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Effect of Stocking Density with Supplementation Probiotic on Productive and Economical Performance in Local Growing Rabbits

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ABSTRACT

An indoor experiment with factorial design (3 × 2) was conducted to investigate the effect of different levels of dietary Saltose Ex® probiotic on productive performance and economic efficiency parameters of growing black Balady rabbits reared under different stocking density (SD) rates for 7 weeks. A total of 72 rabbits (7 weeks old) were divided into six treatments. The experimental treatments were being as follow; T_1: rabbits fed basal diet (BD) + 0.0 g probiotic / Kg diet and reared under SD of 3 rabbits / cage, T_2: rabbits fed BD + 0.4 g probiotic / Kg diet and reared under SD of 3 rabbits / cage, T_3 : rabbits fed BD + 0.0 g probiotic / Kg diet and reared under SD of 4 rabbits / cage, T_4: rabbits fed BD + 0.4 g probiotic / Kg diet and reared under SD of 4 rabbits / cage, T_5: rabbits fed BD + 0.0 g probiotic / Kg diet and reared under SD of 5 rabbits / cage and T_6: rabbits fed BD + 0.4 g probiotic / Kg diet and reared under SD of 5 rabbits / cage during the period from 7 to 14 weeks of age. The obtained results revealed that addition of 0.4 g Saltose Ex®/Kg diet not only alleviated the drastic effects of high SD on rabbits, but also significantly improved of growth performance, nutrients utilization, carcass quality traits, microbial activity, hematological parameters, besides it led to slightly increased of economic efficiency parameters of growing rabbits especially those reared under high SD (5 rabbits / cage). Thus, it could be concluded the addition of 0.4 g Saltose Ex® / Kg diet had useful and practical effects on performance of growing black Balady rabbits reared under high stocking density, specifically reared in cages.

Keywords: Rabbits, Density, Probiotic, Growth performance, Microbial activity

INTRODUCTION

Nowadays, the requirement of efficient and safe production of livestock welfare and environmental viewpoints into account is taking importance world-wide (Jekkel *et al*., 2008). Rabbit can play a major role in enhancing animal protein production in developing countries due to its various biological advantages (Bhattacharjya *et al*., 2017). Fast-paced and moderately developed rabbit industries are recognized in some African countries, such as Egypt, Tunisia and Algeria (Oseni and Lukefahr, 2014).

Commercial rabbit production is an important industry for meat, fur and leather production (Abdelhady and El-Abasy, 2015). In the rabbit production enterprise, farmers have to raise and produce the maximum number of marketable rabbits per year to attain high profitability (Prawirodigdo *et al*., 1985). One way of doing this is to increase the number of rabbits stocked in a cage or house thereby maximizing available space. A high cage density reduces production costs, but this might influence the performance and increase the mortality rate of rabbits (Maertens and De Groote, 1984; Prawirodigdo *et al*., 1985). The main welfare indicators to assess rabbit housing are mortality, morbidity, physiological parameters in the species-specific standard, species-specific behavior and performance on a high level (Hoy *et al*., 2006).

For numerous decades, dietary supplementation with antibiotics and chemotherapeutic in prophylactic dosages have been used to improve animal welfare and to obtain economic benefits in terms of improved animal performance. However, nowadays probiotics were intensively used in animal production, including rabbits due to the resistances in pathogenic bacteria in both human and livestock related to the therapeutic and sub therapeutic use of antibiotics, besides antibiotic-residues in rabbit meat is potentially annoyance to consumer (Flickinger and Fahey, 2002). Consequently, probiotics have been introduced as an alternative to antibiotics, which come under the category as safe ingredients classified by Food and Drug Administration (FDA) (Bansal *et al*., 2011). Probiotics are nonpathogenic bacteria that exert beneficial effects on the host, it neither has any residues in animal production nor exerts any antibiotic resistance by consumption (Rajput and Li, 2012). The possibility to use feed supplements, including probiotics to achieve better animal health, welfare and productivity through manipulation of the gut micro biota ecosystem has gained considerable attention in the last three decades (Ahasan *et al*., 2015). Different types of probiotics were widely used in animal feeding, including rabbits. Using of dietary probiotics lead to improve growth performance and carcass traits of growing rabbits (Amber *et al*., 2014; El-Sagheer and Hassanein, 2014; Abdelhady and El-Abasy, 2015), physiological and immune responses (Onbasilar and Yalcin, 2008; El-Katcha *et al*., 2011 and Sarat Chandra *et al*., 2015), and achieved the highest economic efficiency (Ezema and Eze, 2015), which consequently lead to maximize the profitability of rabbits production systems.

From the animal welfare point of view, providing of adequate space and an appropriate environment is seriously important for providing freedom of movement and comfort. The number of rabbits in a cage or pen is one of the most important factors from the well-being and production aspects (Szendrő *et al*., 2009). It is well-known that increasing stocking density (SD) reduces the cost of production in any livestock enterprise. However, extreme density might affect the productivity of animals (Bhattacharjya *et al*., 2017). In this respect, many attempts were conducted to investigate the effects of SD rates on rabbits or to determine the optimum SD rate for different rabbits' strains (Villalobos *et al*., 2008; Kalaba, 2012 and Bhattacharjya *et al*., 2015 and 2017).

However, rarely attempts were conducted, regarding to determine the suitable SD rate for growing black Balady rabbits in cages by feeding of probiotic. Thus, the present study was conducted to evaluate the effects of dietary Saltose Ex® probiotic on growth, carcass traits, microbial activity, blood biochemical parameters and economic efficiency of newly local growing black Balady rabbits reared under different SD rates in cages for 7 weeks.

MATERIALS AND METHODS

This study was conducted at El-Serw Poultry Research Station, Animal and Poultry Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt.

