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EFFECT OF DIETARY PROBIOTIC AND SEX ON PRODUCTIVE PERFORMANCE, NUTRIENT DIGESTIBILITY, CARCASS CRITERIA, BLOOD BIOCHEMISTRY OF GROWING NEW ZEALAND WHITE RABBITS

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SUMMARY

The experiment was conducted to investigate the effect of dietary probiotic levels and sex on growth performance, nutrient digestibility, carcass criteria, blood biochemistry and meat quality of growing rabbits. A total of sixty four growing male and female New Zealand White rabbits (45 days old), were assigned to four experimental diets including added probiotic at 0, 150, 300 and 450 m g/kg, respectively, for six weeks. Each treatment had 16 replicates (50% males and 50% females). Sex of rabbits did not affect the body weight gain, feed intake, feed conversion ratio and nutrient digestibility during the experimental periods. Body weight gain increased and feed conversion ratio decreased significantly from 0 to 450 mg/kg during the period from 0 to 2, 4 to 6 and 0 to 6 weeks of age, respectively. Feed intake exhibited a noticeable significance ($p<0.05$) in interaction between probiotic treatment levels and sex in the period from 4 to 6 weeks only. Supplementation of probiotic improved the nutrient digestibility during the experimental period. Cecum and gut weights increased significantly from 150 to 450 mg/kg. Interestingly, serum glucose and cholesterol levels showed a significant decrease ($P<0.001$) among probiotic treatments in comparison with the control group. Serum total protein, testosterone, estrogen, progesterone and tri-iodothyronine showed a higher significance ($P<0.001$) in the probiotic levels compared to control group, however thyroxin showed no significant increase or decrease where the tested groups exhibits nearly the normal levels. It could be concluded that supplementation of probiotics to the rabbits diet improved nutrient digestibility and blood glucose, cholesterol and hormones level without any reflection of probiotics on body weight and carcass criteria including carcass weight, hot carcass, liver, kidney, spleen, lungs, heart and head on either male or female growing rabbits.

Keyword: Probiotics, performance, nutrient digestibility, some serum biochemical profile, meat quality, rabbits

INTRODUCTION

Rabbit production in Egypt has developed rapidly, most notably to meet an increased demand of fresh meat for human consumption as well as a source of extra income for families or small farmers in Egypt (Abdel-Wareth *et al*., 2015). Rabbit meat is characterised by convenient sources of palatable and nutritious meat, high in protein, and contain low fat and cholesterol, hence its suitability as special diet (Owen, 1981). However, its production may be limited by inadequate nutrient diet contents (Chantalakhana, 1990). Consequent to the prohibition of in-feed use of antibiotics and other chemical growth promoters in many parts of the world, there has been an increased interest in alternative approaches, which include herbs, probiotics, prebiotics and acidifiers, which were successfully used by many investigators (Tokic *et al*., 2007; Yang *et al*., 2007; Awaad, *et al*., 2011; Abdel-Wareth *et al*., 2012; Abdel-Wareth and Lohakare, 2014; and Abdel-Wareth, 2016). Therefore, probiotics which are mono-or mixed culture of living micro-organisms, induce beneficial effects on the host by improving the properties of the indigenous microflora (Ghadban, 2002). Biotechnological treatments including either the direct use of microorganisms or microbial enzyme to improve the nutrient digestibility of feed ingredients (Shaiful, 1992). Inclusion of probiotics in animal feed has been shown to improve the growth performance, nutrient digestibility and feed efficiency of animals (Martin *et al*., 1989; Santin *et al*., 2001; Ezema and Eze, 2012). Bioactive yeast secretes enzymes which increase digestibility and efficiency of feed utilization (Ozcan, 2001). Serum biochemical analysis is important indicators of physiological, nutritional, pathological status and the health status of animal (Archetti *et al*., 2008 and Melillo, 2007).

Probiotics have been reported to have favourable effects on performance, beneficial effects upon supplementation to animals feed since it has many values such as enhancement of the immune system, antimicrobial effects gut functions cholesterol concentrations, management of diabetes and prevention of osteoporosis, improved nutrition through the enhanced breakdown of nutrients and their absorption through the intestinal walls, cleansing of the blood stream by making it freer of toxins and prevention of infection by harmful bacteria (Rautray *et al*., 2011).

Recent research on the molecular biology and genomics of *Lactobacillus* has focused on the interaction with the immune system, anti-cancer potential, and potential as a biotherapeutic agent in cases of antibiotic-associated diarrhoea, travellers' diarrhoea, paediatric diarrhoea, inflammatory bowel disease and irritable bowel syndrome and ability of modifying the gut microflora (Ghadban, 2002). The mechanism of action of probiotics has not been elucidated, but might include reduction of toxin production, stimulation of enzyme production, 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 stress in rabbits (Falcão-e-Cunha *et al*., 2007). Inclusion of probiotic feed additives in feed may enhance reproductive performance of rabbits. Therefore, the objective of this study was to evaluate the effect of dietary probiotics levels and sex on growth performance, nutrient digestibility, carcass characteristics, serum biochemical profile, and meat quality of growing rabbits.

