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EFFECTS OF DIETARY SUPPLEMENTATION WITH TOYOCERIN PROBIOTIC (*Bacillus cereus* var. *Toyoi*) ON SOME PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF CALIFORNIAN RABBITS

K. H. El-Kholy [1], El-Damrawy S.Z.[2], Seleem T.S.T.[1]

[1] Animal Production Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.

[2] Department of Animal Production, Faculty of Agriculture, Tanta University, Egypt.

Three hundred and ninety eight Californian (CAL) rabbits were used in the present study to determine the effects of dietary supplementation with 100 mg Toyocerin® /kg feed on some productive and reproductive performance of growing and mature rabbits.

The present study included two experiments; the first experiment was lasted for 40 days and was carried out during growing period (from weaning to marketing age at 70 days) by using 250 weaned Californian rabbits aged 30 days. The second experiment was lasted for 4 months and was carried out on 128 multiparous does and 20 sexual mature bucks of CAL rabbits aged nine months. The animals of each experiment were divided into two experimental groups. The first group was used as a control and fed a commercial diet without supplementation. The second group was fed a control diet supplemented with 100 mg Toyocerin® probiotic per kg of feed.

The results obtained revealed that, daily body weight gain; feed efficiency and final body weight values of growing CAL rabbits were significantly (P≤0.05) higher with Toyocerin® fed rabbits compared to the control one. Supplementation with Toyocerin® increased significantly (P≤0.05) spleen and thymus indexes and cell mediated immunity. Rabbits received Toyocerin® recorded the highest (P≤0.01) values of caecal bacteria count (total microbial count, lactobacilli and celluloytic bacteria), except for ureolytic bacteria. Blood picture of growing CAL rabbits including red and white blood cells count; hemoglobin concentration and hematocrite percentage and some blood serum constituents including total protein and its fractions (albumin and globulin) were within the normal physiological range, however it recorded the higher values for treatment group. Liver activity of growing CAL rabbits represented by values of each of aspartate *aminotransferase (AST), and alanine aminotransferase (ALT) also increased significantly (P≤0.05) by Toyocerin® supplemented diet.*

Adding Toyocerin® to the diet of CAL buck rabbits significantly (P≤0.05) improved their reproductive traits represented by libido and physical semen quality (semen-ejaculate volume; advanced-sperm motility; live and normal spermatozoa; acrosome status and sperm-cell concentration per ml and per ejaculate);. No significant for Toyocerin® inclusion on scrotal circumference, testicular index and testosterone concentration. Mating activity of CAL buck rabbits received Toyocerin® were significantly (P≤0.05) higher than those fed the control diet. Kindling rate, litter size and weight at birth; milk yield and pre-weaning mortality rate were significantly better (P≤0.05) for those fed Toyocerin® diet than those fed the control diet.

Conclusively, *it can be concluded that dietary supplementation with Toyocerin® probiotic at level of 100 mg per kg of feed improve growth and reproductive performance of Californian rabbits.*

Key words: Rabbit; probiotics; productive; reproductive.

Probiotics are preparations contain non-pathogenic micro-organisms given to animals as feed additives to stimulate their growth and increase the efficiency of feed utilization (Soccol *et al.*, 2010 and El-Kholy *et al.*, 2012). Many researches attributed the beneficial effects of probiotics for animals to a change in enteric flora and decrease in intestinal pH which inhibit the growth of undesirable bacteria (Hollister *et al.*, 1990; Rautava and Isolauri, 2002; Amit-Romach *et al.*, 2010 and Fink, 2010)

Several microorganisms (*Bacillus cereus*, *Bacillus substilis*, *Enterococcus faecium*, *Lactobacillus farciminis*, etc) have been authorized as new additive for feedstuffs (Auclaiar, 2011) and all these strains have been reported to demonstrate positive influence on different animal models namely broiler chicken, beef cattle, dairy cow, piglets, and rabbits. The proposed mechanisms for probiotic effects of the Bacillus spores are based on immunomodulation, which occurs through the stimulation of the gut-associated lymphoid tissue (GALT) by production of cytokines, competitive exclusion of gastrointestinal pathogens (e.g. competition for adhesion sites) and secretion of antimicrobial substances (Fuller, 1991; Corcionivoschi *et al.*, 2010 and Malago and Koninkx, 2011). *Bacillus cereus* var. *toyoi* (Toyocerin®), has been found to improve the performance of growing rabbits (Trocino *et al.*, 2005) and some reproductive rabbit does (Nicodemus *et al.*, 2004).

No studies have been performed yet in Egypt to evaluate the effects of dietary inclusion the preparation of Toyocerin® probiotic on performance of growing rabbits.

Therefore, the aim of the present work was to study the effect of dietary supplementation of Toyocerin® (*Bacillus cereus* var. *toyoi*) at level of 100 mg/kg on some productive and reproductive performance of growing and mature Californian rabbits.

MATERIALS AND METHODS

Farm and animals

The present study was conducted in a Private Rabbitry, located in Sakkara, Giza governorate, Egypt, during the period from March till June, 2012.

Animals were individually housed in wired battery cages supplied with feeders and stainless steel nipples for feeding and drinking. All batteries were located in a windowed rabbitry with natural ventilation. Fresh tab water was automatically available all the time in each cage. All the experimental animals were healthy and clinically free from internal and external parasites and were kept under the same managerial and hygienic conditions.

