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EFFECT OF FASTING PERIOD AND ZINC SUPPLEMENTATION ON THYROID AND ADRENAL ACTIVITY, ANTIOXIDANT STATUS AND GUT LENGTH OF GROWING RABBITS

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Ninety weaned males V-line rabbits (35 days old) with an average live body weight of 525±8.34 g. were randomly distributed into six experimental groups (n=15 each). All treated groups were received a basal diet; 1st experimental group was served as control group (AD), 2nd group was supplemented with Zn (AD+Zn), 3rd group was fasted one day weekly (F24), 4th group was fasted two days weekly (F48), 5th group was supplemented with Zn and fasted one day weekly (F24+Zn) and 6th group was supplemented with Zn and fasted two days weekly (F48+Zn). Serum concentrations of T_3, T_4, corticosterone, cortisol, total antioxidant capacity (TAC) and malonyaldehyde (MAD) were determined. Lengths of small and large intestine and caecum were measured.

Results showed that: *highly significant increase in serum levels of cortisol and corticosterone hormones were recorded in fasted growing rabbits (F24 and F48) groups and fasted with Zinc supplementation (F24+Zn and F48+Zn) groups, while serum levels of T_3 and T_4 were decreased compared with those in AD and AD+Zn groups. Serum level of TAC was significantly reduced in fasted growing rabbits supplemented or non-supplemented with Zn compared with AD and AD+Zn groups and more reduction was related to fasting. Deleterious effect of fasting was related with increasing serum levels of MAD, where all fasted groups had a significant increase in serum MAD concentration. Length of stomach, small and large intestines and caecum was significantly increased in fasted and Zn treated groups than control group.*

Conclusively*, fasting regimen and dietary Zn supplementation had a role in modulating hormonal profile, antioxidant status, stomach and intestine length.*

Key words: Fasting, Zinc, hormonal profile, digestive tract length,

Kit's rabbit exposed to stressful trouble on digestive tract and microbial count as soon as change feed to solid phase and abstention to suckling. Weaning stage is a critical phase during the development of digestive tract and digestive disorders caused by insufficient development of digestive enzymatic capability at an early age. Moreover, the weaning and the post weaning period are particularly important for the growth and feed efficiency (De Blas, 2013 and Tůmová *et al*., 2016). Debray *et al*., (2003) recorded that the quantitative feed regimen and period restriction for rabbits reduce the incidence of digestible disorders. Gidenne *et al*., (2012) stated that the absorption of nutrients depends on gut status. Also, quantitative and period of feed restriction can modify the morphology of the small intestine mucosa. Absorption may be affected by changing in the small intestine length an entire spectrum of mucosal changes, that containing the amount of villi extending into the lumen. Moreover, feed restriction might positively affect the several changes on metabolic disorders that lead to hormonal changes, immune depression and alter the digestive system functions, especially the liver and small intestine (Tůmová *et al*., 2016). Furthermore, fasting period could rapidly restore the morphology and functions of the intestine (Maria *et al*., 2013). Increasing the endogenous production of adrenal corticoids from the adrenal glands, following the action of stress factors such as fasting was demonstrated (Kock *et al*., 1987). Zinc is a vital trace element that plays an essential role in several biological functions such as protein and DNA synthesis, metabolic activities and growth (Tapiero and Tew, 2003). Dietary Zn has an important component for hormonal functions 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 (Alikwe *et al.*, 2011).

Therefore, the aim of the present study was performed to evaluate the influence of fasting period and dietary zinc supplementation on thyroid and adrenal activities, antioxidant status and morphological changes of digestive tract of growing rabbit.

MATERIALS AND METHODS

Housing and management:

This work was carried out at a Private Rabbit Farm at Qaluobia Governorate, during the period from February to April 2017.

Ninety weaned males V-line rabbits (35 days old) with an average live body weight of 525±8.34 g. were randomly distributed into six experimental groups (n=15 each) (Table 1). Rabbits were housed in a naturally ventilated building and kept in individual wire galvanized battery. The basal diet was formulated to meet the recommended nutrient requirements of growing rabbits according to De Blas and Mateos (1998). They were allowed to a standard pellet diet (17% crude protein, 2.56% crude fat and 2500 Kcal/kg-ration DE and 13% crude fiber). Feed and water were available *ad libitum.* The lighting program provided 16 h of light per day.