Un-sexed black Balady growing rabbits were obtained from Animal and Poultry Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Seventy-two growing rabbits 7 weeks of age were randomly weighed and assigned to six experimental treatments based on their body weight. Each treatment had three replicates. All rabbits were kept under the same managerial conditions. The experimental treatments were planned with factorial design (3×2) as follow: T_1: rabbits fed basal diet (BD) + 0.0 g probiotic / Kg diet and reared under stocking density (SD) of 3 rabbits / cage, T_2: rabbits fed BD supplemented with 0.4 g probiotic / Kg diet and reared under SD of 3 rabbits / cage, T_3 : rabbits fed BD + 0.0 g probiotic / Kg diet and reared under SD of 4 rabbits / cage, T_4: rabbits fed BD supplemented with 0.4 g probiotic / Kg diet and reared under SD of 4 rabbits / cage, T_5: rabbits fed BD + 0.0 g probiotic / Kg diet and reared under SD of 5 rabbits / cage and T_6: rabbits fed BD supplemented with 0.4 g probiotic / Kg diet and reared under SD of 5 rabbits / cage during the period from 7 to 14 weeks of age. The rabbits in each replicate were kept on galvanized wire grower cages measuring (50 cm, length × 50 cm, width × 30 cm, height) and fed their respective experimental diets (Table 1). The cages are equipped with drinkers and feeders.

Table 1. Composition and calculated analysis of the basal diet

Items	%
Ingredients	
Barley grain	24.60
Alfalfa hay	31.00
Soybean meal	13.25
Wheat bran	28.00
Di-calcium phosphate	1.60
Limestone	0.95
Sodium chloride	0.30
Mineral-vitamin premix[1]	0.30
Total	100
Calculated analysis[2] (% on dry matter basis)	
Crude protein (CP, %)	17.08
Crude fiber (CF, %)	12.55
Ether extract (EE, %)	2.20
Digestible energy (DE, Kcal / Kg)	2416
Metabolizable energy (ME, Kcal / Kg)[3]	2219
Calcium (%)	1.20
Total phosphorus (%)	0.761
Lysine (%)	0.84
Methionine (%)	0.23
Price (LE / Kg)[4]	4.68

(1) One kilogram of mineral–vitamin premix provided: Vitamin A, 150,000 UI; Vitamin E, 100 mg; Vitamin K3, 21mg; Vitamin B1, 10 mg; VitaminB2, 40mg; Vitamin B6, 15mg; Pantothenic acid, 100 mg; Vitamin B12, 0.1mg; Niacin, 200 mg; Folic acid, 10mg; Biotin, 0.5mg; Choline chloride, 5000 mg; Fe, 0.3mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1mg; and Zn, 450mg.

(2) Calculated analysis according to feed composition tables for rabbits' feedstuffs used by De Blas and Wiseman (2010); (3) ME (Kcal / Kg diet) estimated as 0.95 DE according to Santoma *et al.* (1989). (4) Price at 2018 in Egypt.

The experimental diets:

The ingredients and the nutrient composition of BD are presented in Table 1, calculated analysis of basal diet was used based on feed composition tables for rabbit's feedstuffs according to De Blas and Wiseman (2010) and Villamide *et al.* (2010) and the requirements of digestible energy (DE Kcal / Kg diet) and crude protein % according to FEDNA (2013). Saltose Ex® is a commercial thermo stable probiotic used in the present study, where each 1 Kg of probiotic contains lactic acid bacteria (*Lacobacillus lactis*) 2.5 × 10^8 Colony-forming unit (CFU), *Bacillus subtilis* 1.8 × 10^9 CFU and calcium carbonate up to 1 Kg as a carrier. This probiotic produced by Pic-Bio, Inc. company, Japan and it was purchased from El-Yousr company for medicine trade, Cairo, Egypt. Tested probiotic was firstly mixed with premix, then gradually mixed with the other ingredients of the experimental diet. Rabbits fed the pellets once a day *ad-libitum*.

Growth performance traits:

Live body weight (BW), feed intake (FI), feed conversion ratio (FCR) and relative growth (RG) performance of rabbits were recorded. Daily weight gain (DWG), and mortality rate were estimated too. RG = (Final BW - initial BW) × 100 / 0.5 × (Final BW + initial BW), also the performance index (PI) was calculated on a group basis; PI (%) = (final BW (Kg) / FCR from 7 to 14 weeks of age) × 100 according to North (1981).

Microbial diagnosis:

The microbial diagnosis examination was carried out on samples of caecum contents (3 rabbits in each treatment) according to Mackie and McCartney (1953), American Public Health Association, APHA (1960) and Difco Mannual (1977).

Carcass parameters:

At the end of the experiment, three rabbits (*n* = 3) were randomly taken from each treatment, fasted for 12 hrs. of feed only, weighed and slaughtered to estimate some of carcass traits. Carcass parts, included carcass, heart, liver, giblets, kidney, spleen and cecum were presented as a percent of live BW.

Hematological and serum biochemical parameters:

At the end of the experiment three rabbits (*n* = 3) were randomly chosen from each treatment to collect the blood samples with or without anticoagulant. Blood samples were collected without anticoagulant and kept at room temperature, then the tubes were centrifuged at 3500 rpm for 20 minutes to separate clear serum. Serum samples were used to determine total protein, triglycerides, total cholesterol and liver enzymes activities using the commercial calorimetric kits, produced by Bio-diagnostic, Egypt. Other blood samples were taken in vial tubes containing EDTA as an anticoagulant from three rabbits (*n* = 3) per treatment to determine some hematological traits in the whole blood. All hematological and serum biochemical parameters were conducted in the Animal Health Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt

Economic efficiency:

At the end of the study, economical efficiency for weight gain was expressed as rabbit-production through the study and calculated using the following equation:

Economic efficiency (%) = (Net return LE/Total feed cost LE) × 100.

Where, net return = Total return- the cost of feeding

Statistical analysis:

All data were statistically analyzed using General Linear Models (GLM) procedure of the Statistical Package for Social Sciences version 17.0 (SPSS, 2008). Differences between mean among treatments were subjected to Duncan´s Multiple Range-test (Duncan, 1955). A factorial design (3×2) was planned, where the following statistical model was used to evaluate the effect of main factors and interaction between SD rates and probiotic (PR) levels on the experimental parameters as following:

$$Yijk = \mu + Ti + Rj + (TR)\,ij + eij$$

Where: Yijk = an observation; μ = overall mean; T= effect of SD rates; i = (1, 2 and 3); R= effect of PR levels; j= (1 and 2); TR= effect of interaction between SD and PR (ij (1, 2….6); and ejik = the experimental error.

