

EFFECT OF FASTING REGIMEN AND DIETARY ZINC SUPPLEMENTATION ON HEMATOLOGICAL PARAMETERS, HORMONAL PROFILES AND ANTIOXIDANT PROPERTIES IN MALES OF GROWING RABBITS

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SUMMARY

The objective of the present study was to evaluate the effect of fasting regimen and dietary zinc (Zn) supplementation on hematological parameters, blood biochemical components, hormonal profiles and the antioxidative properties in males of growing rabbits. A total of 60 weaned male V-Line rabbits (35 days old) were randomly divided into six experimental treatments (10 each): (1) control (basal diet *ad libitum* without any supplementation of Zn); (2) Ad+Zn (basal diet *ad libitum* +100 mg Zn/kg diet); (3) F24 (24 hr fasting regimen per week); (4) F48 (48 hr fasting regimen per week); (5) F24+Zn (24 hr fasting regimen per week+100 mg Zn/kg diet) and (6) F48+Zn (48 hr fasting regimen per week+100 mg Zn/kg diet). All experimental treatments were provided from 35 to 84 day of age. The obtained results showed that fasting regimen (in experimental groups F24 and F48) decreased significantly red blood cells (RBCs) compared with those of control (Ad) or Ad+Zn groups in growing rabbits ($P \leq 0.0001$). While, group F48 recorded the lowest value of blood hemoglobin concentration (Hb) and packed cell volume (PCV). Dietary Zn supplementation relieved the effect of fasting in groups F24+Zn and F48+Zn. Regarding to white blood cells (WBCs) count, the lowest WBCs number was observed in F48 group compared with other treatments. The highest neutrophils percentages were recorded in groups supplemented with Zn (in groups Ad+Zn, F24+Zn and F48+Zn) compared with the other groups. No significant differences were observed in lymphocytes (%) monocytes (%) eosinophils (%) and basophils (%) among the experimental groups. Feeding growing rabbit's *ad libitum* diet supplemented with Zn (Ad+Zn) increased significantly blood plasma total protein and albumin concentration comparing with the other experimental groups. However, fasting regimen groups with or without Zn supplementation decreased significantly plasma total protein, albumin, glucose, total lipids, cholesterol and numerically triglycerides concentrations comparing with those fed *ad libitum* diet with or without Zn supplementation. Growing rabbits exposed to fasting (F24, F48, F24+Zn and F48+Zn groups) had a significant increase in blood plasma aspartate aminotransferase (AST), while, growing rabbits exposed to fasting with Zn (F24 and F48 groups) had a significant increase in blood plasma alanine aminotransferase (ALT) compared with those fed *ad libitum* diet with or without Zn supplementation (Ad and Ad+Zn groups). Total antioxidant capacity (TAC) was significantly increased in the group fed *ad libitum* diet supplemented with Zn (Ad+Zn) compared with the other experimental groups. However, TAC was significantly reduced in growing rabbit subjected to fasting regimen (24 or 48 h) and dietary Zn supplementation alleviated this effect and improved TAC significantly. Growing rabbits exposed to fasting (F24, F48, F24+Zn and F48+Zn groups) had a significant increase in plasma malonyldialdehyde (MDA) concentration, cortisol, corticosterone and significantly decrease in blood plasma triiodothyronine (T₃), thyroxin (T₄) compared with those fed *ad libitum* diet (Ad) and *ad libitum* diet + Zn (Ad+Zn). It could be concluded that growing rabbits exposed to fasting and fed diet supplemented with or without Zn had a significantly decrease in RBCs, Hb concentration, PCV, plasma total protein, albumin, glucose, total lipids, cholesterol, triglycerides, T₃ and T₄ concentrations. Interestingly, dietary Zn supplementation improved the antioxidative properties and reduced the rate of lipid peroxidation in growing rabbits subjected to fasting regimen.

Key words: Fasting, zinc, blood parameter, hormonal profile, antioxidative properties, growing rabbits.

INTRODUCTION

Weaning period is stressful for the kits due to separation from the dam, milk withdrawal and the change to solid feed. These changes can increase the susceptibility of rabbits to trouble on digestive tracts and the microbial count diseases (Gallois et al., 2008). De Blas (2013) stated that the weaning stage is a critical phase of development of digestive disorders, presumably caused by insufficient development of digestive enzymatic capability at an early age. Moreover, for the young mammal, the weaning and the post weaning period are particularly important for the growth performances and feed efficiency (Gidenne et al., 2009; Tůmová et al., 2016). The quantitative feed restriction for growing rabbits reduces the incidence of digestible disorders, particularly epizootic rabbit enteropathy (Debray et al., 2003). In rabbit production, specific and nonspecific enteropathies are always a concern, leading to animal losses of approximately 30% from birth to slaughter (Gidenne and Fortun-Lamothe, 2002). Moreover, feed restriction might positively affect the several changes on metabolic disorders that lead to lower body weight, hormonal changes, immune depression and alter function of the digestive system, especially the liver and small intestine (Tůmová et al., 2016). Furthermore, fasting period could rapidly restore the morphology and functions of the intestine, repairing the intestinal atrophy and normalizing the permeability of the mucosa (Maria et al., 2013).

