

## **EFFECT OF ADDING PROBIOTICS TO GROWING RABBITS RATION ON BLOOD CONSTITUENTS AND IMMUNOLOGICAL RESPONSES**

**Mariz, M. Moneir<sup>1\*</sup>; H.M. Gado<sup>1</sup>; A.S. El-Hawy<sup>2</sup> and H.M. Metwally<sup>1</sup>**

<sup>1</sup> *Animal Production Dept, Fac of Agric, Ain Shams Univ P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt*

<sup>2</sup> *Animal and Poultry Physiology Department, Desert Research Center (DRC), Cairo 11753, Egypt*

\*Corresponding author: [Lizamilad3@gmail.com](mailto:Lizamilad3@gmail.com)

*(Received 27/3/2024, accepted 17/5/2024)*

### **SUMMARY**

The purpose of the current study was to assess the impact of various probiotic dietary additives on the blood components and immunological parameters of Hi-Plus developing rabbits in the specific environmental conditions of North Sinai. A group of 48 Hi-Plus rabbits, 5 weeks age and  $811.00 \pm 20$  g average body weight, were allocated into four experimental groups. Each group was randomly allocated into three equal replicates, with four rabbits each. The first treatment group was administered the basal diet (control). In the second, third, and fourth groups, were administered the basal diet supplemented with 10 ml ZAD<sup>®</sup> per kilogram of diet, 10 g ZADO<sup>®</sup> per kilogram of diet, and 10 g YEAST (*saccharomyces cerevisiae*) per kilogram of diet, respectively. The experiment lasted to 7 weeks. The findings of this study indicate that the rabbits who were administered probiotic treatments exhibited a significant increase ( $P < 0.05$ ) in plasma total protein and globulin concentrations when compared to the control group. The rabbits that were treated with ZADO<sup>®</sup> and YEAST exhibited a significant drop ( $P < 0.05$ ) in plasma triglyceride levels when compared to the control group. Total antioxidant capacity were increased ( $p < 0.05$ ) for different probiotic treatments when compared to the control group. The concentration of ALT level was found to be significantly lower ( $P < 0.05$ ) in the rabbits who were fed YEAST as compared to the control group. The rabbits who were fed YEAST exhibited a statistically significant rise ( $P < 0.05$ ) in plasma levels of IgG and IgA when compared to the control group. In conclusion, the addition of probiotics ZADO<sup>®</sup> and YEAST at a concentration of 1% to the diets of Hi-Plus rabbits during the growing phase may have beneficial effects on blood components and immunological condition.

**Keywords:** *probiotics, rabbits, blood constituents, immunity*

### **INTRODUCTION**

Probiotics are often employed as growth promoters to mitigate the occurrence of antibiotic residues in animal products intended for human consumption. Previous studies indicated beneficial effects of probiotics on the growth performance and overall health of rabbits (Ezema and Eze, 2010; Bhatt *et al.*, 2017). According to Gado *et al.* (2017), the utilization of anaerobic probiotic technology (ZAD)<sup>®</sup> has the potential to serve as an alternative to antibiotic growth promoters in animal feed. The ZAD<sup>®</sup> is a patented product developed by the Academy of Scientific Research and Technology in Egypt. It is a probiotic biotechnology product derived from natural sources, specifically designed to enhance the levels of anaerobic bacteria cellulase enzymes. This probiotic plays a crucial role in the catalytic process of converting polysaccharides into monosaccharides.

The nutritional additive known as ZAD<sup>®</sup> (liquid form), which is an anaerobic probiotic, has been developed specifically for rabbits. This additive, when consumed in a quantity of 1.5 liters, has been found to aid rabbits in mitigating the effects of heat stress. According to Abdel-Azeem *et al.* (2018), the intervention resulted in an increase in milk production for rabbits, an improvement in the daily average weight gain, enhancements in many physiological aspects, and a decrease in rabbit mortality rate.

The addition of ZADO<sup>®</sup> (the powder form of ZAD) has been found to enhance the immune response in broiler chickens. According to Hosam (2013) and El-Sanhoury and Ahmed (2017), it has been shown that ZADO<sup>®</sup> products have a high level of safety when used as feed additive for birds. Consequently, these findings suggest that birds treated with ZADO<sup>®</sup> product pose no significant risks to human consumption. According to a study conducted by Hosam *et al.* (2013), the addition of ZADO<sup>®</sup> to the diets of laying hens may lead to a rise in plasma protein levels and an enhancement of enzyme activity. These changes in physiological parameters could potentially result in a modest improvement in the productivity of the hens. In their study, Abdel-Azeem *et al.* (2018) found that the anaerobic probiotic ZAD<sup>®</sup> had a positive impact on some blood biochemical parameters, specifically leading to a reduction in serum lipid profile.

According to the findings of Fathi *et al.* (2017), the inclusion of probiotic additives in the diet of rabbits resulted in a greater reduction in cholesterol levels compared to the control group. The rabbits that were fed a diet containing 400 g of probiotic per ton of feed exhibited the lowest value of cholesterol. Similarly, the rabbits that were fed a low level of probiotics (200g) also showed lower values compared to the control group. This effect can be attributed to the inhibition of an enzyme involved in cholesterol synthesis. Additionally, there was a numerical increase in triglyceride levels associated with the dietary probiotic treatment. A marginal augmentation in the overall protein concentration was noted in rabbits that were on a diet including probiotic additives, with a corresponding rise specifically identified in the globulin fraction. This implies that the elevated concentration of globulin observed in response to probiotic interventions is regarded as a positive indicator for the augmentation of immunoglobulin levels, hence bolstering the overall state of immunity.

Hence, the objective of this study was to investigate the impact of dietary additive with various probiotics on the blood components and immunological parameters of growing rabbits in the specific environmental conditions of North Sinai.

