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Full Length Research Paper

Economic Performance and Immunological Response of Sheep in Rapport to *Lactobacillus acidophilus.*

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Abstract
This study was objective to evaluate economic, Productive Performance and immunological assessment of Probiotic (L. acidophilus) in male Rahmani sheep in Sharkia Province. A total number of 24 male Rahmani sheep were used. The sheep were ranked into 3 groups. The 1st group was used as control (non-treated and non-vaccinated). The 2nd group administered clostridia polyvalent vaccine. The 3rd group was vaccinated and afford with the probiotic for successive 90 days of experiment (3 gm /head). Immunological parameters (Total and differential leucocytic count, serum lysozyme activity and serum nitric oxide), productive performance parameters (finished body weight, feed conversion ratio and efficiency) and Economical measures (Net profit (LE/ head) were evaluated in different groups. The obtained results divulged that the total leukocytic count, lysozyme activity and serum nitric oxide were significantly elevated in the 3rd group. Productive performance was significantly improved especially total feed intake, feed efficiency and finished body weight in the 3rd group. The most profit (LE/head) was found also in the group that vaccinated and given the probiotic. So we recommend from our economic and immunologic results that using Lactobacillus acidophilus in male Rahmani sheep daily for consecutive 90 days in a dose of 3gm /head would be beneficial.

Key words: *Male Rahmani sheep, Lactobacillus acidophilus, Immunological parameters, productive performance, Economic Evaluations.*

Introduction

Probiotics are living microorganisms that, when delivered in adequate amounts, confer benefits to host health, through a positive action on the intestinal microbiota [1].Additionally, the probiotics are related to decrease lactose intolerance, cancer, allergies, hepatic disease, helicobacter pylori infections, urinary tract infections, hyperlipidemia and increased immunity [2-4]. Probiotics are living microorganisms or microbial mixtures that impact the host in an advantageous manner and increasing its microbial balance, particularly the environment of the gastrointestinal tract [4, 5]. FAO (Food and Agriculture Organization) mentioned probiotics when administered in adequate amounts confers a health benefit on the host and improvement of productive performance and hence improve economic efficiency [6]. An ideal probiotic should have the following, a) the ability to adhere to cells. b) Decrease pathogenic adherence. c) Persist and multiply, d) produce acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth, e) be safe, noninvasive; non-carcinogenic and f) coaggregate to form a normal balanced flora [7]. The beneficial effect of probiotics could be produced in two ways. They could operate by: (a) Suppressing harmful bacteria, this could manifest itself in reduced numbers of bacteria or in a decreased concentration of harmful metabolites such as enterotoxin. (b) Stimulation of bacteria which are engaged in beneficial activities such as the production of essential nutrients like vitamins or in digestion of food components. [8].

The objectives of this research work is to evaluate the immunological effect of *Lactobacillus acidophilus* in sheep vaccinated with *clostridia* vaccine as well as the productive performance and economical evaluation of using *Lactobacillus acidophilus* in male Rahmani sheep in Sharkia Province.

Material and Methods

Data collection

This work was carried out during the period from September 2015 till January 2016.

A. Drugs: Probiotic (Probax®), produced by Microbax (India). It is water soluble powder, a probiotic for poultry and animal health. Each one Kg. haves *Lactobacillus acidophilus* not less than $1x10^{11}$ CFU.

B. Vaccine: Ultrabac® 8. A polyvalent clostridia vaccine, manufactured by Pfizer Animal Health Technical Services, Exton, PA, USA a Division of Pfizer Inc., Ny, 10017.

C. Animals: A twenty-four apparently healthy Rahmani sheep average body weight 25-30 kg and their ages ranged between 6-8 months were used in this study. They were purchased from local markets.

D. Housing: They were kept loose in a shaded yard with a concrete floor covered with chopped rice straw as bedding (3.2mx2.2m floor area). The animals were fed and watered ad-libitum.
E. Feeding and watering: They were provided with a concentrate mixture which was composed of (maize 30%, cotton seed cake 25%, bran20%, rice polish 17%, Mollase 5%, Ca-carbonate 2% and common salt 1%) according to [9]. The animals were provided with the rice straw and clean water ad-libitum from permanent troughs in front of them in the yard. Pre-experiment period extended for one week where animals were subjected to thorough clinical as well as laboratory examination to ensure sound physiological activities.

Experimental Design
The Rahmni sheep (24) were classified into 3 equal groups, each of eight and having nearly the same body weight.
The first group was used as control (non-treated and non-vaccinated). The second group was vaccinated by clostridia polyvalent vaccine (3 ml- subcutaneous. The third group was vaccinated and having the probiotic (3 gm /head) throughout the experiment.

