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 T₃, T₄, corticosterone, cortisol, total antioxidant capacity (TAC) and malonylaldehyde (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 T₃ and T₄ 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

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Basal diet plus</th>
<th>Fasting days</th>
<th>Fasting weekly</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>AD + Zn</td>
<td>Zn**</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>F24</td>
<td>--</td>
<td>Sunday</td>
<td>--</td>
</tr>
<tr>
<td>F48</td>
<td>--</td>
<td>Sunday</td>
<td>Thursday</td>
</tr>
<tr>
<td>F24 + Zn</td>
<td>Zn</td>
<td>Sunday</td>
<td>--</td>
</tr>
<tr>
<td>F48 + Zn</td>
<td>Zn</td>
<td>Sunday</td>
<td>Thursday</td>
</tr>
</tbody>
</table>

**Zinc was added as a ZnSO₄ (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 (T₄) and triiodothyronine (T₃) were measured according to Abdel-Fattah et al., (2011) using radioimmunoaoassay (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; \( \mu \) = Overall mean; \( T_i \) = Treatment effect, and \( e_{ij} \) = Experimental error. When significant differences among means were tested by using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of fasting and zinc supplementation on:
1-Thyroid 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 (e.g., 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 T3 and T4 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 T3 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. Duzguner and 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cortisol (ug/dl)</th>
<th>Corticosterone (nmol/ml)</th>
<th>T₃ (ng/ml)</th>
<th>T₄ (ug/dl)</th>
<th>TAC (mM/L)</th>
<th>MAD (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, AD</td>
<td>5.20 ±0.33</td>
<td>5.25 ±0.08</td>
<td>2.20 ±0.22</td>
<td>4.43 ±0.09</td>
<td>125.87 ±0.52</td>
<td>59.03 ±1.75</td>
</tr>
<tr>
<td>AD +Zn</td>
<td>4.53 ±0.15</td>
<td>5.05 ±0.07</td>
<td>2.70 ±0.07</td>
<td>5.87 ±0.22</td>
<td>135.23 ±0.48</td>
<td>47.93 ±0.80</td>
</tr>
<tr>
<td>F24</td>
<td>8.23 ±1.95</td>
<td>7.18 ±0.06</td>
<td>1.50 ±0.90</td>
<td>1.80 ±0.04</td>
<td>98.20 ±0.79</td>
<td>67.67 ±1.11</td>
</tr>
<tr>
<td>F48</td>
<td>10.87 ±0.16</td>
<td>7.88 ±0.02</td>
<td>1.23 ±0.10</td>
<td>1.57 ±0.06</td>
<td>88.07 ±0.97</td>
<td>78.10 ±1.34</td>
</tr>
<tr>
<td>F24+Zn</td>
<td>6.83 ±0.13</td>
<td>6.52 ±0.06</td>
<td>1.80 ±0.10</td>
<td>2.73 ±0.06</td>
<td>118.63 ±0.99</td>
<td>68.90 ±1.90</td>
</tr>
<tr>
<td>F48+Zn</td>
<td>6.93 ±0.24</td>
<td>7.08 ±0.06</td>
<td>1.43 ±0.34</td>
<td>2.23 ±0.06</td>
<td>108.37 ±1.02</td>
<td>67.93 ±1.59</td>
</tr>
</tbody>
</table>

a,b,c,d Means with different superscripts in the same column differ significantly (P≤0.01), T₃=Triiodothyronine, T₄=Thyroxin, TAC=Total antioxidant capacity, MAD=Malonyaldehyde.

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, Wijten 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).
Table 3. 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Stomach (cm)</th>
<th>Small intestine (cm)</th>
<th>Large intestine (cm)</th>
<th>Caecum (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, AD</td>
<td>9.72 ±0.01</td>
<td>228.60 ±0.56</td>
<td>88.967 ±0.58</td>
<td>9.20 ±0.16</td>
</tr>
<tr>
<td>AD +Zn</td>
<td>10.59 ±0.04</td>
<td>281.33 ±0.41</td>
<td>170.30 ±0.29</td>
<td>12.40 ±0.24</td>
</tr>
<tr>
<td>F24</td>
<td>10.09 ±0.02</td>
<td>246.33 ±0.56</td>
<td>144.30 ±0.66</td>
<td>9.70 ±0.12</td>
</tr>
<tr>
<td>F48</td>
<td>9.99 ±0.01</td>
<td>243.00 ±0.71</td>
<td>139.13 ±0.64</td>
<td>10.07 ±0.16</td>
</tr>
<tr>
<td>F24+Zn</td>
<td>10.49 ±0.05</td>
<td>253.67 ±0.56</td>
<td>141.13 ±0.39</td>
<td>10.90 ±0.21</td>
</tr>
<tr>
<td>F48+Zn</td>
<td>10.41 ±0.07</td>
<td>257.67 ±0.67</td>
<td>143.90 ±0.65</td>
<td>10.43 ±0.24</td>
</tr>
<tr>
<td>Sig</td>
<td>***</td>
<td>***</td>
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a,b,c, d Means with different superscripts in the same column differ significantly (P≤0.001).