Experimental design

The present work included two experiments; the first experiment was lasted for 40 days and was carried out during the growing period by using 250 weaned Californian (CAL) rabbits aged 30 days. The second experiment was lasted for 4 months and was carried out on 128 multiparous does and 20 sexual mature bucks of Californian (CAL) rabbits aged nine months.

The animals in the two experiments were divided into two equal comparable experimental groups, 125 growing rabbits for each in the 1st experiment and 10 bucks and 64 does in the 2nd experiment. The first group was fed un-treated diet (control), while the other group was fed diet treated with Toyocerin® preparation (concentration: 1 x 10^9 *Bacillus cereus* var. *toyoi* spores/g of Toyocerin®). The Toyocerin® preparation was produced by Rubinum S.A. (Barcelona, Spain) in powder form. The preparation was used by mixing it with the vitamin and mineral premix and then included in the treated diet before pelleting. The diets were pelleted to a diameter of 3.5 mm and a length of 1.0-1.1 cm. In general, the pelleted diets covered the nutritional requirements of the growing and mature phase of rabbits according to NRC (1977) recommendations. Ingredients and chemical composition of the pelleted diets are shown in Table 1.

Table 1: Ingredients and chemical composition of diets used during the experimental periods

Ingredients	Growing diet	Mature diet
Artificially dried Alfalfa (IBEX Alfalfa)	29.00	26.95
Wheat bran	35.45	27.50
Yellow corn	20.35	20.70
Soybean meal (44%)	10.00	18.00
Molasses	3.25	3.40
Calcium carbonate (lime stone)	0.80	0.75
Sodium chloride	0.30	0.30
Vitamins & Mineral Premix [1]	0.30	0.30
DL-Methionine	0.10	0.10
Di-Calcium phosphate	0.45	2.00
Total	**100.00**	**100.00**
Calculated chemical composition [2]		
Crude protein (CP)	16.0	18.0
Ether extract (EE)	2.98	2.87
Crude fiber (CF)	12.8	10.52
Digestible energy (Kcal/Kg)	2625	2600

[1] Vitamins and minerals premix per kilogram diet contains:
Vit. A, 6000 IU; Vit. D, 900 IU; Vit. E, 40 mg; Vit. K3, 2 mg; Vit. B1, 2 mg; Vit. B2, 4 mg; Vit. B6, 2 mg; Vit. B12, 10 µcg; Nicotinic acid, 50 mg; Biotin, 50 µcg; Folic acid, 10 mg; Choline chloride, 250 mg; Zinc, 50 mg; Manganese, 85 mg; Iron, 50 mg; Copper, 5 mg; Iodine, 0.2 mg; Selenium, 0.1 mg; Cobalt, 0.1 mg.
[2] According to NRC (1977) for rabbits.

Recordings, measurements and sampling procedures:

The averages of daily weight gain, daily feed intake and feed efficiency ratio, for growing CAL rabbits, were calculated and recorded weekly, during fattening period. The performance index (PI) was calculated according to North (1981) as the following equation:

PI= (Final body weight / Feed conversion) × 100.

At the end of 1St experimental period, five representative rabbits from each group were randomly selected to determine cell mediated immunity (CMI). Fifty µg of PHA (Phytohemagglutinin-P; Difco, Detroit, MI) in 0.1 ml of sterile pyrogen-free physiologic saline or saline only was injected intradermally (ID) into the right and left ear, respectively. Central ear thickness of each rabbit was measured with a constant-tension dial micrometer (Mitutoyo Co., Tokyo, Japan) just before the injection and again every 3 hrs later (from 0-24 hrs). The response was recorded in millimeters as the difference between PHA response (right ear) and the saline response (left ear) after injection (Heba El-Lethey *et al.*, 2003).

At marketing age (70 days), twenty growing rabbits from each group of the first experimental group were randomly taken for slaughter after being fasted for 12 hours. After complete bleeding, spleen and thymus weight was weighed after the rabbits were slaughtered and spleen index and thymus index were calculated according to the following formulae of Fu-Chang *et al.* (2004): spleen index=spleen weight/body weight, thymus index=thymus weight/body weight. Also, the microbial content of the caecum of slaughtered rabbits (6 rabbits/group) was estimated in their selective media, as described by Harrigan *et al.* (1976) for total microbial, lactobacilli bacteria and cellulolytic bacteria and Difco (1971) for ureolytic bacteria. Technique of colony forming unit (CFU) was adopted. Incubation took place at 30° C - for 2-7 days.

At the end of experiment 1 (70 days age), blood samples were collected during slaughter of 25 rabbits within each experimental group. Blood samples were collected into dry clean centrifuge tubes. Blood serum was separated by centrifugation at 3000 r.p.m. for 20 minutes and kept in a deep freezer at (-20 ^{0}C) until biochemical analysis. Non-coagulated blood was tested shortly after collection for estimating blood pictures. Red and white blood cells were counted according to Feldman *et al.* (2000). Hemoglobin concentration and Hematocrite percentage were measured according to Drew *et al.* (2004). Total protein was determined by the method described by Henary *et al.* (1974) using Biuret reagent kit. Albumin was determined by the method described by Doumas *et al.* (1971) using Bromocresol green kit. Globulin level values were obtained by subtracting the values of albumin from the corresponding values of total protein. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated according to Huang *et al*. (2006). During the 2nd experiment, blood samples of buck rabbits were taken from the marginal ear vein of six rabbit bucks within each experimental group monthly. Blood serum testosterone concentration of the buck rabbits was determined using RIA Kits (Immunotech, A Coulter Co., France) according to the manufacturer information.

