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Effect of Probiotic Narine on Gut Microflora of sheep and lambs

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ABSTRACT

An experiment was conducted on sheep and lambs to investigate the influence of Narine probiotic on some intentional microflora counts. Each individual animal received 1 capsule of narine probiotic, orally for 10 successive days. Results showed substantial decline in *E.coli*, *Staphylococcus aureus* and *Salmonella* next day of the probiotic administration. For *E.coli* spp. the bacterial concentration was minimized to reach 28% in sheep and 33% in lambs by day 10 of the experiment. Data of *Staph.aureus* indicated that the effect of the probiotic was so obvious on the 2nd day post probiotic administration, by day 8th of the experiment, the *Staphylococcus* concentration was zero in sheep and lambs intestinal fecal material. *Salmonella* response to the probiotic was also profound where over 90% reduction in bacterial counts on day 5 of the experiment. The zero level of *Salmonella* was reached on day 9 and 10 in sheep and lambs, respectively. On the other hand, *Lactobacillus* development due to the probiotic administration was more clear tast in lambs than in sheep. However both types of animals gained millions of bacilli by day 10 of the experiment.

Keywords: **Probiotic, Microflora, Bacterial count, Enterobacteria, Staphylococcus, Sheep**

1. Introduction

It is important to observe the animals flock daily, so that sudden changes can be identified according to (Turton, 2002; Lynn, 2010) and to keep the animal and whole herd or flock healthy and productive .The word micro flora refer to the collection of live microscopic organisms that flourish inside of living creator (Synthin et al., 2012).The gastro intestinal micro flora have been shown to play a number of vital roles in maintaining gastro intestinal tract functions and over all physiological health, for example the growth and metabolism of many individual bacteria species inhibiting by gastro intestinal tract (Mintz et al., 2008)) .It has been reported that normal flora play a vital role in rein forcing animal immunity and improving animal physiological function compared with germ-free animals , (Jianwen et al., 2002). The term probiotic is derived from the Greek meaning for life.Probiotic currently is defined as live microorganisms which consumed in adequate amounts confer a health of host,

or defined as viable bacteria used as feed additives which produce beneficial effects to promoting the equilibrium of intestinal flora . Common description for probiotic includes friendly, beneficial or healthy bacteria (Zani et al., 1998). The potential probiotic strain was characterized as normal inhabitants of target species have ability to adhere and colonized the epithelial cells of gut and to survive and grow in the respective ecological unit (Mosa et al., 2009).The concept that lactobacilli might be useful in displacing and replacing harmful microorganism on mucosal surface was presented a century ago. The competition among bacteria for nutrients and spaces contributes to the microbial composition of ecosystem. (Lebenthal and Lebenthal, 2002). *Lactobacillus acidophilus* is a member of one of the eight main genera on lactic acid bacteria ,The genera, *lactobacillus,Streptococcus, Lactococcus, Leuconstot, Biofidobacterieases, Carbobacteriease, Enterococcus and Sporolactobacillus* genus and species have different characteristics ,(Salminen and Von Wrogh, 1993) . Probiotic Narine is lyophilized product prepared via fermentation of milk mixture by the Lactobacteria. Each one capsule/tablet contains 10^9 of c.f.u/g of minimal quantity of *Lactobacillus acidophilus* strain Er-2 317/402. *L.acidophillus* is probably the best well-known species of *Lactobacillus* .It is naturally found in the human and animal gastro intestinal tract. *L.acidophillus* is characterized by rod -shaped, motile bacteria, can be grown with or without oxygen having a characterized feature as a homo fermentative that only produces lactic acid as its sole product. (Faro, 1999). *Escherichia coli* belong to the family *Enterobacteriacae* which is the normal inhabitants in gut flora, gram negative, none sporulated, motile by peritrichus flagella, aerobic or facultative anaerobic bacteria. Endo agar and Eosin methylene blue are selective media for this bacteria producing metallic sheen when growth on these media. Fermented to many of sugars like glucose, sucrose, maltose, manitol, arabinose and trihalose producing acid and gas. Positive for indol, methyl red test and negative for VocusProschaur, citrate, urease test, not liquefied gelatin. (Koneman et al., 1997; Brooks et al., 2001; Hamed, 2011). *Salmonella* also belongs to the family *Enterobacteriacae*.A total of 2501 different salmonellae serotypes have been identified up to 2004 according to the statistics from world health organization WHO. (AL talibi, 2005; ALChalaby, 2002). *Salmonella* have rod shape, gram negative, aerobic or facultative anaerobic bacteria, not fermentative for lactose, sucrose, negative for oxidase, urease, utilization of citrate, Most of *Salmonella* species are H_2S positive for triple sugar iron agar and growth on selective media like *Salmonella-Shigella* agar, Bismoth sulphate agar. *Salmonellosis* is zoonotic disease which spread all over the world, (Brooks et al., 2001; AL talibi, 2005 ; ALChalaby, 2002). Staphylococcus species are in the bacterial family Micrococceae but they are phylogenetically unrelated to any other genera in this family.Staphylococcus aureus are gram positive cocci , organized in grape like clusters , blood hemolytic , coagulation of plasma which produce extra cellular toxin .Manitol salts agar is a selective medium for *Staphylococcus aureus* exhibiting yellow colour (Baron and Fingold 1990; Brooks et al., 2001; ALChalaby, 2002).

