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EFFECT OF USING *Pediococcus acidilactici* ON PRODUCTIVE PERFORMANCE OF GROWING RABBITS.

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Sixty growing New Zealand White (NZW) rabbits at 6 weeks of age and average body weight of 702±1.71g were used in the present study. The rabbits were randomly assigned to 5 equal treatment groups (n=12/group). Rabbits in treatment group 1 were fed basal diets without Pedicoccus acidilactici (T_1), while rabbits in treatment groups 2, 3, 4 and 5 were fed diets containing Pedicoccus acidilactici 0.1 g ($1x10^9$ cfu/g) /kg basal diet for different periods once a week (T_2), twice a week (T_3), skip a day (T_4) and through the experimental days (T_5). The experimental lasted for 8 weeks.

***Result show that** rabbits in T_2 and T_3 groups recorded significantly ($P \leq 0.01$) and ($P < 0.05$) higher final body weight, daily weight gain and improved feed conversion ratio. Once a week system (T2) had significantly the best values of CP, CF, EE, DCP, TDN, DE digestibility values, carcass weight and dressing percentages as compared to other treatment groups. Moreover, total protein, AST, albumin, total cholesterol, total lipids and LDL were significantly ($P \leq 0.01$) decreased in T_2. However, there were a significant increase in WBCs, IgG and IgM with T_2 group. Pediococcus acidilactici (PA) supplementation increased significant the population of total bacterial count in T_2, T_3 and T_4 as compare toT_1 and T_5 while, total volatile fatty acid and ammonia concentration were not significantly affected by the different treatments.*

Rabbits in T_2 and T_3 have more villi and intestinal glands of the tunica mucosa, also T_2, T_3 and T_4 groups were recorded high economic efficiency.

***Conclusively,** it is concluded that supplemented 0.1g Pedicoccus acidilactici ($1x10^9$ cfu/g) /kg rabbit diet for once, twice a week improve growth performance traits and economic efficiency, without any adverse side effect of growing rabbits.*

Key words: Rabbits, probiotic, Pedicoccus acidilactici, growth performance, digestibility, microbial activity.

Commercial rabbit production is an important industry for meat and fur. Weaning crucial period for all young animals is associated with a lot of stress and increased sensitivity to diseases (Kritas *et al*., 2008).

Using of antibiotics as growth stimulators is being discouraged. The use of microorganism (probiotics) as feed additives is currently widely promoted as an "alternative" to antibiotic growth promoters (Kamra *et al*., 1996). Several studies have been shown the positive effect of probiotics on the control of certain pathogens in animals, where they appear to control enteric diseases associated with *E. coli* or other enteric pathogens (Kritas *et al*., 2008). The mechanism of action of probiotics might included: 1- reduction of toxin production, 2- stimulation of enzyme production by the host, 3- production of some vitamins or antimicrobial substances, 4- competition for adhesion to epithelial cells and increased resistance to colonization, 5- stimulation of immune system of the host and 6-reducation of stress (Falcao-e-Cunha *et al*., 2007; Shehata and Tawfeek, 2010).

Pedicoccus acidilactici a product from lactic acid bacteria (LAB) and it is a probiotics. It is a live harmless bacterium that help well the host animal and contribute, directly or indirectly to the host animal against harmful bacterial pathogens. Lactic acid bacteria when consumed in adequate amounts produce antagonistic compounds against pathogens and enhancement of the immune response and diseases resistance (Gomez and Balcazar, 2008 and Ferguson *et al*., 2010).

Pediococcus acidilactici has a wide range of potential benefits of which are still being studied. Though it is being used as probiotic supplements in treating constipation, diarrhea, relieving stress, enhancing immune response among birds and small animals. Also *Pediococcus acidilactici* known to prevent colonization of the small intestine by pathogens like *Shigella, Salmonella, Clostridium difficile* and *Escherichia coli* among small animals. I needed it has not been stated in any literature to have toxic effects. *Pediococcus acidilactici* in conjunction with *Saccharomyces boulardii* stimulates humeral immune response to produce high *Eimeria*-specific antibody levels and reducing the number of oocytes shed by possible competitive inhibition which inhibit pathogenic bacteria and other gram-positive spoilage (Lee SH *et al*., 2007). *Pediococcus acidilactici* can function as immune modulators. Animals fed with *P. acidilactici* have shown enhanced immune responses against infectious coccidioidal diseases (Lee SH *et al*., 2007). The supplementation of *Pediococcus acidilactici* to laying hens has been found to improve hen performance including feed efficiency (Ba levi *et al*., 2001), nutrient digestibility (Jin *et al*., 1997) immunomodulation and mucosal immunity (Koenen *et al*., 2004). However, Fadl *et al*., (2013) who reported that the long

duration on consequent dietary supplementation of 2g/kg diet *Pediococcus acidilactici* leading to exhaustion of immune system in tilapia finger lings.

Therefore, this study was undertaken to investigate the effects of probiotics *Pediococcus acidilactici* (PA) supplementation in rabbit diets on some growth performance traits, nutrient digestibility coefficients, some blood parameters, immunological constituents, caecal microbial activity and histological examination of the rabbits for different periods, as well as, economical efficiency. The appropriate period of its inclusion for optimum health status was determined.

MATERIALS AND METHODS

This work was carried out at Sakha (Kefir EL-Sheik), Experimental Station, Animal Production Research Institute. The analysis of blood samples, feces, caecal microbial activity were conducted in Laboratory of Department belonging to Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, and the analysis of immunological constituents, total cell count bacteria and histological examination were conducted in Laboratory of Department belonging to Animal Health Research Institute, Agriculture Research Center, Ministry of Agriculture.

Sixty weaned New Zealand White (NZW) rabbits, six weeks of age with an average live body weight 702±1.71g. The rabbits were randomly assigned to 5 treatment groups (n=12/ treatment group). Rabbits in treatment group 1 were given the basal diet without *Pediococcus acidilactici* (PA) (T_1), while rabbits in treatment groups 2, 3, 4 and 5 were fed diets containing (PA) as Bactocell ® (a commercial product each 1g contains $1x10^9$ CFU, as recommended by Biotal Company, UK) by the concentration of at 0.1 g/kg for the basal diet for different period once a week (T_2), twice a week (T_3), skip a day (T_4) and thorough out the experimental days (T_5) as shown in Table 1.

The commercial ration was formulated to cover the requirements of growing rabbits according to Agriculture Ministry Decree (1996).

Experimental parameters:

Feed intake and live body weight were recorded weekly, while feed conversion was calculated as gram of feed per gram of gain, relative growth rate was calculated according to the following equation:

Relative growth rate = $[(W_2 - W_1) x100] / [1/2 (W_2 + W_1)]$, Where: W_1= Initial body weight (g), and W_2 = Final body weight (g).