In the experiment 2, the measurements of mature bucks including scrotal circumference (10 rabbits/group) which was measured by the method described by Boiti *et al*. (2005). Testicular index (length × width × depth) (10 rabbits/group) was calculated in cubic centimeters as recorded by Castellini *et al*. (2006). Semen was collected artificially twice a week from ten bucks from each group during the experimental period by means of an artificial vagina as described by Boiti *et al*. (2005). Immediately after collection, semen ejaculate volume (mL), advanced sperm motility (%), alive spermatozoa (%), morphological normal spermatozoa (%), acrosomal damages (%), sperm-cell concentration (N×10^6/mL) and total-sperm output (N×10^6/ejaculate) were estimated according to Boiti *et al*. (2005) and Castellini *et al*. (2006). Libido (sexual desire) was assessed in terms of reaction time in seconds estimated from the time of introducing doe to the buck until the buck started to mount (Castellini *et al*., 2006). Mating activity (frequency of mating within 15 minutes) of ten bucks was determined using sexually receptive does.

To determine the fertility traits, 128 CAL doe rabbits in two experimental groups (64 in each) were naturally mated in two sequence parities using the experimental groups of rabbit bucks. Natural mating was carried out by transferring each doe to the buck's cage to be mated and return back to its cage after mating. Palpation of all rabbit does was carried out 12 days post mating to determine pregnancy. At kindling, kindling rate and litter size and weight at birth values were recorded. Pre weaning mortality rates and milk yield per doe were estimated also during the suckling period. Milk yield was estimated after deprivation of pups from suckling their mothers at 8 a.m. daily, then the pups were weighed before and after suckling, the increase in pup's weight was used as the doe milk yield.

Statistical analysis

Data of the first and second experiments were statistically analyzed according to SPSS (2012) computer program using the following fixed model :-

$$Y_i = \mu + B_i + e_i$$

Where: Y_i = Observation of the i^{th} rabbit; μ = Overall mean, common element to all observations; B_i = Effect of the treatments (i = 1 & 2); e_i = Random error component assumed to be normally distributed.

Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory, 2005) before being statistically analyzed. All data are presented least squares means.

RESULTS AND DISCUSSION

1- Growing rabbits performance (Experiment 1)

1.1- Growth performance traits:

Inclusion of Toyocerin® significantly ($P \leq 0.05$) improved final body weight at 70 days; daily weight gain; feed efficiency and feed conversion values of growing CAL rabbits (Table 2). Final body weight and daily body weight recorded 14.8 and 12.0% respectively higher in the treated rabbits than the control one. These results are in agreement with those stated by Trocino *et al.* (2005) and Matusevicius *et al.* (2011). On the other hand, Pascual *et al.* (2008) showed that dietary inclusion of this probiotic did not affect the growth rate, feed intake and feed conversion rate during the growing period. Performance index values in Table (2) showed that PI in treated group was significantly higher than control group; this may be due to the higher daily gain and feed conversion. The improvement in growth performance resulted from the addition of Toyocerin® could be due to the beneficial microbes which improve nutrient utilization by a) increase on intestinal metabolic activity, b) modification of intestinal microbiota by the exclusive competition with intestinal pathogenic bacteria, c) modification of the structure and function of

Table 2: Influence of dietary supplementation with Toyocerin® on some growth performance traits and economic efficiency of growing Californian rabbits during fattening period (Means ± SE.)

Variables	Treatment groups: Control	Treated	Improvement (%)
Body weight (g)			
Initial at 30 days	568.2 ± 19.9	551.3 ± 21.4	- 2.97
Final at 70 days	1451.2 ± 28.6^{B}	1665.4 ± 33.8^{A}	+ 14.76
Daily gain	27.92 ± 1.5^{B}	31.26 ± 1.2^{A}	+ 11.96
Feed intake (g)			
Daily feed intake	78.61 ± 5.7	75.92 ± 4.8	- 3.42
Feed efficiency, weight gain/feed intake	0.355 ± 0.02^{B}	0.412 ± 0.03^{A}	+ 16.06
Feed conversion, feed intake /weight gain	2.82 ± 0.2^{A}	2.43 ± 0.3^{B}	- 13.83
Performance Index (%)	51.46 ± 3.16^{B}	68.53 ± 3.88^{A}	+33.17
Economic efficiency			
Price of diet (L.E/ ton)[1]	2300	2320	+ 0.86
Daily cost of feed (L.E)[2]	0.181	0.176	- 2.76
Cost of feeding, during fattening period[3]	7.23	7.04	+ 2.63
Cost of each 1 Kg rabbit meat Only, regarding converting diet to meat	6.49	5.64	- 13.10
Selling price (L.E/ head)[4]	30.48	34.98	+ 14.76
Net profit of each head[5]	18.25	22.94	+ 25.70

Means within the same row bearing different letter superscripts (A, B) are significantly (P≤0.05) different.
[1] The price was calculated on the base of ingredients price through the Experimental period; L.E = Egyptian pound.
[2]Daily cost of feed (L.E/ head) = price of diet × daily feed intake
[3]Cost of feeding, during fattening period = daily cost of feed × 40 (fattening period)
[4]Selling price of 1 Kg = 21.00 L.E.
[5]Net profit of each head = [Price of rabbit meat/ head – cost of feed during fattening period + 5 (Considering each head coasted 5 L.E included rent, mortality, all managerial efforts, …..etc.)].

the intestinal epithelium, stimulating the immune system (Corcionivoschi *et al.*, 2010 and Malago and Koninkx, 2011). Quigley (2010) demonstrated that these effects are mainly metabolic in nature at specific sites with specific metabolic activity (eg. in the gut). Another reported effect of probiotics is due to their action in the translocation of pathogens, which will be able to prevent enteric infections (Nakamura *et al*., 2002). On the other hand, decreased in daily feed intake by adding Toyocerin® may be due to an increase in the efficiency of nutrients utilization.

