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# EFFECT OF MULTI-ENZYMES ORAL ADMINISTRATION ON OXIDATIVE STRESS, ANTIOXIDANT ENZYMES, AND BLOOD INDICES OF GROWING RABBIT MALES

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**ABSTRACT:**The present research aimed to study the impacts of diverse doses of multi-enzymes (ME) ZAD® oral administration on oxidative stress, some antioxidant enzymes, and blood indices of growing rabbit males. At 4 weeks (average body weight 527.64±15.78 g), forty-five weaned rabbit males from V-line (VL) were randomly distributed to three groups. The control group received orally 1.0 ml distilled water (0.0 ZAD®), the experimental groups administrated orally with 0.75, and 1.25 ml ZAD®/rabbit/day, respectively. The experimental period lasted for 6 weeks. The values of derived compounds of reactive oxygen metabolites were decreased significantly (P≤0.01) with ME oral administration (ZAD®) levels, however, the values of total antioxidant capacity, superoxide dismutase, and glutathione S-transferase were enhanced significantly (P≤0.05). The results indicated that improvement of the scavenging capacity of the antioxidant defense system against oxidative stress processes in the treatment groups. The parameter hematological values containing red blood cell counts, hemoglobin, packed cell volume and mean corpuscular volume were improved significant (P≤0.05) with V-line rabbits groups which treated with ME oral administration (ZAD®) comparing with the control group. Some values of hematological blood in treated groups as mean corpuscular hemoglobin, and Mean corpuscular hemoglobin concentration and platelets count did not differ from those of the control group. There were highly significant differences (P≤0.001) between treatments on white blood cell counts, lymphocytes%, and neutrophils (N): lymphocytes (L) also were significant differences (P≤0.01) between treatments on neutrophils%. The highest value of WBC count and L% (12.11x103/µL and 63%), respectively noted with rabbits received 1.25 ZAD®/rabbit/day, while recording the lowest value of N% and N: L (25.9% and 0.41), respectively.

**Key Words:** antioxidant-hematological indices-oxidative stress-rabbit**.**

## INTRODUCTION

Oxidative stress is one of the main reasons for weakened livestock growth (Meineri et al., 2017). However, it is becoming popular to utilize herbal antioxidants to alleviate stress (Gupta et al., 2006). Oxidative stress may be described as a physiological disorder with an unbalance between antioxidants (enzymatic or non-enzymatic) and free radicals, particularly the concentrations of reactive oxygen species (ROS) (Abudabos et al., 2016; Skowron et al., 2018), and reactive nitrogen species (Toro and Rodrigo, 2009; Ito et al., 2017), causing molecular deterioration and/or disruption of redox control and signaling (Sies and Jones, 2007; Sies, 2018). The ROS comprise hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$), lipid peroxyl radicals ($LOO^-$), singlet oxygen ($O_2$), and hydroxyl radical (OH) (Ito et al., 2017). Free radicals are oxygen-comprising molecules or atoms with an unequal number of electrons. The unequal number makes it easy for them to interact with other molecules. These interactions are named oxidation. It can be beneficial or harmful (Lushchak, 2014), also the outcome of oxidative stress contributes to disease and health status (Sies, 2018). Some ROS are created endogenously through normal metabolic processes, but exogenous factors can significantly increase amounts (Sies, 2018). The causal factor of oxidative stress is weak the production of endogenous antioxidant and undue accumulation of ROS results in cell damage, including lipid membrane spoilage, protein deterioration, and DNA impairment (Sahin et al., 2002). Antioxidants postpone or stop carbohydrates, lipid, protein, and DNA oxidation significantly (Oboh and Rocha, 2007). Alterations in either direction can easily disrupt the balance between oxidant production and oxidant removal to create either an oxidant or reduced stress (Handy and Loscalzo, 2017). The definition of antioxidants is any substance that considerably postpones or halts the oxidation of that substrate when existing at low concentration compared to it of an oxidizable substrate (Halliwell et al., 1995). They can be categorized as antioxidants that are enzymatic or non-enzymatic. Oxidative stress assessment can have great importance to identify negative situations within the farm and suggest the most suitable interventions to create optimal conditions for the animals. Oxidative stress can be directly gauged by revealing free radical production, or indirectly by revealing the organism's antioxidant defenses (Meineri et al., 2017). It would be actually beneficial to evaluate plasma oxidative levels in animals to consider animals' health status (Brambilla et al., 2002; Pasquini et al., 2008).