2. Materials and Methods

Sheep samples

For the 10 day experimented, 5 adult sheep and 5 lambs in the same breed were used

Fecal samples

We took fecal sample using sterilized test tubes. One gram of feces was put in 9ml of physiological water. Nine sterilized tubes were taken and added in each one 9 ml of physiological water. Tenfold dilution was carried out for the basic sample.

Bacterial isolates

0.1 ml of each diluted tube “contains diluted feces" was taken which was put into 4 selective culture media according to each bacterium as follows:

1-**Endo agar, Eosine methyline blue agar** → selective media for *E.coli* .
2-**Bismuth sulphate agar**, ***Salmonella Shegilla*** **agar** → selective media for *Salmonella*

3-**Manitol salts agar** → selective media for *Staph. aureus.*

4-**M.R.S agar** → selective for *Lactobacillus* bacteria

Three petri dishes from each tube were cultured fo r each bacteria .Then, the dishes were incubated on 37°C for 14-16 hours. Counting of the bacteria was done by using the following equation. Bacterial count = (Total number of bacteria in Petri culture media X 10 X inverse dilution)**.**Spreading method was done using L shape spreader in all over the agar which was left for 5 minutes to absorb the suspension from the agar. These media were put in incubator for 14-16 hours at 37°C.The results were read later.

Probiotic Narine

Narine probiotic formula is Lactobacillus acidophilus strain Er-2 317 / 402.

Statistical analysis

To determined the effect of the probiotic on the declination of harm bacteria species, a pair T-test based on before and after data was used according to (Katz.mitchell , 2009)**.**

3. Result & Discussion

The effect of Narine probiotic administration in Armenian sheep and lambs showed a great deal of decline in the levels of intestinal *E.coli* , *Staph.aureus,* and *Salmonella* microbes. On the other hand, *Lactobacillus* level was highly increased as a result of the probiotic administration. Table 1 show the path of E-coli decline in the sheep and lambs, under study, over the 10 successive days of the experimental period the concentration of *E.coli* were