Data in Table 2 show that the total feeding cost increased by 0.68% with adding Toyocerin® to growing diet as compared with control diet. However, selling price of total meat yield increased by 14.76% in the treated group. Economically, net profit values of each head of CAL growing rabbits were improved by 25.70 % for the group fed diet contained Toyocerin® as compared to the control group during overall fattening period. The results showed that the feed additive had a positive effect on the economic efficiency.

1.2- Lymphatic organs system:

Immune organs are those whose functions help maintain the normal immune status of the bodies of animals (Feng *et al.*, 2007). In this regards, the weight of lymphoid organ serve as a measure of the immune status (Pope, 1991). The results of the present work as presented in Table 3 showed that treatment with Toyocerin® caused significant ($P \leq 0.05$) increase in spleen and thymus indexes. However, Wallace *et al.* (2012), showed no significant improvement in spleen and thymus indexes due to probiotic treatment. Spleen is peripheral immunity organ and is the biggest immunity organ in animal body, which larges when the body weight increase. Spleen is identified as the secondary lymphoid tissue (Stephen, 2007). Thymus is main immunity organ. It also is the main place of differencing, maturing T lymphocyte and excreting thymus hormone (Fu-Chang *et al.*, 2004). The bigger the immunity index is, the stronger the immunity capability is (Fu-Chang *et al.*, 2004). They added that when nutrient intake increase, organ is becoming mature and function is becoming perfect, and it is consistent with the growth rule of animal. Also, this improvement can be due to the improvement in immune responsiveness where the proposed mechanisms for probiotic effects of the Bacillus spores are based on immunomodulation, which occurs through the stimulation of the gut-associated lymphoid tissue (GALT) by production of cytokines, competitive exclusion of gastrointestinal pathogens (e.g. competition for adhesion sites) and secretion of antimicrobial substances (Fuller, 1991 and Oelschlaeger, 2010). In this context some recent literature shows that *B. cereus var. toyoi* alters the immune status as well as functionalities of systemic immune cell populations (Schierack *et al.*, 2007).

Probiotic micro-organisms in the gut reportedly have the capacity to stimulate the immune system either by migrating through the gut wall as viable cells which multiply to a limited extent or antigens released by the dead organisms get absorbed and stimulate the immune system directly (Wallace *et al.*, 2012). It seems that the literature is still sparse on the effect of different probiotics on rabbit's lymphatic organs system.

1.3- Blood parameters :

Data presented in Table (4) show that all blood parameters were within the normal physiological range in the control and treated animals. However, the ranges of these blood parameters were higher in the treated animals than in the control. Probiotic may improve immune function by increasing the number of IgA producing plasma cells, improving phagocytosis as well as increasing the proportions of T- lymphocytes and natural killer cells (Srividya and Vishnuvarthan, 2011). Also, addition of Toyocerin® as probiotics increasing the bio-availability of iron which in turn affected blood hemoglobin (Fuller, 1991).

Table 3: Influence of dietary supplementation with Toyocerin® on immunity index of growing Californian rabbits at 70 d of age (Means ± SE.)

Variable	Treatment		
	Control	**Treated**	**Improvement (%)**
Live body weight, g (BW)	1451.2 ± 28.6^{B}	1665.4 ± 33.8^{A}	+ 14.76
Spleen index (weight % BW)	0.085±0.011^{B}	0.098±0.009^{A}	+15.29
Thymus index (weight % BW)	0.105±0.012^{B}	0.118±0.015^{A}	+12.38

Means within the same row bearing different letter superscripts (A, B) are significantly (P≤0.05) different.

Table 4: Influence of dietary supplementation with Toyocerin® on blood picture and some blood serum constituents of growing Californian rabbits (Means ± SE.)

Variable	Treatment		
	Control	**Treated**	**Improvement (%)**
Red blood cells (N × 10^{6}/mm^{3})	5.62 ± 0.51^{B}	6.43 ± 0.44^{A}	+ 14.41
White blood cells (N × 10^{3}/mm^{3})	6.74 ± 0.82^{B}	7.91 ± 0.87^{A}	+ 17.36
Hemoglobin (g/dl)	10.31 ± 1.06^{B}	11.89 ±1.01^{A}	+ 15.32
Hematocrite (%)	35.41 ± 1.12^{B}	37.64 ± 0.92^{A}	+ 6.30
Total protein (mg/ 100 ml)	6.49 ± 0.96^{B}	7.86 ± 0.99^{A}	+ 21.11
Albumin (mg/ 100 ml)	3.36 ± 0.87^{B}	4.54 ± 0.69^{A}	+ 35.12
Globulin (mg/ 100 ml)	3.13 ± 0.14^{B}	3.32 ± 0.12^{A}	+ 6.07
Albumin/ Globulin ratio	1.07 ±0.09^{B}	1.37 ± 0.13^{A}	+ 28.04
AST (U/ L)	25.41 ± 1.99^{B}	28.39 ± 1.58^{A}	+ 11.73
ALT (U/ L)	15.66 ± 0.95^{B}	18.09 ± 1.07^{A}	+ 15.52