Zinc (Zn) is a vital trace element found in small amounts in a variety of cells and tissues of organisms and plays an essential role in several biological functions such as protein synthesis, DNA synthesis (Prasad, 1995), immune function (Solomons, 1998), metabolic activities, productive performance like growth (Underwood and Suttle, 1999) and it is a cofactor of more than 300 enzymes (Tapiero and Tew, 2003). Zlotkin et al. (1995) found that dietary Zn has an important component of their functions of hormones and special physiological process including catalytic, structural and regulatory activities in which they interact with macromolecules such as enzymes, pro-hormones, pre-secretory granules and biological membranes.

Research on the changes in the blood laboratory indicators under the conditions of feeding stress has been done in rabbits (Peng and Coon, 1998). Most of the reported data about the observed changes under fasting conditions are controversial, since the factors of interest are appreciably effectiveness from the duration of the fasting period (starvation), the different types of digestion in mono- and polygastric animals, breed and age related peculiarities as well as various other factors (Aro et al., 2013). Therefore, the objective of the present study was to evaluate the effect of fasting regimen and dietary Zn supplementation on hematological parameters, blood biochemical components, hormonal profiles and antioxidative properties in males of growing rabbits.

MATERIALS AND METHODS

Housing and management

This study was carried out at the Rabbits Research Farm of El-Sabahia, Animal Production Research Institute, Agriculture Research Center, Egypt. Sixty weaned males V-line rabbits (35 days old) with an average live body weight of 535 ± 11.25 g, were distributed randomly into six treatments (n=10 each). Rabbits were housed in a naturally ventilated building and kept in individual wire galvanized battery (60 × 55 × 40 cm). Batteries were accommodated with feeders for pelleted rations and automatic drinkers. Fresh water was offered *ad libitum*. The basal diet was formulated to meet the recommended nutrient requirements of growing rabbits according to De Blas and Mateos (1998). Rabbits were fed *ad libitum* the standard pellet basal diet containing 17% crude protein, 2.56% crude fat, 12.5% crude fiber and 2500 Kcal digested energy/ kg-ration. Chemical analyses of the basal diet were carried out according to AOAC (2000) for crude protein, crude fiber, organic matter and ether extract. Animals were kept under similar management and hygienic conditions. Animals were healthy and clinically free of external and internal parasites. The lighting program provided 18 hrs of light per day.

Experimental design

Group 1: Rabbits were fed commercial diet *ad libitum* and served as control group (Ad).

Group 2: Rabbits were fed commercial diet *ad libitum* +100 mg Zn) Zinc Sulfate, El-Nasr for pharm chemical, Egypt) kg diet (Ad+Zn).

Group 3: Rabbits were fasted for 24 hr each week (F24).

Group 4: Rabbits were fasted for 48 hr (24hr at start of the week and the other 24hr after two days at the same week) each week throughout the experimental period (F48).

Group 5: Rabbits were fasted for 24 hr each week +100 mg Zn/kg diet (F24+Zn).

Group 6: Rabbits were fasted for 48 hr (24hr at start of the week and the other 24hr after two days at the same week) each week +100 mg Zn/kg diet (F48+Zn).

At the last week of the experiment, all groups were fed freely.

Blood sampling

Blood samples were collected from marginal vein of each rabbits to evaluate hematological parameters and blood biochemical measurements. Blood samples were taken in heparinized tubes. Blood samples were centrifuged at 3000 rpm for 15 minutes; clear plasma was separated and then stored at -20°C until analyses.

Hematological parameters

Red blood cells counts (RBC's), white blood cell counts (WBC's), packed cell volume (PCV, %) and hemoglobin concentration (Hb) were measured according to the method of Helper (1966). Differential leukocytes percentages were determined according to the method described by Lucky (1977).

Blood biochemical constituents

Blood plasma concentrations of total proteins and albumin was spectrophotometrically determined using commercial kits according to Gornall et al. (1949) and (Weichselaum 1946); respectively. While, globulin concentration was calculate by subtracting albumin concentration from total proteins. Plasma glucose was measured using the method of Hyvarinen and Nikkila (1962). Plasma creatinine was measured according to Cabaud and Wroblewski (1958). Plasma total lipids, triglycerides and cholesterol were measured using the method of Chabrol and Charonnat (1973), Fasati and Prencipe (1982) and Stein (1986); respectively. Blood ALT and AST were measured using the method of Reitman and Frankel (1957)

Hormones assay

Blood plasma concentrations of thyroxin (T₄) and triiodothyronine (T₃) were measured according to Abdel-Fattah et al. (2011) using radioimmunoassay (RIA) technique. Blood plasma concentrations of corticosterone and cortisol were evaluated using RIA, using the CORT kit (ICN Biomedical Inc., Costa) according to Palme et al. (1996).

