

IMPACT OF PROBIOTIC (*Lactobacillus planterium* L) SUPPLEMENTATION ON PRODUCTIVE AND PHYSIOLOGICAL PERFORMANCE OF GROWING RABBITS UNDER EGYPTIAN CONDITIONS

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ABSTRACT

*The aim of this work was to study the effect of probiotic (*Lactobacillus planterium*) supplementation in diet on productive and physiological performance, intestinal microbial examination, histomorphology and economic efficiency of growing rabbits. A total of 36 four weeks old White New Zealand (NZW) rabbits were randomly divided into 3 groups with 3 replicates (4 rabbit in each). The first group was fed a basal diet as a control group according to NRC (1977). The 2nd and 3rd groups were fed on basal diet supplemented with 0.25 and 0.5 g of probiotic / kg diet (*Lactobacillus planterium* 1×10^6 CFU/g), respectively during the experimental period (4-12 weeks of age).*

*The Results revealed that by supplementation of rabbit diets with probiotic (*Lactobacillus planterium*) has positive effects ($P \leq 0.05$) on both body weight and feed conversion ratio. Moreover, the results indicated that there was significant ($P \leq 0.05$) increase in serum total protein, serum albumin and serum globulin of the treated groups as compared to the control group.*

*Addition of probiotic to rabbit diets caused to improve production of mucus, beneficial microflora count and clearly decreased *Escherichia coli* count in intestine when compared with control group.*

***Conclusively**, it can be concluded that probiotic (*Lactobacillus Plantarium*) at the tested levels can be applied in rabbit rations to improve the productive and physiological performance, as well as, gut health under Egyptian environmental conditions.*

Key words: Growing rabbits, Probiotic levels, Productive & Physiological Performance.

Rabbit is considered as superior to the other livestock because it is one of the fast growing and its high prolific breeds. It has a high production capacity and high feed conversion efficiency when compared to the other meat animals. Rabbit meat is one of the most delicious, low in fat and cholesterol (4%), easy digestible, high in protein content (25%) and low in caloric value (160 Kcal/100g meat) (Risam *et al.*, 2005). Therefore, farms of rabbits play a very important role in the national economics of European and North African countries (Ayyat *et al.*, 2018).

The mode of action of probiotics may be enteric diseases of rabbits can be prevented by probiotics that contain yeast, live bacteria or bacterial spores. Although, the mechanism of useful probiotic has not been elucidated, it might include reduction of toxin production, stimulation of enzyme production by the host, production of some vitamins or antimicrobial substances, competition for adhesion to epithelial cells, increase resistance to colonization, stimulation of the immune system of the host and reduction of stress in rabbits (Shehata and Tawfeek., 2010).

The mode of action of probiotics may be by decreasing population of harmful bacteria by decreasing intestinal pH (Makled, 1991). Many other beneficial effects of probiotics were suggested, such as reduced inflammatory reactions, decreased ammonia and urea excretion, lower serum cholesterol and improved mineral absorption. Also, probiotics may have an indirect positive impact on performance parameters and production profitability (Ferreira *et al.*, 2011 and Hutkins, *et al.*, 2016).

Therefore, this study aimed to investigate the effect of probiotic supplementation to rabbit diets on the productive and physiological performance, during the experimental period (4-12 weeks of age) under Egyptian environmental conditions.

MATERIALS AND METHODS

A total number of thirty six, four weeks old male White New Zealand weaned male rabbits were obtained from Poultry Experimental Station, Faculty of Agriculture, Al-Azhar University, Naser City, Cairo, Egypt from December 2017 till January 2018. Rabbits were randomly selected and distributed into three groups each group contained 12 rabbit in 3 replicates (4 rabbits in each). The average initial live body weights of different groups

were nearly similar at 28-30 day of age average weight was 548g. Animals were kept in pyramid shape cages in opened house system and the same managerial environmental and hygienic conditions.

The experiment was designed to evaluate the impact of probiotic (*Lactobacillus planterium*) on body weight (g), total body weight gain (g), total feed intake (g), feed conversion ratio (g feed /g gain), mortality rate (%), carcass characteristics, some blood parameters, intestinal bacteria contents and histomorphological examination of duodenum and cecum, as well as, economical efficacy of New Zealand White male rabbits, during the experimental period (4-12 weeks of age) under Egyptian environmental Conditions.

Experimental treatments and diets

The experiment design was consisted of three dietary treatments as follows:

T1: A basal diet (No probiotic supplementation) according to NRC (1977).

T2: A basal diet supplemented with a probiotic (*Lactobacillus plantarum*) at a level of 0.25 g (1×10^6 CFU/g) /kg diet.

T3: A basal diet supplemented with a probiotic (*Lactobacillus plantarum*) at a level of 0.5 g (1×10^6 CFU/g) /kg diet.