Means within the same row bearing different letter superscripts (A, B) are significantly (P≤0.05) different

Wallace *et al.* (2012) showed that increased of WBC in growing rabbits by dietary inclusion of probiotics could be due to the stimulating and boosting the immune status of the rabbits. The increase of blood total protein in treated rabbits may be attributed to the improvement in crude protein digestibility and decrease in intestinal pH that accordingly suppress the growth of most pathogenic bacteria, which leads to optimal enzyme activity (Hoyos and Cruz (1990). The significant increase (P≤0.05) in the concentrations of serum transaminase enzymes (AST and ALT), serum total protein and their fractions (Alb and Glb) in the treated animals compared to control may reflect an increase in the hepatic function as mentioned by El-Harairy *et al.* (2003).

1-4. Lipids profile:

In Table 5, the probiotic treated rabbits had significantly (P≤0.05) lower total lipids, phospholipids, triglycerides, HDL-cholesterol and total cholesterol compared with those from the control group. These findings agree with the results of Ghoneim and Moselhy (2012) who reported that, the decrease in cholesterol levels could be associated with a reduction in cholesterol biosynthesis in the liver and an increase in the degradation of bile acids by

Table 5: Influence of dietary supplementation with Toyocerin® on serum lipid profile of growing Californian rabbits (Means ± SE.)

Variable	Treatment		
	Control	Treated	Improvement (%)
Total lipids (ng/L)	181.32±9.41^{A}	163.29±7.77^{B}	- 9.95
Phospholipids (ng/L)	2.01±0.6^{A}	1.14 ± 0.04^{B}	- 43.28
Triglycerides (ng/L)	406.41±24.31^{A}	371.52±21.18^{B}	- 8.58
HDL-cholesterol (ng/L)	9.17±0.96^{A}	6.82 ± 0.54^{B}	- 25.63
Total cholesterol (ng/L)	136.68±6.12^{A}	128.69 ± 4.67^{B}	- 5.85

Means within the same row bearing different letter superscripts (A, B) are significantly ($P \leq 0.05$) different.

probiotic. Studies in the literature have demonstrated that probiotics are able to decrease the serum cholesterol levels by breaking down the bile juice in the gut, thus inhibiting its re-absorption which enters the blood as cholesterol (Srividya and Vishnuvarthan, 2011). In addition, evidence has indicated that probiotics (*lactobacilli* and *bifidobacteria*) may cause, by ingestion, a significant reduction in serum cholesterol. This is because cholesterol synthesis mainly occurs in the intestines, hence the gut microflora promote effects on lipid metabolism. Some studies demonstrated that probiotics could promote a decrease in the blood cholesterol levels and increase the resistance of low-density lipoprotein to oxidation, therefore leading to a reduced blood pressure (Goel *et al.*, 2006).

It was also indicated in the literature that thyroid hormones greatly affect enzymes involved in the fatty acid and glycerolipid synthesis in tissues (Ito *et al.*, 2009).

1.5- Phytohemagglutinin-elicited skin reaction:

The present experiment demonstrates that Toyocerin® treatment impulses cell-mediated immunity in growing CAL rabbits (Figure 1). This result may be due to the effect of Toyocerin® improving thymus index which lead to produced more T-cells. T-cells undergo maturation in the thymus gland and play a major role in cell-mediated immunity (Stephen, 2007). The present results are in agreement with those of Al-Ammar *et al.* (2012). There were good indications that cell-mediated immunity plays an important role in controlling and cleaning intracellular bacterium (Kougt *et al.*, 1995). Many studies have demonstrated that probiotic can be stimulate the immune system by modification of the structure and function of the intestinal epithelium (Corcionivoschi *et al.*, 2010 and Malago and Koninkx, 2011).

The changes in the cell mediated immunity and lymphatic organs system are due to Toyocerin® inclusion, provided evidence of improved growing rabbit's immunity. Thus, protective immune responses require a supply of nutrients at the appropriate times and amounts (Humphrey *et al.*, 2002).

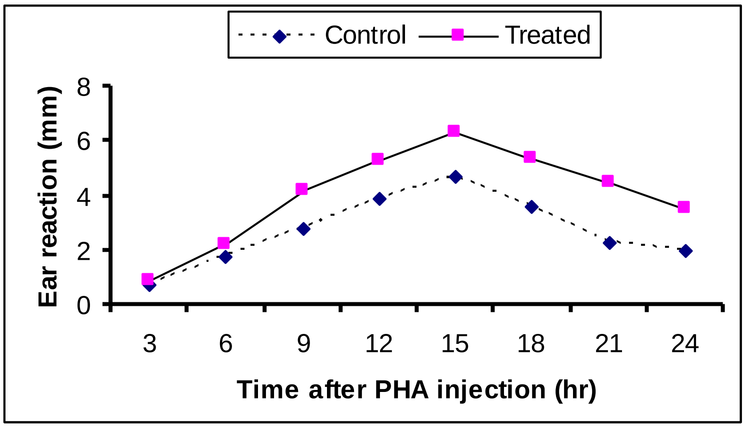

Figure 1. Cutaneous response of Californian growing rabbits as affected by Toyocerin® treatment to phytohemagglutinin-P (PHA) expressed as difference in ear thickness (mm).