RESULTS AND DISCUSSION

Results

Body weight and daily body weight gain:

Growing rabbits reared under low SD (3 rabbit / cage) led to significantly ($P \leq 0.05$) increased of BW and DWG compared to those reared under high SD rates (4 and 5 rabbits / cage) during different experimental periods (Table 2). From other side, there are no significant ($P \geq 0.05$) differences in both of BW or DWG among rabbits fed probiotic supplemented diet (0.4 g Saltose Ex® / Kg diet) and those fed free probiotic-BD during all experimental periods. The obtained results in Table 2 also revealed that the interaction effect between SD and dietary addition of tested probiotic (Saltose Ex®) led to significantly increased of both BW and DWG in case of rabbits reared in high SD and fed Saltose Ex® among all treatments, particularly at the end of the experiment (7-14 weeks).

Table 2. Effect of stocking density, probiotic and their interaction on body weight and daily weight gain of growing rabbits from 7 to 14 weeks of age

Traits		Body weight (g/rabbit)			Daily weight gain (g/rabbit/day)		
		7	10	14	7-10	10-14	7-14
Stocking density (SD, rabbits / cage)							
3		937.5	1485.0^{a}	2046.9^{a}	19.6^{a}	20.1	19.8^{a}
4		913.5	1318.4^{b}	1879.7^{b}	14.5^{b}	20.1	17.3^{b}
5		896.9	1278.4^{b}	1896.0^{b}	13.6^{b}	22.1	17.9^{b}
Pooled ± SE		14.26	31.01	36.84	1.04	1.17	0.58
Probiotic (PR, g / Kg diet)							
0.0		905.7	1348.3	1880.9	15.8	18.6	17.2
0.4		926.2	1372.9	2012.3	16.0	22.8	19.4
Pooled ± SE		11.64	25.32	30.08	0.85	0.96	0.48
Interaction effect (SD × PR)							
3	0.0	925.6	1515.0^{a}	2005.6^{a}	21.1^{a}	17.5^{b}	19.3^{a}
	0.4	949.4	1455.0^{ab}	2088.3^{a}	18.1^{ab}	22.6^{ab}	20.3^{a}
4	0.0	899.6	1278.2^{c}	1797.0^{b}	13.5^{bc}	18.5^{ab}	16.0^{b}
	0.4	927.5	1358.6^{bc}	1962.2^{ab}	15.4^{bc}	21.6^{ab}	18.5^{ab}
5	0.0	892.1	1251.7^{c}	1806.8^{b}	12.8^{c}	19.8^{ab}	16.3^{b}
	0.4	901.8	1305.1^{c}	1986.3^{a}	14.4^{bc}	24.3^{a}	19.4^{a}
Pooled ± SE		20.16	43.86	52.10	1.48	1.66	0.82

a, b, c: Mean in the same column bearing different superscripts are significantly different ($P \leq 0.05$).

Feed intake and feed conversion ratio:

Rabbits reared under low SD (3 rabbits / cage) achieved significantly increased of FI and the best ($P \leq 0.05$) FCR compared to those reared in other SD (4 and 5 rabbits / cage) during the experimental period (7 - 10 weeks), while no significant ($P \geq 0.05$) differences of FI or FCR among all treatments in other experimental periods (Table 3). Results also revealed that rabbits fed 0.4 g Saltose Ex® / Kg diet led to significantly increased of FI compared to those fed free probiotic-BD, partially during the experimental periods (10 – 14 and 7 – 14), while no significant ($P \geq 0.05$) differences of FCR of both rabbits fed BD supplemented with or without probiotic during all the experimental periods were recorded (Table 3). The interaction between different SD rates and dietary addition of Saltose Ex® probiotic levels led to insignificant ($P \geq 0.05$) improved of FI and FCR, especially in case of rabbits reared under high SD (5 rabbits / cage) and fed 0.4 g Saltose Ex® / Kg diet during different experimental periods (Table 3).

Table 3. Effect of stocking density, probiotic and their interaction between them on feed intake and feed conversion ratio of growing rabbits from 7 to 14 weeks of age

Traits		Feed intake (g/rabbit)			Feed conversion ratio		
		7-10	10-14	7-14	7-10	10–14	7-14
Stocking density (SD, rabbits / cage)							
3		70.4^{a}	98.1	84.0	3.6^{b}	4.9	4.2
4		55.5^{b}	92.2	73.9	3.9^{b}	4.7	4.3
5		65.2^{b}	84.2	74.7	4.9^{a}	3.9	4.2
Pooled ± SE		3.77	5.91	3.62	0.27	0.17	0.16
Probiotic (PR, g / Kg diet)							
0.0		64.0	83.8^{b}	73.9^{b}	4.3	4.6	4.9
0.4		63.5	99.2^{a}	81.2^{a}	4.0	4.4	4.5
Pooled ± SE		3.08	4.83	2.95	0.22	0.28	0.13
Interaction effect (SD × PR)							
3	0.0	72.2	89.3^{ab}	80.7	3.4^{c}	5.1	4.2
	0.4	68.5	106.8^{a}	87.3	3.8^{bc}	4.7	4.3
4	0.0	56.8	84.0^{ab}	70.4	4.3^{abc}	4.6	4.4
	0.4	54.3	100.5^{ab}	77.4	3.6^{bc}	4.7	4.2
5	0.0	62.9	78.1^{b}	70.5	5.1^{a}	4.0	4.3
	0.4	67.6	90.2^{ab}	78.9	4.7^{ab}	3.7	4.1
Pooled ± SE		5.33	8.36	5.11	0.38	0.48	0.22

a, b, c: Mean in the same column bearing different superscripts are significantly different ($P \leq 0.05$).

Carcass traits:

Results in Table 4 showed that the effect of SD rates, supplemented probiotic levels and their interaction on carcass quality parameters as % of live body weight of growing rabbits at 14 weeks of age. Rabbits carcass quality parameters were significantly ($P \leq 0.05$) decreased by increased SD (5 rabbit / cage), except heart, spleen and cecum (%) compared to those reared in low SD (3 rabbits / cage). No significant ($P \geq 0.05$) differences were recorded in all carcass quality parameters (%) of both growing rabbits fed tested probiotic or those fed free probiotic-BD. The interaction effects between SD rates and different levels of probiotic significantly improved all carcass quality parameters, specifically with increasing SD rates of rabbits per cage, except spleen and cecum (%).