## **MATERIALS AND METHODS**

The experiments were conducted in adherence to the rules established by the Institute of Animal Ethics Committee for the utilization of animals (European Union 2010/63/EU of the European Parliament and of the Council, September 22, 2010).

The current investigation was conducted at a privately owned rabbit farm located at Latitude 31° 29' N and Longitude 32° 34' E in the North Sinai Governorate. The study was conducted in collaboration with the Department of Animal and Poultry Physiology at the Desert Research Center, Ministry of Agriculture in Cairo, Egypt, as well as Systel Telecom Company and the Egyptian Center of Excellence for Bio-Saline Agriculture. The study period spanned from December 2021 until the end of January 2022.

### ***The management and feeding of rabbits:***

The rabbits were housed in wire cages and provided with clean water and a commercial concentrate pelleted diet. The diet chemical compositions were 17.0% crude protein, 13.0% crude fiber, 2.0% fat, 0.3% minerals mixture, and 2700 kcal/kg digestible energy, as specified by the NRC (1994) Table 1. The feeding regimen was maintained until the rabbits reached 12 weeks of age, which marked the end of the experiment (7 weeks' experimental period).

The commercial probiotic ZAD is composed of a blend of anaerobic bacteria, specifically *Ruminococcus flavefaciens*, at a concentration of  $1 \times 10^9$  colony-forming units per liter (CFU/ml). Additionally, the probiotic contains exogenous enzymes that have been formulated to serve as a supplement in liquid form for ruminant diets. The commercial probiotic ZADO comprises a blend of anaerobic bacteria, specifically *Ruminococcus flavefaciens* at a concentration of  $1 \times 10^{10}$  CFU/g along with exogenous enzymes. This formulation is intended to serve as a supplement for ruminant feeds and is available in powder form. The ZAD and ZADO were generously supplied by the Bactizad Company. The *Saccharomyces cerevisiae* (yeast) utilized in the present investigation was of commercial life yeast with concentration of  $3 \times 10^9$ .

The Hi-Plus rabbits were maintained under identical management and hygienic circumstances. The rabbits were assessed for their overall health and subjected to thorough examinations to detect any potential infections. Additionally, they were administered vaccines as a preventive measure against several illnesses.

**Experimental design:**

A cohort of 48 Hi-Plus rabbits, which had been weaned at 5 weeks of age and had an average body weight of 811.00±20g, were divided into four experimental groups of equal size, with 12 rabbits in each group. Each group was randomly allocated into three equal replicates, with each replicate consisting of four rabbits. The initial treatment was designated as the control group (C) and was provided with the basal diet without any further additives. The 2<sup>nd</sup> group (Tr2) received the basal diet + 10 ml ZAD/kg diet (1.0 %). The 3<sup>rd</sup> group (Tr3) received the basal diet + 10 g ZADO / kg diet (1.0 %), while the 4<sup>th</sup> group (Tr4) received the basal diet + 10 g saccharomyces cerevisiae / kg diet.

**Table (1): Composition and chemical analysis of experimental diet (% DM basis).**

Ingredients (%)	Basal diet	ZAD	ZADO	YEAST
ZAD	0.0	1.0	0.0	0.0
ZADO	0.0	0.0	1.0	0.0
YEAST	0.0	0.0	0.0	1.0
Soybean meal (48% CP)	9.0	9.0	9.0	9.0
Barley	11.0	11.0	11.0	11.0
Wheat bran	14.0	14.0	14.0	14.0
Corn	19.0	19.0	19.0	19.0
Clover hay	29.0	29.0	29.0	29.0
Fennel hay	13.0	13.0	13.0	13.0
Molasses	3.0	3.0	3.0	3.0
Di- calcium phosphate	1.0	1.0	1.0	1.0
DL-Methionine	0.40	0.40	0.40	0.40
Sodium chloride	0.30	0.30	0.30	0.30
Vit. Min. premix*	0.30	0.30	0.30	0.30
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>Chemical analysis</b>				
Dry matter (DM)	94.52	94.95	95.22	95.23
Organic matter (OM)	90.88	89.99	89.33	89.54
Crude protein (CP)	17.00 <sup>c</sup>	18.30 <sup>ab</sup>	18.50 <sup>a</sup>	17.60
Crude fiber (CF)	13.30	13.31	13.50	12.52
Ether extract (EE)	4.61	4.43	4.75	4.43
Nitrogen free extract (NFE)	54.82	54.25	52.58	58.08
Ash	10.27	10.01	10.67	10.46

\*Vitamins and mineral mixture supplied per kg of diet: Vitamin A 10,000 IU, Vitamin D3,1,800 IU; Vitamin E, 15 mg; vitamin K3, 4.5 mg; Vitamin B1, 0.5 mg; Vitamin B2, 4 mg; Vitamin B12, 0.001 mg; Folic acid, 0.1 mg; Pantothenic acid, 7 mg; Nicotinic acid, 20 mg; I, 1 mg; Mn, 60 mg; Cu, 5.5 mg, Zn, 75 mg; Fe, 40 mg; Co, 0.3 mg; Se, 100 mg, Robenidine, 52.8 mg.

**Blood plasma parameters:**

Blood samples were taken (3 times) throughout the experiment period into tubes containing EDTA as an anticoagulant (5 rabbits/treatment). Plasma was then harvested after centrifugation at 5000 g for 15 min and then stored at -20°C for later analysis. The bioanalysis of plasma was carried out for quantitative determination of blood parameters by spectrophotometer. Plasma total protein (TP), albumin (ALB), glucose (GLC), cholesterol (CHOL), triglycerides (TG), creatinine (CRA), urea, total antioxidant capacity (TAC) were analyze using commercial kits (Biomed diagnostics, Egypt and Biodiagnostic Research, Egypt).