1.6- Caecal bacteria count:

The supplementation of Toyocerin® had significant effect on the caecal contents (Table 6). Total microbial count, *Lactobacilli* and cellulolytic bacteria were significantly increased by adding dietary probiotic., but ureolytic bacteria count decreased ($P \leq 0.05$) by 61.01% for rabbits fed diet with Toyocerin®. These results are in agreement with observed by Amber *et al.* (2004). Ureolytic bacteria was decreased ($P \leq 0.01$) by 61.01% for rabbits fed diet with Toyocerin®. This may be due to suppress the growth of bacteria as a result to moderate pH of the media (Yeo and Kim, 1997). Competition has been shown to exist between *Bacillus* sp. and the pathogenic flora at the gastro-intestinal level, which could help in maintaining a positive flora and good health condition (Trocino *et al.*, 2005**)**. A healthy GIT microflora may provide an optimal precondition for effective protection against pathogenic microorganisms, ultimately resulting in improved performance in animals (Niba *et al.*, 2009).

Table 6: Influence of dietary supplementation with Toyocerin® on caecal bacteria count ($N \times 10^{5}$ $CFUml^{-1}$) of growing Californian rabbits

Variable	Treatment groups		
	Control	**Treated**	**Improvement (%)**
Total microbial count	14.27 ± 5.54^{B}	76.54 ± 18.22^{A}	+ 436.37
Ureolytic bacteria	3.18 ± 0.37^{A}	1.24 ± 0.21^{B}	- 61.01
Celluloytic bacteria	4.62 ± 0.95^{B}	8.94 ± 1.47^{A}	+ 93.51
Lactobacilli	3.87 ± 1.14^{B}	41.68 ± 9.55^{A}	+ 981.65

Means within the same row bearing different letter superscripts (A, B) are significantly ($P \leq 0.05$) different

2- Mature rabbits performance (Experiment 2)

2.1- Reproductive performance of bucks

Data presented in Table 7 indicate that, libido (sexual desire) and physical semen characteristics (semen-ejaculate volume; advanced-sperm motility; live and normal spermatozoa; acrosome status; sperm-cell concentration and total-sperm output) were significantly higher ($P \leq 0.05$) in bucks fed Toyocerin® diet compared to the control one. These results are in agreement with findings of Humphrey *et al.* (2002) and Metwally *et al.* (2002) who found that probiotics supplementation lead to improvement in semen quality in breeding males. Al-Daraji (2012) attributed the improvement of semen quality to the powerful role of probiotic in enhancing the process of sperm formation, sperm maturation, and other parameters of sperm quality.

In this connection, Dhami and Kodagali (1987) reported a positive correlation between AST activity in seminal plasma and sperm concentration, live sperm percent, motility, semen volume and fertility rate of semen. Also, they reported that AST enzyme plays an important role in sperm metabolism through its involvement in the vital cellular process. So, it is suggested that increased AST enzyme in blood (Table 4) may be responsible for the increase of AST in seminal plasma which would explain the improvement of semen quality recorded in the present study. Accordingly, it seems that Toyocerin® probiotic may play an indirect role in rabbit spermatogenesis.

Table 8 shows that, scrotal circumference, testicular index and testosterone concentration were not affected by the treatment. It is so clear that mating activity (number of mating within 15 minutes) significantly improved ($P \leq 0.01$) by +38.69% in the treated group compared to the control. This effect seems to be related to an increase on health status by Toyocerin®.

2.2- Reproductive performance of does:

Data presented in Tables 9 and 10 show that, kindling rate, litter size and weight at birth; and milk yield/doe were significantly higher ($P \leq 0.05$) in doe rabbits fed the Toyocerin® diet than the control. These results support previous reports on using commercial probiotics by Ashour *et al.* (2004) and Pinheiro *et al.* (2007). In this respect, Cheek (1989) reported that probiotic dietary additives lead to increase in appetite and feed efficiency of rabbits; this may leads to an increase in milk secretion and its yield. Beside that, the increase in milk production may lead to an increase in litter size at birth where there was a positive correlation between the litter size and milk yield (Lebas *et al.*, 1997 and Rommers *et al.*, 2001). The improvement in litter traits prove that the Toyocerin® treatment improve doe milk yield.

Table 7: Influence of dietary supplementation with Toyocerin® on libido and physical semen characteristics of Californian rabbit bucks (Means ± SE.)

Variable	Treatment groups		
	Control	Treated	Improvement (%)
Libido- (sec.)	39.41 ± 6.42^{A}	25.67 ± 5.64^{B}	- 34.86
Semen-ejaculate volume (ml)	0.49 ± 0.05^{B}	0.87 ± 0.04^{A}	+ 77.55
Advanced-sperm motility (%)	57.64 ± 4.19^{B}	78.05 ± 4.98^{A}	+ 35.40
Dead spermatozoa (%)	24.19 ± 3.99^{A}	18.40 ± 2.87^{B}	- 23.92
Sperm abnormalities (%)	18.94 ± 3.43^{A}	15.22 ± 2.07^{B}	- 19.64
Acrosomal damages (%)	14.41 ± 1.88^{A}	11.21 ± 1.60^{B}	- 22.20
Sperm-cell concentration ($N\times10^{6}$/ml)	397.29±41.53^{B}	591.33±36.61^{A}	+ 48.84
Total-sperm output ($N\times10^{6}$/ejaculate)	194.65±26.32^{B}	514.41±31.62^{A}	+ 164.24

Means within the same row bearing different letter superscripts (A, B) are significantly ($P\leq0.05$) different.