Blood samples
Two blood samples were obtained from each animal at different ages (at zero time 3,7,28, 60, 90 days post vaccination).

a. Whole blood samples: 3 ml of blood were allowed to flow freely and gently into a clean and dry sterile vials containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. These samples were used for counting total and differential leukocyte counts.
b. Serum samples: An equal number of blood samples were collected in the same manner in centrifuge tubes without anticoagulant, then allowed to be clotted at room temperature and centrifuged at 3000 r.p.m. for 15 minutes. Clear non-haemolysed sera were obtained and transferred into clean dry and sterile vials and kept at -4 C^{o} until used for estimating nitric oxide (NO) production and lyzosomal activity.

Methods
Determination of Total and Differential leukocyte count (WBCs count): Total and differential Leukocyte count was done by using the improved Neubauer chamber according to the method of [10].

Nitric oxide production assay: Was evaluated according to [11].

Lysosomal activity assay: Was performed according to [12].

Productive Performance Measures.
Live body weight (LBW):- Rahmani sheep were weighted in each group during experimental period. Total individual live weights in each group were divided by the number of Rahmani sheep the group to obtain the average live body weight per sheep (LBW).

Feed intake: - Rahmani sheep in each group were provided with a weighed amount of feed day, the residual were obtained at the end of the day and the amount consumed was calculated by the difference.

Feed conversion rate (FCR):- was calculated according to [13].

Feed efficiency (FE): - was calculated as follow: Feed efficiency = (Gain in live body weight in this period / Feed intake in certain period cited by [14, 15].

Economic measures
Costs of male sheep's production (LE/Animal): Variable, fixed and total costs include all ingredient of variable cost includes Feed, labor, veterinary and others related to production cited by [16]. Also Depreciations of Building and equipment were calculated according to [17, 18].

Income parameters of Rahmani sheep in production (LE/ animal): that includes Variable factors of return from sale animals and litter. According to [19].

Net income: That includes total returns minus total costs according to [20].

Statistical analysis: the data analyzed by SPSS/PCT, 2001 [21] using ANOVA Post-hoc analysis by The Duncan multiple range test are also used [22].

Results and Discussion
Effect on total and differential leukocyte count
The present study was conducted to study the immunological profile of the probiotic (*Lactobacillus acidophilus*) in Rahmani sheep. The obtained results revealed that total leukocytic count in vaccinated and probiotic treated group was significantly increased at the 60th and 90th days post vaccination when compared to control groups similar results were obtained in poultry by [23]. Vaccination of sheep with *Clostridia* polyvalent vaccine without the probiotic evoked a significant increase in total leukocytic count at the 3rd and 7th day

post vaccination. The values tend to decrease gradually to reach normal levels by the end of the trial. The present study showed non-significant increase in neutrophil count at the 7th and 28th, 60th and 90th days after vaccination in vaccinated, probiotic treated sheep when compared to the control animals. The result concerning lymphocyte count revealed a non-significant change throughout the entire trial in the animals under investigation. Monocyte count showed a significant decrease in the vaccinated probiotic treated group at the 28th, 60th and 90th days post vaccination.

Table (1): Effect of vaccination alone and /or in combination with probiotic (Lactobacillus acidophilus) on total and differential Leukocyte count in Rahmani sheep (Means±SE) (n=8)