dropped down from 54×10^4 in sheep and from $33x10^4$ in lamb to $39x10^4$ and 29×10^4, in the two respective types of animals, right one day after the probiotic administration. Daily supplimentation of the probiotic showed significantly high decline in *E.coli* level where it reached 15×10^4 and 11×10^4 in sheep and lambs, respectively, by day 10 past the probiotic administration. In term of percentages, the decline in *E.coli* counts formed above 90% compared with the mean bacterial count prior to the drug administration, this ratio was achieved on day 4 in sheep and day 5 in lambs. In regard off all reduction rates of *E.coli* levels, it is worth to mention that all decreases were statistically significant ($p < 0.05$) (Fig. 1). As with *Staphylococcus*, table 2 shows that basal levels of the microbe were 40×10^4 in sheep and 38×10^4 in lambs. An a result of imposing the Narine probiotic orally, the bacterial counts dropped slightly on the next day of treatment with no significant differences were observed, however, 2 days, after giving the probiotic significant decline in microbes levels was noticed in both sheep and lambs. In term of percentage, sheep showed much higher reduction in the microbe's counts than lambs did, however, both were100% tree of Staphylococcus. On day 8^{th}, post the probiotic administration (Fig. 2). Data of Salmonella revealed that only one sheep and one lamb were carries. Thus, no statistical analysis was done accordingly by following up the level of the Salmonella in the intestinal fecal material of their two animals, it was found that the probiotic wan mathematically effective in reducing the microbe level from 36×10^3 in the sheep and from 40×10^3 in the lamb to 0.40×10^3 and zero on day 8^{th} in respective types of animals (table 3) (Fig. 3). Lactobacillus counts, 10^5 cfu, in sheep and lambs are shown in table 4. The basal levels were very nil, 0.014 and 0.240×10^5 in sheep and lambs respectively. Significant ($P > 0.05$) increases were noticed starting the 2^{nd} day of the probiotic administration. The significant in Lactobacillus counts were 39.57×10^5 and 66.76×10^5 in the sheep and lambs by day 10 post treatment. When the increase in lactobacillum is considered so beneficial to the animal hygiene, the millions of bacillus reproduced on day 10 post treatment should be appreciated (Fig. 4).

Table 1 Effect of probiotic administration (10 day treatment) on E.coli 10^4 CFU means ± se in sheep and lambs

Sheep				Lambs			
Days	Means ± se	Differences ± se	Decline, %	Days	Means ± se	Differences ± se	Decline, %
Day 0 or Before treatment	54.0 ± 5.29			Day 0 or Before treatment	33.0 ± 4.848		
Day1	39.0± 5.07	15.0 ± 3.51 *	27.7	Day1	29.00± 4.708	4.0 ± 1.472 NS	12.12
Day 2	19.25 ± 2.56	34.75 ± 3.95 *	64.3	Day 2	21.00 ± 4.564	12.0± 0.408 *	36.36
Day 3	7.80 ± 2.46	46.20 ± 4.74 *	85.5	Day 3	5.65 ± 0.538	27.35 ± 5.262 *	82.87
Day 4	3.70 ± 0.63	50.3 ± 5.23 *	93.1	Day 4	5.10 ± 0.506	27.9 ± 5.326 *	84.45
Day 5	2.50 ± 0.68	51.5 ± 5.27 *	95.3	Day 5	2.90 ± 0.526	30.1 ± 5.360 *	91.21
Day 6	1.60 ± 0.38	52.40 ± 5.26 *	97	Day 6	1.90 ± 0.47	31.1 ± 5.311 *	94.24
Day 7	0.72 ± 0.26	53.28 ± 5.19 *	98.62	Day 7	0.48 ± 0.028	32.52 ± 4.863 *	97.45
Day 8	0.36 ± 0.04	53.64 ± 5.28 *	99.33	Day 8	0.30 ± 0.041	32.70 ± 4.874 *	99.44
Day 9	0.26 ± 0.03	53.74 ± 5.29 *	99.52	Day 9	0.19 ± 0.028	32.81 ± 4.852 *	99.42
Day 10	0.15 ± 0.02	53.85 ± 5.29 *	99.72	Day 10	0.11 ± 0.0372	32.89 ± 4.868 *	99.62

Indicated the differences significant ($p < 0.05$)*

Fig. 1: shows the decrease of *E.coli c*ount after administration of probiotic Narine for 10 days in sheep and lambs