Performance index, viability, relative growth and microbial activity:

Data in Table 5 described the effect of SD, addition of tested probiotic and their interaction effects on PI, viability, RG, and microbial activity parameters of growing rabbits at 14 weeks of age. Where, there are no significant ($P \geq 0.05$) differences of both PI and viability of growing rabbits reared under different SD rates were observed. While, RG was significantly ($P \leq 0.05$) decreased by increasing SD rates of rabbits compared to those reared under low SD (3 rabbits / cage). The microbial activity parameters revealed significantly increased of TBC, while

E. coli, LBA, and LBA/TBC were significantly decreased by increasing SD rates of growing rabbits compared to those reared under low SD (3 rabbits / cage). From other hand, rabbits fed 0.4 g Saltose Ex® / Kg diet led to significantly increased of PI and RG compared to those fed free probiotic-BD, while there are no significant differences of viability among of both rabbits fed tested probiotic or those fed free probiotic-BD (Table 5).

Table 4. Effect of stocking density, probiotic and their interaction between them on carcass quality traits as % of live body weight of growing rabbits at 14 weeks of age

Traits		Carcass	Heart	Liver	Giblets	Kidney	Dressing	Spleen	Cecum
Stocking density (SD, rabbits / cage)									
3		49.8^{a}	0.28	4.2^{b}	5.3^{a}	0.86^{a}	55.1^{a}	0.06	6.1
4		46.6^{b}	0.29	4.4^{a}	5.5^{a}	0.79^{a}	52.1^{b}	0.07	6.7
5		47.6^{ab}	0.23	3.5^{b}	4.4^{b}	0.66^{b}	52.0^{b}	0.04	6.9
Pooled ± SE		0.94	0.02	0.28	0.27	0.03	0.88	0.01	0.40
Probiotic (PR, g / Kg diet)									
0.0		47.6	0.28	4.0	5.0	0.77	52.6	0.06	6.5
0.4		48.5	0.25	4.1	5.1	0.76	53.5	0.06	6.7
Pooled ± SE		0.76	0.02	0.23	0.11	0.02	0.72	0.01	0.33
Interaction effect (SD × PR)									
3	0.0	48.5^{ab}	0.31^{a}	4.1^{ab}	5.2^{ab}	0.83^{a}	53.6^{ab}	0.07	6.0
	0.4	51.2^{a}	0.26^{ab}	4.3^{ab}	5.5^{a}	0.87^{a}	56.6^{a}	0.06	6.2
4	0.0	47.7^{ab}	0.33^{a}	4.1^{ab}	5.3^{ab}	0.84^{a}	53.0^{ab}	0.06	7.1
	0.4	45.6^{b}	0.25^{ab}	4.6^{a}	5.6^{a}	0.74^{ab}	51.2^{b}	0.08	6.8
5	0.0	46.6^{b}	0.29^{b}	3.8^{ab}	4.6^{ab}	0.65^{b}	51.2^{b}	0.05	6.4
	0.4	48.7^{ab}	0.26^{ab}	3.2^{b}	4.1b	0.68^{b}	52.8^{ab}	0.03	7.0
Pooled ± SE		1.32	0.03	0.12	0.39	0.04	1.25	0.02	0.57

a, b, c: Mean in the same column bearing different superscripts are significantly different (P ≤ 0.05). Carcass weight (%) = empty body weight / preslaughter × 100; Dressing weight = Carcass weight % + giblets weight % (Liver + Heart + Kidneys weight %).

Regarding, the microbiol activity of rabbits fed 0.4 g Saltose Ex® / Kg diet led to significantly decreased of TBC and *E. coli* compared to those fed free probiotic-BD, while there are no significant (P ≥ 0.05) differences of both LAB and LBA/TBC among rabbits fed tested probiotic or those fed free probiotic-BD (Table 5). The interaction effects between SD rates and different levels of dietary tested probiotic significantly increased RG of rabbits reared in high SD (5 rabbits / cage) and fed the tested probiotic compared to those reared under the same SD and fed free probiotic-BD. Meanwhile, insignificant effects on PI and no significant differences of viability of growing rabbits by the interaction between SD rates and different levels of tested probiotic were detected. In case of the microbiol activity of rabbits the interaction between SD rates and different levels of tested probiotic led to significantly decreased of TBC of rabbits fed 0.4 g Saltose Ex® / Kg diet and reared under all SD rates. Also, addition of tested probiotic led to significantly decreased of *E. coli*, LAB, and LAB/TBC of rabbits reared under low SD (3 rabbits / cage) compared to those reared under high SD (5 rabbits / cage).

Table 5. Effect of stocking density, probiotic and their interaction between them on performance index, viability, relative growth performance and microbial activity parameters of growing rabbits at 14 weeks of age

Traits		PI and viability (%)				Microbial activity			
		PI[1]	N. R[2]	V[3]	RG[4]	TBC[5]	*E. coli*[6]	LAB[7]	LAB /TBC
Stocking density (SD, rabbits / cage)									
3		48.4	3	88.9	66.7^{a}	4.2^{b}	2.0^{b}	1.8^{b}	0.4^{b}
4		44.5	4	75.0	54.2^{b}	2.6^{c}	2.3^{a}	2.3^{a}	0.9^{a}
5		45.3	5	70.0	56.1^{b}	4.4^{a}	0.4^{c}	0.8^{c}	0.3^{c}
Pooled ± SE		1.96	-	6.42	26.26	0.06	0.07	1.6	0.02
Probiotic (PR, g / Kg diet)									
0.0		43.9^{b}	4	77.6	53.7^{b}	4.6^{a}	2.8^{a}	1.6	0.5
0.4		48.3^{a}	4	78.3	64.0^{a}	2.8^{b}	0.3^{b}	1.7	0.6
Pooled ± SE		1.60	-	5.10	21.44	0.05	0.06	0.06	0.01
Interaction effect (SD × PR)									
3	0.0	48.1	3	75.8^{ab}	63.3^{a}	4.8^{b}	3.9^{a}	2.0^{b}	0.4^{d}
	0.4	48.7	3	100.0^{a}	69.2^{a}	3.5^{c}	0.07^{c}	1.7^{c}	0.5^{d}
4	0.0	41.5	4	75.0^{ab}	48.5^{b}	2.1^{e}	4.0^{a}	2.2^{ab}	1.0^{a}
	0.4	47.5	4	75.0^{ab}	60.0^{ab}	3.1^{d}	0.5^{b}	2.4^{a}	0.8^{b}
5	0.0	41.9	5	80.0^{ab}	49.5^{b}	6.9^{a}	0.5^{b}	0.6^{e}	0.1^{e}
	0.4	48.8	5	60.0^{b}	62.8^{a}	1.9^{e}	0.2^{bc}	1.1^{d}	0.6^{c}
Pooled ± SE		2.78	-	8.81	37.13	0.09	0.10	0.08	0.02