Conclusively, 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.

REFERENCES


تأثير التصويم وإضافة الزنك على نشاط الدرقي وادرينال والمضادات الأكسدة وطول القناة الهضمية في الأرانب النامية

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استخدمت هذه التجربة عدد 90 أرنب ذكر مقطوم عمر 35 يوم بمتوسط وزن 255 ± 8.34 جرام من خط V-Line، حيث تم توزيعهم عشوائيا إلى 6 مجموعات تجريبية (15 أرنب بكل منها) كالتالي:

المجموعة الأولى: تغذية حرة على العلبق الأساسية كمجموعة مقارنة (AD).
المجموعة الثانية: تغذية حرة على العلبق الأساسية مضافة إليها 100 مجم زنك/كم².
المجموعة الثالثة: تغذية حرة على العلبق الأساسية مع التصويم لمدة 24 ساعة.
المجموعة الرابعة: تغذية حرة على العلبق الأساسية مع التصويم لمدة 48 ساعة.
المجموعة الخامسة: تغذية حرة على العلبق الأساسية مضافة إليها 100 مجم زنك/كم² مع التصويم لمدة 24 ساعة أسبوعيا.
المجموعة السادسة: تغذية حرة على العلبق الأساسية مضافة إليها 100 مجم زنك/كم² مع التصويم لمدة 48 ساعة أسبوعياً.

وقد أوضح النتائج المالي:

- زاد معنوي مستوي هرمون ال kortizوl والكروتيسين ميرو في ميرو دم الأرانب التي تعرضت للتصويم سواء يوم أو يومين أسبوعياً مقارنة بالمجموعتين الأولي والأيادي التي لم تتعرض للتصويم.
- إنخفض معنوي مستوي هرمونات الغدة الدرقي (T3 و T4) في ميرو دم الأرانب التي تعرضت للتصويم سواء يوم أو يومين أسبوعياً مقارنة بالمجموعتين الأولي والأيادي التي لم تتعرض للتصويم.
زاد معدنباً مستويًّا TAC في سيرم دم الأئربات التي تغرد على علبق ماضفاً إليها 100 مجم زنك/كم غليقة.

إنخفض معنباً مستويًّا TAC في سيرم دم الأئربات التي تعرضت للتصوير سواء يوم أو يومين أسبوعياً مقارنة بالمجموعة الأولى والثانية التي لم تتعرض للتصوير.

أدت إضافة الزنكيُّ بمستوى 100 مجم/كم غليقة ليس لمعدلات تأثير التصوير فقم الذي خفض معدنباً مستويًّا TAC بل تحسن مستواها في سيرم دم الأئربات التي تعرضت للتصوير سواء يوم أو يومين أسبوعياً.

زاد معدنباً مستويًّا MAD في سيرم دم الأئربات التي تعرضت للتصوير بينما كان أقل MAD مستوىً للسيرم دم الأئربات التي تعرضت على علبق ماضفاً إليها من مجم زنك/كم غليقة (AD+Zn).

بالمقارنة بين المجموعات التجريبية يتضح أن الأئربان النانية التي تغرد على علبق الأساسية ماضفاً إليها 100 مجم زنك/كم غليقة، وكذلك الأئربان التي تعرضت للتصوير وغرد على علبق ماضفاً إليها أو بدون إضافة زنكيُّ حذرت فيها زيادة معنباً في طول العبقاق الهضمي ونسب المنيوية لأجزائها.

أدت إضافة الزنكيُّ لزيادة معنباً في طول ونسب أجزاء العبقاق الهضمي سواء تعرضت التصوير أم لم تتعرض.

التوصية: التصوير وإضافاناً الزنكي لعلبقة الأئربان النانية يؤدي إلى تحسن مستوي هرمونات الإجهاد والغدة الدرقية ومضادات الأكسدة وكذلك طول العبقاق الهضمي ونسب أجزائها.