Performance index (PI) = (Final live body weight (kg)/ Feed conversion ratio) × 100, according to North (1981).

Table 1. Ingredients and chemical composition of the basal experimental diet.

Ingredients	**%**
Barely	30.28
Clover hay	27.05
Wheat bran	18.40
Soybean meal (44% CP)	18.10
Molasses	3.00
Di calcium phosphate	2.20
NaCl	0.30
premix*	0.30
Lime stone	0.22
DL-Methionine	0.10
Anticoccidia (Diclazuril)	0.05
Total	**100**
Calculated analysis%[1]	
OM%	90.34
CP%	17.76
CF%	12.97
EE%	2.18
NFE%	57.43
Ash%	9.66
DE(kcal/kg)	2518.4
Calcium	1.11
Total phosphorus	0.85
Methionine+ cyct.	0.65
Lysine	0.91

**Each1.5 kg of vitamins and minerals mixture contains: Vit. A 10.000.000 IU, Vit.B_1 1000mg, Vit.B_2 5000mg, Vit.D_3 2.000.000 IU, Vit E 10.000mg, Vit. $K_3$1000 mg, Pantothenic acid 10.000mg; Nicotinic acid, 30.000g; Vit. B_6 15000mg; Vit. B_{12} 10 mg, Folic acid 1.0g, Biotin 50 mg, Cu 4g, choline chloride 200mg,Mn 60g, Fe 30g, , Co 0.1 g, Se 0.1 g, Zn 50 g,Iodine 0.3 g and Antioxidant 10.000mg.*

[1] according to Feed composition for animal and poultry feed stuff used in Egypt (2001).

The experimental period lasted for 8 weeks. At the end of the experimental period, a digestibility trial was conducted to determine the digestibility coefficient of the nutrients according to Fekete (1985). Digestibility trial was carried out using four male rabbits from each experimental group at the last week of the growth experiment (14 weeks of age). The rabbits were housed in metabolic cages, where feces and urine samples were collected separately for 6 days as a collection during period, which the feces were collected daily and sprayed with 2% boric acid solution for trapping any ammonia released from feces. At the end of that period, the feces were dried at 60^{o} C for 48 hours (till constant weight), finely grounded and thoroughly mixed to ensure sample uniformity and then stored until being

analyses. Proximate analysis of the diets and feces were carried out according to the methods of A.O.A.C (2000).

At the end of the experimental period (14 weeks of age), four rabbits of each group were randomly taken and fasted for 12 hours before being slaughtered to determine carcass traits according to Steven *et al*., (1981).

Also, sections of the cecum were collected for to histology investigation, total bacterial counts by Pour Plate Count Technique according to British Standard Institution (1991), total volatile fatty acids were determined according to Eadie *et al*., (1967) and ammonia was determined by applying Conway method (1958). Blood serum samples were taken from the same rabbits of each treatment after slaughter and collected in 5 ml. heparinized test tubes and centrifuged at 3000 r.p.m for 20 minutes then plasma were transferred and stored in deep freezer at approximately -20ºC till the time to determine total protein (TP, g/ dl) and albumin (Alb, g/ dl) levels were determined using commercial kits supplied by Randox (Randox laboratories Ltd, Crumlin, Co, Antrim, UK) according to Doumas *et al*., (1981). Plasma samples were analyzed for determinations of aspartate aminotransferase (AST, U/L), alanine transaminase (ALT, U/L) using a commercial kits (Linear Chemicals, Barcelona, Spain) according to the manufacturer procedure. Total cholesterol was determined according to Richmond (1973), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined according to the methods of Warnick *et al.,* (1983) and Bergmenyer (1985), respectively. Total lipids were determined according to Feteris (1965), plasma total immunoglobulin traits were determined according to Van der Zipp *et al*., (1983). Some fresh blood were taken after collection for determination of blood pictures including red blood cells count (RBCs, / (10^3/ µl), Platelet count (10^3/ µl), white blood cells count (WBCs, (10^3/ µl) (lymphocytes, neutrophils, monocytes, eosinophils and basophils), hemoglobin (Hb, g/dl) and hematocrite concentration according to Drew *et al*., (2004).

Economic efficiency

The economic efficiency (EEF) was calculated according to the following equation:

EEF = Net revenue / total costs. Where, the total cost calculated by Egyptian pound (L.E.) in the local market at the time of experiment.

Statistical analysis:

All data were subjected to analysis of variance using the general linear models (GLM) Procedure of SAS (2004) by applying the following model:

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

Where: μ= Overall mean of Y_{ij}, T = Effect of treatment groups, i = (1, 2…, etc) and e_{ij}= Experimental error.

The significant differences between treatment means were separated at alpha level (P≤0.05) by Duncan's Multiple Range Test (1955).

RESULTS AND DISCCUSION

Growth performance traits

Results in Table 2 present, the effect of *Pediococcus acidilactici* (PA) on some growth performance traits of growing rabbits. It showed final body weight was significantly ($P \leq 0.01$) higher with (PA) supplementation for once a week (T_2) and twice a week (T_3) as compared to throughout experimental days (T_5). Daily weight gain was significantly *($P \leq 0.01$)* higher with (PA) supplementation during 6-10 weeks of age period for once a week (T_2) and twice a week (T_3) compared to T_1 and T_5 also, during the whole growing period with T_2, T_3 and T_4 (skip a day) compared to T_1 and T_5. While, during 10-14 week of age period, there was no significant effect on weight gains. The improvement in daily live weight gain was accounted to be 8.91, 5.24 and 4.86% with once a week (T_2), twice a week (T_3) and skip a day (T_4) feeding system of (PA) respectively, when compared to the control.

Feed conversion ratio was significantly ($P \leq 0.05$) improved with (PA) supplementation, during 6-10 weeks of age period for once a week (T_2) and twice a week (T_3) as compared to T2, T3 and T4 also, during the whole growing feed conversion ratio was significantly ($P \leq 0.0.5$ improved with once a week (T_2) compared to (T_5). While, during 10-14 week of age period, there was no significant effect among treatment groups on feed conversion ratio. The improvement in feed conversion was accounted to be 11.83 and 7.40% with once a week and twice a week feeding system of (PA) respectively, compared to the control (T_1). Although, there is no significant difference in feed intake all the growth period and relative growth rate (%) in rabbits fed (PA) in all different periods. While, relative growth rate (%) in rabbits fed (PA) with T_2, T_3 and T_4 recorded high values than T_1 and T_5.
Performance index (%) increased significantly ($P \leq 0.0.01$) in rabbits fed (PA) with once a week (T_2). Total mortality recorded during the period from 6-14 weeks of age for different experimental treatments are given in Table 2. Rabbits in once a week (T_2) and twice a week (T_3) recorded significantly lowest values of mortality during the experimental period, as compared with T_1 and T_5 (5% and 5% vs. 25%) respectively). This may be due to rabbits fed *Pediococcus acidilactici* obtained high resistance against bacterial challenge in addition to that (PA) the improved rabbit immunity through

Table 2. Effect of *Pediococcus acidilactici* supplementation on growth performance of growing rabbits during period from 6 to 14 weeks of age.