a, b, c, d, e: Mean in the same column bearing different superscripts are significantly different (P ≤ 0.05). [1]Performance index; [2] Number of rabbits; [3] viability; [4] Relative growth performance; [5] total bacterial count (× 10^5) germ counts expressed in CFU/g caecal digesta; [6] *Escherichia coli* (× 10^3) germ counts expressed in CFU/g caecal digesta; [7] Lactic acid bacteria (× 10^5) germ counts expressed in CFU/g caecal digesta.

Serum biochemical parameters:

Both of SD rates or tested probiotic levels led to insignificant effects on all serum biochemical parameters, except albumin and globulin, which insignificantly (P ≥ 0.05) decreased and significantly (P ≤ 0.05) increased, respectively by increasing SD rates of growing rabbits compared to low SD rate (3 rabbits / cage; Table 6). The interaction between SD rates and different levels of probiotic led to significantly decreased of total protein, globulin, and ALT, particularly by increasing SD rates. Meanwhile, there are not clear significant trends of other serum biochemical parameters related with the experimental treatments (Table 6).

Hematological parameters:

Increasing SD of growing rabbits led to significantly (P ≤ 0.05) decreased of neutrophils (%), and neutrophils/ lymphocytes ratio (N / L), and significantly increased of lymphocytes (%) compared to those reared under low SD (3 rabbits / cage, Table 7). Meanwhile, rabbits fed 0.4 g Saltose Ex® / Kg diet or those fed free probiotic-BD did not significantly affects on all hematological parameters (Table 7). The interaction between SD rates and different levels of tested probiotic led to insignificant (P ≥ 0.05) increased of lymphocytes and insignificant decreased of neutrophils (%), and N / L.

Table 6. Effect of stocking density, probiotic and their interaction between them on serum biochemical parameters of growing rabbits at 14 weeks of age

Traits		Serum biochemical parameters						
		Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	AST (U/L)	ALT U/L)
Stocking density (SD, rabbits / cage)								
3		5.3	3.6^{a}	1.7^{b}	153.3	130.9	5.8	12.6
4		5.4	2.7^{b}	2.8^{a}	163.3	116.0	6.0	9.8
5		5.8	3.4^{a}	2.5^{a}	152.5	107.1	7.3	9.2
Pooled ± SE		0.16	0.10	0.16	3.64	8.01	0.62	1.33
Probiotic (PR, g / Kg diet)								
0.0		5.7	3.1	2.6	153.8	122.0	6.0	10.8
0.4		5.4	3.3	2.1	158.8	114.0	6.7	10.3
Pooled ± SE		0.13	0.08	0.13	2.97	6.54	0.51	1.08
Interaction effect (SD × PR)								
3	0.0	5.1^{b}	3.4^{a}	1.7^{c}	161.0	143.0	5.5	8.3^{b}
	0.4	5.6^{b}	3.8^{a}	1.8^{c}	145.5	119.0	6.0	17.1^{a}
4	0.0	5.6^{b}	2.6^{b}	3.1^{a}	155.0	115.0	5.5	11.8
	0.4	5.3^{b}	2.8^{b}	2.5^{ab}	171.5	116.9	6.5	7.9^{b}
5	0.0	6.4^{a}	3.4^{a}	3.0^{a}	145.5	108.1	7.0	12.2^{ab}
	0.4	5.3^{b}	3.4^{a}	2.0^{bc}	159.5	106.1	7.5	6.1^{b}
Pooled ± SE		0.23	0.14	0.22	5.15	11.33	0.88	1.87

a, b, c: Mean in the same column bearing different superscripts are significantly different (P ≤ 0.05). AST = Aspartate aminotransferase; ALT = Alanine aminotransferase.

Table 7. Effect of stocking density, probiotic and their interaction between them on hematological parameters of growing rabbits at 14 weeks of age

Traits		Neutrophils %	Lymphocytes %	N/L ratio*
Stocking density (SD, rabbits / cage)				
3		41.5^{b}	46.5^{b}	0.9^{b}
4		53.0^{a}	33.0^{c}	1.6^{a}
5		40.3^{b}	49.0^{a}	0.8^{b}
Pooled ± SE		1.32	0.80	0.09
Probiotic (PR, g / Kg diet)				
0.0		42.7	44.3	1.0
0.4		47.2	41.3	1.3
Pooled ± SE		1.08	0.65	0.08
Interaction effect (SD × PR)				
3	0.0	40.0^{c}	49.0^{a}	0.8^{c}
	0.4	$43.0b^{c}$	44.0^{b}	1.0^{c}
4	0.0	47.0^{b}	36.0^{c}	1.3^{b}
	0.4	59.0^{a}	30.0^{d}	2.0^{a}
5	0.0	41.0^{bc}	48.0^{a}	0.9^{c}
	0.4	39.5^{c}	50.0^{a}	0.8^{c}
Pooled ± SE		1.86	1.13	0.06

a, b, c, d: Mean in the same column bearing different superscripts are significantly different (P ≤ 0.05). * N/L = Neutrophils / Lymphocytes ratio

Economic efficiency parameters:

Economic efficiency parameters of growing rabbits at 14 weeks of age reared under different SD rates and fed different levels of tested probiotic are presented in Table 8. Rabbits reared under different SD rates, and fed tested probiotic insignificantly (P ≥ 0.05) improved of economic efficiency parameters (Table 8).