Table 8: Influence of dietary supplementation with Toyocerin® on scrotal circumference, testicular index, testosterone concentration and mating activity of Californian rabbit bucks (Means ± SE.)

Variable	Treatment		
	Control	Treated	Improvement (%)
Scrotal circumference (cm)	7.87± 1.54	7.94 ± 1.31	+ 0.89
Testicular index (cm^{3})	7.84 ± 0.91	7.91 ± 0.86	+ 0.89
Testosterone concentration (ng/ ml)	4.72 ± 0.68	4.54 ± 0.61	- 3.81
Mating activity (frequency of mating/15 minutes)	3.67 ± 0.36^{B}	5.09 ± 0.69^{A}	+ 38.69

Means within the same row bearing different letter superscripts (A, B) are significantly ($P\leq0.05$) different.

Table 9: Influence of dietary supplementation with Toyocerin® on some reproductuve traits of Californian doe rabbits naturally mated (Means ± SE.)

Variable	Treatment groups		
	Control	Treated	Improvement (%)
Number of mated does	64	64	-
Number of conceived does	42 ^{B}	56 ^{A}	+ 33.33
Kindling rate (%)	65.63 ^{B}	87.50 ^{A}	+ 33.32
Litter size at birth	5.94 ± 0.49 ^{B}	8.29 ± 0.74 ^{A}	+ 39.56
Litter weight at birth (g)	243.24±29.24.5^{B}	373.57±27.35^{A}	+ 53.58
Bunny weight at birth (g)	40.96 ± 1.71 ^{B}	45.06 ± 2.09 ^{A}	+ 10.01
Bunny weight at weaning (g)	561.45 ± 27.34 ^{B}	697.47 ± 31.42 ^{A}	+ 24.23

Means within the same row bearing different letter superscripts (A, B) are significantly ($P\leq0.05$) different.

Table 10: Influence of dietary supplementation with Toyocerin® on milk yield of Californian doe rabbits naturally mated (Means ± SE.)

Variable	Period		Treatment groups		
	From	Till	Control	Treated	Improvement (%)
Milk yield (g/ doe)	Birth	7 days	541.09 ± 41.52^{B}	715.22± 37.25^{A}	+ 32.18
	Birth	14 days	1187.12 ± 53.25^{B}	1492.56 ± 58.19^{A}	+ 25.71
	Birth	21 days	2101.08 ± 58.64^{B}	2375.22 ± 61.18^{A}	+ 13.05
	Birth	28 days	2762.44 ± 57.14^{B}	3021.29 ± 54.96^{A}	+ 9.35

Means within the same row bearing different letter superscripts (A, B) are significantly ($P \leq 0.05$) different.

Data in Table (11) recorded a significantly ($P \leq 0.05$) decrease in pre-and post-weaning mortality rate (%) of Toyocerin® treated group. These results agree with those of Ashour *et al.* (2004) and Pinheiro *et al.* (2007) who reported that probiotics reduced mortality rate by about 50% compared with the control. The authors added that the reduction in mortality rate in growing rabbits may be as a result of production of enough hydrogen peroxide in the gut that inhibit several micro-organisms (Fox, 1988), inhibition of *E. coli* enterotoxemic activity (Malago and Koninkx, 2011). In addition, Lee *et al.* (2000) reported that *E. coli* counts, in small intestine, were reduced by 25% of neonatal rabbits fed probiotics in their diet.

On the other hand, the milk available per kit may also have a pronounced effect on the mortality of young rabbits (Rommers *et al.*, 2001 and Szendro *et al.*, 2002).

Table 11: Influence of dietary supplementation with Toyocerin® on mortality rate (%) of Californian doe rabbits naturally mated (Means ± SE.)

Variable	Period		Treatment groups		
	From	Till	Control	Treated	Improvement (%)
Pre-weaning mortality rate (%)	Birth	7 days	8.00±1.5^{A}	6.40±1.2^{B}	- 20.00
	8 days	14 days	6.96±1.8^{A}	5.98±1.1^{B}	- 14.08
	15 days	21 days	5.61±1.1^{A}	5.45±1.3^{B}	- 2.85
	22 days	30 days	4.95±1.3^{A}	4.81±1.4^{B}	- 2.83
Total Pre-weaning mortality rate (%)			25.14±1.1^{A}	22.33±0.9^{B}	- 11.18
Post-weaning mortality rate (%	31 days	40 days	3.13±1.2^{A}	3.03±1.2^{B}	- 3.19
	41 days	50 days	3.23±0.9^{A}	2.08±1.1^{B}	- 35.60
	51 days	60 days	2.22±0.8^{A}	1.06±0.8^{B}	- 52.25
	61 days	70 days	1.14±0.7^{A}	0.00±0.9^{B}	- 100.00
Total Post-weaning mortality rate (%			9.65±1.8^{A}	6.17±1.7^{B}	- 36.06
Total mortality rate (%)			34.40±2.2^{A}	28.52±2.0^{B}	- 17.09

Means within the same row bearing different letter superscripts (A, B) are significantly ($P \leq 0.05$) different.