MATERIALS AND METHODS

Experimental animals, design and management:

A total of sixty four, 45-days-old, New Zealand rabbits (50% males and 50% females) were randomly allocated into four treatment groups of 16 rabbits each (n = 16). The rabbits of the control group were fed *ad libitum* from 45 to 87 days of age; the other three groups received the control diet supplemented with 150 g/kg, 300g/kg and 450 g/kg probiotics mix (1:1 *Bacillus subtilis* and *Lactobacillus acidophilus)*, respectively.

Rabbits were individually reared in cages of galvanised wire net (width × length × height: 50 cm × 60 cm × 40 cm), equipped with fresh tap water, which was available for *ad libitum* intake via stainless steel nipples located inside each cage. Ambient temperature was maintained at 22°C with a 12 h light/dark cycle. During the total experimental period of 6 weeks (wks). Rabbits were housed under the same managerial, hygienic and environmental conditions, and throughout the trial, the rabbits were handled according to the principles of care for experimental animals (Lebas *et al.*, 1984), and the experiment was approved by the Committee of Ethics for the Animal and Poultry Production Department of the South Valley University, Egypt.

The rabbits were subjected to regular inspections for health and body conditions. The assessments of body conditions were carried out by touching the ribs, pelvis and spine of the rabbits (Abdel-Wareth *et al.*, 2015).

Ingredients and chemical composition of the experimental diets are presented in Table (1). Animals of all treatments received the same diet that was formulated to meet the standard nutritional requirements of growing meat-type rabbits (Lebas, 2004). The mean gross energy and crude protein concentrations were 18.2 MJ/kg and 170 g/kg, respectively. Individual feed intake was recorded weekly at 8:00am. Each rabbit was weighed weekly at the same day at 7:00 am. Feed conversion ratio (FCR) was calculated by dividing daily feed consumption by average daily body weight gain (BWG). Mortality rate was recorded as it occurred and any signs of diarrhea were documented daily.

Table 1. Ingredients and chemical composition of the experimental diets of growing Nzw white rabbits

Ingredients, g/kg	Control	150%Pro	300%Pro	450%Pro
Yellow maize grain	320.0	320.0	320.0	320.0
Wheat bran	200.0	200.0	200.0	200.0
Soybean meal (44% CP)	180.0	180.0	180.0	180.0
Wheat straw	120.0	120.0	120.0	120.0
Lucerne hay	50.0	50.0	50.0	50.0
Rice bran	50.0	50.0	50.0	50.0
Linseed straw	28.0	28.0	28.0	28.0
Sunflower meal	25.0	25.0	25.0	25.0
Lime stone	20.0	20.0	20.0	20.0
Sodium chloride	3.0	3.0	3.0	3.0
Vitamin-mineral mix[a]	3.0	3.0	3.0	3.0
DL-Methionine	1.0	1.0	1.0	1.0
Chemical composition analysed, g/kg				
Dry matter	914.0	915.0	914.0	915.0
Ash	98.20	98.23	98.26	98.28
Crude protein	18.6	18.6	18.8	19.0
Crude fiber	11.9	11.9	11.9	11.8
Ether extract	29.2	29.1	29.4	29.6
Gross energy, MJ/kg	18.24	18.26	18.28	18.40

Notes: §Vitamin and mineral premix provided per kg of diet: vitamin A, 10.000 IU; vitamin D_3, 900 IU; vitamin E, 50 mg; vitamin K, 2 mg; vitamin B_1, 2 mg; folic acid, 5 mg; pantothenic acid, 20 mg; vitamin B_6, 2 mg; choline, 1.2 g; vitamin B_{12}, 10 µg; niacin, 50 mg; biotin, 0.2 mg; Cu, 0.1 mg; Fe, 75 mg; Mn, 8.5 mg; Zn, 70 mg

Digestibility trial:

At the end of the experiment, a digestibility trial was conducted in the last three days where the rabbits were individually housed in metabolic cages which allowed separation of faeces and urine. Every day at the same time, the animals were fed the pelleted experimental diet and always had free access to clean drinking water. Feed residues and faeces were collected every day and weighed to the nearest one g using an analytical scale. Faeces were stored frozen, and after thawing, samples were dried and ground. The samples prepared in this way were analysed for chemical composition and energy value to calculate digestibility coefficients of nutrients and energy. The digestibility coefficient (DC) of nutrients was calculated according to the following equation:

DC [%] = [(t-f)/t] 100;

Where, (t) is expressing the nutrient intake during the collection period [g], and (f) express the amount of nutrient excreted in faeces [g].

Chemical analysis:

The diet and faces were analyzed for estimation of moisture by oven drying (930.15), ash by incineration (942.05), protein by Kjeldahl (984.13), and ether extract by Soxhlet fat analysis (920.39), Crude Fiber was determined by the Weende method as described by the AOAC International (2006). Gross energy was determined by Parr adiabatic bomb (Moline, IL, USA).