Table 1. Experimental design

Treatment groups	Basal diet plus	Fasting	
		days	weekly
AD	--	--	--
AD + Zn	Zn**	--	--
F24	--	Sunday	--
F48	--	Sunday	Thursday
F24 + Zn	Zn	Sunday	--
F48 + Zn	Zn	Sunday	Thursday

** Zinc was added as a $ZnSO_4$ (100 mg Zn/kg diet), purchased from El-Gomhoria for chemical Company, Cairo, Egypt.

Blood samples:

At the end of experiment (84 days of age), blood samples were obtained from the ear vein using 3 ml syringe, centrifuged at 3000 r.p.m. for 20 min., serum was collected and stored at -20°C until analyses.

Hormonal assay:

Serum concentrations of thyroxin (T_4) and triiodothyronine (T_3) were measured according to Abdel-Fattah *et al.,* (2011) using radioimmunoassay (RIA) technique. Blood serum concentrations of corticosterone and cortisol were measured using RIA, using the CORT kit (ICN Biomedical Inc., Costa) according to Palme *et al*., (1996).

Antioxidative status:

Total antioxidant capacity (TAC) and malonyaldehyde (MAD) were measured according to Koracevic *et al.,* (2001) and Richard *et al*., (1992), respectively.

Gastrointestinal measurements:

At the end of experiment, rabbits were slaughtered, eviscerated and the length of small and large intestine and caecum were measured. Small intestine was measured from duodenum to junction of ileum, caecum (without appendix), and colon, large intestine involved proximal and distal colon.

Statistical analysis:

Data were subjected to ANOVA using the general linear models procedure of SAS (2002) as following model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

Where: Y_{ij} = An observation; μ = Overall mean; T_i = Treatment effect, and e_{ij}= Experimental error. When significant differences among means were tested by using Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of fasting and zinc supplementation on:

1-Thyriod and adrenal hormones:

Results concerning the effect of fasting regimen and dietary Zn supplementation on serum glucocorticoid hormones and thyroid hormones concentrations in growing rabbits are presented in Table 2. It is noteworthy to note that growing rabbits exposed to fasting (F24, F48, F24+Zn and F48+Zn groups) had a significant increase in serum levels of cortisol and corticosterone compared with those in control groups (AD and AD+Zn). These results are in agreement with Zhang *et al.,* (2011) who reported that plasma corticosterone is considered the main glucocorticoid involved in regulation of stress responses in rodents (eg, heat stress, fasting,..etc), serum cortical hormones level can be used as an indicator for activation of stress in rabbits. Cortisol secretion is a generic response to stress as well as a specific adaptive response to fasting and nutritional stress. Wu *et al.*, (2012) observed that the plasma corticosterone concentration in mice subjected to chronic stress was significantly higher than control. Also, Liu *et al.,* (2013) observed a significant increase of corticosterone levels in mice following 24 h of acute restraint stress.

As shown in Table 2, fasting regimen and fed diet supplemented with or without Zn (F24, F48, F24+Zn and F48+Zn groups) had significantly decrease in blood serum T_3 and T_4 compared with those in non fasting groups (AD and AD+Zn).

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 T_3 is caused by a reduction from T_4 rather than by an acceleration in its metabolic clearance rate. The decrease in T_3 concentration is accompanied by a concomitant and reciprocal change in the concentration of total and free T_3. Rommers *et al.,* (2002) reported that long-term nutrient deficiency during development has major neuro-endocrine consequences trigger prominent homeostatic reactions of the corticotrophin, somatotropic and thyrotrophic axes. Chan *et al.,* (2006) reported that fasting 72 h caused to decrease a significant in free T_3, and increased reverse T_3, whereas free T_4 remained stable.

Zinc may play a role in thyroid hormones metabolism. Maxwell and Volpe (2007) reported that Zn supplementation appeared to have a favorable effect on thyroid hormones levels, particularly total T_3. Moreover, Ertek *et al.,* (2010) showed that Zn levels were significantly positively correlated with free T_3 levels. Similarly, Zearah *et al.,* (2016) showed that the elevation of total T_4 and total T_3 levels after the intake of Zn supplementation.