Antioxidative status and lipid peroxidation

Total antioxidant capacity (TAC) and malonyaldehyde (MDA) were measured according to Koracevic et al. (2001) and Richard et al. (1992); respectively. All biochemical parameters were analyzed by commercially available kit methods. GNW-Model: SM-721Spectrophotometers, Absorbance Microplate Reader and other laboratory equipment aids were used for biochemical analysis. Moreover, each parameter was done according to the instructions of its

Statistical analysis

Data were subjected to ANOVA using the general linear models procedure of SAS (Statistical Analysis System, 2002). The differences among groups means were Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Hematological parameters

Data presented in Table (1) illustrated the overall means of red blood cell count (RBCs), blood hemoglobin concentration (Hb), packed cell volume (PCV) and white blood cell count (WBCs) of growing rabbits as affected by fasting period and dietary Zn supplementation. Fasting regimen in groups F24 and F48 decreased significantly RBCs—and Hb concentration compared with those of control (Ad) or Ad+Zn groups in growing rabbits ($P \leq 0.0001$).

Table (1): Effect of fasting (feed regimen) and dietary zinc supplementation on some blood hematological parameters of V-line male rabbits at 84 days.

Item	C(Ad)	Ad+Zn	F24	F48	F24+Zn	F48+Zn	Sig
RBCs (10 ⁶ /mm ³)	6.33 ^A ±0.08	6.47 ^A ±0.08	5.53 ^C ± 0.11	5.33 ^C ±0.09	5.87 ^B ±0.04	5.80 ^B ±0.04	***
Hb (g/dl)	12.67 ^A ±0.09	12.87 ^A ±0.02	11.97 ^B ±0.24	10.73 ^C ±0.08	12.10 ^B ±0.07	12.03 ^B ± 0.04	***
PCV (%)	40.30 ^B ±0.04	43.37 ^A ±0.42	39.33 ^{CD} ±0.09	37.47 ^E ±0.22	39.90 ^{BC} ±0.07	39.03 ^D ±0.08	***
WBCs (10 ³ /mm ³)	7.39 ^A ± 0.09	7.80 ^A ±0.17	7.33 ^A ±0.20	6.97 ^B ±0.08	7.67 ^A ±0.06	7.78 ^A ±0.10	***

A, B, C., D Means with different superscripts in the same row, differ significantly ($P < 0.0001$). C(Ad): free feeding (*ad libitum*), Ad+Zn: free feeding (*ad libitum*) +100mg zinc /kg diet , F24:Fasting feed 24 hr each week, F48 :Fasting feed 48 hr each week, F24+Zn : Fasting 24hr each week +100mg zinc/kg diet, F48+Zn: Fasting 48hr each week +100mgzinc/kg diet.

Fasting regimen in groups F48 with or without Zn decreased PCV%. Regarding to WBCs, the lowest WBCs count were observed in F48 compared with other treatments. These results are in agreement with Ebeid et al. (2012) who indicated that due to feed restriction, erythrocyte number and Hb concentration were significantly reduced while mean cell volume (MCV) was significantly increased in growing rabbits. This reduction in Hb content was suggested to be due to the observed reduction in erythrocytes' counts. Also, Tůmová et al. (2007) concluded that haematocrit was significantly decreased by fasting (feed regimen) in rabbits. Similarly, Matsuoka et al. (2006) found that hematological examination of pregnant does (on gestation day 19) documented variations in several blood parameters only in animals subjected to regimen feeding at 20 g/head/day. In the present study, it could be observed that the values of the haematological characteristics were within the physiological range described by Tůmová et al. (2007). Generally, hematological parameters are good indicator for productive performance and the physiological status of animals (Khan and Zafer, 2005), and for determine environmental stresses (Nse-Abasi et al., 2014). The reduction in Hb content was suggested to be due to the observed reduction in erythrocytes' counts (Table 1). On the other hand, Nafeaa et al. (2011) mentioned that no significant differences in RBCs, Hb and PCV values between the control and feed-restricted groups. Also, El-Speiy et al. (2015) recorded that feed restriction had no significant effect on WBCs but erythrocyte count and Hb concentration were significantly reduced by feed restriction compared with the control group.

Results of the present study are in correspondence with those of El Hendy et al. (2001) who postulated that hematological parameters (included Hb, PCV and total erythrocyte count) were significantly affected by Zn insufficiency. Similarly, Younas et al. (2015) resulted that Hb content significantly affected due to treatments rabbits with Zn and non- significant behavior for leukocytes was observed. Contrarily, Mahmood and Sarmad (2016) reported that no significant differences were observed in WBCs and RBCs among treated group with pure Zn and the control group. Bovera et al. (2012) reported that total WBCs count was not different among groups treated with Zn-bacitracin.