Items		T.L.C (*10^3/ml)	NEUT (%)	Lymph (%)	MON (%)
	Zero time	8.56 ± 0.34^{b}	53.7 ± 2.5^{ab}	41.5±1.7	3.8 ± 0.2^{a}
Control Group	3 days	8.7 ± 0.33^{b}	54.8 ± 2.1^{ab}	42.0±1.9	3.7 ± 0.3^{a}
	7day	8.7 ± 0.32^{b}	54.25 ± 2.3^{ab}	42.0±1.7	3.7 ± 0.2^{a}
	28day	8.7 ± 0.32^{b}	53.9 ± 2.3^{b}	41.9±1.5	3.6 ± 0.1^{a}
	60 days	8.68 ± 0.31^{ab}	52.3 ± 2.4^{ab}	41.3±1.7	3.8 ± 0.2^{a}
	90 days	8.73 ± 0.4^{ab}	53.1 ± 2.5^{ab}	41±1.6	3.7 ± 0.3^{a}
Vaccinated Group	Zero time	7.99 ± 0.21^{b}	53.3 ± 2.2^{ab}	42.3±2.1	3.7 ± 0.8^{a}
	3 days	9.17 ± 0.23^{ab}	53.5 ± 2.4^{ab}	41.2±2.2	3.6 ± 0.7^{a}
	7day	9.73 ± 0.28^{a}	54.2 ± 2.7^{a}	41.7±2.3	3.1 ± 0.3^{ab}
	28day	9.13 ± 0.32^{ab}	55.1 ± 2.8^{a}	41.3±2.6	3.1 ± 0.8^{ab}
	60 days	8.25 ± 0.35^{ab}	55.3 ± 3.1^{ab}	42.9±2.5	3.0 ± 0.7^{ab}
	90 days	8.17 ± 0.29^{ab}	53.6 ± 2.2^{ab}	42.3±2.5	3.2 ± 0.8^{a}
Vaccinated and probiotic treated group	Zero time	8.34 ± 0.30^{b}	53.4 ± 2.5^{ab}	42.1±2.0	3.5 ± 0.7^{a}
	3 days	8.41 ± 0.32^{b}	53.7 ± 2.3^{ab}	42.5±2.3	3.1 ± 0.6^{ab}
	7days	8.92 ± 0.42^{ab}	54.3 ± 2.7^{ab}	42.3±2.9	2.8 ± 0.6^{ab}
	28day	9.15 ± 0.4^{ab}	55.6 ± 2.9^{a}	41.7±2.3	2.5 ± 0.2^{b}
	60 days	9.73 ± 0.4^{a}	55.5 ± 2.3^{a}	41.5±3.7	2.6 ± 0.3^{b}
	90 days	9.8 ± 0.42^{a}	55.9 ± 3.2^{a}	41.7±2.9	2.2 ± 0.2^{b}

Means within the same columns in each category carrying different litters are significant at ($P \leq 0.05$).

Effect on Lysozymes activity

It was apparent from Table (2) that a significant high level of serum lysozyme was detected in serum samples obtained from vaccinated, probiotic treated sheep at the 28[th] 60th and 90[th] days post vaccination when compared to the control group, these results were in agreement with those obtained from [24] as they recorded a significant increase in serum lysozyme activity in *Catla catla* fish following administration of the probiotic *Bacillus amyloliquifaciens*. Lysozyme is an enzyme present in mucus secretion, blood and other areas of virtually all eukaryotic organisms. It is present in cytoplasmic granules at the macrophage and the polymorph nuclear neutrophils (PMNs), thereby, play an important role in innate immune defense. It is a hydrolytic enzyme able to cleave the cell wall of gram +ve and some gram -ve bacteria. Cleavage of peptidoglycan wall at the B1-4 Acetyl-galactosamine (muramate) results in lysis of the bacteria [25]. In the same area of interest, *Palmer et al,* [26] found an Increased IgA producing cells, CD4 cells in the lamina propria of the small intestine and secreting IgA in the lumen after Influence of a probiotic *Lactobacillus* strain.

Table (2): Effect of vaccination alone and/or in combination with probiotic (*Lactabacillus acidophilus*) on serum lysozymes of sheep. (Mean + S.E), n = 8 .

Group	Serum lysozymes (µg/mL) post treatment					
	Zero time	Three days	Seven days	28 days	60 days	90 Days
Control	170.26±8.24	174.7±5.39	176.5±7.21	147.21±8.5	163.8±11.6	167.33±12.3
Vaccinated	174.52±5.28	196.34±8.25	199.4±7.34	213.81±11.75	210.7±9.18	207.14±12.34
Vaccinated + probiotic	164.70±5.39	186.70±7.27	195.21±10.5	248.17±11.63	261.3±12.4	219.62±11.78

Means within the same columns in each category carrying different litters are significant at ($P \leq 0.05$)

Effect on Nitric oxide production

Table (3) illustrates that vaccination of sheep with *Clostridia* polyvalent vaccine elicited a significant increase in serum nitric oxide production along the entire period of the study except at the 90[th] day post vaccination, where there was a non-significant increase when compared to the control group. The administration of probiotic to the vaccinated group afforded a significant increase in serum nitric oxide production along the course of study when compared to control animals. On the 28[th] and 60[th] day post vaccination, values of nitric oxide showed a significant increase when compared to the vaccinated animal. Undoubtedly, our data were in accordance with those reported by [24] they recorded a significant increase in nitric oxide production in *Catla catla* dietary supplementation of 10[th] CFU/gm *Bacillus amyloliquifaciens*, suggested that, the use of food containing *Lactobacillus* may work as a palliative to reinforce immune system and improve feed efficiency. Nitric oxide produced by inducible nitric oxide synthetase (iNOS) has an important role in antimicrobial host defense and immune- surveillance, low physiologic concentration of

NO can inhibit apoptosis but higher concentration may be toxic. High NO concentration leads to the formation of toxic product like dinitrogen trioxide and peroxynitrite, which induce cell death, if not by apoptosis then by necrosis [27].