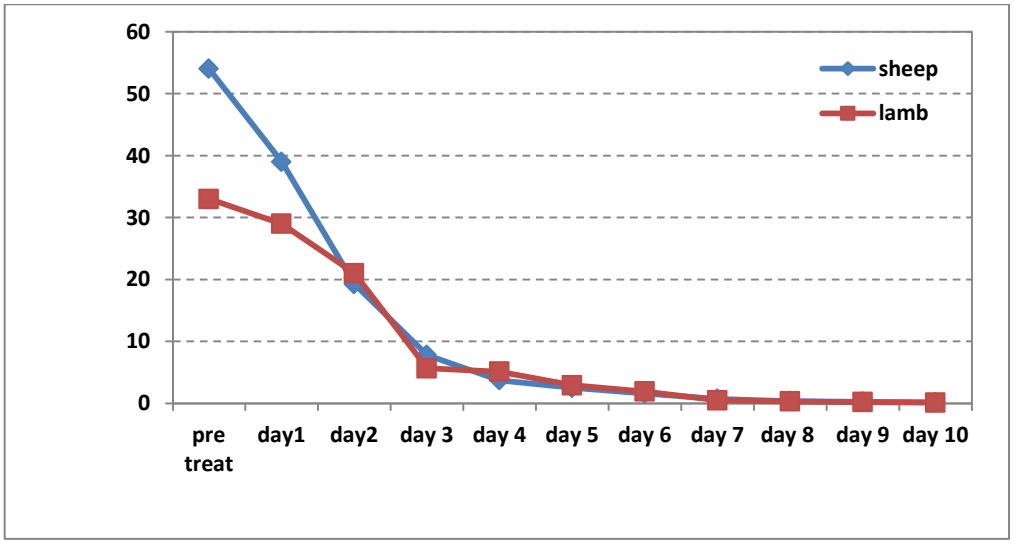


Table 2: Effect of Probiotic administration (10 day treatment) on *Staphylococcus* 10^4 CFU means ± se in sheep and lambs

Sheep				Lambs			
Days	**Means ± se**	**Differences ±se**	**Decline,%**	**Days**	**Means ± se**	**Differences ±se**	**Decline, %**
Day o or Before treatment	**40.0 ± 5.0**			**Day o or Before treatment**	**38.00 ±6.0**		
Day1	**34.0 ± 6.00**	**6.0 ± 1.0**	**15**	**Day1**	**34.0 ±6.0**	**4.00 ± 6.00**	**10.52**
Day 2	**18.0 ± 4.00**	**22.0 ± 1.0**	**55**	**Day 2**	**22.0 ±8.0**	**16.00 ± 8.00**	**42.10**
Day 3	**3.4 ± 0.30**	**36.6 ± 4,7**	**91.5**	**Day 3**	**14.0 ±2.0**	**24.00± 5.5**	**63.15**
Day 4	**2.8 ± 0.80**	**37.2 ± 4.2**	**93**	**Day 4**	**8.4 ±0.50**	**29.6 ± 5.5**	**77.89**
Day 5	**0.36 ± 0.05**	**39.6 ± 5.05**	**99**	**Day 5**	**4.40 ±0.10**	**33.6 ±**	**88.42**
Day 6	**0.18 ± 0.02**	**39.82± 5.02**	**99.55**	**Day 6**	**2.20 ±0.10**	**35.8 ±**	**94.21**
Day 7	**0.12 ± 0.02**	**39.88 ± 4.98**	**99.70**	**Day 7**	**1.25 ±0.05**	**36.75±**	**96.71**
Day 8	**0.00**	**40.00**	**100**	**Day 8**	**0.00**	**38.00±**	**100**
Day 9	**0.00**	**40.00**	**100**	**Day 9**	**0.00**	**38.00**	**100**
Day 10	**0.00**	**40.00**	**100**	**Day 10**	**0.00**	**38.00**	**100**

***Indicated the differences significant ($p < 0.05$)**

Fig. 2: shows the decrease of *Staphylococcus* count after administration of Probiotic Narine for 10 days in sheep and lambs