White blood cells differential

Data shown in Table (2) illustrated that the lowest significant neutrophils % was detected in control group compared with the other experimental groups ($P \leq 0.01$). It could be observed that no significant differences in the neutrophils percent between Ad+Zn, F24+Zn and F48+ Zn groups. No significant differences were observed among the experimental treatments in lymphocyte, monocyte, esinophils and basophilis percentages. It might be speculated that fasting regimen and dietary Zn had a role in modulating the immune system in growing rabbits. Frakock et al. (1987) reported that feeding stress (fasting status) cases change in percentage of neutrophilia, eosinophilia, and monocytopenia are typical for the so-called "stress leukogramme", which is due to the increased endogenous production of cortisol from the adrenal glands. It has also been observed that Zn reduction deactivates the T lymphocytes thus reduces the phagocytic action of macrophages, failing the immunity and play important role in the formation of superoxide dismutase (SOD) and catalase (CAT) in liver that plays disease modification role (Faa et al., 2008). Also, Fukada et al. (2011) demonstrated that Zn inadequate intake in animals results in serious immunodeficiency, increased numbers of infections, increased severity of infections, neuronal and sensory dysfunctions. Therefore, maternal Zn level is one of the basic requirements for the normal function of concepts and trophoblast (Mistry and Williams, 2011). The deficiency of Zn impaired functions of NK (natural killer) cells, T and B cells, neutrophils and macrophages (phagocytosis).

Table (2): Effect of fasting (feed regimen) and dietary zinc supplementation on leukocytes differentiation of V-line male rabbits at 84 days.

Item	C(Ad)	Ad+Zn	F24	F48	F24+Zn	F48+Zn	Sig
Neutrophils (%)	44.50 ^C	46.19 ^{AB}	45.57 ^B	45.57 ^B	46.73 ^A	47.93 ^A	**
	±0.29	±0.28	±0.15	±0.21	±0.40	±0.42	
Lymphocytes (%)	44.10	43.13	43.59	43.68	43.37	43.70	NS
	± 0.19	±1.59	±0.11	±0.23	± .31	±0.40	
Monocytes (%)	4.66	4.97	4.81	4.90	4.67	4.87	NS
	± 0.04	± 0.19	±0.23	±0.04	±0.08	±0.02	
Eosinophils (%)	1.91	1.87	1.88	1.85	1.93	1.83	NS
	±0.04	±0.02	±0.02	± 0.04	±0.17	±0.09	
Basophils (%)	4.40	4.27	4.15	4.40	4.26	4.37	NS
	±0.06	± 0.06	± 0.04	± 0.13	±0.19	±.16	

A, B, C Means with different superscripts in the same row, differ significantly ($P < 0.0001$). C(Ad): free feeding (*ad libitum*), Ad+Zn: free feeding (*ad libitum*) +100mg zinc /kg diet , F24:Fasting feed 24 hr each week, F48 :Fasting feed 48 hr each week, F24+Zn : Fasting 24hr each week +100mgzinc/kg diet, F48+Zn: Fasting 48hr each week +100mgzinc/kg diet.

This situation induces lymphopenia (less lymphocyte production) hence attacked by chronic pathogens, compromising immunity (Blewett and Carla, 2012). However, it has been documented that Zn helps in the formation and maturation of T-cells subsequently synthesizes IgA and IgG. Similarly, Zn supplementation was also found effective in restoring the function of T helper cells, if treated three weeks before immunization whilst, side effects were detected if consumed after vaccination (Haase et al., 2008).

Blood biochemical constituents

Results concerning the effect of fasting regimen on blood plasma biochemical constituents in growing rabbits are tabulated in Table (3). Growing rabbits fed *ad libitum* and supplemented with Zn (Ad+Zn) had a significant increase in blood plasma total protein, albumin and glucose. These improvements due to Zn supplementation are in agreement with Kalafova et al. (2008) who noticed that higher average concentrations of total proteins were measured in groups with Zn supplementation. However, Atakisi et al. (2009) documented that glucose and total protein levels were decreased in Zn-supplemented animals compared to the control. Al-Mousawi (2013) stated that Zn administration significantly reduced the serum glucose.

In Table (3), fasting regimen groups with or without Zn supplementation decreased significantly blood plasma total protein, albumin, glucose, total lipids and cholesterol concentrations comparing with those fed *ad libitum* diet with or without Zn supplementation. These results are in agreement with several previous studies (Chilliard et al., 1998; Abeer et al., 2008; Matsuoka et al., 2009; Ebeid et al., 2012; El-Speiy et al., 2015). Abeer et al. (2008) reported that total protein was significantly reduced in serum at late gestation period during feed restriction in NZW rabbits does. Matsuoka et al. (2009) noted that restricted feeding showed significantly lower values in many parameters such as total protein and albumin reflecting low nutritive condition. Also, Daoud et al. (2012) documented that the decreased levels of total protein, albumin and increasing creatinine concentration in NZW rabbits might be connected with feed restriction. These results are in accordance with El-Speiy et al. (2015) who confirmed that feed restriction caused significant decrease in blood plasma protein values compared with control group in growing rabbits.