The experimental diets were formulated to contain 18.3% crud protein and 2462 Kcal DE/Kg feed according to NRC (1977). The composition of the basal growing diet is presented in (Table 1).

Blood sampling and biochemical analysis in serum:

Blood samples were withdrawn from two rabbits within each replicate at the end of experiment (12 weeks of age) from ear vein. The blood samples were collected into dry clean centrifuge tubes without anticoagulants and centrifuged. Serum samples were stored in the deep freezer at approximately $-20^{\circ}\text{C} \pm 1$ until used to analyze.

The bio-analysis of serum was carried out for quantitative determination of blood parameters by spectrophotometer. Concentration of IgG in serum was measured according to (Fahey and Mckelvey, 1965). Total protein was determined according to (Henry *et al.*, 1974). Albumin was determined according to (Dumas and Biggs, 1972). Globulin was calculated by subtraction of serum total protein and serum albumin. A/G ratio was

Table (1): Composition and chemical analysis of the basal diet.

Ingredients	Quantity (Kg)
Soybean meal (44%, CP)	17.50
Alfalfa hay (17 %, CP)	30.05
Barley grains	24.60
Wheat bran	21.50
Molasses	3.00
Limestone	0.95
Dicalcium phosphate	1.60
Sodium chloride	0.30
Mineral-Vitamin Premix*	0.30
DL-Methionine	0.20
Total (Kg)	100
Calculated analysis (NRC, 1977)	
Crude protein (%)	18.30
Ether extract (%)	2.26
Crude fiber (%)	13.40
Calcium (%)	1.18
Total Phosphorous (%)	0.83
Lysine (%)	0.90
Methionine (%)	0.46
Methionine + Cystine (%)	0.76
Digestible energy (kcal/kg feed)	2462

*Each 3 kilogram of mineral–vitamin premix contains: Vitamin A, 10000000IU; Vitamin D3, 9000000IU; Vitamin E, 50000 mg; Vitamin K3, 2000mg; Vitamin B1, 2000 mg; Vitamin B2, 4000mg; Vitamin B6, 2000mg; Pantothenic acid, 10000 mg; Vitamin B12, 10mg; Niacin, 50000 mg; Folic acid, 3000mg; Biotin, 100mg; Choline chloride 250000mg; Fe, 50000mg; Mn, 8500 mg; Cu, 5000 mg; Co, 100 mg; Se, 200mg; and Zn, 50000mg.

calculated by dividing the value of albumin on the value of globulin. Alanine and aspartate amino transaminase (ALT and AST) activities were determined according to (Retiman and Frangle, 1957). Serum creatinine and urea were measured according to (Husdan and Rapoport, 1968). Total lipids, triglycerides and total cholesterol were determined according to (Zollner and Kirsch, 1962). High density lipoprotein (HDL) was determined according to (Siedel, 1983). The serum low density lipoprotein cholesterol and very low density lipoprotein cholesterol were estimated Friedewald equation (Low

density lipoprotein cholesterol = Total cholesterol – High density lipoprotein cholesterol – (triglycerides/5), VLDL = (triglycerides/5) (Friedewald *et al.*, 1972). Serum glucose was determined according to (Doumas *et al.*, 1971). Concentrations of total T3 and T4 were analyzed by Radioimmunoassay (RIA) method using RIA kits (Amersham International Ltd., Amersham, United Kingdom).

Microbial populations:

Cecum microbial samples were collected from two rabbits per replicate at the end of experiment (12 weeks of age). The mentioned organ was removed and placed in a sterile sample bag and put in an ice box. Then the samples were kept at (-20°C), Lactic acid bacteria, *Enterococci*, *E. Coli*, *Salmonella*, *Clostridia* and *Listeria* bacteria were counted as following: Content of cecum samples were then diluted serially from 10⁻¹ to 10⁻⁷. One-tenth milliliter of each diluted sample was immersed on the appropriate agar media, in duplicate for enumeration of the selected microbial populations. Bacterial counts were performed using the appropriate dilution and plate culture techniques under aerobic or anaerobic conditions according to Rada *et al.*, (1999).

Histomorphological examination:

At the end of experiment target organs were collected from 6 rabbits of each group for histopathological examination. Specimens were collected from the duodenum and cecum of the sacrificed rabbits and directly fixed in 10% neutral buffered formalin. Five micron paraffin sections were prepared, stained with hematoxylin and eosin according to Bancroft and Gamble, (2008) and examined microscopically.

Economic efficiency (%):

The economic efficiency (EEF) was calculated according to the following equation: $EEF = \text{Net revenue} / \text{Total costs}$.