Immunoglobulin G (IgG), Immunoglobulin A (IgA), Immunoglobulin M (IgM) were analyzed using commercial kits (Reactivos GPL Barcelona, España).

**Statistical analysis:**

Blood plasma analysis was conducted at the Animal and Poultry Physiology Laboratory, a facility affiliated with the Animal and Poultry Production Division of the Desert Research Center, under the

auspices of the Ministry of Agriculture and Reclamation in Cairo, Egypt. The Faculty of Agriculture at Ain-Shams University conducted proximate analysis and examinations.

Data were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004). The design was one-way analysis, and the model was as follows:

$$Y_{ij} = \mu + \text{Tri} + e_{ij} \text{ Where,}$$

$Y_{ij}$  = any observation of  $j$ th rabbit within  $i$ th treatment.

$\mu$  = overall mean.

Tri = effect of  $i$ th treatment ( $i$ : 1-4).

$e_{ij}$  = experimental error.

Duncan Multiple Range Test (Duncan, 1955) was used to test the level of significance among means.

## RESULTS AND DISCUSSION

### *Blood plasma of total protein and its constituent fractions:*

The rabbits that were fed ZAD®, ZADO®, and YEAST exhibited a considerable rise in plasma total protein concentration. Specifically, the concentrations were increased ( $P < 0.05$ ) by 15.61%, 17.09%, and 15.40% correspondingly, as compared to the control group. Furthermore, the rabbits that were fed ZADO and YEAST exhibited a significant increase ( $P < 0.05$ ) in globulin concentration. Specifically, the globulin concentration was increased by 59.72% and 44.44% in the ZADO and YEAST groups, respectively, as compared to the control group. However, the rabbits were fed ZAD had a statistically insignificant increase in globulin levels, with an increase of 31.25% compared to the control group. However, it should be noted that there were no statistically significant changes observed among the experimental treatments in terms of albumin and albumin/globulin ratio, as indicated in Table 2.

**Table (2): Effect of biological additives on blood total protein and its fractions of Hi-Plus growing rabbits.**

Traits	Treatments				±SE	P value
	C	ZAD	ZADO	YEAST		
Total protein (g/dl)	4.74 <sup>b</sup>	5.48 <sup>a</sup>	5.55 <sup>a</sup>	5.47 <sup>a</sup>	0.22	0.037
Albumin (g/dl)	3.29	3.58	3.24	3.39	0.15	0.434
Globulin (g/dl)	1.44 <sup>b</sup>	1.89 <sup>ab</sup>	2.30 <sup>a</sup>	2.08 <sup>a</sup>	0.18	0.014
Albumin/globulin ratio	2.28	1.89	1.40	1.62	0.58	0.103

C= control group, ZAD= rabbits received 10 ml ZAD/kg diet, ZADO= rabbits received 10 g ZADO/kg diet, YEAST= rabbits received 10 g YEAST/kg diet.

<sup>a, b</sup> Means bearing different superscripts within the same rows are significantly different ( $P < 0.05$ ).

The findings presented in this study are consistent with the results reported by Makled *et al.* (2005) and Abdel-Azeem *et al.* (2018). The levels of total protein, globulin, and albumin were found to fall within the normal ranges for rabbits, as reported by Ajayi and Raji (2012). This statement pertains to the enhancement of dietary protein quality and availability by the utilization of an anaerobic probiotic. Proteins and globulins are integral components of the immune system. Albumin-based antibodies, which are the primary protein constituents of serum, are generated in hepatic tissues. These antibodies play a crucial role in the humoral immune response and contribute to the enhancement of immunological organs. In line with the objectives of the current investigation, the research conducted by Gao *et al.* (2007) provides corroborating evidence regarding the amounts of globulin in plasma and the decrease in the albumin/globulin ratio may suggest an enhancement in the immune response of rabbits and this compatible with our results showed in Table 2.

### *Metabolites in the blood:*

The findings of the study revealed a substantial reduction ( $P < 0.05$ ) in triglyceride (TG) levels among rabbits that were fed ZADO and YEAST, with decreases of 36.73% and 51.07% respectively, in

comparison to the control group Table 3. The present results showed a positive effect on the decrease of plasma cholesterol and TG from probiotic addition, indicating that supplementation of probiotics could play a role in the metabolism of rabbit. Few studies on the influences of enzyme supplementation on blood lipid metabolites in rabbit diets published. By supplementing enzymes, it can inhibit the merger of <sup>14</sup>C-labeled acetate to the non-saponifiable lipid fraction and therefore decrease the biosynthesis of lipid profiles and /or may have indirect inhibitory effects in the lipid biosynthesis enzyme hydroxymethylglutaryl coenzyme-A reductase levels (Fukushima and Nakano, 1995). This would indicate to the reduce the lipid profile of plasma, hence, aiding to reduce total cholesterol and TG depositins in the muscles and skin.

Furthermore, the rabbits treated with ZAD and ZADO exhibited a substantial rise in glucose concentration ( $P<0.05$ ). Specifically, the glucose concentration was increased by 28.77% and 35.56% in the ZAD and ZADO groups, respectively, as compared to the control group. In contrast, the probiotic treatments (ZAD, ZADO, and YEAST) exhibited a statistically significant increase ( $P<0.05$ ) in total antioxidant capacity (TAC) compared to the control group (Fig.1). In particular, the TAC was increased by 43.59% with the ZAD treatment, 76.92% with the ZADO treatment, and 56.41% with the YEAST treatment. Significant variations in total antioxidant capacity (TAC) levels were identified between the ZADO and ZAD groups, as depicted in Figure 1.