Carcass measurements and meat quality:

Six representative rabbits from each treatment were selected for similar final body weight (slaughter weight) and sacrificed at termination of the experimental period of 87 days of age. The sacrificed rabbits were bled and then the skin, genitals, head, urinary bladder, gastrointestinal tract and the distal part of legs were removed. Head and full gastrointestinal tract were weighed and expressed as percentage of slaughter weight. Also, the length of full gastrointestinal tract was measured. Carcasses, with liver, heart, spleen, lungs, kidneys, as well as perineal and scapular fat were weighed and calculated as percent of hot carcass. Carcass yield was calculated as per the following:

Carcass yield [%] = (Hot carcass weight [g]/Body weight [g]*100.

The ratio of the internal organs including carcass weight, liver, kidney, spleen, lungs and heart (each separately) to the hot carcass weight was calculated as required.

To evaluate meat quality Ultimate pH (pHu) was measured at 24 h *post mortem* with a Knick digital pH meter (Broadly Corp., Santa Ana, CA, USA) after homogenization of raw muscles (5g) with iodoacetate (Korkeala *et al.*, 1984). To evaluate cooking loss, muscle samples of about 5 g were placed in an open aluminum pans and cooked in an electric oven (pre-heated at 200 °C) for 15 min to an internal temperature of 80 °C (Cyril *et al*., 1996). Cooking loss was estimated as the difference between the weight of the cooked samples (cooled for 30 min at 15 °C) and the weight of the raw samples expressed in percentage of the raw sample. The Water Holding Capacity (WHC) was estimated by centrifuging 5g of muscle placed on tissue paper inside a tube, for 4 min at 1.500 x g (Nakamura and Katoh, 1985). The water remaining after centrifugation was quantified by drying the samples at 70 °C overnight. The WHC was calculated as follows: (weight after centrifugation - weight after drying) x 100/initial weight.

Blood sampling and laboratory analyses:

At the end of the experimental period (87 days age), blood samples of growing rabbits (6 rabbits within each experimental group) were collected during slaughter. Blood samples were kept in dry clean centrifuge tubes. After the serum was separated naturally, it was centrifuged for 10 min (3000 rpm) at room temperature. Serum was collected in tubes and stored at −20°C until further analysis. Serum glucose, total cholesterol and total proteins were measured spectrophotometrically by using commercial kits (Spectrum chemical company, Obour City – Cairo, Egypt). Serum total testosterone was assayed colorimetrically by a spectrophotometer according to the method of Ismail *et al*. (1986), using a competitive chemiluminescent enzyme immunoassay kit. Serum progesterone and estradiol were determined using method reported by Burtis and Ashwood (1994) using a competitive chemiluminescent enzyme immunoassay kit. Serum thyroxine (T4) and tri-iodothyronine (T3) were measured according to the method of Witherspoon and Shuler (1984) and Felig *et al*. (1987), respectively, using chemiluminescent microparticle immunoassay (CMIA) kit.

Statistical analysis:

Experimental data were analyzed as a randomized complete block design, with 2×4 factorial arrangement of sex and dietary treatments, using the General Linear Model procedures of SAS (2009). Treatment, sex, and interaction between treatment and sex were defined as sources of variation. Significant differences between means were evaluated by Duncan multiple range test (Duncan, 1955) after a significant F-test. Significance was declared at $P<0.05$; *P*-values less than 0.001 are expressed as "<0.001" rather than the actual value. For response of probiotics hormones concentrations, data were expressed as mean standard error of the mean (SEM), which represents the pooled SEM for the model. Moreover, data were analyzed One-Way ANOVA using GraphPad Prism (Version 5) and presented as mean ± SEM of four independent biological replicates.

RESULTS

Growth performance:

The growth performance, as affected by probiotics supplementations to the diets at 150 g/kg, 300g/kg and 450 mg/kg, are presented in Table (2). Body weight gain was significantly increased by supplementing probiotic levels during 0-2 wk, 4-6 wk and 0-6 wk (at the start, end and the whole experimental period) of the experimental period. On the other hand, feed intake exhibited a noticeable significance (P<0.05) in interaction between treatments and sex in the period from 4-6 wk only (at the end); whereas for sex and treatments there was absence of any significance difference. Additionally, total feed conversion ratio indicated significant improvement (P<0.05) for rabbit fed on probiotics levels during period (0-2 and 0-6 weeks), compared to the control.