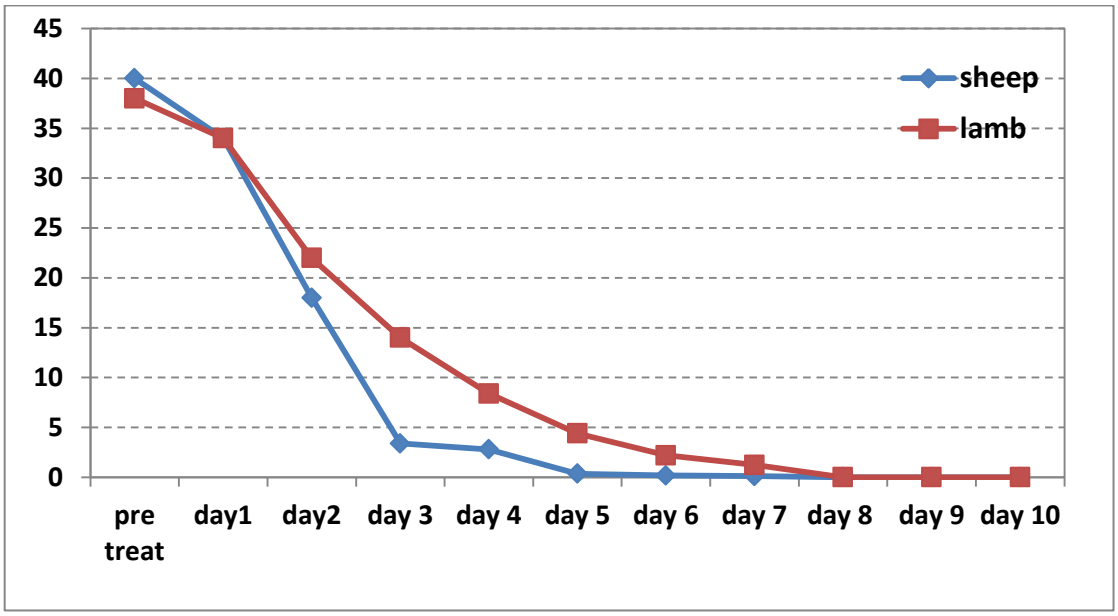


Table 3: Effect of probiotic administration (10 day treatment) on *Salmonella* 10^3 CFU

Means ± se in sheep and lambs.

Sheep				Lambs			
Days	Means ± se	Differences ±se	Decline,%	Days	Means ± se	Differences ±se	Decline,%
Day 0 or Before treatment	36.00			Day o or Before treatment	40.00		
Day1	32.0	4.0	11.11	Day1	24.0	16.0	40
Day 2	20.0	16.0	44.44	Day 2	16.0	24.0	60
Day 3	12.0	24.0	66.66	Day 3	8.0	32.0	80
Day 4	4.0	32.0	88.88	Day 4	5.2	34.8	87
Day 5	3.2	32.8	91.11	Day 5	3.6	36.4	91
Day 6	2.2	33.8	93.88	Day 6	2.0	38.0	95
Day 7	2.0	34.0	94.44	Day 7	1.2	38.8	97
Day 8	1.0	35.0	97.22	Day 8	1.0	39.0	85
Day 9	0.40	35.60	98.88	Day 9	zero	40.0	100
Day 10	zero	36.0	100	Day 10	zero	40.0	100

No statistical analysis was done, due to the availability of only one observation.

Fig. 3: shows the decreases of Salmonella count after administration of probiotic Narine for 10 days in sheep and lambs.