Items	T_1	T_2	T_3	T_4	T_5	SEM	Sign.
Initial body weight(g)	702	703	701	703	700	**1.71**	**NS**
Final body weight (g)	2020^{bc}	2146^{a}	2089^{ab}	2085^{abc}	1985^{c}	**16.32**	**
Daily weight gain (g)							
6-10 weeks of age	24.1^{b}	25.8^{a}	25.7^{a}	25.3^{ab}	23.8^{b}	**0.28**	**
10-14 weeks of age	22.5	24.3	23.5	23.4	22.4	**0.33**	**NS**
6-14 weeks of age	23.5^{b}	25.8^{a}	24.8^{a}	24.7^{a}	22.9^{b}	**0.26**	**
Daily feed intake(g)							
6-10 weeks of age	61.5	59.5	59.8	63.9	63.2	**0.62**	**NS**
10-14 weeks of age	99.4	96.7	98.0	99.9	100.47	**0.76**	**NS**
6-14 weeks of age	79.4	76.8	77.6	80.2	79.6	**0.81**	**NS**
Feed conversion ratio							
6-10 weeks of age	2.55^{a}	2.31^{b}	2.33^{b}	2.52^{a}	2.65^{a}	**0.03**	*
10-14 weeks of age	4.42	3.98	4.17	4.27	4.48	**0.11**	**NS**
6-14 weeks of age	3.38^{ab}	2.98^{b}	3.13^{ab}	3.25^{ab}	3.47^{a}	**0.06**	*
Relative growth rate (%)	96.84	101.29	99.50	99.14	95.72	**1.69**	**NS**
Performance index (%)	59.76^{b}	72.01^{a}	66.74^{ab}	64.15^{b}	57.20^{b}	**1.72**	**
Mortality(%)	25.00^{a}	5.00^{c}	5.00^{c}	10.00^{bc}	15.00^{ab}	**1.84**	**

a,b,--- Means in the same row with different superscripts are significantly different.
NS: Not significant, *:(P<0.05), and **:(P<0.01).
$T_{1:}$ Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, $T_{4:}$ Group fed diet containing *Pediococcus acidilactici*, skip a day, $T_{5:}$ Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.

improvements of the differential leucocytic count and activity of bacteria. The high mortality rate in throughout experimental days (T_5) may be due to

using the large doses, which leads to decreased immunity. This result confirmed by Fadl *et al*., (2013) who reported that the long duration on consequent supplementation of 2g/kg diet (PA) leading to exhaustion of immune system. In these connection, Abdel-Azeem *et al.*, (2009) who found an improvement in the performance index (%) for rabbits fed diets dietary supplementation 200 or 400 mg Bio-Plus®/kg (*Bacillus subtilis* and *Bacillus licheniformis*) diet as compared with received un-supplemented diet. Moreover, Alkhalf *et al*., (2010) found that probiotic (Bactocell) dietary supplementation (1.6 g, 1 g and 0.8 g of Bactocell per kg feed) increased significantly body weight and daily weight gain of broiler chicks at late ages (3–6 weeks). Abdelhady and El-Abasy (2015) found that rabbits fed on basal diet dietary supplemented with 0.4 g/kg diet Bio-Plus® (*Bacillus subtilis* and *Bacillus licheniformis*) significantly improved body weight gain and feed conversion when compared with control group. Also, Bhatt *et al*., (2016) found that *Lactobacillus acidophilus* (10^7 CFU/g) dietary supplementation in rabbit diets enhanced weight gain and feed conversion ratio compared to control group.

The benefits have generally been attributed to improvements in health status of the animal, which may be due to *Pedicoccus Acidilactici* help well the host animal and contribute, directly or indirectly to the host animal against harmful bacteria pathogens. Lactic acid bacteria when consumed in adequate amounts produce antagonistic compounds against pathogens and enhancement of the immune response and diseases resistance (Gomez and Balcazar, 2008, Ferguson *et al*., 2010).

Digestibility coefficients and nutritive value of the diets:

Data of digestibility coefficients of nutrients are shown in Table 3. Results indicate that apparent digestibility of DM, OM and NFE were not significantly affected by (PA) supplementation. On the other hand, digestibility coefficients of CP and nutritive value of DCP were increased significantly in T_2, T_3 and T_4 groups when compared to T_5 group. Digestibility coefficients (%) of CF increased significantly in T_2 and T_3 compared to T_5 and T_1 groups. Also, digestibility coefficients of EE increased significantly ($P \leq 0.001$) in T_2 and T_3 compared to T_5. There were significant ($P \leq 0.0.05$) and ($P \leq 0.001$) increase in the nutritive values of TDN and DE in T_2 as compared to T_5 and T_1 groups.

Table 3. Effect of *Pediococcus acidilactici* supplementation on apparent digestibility and nutritive values% of diets.

Items	T_1	T_2	T_3	T_4	T_5	SEM	Sign.
DM	61.8	65.3	63.0	63.7	60.1	**1.88**	**NS**
OM	66.9	73.7	72.6	67.7	65.2	**1.87**	**NS**
CP	69.4^{ab}	78.1^{a}	75.2^{a}	74.6^{a}	65.3^{b}	**1.57**	**
CF	25.7^{bc}	38.6^{a}	35.5^{a}	31.1^{ab}	22.9^{b}	**1.84**	**
EE	69.4^{ab}	76.7^{a}	76.0^{a}	72.3^{ab}	67.0^{b}	**1.27**	**
NFE	75.4	80.6	78.7	76.8	70.7	**3.41**	**
DCP	12.3^{bc}	13.9^{a}	13.3^{ab}	13.2^{ab}	$11.\ 6^{c}$	**0.28**	**
TDN	55.5^{b}	60.3^{a}	58.9^{ab}	57.7^{ab}	54.7^{b}	**0.84**	*
***DE kcal/kg**	2459^{b}	2671^{a}	2609^{ab}	2556^{ab}	2423^{b}	**34.18**	**

a,b and c--- Means in the same row with different superscripts are significantly different : NS: Not significant, *:(P<0.05), and **:(P<0.01).