Hematological studies are essential because of blood is the body's major transportation system, and hematological profile assessments usually provide fundamental information about the body's response to all forms of harms, including toxic injury (Ihedioha et al., 2004; Etim, et al., 2014). Hematological indicators are a guide and a reflexion of an animal's blood status to fulfill its metabolic, physiological and biochemical needs (Ewuola et al., 2004; Ayo-Ajasa et al., 2015). Blood testing provides an opportunity to investigate the presence of several metabolites and other components and helps to detect stress conditions that may be nutritional, environmental or physical (Aderemi, 2004).

The ZAD® is a patented product manufactured by the Academy of Scientific Research and Technology,

Egypt and an enzyme biotechnology product from natural sources to raise the anaerobic bacteria cellulase enzymes levels that can transform polysaccharide by the enzyme catalytic process to monosaccharide. It is designed in form liquid to provide tools to improve the nutritional value of fibrous materials and to improve the overall digestion of animals (Abdel-Azeem et al., 2018). The product includes the subsequent activity of enzymes such as 12.3 U / g protease, 64.4 U / mg amylase as well as 6.2 U / g hemicellulase and 8.2 U / g cellulose (Gado et al., 2011; Gado and Salem, 2013; Gado et al., 2017), besides the anaerobic bacteria that create these enzymes (Abdel-Aziz et al., 2014, 2015).

Enzyme supplementation has increased dietary digestion and the performance of young rabbits on starter diets (Gutiérrez et al., 2002; Abdel-Aziz et al., 2014 and 2015), where fiber and starch digestion rates are restricted in young rabbits (Abdel-Aziz et al., 2014 and 2015). The mechanism of action of exogenous enzymes on different sections of the rabbit gut had previously been proved by scientists. Sequeira, et al. (2000) showed a substantial decrease in gastric pH due to the addition of enzymes, while showed no important effect on gastric, intestinal and caecal contents, even in the era following early weaning (Falcão-e-Cunha et al., 2007).

Despite the study of the effect of ZAD® on rabbits and referring to positive growth results, declined mortality rates of NZW rabbits, enhanced the nutrient digestible coefficient, nitrogen balance, increased concentration of Lactobacillus in the caecum, and enhanced some biochemical parameters of the blood such as lowered serum lipid profile (Abdel-Azeem et al., 2018), but its impact on oxidative stress, antioxidant enzymes and blood indicators has not been researched.

To complete the knowing of effects of synergistically acting blend enzymes with anaerobic probiotic on the rabbit this report is hence to study the effect of anaerobic probiotic ZAD® oral intake on oxidative stress, some antioxidant enzymes, and blood indices of growing rabbit males.

## MATERIALS AND METHODS

### Experimental design:

This study took place at a private farm which called "Baldi Farm", Intelligence land, Fayoum, Egypt, to study the effect of multi-enzymes (ME) oral administration (ZAD®) on oxidative stress, antioxidant enzymes, and blood indices of growing rabbit males of V-line (VL) rabbits for 6 weeks. In a simple randomized design experiment, forty-five weaned rabbit males at 4 weeks old (average body weight 527.64±15.78 g) from VL were distributed randomly to three groups (15 in each group; three replicates 5 for each replicate). The control group was administered 1.0 ml distilled water (0.0 ZAD®) orally once daily, the trial groups were administered multi-enzymes (ME) oral administration (ZAD®) at levels 0.75, and 1.25 ml/rabbit/ day (Groups 0.75 ZAD®, and 1.25 ZAD®, respectively) were oral gavage administration/each rabbit. Doses were given once daily via gavage for 6 consecutive weeks. The oral administration is very useful and important to eliminate the risk of variability in intake among individual animals that may arise when giving substances through water delivery.