Table 2. Effects (Mean±SEM) of probiotic levels on growth performance of New rabbits

Items	SEX		Probiotic levels				SEM[a]	P-VALUE		
	M[b]	F[c]	0	150	300	450		S	T	SXT
Body weight gain, g										
0-2 week	405	399	368[b]	385[b]	360[b]	496[a]	19.42	0.870	0.049	0.593
2-4week	475	415	438	433	440	471	12.70	0.014	0.626	0.150
4-6week	319	335	368[a]	376[a]	370[b]	381[ab]	18.16	0.579	0.015	0.036
0-6week	1200	1151	1192[ab]	1173[b]	1210[ab]	1348[a]	32.35	0.379	0.038	0.074
Feed intake ,g										
0-2week	1186	1184	1200	1200	1196	1146	9.35	0.922	0.156	0.998
2-4week	1558	1493	1541	1455	1566	1542	26.08	0.204	0.436	0.192
4-6week	1142	1112	1117	1106	1141	1146	22.44	0.984	0.484	0.037
0-6week	3888	3791	3858	3762	3903	3835	45.42	0.266	0.266	0.061
Feed conversion ratio										
0-2week	3.20	3.00	3.31[a]	3.18[a]	3.12[a]	2.49[b]	0.11	0.371	0.021	0.517
2-4week	3.62	3.33	3.53	3.48	3.28	3.32	0.09	0.160	0.807	0.659
4-6week	3.34	3.47	3.04	3.21	3.63	3.74	0.13	0.584	0.177	0.228
0-6week	3.36	3.31	3.25[b]	3.23[b]	3.84[a]	3.02[b]	0.09	0.819	0.026	0.680

[a-b] Means bearing different superscripts within a row differ significantly ($P < 0.05$)
[a]SEM, standard error of means [b]M, Male; [c]F, Female

Nutrient digestibility:

Effects of sex and probiotics levels on nutrient digestibility of rabbit are presented in (Table 3). No significant differences were observed on digestibility between males and females and also the interaction between sex and treatments.

Concerning digestibility coefficient of crude protein, nitrogen free extract, dry and organic matter digestibility, they were significantly (P<0.01) increased with probiotic supplementation from 0 to 450 mg/kg. In addition, noticeable significant (P<0.01) improvements were observed in digestibility of gross energy at level 150g/kg, and crude fiber at level 300 g/kg, compared to the other treatments and the control, also no significant difference was found in ether extract.

Table 3. Effect (Mean±SEM) of probiotic on nutrient digestibility of growing New rabbits

Items	Sex		Probiotic levels				SEM[a]	P-VALUES		
	M[b]	F[c]	0	150	300	450		S	T	SXT
Crude protein	83.94	84.01	78.37[c]	82.66[b]	85.59[b]	89.45[a]	1.04	0.899	0.0001	0.954
Crude fiber	53.85	48.88	38.49[b]	56.32[a]	56.62[a]	54.04[a]	2.56	0.297	0.039	0.896
Gross energy	76.34	74.45	66.52[b]	79.29[a]	77.63[a]	78.32[a]	1.38	0.326	0.008	0.903
Ether extract	89.27	89.49	86.29	93.29	86.30	90.99	1.25	0.929	0.169	0.375
Dry matter	65.19	66.72	50.01[c]	64.06[b]	66.87[b]	82.88[a]	2.89	0.680	0.0001	0.821
Organic matter	63.23	64.32	47.73[c]	62.14[b]	64.77[b]	80.45[a]	2.90	0.775	0.0002	0.884
N-free extract	78.12	76.96	70.23[b]	79.31[a]	78.48[a]	82.15[a]	1.29	0.594	0.0007	0.203

[a-b] Means bearing different superscripts within a row differ significantly ($P < 0.05$)
SEM[a], standard error of means
M[b], Male; F[c], Femal

Carcass criteria:

Results indicated that there were no significant differences in carcass characteristics including carcass weight, hot carcass, liver, kidney, spleen, lungs, heart and head among probiotic treatments, sex and their interaction (Table 4). However, abdominal fat was significantly different in sex, where the fat ratio was greater in females than males. The tests, gut and caecum weight exhibited a high significance (P<0.001) among treatments compared to the control group.

Table 4. Effect (Mean±SEM) of probiotic levels on carcass criteria of growing New rabbits

Items	Sex		Probiotic levels				SEM	P-VALUES		
	M	F	0	150	300	450		S	T	SXT
Live wt[d]	2202[a]	2089[b]	2157	2192	2129	2104	24.19	0.018	0.533	0.333
Carcass wt	1168	1124	1137	1198	1135	1116	18.82	0.233	0.402	0.164
Hotcacarcasswt	1284	1238	1242	1319	1258	1229	19.11	0.198	0.286	0.127
Carcass %	58.37	59.31	58.23	60.11	59.51	58.26	0.704	0.531	0.674	0.434
Liver %	4.96	4.95	4.61	5.02	5.35	4.83	0.163	0.974	0.541	0.899
Heart%	0.492	0.487	0.351	0.537	0.497	0.575	0.035	0.939	0.161	0.589
Kidney %	1.421	1.443	1.403	1.364	1.434	1.527	0.065	0.885	0.881	0.758
Spleen %	0.152	0.170	0.155	0.150	0.180	0.159	0.007	0.226	0.501	0.366
Lunges%	1.060	1.230	1.059	1.011	1.492	1.019	0.082	0.288	0.123	0.585
Fat abdomen %	0.561[b]	0.972[a]	0.703	0.919	0.715	0.730	0.0997	0.046	0.834	0.324
Head %	5.728	5.045	5.185	4.823	4.803	6.733	0.380	0.377	0.257	0.528
Cecum %	0.347	0.383	0.278[b]	0.489[a]	0.359[b]	0.334[b]	0.020	0.181	0.0003	0.230
GUT %	16.40	17.27	17.94[a]	14.83[b]	18.700[a]	15.86[ab]	0.551	0.368	0.036	0.313
CECUM(cm)	10.50	10.33	10.16	10.50	10.66	10.33	0.020	0.736	0.897	0.166
GUT (cm)	3.241	3.225	3.383	3.233	3.233	3.083	0.551	0.938	0.804	0.627