Where: Total average weight(g) x Price of 1 kg live body weight (42 LE) = Selling price/rabbit (LE) as (A), Total feed cost/rabbit (LE) = Total feed intake x, Price/kg feed (LE) = (B), (1) Net revenue = A – B, Economic efficiency = (A-B/B) and Relative Economic Efficiency (%) = Economic efficiency of treatments other than the control/ Economic efficiency of the control group.

Statistical analysis:

Data were subjected to analysis of variance using the General Linear Models procedure of SPSS software program package (SPSS, 2001). Data were analyzed by one way method using the following model.

$$Y_{ijk} = u + N_i + e_{ijk}$$

Where Y_{ijk} = Any observation value, u = Overall means, N_i = Effect of treatment groups ($i=1,2$ and 3), e_{ijk} = the standard error.

All percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA. Also, significant differences among means were determined by Duncan's multiple range test (Duncan, 1955) at 5% level of significant.

RESULTS AND DISCUSSION:***Effect of probiotic supplementation levels in growing rabbit diets on:******1. Some productive performance***

The effects of probiotic supplementation in growing rabbit diets on body weight, final body weight gain, total feed intake, feed conversion ratio and mortality rate during the experimental period (4-12 weeks of age) are presented in Table (2). In general, the differences of body weight and body weight gain among the experimental groups were significantly ($P \leq 0.05$) increased with increasing probiotic level in the diet. However, supplementation of rabbit diets with probiotic led to clear significant increase in the body weights of treated groups compared to control group. These results revealed that the dietary treatments had significant effect on the mentioned parameters. Whilst the group fed 500 mg probiotic /kg diet showed significant ($P \leq 0.05$) increase in the body weight and body weight gain compared to the control group and group fed 0.25 mg probiotic/kg diet.

Concerning feed intake (FI), the group fed 500 mg probiotics/kg diet was significantly ($P \leq 0.05$) lower in FI compared to the other groups. The obtained results showed that the group fed 500 mg probiotic /kg diet was significantly ($P \leq 0.05$) improved in feed conversion ratio (FCR) compared to control group. In spite of, there were no significant effect in FCR between group T2 and T3.

The improvement noticed in the above parameters may be due to that probiotic lowering the intestinal pH and this could be a suitable environment

Table (2): Effect of different levels of probiotic on initial body weight, final body weight, body weight gain, total feed intake, feed conversion ratio and mortality rate of growing rabbits during the whole period of experiment (4-12 weeks of age).

Treatments	Initial body weight (g)	Final body weight (g)	Total Body weight gain (g)	Total feed intake (g)	Feed conversion ratio	Mortality rate (%)
T1 (Control)	548.58 ^{1A} ±1.00	1666.60 ^C ±5.97	1118.02 ^C ±5.80	3960.70 ^A ±5.21	3.54 ^A ±0.03	Nil
T2 (250 mg/kg diet)	548.42 ^A ±1.10	1762.80 ^B ±8.95	1214.40 ^B ±8.56	3926.70 ^{AB} ±12.02	3.23 ^B ±0.05	Nil
T3 (500 mg/kg diet)	549.75 ^A ±1.73	1783.50 ^A ±4.01	1233.80 ^A ±4.82	3895.70 ^B ±12.33	3.16 ^B ±0.02	Nil

Mean ± Standard error.

A,B,C Means having different letter exponents among rows are significantly different ($P \leq 0.05$).

for working of lactic acid bacteria as reported by (De Keersmaecker *et al.*, 2006). Furthermore, these results may refer to the fact that probiotics have ability to improve the microbial ecology of the intestine, reduce passage rate of the digesta and improve the digestibility of amino acids (Biggs and Parsons, 2007).

In addition, the changes in mucosal architecture, changes in gut environment have been reported to be due to the supplementation of probiotics (Yang and Choct, 2007; Houshmand *et al.*, 2012). The beneficial effect of probiotic supplementation to rabbit diets in terms of improved FCR and body weight gain may be due to better nutrient digestion, nitrogen retention and absorption, as well as, reduction of gastrointestinal pH which led to a reduction of pathogenic microbes and thus improved the efficiency of feed utilization by rabbits (Phuoc and Jamikorn, 2017; Sherif, 2018). The action mechanism of probiotics which led to improve the general performance of animals may refer to many sites such as reduction of toxin production, stimulation of enzyme production by the host, production of some

vitamins or antimicrobial substances, competition for adhesion to epithelial cells and increased resistance to colonization, stimulation of the immune system of the host and reduction of rabbits stress (Falcão-e-Cunha *et al.*, 2010). These results are agreed with (Amber *et al.*, 2014; El-Sagheer and Hassanein, 2014) who reported that when rabbits fed basal diet supplemented with probiotic alone or mixture of probiotic and prebiotics the body weight and FCR were significantly improved than those of the control group.

