it was shown that activity of LDH decreased in serum in a dose dependent manner with zinc deficiency. The authors concluded that dietary zinc deficiency exerts alterations in the serum enzymatic activity (Patel et al., 2018).

It was found that supplementation of the rabbit's diets with Zn-Met at 10 and 20 mg/kg did not significantly change the total calcium, inorganic phosphorus content and their ratio in serum, which was consistent with Chrastinová et al. (2016). The author observed that feeding of rabbits with inorganic or organic (Glycinoplex-Zn and Bioplex-Zn) zinc sources did not influence in Ca and P content. Increase in the supplemental Zn level to 100 $mg.kg^{-1}$ diet leads to improving live body weight gain and significantly improves feed conversion ratio of the rabbit.

Haematological studies provides the opportunity for assessing the physiological and health status of animals. Reports by different researchers indicated that dietary contents have effect on blood profile of farm animals (El Hendy et al., 2001; Elmas Ulutas et al., 2020). The ingestion of dietary components have meanrable effects on blood composition, so the evaluation of the haematological profileand may be considered as appropriate measure of effectivity of animals feeding (Prasad, 2009).

The zinc status affects the majority of immunological events such as hematopoiesis, immune cell function and survival, humoral immunity, and cytokine secretion (Sauer et al., 2009). Ulutas (2020) reported in their study that the amount of zinc in the RBCs is approximately ten times higher than the zinc amount in the plasma, because RBCs are rich in enzymes such as zinc-containing carbonic anhydrase.

All hematological parameters assayed in this study fall, within the normal range reported for rabbits, and it is an indication that the diets did not show any adverse effects on hematological parameters during the experimental period. Such of the erythrocyte parameters as RBC count and hemoglobin content, were not significantly changed in the control rabbits and animals of experimental groups fed dietary supplemented with Zn-Met. Similarly, it was reported that the 250-ppm zinc supplementation to the feed of goats for six months was no change in the RBC, Hb, and Hct levels until the third month (Ulutas et al., 2020). It was also reported that the supplemental zinc intake did not affect either the hemoglobin concentration or the total RBC count in the blood of the men fed a diet in low zinc. Nevertheless, the serum zinc and RBC zinc concentrations, total carbonic anhydrase activity, and the activities of the carbonic anhydrase I and II isoforms in RBCs were significantly increased at the 9 weeks (Henry, 2005).

However, the analysis of erythrocyte morphometric parameters showed statistically significant differences in control and experimental rabbits. The significantly lower mean value recorded for mean corpuscular volume (MCV), mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in the rabbits, which received zinc-methionine supplementation at the higher dose. Such differences observed in these indices may be attributed to the Zinc influence in the oxygen transport and the distribution of metalloprotein in the body of the experimental animals. Similarly, Noha &Tag-El Din (2019) resulted that hematological parameters significantly affected due to treatments rabbits with Zn. Also, Bartlett & Smith (2003) reported that dietary Zn supplementation improved. This result is similar to observations El Hendy et al. (2001), which reported that Hb, PCV and total erythrocyte count, were significantly affected by Zn insufficiency. Furthermore, in our research were observed the relation between the decreasing MCHC values and increasing in the level of protein in blood serum of rabbits fed zinc reported also by Nse Abasi et al. (2014). The results revealed that supplementation of diet with Zn-Met at level of 10 and 20 mg/kg enhance the nutrients digestibility of growing rabbits.

Conclusion

Dietary supplementation of Zn-methionine during growing rabbits was accompanied with significantly elevated in the activity of alkaline phosphatase and serum albumin concentration on the 80th day of experiment and decreased the creatinine content on the 90th day, the total cholesterol and TAG concentrations, during all period of feeding in both experimental groups. The results assayed in this study showed supplementation of Zn-Met to basal diet had significant effected on the metabolic processes and may be use to increase the productivity and profitability of growing rabbits during fattening period. Improvement in growth performance parameters of rabbits for 10 mg Zn-Met addition to diet showed that the lower amount of organic compound of zinc have positive effect on the processes of growth and development of the organism of rabbits after weaning.

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