[a-b] Means bearing different superscripts within a row differ significantly ($P < 0.05$).
SEM[a], standard error of means M[b], Male; F[c], Female

Blood parameters:

Supplementation of dietary probiotic showed a significant decrease in blood glucose levels, followed by an increase, in the probiotic level of 450 mg/kg. This increase may be attributed to the higher digestibility of crude protein and decrease in cholesterol (P<0.001) at probiotic levels of 150, 300 and 450 mg/kg, respectively. With respect to the total protein and T3, significant increases (p<0.05 and p<0.001 respectively) were observed for all treatments (Table 5), whereas sex and interactions between sex and treatments did not show any significance. On the other hand, T4 did not show any significance at any treatment level, sex and their interaction.

Finally, sex hormones, as represented by levels of testosterone (Fig. 1), and estrogen (Fig. 2) exhibited a high significance (P<0.05) at the level of 150 and 450 mg/kg than control and the first treatment level, on the other hand progesterone (Fig. 3) showed high significance (P< 0.05) at level 300 mg/kg compared to control and other treatment levels.

Table 5. Effect (Mean±SEM) of probiotic on serum biochemical profile of growing New rabbits

Items	SEX		Probiotic levels				SEM[a]	P-VALUE		
	M[b]	F[c]	0	150	300	450		S	T	SXT
Glucose(mg/dl)	78.91	82.08	88.66[b]	57.50[c]	80.16[b]	95.66[a]	2.29	0.415	0.0001	0.003
Cholesterol(mg/dl)	72.66	74.08	93.66[a]	73.83[b]	42.83[c]	83.16[ab]	1.82	0.803	0.0001	0.047
Total protean(g/l)	6.67	6.57	6.66[a]	6.75[a]	6.16[b]	6.91[a]	0.090	0.490	0.010	0.191
T3(ng/dl)	66.75	56.66	57.33[b]	81.33[a]	74.33[b]	58.16[b]	3.675	0.082	0.006	0.243
T4(ng/dl)	3.53	3.49	3.48	3.72	3.54	3.28	0.139	0.897	0.805	0.896

[a-b] Means bearing different superscripts within a row differ significantly ($P < 0.05$).
SEM[a], standard error of means M[b], Male; F[c], Female

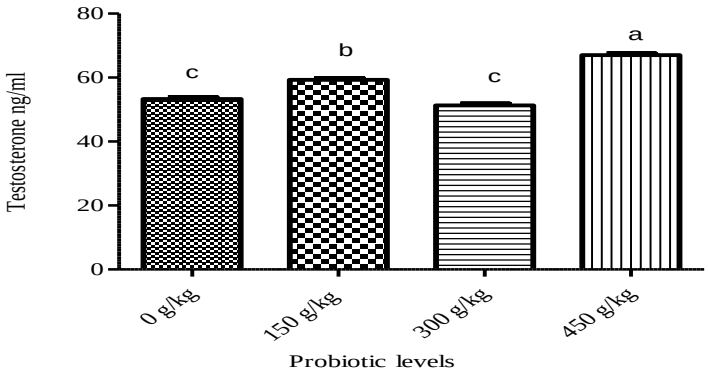

Figure 1. Serum levels (ng/ml) of total testosterone secretion in response to Probiotic supplementation of 150, 300 and 450 g/kg, or a control (0 g/kg) in male New rabbits. Letters on the bars a, b, c denote the significant difference among the different treatments (P<0.05)

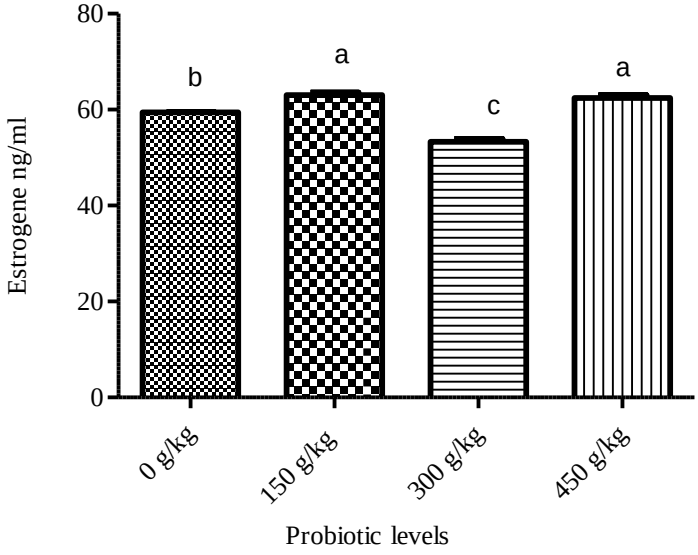

Figure 2. Serum levels of estrogen secretion in response to Probiotic supplementation of 150, 300 and 450 g/kg, or a control (0 g/kg) in female New rabbits. Letters on the bars a, b, c denote the significant difference among the different treatments (P<0.05)