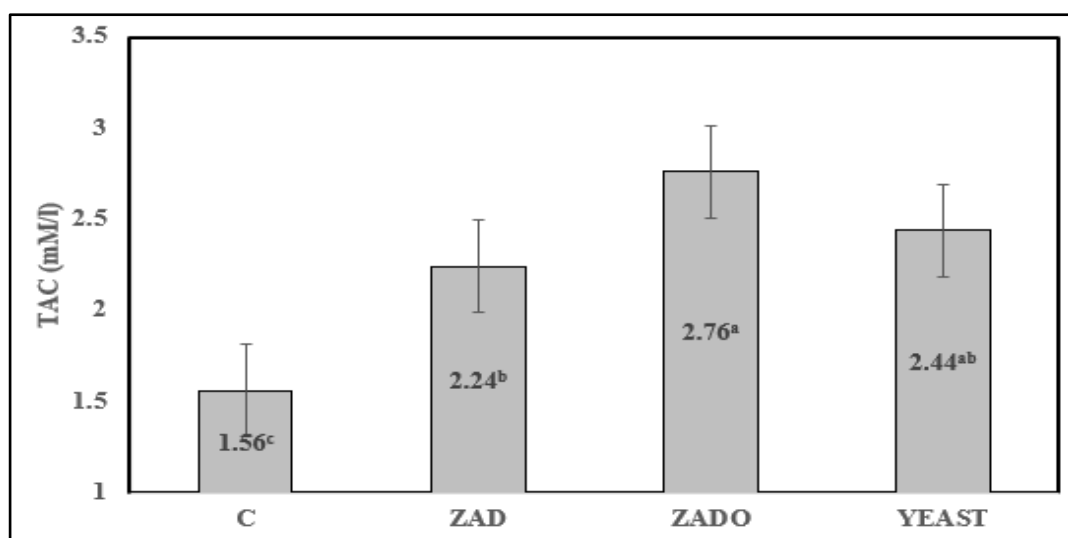
**Table (3): Effect of biological additives on blood metabolites parameters of Hi-Plus growing rabbits.**

Traits	Treatments				±SE	P value
	C	ZAD	ZADO	YEAST		
Cholesterol (mg/dl)	60.94 <sup>a</sup>	48.68 <sup>b</sup>	51.36 <sup>b</sup>	56.46 <sup>ab</sup>	4.16	0.057
TG (mol/l)	109.26 <sup>a</sup>	105.94 <sup>a</sup>	69.13 <sup>b</sup>	53.46 <sup>c</sup>	4.90	0.001
Glucose (mg/dl)	101.03 <sup>b</sup>	130.10 <sup>a</sup>	136.96 <sup>a</sup>	120.57 <sup>ab</sup>	9.29	0.047

C= control group, ZAD= rabbits received 10 ml ZAD/kg diet, ZADO= rabbits received 10 g ZADO/kg diet, YEAST= rabbits received 10 g YEAST/kg diet.

TG=tri-glycerides

<sup>a-b</sup> Means bearing different superscripts within the same row are significantly different ( $P<0.05$ ).



**Figure (1): Effect of biological additives on total antioxidant capacity of Hi-Plus growing rabbits.**

C= control group, ZAD= rabbits received 10 ml ZAD/kg diet, ZADO= rabbits received 10 g ZADO/kg diet, YEAST= rabbits received 10 g YEAST/kg diet.

TAC=total antioxidant capacity

<sup>a-b</sup> Means bearing different superscripts are significantly different ( $P<0.05$ ).

The present results showed a positive effect on the decrease of plasma cholesterol and TG from probiotic addition, indicating that supplementation of probiotics could play a role in the metabolism of rabbit. This

means the possibility of incorporating the probiotics which will lead to animal products with lower cholesterol Abdel-Azeem *et al.* (2018) reported that in rabbits received probiotic, The decline in rabbit serum cholesterol levels received probiotic is likely to indicate an overall decline in the mobilization of lipid (Abdel-Azeem *et al.*, 2018). It has been postulated that certain microbes, such as *Lactobacillus* and *Bifidobacterium*, may have a probiotic influence on lipid metabolism. This is due to their ability to exhibit activity in bile salt hydrolase and cholesterol assimilation, as well as their capacity to produce short-chain fatty acids. Similarly, the reduction in serum cholesterol levels observed in broiler chicks that were given a probiotic diet can be attributed to a decrease in the production and/or absorption of cholesterol in the gastrointestinal tract (Mohan *et al.*, 1995 and 1996 and Safaa, 2013).

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 and Abdel-Azeem *et al.*, 2018). Antioxidant enzymes can stop the oxidation either by scavenging the mainly reactive free radical in vivo or by steadying move metal radicals such as Cu<sup>+</sup> or Fe<sup>2+</sup> (Afolabi and Oloyede, 2014). The current results may reveal that TAC in blood plasma of rabbits fed biological probiotics treatments (ZAD<sup>®</sup>, ZADO<sup>®</sup> and YEAST) were significantly increased compared with control group indicating improved scavenging capacity of the antioxidant defense system against oxidative stress processes in these groups.

**Liver and kidney functions:**

Alanine aminotransferase (ALT) concentration was decreased (P<0.05) in the rabbits fed YEAST by 26.51 % compared to rabbits in control group Table 4.

**Table (4): Effect of biological additives on liver and kidney parameters of Hi-Plus growing rabbits.**

Traits	Treatments				±SE	P value
	C	ZAD	ZADO	YEAST		
ALT (i.u./l)	12.60 <sup>a</sup>	10.66 <sup>ab</sup>	10.73 <sup>ab</sup>	9.26 <sup>b</sup>	0.73	0.022
AST (i.u./l)	35.16 <sup>a</sup>	28.16 <sup>b</sup>	32.63 <sup>ab</sup>	30.23 <sup>ab</sup>	2.53	0.051
Creatinine (mg/dl)	0.74	0.75	0.73	0.76	0.03	0.906
Urea (mg/dl)	39.23	39.28	37.72	36.03	1.66	0.472

C= control group, ZAD= rabbits received 10 ml ZAD/kg diet, ZADO= rabbits received 10 g ZADO/kg diet, YEAST= rabbits received 10 g YEAST/kg diet.