$T_{1:}$ Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, $T_{4:}$ Group fed diet containing *Pediococcus acidilactici*, skip a day, $T_{5:}$ Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.

*DE = TDN X 44.3, according to (Schneider and flatt, 1975).

In this respect, Amber *et al.*, (2004) showed that apparent digestibility of CF was improved (P<0.05) by 13% for rabbits received diet containing probiotic (0.5 gm/kg *Lactobacillus acidophilus*) as compared to control diet, due to increase the number of cellulolytic bacteria as a result to enhancing lactate utilization and moderating pH of the media (Dawson, 1987). Bovera *et al*., (2010) who found that rabbits fed dietary Bio-Mos®, mannoligosacchride at 1.0 g/kg showed significantly better nutrient digestibility (OM, CP and CF) than those fed 1.5 g Bio-Mos® /kg diet or control diet. Amber *et al*., (2014) who found that digestion coefficients of DM, OM, CP, CF ,NFE, DCP and TDN values were increased significantly, but the EE digestibility decreased significantly and DE were not significantly affected by dietary supplementation of probiotic 0.4 g/kg diet Bio plus® (*Bacillus subtilis* and *Bacillus licheniformis*). Bhatt *et al*., (2016) found that dietary supplementation of *lactobacillus acidophilus* (10^7CFU/g) in rabbit diets enhanced digestibility and utilization of nutrients. The improvement in nutrients digestibility may be due to reducing the surface tension of cell membranes which could aid in better absorption of nutrients across the cell membranes (Johnston *et al.*, 1982).

Carcass characteristics

The effect of (PA) supplementation on some carcass traits are showed in Table 4. Results indicated that carcass weight and dressing weight

Table 4. Effect of *Pediococcus acidilactici* supplementation on carcass quality traits of growing rabbits.

Items	T_1	T_2	T_3	T_4	T_5	SEM	Sign.
Carcass wt, %	57.44^{ab}	59.24^{a}	58.60^{ab}	57.48^{ab}	56.54^{b}	0.49	*
Edible giblets,%:	2.98	3.19	3.06	3.02	2.90	0.04	NS
Liver, %	2.06	2.20	2.12	2.09	2.01	0.02	NS
Heart, %	0.27	0.30	0.28	0.28	0.27	0.005	NS
Kidney,%	0.60	0.64	0.61	0.60	0.57	0.011	NS
Spleen, %	0.052	0.053	0.053	0.052	0.052	0.0002	NS
Dressing wt.,%	60.42^{bc}	62.43^{a}	61.66^{ab}	60.50^{abc}	59.44^{c}	0.35	**
Cecum length(cm)	37.7	40.2	39.2	38.2	37.2	0.74	NS
Intestinal length (cm)	256	262	258	258	252	3.03	NS

a,b and c--- Means in the same row with different superscripts are significantly different.
NS: Not significant, *:(P<0.05), and **:(P<0.01).
T_1 $T_{1:}$ Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, $T_{4:}$ Group fed diet containing *Pediococcus acidilactici*, skip a day, $T_{5:}$ Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.

percentages were significant (P<0.05) and (P<0.01) increase with T_2 compared to T_5 group. However, edible giblets (liver, heart, kidney, spleen percentages), caecum length and intestinal length were not significantly affected by (PA) supplementation. In this connection, Omer *et al*., (2010) showed that dressing percentage was not affected by dietary supplementation 0.5% of dried yeast (*Saccharomyces cerevisiae*) in rabbit diets compared with the control group. Also, Wallace *et al*., (2011) found that liver weight, full stomach, full gastrointestinal and carcass length did not affected by dietary supplementation RE_3 as a probiotic to rabbit diets. Rotolo *et al*., (2014) observed that the measure of caecum was relatively longer and significantly increase in the probiotics supplemented group than control group. Bhatt *et al*., (2016) observed found that dietary supplementation of *lactobacillus acidophilus* (10^7CFU/g) in rabbit diets had no significant effect on carcass traits.

These increments on carcass traits were in the same trend, that due to the effect of (PA) supplementation in treatments T_2 and T_3 improved digestibility and utilization of nutrient, body weight gain and feed conversion. Also, this increment in T_2 and T_3 especially can be due to the right dose and suitable period (once or twice a week) to the rabbits, so effect well on body weight, body gain and the animals health status.

Blood constituents

Blood constituents of growing rabbits fed the experimental diets are shown in Table 5. Total protein and albumin values were decreased significantly (P<0.01) in T_2 and T_3 groups compared to T_1 and T_5 groups. Also, there was significantly (P<0.01) decreased in AST with T_2 group as compared to T_1 .

There were significantly decreased in values of total cholesterol and total lipids especially in T_2, T_3 and T_4 compared to T_1 and LDL was significantly decreased with T_2 and T_3 compared to T_1. While, significantly increased in HDL for rabbit groups fed T_2, T_3 and T_4 as compared to T_5 were observed.

Table 5. Effect of *Pediococcus acidilactici* supplementation on some blood parameters (With in normal range) of growing rabbits.

Items	T_1	T_2	T_3	T_4	T_5	SEM	Sign.
Total protein (g/dl)	6.9^{a}	6.1^{b}	6.5^{b}	6.6^{ab}	7.3^{a}	0.16	**
Albumin (g/dl)	4.2^{a}	3.3^{b}	3.4^{b}	3.9^{a}	4.2^{a}	0.11	**
ALT(U/L)	33.3	28.6	28.3	32.0	36.0	1.46	NS
AST(U/L)	44.0^{a}	27.0^{b}	31.0^{ab}	36.0^{ab}	42.3^{ab}	2.54	**
Total cholesterol (mg/dl)	185^{a}	138^{c}	147^{bc}	158^{b}	173^{a}	5.49	NS
HDL(mg/dl)	155^{bc}	164^{a}	162^{a}	161^{ab}	154^{c}	1.35	**
LDL(mg/dl)	74^{a}	54^{b}	58^{b}	60^{ab}	61^{ab}	2.54	**
Total Lipids(mg/dl)	360^{a}	258^{b}	261^{b}	291^{b}	314^{ab}	12.72	**

a,b and c-- Means in the same row with different superscripts are significantly different.
NS: Not significant, **:(P<0.01).
T_1: Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, T_4: Group fed diet containing *Pediococcus acidilactici*, skip a day, T_5: Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.
ALT, alanine amiotransferase; aspartate aminotransferase HDL: High density lipoprotein, LDL: Low density lipoprotein.