As presented in Table (3) blood plasma glucose concentration was significantly reduced by feed restriction in growing rabbits. These results are in agreement with those of Dewil et al. (1999) who found that decrease in plasma glucose motivate by the short food restriction (24 hr) in chickens. Similarly, Rommers et al. (2004) reported that during the restricted period, plasma glucose was constantly lower ($P < 0.05$) in feed restriction groups. On the other hand, Ebeid et al. (2012) noted that blood plasma glucose concentration was not significantly affected by feed restriction in growing rabbits.

Data tabulated in Table (3) declare that feed restriction resulted in reducing blood plasma total lipids and cholesterol concentrations indicating lipid depletion of these experimental animals in the present study.

Table (3): Effect of fasting (feed regimen) and dietary zinc supplementation on blood biochemical parameters of V-line male rabbits at 84 days.

Item	C (Ad)	Ad+Zn	F24	F48	F24+Zn	F48+Zn	Sig
Total protein (g/dL)	6.16 ^B ±0.09	6.60 ^A ±0.06	5.60 ^C ±0.06	5.57 ^C ±0.10	5.73 ^C ±0.06	5.70 ^C ±0.04	***
Albumin (g/dL)	3.83 ^B ±0.11	4.20 ^A ±0.04	3.27 ^C ±0.10	2.99 ^E ±0.06	3.43 ^D ±0.06	3.33 ^D ±0.04	***
Globulin (g/dL)	2.33 ±0.02	2.40 ±0.09	2.33 ±0.04	2.58 ±0.04	2.30 ±0.02	2.37 ±0.02	NS
Glucose (mg/dL)	114.67 ^B ±0.76	119.33 ^A ±0.56	105.33 ^C ±0.92	104.10 ^C ±0.37	106.00 ^C ±0.37	109.00 ^C ±0.37	***
Creatinine (mg/dL)	0.51 ±0.04	0.53 ±0.02	0.53 ±0.02	0.54 ±0.04	0.50 ±0.02	0.51 ±0.03	NS
Total lipids (mg/dL)	105.03 ^A ±0.61	99.33 ^B ±0.29	95.40 ^C ±0.33	90.40 ^D ±0.86	95.80 ^C ±1.93	94.53 ^C ±1.92	***
Cholesterol (mg/dL)	195.93 ^A ±2.12	166.57 ^B ±2.59	147.9 ^{CD3} ±0.86	145.63 ^D ±1.70	154.53 ^C ±5.10	143.57 ^D ±2.02	***
Triglycerides (mg/dL)	39.30 ^A ±0.45	33.90 ^B ±0.49	33.80 ^B ±0.19	31.23 ^C ±0.40	30.90 ^C ±0.16	30.63 ^C ±0.49	***
ALT (U/L)	36.66 ^B ±0.56	30.00 ^E ±0.37	47.00 ^A ±0.37	49.67 ^{A±} 0.76	41.00 ^{B±} 0.37	42.00 ^B ±0.37	***
AST (U/L)	25.33 ^C ±0.56	17.66 ^D ±0.56	32.00 ^A ±0.37	34.67 ^A ±1.48	27.33 ^A ±0.76	29.00 ^B ±0.37	***

A,B,C,D,E Means with different superscripts in the same row, differ significantly ($P < 0.0001$). C(Ad): free feeding (*ad libitum*), Ad+Zn: free feeding (*ad libitum*) +100mg zinc /kg diet, F24: Fasting feed 24 hr each week, F48 :Fasting feed 48 hr each week, F24+Zn : Fasting 24hr each week +100mgzinc/kgdiet, F48+Zn: Fasting 48hr each week +100mgzinc/kg diet.

These results are in accordance with several previous studies (Rajman et al., 2006; van Harten and Cardoso, 2010; Ebeid et al., 2012). van Harten and Cardoso (2010) stated that feed restriction reduced significantly triacylglycerols, non-esterified fatty acids and free fatty acids in rabbits and induced a higher lipidic depletion in these animals. Also, Rajman et al. (2006) confirmed that feed restriction decreased plasma concentrations of total lipids, triacylglycerols, cholesterol, and high density lipids. Similarly, Yassein et al. (2011) founded that feed restriction had dramatic changes in lipoprotein metabolism characterized by decreased triglycerides and remodeling of LDL and HDL cholesterol to form larger particles. In this context, Turturro et al. (1993) suggested that fat metabolism was significantly modified by feed restriction.

The main effects of fasting regimen and dietary Zn supplementation on blood plasma ALT and AST are shown in Table (3). Growing rabbits subjected to fasting (F24, F48, F24+Zn and F48+Zn groups) recorded significant increase in blood plasma AST compared with those fed *ad libitum* diet with or without Zn supplementation (Ad and Ad+Zn groups). The reduction in AST concentrations recorded are in harmony with the results obtained by Al-Mousawi (2013) who showed that supplemented with Zn significantly ($P < 0.05$) reduced the serum ALT and AST concentration in diabetic rabbits.