2. Physiological performance:

2.1. Serum total protein, albumin, globulin, A/G ratio and immunoglobulin type G

Effect of probiotics supplementation in rabbit diets on serum total protein (STP), serum albumin, serum globulin and serum immunoglobulin type G (SIgG) and A/G ratio are shown in Table 3.

Results of STP, serum albumin, serum globulin and SIgG suggested that there were insignificant numerical increase in STP, serum albumin, serum globulin and SIgG of treated groups compared with the control group. These results are in agreement with those obtained by (Chunyang *et al.*, 2012 and Abd El-Hady *et al.*, 2013) found that the values of serum total protein, albumin, globulin and serum SIgG in growing rabbits fed on diet supplemented with probiotic were insignificant in all groups. Parameters measured in this study related to immunity such as globulin and SIgG were higher in the groups supplemented with probiotic compared with control group. Increase concentration of serum total protein, serum albumin, serum globulin and SIgG in the rabbit by inclusion of probiotic in the rabbit diets indicated that the probiotic has affected protein metabolism, which is harmonious with the enhancement observed in the body weights of treated groups but the enhancement was not significant. The enhancement noticed in above parameters of this study may be due to that the probiotics have the potential to affect the balance of gut flora and influence the pathogenesis of diseases, which occur in tissues removed from the intestinal tract (Berg, 1983). Increased of SIgG in the probiotic groups could be due to the persistent of probiotic bacteria in the intestinal tract and acting as immunological enhancer to the immune system and therefore stimulating IgG production (Naqid *et al.*, 2015).

On the other hand, improvements in immunity parameters (globulin and IgG) observed in the supplemented treatments with probiotic in the present work might be related to the lack of *Lactobacilli* count increases in the gastrointestinal

Table (3): Shows the effect of different levels of probiotic in rabbit diets on some serum constituents of growing NZW rabbits at 12 weeks of age.

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	IgG (mg/dl)
T1 (Control)	5.93 ^{1A} ±0.10	3.08 ^A ±0.06	2.85 ^A ±0.04	1.08 ^A ±0.03	52.50 ^A ±2.77
T2 (250 mg/kg diet)	6.17 ^A ±0.16	3.17 ^A ±0.08	3.00 ^A ±0.10	1.06 ^A ±0.03	58.33 ^A ±2.46
T3 (500 mg/kg diet)	6.30 ^A ±0.18	3.18 ^A ±0.08	3.12 ^A ±0.10	1.02 ^A ±0.01	58.01 ^A ±2.21

¹Mean ± standard error.

^{A,B} Means having different letter exponents among rows are significantly different ($P \leq 0.05$).

tract (Tables 3 and 7), since those bacteria have been reported to have beneficial effects on the host's immune system (Xu *et al.*, 2003). The improvement in immune response can be explanation through several tracks; a) the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes, b) the direct effect might be related to stimulate the lymphatic tissue, c) whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract (Abdelhady and El-Abasy 2015).

2.2. Serum glucose, triiodothyronine and thyroxin:

Effect of probiotic supplementation in rabbit diets on serum glucose, triiodothyronine (T3) and thyroxin (T4) are given in Table (4). Results of glucose, T3 and T4 showed that there were significant ($P \leq 0.05$) increase in treated groups in the above parameters compared with the control group. The changes in glucose values could be due to the lower digestibility of ether extract (EE) in these diets as reported by (Palermo *et al.*, 2011; Amber *et al.*, 2014). The positive effect of probiotic on glucose, albumin, T3 and T4 values in the present study could be explained by a higher absorptive capacity of the intestinal mucosa due to histo-morphological changes (Awad *et al.*, 2009;

Table (4): Displays the effect of different levels of probiotic in rabbit diets on serum glucose, triiodothyronine (T3), and thyroxin (T4) of growing NZW rabbits at 12 weeks of age.

Treatments	Glucose (mg/dl)	T3 (ng/ml)	T4 (ng/ml)
T1 (Control)	107.33^{1B} ±0.67	1.25^B ±0.03	4.81^B ±0.17
T2 (250 mg/kg diet)	111.17^A ±0.79	1.76^A ±0.12	5.78^A ±0.15
T3 (500 mg/kg diet)	112.50^A ±0.76	1.77^A ±0.12	6.13^A ±0.10

¹Mean ± standard error.

^{A,B} Means having different letter exponents among rows are significantly different ($P \leq 0.05$).

Aliakbarpour *et al.*, 2012) and/or a more effective digestion of the diet due to a higher intestinal enzyme activity (Mountzouris *et al.*, 2007; Wang and Gu, 2010), thus increasing the nutrients available to the animals.