Cholesterol decreases may be due to supplementation of probiotic in growing rabbits could be reducing in absorption and synthesis of cholesterol in the gastrointestinal tract. No clear mechanisms have been reported responsible for the reduction of lipid synthesis by prebiotics. It might in part be due to increasing beneficial bacteria such as lactobacillus that decrease the activity of acetyl-CoA carboxylase, which is the rate-limiting enzyme in fatty acids synthesis (Toghyani *et al*., 2011). In this connection, Fadl *et al*., (2013) found that the immune-modulatory effect of dietary supplementation 2g/kg diet PA on the liver cells

activating the anabolic capacity to produce blood proteins and this also, supported by the results of hepatic enzymes analysis, which decreased especially in T_2 and T_3 groups indicating a positive effect on the integrity of hepatocytes. Abdelhady and El-Abasy (2015) showed no significant change in albumin, ALT, AST, but revealed significant increase ($P<0.05$) of total proteins concentration for rabbit received 0.4 g Bio-Plus® (*Bacillus subtilis* and *Bacillus licheniformis*) / kg diet while, total cholesterol was significantly decreased in all treated rabbit groups when compared with control group.

Hematological parameters:

Hematological values in Table 6 show no significant differences in hemoglobin, RBCs count, hematocrit %, platelet count, neutrophil, lymphocyte, monocyte, eosinophyil and basinophiles between the experimental treatments. However, there was a significant increase ($P<0.05$) in WBCs in T_2 compared to T_1, T_4 and T_5. This result is supported by Khaksefidi and Ghoorchi (2006) reported that probiotic caused increase in total erythrocyte and leukocyte cell counts and increase in percentage of lymphocytes and monocytes. Also, Fadl *et al*., (2013) who found that WBCs was significantly increased with fish fed diets supplementing with 2g

Table 6. Effect of *Pediococcus acidilactici* supplementation on some blood hematological values (With in normal range) and different leukocyte count of growing rabbits.

Items	T_1	T_2	T_3	T_4	T_5	SEM	Sign.
Hemoglobin (g/dl)	12.16	12.56	12.20	11.23	11.93	0.22	NS
RBCs count (10^6/ µl)	5. 22	5.39	5.33	5.34	4.70	0.12	NS
Hematocrit, %(PCV)	40.46	43.00	42.10	40.86	37.90	0.80	NS
Platelet count (10^3/ µl)	441.7	523.7	490.3	409.3	394.7	35.58	NS
WBCs count (10^3/ µl)	6.46^b	13.03^a	8.00^{ab}	7.56^b	5.90^b	0.91	*
Different leukocyte count%							
Neutrophil	49.3	50.2	52.2	52.0	54.2	0.83	NS
Lymphocyte	41.3	42.3	40.2	41.3	39.7	1.13	NS
Monocyte	4.3	4.7	4.6	4.3	4.0	0.13	NS
Eosinophyil	1.7	1.9	2.0	2.0	1.7	0.21	NS
Basinophiles	0.33	0.98	0.94	0.33	0.33	0.12	NS

a,b,--- Means in the same row with different superscripts are significantly different.

NS: Not significant, *:($P<0.05$).

T_1: Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, T_4: Group fed diet

containing *Pediococcus acidilactici*, skip a day, $T_{5:}$ Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.

Pediococcus acidilactici / kg diet. Amaravadh *et al*., (2012) showed that total erythrocyte count, total leucocyte count, lymphocytes, neutrophils, eosinophils, monocytes, hemoglobin and packed cell volume were not significantly affected by supplement probiotic to rabbit diets. Wallace *et al*., (2012) showed significant higher ($P<0.05$) white blood cells and lymphocytes when rabbits fed diet supplementing with probiotic 1.0 ml per kg/ feed compared to control group. Abdelhady and El-Abasy (2015) reported significant increase in RBCS count, PCV, Hb concentration, and number of lymphocytes for rabbit received 0.4 g Bio-Plus® (*Bacillus subtilis* and *Bacillus licheniformis*) / kg diet. The effect may be direct on the lymphatic tissues (Kabir *et al.,* 2004) or indirect through the impact of live yeast on microbial population of gastrointestinal lumen (Jin *et al*., 1997).

Immunological response:

Data presented in Table 7 show that immunoglobin G (IgG) was significantly ($P<0.01$) increased in T_2, T_3 and T_4 compared with T_5 and there was significant increase ($P<0.01$) in immunoglobin M (IgM) in T_2 compared to T_5. Results of immunoglobin A (IgA) showed no significant difference between groups. Chunyang *et al*., (2012) found that supplementation of *Lactobacillus zeae* in rabbit diets significantly increased IgG and IgM. Haghighi *et al*., (2006) found that increase in IgA and IgG antibodies when chicken treated with dietary commercial probiotic contain *Lacto bacillus acidophilus, Bifidobacterium* and *streptococcus faecalis*.

Table 7. Effect of *Pediococcus acidilactici* supplementation on immunological assay of growing rabbits.

Items	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**	**Sig.**
IgG(mg/dl)	526^{ab}	627^{a}	576^{a}	559^{a}	435^{b}	22.14	**
IgM(mg/dl)	38.7^{ab}	46.0^{a}	40.3^{ab}	39.3^{ab}	36.7^{b}	1.20	**
IgA(mg/dl)	103	111	110	103	83	4.39	NS

a,b,--- Means in the same row with different superscripts are significantly different .
NS: Not significant, *:($P<0.05$), and **:($P<0.01$).
IgG , Immunoglobin G; IgA, Immunoglobin A ; IgM, Immunoglobin M.
$T_{1:}$ Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, $T_{4:}$ Group fed diet

containing *Pediococcus acidilactici*, skip a day, $T_{5:}$ Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.

Microbiological assay

Caecum content of total bacterial count, total volatile fatty acid and ammonia concentration of growing rabbits are presented in Table 8. *Pediococcus acidilactici* (PA) supplementation increased significant ($P<0.05$) the population of total bacterial count in T_2, T_3 and T_4 as compare to T_1 and T_5. However, total volatile fatty acid and ammonia concentration were not significantly affected by (PA) supplementation. In this connection, Maertens *et al.*, (1994) reported that 0.01 % Paciflor® (*Bacillus CIP* 5832, 1.0 x 10^6 CFU/g) had no effect on total volatile fatty acid and ammonia concentration.

Table 8. Effect of *Pediococcus acidilactici* supplementation on caecum content and total bacterial activity of growing rabbits.