2-Antioxidant status:

Respecting to the influence of fasting regimen and dietary Zn supplementation on serum TAC and MAD (Table 2), it could be noted that TAC was significantly increased in the group fed 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 serum MAD concentration which used as lipid peroxidation index; however, the lowest value was recorded for the group fed basal diet supplemented with Zn (AD+Zn). One of Zn important functions is participation in the antioxidant defense system (Powell, 2000). Oxidative stress is manifested primarily via changes of antioxidant enzyme activities and the reductions of some non-enzymatic antioxidants. Bun *et al.,* (2011) noted that activities of superoxide dismutase and glutathione peroxidase were increased with increasing dietary Zn levels in broiler chickens, while, lipid peroxidation tended to be reduced at Zn inclusion of 20 and 40 mg/kg diet.

Shaheen and Abdel-Fattah (1995) reported that Zn deficiency caused increased lipid peroxidation and that this was overcome by Zn supplementation. Juda *et al.,* (2009) found that orally Zn supplementations significantly decrease in serum concentration of MAD in comparison with control. Duzgunerand Kaya (2007) concluded that daily Zn supplementation could reduce the harmful effects of oxidative (by reduce MAD) stress in diabetics rabbit.

Table 2. Effect of fasting and dietary Zn supplementation on hormonal profile and antioxidants status of growing V-line male rabbits at 84 days of age.

Treatments	Cortisol (ug/dl)	Corticosterone (nmol/ml)	T_3 (ng/ml)	T_4 (ug/dl)	TAC (mM/L)	MAD (nmol/ml)
Control, AD	5.20 ^{d} ±0.33	5.25 ^{d} ± 0.08	2.20 ^{b} ±0.22	4.43 ^{b} ±0.09	125.87 ^{b} ± 0.52	59.03 ^{c} ±1.75
AD +Zn	4.53 ^{de} ± 0.15	5.05 ^{d} ± 0.07	2.70 ^{a} ±0.07	5.87 ^{a} ±0.22	135.23 ^{a} ± 0.48	47.93 ^{d} ±0.80
F24	8.23 ^{b} ±1.95	7.18 ^{b} ±0.06	1.50 ^{cd} ±0. 09	1.80 ^{e} ±0.04	98.20 ^{d} ±0 .79	67.67 ^{b} ±1.11
F48	10.87 ^{a} ± 0.16	7.88 ^{a} ± 0.02	1.23 ^{d} ±0.10	1.57 ^{e} ± 0.06	88.07 ^{e} ±0.97	78.10 ^{a} ±1.34
F24+Zn	6.83 ^{bc} ±0.13	6.52 ^{c} ± 0.06	1.80 ^{c} ±0.10	2.73 ^{c} ±0.06	118.63 ^{bc} ±0.99	68.90 ^{b} ± 1.90
F48+Zn	6.93 ^{bc} ±0.24	7.08 ^{b} ± 0.06	1.43 ^{cd} ± 0.34	2.23 ^{d} ± 0.06	108.37 ^{c} ±1.02	67.93 ^{b} ± 1.59
Sig	**	**	**	**	**	**

a,b,c,d Means with different superscripts in the same column differ significantly ($P \leq 0.01$).,
T_3=Triiodothyronine,**T_4**=Thyroxin, **TAC**=Total antioxidant capacity, **MAD** =Malonylaldhyed.

3- Gastrointestinal tract measurements:

Table 3 shows the overall means of relative length and weight percentage of digestive tract in rabbit slaughtered at 84 days of age. Data revealed that growing rabbits fed diet+Zn (AD+Zn) and those subjected to feeding regimen and consumed diet with or without Zn (F24, F48, F24+Zn and F48+Zn groups) had a significant increase in length and percentage of studied digestive tract parts. There was a great variant in the results found in the literature, probably due to the differences in the restriction level, duration and period when feeding regimen was performed. Domestic rabbits are herbivores, concentrate selectors, and are classified as hindgut fermentors (Cheeke, 1987 and McNitt *et al.*, 1996).The rabbit caecum is very large, compared with the rest of the gut (Stevens and Hume, 1995) and forms a spiral shape that fills the abdominal cavity. The caecum (Jenkins, 1999) has a capacity 10 times that of the rabbit’s stomach. Mazeti and Furlan (2008) verified a higher relative weight and length of small intestine in regimen animals compared with control. Maria *et al.*, (2013) revealed that rabbits fed *ad libitum* or restricted diets from 33 to 40 days of age showed higher villus and villus perimeter. Also, rabbits restricted from 33 to 40 days had larger villus and higher absorption surface ($P<0.01$) than the non-restricted rabbits or the ones restricted from 54 to 61 days. In the same respect, On the other hand, Tůmová *et al.*, (2007) demonstrated that feeding regimen reduced the intestine weight and size, but one week after re-feeding, intestine weight had increased, at the end of the experiment there was no difference in weight among the animals, but the small intestine was longer in rabbits fed *ad libitum.* Also, Wijtten *et al.*, (2010) reported that feeding regimen can result in lower villus perimeter and lower crypt depth, in addition to decreasing the enzymatic activity in the enterocytes.

However, Southon *et al.*, (1986) reported that Zn deficiency caused impairment of gut architecture and organelles, such as shorter and narrower jejunal villi, reduced absorptive surface area, decreased number of mitochondria, swelling of the endoplasmic reticulum and atrophic Golgi apparatus, accompanied by increased membrane permeability and declined cell mobility. Also, Zn deficiency decreased the number of goblet cells and mucus thickness, inhibited mucin synthesis, which resulted in colonization by pathogenic microorganisms, penetration of mucus layer, and finally infectious diseases (Quarterman *et al.*, 1976 and Quarterman 1987). Also, Liu *et al*., (2013) concluded that dietary Zn supplementation caused changes in colonic morphology, mucin profiles and immunological parameters in piglets after weaning. Starvation induces intestinal epithelial atrophy, which is more closely associated with increased epithelial cell apoptosis (Song *et al.*, 2011).

According to the literatures, food regimen (starvation or fasting) caused increased concentration of corticosterone hormone, including increase the expression of sodium glucose co-transporter 1 (SGLT1) and peptide transporter 1 (PepT1) mRNA in the small intestine of chickens and rats (Naruhashi *et al.*, 2002). All kinds of stressors exist around broiler chickens can be mimicked by glucocorticoids (such as corticosterone) (Lin *et al.*, 2007). Hu and Guo, (2008) revealed that corticosterone administration decreased feed intake and duodenal and jejunal epithelial cell proliferation of young broilers. Villus height is positively related to villus surface area (Mitchell and Carlisle, 1992); reduction of villus height during villus growth would decrease the expansion of small intestinal surface area and subsequently decrease the absorptive capacity (Moran, 1985).

Table3. Effect of fasting days and dietary Zn supplementation on lengths of the digestive tract of growing V-line male rabbits at 84 day of age.

Treatments	Stomach (cm)	Small intestine (cm)	Large intestine (cm)	Caecum (cm)
Control, AD	9.72 [d] ±0.01	228.60 [d] ±0.56	88.967 [c] ±0.58	9.20 [c] ±0.16
AD +Zn	10.59 [a] ±0.04	281.33 [a] ±0.41	170.30 [a] ±0.29	12.40 [a] ±0.24
F24	10.09 [c] ±0.02	246.33 [c] ±0.56	144.30 [b] ±0.66	9.70 [c] ±0.12
F48	9.99 [c] ± 0.01	243.00 [c] ± .71	139.13 [b] ±0.64	10.07 [c] ±0.16
F24+Zn	10.49 [ab] ±0.05	253.67 [b] ±0.56	141.13 [b] ±0.39	10.90 [b] ±0.21
F48+Zn	10.41[b] ±0.07	257.67 [b] ±0.67	143.90 [b] ±0.65	10.43 [bc] ±0.24
Sig	***	***	***	***

a,b,c, d Means with different superscripts in the same column differ significantly (P≤0.001).