2.3. Liver and kidney functions:

Effect of probiotic supplementation in rabbit diets on serum constituents Alanine and aspartate amino transaminase (ALT, AST), creatinine and urea are offered in Table (5). Results of ALT, AST and creatinine showed that there were significant ($P \leq 0.05$) gradually decrease in treated groups compared with the control group. The ALT, AST and creatinine values were decreased with increasing of probiotic level in rabbit diets. Regarding to creatinine the results demonstrated that group of T3 was significantly ($P \leq 0.05$) the lowest in creatinine compared to the control group.

On the other hand, the results of urea did not show any significant differences ($P \leq 0.05$) between all experimental groups due to addition of probiotic in rabbit diets. However, there was a tiny decrease in urea with increasing probiotic in diet without significant. The decreasing noticed in

Table (5): Presents the effect of different levels of probiotic in rabbit diets on liver and kidney functions of growing NZW rabbits at 12 weeks of age.

Treatments	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	Urea (mg/dl)
T1 (Control)	74.33 ^{1A} ±3.72	50.83 ^A ±1.85	0.76 ^A ±0.01	34.61 ^A ±0.14
T2 (250 mg/kg diet)	62.83 ^B ±3.84	47.67 ^A ±1.00	0.75 ^{AB} ±0.01	34.40 ^A ±0.32
T3 (500 mg/kg diet)	50.33 ^C ±1.76	41.50 ^B ±1.00	0.73 ^B ±0.01	34.21 ^A ±0.28

¹Mean ± standard error.

^{A,B,C} Means having different letter exponents among rows are significantly different ($P \leq 0.05$).

ALT and AST activities in the present study agrees with those observations by (Kopp-Hoolihan, 2001 and Praveen *et al.*, 2009) found that probiotic, prebiotic and symbiotic supplementation resulted in decreased bacterial translocation in the liver of mice challenged with *Salmonella typhimurium* and decreased levels of serum aminotransferases (ALT and AST), suggesting the protection role against *Salmonella* infection.

Regarding to creatinine, the results demonstrated that group of T3 was significantly ($P \leq 0.05$) the lowest in creatinine compared to the control group which mean that the probiotic in rabbit diets may be improves kidney function by indirect way.

2.4. Lipids profile:

Effect of probiotic supplementation in rabbit diets on serum total lipids, triglyceride, total cholesterol, LDL and VLDL are given in Table (6). Results of mentioned measurements suggested that there were significant ($P \leq 0.05$) decrease in treated groups compared with the control group. While the group fed 500 mg probiotic /kg diet showed significant reduction in total lipids, triglyceride, and total cholesterol, LDL and VLDL compared to the control group, while HDL value was significantly ($P \leq 0.05$) increased.

Table (6): Effect of different levels of probiotic in diet on total lipids, triglyceride, and total cholesterol, HDL, LDL and VLDL of growing NZW rabbits at 12 weeks of age.

Treatments	Total lipids (mg/dl)	Triglyceride (TAG) (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	254.00 ^{1A} ±1.71	65.17 ^A ±1.35	67.83 ^A ±0.98	30.33 ^C ±0.42	24.47 ^A ±1.01	13.03 ^A ±0.27
250 mg/kg diet	239.17 ^B ±0.87	57.00 ^B ±0.63	61.17 ^B ±0.75	35.17 ^B ±0.31	14.60 ^B ±0.71	11.40 ^B ±0.13
500 mg/kg diet	239.50 ^B ±1.15	56.83 ^B ±0.61	61.67 ^B ±0.92	38.33 ^A ±0.57	11.97 ^B ±1.20	11.37 ^B ±0.12

¹Mean ± standard error.^{A, B} Means having different letter exponents among rows are significantly different (P≤0.05).

The mechanisms involved for improving lipid profile particularly cholesterol and its fractions are not fully understood, it could be that some bacterial probiotic strains are able to incorporate cholesterol into the bacterial cells, hydrolyze bile salts or inhibit hydroxyl methylglutaryl-CoA, the rate limiting enzyme of cholesterologenesis, thus reducing cholesterol in the body pool (Kalavathy *et al.*, 2003). The decreasing found in LDL level may be due to total cholesterol, HDL-c and triacylglycerol significantly reduced the levels of VLDL, LDL in addition to having increased HDL as reported by (Pereira *et al.*, 2013 and Tarek *et al.*, 2017). While, the increasing in HDL levels could be due to the types of microorganisms in the probiotic used. Each strain may have a shared role in the hypocholesterolemic effect and lowering action of triacylglycerol as reported by (Ghoneim and Moselhy, 2012). The effect of probiotic microorganism on lipid metabolism can be explain as: posing bile salt hydrolase activity and precipitation of cholesterol by some microorganisms such as Lactobacillus and Bifidobacterium, incorporation of cholesterol or binding to bacteria and making of short-chain fatty acids by probiotic bacteria (Liong and Shah, 2005; Sudha *et al.*, 2009; Ooi and Liang,

2010). In addition to that, the mechanism by which a probiotic can lower the serum cholesterol has been demonstrated by Fukushima and Nakano (1995).