Items	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**	**Sig.**
Total bacterial count(10^6)(1)	7.46^b	12.50^a	11.73^a	11.33^a	6.06^b	0.70	*
***TVFA (mg/100ml)**	3.66	4.11	3.73	3.68	3.50	0.13	NS
Ammonia(mg/100ml)	10.41	8.58	8.95	9.02	10.00	0.33	NS

a,b,--- Means in the same row with different superscripts are significantly different.
NS: Not significant, *:($P<0.05$).
(1) Germ counts expressed in CFU/g of caecal digesta.
* TVFA : Total Volatile Fatty Acid.
$T_{1:}$ Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, $T_{4:}$ Group fed diet containing *Pediococcus acidilactici*, skip a day, $T_{5:}$ Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.

Moreover, Amber *et al*., (2004) found that values for total count were increased, while ammonia levels in the caecal decreased for rabbits received diet containing as probiotic (0.5 gm/kg *lactobacillus acidophilus*). Abdel-Azeem *et al.,* (2009) who found that dietary supplementation (400 mg/ kg diet) of Bio plus®(*Bacillus subtilis* and *Bacillus licheniformis*) in rabbit diets reduced number of total bacterial count (especially pathogenic bacteria) in caecum content of rabbits. Amber *et al*., (2014) who found that Pathogenic bacteria (*Escherichia coli* and *Clostridium spp*.) in caecum

content decreased (P<0.001) by dietary supplementation 0.4 g/ kg diet Bio plus® (*Bacillus subtilis* and *Bacillus licheniformis*). This result may due to that PA provide a better environment for bacterial and that the intestinal microbiota plays a vital role in nutritional and immological function of the host animals as reported by Fadl *et al*., (2013).

Histological examination:

These results are confirmed by histological examination in cecum, which presented in Figures 1 to 5. Figure 1 showed that T_1 revealing normal tunica mucosa with normal intestinal gland and villi, while with (PA) supplementation as in T_2 and T_3 increased of villi and intestinal glands of the tunica mucosa were observed (Figures 2 and 3) This interpret at improvement of digestion coefficient and growth performance in T_2 and T_3. In T_4 normal histological picture of intestinal gland with mild hyperplasia of mucous secreting cells can be seen (Figure 4). However, with continues supplementation of (Pa) it can be notice a few area showing aggregation of mononuclear cells and area of hyperplasia of living epithelium of intestinal glands due to shortening of intestinal villi and glands, (Figure 5). These interpret at adverse effect on digestion coefficient and growth performance in T_5. These results are in agreement with Seyidoglu and Peker (2015) found that addition 2g/kg *Saccharomyces cerevisiae* in rabbit diets increased thickness of the mucosa, villus heights, crypt depths and gland depths. Peker *et al.*, (2014) reported increased total mucosa, villus height, and gland depth with dietary addition of 3 g/kg *Saccharomyces cerevisiae* in rabbit diets. Giancamillo *et al*., (2008) who showed that villi height and crypts depth increased with *Pediococcus acidilactici* supplementation in piglet diets. Harper *et al*., (2011) found that histological examination did not cause damage to the gut of the rainbow trout as well as no signs of translocation of either the probiotic or pathogen were observed due to *Pediococcus acidilactici* supplementation.

Economic efficiency

Effect of PA supplementation at different periods are shown in Table 9. Growing rabbits fed diets supplemented with PA either in T_2 and T_3 recorded high economic efficiency followed by T_4 (2.98, 2.85 and 2.69 %), respectively. Total feed cost decreased in T_2 and T_3 as a result to decreasing of feed intake than control and T_5. Also, selling price was increased in T_2, T_3 and T_4 this increase may due to increase in total average weight (kg/head). The same trend was found in net revenue and relative economic, where the best values were recorded in T_2, T_3 and T_4 groups (42.63, 41.10 and 40.10) and (118.25 113.09 and 106.74%), respectively, while the poorest value was

recorded in T_5 and T_1 groups. These results are in agreement with the results of Abdel-Azeem *et al*., (2009) who found an improvement in the economical efficiency (%) for rabbits fed diets supplemented with 200 or

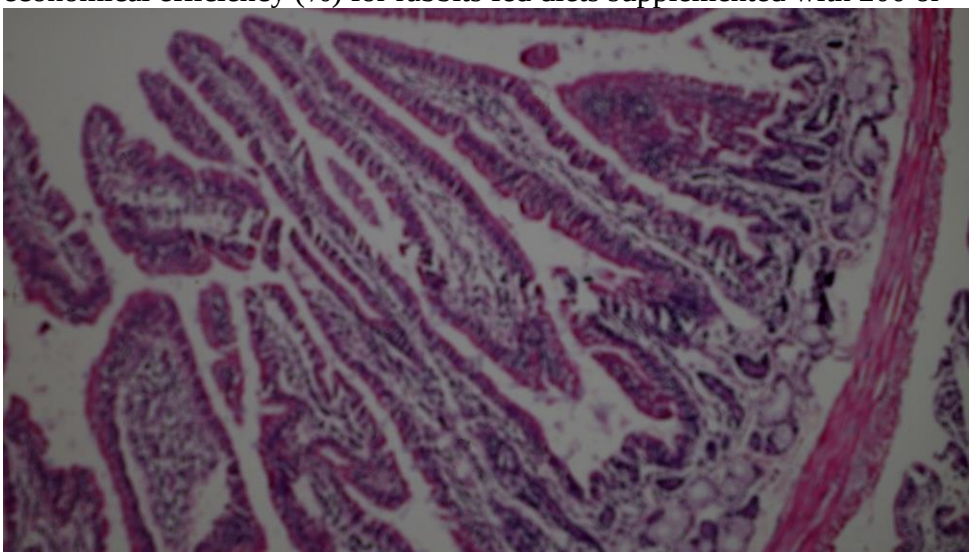

Figure 1. T. S. in rabbits caecum of control group (T_1) revealing normal tunica mucosa with normal intestinal gland and villi. (H × E × 100).