### Diets and housing of the experiment:

The rabbits were kept in cages from galvanized wire individually (50×40×35 cm) under a 8: 16 h dark–light cycle until marketing at 10 weeks of age. Pelleted

feed ad libitum was fed to all rabbits. According to Lebas (2004), the experimental diets were prepared to fulfill the nutrient needs of growing rabbits. All rabbits were reared under the same conditions of hygiene, management, and the environment. Rabbits were kept in a building was well-ventilated; through nipples from stainless-steel fastened in every cage, fresh water was automatically obtainable the whole time. The composition and calculated analyses of the experimental diets and feed ingredients are calculated according to NRC (1977), and presented in Table (1).

**Blood samples**

At the end of the experiment the blood samples collected from five randomly selected rabbits per treatment group via the external ear vein and split into two parts, the first one was immediately placed for hematological parameters with ethylene-diamine-tetra-acetic acid (EDTA), the second one was put in centrifuge tubes, left to clot and centrifuged for separation from the serum, up to the biochemical parameters, serum samples were kept at -20°C.

**Serum biomarkers of oxidative stress**

Reactive oxygen species-derived hydroperoxides, as an indicator of ROS production, have been measured by using the derived compounds of reactive oxygen metabolites (d-ROMs) test (Diacron s.r.l., Grosseto, Italy) on rabbit serum (Pasquini et al. 2008; Meineri et al. 2017). This test depends on the basis that oxygen-free radicals are atoms that have one or more unpaired electrons in one of their outer orbitals; these free radicals head for reacting with specific organic molecules and produce ROMs due to their excessive reactivity. The final is steadier and can, therefore, be measured than their predecessors. In the d-ROM test, ROMs (mainly hydroperoxides) are produced (via the Fenton reaction), in the existence of iron (through an acid buffer released from plasma proteins), accompanied by some alkoxy radicals and peroxy radicals. These radicals interact with aromatic amine (N, N dietlparaphenylendiamine), that is oxidized and transformed to a photometrically quantifiable pink derivative, at a 550 nm wavelength (Giongo et al., 2011). According to the Beer-Lambert law, the strength of the color is directly proportional to the ROMs concentration. The d-ROM test outcomes are stated in qualitative units known as 'Carratelli Units' (1 CARR U = 0.08 mg hydrogen peroxide/dl) using the following formula: CARR U = F (ΔAbs/minutes) where F is an assigned value correction factor (about 9000 at 37° C, according to the standard results) and (ΔAbs/min) the mean absorption differences recorded at 1, 2, and 3 min.

**Serum enzymatic activities of antioxidant**

According to Misra and Fridovich (1972), the activity of superoxide dismutase (SOD) was tested. Based on (Koracevic et al., 2001) method total antioxidant capacity (TAC) was determined, also, activitiy of glutathione S-transferase (GST) was measured according to (Habig et al., 1974).

**Blood hematological parameters**

The determined hematological parameters were also red blood cell counts (RBC), gauged according to Perkins (2009) also, calculated the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Furthermore, the counts of white blood cells (WBC), packaged cell volume (PCV), differential leukocytes were determined according to (Voigt and Swist,

2011 and Harvey, 2012). The hemoglobin (Hb) % analyzed according to Van Kampen and Zillstra (1983).

**Statistical analysis**

The experiment data were analyzed using General Linear Models (GLM) procedure of SPSS (2007), studied traits were subjected to a one-way ANOVA, using the following model: $y_{ij}=\mu+T_i+e_{ij}$, Where: $y_{ij}$= Observed value, $\mu$= Overall mean, $T_i$= Treatment effect (i: 1 to 3), and $e_{ij}$= Random error. Using Duncan multi-range test (Duncan, 1955), the significant differences between means were detected.