Indicated that probiotic microorganisms inhibits hydroxymethyl-glutaryl-coenzymeA; an enzyme involved in the cholesterol synthesis pathway thereby decrease cholesterol synthesis. Similarly, reduction in serum cholesterol of broiler chickens (Mohan *et al.*, 1995 and 1996) or rabbits (Abdelhady and El-Abasy, 2015). Fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastrointestinal tract by probiotic supplementation. Furthermore, it was considered that *Lactobacillus acidophilus* decreases the cholesterol in the blood by deconjugating bile salts in the intestine, thereby prohibiting them from working as precursors in cholesterol synthesis (Abdulrahim *et al.*, 1996). Concerning to lower serum triglyceride observed in the present study with the supplemented groups (T2 and T3; Table 7) compared with control group. It could be related to abdominal fat that can decreasing the activity of acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid synthesis, after *Bacillus subtilis* culture supplementation, (Santoso *et al.*, 1995 and Mansoub, 2010) which in turn could explain the decreasing occurred in blood triglyceride level.

3. Microbial results:

3.1. Microbial populations in rabbit cecum:

Effect of probiotics supplementation in rabbit diets on cecum microbial populations such as total bacterial count *Lactobacillus*, *Escherichia coli*, *Salmonella*, *Clostridia* and *Listeria* are shown in Table (7). Results of mentioned bacteria showed that there were significant ($P \leq 0.05$) improvement in treated groups compared with the control group. Meanwhile, the group fed 500 mg probiotic /kg diet showed significant improves in bacteria count of total bacteria count particularly with *Lactobacillus*, *Escherichia coli* compared to the control group. The addition of probiotic to rabbit diets may be led to improve the microbial ecosystem of rabbit guts.

The improvement noticed in microbial population in this study could be due to increased count of lactic acid bacteria and certain other microorganism's that helps in increasing the resistance to *Salmonella* and *E. coli* colonization (Panda *et al.*, 2000). Moreover, gut function might have been improved by feeding diet supplemented with *L. acidophilus* due to the increase of lactase and sucrase activities in the small intestinal mucosa.

Table (7): Indicates the effect of probiotic supplementation in diet on Cecum microbial populations of growing NZW rabbits at 12 weeks of age.

Treatments	Total bacterial count (x10 ⁷) ²	Lactobacillus (x10 ⁴) ²	Escherichia Coli (x10 ⁴) ²	Salmonella	Clostridia	Listeria
T1 (Control)	22.67 ^{1A} ±0.84	2.50 ^B ±0.18	7.83 ^A ±0.31	ND	ND	ND
T2 (250 mg/kg diet)	11.97 ^B ±0.36	10.17 ^A ±0.99	4.67 ^B ±0.17	ND	ND	ND
T3 (500 mg/kg diet)	10.65 ^B ±0.22	10.58 ^A ±0.89	3.92 ^C ±0.20	ND	ND	ND

¹Mean ± standard error.

^{A, B} Means having different letter exponents among rows are significantly different (P≤0.05).

²Germ counts expressed in CFU/g caecal digesta. ND = Not detected

Likewise, an increase of the cecal lactobacilli population in the rabbits supplemented with *Lactobacillus acidophilus* lead to an increase the cecal acetic acid and total volatile fatty acids (VFA) concentration and a reduced intestinal coliform population (Jin *et al.*, 2000). Acetic acid has been shown to penetrate into the bacterial cytoplasm resulting in a reduced internal bacterial pH and collapse the electrochemical proton gradient, leading a bacteriostasis and death of susceptible bacteria such as cecal coliforms (Eklund, 1989). The decrease of the intestinal coliform population could contribute to a reduction of the gastrointestinal problems in the weaning animals (Oglesbee and Jenkins, 2012).

Also, *Lactobacillus acidophilus* fed rabbits indicated that the intestine was predominantly colonized by non-pathogenic bacteria, and toxic substances of intestinal pathogens were inhibited by gut beneficial bacteria (Phuoc and Jamikorn, 2017).

Probiotics are mainly used to reinforce or reestablish the gut microbial balance, especially when hosts are confronted with challenges or stress (Vanbelle, 2001), generally associated with poor growth rate and immunity. It

has been reported that probiotic bacteria maintain normal gut microflora in two ways: competitive exclusion or antagonism. Once established in the gut, probiotic bacteria may produce compounds with bactericidal or bacteristatic properties (bacteriocins) such as organic acids, hydrogen peroxide, lactoferrin. These substances are thought to inhibit the growth of pathogenic bacteria by reducing the pH in the gut (Jin *et al.*, 1997; Ahmed *et al.*, 2014).