ALT= alanine aminotransferase, AST= aspartate aminotransferase

<sup>a-b</sup> Means bearing different superscripts within the same rows are significantly different (P<0.05).

Nevertheless, the findings indicated that the concentration of ALT exhibited a statistically insignificant decline in rabbits fed on ZAD<sup>®</sup> and ZADO<sup>®</sup>, with reductions of 15.40% and 14.84% respectively, in comparison to the control group of rabbits. In contrast, there were no notable variations detected in AST, creatinine, and urea levels across the different therapy groups. The plasma levels of ALT, AST, creatinine, and urea in the present investigation are laying within the normal range (Ajayi and Raji 2012). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are frequently employed in the diagnosis of hepatic injury in domestic animals and are also used to identify biliary blockage (McGill, 2016). The absence of any alterations in blood metabolites among the biological treatments ZAD<sup>®</sup>, ZADO<sup>®</sup>, YEAST, and their conformity to the established normal levels for rabbits indicate the absence of liver injury.

The rabbits who were administered probiotics in this trial did not exhibit any clinical indicators of toxicity or morbidity. Liong and Shah (2005), Sudha *et al.* (2009), Ooi and Liong (2010), Abdelhady and El-Abasy (2015), and Sherif (2017) reported similar findings.

**Parameters of immunoglobulins:**

The rabbits fed on YEAST exhibited a substantial rise in plasma IgG and IgA levels (P<0.05), with a respective increase of 18.10% and 72.96% compared to the control group (Figures 2 and 3). Furthermore, the levels of IgG and IgM exhibited a statistically insignificant rise in the rabbits that were administered ZAD (by 9.92% and 56.38%, respectively) and ZADO (by 7.86% and 55.40%, respectively) as compared to the control group. In contrast, the rabbits that were administered probiotic treatments (ZAD, ZADO, and YEAST) exhibited a statistically significant rise in IgM levels. Specifically, the IgM level increased by 41.93%, 17.81%, and 44.49% for the ZAD, ZADO, and YEAST treatments, respectively, as compared to the control group of rabbits (Figure 4). There were no statistically significant variations were observed in the levels of IgG, IgA, and IgM among the groups who received probiotic treatments.

The inclusion of probiotics in the meals of rabbits resulted in an elevated concentration of immunoglobulins (namely IgG, IgA, and IgM) in their blood. This observation suggests that the probiotics may have an impact on protein metabolism, as seen by the increased body weights observed in the treated groups. The findings of the current study are consistent with those of Abdel-Khalek *et al.* (2012), who observed that the administration of probiotics had a positive impact on the growth performance of rabbits by boosting both feed efficiency and immunological response. According to Edens (2003), the significance of this stimulator is in the fact that it enables healthy animals to fully manifest their productive potential.

The utilization of probiotics has been associated with numerous potential advantages, including the alteration of host metabolism, stimulation of the immune system, initiation of anti-inflammatory responses, prevention and eradication of pathogens within the gastrointestinal tract, and improvement of nutritional absorption and overall performance. However, it reduced disease incidence and animal death, suggesting that it could improve disease resistance in rabbits Colombino *et al.* (2022).

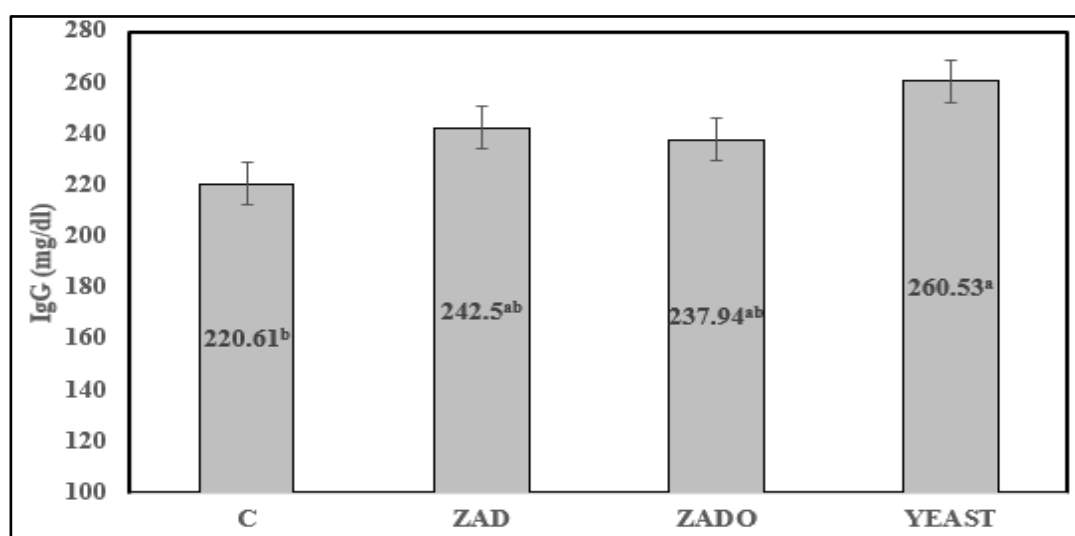


Figure (2): Effect of biological additives on immunoglobulins IgG of Hi-Plus growing rabbits.

C= control group, ZAD= rabbits received 10 ml ZAD/kg diet, ZADO= rabbits received 10 g ZADO/kg diet, YEAST= rabbits received 10 g YEAST/kg diet. <sup>a-b</sup> Means bearing different superscripts are significantly different ( $P < 0.05$ ).