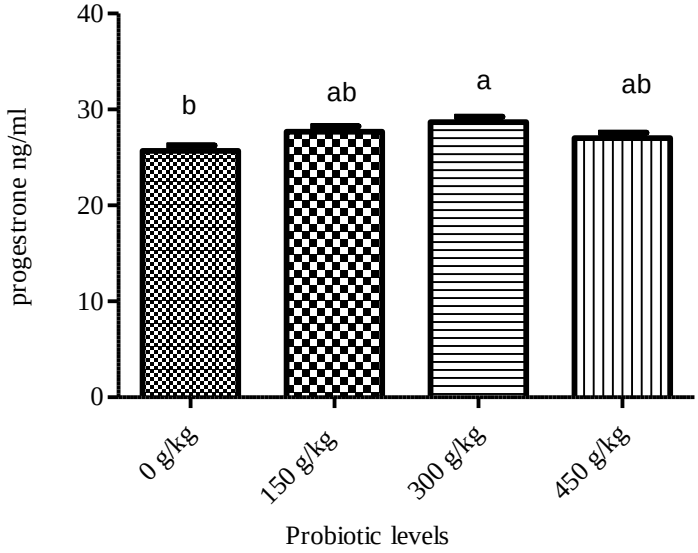

Figure 3. Serum levels of progesterone secretion in response to Probiotic supplementation of 150, 300 and 450 g/kg, or a control (0 g/kg) in female New rabbits. Letters the bars a, b, c denote the significant difference among the different treatments (P<0.05)

Meat quality:

The effects of probiotic supplementation on meat quality characteristics of rabbits at the end of experimental period are shown in Table (6). Probiotic supplemented groups resulted in a significant decrease (P<0.05) in water holding capacity at level of 150 and 300 mg/kg, followed by an increase in the last level 450 mg/kg, compared to the control. Also, significance was observed in the interaction between probiotic levels and sex; meanwhile, there was no significance for sex. Concerning the water holding capacity (WHC), and pH values, no observable significance was found. On the other hand, females exhibited a significant increase (P<0.001) in the cooking loss than males. Again, observed significance for sex in cooking loss was not significant in the treatments and interaction. Finally, there were no significant differences in PH value at probiotic treatments, sex and their interaction.

Table 6. Effect (Mean±SEM) of probiotic on meat quality of growing rabbits

Items	Sex		Probiotic levels				SEM	P-VALUE		
	M	F	0	150	300	450		S	T	SXT
Water capcity	32.28	30.40	34.30[a]	24.86[b]	31.2[a]	35.00[a]	1.39	0.379	0.013	0.046
PH	6.25	6.17	6.29	6.46	6.12	5.98	0.08	0.578	0.174	0.228
Cooking loose	18.08[b]	22.22[a]	21.39	18.41	21.2	19.51	0.78	0.004	0.291	0.193

[a-b] Means bearing different superscripts within a row differ significantly ($P < 0.05$).
SEM, standard error of means M[b], Male; F[c], Female

DISCUSSION

Productive performance:

Effects of probiotics supplementation on growth performance indices of growing rabbits during the experiment are shown in Table (2). The results showed that the groups supplemented with 300 and 450 mg/kg of probiotic exhibited significantly ($p<0.05$) higher body weight gain than the control. The increased body weight gain could be attributed to the improved protein digestibility and increased efficiency of feed utilization (Martin *et al*., 1989; Numan, 2001; and Ezema, 2007). Shrivastava *et al*. (2012) reported that the increase in growth performance in rabbits fed a ration supplemented with probiotic may be due to increased digestion and absorption through the intestine. In addition, Chandra *et al*. (2014) attributed the improvement in growth performance to good digestibility and absorption in the ilea. Nevertheless, Attia *et al*. (2012) concluded that the positive effect of probiotics on growth performance resulted from change in gut microbiota. The present results showed feed intake were slightly higher in probiotic supplemented groups, which is in agreement with observation made by earlier workers such as (Onifade *et al*., 1999; Adejumo *et al*., 2005; Shehu *et al*., 2014; Amber *et al*., 2014; and Iwu *et al*., 2015).