4. Histomorphological results

Histomorphological examination of rabbit duodenum and cecum:

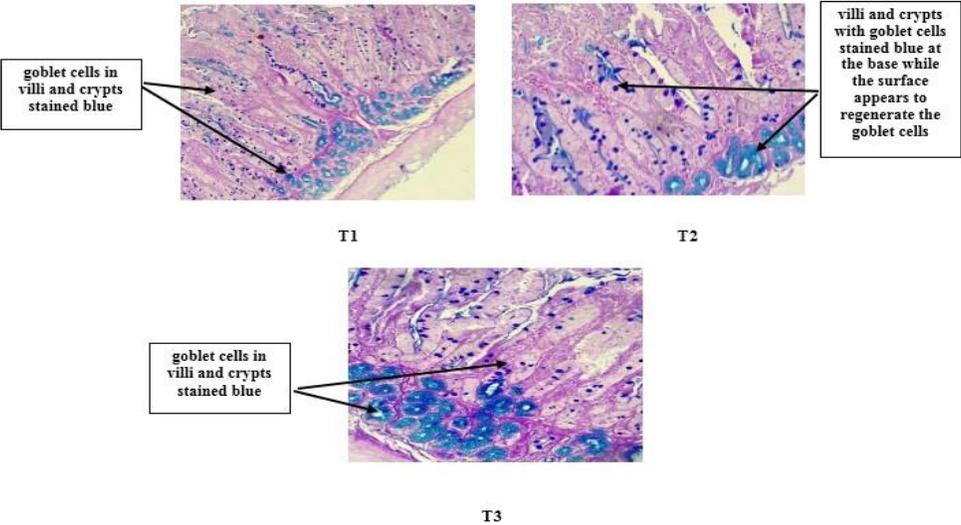
The effect of probiotic supplementation in growing rabbit diets on the histo-pathological of (duodenum, cecum) are shown in Figures (1 & 2). The goblet cells appeared in treated groups T2 and T3 suggested that there were improvement in production of mucus compared with control group. Meanwhile, the group fed 500 mg probiotic/kg diet (T3) showed improvement in goblet cells and crypts in the base of the tissue or surface compared to control group.

The enhanced mucus layer overlying the epithelial lining of the gut can serve as an antibacterial shield that prevents the binding of enteric pathogens, such as enter pathogenic *E. coli* (Mack *et al.*, 2003), to mucosal surfaces and increase clearance of the pathogen from the gastrointestinal tract (Linden *et al.*, 2008).

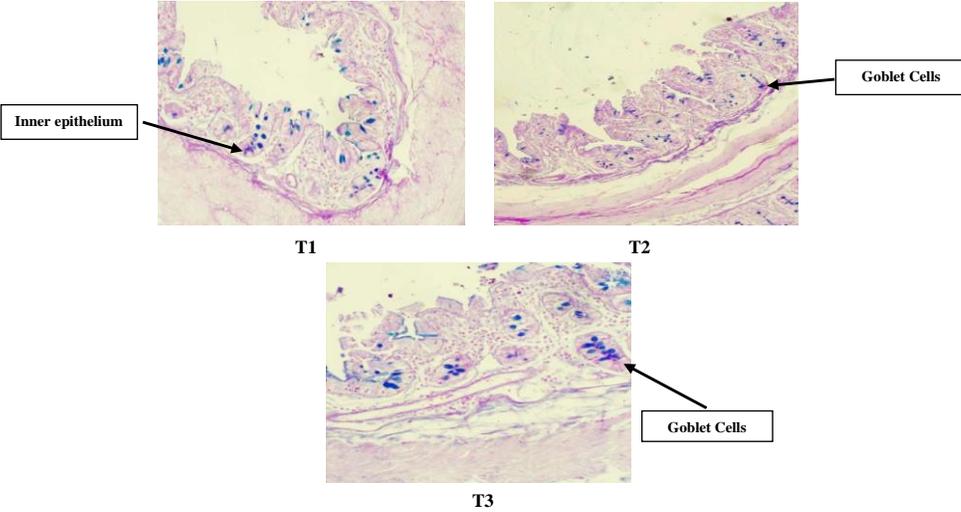
Bacillus and Lactobacillus are also known to increase the rate of glucose transport, intestinal villous height, and crypt depth ratio (Breves *et al.*, 2000; Rao and Wang, 2010), which may have contributed to improved FI in animals. Moreover, probiotic products may compete with other intestinal microorganisms for nutrients or result in production of antibacterial substances (Hentges, 1992). On the other hand, goblet cells have a role in defense at the intestinal mucosa, as reported by (Birchenough *et al.*, 2015).