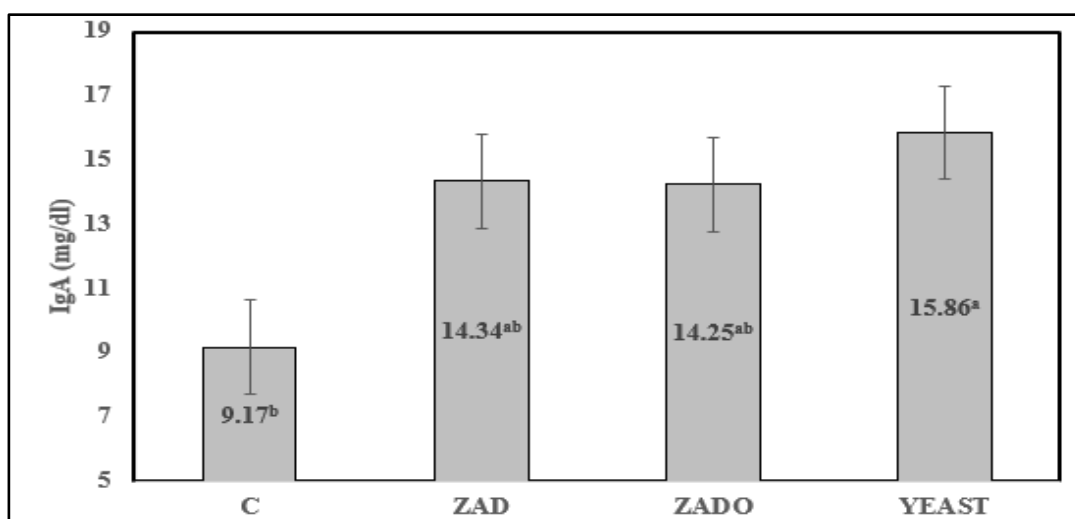
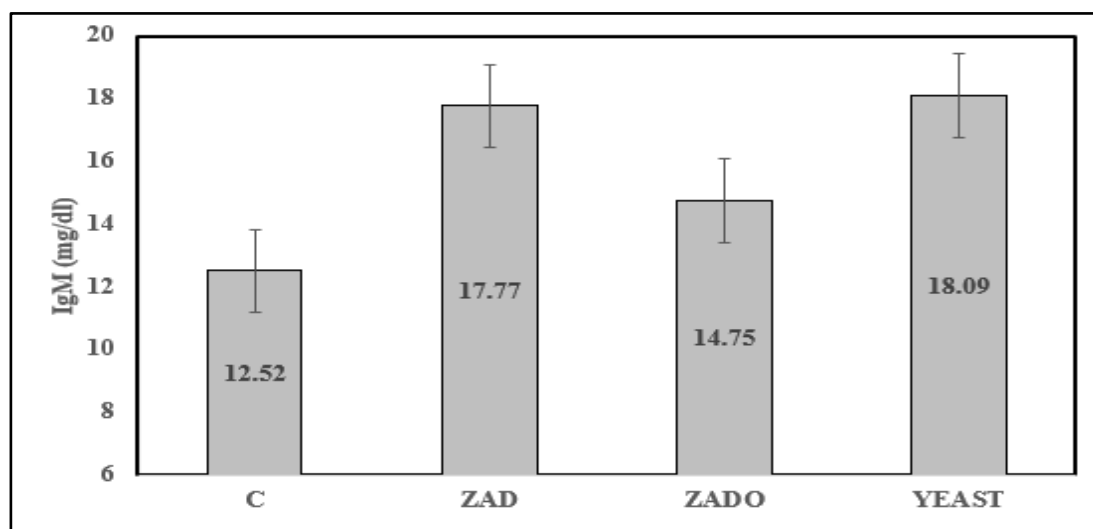


Figure (3): Effect of biological additives on immunoglobulins IgA of Hi-Plus growing rabbits.

C= control group, ZAD= rabbits received 10 ml ZAD/kg diet, ZADO= rabbits received 10 g ZADO/kg diet, YEAST= rabbits received 10 g YEAST/kg diet. <sup>a-b</sup> Means bearing different superscripts are significantly different ( $P < 0.05$ ).

Probiotics have been observed to elicit immune system stimulation through two mechanisms in rabbits. Firstly, the probiotic flora migrates across the gut wall and undergoes limited multiplication. Secondly, the immune system is stimulated by the absorption of antigens produced by the deceased probiotic organisms. The enhancement of the immune system can be categorized into three distinct methods. The immune response can be characterized by several key features. Firstly, there is an augmentation in macrophage activity, resulting in an improved capacity to engulf microorganisms or carbon particles. Secondly, there is an elevation in the production of antibodies, predominantly of the IgG and IgM classes, as well as interferon, which acts as a nonspecific antiviral agent. Lastly, there is an increase in the presence of antibodies at mucosal surfaces, specifically the gut wall (Havenaar and Spanhaak, 1994).



**Figure (4): Effect of biological additives on immunoglobulins IgM of Hi-Plus growing rabbits.**

C= control group, ZAD= rabbits received 10 ml ZAD/kg diet, ZADO= rabbits received 10 g ZADO/kg diet, YEAST= rabbits received 10 g YEAST/kg diet.

The inclusion of yeast in the diets of rabbits has been found to have several beneficial effects on their mucosal immunity. This includes an increase in the activity of immunoglobulins IgM and IgA against pathogens. Additionally, yeast supplementation has been shown to enhance the development and function of the intestines in rabbits. Yeast also has the ability to adsorb mycotoxins, which are harmful substances produced by certain fungi. Furthermore, yeast has been found to modulate the composition of the gut microbiota in rabbits. This modulation of the gut microbiota has been associated with a reduction in the occurrence of post-weaning diarrhea in rabbits (Elghandour *et al.*, 2019). According to the findings of Azoz and Al-Kholy (2006), the measurement of globulin levels in the blood has been identified as a reliable biomarker of the immune response.