Table 8. Effect of stocking density, probiotic and their interaction between them on economic efficiency parameters of growing rabbits at 14 weeks of age

Traits		Economic efficiency parameters							
		TFI/ rabbit[1]	Price/Kg feed[2]	TFC/ rabbit[3]	WG/ rabbit[4]	Price/ Kg BW[5]	Total return	Net return	EEF[6]
Stocking density (SD, rabbits / cage)									
3		4.7	4.72	22.2	1109.4	28	38.8	16.6	75.4
4		4.1	4.72	19.5	966.1	28	33.8	14.3	75.3
5		4.2	4.72	19.8	999.6	28	35.0	15.2	76.7
Pooled ± SE									6.16
Probiotic (PR, g / Kg diet)									
0.0		4.1	4.68	19.4	964.0	28	33.7	14.4	75.3
0.4		4.6	4.76	21.6	1086.0	28	38.0	16.4	76.4
Pooled ± SE									5.03
Interaction effect (SD × PR)									
3	0.0	4.5	4.68	21.2	1080.0	28	37.8	16.6	79.4
	0.4	4.9	4.76	23.3	1138.9	28	39.9	16.6	71.4
4	0.0	3.9	4.68	18.4	897.5	28	31.4	13.0	73.3
	0.4	4.3	4.76	20.6	1034.7	28	36.2	15.6	77.4
5	0.0	4.0	4.68	18.5	914.8	28	32.0	13.5	73.1
	0.4	4.4	4.76	21.0	1084.5	28	38.0	17.0	80.3
Pooled ± SE									8.71

a. b. c Mean in the same column bearing superscripts are significantly different (P ≤ 0.05). [1] = Total feed intake/rabbit/overall period; [2]Price / Kg feed= the price of 1 Kg feed by Egyptian pound; [3]=Total feed cost/rabbit; [4]= Total weight gain/rabbit; [5]= the price of 1 Kg of live body weight by Egyptian pound; [6]EEF= Economic efficiency (%) = (Net return / Total feed cost) ×100.

Discussion

Increasing SD in rabbit house worsens their productivity (decrease feed intake and body weight gain) (Abd El-Monem *et al*., 2009 and Baiomy, 2012). Where, SD had a significant effect on growth performance of growing rabbits in the present study. These findings herein are consistent with those reported earlier by Kalaba (2012); El-Samra *et al*. (2013) and Bhattacharjya *et al*. (2015). Also, Bhattacharjya *et al*. (2017) revealed that New Zealand White (NZW) rabbits providing 0.38 m^2 floor spaces per animal showed better performance in terms of BW, body weight gain and FCR. More recently, El-Bayoumi *et al*. (2018) reported that NZW rabbits housed at high SD led to the lowest weight gain and FI with highest FCR. Controversy with the present findings, Garcia *et al*. (2005) and Neto *et al*. (2007) stated that the SD had no effect on live body weight and gain of growing rabbits. In this respect, Oliveira and Almeida (2002) and Trocino *et al*. (2004) also reported that SD had no overall effect on feed intake, which is disagreement with the present findings. According to these results the effect of SD depends on cage size and the final weight (age) of the rabbits. The growth rate is in close connection with FI and BW of the rabbits. In most cases SD did not influence FCR (Matics *et al*., 2004).

Regarding the positive effects of tested probiotic on experimental rabbits reared under different stocking rates in the present study. Similarly, results were obtained by Mountzouris *et al*. (2010) and Bansal *et al*. (2011) as they reported beneficial effects of probiotic supplementation to broiler diet in terms of increased BW and FCR through a natural physiological way and educating the digestion by improving the integrity of the intestinal mucosal barrier, digestive and immune functions of intestine, which leads to increase the immune resistance and productivity of rabbits (Rajput and Li, 2012). Similar findings in agreement with our obtained results regarding the valuable effects of probiotic on growth performance parameters of growing rabbits have been well recently documented by El-Badawi *et al*. (2017) who stated that body weight gain and feed efficiency of growing NZW rabbits were obviously improved ($P < 0.05$) with diets supplemented with yeast, bacteria or their mixture than the control. The same conclusion was also stated by Thanh and Jamikorn (2017) of weaning rabbits fed supplemented *B. subtilis* and *L. acidophilus*. Similar results were recently reported in growing rabbits (Bhatt *et al*., 2017) or weaning rabbits (Phuoc and Jamikorn, 2017) fed probiotics containing beneficial bacterial strains. More recently, the same trends of growth performance and nutrients utilization parameters of Chinchilla rabbits fed 1.0 g probiotic / Kg of feed were reported also by Kalma *et al*. (2018). Generally, the enhancement in body weight gain of experimental rabbits by supplemented probiotic in the present study could be due to beneficial effects of retained microbiota in the gut, which helped in improving feed digest ion and absorption. In addition, probiotics may act on non-digestible carbohydrates and give rise to short-chain fatty acids (Simonová *et al*., 2015). Moreover, Kritas *et al*. (2008) explained that feeding probiotics may have a growth promoting activity by competing with harmful flora and stimulating the immune system. Also, Copeland *et al*. (2009) clarified that probiotics fortified diets were effective in decreasing pathogenic bacteria colonization.

The cage area per rabbit specified in the act and regulation on conditions for housing livestock ensures the animals suitable living space (Brzozowski and Łukaszewska, 2015). In the current study, high SD significantly reduced the carcass quality parameters of growing rabbits than those reared under low SD, where the SD of cage had bad effect on carcass traits. Also, Abd El-Monem *et al*. (2009) and Trocino *et al*. (2015) reported the same findings as in the current study. Partially with the obtained results, El-Samra *et al.* (2013) found that there was non- significant ($P > 0.05$) effect of cage density on carcass weight of rabbits under different SD rates. More recently, El-Bayoumi *et al*. (2018) reported that SD had a significant effect on hot carcass weight, dressing out percentage, liver and kidney weight, in addition to head percentage of NZW rabbits. Inversely with the obtained findings, a non-significant effect of SD on carcass traits was earlier reported by Villalobos *et al*. (2008); Yakubu and Adua (2010) and Dorra *et al*. (2013).