In view of the obtained results on FCR, it may be concluded that they were in close harmony with the results of Adeniji and Adewole (2015) who reported a significant effect on FCR upon replacing brewers dried grains for groundnut cake with or without probiotics supplementation.; Amber *et al*. (2014) in supplementing rabbits basal diet with a mixture of probiotics (i. e. Bio-MOS Reg; mannan oligosaccharide at 1 g/kg diet) and a probiotic (i.e. Bio-Plus Reg.2B, *Bacillus subtilis*and *Bacillus licheniformis* at 0.4 g/kg diet) had significant effects on feed efficiency among the group. Adeniji *et al*. (2013 and 2014) observed significant influence on the feed to gain ratio upon supplementation of rabbits with probiotic. El-Kholy *et al*. (2012) also reported significantly higher FCR in case of diet supplemented with *E. Faecalis* as compared to control group of NZW rabbits. Additionally, Karima *et al*. (2011) also confirmed an improved FCR (about 13.0% and 13.1%) with dietary supplementation by *Lactobacillus* strains. Chrastinova *et al*. (2010) reported better FCR of New Zealand white rabbits in comparison with the control group upon supplementation with Phyto-additives and probiotics. On the other hand, Ezema *et al*. (2015 and 2012) reported that there was no significant difference in FCR among rabbits fed at different probiotic diet levels. However, Thanh and Jamikorn (2012) noted that the FCR was reduced significantly in weaning New Zealand White rabbits diets supplemented with *L. acidophilus* and *B. subtilis*as compared to the control diet.

Digestibility:

The nutrient digestibility of the rabbits was significantly ($P<0.05$) increased by the dietary treatments. The effect of supplementing probiotics on digestibility of rabbits has not been fully addressed by researchers. Yamani *et al*. (1992) stated that using lacto-sacc (a complex product containing not only a mixture of the micro-organisms Lactobacillus acidophilus, Streptococcus faecium and yeasts, but also the enzyme protease, cellulases, and amylase) improved crude fibre digestibility. Also, Amber *et al*. (2004) worked with Lact-A-Bac (*Lactobacillus acidophilus*) and reported an improvement in the digestibility of energy and most analytical fractions (dry matter, crude protein, ether extract) including crude fibre which corroborates the results obtained in this study.

In general, probiotic supplementation has been reported to show a positive influence on nutrient utilization due to its ability to fortify beneficial members of the intestinal microbiota, thereby improving efficiency of nutrient digestion and absorption processes of the host (Mountzouris *et al*., 2010). In probiotics-supplemented groups, the higher digestibility of crude protein and fiber components (NDF, hemicelluloses) could be the result of maintaining a relatively better health and environmental gut microbiota that supported improvement in nitrogen utilization and growth with efficient FCR (Combes *et al*., 2012).

Carcass characteristics:

Results indicated that rabbits were insignificantly ($P<0.05$) influenced by probiotics dietary supplements except the testicles, caecum and gut weights which were statistically ($P<0.001$) impacted. Meanwhile no significant differences were noted in dressing percentage at 150 and 300 mg/kg probiotic levels. These results are in agreement with those reported by Ewuola *et al*. (2011) who observed no significant differences in carcass characters among

all treatment groups upon supplementation of growing New Zealand white rabbits with enzymes and probiotic mixture (Veta-zyme), prebiotics and symbiotics. An increased caecum weight in weaning rabbits upon dietary inclusion of a probiotic was reported (Rotolo *et al*., 2014).

Serum biochemical parameters:

Biochemical blood parameters are usually good indicators for physiological, pathological, and nutritional status of an animal and have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet (Ashour *et al*., 2014). In the current study, blood constituents indicate significant decrease in glucose at 150 and 300 mg/kg probiotic levels followed by an increase at 450 mg/kg, compared to the control. Concerning cholesterol values, all levels at 150, 300 and 450 mg/kg showed a significant decrease ($p<0.001$) in comparison with the control group. Our results also were in line, and in physiological normal range, with those obtained by Abd-El-hady *et al*. (2015) who reported that rabbits fed with prebiotic, probiotic had significantly reduced glucose ($P<0.05$), cholesterol and triglycerides ($P<0.001$), compared with control group. Also, Simonova *et al*. (2013) noted that dietary supplementation with Enterococcus faecium CCM7420 (EF) and E. senticosus extract (ES) or their combination (EF+ES) had a significant decrease ($P<0.05$) in glucose among groups. Furthermore, rabbit groups fed probiotic supplemented diet showed a significant ($P<0.05$) decrease in cholesterol and triglyceride concentrations when compared to the control. Similar results were reported by other workers (Arun *et al*., 2006; Ashayerizadeh *et al*., 2011; Amer and Khan, 2012; and Mokhtar, 2013) who reported significant reduction in serum total cholesterol and triglycerides by dietary supplementation of probiotic. The significant reduction in serum cholesterol of rabbits fed probiotic supplemented diet could be attributed to reduced absorption or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan *et al*., 1996). Also, it was speculated that *Lactobacillus acidophillus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis. (Abdulrahim *et al*., 1996). Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and is more likely to be excreted in faeces. (Klaver and Van der Meer, 1993). This could be the case in the present study, thus forcing de novo bile synthesis in the liver from body cholesterol pool, causing a net reduction in total blood cholesterol level as one of the probiotic microbes utilized in the study (*Lactobacillus acidophillus*) is acidophilic which lowers the pH of the surrounding environment. Another explanation of the mechanism by which a probiotic can lower the serum cholesterol has been declared by Fukushima and Nakano (1995). The authors demonstrated that probiotic microorganisms inhibit hydroxymethyl-glutaryl-coenzyme A; an enzyme involved in the cholesterol synthesis pathway thereby decrease cholesterol synthesis. Corcoran *et al*. (2005) reported that fat digestion rate is linked to the rate of gall bladder acids in digestion latex and subsequently the lipid concentration. Supplementation of probiotics in diet cause a decrease in gall bladder acids in digestion latex and this resulted in a reduction of fat digestion and therefore decreasing lipid level of blood (Corcoran *et al*., 2005).