## CONCLUSION

In summary, the addition of probiotics ZADO® and YEAST® at a concentration of 1% to the meals of Hi-Plus rabbits during their growth phase may have beneficial effects on blood constituents and immunological condition.

## REFERENCES

Abdel-Azeem A. S., Hassan A. A., Basyony .M. M. and Salma H. Abu Hafsa. (2018). Rabbit growth, carcass characteristic, digestion, caecal fermentation, microflora, and some blood biochemical



- components affected by oral administration of anaerobic probiotic (zad). *Egyptian J. Nutrition and Feeds*, 21 (3): 693 - 710. Doi: [10.21608/EJNF.2018.75774](https://doi.org/10.21608/EJNF.2018.75774)
- Abdelhady D. H. and El-Abasy M. A. (2015). Effect of prebiotic and probiotic on growth, immunohematological responses and biochemical parameters of infected rabbits with *Pasteurella multocida*. *Benha Vet. Med. J.*, 28 (2): 40 - 51, Conference Issue.
- Abdel-Khalek A. E., Abdelhamid A. M., Mehrez A. F. and El-Sawy I. (2012). Growth performance, digestibility coefficients, blood parameters and carcass traits of rabbits fed biologically treated diets. *J. Animal and Poultry Prod.*, Vol. (5): 227 - 239. DOI: [10.21608/JAPPMU.2012.82790](https://doi.org/10.21608/JAPPMU.2012.82790)
- Afolabi A. B. and Oloyede O. I. (2014). Antioxidant properties of the extracts of *Talinum triangulare* and its effect on antioxidant enzymes in tissue homogenate of Swiss albino rat. *Toxicol Int.*, 21 (3): 307 - 313. doi: [10.4103/0971-6580.155377](https://doi.org/10.4103/0971-6580.155377)
- Ajayi A. F. and Raji Y. (2012). Hematological and serum biochemical indices of pre-pubertal male rabbits fed with graded level of blood-wild sunflower forage meal mixture. *Afri. J. Biot.*, 11 (35): 8730 - 8734. DOI: [10.5897/AJB10.2169](https://doi.org/10.5897/AJB10.2169)
- Azoz A.A. and Al-Kholy K. H. (2006). Reproductive performance and blood constituents of V-line and Bauscat female rabbits under middle Egypt conditions. *Egyptian Journal of Rabbit Science* 16: 139 - 160.
- Bhatt R. S., Agrawal A. R. and Sahoo A. (2017). Effect of probiotic supplementation on growth performance, nutrient utilization and carcass characteristics of growing Chinchilla rabbits. *Journal of Applied Animal Research*, 45 (1): 304 - 309. <https://doi.org/10.1080/09712119.2016.1174126>
- Colombino E., Biasato I., Michetti A., Rubino M. G., Franciosa I., Giribaldi M. and Capucchio M. T. (2022). Effects of Dietary Supplementation of *Lactobacillus acidophilus* on Blood Parameters and Gut Health of Rabbits. *Animals*, 12 (24): 3543. <https://doi.org/10.3390/ani12243543>
- Duncan D. B. (1955). Multiple Range and Multiple F-Test. *Biometrics*, 11: 1 - 5. <https://doi.org/10.2307/3001478>
- Edens F. W. (2003). An alternative for antibiotic use in poultry: probiotics. *Nutritional Abstract*, 74: 628 - 632. <https://doi.org/10.1590/S1516-635X2003000200001>
- Elghandour M. M. Y, Tan Z. L, Abu Hafsa S. H., Adegbeye M. J., Greiner R., Ugbogu E. A. f, Cedillo Monroy J. and Salem A. Z.M. (2019). *Saccharomyces cerevisiae* as a probiotic feed additive to none and pseudo-ruminant feeding. *Journal of Applied Microbiology*, Vol. 3: 1364 - 5072. <https://doi.org/10.1111/jam.14416>
- El-Sanhoury M. H. S. and Ahmed A. M. H. (2017). Broiler performance, enzymes activity and histological observations affected by multi enzymes complex (ZADO®). *Egyptian J. Nutrition and Feeds*, 20 (2): 251 - 262.
- European Union. (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* L276/33
- Ezema C. and Eze D. C. (2010). Determination of the effect of probiotic (*Saccharomyces cerevisiae*) on growth performance and hematological parameters of rabbits. *Comparative and Clinical Pathology*, 21: 73 - 76. <https://doi.org/10.1007/s00580-010-1066-6>
- Fathi M., Abdelsalam M., AL-Homidan I., Ebeid T., EL-Zarei M. and Abou-Emera O. (2017). Effect of probiotic supplementation and genotype on growth performance, carcass traits, hematological parameters and immunity of growing rabbits under hot environmental conditions. *Animal Science Journal*, 88: 1644 - 1650. <https://doi.org/10.1111/asj.12811>
- Fukushima M. and Nakano M. (1995). The effect of probiotic on faecal and liver lipid classes in rats. *British journal of nutrition*, 73 (5): 701 - 710. <https://doi.org/10.1079/BJN19950074>
- Gado H. M., Khusro A. and Salem A. Z. M. (2017). Role of probiotics in animal nutrition. *Anim. Rev.*, 4 (1): 8 - 20. DOI: 10.18488/journal.ar.2017.41.8.20.
- Gao F., Jiang Y., Zhou G.H. and Han Z. K. (2007). The effects of xylanase supplementation on growth, digestion, metabolite circulating hormone and levels, immunity and gut microflora in cockerels fed on wheat-based diets. *Br. Poult. Sci.*, 48: 480 - 488. <https://doi.org/10.1080/00071660701477320>