Antioxidative status and lipid peroxidation

Respecting to the influence of fasting regimen and dietary Zn supplementation on plasma TAC and MDA (Table 4), it could be noted that TAC was significantly increased in the group fed *ad libitum* diet supplemented with Zn (Ad+Zn) compared with the other experimental groups. However, TAC was significantly reduced in growing rabbit subjected to fasting regimen (24 or 48 h) and dietary Zn supplementation alleviated this effect and improved TAC significantly. It is noteworthy to indicate that growing rabbits exposed to fasting (F24, F48, F24+Zn and F48+Zn groups) had a significant increase in plasma MDA concentration which used as lipid peroxidation index; however, the lowest value was recorded for the group fed *ad libitum* diet and supplied with Zn (Ad+Zn). Zn is a biologically important trace mineral with multiple functions because it is a cofactor of more than 300 enzymes in metabolic systems (Vallee and Falchuk, 1993). One of its important functions is participation in the antioxidant defense system (Powell, 2000). Dietary Zn status exerts a powerful influence on the degree of oxidative damage caused by free radicals (Tupe et al., 2010). Oxidative stress is manifested primarily via alterations of antioxidant enzyme activities and the reductions of some nonenzymatic antioxidants. Lipid peroxidation is an indication of oxidative damage of cells.

Table (4): Effect of fasting (feed regimen) and dietary zinc supplementation on antioxidative properties and lipid peroxidation of V-line male rabbits at 84 days.

Item	C (Ad)	Ad+Zn	F24	F48	F24+Zn	F48+Zn	Sig
TAC (mM/L)	140.87 ^B ± 0.52	150.23 ^A ± 0.48	113.20 ^D ±0.79	103.07 ^E ±0.97	133.63 ^{BC} ±0.99	123.37 ^C ±1.02	**
MDA (nmol/ml)	54.33 ^C ±1.75	43.23 ^D ±0.80	62.97 ^B ±1.11	73.40 ^A ±1.34	64.20 ^B ± 1.90	63.23 ^B ± 1.59	**

A,B,C,D Means with different superscripts in the same row, differ significantly ($P < 0.0001$). C: free feeding (*ad libitum*), R1: free feeding (*ad libitum*) +100mg zinc /kg diet, R2: Fasting feed 24 hr each week, R3 :Fasting feed 48 hr each week, R4: Fasting 24hr each week +100mgzinc/kgdiet, R5: Fasting 48hr each week +100mgzinc/kg diet. TAC= Total antioxidant capacity, MDA = Malonyldialdehyd.

Zn can compete with iron and copper to bind to the cell membrane and decrease the production of free radicals, thus exerting a direct antioxidant action (Tate et al., 1999). Dietary Zn was reported to increase the Cu-Zn-superoxide dismutase activity in piglets (Wang et al., 2012). Bun et al. (2011) noted that activities of superoxide dismutase and glutathione peroxidase were increased ($P < 0.001$) with increasing dietary Zn levels in broiler chickens, while, lipid peroxidation tended to be reduced ($P = 0.08$) at Zn inclusion of 20 and 40 mg/kg. Shaheen and El-Fattah (1995) reported that Zn deficiency caused increased lipid peroxidation and that this was overcome by Zn supplementation. Juda et al. (2007) found that orally Zn supplementations significantly decrease in serum concentration of MDA in comparison with control. Duzguner and Kaya (2007) concluded that daily Zn supplementation could reduce the harmful effects of oxidative (by reduce MDA) stress in diabetics rabbit.

Hormones profile

Results concerning the effects of fasting regimen and dietary Zn supplementation on serum adrenal cortex hormones (cortisol and corticosterone) and thyroid hormones (T_3 and T_4) concentrations in growing rabbits are presented in Table (5). It is noteworthy to note that growing rabbits exposed to fasting (F24, F48, F24+Zn and F48+Zn groups) had a significant increase in blood plasma cortisol and corticosterone compared with those fed *ad libitum* diet (Ad) and *ad libitum* diet + Zn (Ad+Zn). These results are agreement with the results reported by Mastorakos and Ilias (2003) and Zhang et al. (2011) who reported that plasma corticosterone is considered the main glucocorticoid involved in regulation of stress (eg., heat stress, fastingetc) responses in rodents, the presence of plasma cortical hormones and whether its level can be used as an indicator for rodent activation of stress remain to be determined. Cortisol secretion is a generic response to stress as well as a specific adaptive response to fasting and nutritional stress recruiting all available energy sources in the body. In the same line, Wu et al. (2012) observed that the plasma corticosterone concentration in mice that had been subjected to chronic unpredictable stress for 30 days was significantly higher than that in control mice. Also, Liu et al. (2013) observed a significant elevation of endogenous corticosterone levels in mice following a 24 h acute restraint stress reported that serum corticosterone levels in mice exposed to chronic stress for 21 days (242.55 ng/ml) increased significantly compared with those in unstressed control mice.