In the present study, serum total protein (T.P), significantly increased ($P<0.05$) at 150 and 450 mg/kg levels; whereas it showed a decrease at 300 mg/kg level of supplementation probiotics in diets. This might be due to the higher digestibility of crude protein in these diets (Amber, 2000). Meanwhile, T3 concentrations was significantly ($P<0.001$) increased at 150, 300 and with a slight increase at 450 mg/kg in probiotics supplemented treatments, compared to the control group. In the current study, sex hormones, represented by testosterone, estrogens and progesterone exhibited a high significance ($P<0.05$) compared to control. Our results were in agreement with those reported in the literature that probiotics caused enhancement of testosterone level, which may be interpreted, along with the hypocholesterolemic action of probiotics, by mobilization of blood cholesterol to testosterone synthesis (Huey-Shi *et al*.,2009; Daniela *et al*., 2009; Lay-Gaik and Min-Tze, 2010; Ghoneim and Moselhy, 2012;). In our results the concentration of blood serum testosterone tended to be lower when the probiotic level reached dose of 300 mg/kg compared to 150 and 400 mg/kg. This reduction in testosterone concentration may be due to the hypocholesterolemic action of probiotics at this level. Also, Martarelli *et al*. (2011) who reported that supplementation of probiotic had a beneficial effect of reducing the severity of the changes within the somniferous tubules and improve testicular profile, as observed in histological study.

CONCLUSIONS

Obtained results support the addition of probiotics in the rabbit diets at level of 450 mg/kg to enhance of productive performance, nutrient digestibility, blood biochemistry and sexual hormone without any adverse effect on carcass criteria. Further detailed studies are required to establish the reproductive efficiency of adults rabbits under Upper Egyptian hot summer condition.

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تأثير الجنس و اضافة البروبيوتك للعليقة على أداء النمو وهضم المواد الغذائية وصفات الذبيحه وبيوكيمياء الدم وبعض الهرمونات على جوده اللحم في الأرانب النيوزيلندى النامية

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اجريت هذه التجربه لتقييم تاثير اضافه مستويات مختلفة من البروبيوتك والجنس علي مظاهر النمو و هضم المواد الغذائية ومواصفات الذبيحه وصفات الدم البيوكيميائيه وكذلك جوده اللحم في الارانب الناميه. ولقد استخدم فى هذه التجربة عدد 64 ارنب نامى عمر 45 يوم (50% اناث :50 % ذكور) واستمرت لمدة 6 اسابيع , وقد وزعت عشوائيا الى 4 معاملات تجريبه بمستويات مختلفه من البروبيوتك وهي 0 , 150, 300 و 450 ملجم/كجم من العلف وقد اظهرت النتائج انه لا يوجد فروق معنوية بين الذكور والاناث في وزن الجسم وكميه الغذاء المستهلك او الكفاءة الغذائيه او معاملات الهضم طوال فتره التجربه. بينما كان هناك زياده معنويه في وزن الجسم المكتسب يقابله انخفاضا معنويا في الكفاءة الغذائية لجميع مستويات البروبيوتك من 150, 300 و 450 ملجم/كجم من العلف. واتضح ايضا ان اضافه البروبيوتك اثرت بالانخفاض فى كمية الغذاء المستهلك معنويا بين المعاملات المختلفه للذكور والاناث (الذكور أعلى) خاصة في الفتره مابين 4-6 أسابيع فقط. كذلك كان هناك دورا ايجابيا لاضافه البروبيوتك بالنسبه لمعاملات الهضم حيث انها حسنت من معاملات الهضم خلال التجربة. كما وجد أن اضافة البروبيوتك أدى الى زيادة معنويه في وزن الخصيتين والمبيض وكذلك وزن الامعاء والاعورين . ومن ناحية اخرى اتضح الانخفاض المعنوى لكل من مستويات الجلوكوز والكوليسترول في الدم بين المعاملات مقارنة بمعاملات الكنترول وايضا سجلت اعلى معنوية لبروتينات الدم و هرمونات التستيرون و الاستروجين و البروجيسترون والتراى ايدوثيرونين مقارنه بالكنترول ولم تظهر معنوية علي الثيروكسين بين المجموعات المختبرة. يتضح لنا مما سبق ان اضافه البروبيوتك للعليقه بالمستويات المختلفة يعمل علي تحسين معاملات الهضم وتحسن مستويات الجلوكوز والكوليسترول والهرمونات المختلفة فى ذكور واناث الارانب النيوزلاندي الناميه.