## RESULTS AND DISCUSSION

Table (2) showed that the values of d-ROMs were decreased significantly ($P\leq0.01$) with ME oral administration (ZAD®) levels. However, the values of TAC, SOD, and GST were enhanced significantly ($P\leq0.05$) with ME oral administration (ZAD®) levels. An availability indicator of reduction agents in blood plasma is the TAC, and thus plasma's ability to scavenge free radicals of oxidation (Kambayashi et al., 2009). Antioxidant enzymes such as SOD can stop the oxidation either by scavenging the mainly reactive free radical in vivo or by steadying move metal radicals such as $Cu^+$ or $Fe_2^+$ (Afolabi and Oloyede, 2014). Glutathione S-transferase acts a vital role in the detoxification of xenobiotics within cells, however high concentration in blood refers to cell damage (Sharma et al., 2004). Lower total antioxidant activity in the serum blood of control group might have participated in the disturbances or resulted from other free radical stress that reduced total antioxidant activity. In either case, decreased total antioxidant activity as a result of ROM-reducing reactions increases vulnerability to oxidative stress. The results revealed that TAC, SOD, and GST in blood serum of ME oral administration (ZAD®) groups were significantly high (Table 2), indicating improved scavenging capacity of the antioxidant defense system against oxidative stress processes in these groups. The outcomes indicated that the changes in the activity of numerous antioxidant enzymes can be used in rabbits to evaluate the oxidative stress level and the total antioxidant status. Additionally, a balance between production and safe disposal of ROM may contribute to the improvement of growth and health status in growing rabbits.

These findings are due to direct cause that containing the mixture on the anaerobic bacteria that create these enzymes which increases the number of beneficial bacteria from *Lactobacillus acidophilus* and *Lactobacillus cellobiosus* (Abdel-Azeem et al., 2018), which may be used the free radical and ROS during their metabolic processes and⁄or indirectly because of the mixture of enzymes that positively improved growth performance, nutrient digestible coefficient and nitrogen balance as well as mortality rate (Gado et al., 2017; Bhatt et al., 2017; Abdel-Azeem et al., 2018 and Sherif, 2018), which is reflected on the improvement of the state of public health, increases the activity of cells to produce antioxidants and resistance to increase the production of free radicals. The ZAD® may be called probiotics to contain the mixture on the anaerobic bacteria that create these enzymes. Probiotics antioxidant mechanisms may be attributed to ascorbate autoxidation reduction activity and inhibition, ROS scavenging, enzyme inhibition, and metal ion chelation (Talwalkar and Kailasapathy 2003). Probiotic metabolic operations may have an antioxidant impact by the scavenging or banning of oxidant compounds in the gut (Azcárate-Peril et al.

2011). Moreover, Lin and Yen (1999) assumed that beneficial gut bacteria create several factors able to inhibit cytotoxic activity, remove free radicals, and catch ROS.

Probiotics ZAD® may act a useful role in numerous oxidative stress conditions, comprising decrease of the d-ROM compounds and increase of antioxidant system. Weston et al. (2005) indicated that probiotics impact is used not only through enzymatic activation but also through stimulation of gene expression. Numerous other reports have shown that probiotics positively adjust oxidant and antioxidant dynamics in rats, Japanese quails, chickens, rabbit, and human (Lin and Yen, 1999; Lu et al., 2006; Yadav et al., 2007; Sohail et al., 2011; Ghoneim and Moselhy, 2013).

The findings in Table (3), illustrated that the parameter hematological values containing RBC, Hb, PVC, and MCV were improved significant ($P \leq 0.05$) with V-line rabbits groups which treated with ME oral administration (ZAD®) comparing with the control group. These elevated values, however, stay within the normal range (Campell and Grant, 2010; Taylor et al., 2010). These changes in RBC are linked to an increase in blood oxygen carrying capacity accompanied by an increase in respiratory rate (El-Banna et al., 2005).