In the present study, dietary addition of tested probiotic to growing rabbits not affected on carcass quality parameters compared to those fed the free probiotic-BD. The obtained results herein were in compliance with the findings reported by Rotolo *et al*. (2014) who reported that no effect on the weight of the full and empty caecum of rabbits fed live yeast supplementation. Recently, Bhatt *et al*. (2017) reported that probiotics had non-significant ($P > 0.05$) effect on carcass traits and fatty acid profile of experimental rabbits. Moreover, El-Badawi *et al*. (2017) also stated that carcass characteristics, dressing % calculated relative to pre-slaughter or empty body weight, meat, and bone ratio were not statistically different among groups of growing NZW rabbits fed diets supplemented with live yeast, bacteria or their mixture and even the control. Inversely with previous findings, Maertens *et al.* (1994) observed that caecal weight was higher in rabbits fed the Paciflor® (*Bacillus*) diet. Additionally, Kermauner and Struklec (1996) noted that an effect of 0.5% probiotic Acid Pack Way (*L. acidophilus,* and *Streptococcus faecium*) was only observed in the decreased proportion of stomach and increased proportion of caecum of gastrointestinal weight. Thus, the contradictory results in the literature could be explained by differences in experimental design concerning breeding conditions, breed of rabbit, weaning age, composition of diet and strain, dose and period of administration of microorganisms used, as well as the hygienic levels during the experimental period.

High SD affects the feeding behavior and welfare of animals and therefore adversely effects on the productivity (Martrenchar *et al*., 1997). Thus, increasing SD of growing rabbits in the present study led to significantly decreased of GR, but not affected on both of PI or viability compared to those reared under low SD (Table 5). These findings are seriously related with the severely effects of high SD on growth performance (Table 2), FI, and FCR (Table 3) parameters of growing rabbits than those reared under low SD (3 rabbits / cage). These findings are closely agreed with those obtained by Das *et al*. (2007). Where, mortality was independent of SD (Matics *et al*., 2004). Rearing rabbits at a high density had scarce effects on dressing out percentage and on meat red index (Xiccato *et al*., 1999). The caecal microbiota in the caecum play an important role in the digestion and digestive health of rabbits (Carabaño *et al*., 2006). Hence, increasing SD of growing rabbits in the current study severely effects on the microbiol activity parameters compared to those reared under low SD (Table 5).

Regarding the effect of tested probiotic on PI and RG of rabbits in the present study, addition of tested probiotic led to significantly increased of both PI and RG of rabbits compared to those fed free probiotic-BD (Table 5). Also, addition of tested probiotic significantly reduced the microbiol activity of rabbits than those fed free probiotic-BD. These beneficial effects of tested probiotic may be related with its inclusion of *L. lactis* 2.5×10^8 CFU, and *B. subtilis* 1.8×10^9 CFU, which led to significantly improved of growth performance parameters (Table 2), and FI or FCR of growing rabbits in the present study (Table 3). Where, the direct effect of dietary probiotic in the present study might be related to stimulate the lymphatic tissue as reported by Kabir *et al.* (2004). Whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract. Additionally, Christensen *et al*. (2002) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Since probiotic- and prebiotic- induced health promoting effects are likely to be attributed to their ability to antagonize pathogenic bacteria and to modulate host immune responses (Yan and Polk, 2011). Recently, Phuoc and Jamikorn (2017) reported that weaning rabbits fed diets supplemented with *L. acidophilus* had greater number of intestinal lactobacilli, which could enhance intestinal hydrolytic enzyme activity in these rabbits resulting in an increase of nutrient digestibility and feed efficiency utilization (Fuller, 1989). Inversely, Belhassen *et al*. (2016) stated that supplementation of live yeast *Saccharomyces cerevisiae* did not modify growth traits of growing rabbits and a slightly altered caecal microbiota after weaning. Where, the effect of probiotics on growth performance, several production parameters and health status vary depending on the dose, age, livestock conditions, and even between studies.

The current findings revealed that both of SD rates or tested probiotic levels did not affected on serum biochemical parameters of growing rabbits (Table 6). Similar results were obtained by Seyidoğlu and Galip (2014) when rabbits fed live yeast; and Özsoy and Yalçin (2011) with *S. cerevisiae* in broiler turkey. Recently, Belhassen *et al*. (2016) found that dietary live yeast supplementation did not affect blood biochemical parameters of treated growing rabbits. Inversely, serum cholesterol and triglycerides levels were significantly decreased by supplementing Bio-Mos, Bio-Plus or their mixture in rabbit diets (Abdelhady and El-Abasy, 2015). Similar findings were reported also by Sudha *et al*. (2009) and Ooi and Liong (2010). Likewise, reduction in serum cholesterol of broiler chickens fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastrointestinal tract by probiotic supplementation (Mohan *et al*., 1995 and 1996). In contrary with the obtained results herein, Das *et al*. (2007) and Kalaba (2012) contravene the current findings. More recently, El-Bayoumi *et al*. (2018) reported that SD had a significant effect on serum biochemical parameters of NZW rabbits.

In the present study, high SD led to significantly decreased of neutrophils (%), and N / L, and significantly increased of lymphocytes (%) compared to those reared under low SD (3 rabbits / cage, Table 7). In partial accordance with the obtained findings, Yakubu *et al*. (2008) found higher average of hematological parameters of rabbits reared under different stocking rates. In this respect, Kalaba (2012) stated that rabbits stocked at 8 rabbits/m^2 had the highest ($P < 0.05$) values of tested hematological parameters than those stocked at 4 rabbits/m^2. In harmony with the current findings, El-Samra *et al*. (2013) suggested that rabbits stocked at 4 rabbits/cage had significantly ($P < 0.05$) the highest white blood cells (WBCs) compared to those having 1, 2 and 3 rabbits/cage. More recently, El-Bayoumi *et al*. (2018) concluded that increasing SD up to (20 rabbits/m^2) induce stressful condition in term of increasing WBCs and platelets counts and some disturbance in performance and carcass traits of NZW rabbits. Inversely, De la Fuente *et al*. (2004) reported that platelets was similar in rabbits stocked at 8 and 12 rabbits per cage. Non- significant difference in WBCs in rabbits stocked under different SD rates was also reported by Yakubu *et al*. (2008).