- Havenaar R. and Spanhaak S. (1994). Probiotics from an immunological point of view. *Current Opinion in Biotechnology*, 5 (3): 320 - 325. [https://doi.org/10.1016/0958-1669\(94\)90036-1](https://doi.org/10.1016/0958-1669(94)90036-1)
- Hosam M.S. (2013). Influence of Dietary Enzymes Prepared at Ensiling (ZADO®) from Hatch to 42 Days of Age on Productivity, Slaughter Traits and Blood Constituents in Broiler Chickens. *International Journal of Poultry Science*, 12 (9): 529 - 537.
- Kambayashi Y., Binh N. T., Asakura H. W., Hibino Y., Hitomi Y., Nakamura H. and Ogino K. (2009). Efficient assay for total antioxidant capacity in human plasma using a 96- well microplate. *J. Clin. Biochem. Nut.*, 44: 46 - 51. DOI:<https://doi.org/10.3164/jcfn.08-162>
- Liong M. T. and Shah N. P. (2005). Acid and bile tolerance and cholesterol removal ability of Lactobacilli strains. *J. Dairy Sci.*, 88: 55 - 66. [https://doi.org/10.3168/jds.S0022-0302\(05\)72662-X](https://doi.org/10.3168/jds.S0022-0302(05)72662-X)
- Makled M. N., Metwally M. A., Soliman I. A. and Younis H. A. (2005). Impact of different levels of dietary multi-enzymes supplement on California rabbit performance. 3<sup>rd</sup> International Poultry Conference, Apr. 4 - 7, 2005, Hurgada, Egypt.
- McGill M. R. (2016). The past and present of serum aminotransferases and the future of liver injury biomarkers. *Excli J.*, 15: 817 - 828 doi: [10.17179/excli2016-800](https://doi.org/10.17179/excli2016-800)
- Mohan B., Kadirvel R., Bhaskaran M. and Natarajan A. (1995). Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *Br. Poult. Sci.*, 36 (5): 799 - 803. <https://doi.org/10.1080/00071669508417824>
- Mohan B., Kadirvel R., Natarajan A. and Bhaskaran M. (1996). Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. *Br. Poult. Sci.*, 37 (2): 395 - 401. <https://doi.org/10.1080/00071669608417870>
- NRC (1994). *Nutrient Requirements of Poultry*. National Research Council, National Academy Press, Washington, D. C., 9th Revised Edition. Pp. 234
- Ooi L. G. and Liong M. T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings. *Int. J. Mol. Sci.*, 11 (6): 2499 - 2522. <https://doi.org/10.3390/ijms11062499>
- Safaa H. M. (2013). Influence of dietary enzymes prepared at ensiling (ZADO®) from hatch to 42 days of age on productivity, slaughter traits and blood Constituents in broiler chickens. *Int. J. Poult. Sci.*, 12 (9): 529 - 537
- SAS (2004). *User's Guide: Statics*. Release 9.1. SAS Institute Inc., Cary, NC.2004.
- Sherif S. K. (2017). Effect of dietary additives on rabbit performance, carcass traits and some blood constituents under Egyptation summer season. *Journal of Agricultural Science*, 10 (1) 1: 39. <https://doi.org/10.5539/jas.v10n1p139>
- Sudha M. R., Chauhan P., Dixit K., Babu S. and Jamil K. (2009). Probiotics as complementary therapy for hypercholesterolemia. *Biol. Med.*, 1 (4): 1 - 13, Rev. 4.

## تأثير إضافة البروبيوتيك إلى علائق الأرانب النامية على مكونات الدم والاستجابة المناعية

ماريز ميلاد منير<sup>1</sup>، هانى محمود جادو<sup>1</sup>، أحمد صبحي الحاوى<sup>2</sup> و حمدي موسى متولى<sup>1</sup>

<sup>1</sup> قسم الإنتاج الحيوانى - كلية الزراعة - جامعة عين شمس - القاهرة - مصر

<sup>2</sup> مركز بحوث الصحراء - وزارة الزراعة - المطرية - القاهرة - مصر

الهدف من الدراسة تحديد تأثير البروبيوتيك كإضافات غذائية علي قياسات الدم و المناعة في الارانب النامية من سلالة Hi-Plus تحت الظروف البيئية في شمال سيناء. تمت التجربة علي عدد 48 ارنب بعد الفطام عند عمر 5 اسابيع و متوسط وزن  $20.46 \pm 811$  جم . تم تقسيمهم الي اربع مجموعات متشابهة الاوزان كل مجموعة تحتوي 12 ارنب . كل معاملة تم تقسيمها الي ثلاث تكرارات تحتوي على اربعة ارناب.

المجموعة الاولى كانت تتغذى على العليقة فقط كمجموعة كنترول . و تمت الاضافة في المجموعات الثانية و الثالثة و الرابعة على العليقة بمعدل 10 مل / كجم علف زاد ، 10 جم / كجم علف زادو و 10جم/كجم علف خميرة بالتوالي. و كانت اهم النتائج ان مجموعات الارانب التي تغذت علي البروبيوتك اظهرت فروقا معنوية ( $P<0.05$ ) في بروتين بلازما الدم و الجلوبيولين عند مقارنته بالكنترول .

مجموعة الارانب التي تغذت على الزاد و الخميرة اظهرت فروقا معنوية ( $P<0.05$ ) في مستويات الجلوسريدات الثلاثية مقارنة بالكنترول . اضافة البروبيوتك اظهرت فروقا معنوية في مستويات مضادات الاكسدة مقارنة بالكنترول . مستويات تحليل الكبد (ALT) . انخفضت معنويا في الارانب التي تغذت علي الخميرة مقارنة بالكنترول , بينما ارتفعت مستويات تحاليل المناعة IgA و IgG فى نفس المجموعة مقارنة بالكنترول .

وملخص البحث يشير إلى أن إضافة بروبيوتك الزادو و الخميرة بمستويات 1% من العليقة المركزة لأرانب هاى بلس بعد مرحلة الفطام تحسن من خصائص الدم و المناعة فى الأرانب.