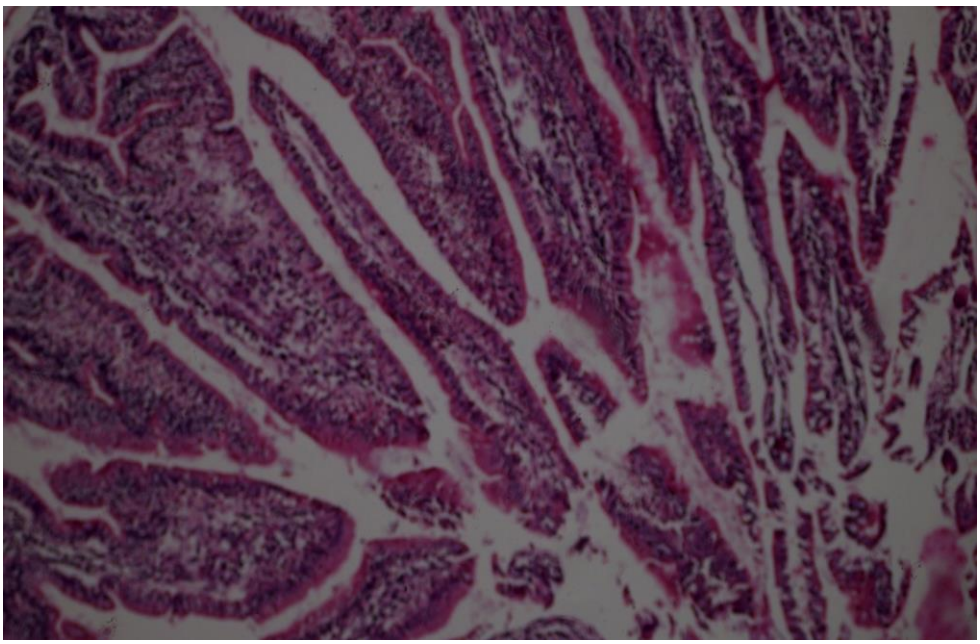

Figure 2. T. S. in rabbits caecum of (T_2) showing normal histological picture with mild increase in length of villi and intestinal glands of the tunica mucosa. (H × E × 100).

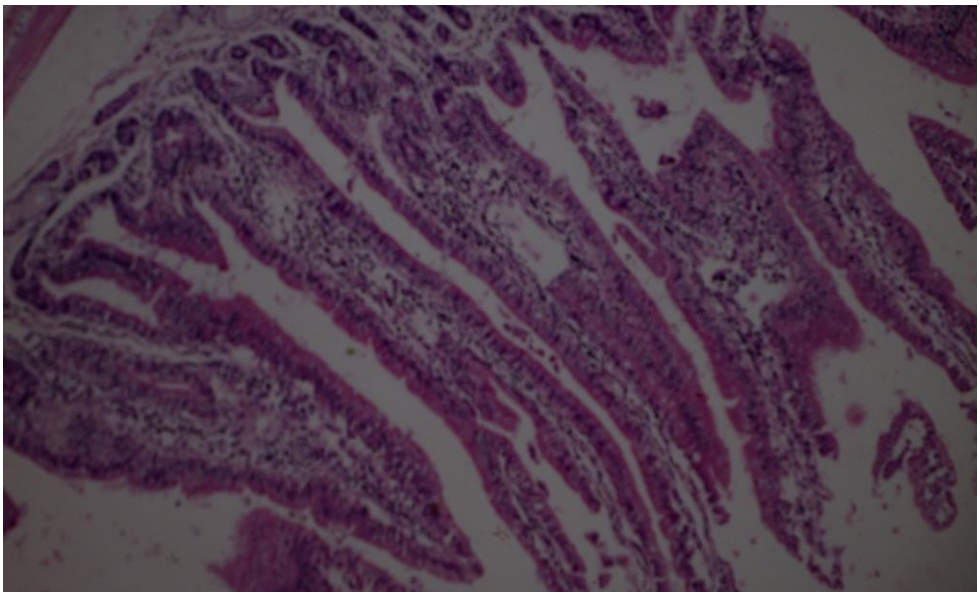

Figure 3. T. S. in rabbits caecum of (T_3) exhibiting improvement in histological picture of the intestinal glands with increase in length of intestinal villi. (H × E × 100).

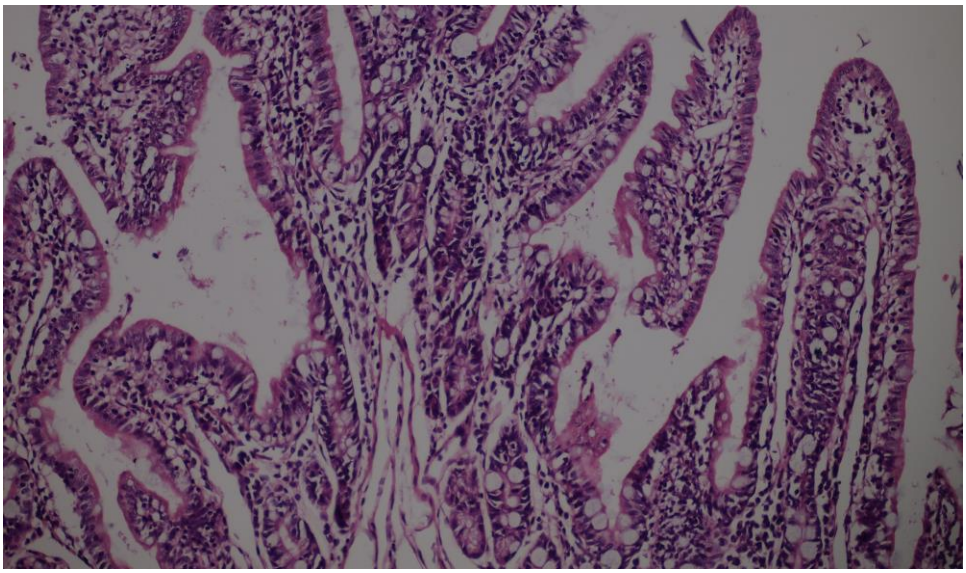

Figure 4 . T. S. in rabbits caecum of (T_4) revealing normal histological picture of intestinal gland with mild hyperplasia of mucous secreting cells (H × E × 400).

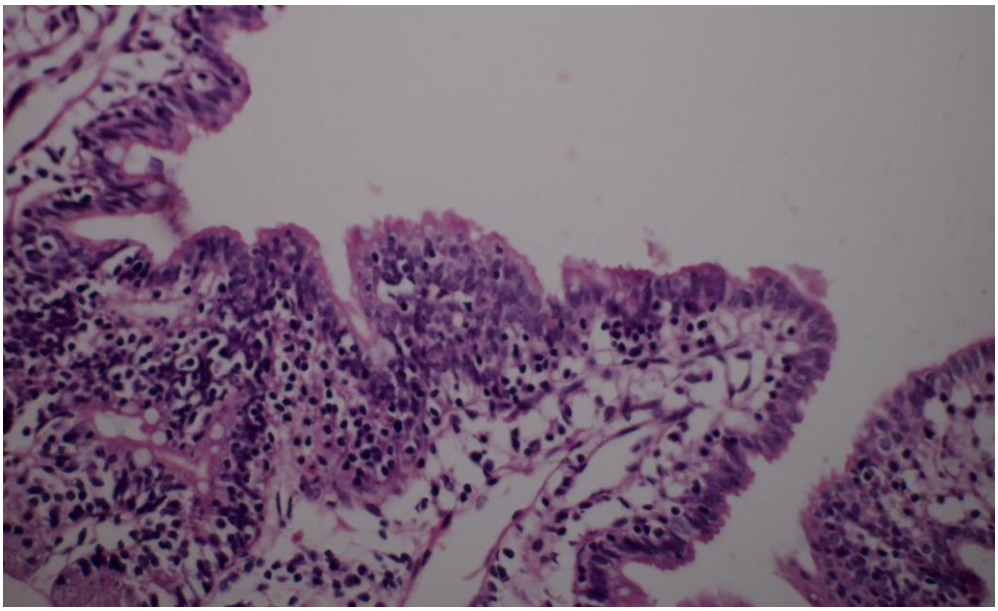

Figure 5. T. S. in rabbits caecum of (T_5) revealing shortening of intestinal villi and glands, few area showing aggregation of mononuclear cells and area of hyperplasia of living epithelium of intestinal glands. (H ×E × 400).