This may be indicated to the high capacity of the rabbits received the dietary enzymes ZAD® supplementation via oral to carry oxygen and the ability to withstand stress. The ME oral administration of ZAD® caused a significant ($P \leq 0.05$) reduction in MCV in comparison with the control group in spite of the ME oral administration (ZAD®) augmented the RBC count, as shown in Table (3), these results indicate that the role of ME oral administration ZAD® is directly or indirectly to boost small-sized RBC synthesis (reticulocyte). Some values of hematological blood in treated groups (MCH, MCHC, and platelets count) did not differ from those of the control group. The role of probiotic oral administration of ZAD® may be because of the mix among enzymes and the basic diets besides the anaerobic bacteria that create these enzymes increasing the total digestible nutrients and the heights of the coefficients of digestion (Gado and Salem, 2013), this is reflected positively in improving the health and physiological status of different rabbit organs and enhancing the body's ability to perform its functions properly and in a healthy.

Effects of the ME oral administration of ZAD® on WBC count and differentiate leukocytes percentage of growing rabbits are presented in Table (4). Results indicated that there were highly significant differences ($P \leq 0.001$) between treatments on WBC count, lymphocytes (L)%, and neutrophils (N): L also was significant differences ($P \leq 0.01$) between treatments on N%. The highest value of WBC count and L% (12.11x103/µL and 63%), respectively noted with rabbits received 1.25 ZAD®/rabbit/day, while recording the lowest value of N% and N: L (25.9% and 0.41), respectively. The rise of neutrophils% may reveal the stress severity in growing rabbits of the control group. Neutrophils can absorb molecular oxygen and, when stimulated, produce reactive oxygen-containing molecules (Bendich, 1990), so noted that the high d-ROM level in the control group (Table 2) and increase the N% in the same group (Table 4). The increased value of WBC count with high L% and decreased N% indicate that rabbit immunity improvement in these treated groups. The

best value of the N: L ratio recorded in rabbits received 1.25 ZAD® /rabbit/ days may be because of an augmentation in the percentage of L and a decrease in the percentage of N. The N: L ratio augment can be reflected in stress severity and can be benefited as an indicator of stress (Abdel-Azeem, 2010). Some hematological parameters in treated groups (percentage of monocytes, eosinophils, and basophils) were no different from control group parameters.

## CONCLUSION

The current study concludes that multi-enzymes oral administration ZAD® positively decreased derived compounds of reactive oxygen metabolites and enhanced the total antioxidant capacity, superoxide dismutase, and glutathione S-transferase, so that, better scavenging capacity of the antioxidant defense system against oxidative stress process. In addition, enhancement of some hematological indices such as red blood cell counts, hemoglobin, packaged cell volume and mean corpuscular volume, white blood cell counts, lymphocytes%, neutrophils%, and neutrophils: lymphocytes, thus, a high capacity of the rabbits to carry oxygen, the ability to withstand stress and increased immune capacity. That reflects positively on animal health and the absence of disease and production increasing.

**Table (1):** Composition and calculated analyses (%) of the experimental diets on dry matter basis.

| **Feed ingredients %** | **Control diet** | **Calculated analyses** | |
|---|---|---|---|
| | | **Item** | **%** |
| Dried Egyptian clover | 35.00 | dry matter | 89.67 |
| Barley | 18.0 | Neutral detergent fiber | 37.49 |
| Soybean meal | 17.5 | Nitrogen-free extract | 56.03 |
| Wheat bran | 15.00 | Digestible energy (Kcal/Kg) | 2519.87 |
| Yellow corn | 10.0 | Crude protein | 17.18 |
| Molasses | 3.00 | Crude fiber | 13.05 |
| DL-Methionine | 0.10 | Ether extract | 3.41 |
| Di- Ca- phosphate | 0.8 | Total sulphur amino acid (%) | 0.68 |
| Vit.-Min. premix[1] | 0.30 | Methionine | 0.36 |
| NaCl | 0.30 | Ash | 10.33 |
| Total | 100 | Calcium | 0.83 |
| | | Phosphors | 0.31 |

[1]Provided per kilogram diet: vitamin E, 40 mg; vitamin $D_3$, 450 IU; vitamin A, 6000 IU; vitamin $B_1$, vitamin K3, 1 mg; ; vitamin $B_2$, 3 mg; 1 mg; niacin, 180 mg vitamin $B_6$, 39 mg; pantothenic acid, 10 mg; folic acid 2.5 mg; biotin, 10 mg; manganese, 15 mg; , 2.5 mg; vitamin $B_{12}$, 1200 mg; zinc, 35 mg; iron, copper, 5 mg; selenium, 0.05 mg; iodine, 0.2 mg; 38 mg; choline chloride,.