5. Economic efficiency:

The effects of probiotic supplementation in growing rabbit diets on economic situation are presented in Table (8). In general, average feed intake/kg gain decreased by increasing probiotic level in the diet. The supplementation of rabbit diets with probiotic led to clear increase in price (LE)/Kg diet, however net revenue and economic efficiency increased compared to control group. These results revealed that the dietary supplementation with probiotic had effect on the economic situation, and the



(Fig. 1): photomicrograph of a section in the duodenum of a rabbit in all groups



(Fig. 2): photomicrograph of a section in the Cecum of a rabbit in all groups

Table (8): Effect of different levels of probiotic on economic efficiency during the whole of experimental period (8-12 weeks of age) of growing NZW rabbits.

Measurements	Probiotic levels		
	Control	250 mg/kg diet	500 mg/kg diet
Average feed intake/Kg gain	3.54	3.23	3.16
Price (LE)/Kg feed ¹	4.80	4.85	4.90
Total feed cost (LE)	16.99	15.67	15.48
Price (LE)/Kg gain ²	42.00	42.00	42.00
Net revenue (LE) ³	25.01	26.33	26.52
Economic efficiency	1.47	1.68	1.71
Relative economic efficiency ⁴	100	114.29	116.33

¹Based on average price of the diets during the experimental diet.

²According to the local market price at the experimental time.

³Net revenue per unit feed cost.

⁴Assuming that the control group 100%.

improvement noticed compared to control group may be due to increasing happened in treated group in body weight, body weight gain and feed conversion ratio.

Conclusively, it can be recommended to apply probiotic in rabbit farms because it were effective on reducing the harmful effects of intestinal bacteria, improving productive and physiological performance and immunoglobulin situations.

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تأثير مستويات البروبيوتيك على الأداء الإنتاجي والفيولوجي للأرانب النامية تحت الظروف المصرية

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اجريت هذه التجريه فى محطة ابحات الدواجن التابعه لقسم الانتاج الحيوانى - كلية الزراعة بالقاهرة جامعة الأزهر. تم تصميم هذه التجربة لدراسة تأثير مستويات أحد المحفزات البيولوجية (البروبيوتيك) المتوفر تجارياً على الأداء الإنتاجي والفيولوجي والعد الميكروبي والاختبارات الهستولوجية للأمعاء فى الأرانب النيوزيلندي الأبيض النامية حيث تم استخدام عدد 36 أرنب نامي عمر أربعة أسابيع (بعد الفطام مباشرة) من سلالة (النيوزيلندي الأبيض) وتم توزيعهم عشوائياً إلى ثلاثة مجموعات (معاملات) تحتوى كل منها على ثلاثة مكررات (4 أرانب نيوزيلندي ابيض عمر أربعة أسابيع لكل مكررة). والمعاملات كانت كالتالى: 1- المعاملة الأولى وهى معاملة بدون أى إضافة على العليقة (الكنترول)، 2- المعاملة الثانية وهى إضافة 0.25 جرام من المحفز البيولوجي (لاكتوباسلس بلانترم) لكل كيلو جرام من العليقة، 3- المعاملة الثالثة وهى إضافة 0.50 جرام من المحفز البيولوجي لكل كيلو جرام من العليقة) واستمرت التجربة لمدة ثمانية أسابيع.

أوضحت النتائج المتحصل عليها أن إضافة المحفزات البيولوجية (المعاملة الثانية والثالثة) كان له تأثير معنوى إيجابي على وزن الجسم الحي ووزن الجسم المكتسب للأرانب النيوزيلندي النامية وكذلك حدث تحسن فى معدلات تحويل الغذاء مقارنة بالمعاملة الكنترول (المعاملة الأولى). أظهرت النتائج أيضاً تأثير معنوى للمحفزات البيولوجية على بعض قياسات الدم حيث زادت قيم كل من البروتين الكلى والألبومين والجلوبيولين وكذلك الجلوبيونات المناعية من النوع G فى سيرم دم الأرانب النيوزيلندي النامية عند عمر 12 أسبوع (نهاية الفترة التجريبية) مقارنة بالمعاملة الكنترول.

كما أظهرت النتائج أيضاً زيادة إنتاج المواد المخاطية فى الأمعاء نتيجة تأثير إضافة المحفزات البيولوجية كما أدت الإضافة إلى انخفاض أعداد الميكروبات القولونية (إيشرشيا كولاي) وزيادة أعداد البكتريا النافعة فى الأمعاء مقارنة بالمعاملة الكنترول.

التوصية: وتوصى الدراسة باضافة المحفز البيولوجي بالمعدلات التى درست الى علائق الأرانب النيوزولاندى بدون خوف من اى تأثيرات عكسية.