Table 9. Effect of *Pediococcus acidilactici* supplementation on economical traits of growing rabbits at 14 weeks of age.

Items	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Total weight gain (kg)	1.297	1.422	1.388	1.376	1.285
Price of 1kg body weight	40	40	40	40	40
Selling price/rabbit (LE) (A)	51.9	56.9	55.5	55.0	51.4
Total feed intake	4.44	4.30	4.34	4.49	4.45
Price/kg feed(LE)	3.32	3.32	3.32	3.32	3.32
Total feed cost/rabbit (LE)(B)	14.74	14.27	14.40	14.90	14.77
Net revenue(LE)[1]	37.16	42.63	41.10	40.10	36.63
Economic efficiency[2]	2.52	2.98	2.85	2.69	2.48
Relative Econ. Eff.[3]	100	118.25	113.09	106.74	98.41

T_1: Control group, T_2: Group fed diet containing *Pediococcus acidilactici*, once a week, T3: Group fed diet containing *Pediococcus acidilactici*, twice a week, T_4: Group fed diet containing *Pediococcus acidilactici*, skip a day, T_5: Group fed diet containing *Pediococcus acidilactici*, throughout experimental days.

(1) Net revenue = A – B.

(2) Economic efficiency = (A-B/B x 100).
(3) Relative Economic Efficiency= Economic efficiency of treatments other than the control/ Economic efficiency of the control group.

400 mg Bio plus®(*Bacillus subtilis* and *Bacillus licheniformis*) / kg diet as compared with rabbits received un-supplemented diet. Also, Amber *et al*., (2014) found that net revenue was increased when supplementing 0.4 g/ kg Bio plus® (*Bacillus subtilis* and *Bacillus licheniformis*) in rabbit diets.

Conclusively, it is recommended that supplemented 0.1g *Pedicoccus acidilactici* /kg rabbit diet for once, twice a week improve growth performance traits and economic efficiency, without any adverse side effect of growing rabbits.

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تأثير إستخدام البيدوكوكس أسيدى لاكتيسى على الأداء الأنتاجى للأرانب النامية .

ولاء عطية سلامة , مرفت نبيل غزال , صفاء عطايا بركات و عنايات حسن أبو العزايم

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أستخدم فى هذه الدراسة ٦٠ أرنب نيوزيلاندى أبيض مفطوم عمر ٦ أسابيع بمتوسط وزن ٧٠٢±١,٧١ جم وقسمت الأرانب عشوائيا على ٥ مجموعات تجريبية (١٢ فى كل مجموعة تجريبية)، غذيت الأرانب المجموعة الأولى على عليقة كنترول بدون **اضافة** البيدوكوكس أسيدى لاكتيسى (T_1)، بينما المجموعات التجريبية الثانية والثالثة والرابعة والخامسة غذيت على عليقة تحتوى على المعاملة بالبيدوكوكس أسيدى لاكتيسى ١ , جم /كجم علف (١x ١٠^{9} خلية/ جم) على فترات مختلفة مرة واحدة فى الاسبوع (T_2) , مرتين فى الأسبوع (T_3) , يوم ويوم (T_4) و طول فترة التجربة (T_5) وأستمرت تجربة النمو لمدة ٨ أسابيع.

أشارت النتائج أن الأرانب التى غذيت مرة واحدة فى الاسبوع (T_2) أو مرتين فى الأسبوع (T_3) سجلت اعلى قيم معنوية *($P\leq 0.01$)* و*($P<0.05$)* فى وزن الجسم ,الزيادة الوزنية اليومية وكفاءة التحويل الغذائى .حقق نظام التغذية مرة واحدة فى الأسبوع أعلى قيم معنوية فى معاملات هضم البروتين الخام ,الألياف , الدهن , القيمة الغذائية للبروتين المهضوم , المركبات الكلية المهضومة, الطاقة المهضومة و ,النسبة المئوية لوزن الذبيحة و النسبة المئوية للتصافى بالمقارنة بالمجموعات الأخرى .لكن وجد نقص معنوى *($P\leq 0.01$)* في البروتين الكلى ,انزيمات الكبد (AST), الالبيومين , كوليسترول , الليبيدات الكلية , LDL مع التغذية مرة واحدة فى الاسبوع . لكن وجد زيادة معنوية فى عدد كرات الدم البيضاء والأجسام المناعية IgG وكذلك IgM مع التغذية مرة واحدة فى الاسبوع .

إضافة البيدوكوكس أسيدى لاكتيسى وجد زيادة معنوية فى قيم العدد الكلى للبكتريا مع التغذية مرة واحدة فى الاسبوع (T_2) , مرتين فى الأسبوع (T_3) و يوم ويوم (T_4) بالمقارنة بالتغذية على عليقة الكنترول أومع التغذية طول فترة التجربة(T_5) . بينما لم يتأثر معنويا تركيز الأحماض الدهنية الطيارة والأمونيا بالمعاملات المختلفة . كانت الأرانب المغذاه مرة واحدة فى الاسبوع (T_2) , مرتين فى الأسبوع (T_3) زيادة فى villi والغدد المعدية. وأيضا التغذية مرة واحدة فى الاسبوع (T_2) , مرتين فى الأسبوع (T_3) و يوم ويوم (T_4) سجلت أعلى كفاءة أقتصادية.

الخلاصة : إضافة البيدوكوكس أسيدى لاكتيسى بنسبة ١ , جم (١x ١٠^{٩} خلية/ جم) / كجم علف أرانب مرة واحدة فى الاسبوع أو مرتين فى الأسبوع حسنت من الأداء الأنتاجى للأرانب و الكفاءة الأقتصادية دون أى تأثير سلبى على الأرانب النامية.