In conclusion, the results of this study indicate that dietary supplementation with Toyocerin® probiotic improved productive and reproductive performance of growing and mature rabbits under Egyptian environmental conditions. However, further studies are needed to determine the effect of this feed additive on reproductive hormones especially in the subsequent parities.

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الإضافة الغذائية بمستحضر بروبيوتك التويوسيرين (الباسليس سيريس فار تويو) على الأداء الإنتاجي والتناسلي لأرانب الكاليفورنيا

خالد حسان الخولى[١]- سعد زغلول الدمراوي[٢] - طارق سليمان توفيق سليم[١]
١- معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية – الدقي - جيزة - مصر.
٢- قسم الانتاج الحيوانى - كلية الزراعة - جامعة طنطا - طنطا - مصر.

أستخدم فى هذه الدراسة ٣٩٨ أرنب كاليفورنيا لدراسة تأثير الإضافة الغذائية لمركب التويوسيرين (*bacillus cereus* var. *toyoi*) كمنشط نمو "بروبيوتك" على بعض معدلات الأداء الإنتاجية والتناسلية لأرانب الكاليفورنيا النامية والبالغة.

إشتملت الدراسة على تجربتين أساسيتين. التجربة الأولى إستغرقت ٤٠ يوماً، وأجريت على ٢٥٠ أرنب نامى مفطوم حديثاً على عمر ٣٠ يوماً، حيث قُسمت الأرانب إلى مجموعتين تجريبيتين (كل مجموعة ١٢٥ أرنب) أُتخذت المجموعة الأولى كمقارنة (كنترول) والثانية تم إعطاؤها ١٠٠مللجم من مركب التويوسيرين/كجم علف. والتجربة الثانية إستغرقت أربعة أشهر وأجريت على ٢٠ ذكر ناضج جنسياً، و ١٢٨ أم ناضجة وضعت أكثر من بطن، قُسمت أيضاً لمجوعتين تجريبيتين، أشتملت كل مجموعة على ١٠ ذكر ناضج جنسياً و ٦٤ أم فى بطنين متتاليين، المجموعة الأولى هى مجموعة الكنترول والمجموعة الثانية تم إعطاؤها نفس عليقة الكنترول مُضاف إليها ١٠٠ مللجم مركب التويوسيرين/كجم عليقة (المجموعة المُعاملة).

أوضحت النتائج المتحصل عليها من هذه الدراسة: أن قيم كل من الزيادة اليومية فى وزن الجسم وكفاءة تحويل الغذاء وكذلك وزن الجسم النهائي عند عمر ٧٠ يوماً لأرانب الكاليفورنيا المعاملة كانت أعلى معنوياً (عند مستوى ٥٪) مقارنة بمجموعة الكنترول. أظهرت أرانب المجموعة المعاملة زيادة معنوية (عند مستوى ٥٪) فى نسبة أوزان الأعضاء الليمفاوية، وأيضاً قيم صورة الدم متمثلة فى كل من (عدد كريات الدم الحمراء والبيضاء، وتركيز الهيموجلوبين، ونسبة الهيماتوكريت)، بالإضافة إلى بعض مكونات الدم الأخرى مثل تركيز كل من بروتين بلازما الدم الكلي ومفرداته (الألبيومين، الجلوبيولين). سجلت مستويات إنزيمي الـ ALT & AST الدالين على نشاط الكبد زيادة غير مرضية (عند مستوى ٥٪) كنتيجة لإضافة مركب التويوسيرين مقارنة بالمجموعة

الكنترول. كما أظهرت المجموعة المعاملة أعلى القيم في العدد الميكروبي الكلي وأعداد كل من ميكروبي اللاكتوباسلاي والسليوليتك وأقل القيم في عدد ميكروب اليوريوليتك.

أدى تقديم العليقة المُضاف إليها مركب التويوسيرين لذكور أرانب الكاليفورنيا الناضجة جنسياً إلى تحسين معنوي (عند مستوى ٥٪) فى رغبتها الجنسية والخصائص الطبيعية للسائل المنوي متمثلة فى قيم كل من حجم قذفة السائل المنوي ونسب كل من الحركة التقدمية للحيوانات المنوية، الحيوانات المنوية الحية والسليمة مورفولوجياً، حالة الأكروسوم وكذلك تركيز الحيوانات المنوية في المل[٣] وفى القذفة. لم يلاحظ أي تأثير بين المجموعتين فى قيم كل من محيط كيس الصفن والدليل الخصوي وتركيز الهرمون الذكري التيستيرون بينما كان النشاط التزاوجي (عدد مرات التلقيح فى ١٥ دقيقة) لذكور أرانب الكاليفورنيا المُعاملة أعلى معنوياً مقارنة بالمجموعة الكونترول. كما وجد أن معاملة إناث أرانب الكاليفورنيا بمركب التويوسيرين والملقحة طبيعياً من ذكور معاملة بنفس المركب سجلت قيم أفضل معنوياً (عند مستوى ٥٪) لكل من معدل ولادات، وعدد ووزن خلفات عند الميلاد، ومحصول لبن لكل أم، وكذلك معدلات نفوق للخلفات قبل الفطام مقارنة بأمهات مجموعة الكنترول.

التوصية: تُشير نتائج الدراسة الحالية في مجملها إلى أن إضافة مركب التويوسيرين إلى غذاء الأرانب بمعدل ١٠٠ مللجرام/كجم علف قد حسنً معنوياً الأداء الإنتاجي والتناسلي لأرانب الكاليفورنيا.