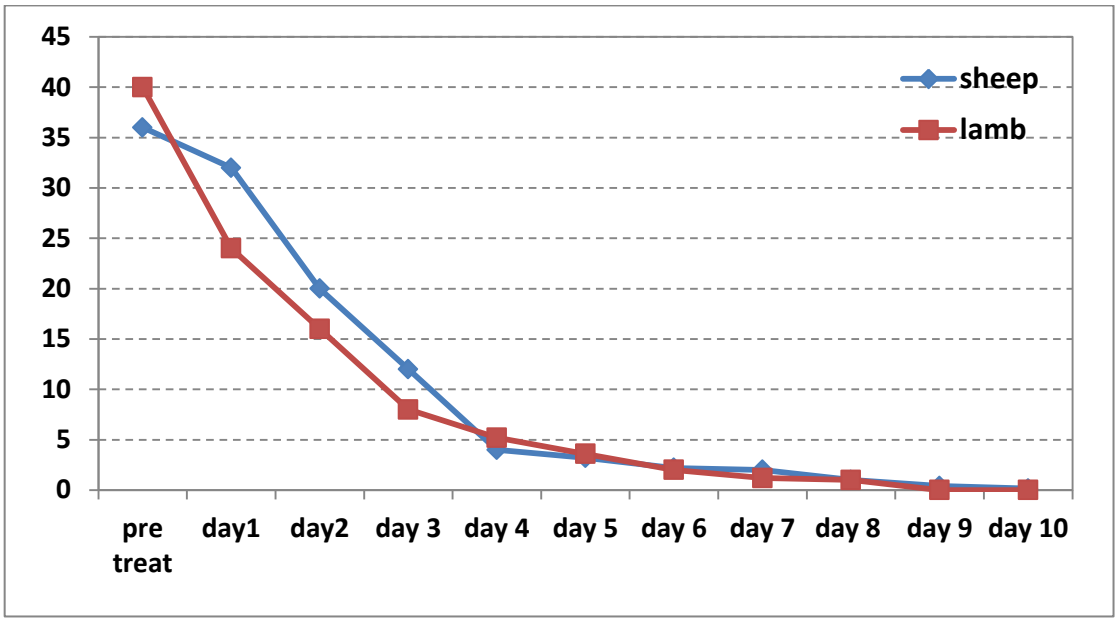


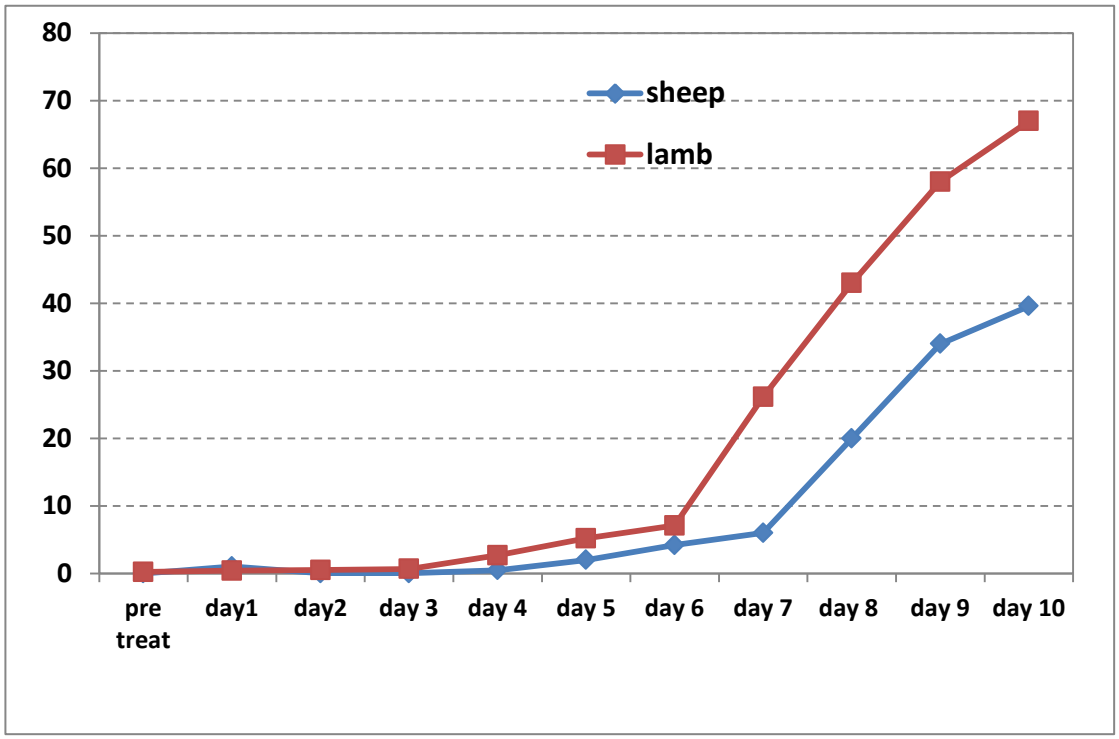
Table 4: Effect of Probiotic administration (10 day treatment) on *Lactobacillus* Means, 10^5 CFU, ± se in sheep and lambs.