Conclusivly, fasting regimen and dietary Zn had a role in modulating hormonal profile of thyroid and adrenal glands, antioxidant status and digestive tract length in growing rabbits.

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تأثير التصويم وإضافة الزنك على نشاط الدرقية والادرينال ومضادات الأكسدة وطول القناة الهضمية فى الأرانب النامية

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** قسم إنتاج الدواجن - كلية الزراعة ـ جامعة عين شمس ـ مصر.

إستخدم فى هذة التجربة عدد 90أرنب ذكر مفطوم عمر 35يوم بمتوسط وزن 525+8.34 جرام من خط V-Line، حيث تم توزيعهم عشوائيا إلى 6 مجاميع تجريبية (15 أرنب بكل منها) كالتالى :

المجموعة الأولى : تغذية حرة على العليقه الأساسية كمجموعة مقارنة (AD).

المجموعة الثانية : تغذية حرة على العليقه الأساسية مضافا إليها 100 مجم زنك/كجم عليقة (Ad+Zn).

المجموعة الثالثة: تغذية حرة على العليقه الأساسية مع التصويم لمدة 24 ساعة أسبوعيا (F24).

المجموعة الرابعة: تغذية حرة على العليقه الأساسية مع التصويم لمدة 48 ساعة أسبوعيا (F48).

المجموعة الخامسة: تغذية حرة على العليقه الأساسية مضافا إليها 100 مجم زنك/كجم عليقة مع التصويم لمدة 24 ساعة أسبوعيا (F24+Zn).

المجموعة السادسة: تغذية حرة على العليقه الأساسية مضافا إليها 100 مجم زنك/كجم عليقة مع التصويم لمدة 24 ساعة أسبوعيا (F48+Zn).

وقد أوضحت النتائج مايلى:

- زاد معنويا مستوى هرمون الكورتيزول والكورتيكوستيرون فى سيرم دم الأرانب التى تعرضت للتصويم سواء يوم أو يومين أسبوعيا مقارنة بالمجموعتين الأولى والثانية التى لم تتعرض للتصويم.
- إنخفض معنويا مستوى هرمونات الغدة الدرقية (T_3 and T_4) فى سيرم دم الأرانب التى تعرضت للتصويم سواء يوم أو يومين أسبوعيا مقارنة بالمجموعتين الأولى والثانية التى لم تتعرض للتصويم.

-زاد معنويا مستوى TAC فى سيرم دم الأرانب التى تغذت على عليقة مضافا إليها 100 مجم زنك/كجم عليقة.

-إنخفض معنويا مستوى TAC فى سيرم دم الأرانب التى تعرضت للتصويم سواء يوم أو يومين أسبوعيا مقارنة بالمجموعتين الأولى والثانية التى لم تتعرض للتصويم.

-أدت إضافة الزنك بمستوى 100 مجم/كجم عليقة ليسلمعادلة تأثير التصويم فقط الذى خفض معنويا مستوى TAC بل تحسن مستواه فى سيرم دم الأرانب التى تعرضت للتصويم سواء يوم أو يومين أسبوعيا.

-زاد معنويا مستوى MAD فى سيرم دم الأرانب التنتعرضت للتصويم بينما كان أقل مستوى للMAD فى سيرم دم الأرانب التى تغذت على عليقة أساسية مضافا إليها 100 مجم زنك/كجم عليقة (AD+Zn).

-بالمقارنة بين المجاميع التجريبية يتضح أن الأرانب النامية التى تغذت على العليقة الأساسية مضافا إليها 100 مجم زنك/كجم علف وكذلك الأرانب التى تعرضت للتصويم وتغذت على عليقة مضافا إليها أو بدون إضافة زنك حدثت فيها زيادة معنوية فى طول القناة الهضمية والنسب المئوية لأجزائها.

-أدت إضافة الزنك لزيادة معنوية فى طول ونسب أجزاء القناة الهضمية سواء تعرضت للتصويم أم لم تتعرض.

التوصية:التصويم وإضافةالزنك لعليقةالأرانب النامية يؤدى إلى تحسن مستوى هرمونات الإجهاد والغدة الدرقية ومضادات الأكسدة وكذلك طول القناة الهضمية ونسب أجزائها.