Table (3): Effect of vaccination alone and/or in combination with probiotic (*Lactabacillus acidophilus*) on Nitric oxide production of sheep. (Mean + S.E). n = 8

Group	Nitric oxide (µM) post treatment					
	Zero time	Three days	Seven days	28 days	60 days	90 Days
Control	19.6±0.6	19.6±0.6[b]	19.6±0.6[b]	19.6±0.6[c]	19.6±0.6[c]	19.6±0.6[b]
Vaccinated	19.7±0.69	26.18±0.45[a]	26.7±1.65[a]	26.3±1.2[b]	25.4±1.8[b]	21.3±1.3[b]
Vaccinated + probiotic	19.4±0.8	26.1 ±0.9[a]	28.9±1.3[a]	31.6±0.9[a]	32.1±1.85[a]	30.7±1.2[a]

Means within the same columns in each category carrying different litters are significant at (P ≤ 0.05).

Effect on Productive performance for different treated groups in Rahmani sheep

Table (4): illustrated the different productive performance for different groups of Rahmani sheep in relation to the vaccination in combination with probiotic. The results showed that there was a significant difference (*P*< 0.05) between different groups at total feed intake and final body weight in comparison with the initial body weight for each group. These results were similar to that obtained with [28] as they stated that diet supplementation with probiotic was improve the performance of sheep as it increases feed intake subsequently final body weight. The feed conversion ratio and the feed efficiency were different among different groups but were non-significant. The feed efficiency was at highest value 0.14 at third group. These results were in agreement with [29, 30] as they mentioned that *L.acidophilus* supplementation stimulate invective behavior, weight gain and feed efficiency of sheep. In contrast [31] found that supplement the diet with probiotic had no effect on feed conversion values and weight gain in growing lambs.

Table (4): Effect of of vaccination in combination with probiotic (*Lactabacillus acidophilus*) on productive performance in Rahmani sheep.

Group	Initial Live body weight (kg/ animal)	Total feed intake (kg/animal)	Final body weight (kg/ animal)	Feed conversion ratio (FCR)	Feed efficiency per animal (FE)
Control	32.8± 2.6[a]	115.8± 9.5[b]	52.6± 3.5[b]	8.57± 0.27	0.121 ± 0.02
Vaccinated	33.6± 3.2[a]	120.2± 10.5[b]	53.1± 4.2[b]	7.70± 0.4	0.129 ± 0.02
Vaccinated+ Probiotic	33.1± 2.4[a]	126.7± 11.5[a]	55.9±4.9[a]	7.03±0.3	0.14 ± 0.01

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05).

Total costs, total returns and net profit (LE/ Animal) for different groups

Table (5): showed different economic parameters for different groups of Rahmani sheep. The total variable costs and the total fixed costs are showed non-significant. Meanwhile there were significant difference (*P*< 0.05) between different groups at total returns and net profits LE/head. The highest total returns and net profit at the third groups that had probiotic and these indicated that the using of probiotic had increased finished body weight that leads to increase net profit.

Table (5): Total costs, total returns and net profit (LE/ Animal) for different groups.

Group	Total Variable costs (LE/Animal)	Total Fixed costs (LE/ Animal)	Total costs (LE/ Animal)	Total returns (LE/ Animal)	Net profit (LE/Animal)
Control	1345.7± 12.6	190.8± 6.5	1536.5± 35.5	1780.1± 82.2[b]	243.6 ± 19.2[b]
Vaccinated	1350.5± 15.2	190.8± 6.5	1541.3± 46.2	1796± 87.4[b]	254.7 ± 26.2[b]
Vaccinated + Probiotic	1352.2± 19.4	190.8± 6.5	1543.1±41.2	1813± 99.3[a]	269.9 ± 23.1[a]

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05).

Conclusion

Finally, from our obtained results, we can conclude that the probiotic *Lactobacillus acidophilus* possesses an immunostimulating properties evidenced by an increase in total leucocytic count, lysozyme activity and serum nitric oxide. This was reflected on the productive performance of Rahmani sheep. So, we recommended from our economic results that using Lactobacillus acidophilus in fattening Rahmani sheep for successive 90 days (3 gm /head) will lead to increase net profit and immunological status of the animal .

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