Table (5): Effect of fasting (feed regimen) and dietary zinc supplementation on some hormones function of V-line male rabbits at 84 days.

Item	C(Ad)	Ad+Zn	F24	F48	F24+Zn	F48+Zn	Sig
Cortisol (ug/dL)	6.00 ^D ±0.33	5.33 ^{DE} ± 0.15	9.03 ^B ±1.95	11.67 ^A ± 0.16	7.73 ^{BC} ±0.13	7.63 ^{BC} ±0.24	**
Corticosterone (nmol/ml)	3.4 ^D ± 0.08	3.20 ^D ± 0.07	5.33 ^B ±0.06	6.03 ^A ± 0.02	4.67 ^C ± 0.06	5.23 ^B ± 0.06	**
T3 (ng/ml)	1.80 ^B ±0.22	2.30 ^A ±0.07	1.10 ^{CD} ±0.09	0.83 ^D ±0.10	1.40 ^C ±0.10	1.03 ^{CD} ± 0.34	**
T4 (ug/dl)	5.13 ^B ±0.09	6.57 ^A ±0.22	2.50 ^E ±0.04	2.27 ^E ± 0.06	3.43 ^C ±0.06	2.93 ^D ± 0.06	**

A, B, C., D Means with different superscripts in the same row, differ significantly ($P < 0.0001$).

C (Ad): free feeding (*ad libitum*), Ad+Zn: free feeding (*ad libitum*) +100mg zinc /kg diet, F24:Fasting feed 24 hr each week, F48 :Fasting feed 48 hr each week, F24+Zn : Fasting 24hr each week +100mgzinc/kgdiet, F48+Zn: Fasting 48hr each week +100mgzinc/kg diet., Corticosterone = Cortic, Triiodothyronine = T3, Thyroxine = T4

As shown in Table (5), fasting regimen and fed diet supplemented with or without Zn (F24, F48, F24+Zn and F48+Zn groups) had significantly decrease in blood plasma T₃ and T₄ compared with those fed *ad libitum* diet (Ad) and *ad libitum* diet + Zn (Ad+Zn) in growing rabbits. It is well known that feed restriction is involved in the regulatory mechanisms of metabolism in animals. In this context, Suda et al. (1978) recorded that calorie deprivation leads to reduction in serum T₃ is caused by a reduction in its generation from T₄ rather than by an acceleration in its metabolic clearance rate. The decline in T₃ concentration is accompanied by a concomitant and reciprocal change in the concentration of total and free T₃. The increase in the serum T₃ concentration tends to begin later and to return to normal at the time serum T₃ is being maintained at a low level with continuous. Also, Rommers et al. (2002) reported that long-term nutrient deficiency during development has major neuro-endocrine consequences trigger prominent homeostatic reactions of the corticotrophin, somatotrophic, leptinergic and thyrotrophic axes. In contrast, Chan et al. (2006) reported that seventy-two-hour fasting significantly decreased free T₃, increased reverse T₃, and markedly suppressed several parameters of TSH pulsatility, whereas free T₄ remained stable. The role of Zn for improvement metabolism and thyroid hormones is reported by Nishiyama et al. (1994) who demonstrated that Zn is a cofactor for iodothyronine iodine (IDI) enzyme, the enzyme that convert T₄ hormone to T₃. Zn may play a role in thyroid hormones metabolism in patients with low levels of T₃ hormone and may control the conversion of T₄ to T₃ in human (Christy and Stella, 2007). Also, Maxwell and Volpe (2007) reported that Zn supplementation appeared to have a favorable effect on thyroid hormones levels, particularly total T₃. Moreover, Ertek et al. (2010) showed that Zn levels were significantly positively correlated with free T₃ levels ($P < 0.001$). Similarly, Zearah et al. (2016) showed that the elevation of total T₄ and total T₃ level after the intake of Zn supplementation and this elevation was highly significant ($P < 0.001$) and it has important role to increase T₃.

CONCLUSION

Based on the data presented above, it could be concluded that growing rabbits exposed to fasting and fed diet supplemented with or without Zn had a significantly decrease in RBCs, Hb concentration, PCV, plasma total protein, albumin, glucose, total lipids, cholesterol, triglycerides, T₃ and T₄ concentrations. Interestingly, dietary Zn supplementation improved the antioxidative properties and reduced the rate of lipid peroxidation in growing rabbits subjected to fasting regimen.