sheep				lambs	
Days	Means ± se	Differences ±se	Days	Means ± se	Differences ± se
Day o or Before treat		0.014 ±0.008	Day o or Before treat		0.240±0.14
Day1	1.024± 0.008	0.01 ± 0.008 Ns	Day1	0.43 ±0.05	0.19 ± 0.008 Ns
Day 2	0.039± 0.001	0.025 ± 0.005 Ns	Day 2	0.52 ±0.04	0.28 ± 0.107 Ns
Day 3	0.370± 0.117	0.356± 0.172 Ns	Day 3	0.68 ±0.047	0.44 ± 0.160 Ns
Day 4	0.50 ± 0.108	0.486 ± 0.101 *	Day 4	2.7 ±0.183	2.46 ± 0.078 *
Day 5	2.0 ± 0.616	1.97 ± 0.305 *	Day 5	5.2 ±0.342	4.96 ± 0.225 *
Day 6	4,20 ± 0.507	4.186 ± 0.505 *	Day 6	7.1 ±0.363	6.86 ± 0.395 *
Day 7	6.00 ± 0.558	5.986 ± 0.565 *	Day 7	26.15 ±9.08	25.91 ± 8.95 *
Day 8	20,00± 8.236	19,99 ± 8.24 *	Day 8	43.00 ±8.88	42.16 ± 8.77 *

Day 9	34.0 ± 11.31	33.99 ± 11.31 *	Day 9	58.00 ±4.92	57.76 ± 4.87 *
Day 10	39.6 ± 13.79	39.57± 13.48 *	Day 10	67.00 ±7.25	66.76 ± 7.24 *

***Indicated the differences significant (p< 0.05)**

Fig. 4: shows the increases of Lactobacillus count after administration of probiotic Narine for 10 days in sheep and lambs.



4. Discussion

To be the main factors for the antagonistic activities of *L. acidophilus* against the *E.coli* .Although the highly complex relationship of food and health is still poorly understood, recent research advances in different disciplines provides promising new approaches to improve our standing (Mosa, 2009). Probiotic lactic acid bacteria are presently the only choice available for replacing the antibiotic used universally by feed industries, they enhance the growth and health of animal and maintain normal intestinal Micro flora through comparative exclusion and antagonistic action against pathogen in the intestines of animal (Ahn, 2002; Fuller, 1989).The current study improve that the probiotic Narine had effective inhibitory action on *E.coli* , *Salmonella* and *Staph.aureus*. the inhibitory effect of this probiotic could be due to presence of *L. acidophilus* in their content which had more than mechanisms, one of them is induce natural antibiotic and hydrogen oxide and also produce antimicrobial substance like *L. acidophilus* and acidolin acting against food and environment of borne pathogen (Zahra, 2008; Barnes and Gross,1997).The result of this study are similar to other studies which done by (Callae and Fraser, 2004) which confirm that the probiotic have a good inhibitory effect by more than one

mechanism such as competitive exclusion, antagonistic action producing antimicrobial substance and by enhance the immuno modulating factor (Ahn, 2002; Fuller, 1989) to the many pathogenic bacteria *E.coli*, *Salmonella and Staph.aureus*.in conclusion of our study can be conducted that probiotic narine (contain lactobacillus acidophilus)) had inhibitory effect of growth proliferation pathogenic bacteria ie *Salmonella, E.coli* and *Staph.aureus* which invaded the gastro intestinal tract of sheep in vivo, Mainly due to low pH and organic acid particularly lactic acid (Ahn, 1997) .the organic acids produced by *L.acidophillus appeari* and *Salmonella* spp and also *Staph. aureus* (Ahn, 2002).

5. Conclusion

probiotic narine contain *lactobacillus acidophilus* which posses inhibitory effect of growth proliferation for pathogenic bacteria like *Salmonella, E.coli* and *Staph.aureus.*

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