The obtained findings herein revealed that rabbits fed 0.4 g Saltose Ex® / Kg diet or those fed free probiotic-BD did not significantly affected on all hematological parameters (Table 7). The obtained results regarding the effects of probiotic on the hematological parameters are in agreement with those reported by Dimcho *et al*. (2005) who found that the probiotic supplementation did not affect the blood constituents comprising, or hemoglobin (Hb) concentrations. Similarly, Ewuola *et al*. (2010) mentioned that weaned rabbits fed dietary prebiotics (Biotronic®) and probiotics (BioVET®-Yc) did not affect the erythrocytes (RBCs) and Hb. Recently, Abdelhady and El-Abasy (2015) stated that non-significant change in RBCs count, Hb concentration, or blood indices (MCV, MCH and MCHC) of growing NZW male rabbits fed prebiotics and probiotics and their mixture.

Rabbit production is relatively important to the economy of some developing countries including Egypt (Colin and Lebas, 1996). In the present study, increasing SD of growing rabbits had non-significant affected on economic efficiency parameters compared with those reared under low SD (3 rabbits / cage, Table 8). Although the tested probiotic significantly achieved highest growth performance (Table 2) and nutrients utilization (Table 3) of growing rabbits than those fed free probiotic-BD, while feeding rabbits of tested probiotic led to slightly insignificant improved of economic efficiency parameters (Table 8). These economically findings herein may be due to the low price of growing black Balady rabbits as a local strain in Egypt, as well as may be due to the shortage of the experimental period (7 weeks), which led to slightly improve of economic efficiency. In this regard, Hoy *et al*. (2006) reported that improved welfare of rabbits might increase economic returns by boosting growth rate or feed conversion efficiency, the introduction of environmental enrichment to farmed rabbits may also improve the public image of animal production in intensive breeding systems. In contrary with the obtained findings, El-deek *et al*. (2013) reported that growing rabbits fed dietary probiotic achieved the highest economic efficiency value among all experimental groups.

Finally, the obtained findings revealed that addition of 0.4 g Saltose Ex® / Kg diet significantly improved the growth performance, FI, FCR, carcass quality parameters, PI, RG, microbiol activity and hematological parameters, as well as it led to slightly affected on serum biochemical and economic efficiency parameters of growing black Balady rabbits, especially those reared under high SD rate (5 rabbits / cage). These superiorities may be related with the beneficial microorganisms of tested probiotic, and highest growth performance of growing rabbits with the little feed consumption compared to those fed free probiotic-BD. Thus, the margin on the feed cost is generally improved by 2% to 10%, when an intake limitation strategy was applied (Dolberg, 2001; Owen *et al*., 2005).

CONCLUSION

Based on the obtained findings it could be concluded the useful addition of 0.4g Saltose Ex®/Kg diet, which significantly improved the productivity parameters of growing black Balady rabbits reared under different SD rates in cages, especially those reared under high SD (5 rabbits / cage). Nevertheless, further studies are required to assess the efficacy of dietary Saltose Ex® or other probiotics alone or by mixing them with prebiotic as symbiotic of rabbits reared under different environmental, stocking density and housing conditions.

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تأثير كثافة التخزين والتغذية التكميلية بالبروبيوتيك على الأداء الإنتاجي والإقتصادي في الأرانب النامية المحلية

ملاك منصور بشاره ، مني أحمد رجب ،عادل السيد الدسوقي ،هاني نبيل فهيم ،عبد الغني محمد الشحات ، أحمد منير العزب و أحمد أحمد الجمل

معهد بحوث الإنتاج الحيواني - مركز البحوث الزراعية – الدقي - الجيزة - مصر

أجريت تجربة داخلية ذات تصميم عاملى (٢ × ٣) لدراسة تأثير مستويات مختلفة من البروبيوتيك Saltose Ex® على الأداء الإنتاجي ومعايير الكفاءة الاقتصادية للأرانب البلدي السوداء النامية والتي تم تربيتها تحت معدلات كثافة تخزين مختلفة لمدة ٧ أسابيع. أجمالى الأرانب ٧٢ أرانب (٧ أسابيع من العمر) تم تقسيمها إلى ستة معاملات تجريبية. كانت المعاملات التجريبية على النحو التالي ، المعاملة الأولي: غذيت الأرانب على العليقة الأساسية + صفر جرام بروبيوتيك / كيلوجرام علف تحت كثافة تخزين ٣ أرانب / قفص ، المعاملة الثانية: غذيت الأرانب على العليقة الأساسية مضاف إليها ٠.٤ جرام بروبيوتيك / كيلوجرام علف تحت كثافة تخزين ٣ أرانب / قفص ، المعاملة الثالثة: غذيت الأرانب على العليقة الأساسية + صفر جرام بروبيوتيك / كيلوجرام علف تحت كثافة تخزين ٤ أرانب / قفص ، المعاملة الرابعة: غذيت الأرانب على العليقة الأساسية مضاف إليها ٠.٤ جرام بروبيوتيك / كيلوجرام علف تحت كثافة تخزين ٤ أرانب / قفص ، المعاملة الخامسة: غذيت الأرانب على العليقة الأساسية + صفر جرام بروبيوتيك / كيلوجرام علف تحت كثافة تخزين ٥ أرانب / قفص والمعاملة السادسة: غذيت الأرانب على العليقة الأساسية مضاف إليها ٠.٤ جرام بروبيوتيك / كيلوجرام علف تحت كثافة تخزين ٥ أرانب / قفص وذلك خلال الفترة من ٧ إلى ١٤ أسبوع من العمر. أظهرت النتائج التي تم الحصول عليها أن إضافة ٠.٤ جرام بروبيوتيك Saltose Ex® / كيلوجرام علف لا تخفف فقط من التأثيرات الحادة لارتفاع كثافة التخزين على الأرانب ، ولكن أيضا تحسن بشكل معنوي من أداء النمو ، وكفاءة العناصر الغذائية ، وخصائص جودة الذبيحة ، والنشاط الميكروبي ، بالإضافة إلى ذلك أدى إلى زيادة طفيفة في معايير الكفاءة الاقتصادية للأرانب النامية وخاصة تلك التي تم تربيتها تحت كثافة تخزين عالية (٥ الأرانب / قفص). وبالتالي يمكن التوصيه بالإستخدام المفيد لـ ٠.٤ جرام بروبيوتيك Saltose Ex® / كيلوجرام علف فى النظم الإنتاجية المختلفة للأرانب البلدي السوداء النامية خاصة تلك التى يتم تربيتها في الأقفاص.