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تأثير التصويم وإضافة الزنك إلى العليقة على صورة الدم والصورة الهرمونية والخصائص المضادة للأكسدة في ذكور الأرانب النامية

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تهدف هذه الدراسة إلى تقييم تأثير نظام تغذية (الصيام) وإضافة الزنك للعليقة على كلا من: صورة الدم ومكوناته الكيميائية والصورة الهرمونية والخصائص المضادة للأكسدة في ذكور الأرانب النامية، تم تقسيم 60 ذكر من أرانب الفي لاين (عمر 35 يوم) بشكل عشوائي إلى 6 مجموعات متساوية (10 ذكور لكل منهما) كالتالي:

(1) مجموعة الضابطة أو الكنترول (تم تغذية الأرانب بنظام التغذية الحرة حتى الشبع على العليقة التجارية)

(2) مجموعة التغذية الحرة حتى الشبع مع الزنك (تم تغذية الأرانب حتى الشبع +100 ملليجرام زنك / كجم من العليقة)

(3) مجموعة التصويم لمدة 24 ساعة،

(4) مجموعة التصويم لمدة 48 ساعة،

(5) مجموعة التصويم لمدة 24 ساعة+ 100 ملليجرام زنك / كجم من العليقة

(6) مجموعة التصويم لمدة 48 ساعة+100 ملليجرام زنك / كجم من العليقة

استمرت التجربة من عمر 35-84 يوم، وخلال الأسبوع الأخير من التجربة تم تغذية الأرانب حتى الشبع.

وأوضحت النتائج ان تصويم ذكور الأرانب النامية لمدة 24 أو 48 ساعة بالأسبوع إلى انخفاض عدد كرات الدم الحمراء مقارنة بأرانب الكنترول أو أرانب التغذية الحرة مع الزنك، بينما سجلت مجموعة التصويم لمدة 48 ساعة أقل قيمة لتركيز الهيموجلوبين وحجم الخلايا المعبأة (PCV) ولقد أدت إضافة الزنك إلى التخفيف من آثار التصويم، أدى التصويم لمدة 48 ساعة إلى انخفاض عدد خلايا الدم البيضاء مقارنة بالمعاملات الأخرى. أدت إضافة الزنك إلى ارتفاع نسبة الخلايا المتعادلة في مجموعة التغذية الحرة حتى الشبع مع الزنك ومجموعة التصويم لمدة 24 ساعة +100 ملليجرام زنك / كجم من العليقة ومجموعة التصويم لمدة 48 ساعة +100 ملليجرام زنك / كجم من العليقة، لم توجد فروق معنوية بين المعاملات في كل من الخلايا الليمفاوية والخلايا الأحادية والخلايا الحامضية والخلايا القاعدية. عموماً أدت تغذية الأرانب حتى الشبع +100 ملليجرام زنك / كجم من العليقة إلى ارتفاع البروتينات الكلية والألبومين بلازما الدم بالمقارنة بالمجاميع التجريبية الأخرى، بينما مجاميع التصويم سواء مع إضافة الزنك أو عدم إضافته أدى إلى انخفاض البروتينات الكلية والألبومين و الجلوكوز مقارنة بمجموعة المقارنة أو مجموعة التغذية حتى الشبع +100 ملليجرام زنك / كجم من العليقة. كما أدى إتباع نظام التصويم أو التغذية علي الزنك إلى انخفاض تركيز الدهون الكلية والكوليسترول ورقماً الدهون الثلاثية في بلازما الدم. عموماً التصويم لمدة 24 أو 48 ساعة مع إضافة الزنك في الأرانب إلى زيادة إنزيمات الكبد مقارنة بمجموعة المقارنة أو التغذية حتى الشبع مع الزنك. وزادت معنويًا الكفاءة الكلية للأكسدة في مجموعة التغذية الحرة المضاف إليها الزنك بالمقارنة بالمجاميع التجريبية الأخرى، بينما أدى التصويم مع التغذية علي الزنك إلى تحسن في الكفاءة الكلية للأكسدة. أدى تصويم الأرانب النامية لمدة 24 أو 48 ساعة مع أو بدون الزنك إلى زيادة تركيز المالونالدهيد (دليل أكسدة الدهون)، وتركيز هرمون الكورتيزول وهرمون الكورتيكوستيرونو هرمونات الغدة الدرقية (T3، T4) في بلازما الدم، إلا أن أقل تركيز للمالونالدهيد قد لوحظ في مجموعة المقارنة أو مجموعة التغذية الحرة حتى الشبع المضاف إليها الزنك.

نخلص من هذه الدراسة إلى أن تصويم الأرانب النامية مع إضافة الزنك أو عدم إضافته إلى انخفاض عدد كرات الدم الحمراء، وتركيز الهيموجلوبين وقيمة حجم الخلايا المعبأة وانخفاض محتوى بلازما الدم من كل من البروتينات الكلية والألبومين والجلوكوز والدهون الكلية والكوليسترول والدهون الثلاثية في بلازما الدم وتركيز هرمونات الغدة الدرقية في بلازما الدم، ومن المثير للاهتمام أن إضافة الزنك قد حسن من الخصائص المضادة للأكسدة ومن معدل أكسدة الدهون في الأرانب النامية عند تصويمها.