**Table (2):** Effects of multi-enzymes oral administration (ZAD®) on serum biomarkers of oxidative stress and antioxidant of growing rabbits.

| Items | Treatments | | | SEM[#] | Sig.[2] |
|---|---|---|---|---|---|
| | Control | 0.75 ml ZAD®/rabbit/day | 1.25 ml ZAD®/rabbit/day | | |
| **Serum biomarkers of oxidative stress:** | | | | | |
| d-ROMs test (CARR U) | 305.67[a] | 292.94[b] | 285.35[c] | 0.05 | ** |
| **Serum enzymatic activities of antioxidant**: | | | | | |
| Total antioxidant capacity (mmol/l) | 1.50[b] | 2.26[a] | 2.54[a] | 0.17 | *** |
| Superoxide dismutase (U/l) | 24.71[b] | 31.97[a] | 34.73[a] | 1.07 | ** |
| Glutathione S-transferase (U/l) | 12.66[b] | 14.76[a] | 15.37[a] | 0.93 | ** |

[#]SEM= standard error of the mean; Sig.= significance.

[a,b,c] Means within a row having different superscripts are significant (P≤0.05).

[2]***= significant at the 0.1% probability level; **= significant at the 1% probability level;

*= significant at the 5% probability level; NS=Not significant.

**Table (3):** Effect of multi-enzymes oral administration (ZAD®) on some hematological parameters of growing rabbits.

| Items | Treatments | | | SEM# | Sig.[2] |
|---|---|---|---|---|---|
| | Control | 0.75 ml ZAD®/rabbit/day | 1.25 ml ZAD®/rabbit/day | | |
| **Hematological parameters:** | | | | | |
| RBC (X $10^6$/µL) | 4.20[c] | 5.72[b] | 6.11[a] | 0.98 | * |
| Hb (g/dL)% | 9.23[c] | 11.34[b] | 12.42[a] | 0.23 | * |
| PCV (%) | 33.50[c] | 38.61[b] | 42.14[a] | 0.52 | * |
| MCV (fL) | 79.76[a] | 67.50[b] | 68.97[b] | 1.12 | * |
| MCH (pg) | 21.98 | 19.83 | 20.33 | 0.59 | NS |
| MCHC% | 27.55 | 29.37 | 29.47 | 0.57 | NS |
| Platelets count | 300 | 318 | 326 | 1.42 | NS |

#SEM= standard error of the mean; Sig.= significance; RBC= red blood cell count;
Hb= hemoglobin; PCV= packed cell volume; MCV= mean corpuscular volume;
MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration.
[a,b,c] Means within a row having different superscripts are significant (P≤0.05).
[2*]= significant at the 5% probability level; NS=Not significant.

**Table (4):** Effects of multi-enzymes oral administration (ZAD®) on white blood cells count (WBC) and differentiate leukocytes percentage of growing rabbits.

| **Items** | **Treatments** | | | **SEM#** | **Sig.[2]** |
|---|---|---|---|---|---|
| | **Control** | **0.75 ml ZAD®/rabbit/day** | **1.25 ml ZAD®/rabbit/day** | | |
| WBC | 8.15[c] | 10.19[b] | 12.11[a] | 0.61 | *** |
| Neutrophils% | 38[a] | 30[b] | 25.9[c] | 1.03 | ** |
| Lymphocytes% | 51[c] | 59[b] | 63[a] | 1.73 | *** |
| N:L | 0.75 | 0.51 | 0.41 | 0.11 | *** |
| Monocytes% | 2.2 | 2.3 | 2.2 | 0.19 | NS |
| Eosinophils% | 2 | 1.9 | 2 | 0.18 | NS |
| Basophils% | 6.8 | 6.8 | 6.9 | 0.46 | NS |

[#]SEM= standard error of the mean; Sig.= significance.
[a,b,c] Means within a row having different superscripts are significant (P≤0.005).
[2***]= significant at the 0.1% probability level; [**]= significant at the 1% probability level; NS=Not significant.

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## الملخص العربى

## تأثير تجريع إنزيمات متعددة بالفم على الإجهاد التأكسدي و إنزيمات مضادات الأكسدة و مؤشرات الدم بذكور الأرانب النامية

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يهدف البحث الحالي إلى دراسة آثار الجرعات المتنوعة من تناول إنزيمات متعددة ZAD® عن طريق الفم على الإجهاد التأكسدي وبعض الإنزيمات المضادة للأكسدة ومؤشرات الدم لدى ذكور الأرانب النامية. تم توزيع خمسة وأربعين من الذكور الأرانب المفطومة عمر 4 أسابيع (متوسط وزن الجسم 527.64 ± 15.78 جم) من الخط الخامس الاسباني {قى لاين} (VL) بشكل عشوائي على ثلاث مجموعات. تلقت المجموعة الضابطة تجريعا بالفم 1 مل ماء مقطر (مستوي صفر من ZAD®) والمجموعات التجريبية تجريعا بالفم مستوي 0.75 و 1.25 مل ZAD®/ أرنب/ يوم، على التوالي. استمرت الفترة التجريبية لمدة 6 أسابيع. قيم المركبات المشتقة من نواتج تمثيل الأكسجين التفاعلي (d-ROM) انخفضت بشكل معنوي ($P\leq0.01$) مع مستويات تجريع الإنزيمات المتعددة ZAD® بالفم، ومع ذلك، فقد تم تحسنت قيم القدرة الكلية لمضادات الأكسدة وانزيم ديسموت فائق الاكسدة، وانزيم الجلوتاثيون-اس الناقل بشكل معنوي ($P\leq 0.05$). أشارت النتائج إلى تحسن قدرة الكسح (التنظيف) بمضادات الأكسدة ضد عمليات الإجهاد التأكسدي في المجموعات المعاملة. قيم مؤشرات الدم المقاسة التي تضم عدد خلايا الدم الحمراء و الهيموجلوبين و النسبة المئوية للمكونات الخلوية و متوسط حجم خلية الدّم الحمراء قد تحسنت معنويا ($P\leq0.05$) مع مجموعات أرانب" الخط الخامس الاسباني (قى لاين)" التي تجرعت إلانزيمات المتعددة ZAD® بالفم بالمقارنة بمجموعة الكنترول. لم تختلف بعض قيم مؤشرات الدم في المجموعات المعاملة كـ متوسطِ حجم الهيموجلوبين و متوسط تركيز الهيموجلوبين بخلية الدم وعدد الصفائح الدموية عن تلك الموجودة في المجموعة الضابطة. كانت هناك اختلافات معنوية ($P\leq0.001$) بين المعاملات في عدد خلايا الدم البيضاء و نسبة الخلايا اللمفاوية و نسبة الخلايا المتعادلة الي الخلايا اللمفاوية وسجلت اختلافات معنوبة عند مستوي ($P\leq0.01$) بين المعاملات في نسبة الخلايا المتعادلة. سجلت أعلى قيمة لعدد خلايا الدم البيضاء ونسبة الخلايا الليمفاوية ($12.11 X 10^3$ لكل ميكرولتر و 63%، على التوالي) مع الأرانب التي تجرعت ZAD® 1.25/أرنب/ يوم و بينما سجلت اقل قيمة لنسبة الخلايا المتعادلة ونسبة الخلايا المتعادلة الي الخلايا الليمفاوية (29.9% و 0.41، على التوالي).

الكلمات الدالة: مضادات الأكسدة، مؤشرات الدم، الإجهاد التأكسدي، الأرنب.