

Safety and protective effect of *Lactobacillus acidophilus* used as probiotic agent in goats

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Abstract

The study was aimed to study the protective effect of *Lactobacillus acidophilus* isolated from fresh sheep milk. the result of this study was revealed that *Lactobacillus* isolates had liver improvement functions. Lactate & Sorbitol dehydrogenase activities of goats dosed *Lactobacillus* isolates alone were lower than the control. There were reduction in the count of enterobacteria in goat dosed with *Lactobacillus* after three days. Protection of gastrointestinal tract by these isolates was also observed.

Introduction

Gastrointestinal disorders are caused by various factors including antibiotic administration (29) or as a result of infectious agents such as toxogenic *Escherichia coli*, *Salmonella enteritidis*, *Entamoeba histolytica* and viruses (27). Innovative approaches have been tried as alternative to antibiotics in treating gastrointestinal diseases and these include using live biotherapeutic agents such as yeast (*Saccharomyces Spp*) and bacterial isolates (*Lactobacillus Spp*). Or fecal animals (14). *Lactobacillus* are important for maintenance of the intestinal microbial ecosystem (26). Colonization of the gut with lactobacilli start within the first week of life (25). The presence of this group of bacteria in the gut is considered to have several potential benefits such as growth promotor of farm animals (2), protection against pathogens (7), alleviation of lactose intolerance (17), relief of constipation (30), anticholesterolemic effect (5) and

immunomodulation (1). Lactobacilli exert their protective or therapeutic effect through production of antimicrobial compounds (10,21), reduction of gut pH by stimulating the lactic acid producing microflora (11), competition with binding receptor sites that pathogens occupy (20,22), stimulating of immunomodulatory cells (24) and competition with pathogens for a viable nutrients (11,24). Walker and Duffy (30) suggested that current perspectives on biotechnological applications of probiotic products require further in vitro and in vivo investigation to evaluate the safety of using wild type organisms or those obtained by genetic engineering. The present study is therefore aimed for understanding the protective effect of *Lactobacillus acidophilus* from fresh sheep milk and their ability to reduce the toxicologic and pathologic consequence associated with enterotoxigenic *E.coli* used to experimentally infected goats.

Materials and Methods

Lactobacillus acidophilus were isolated from fresh sheep milk on MRS agar. The isolates were characterized using colonial morphology and biochemical tests according to (6). These *Lactobacillus* spp were also found to adhere to the ileal epithelial cells of goats. The isolates were inoculated in MRS broth and incubated at 37 C° for 2 days to obtain large cell concentration about 10¹⁰ CFU/ ml. These cells were washed, suspended in rehydrated skim milk lyophilized and

stored at -20 C° until use (13). The concentration of a viable cells was determined by serial dilution techniques (28). Twenty four (24) goats were used in this study they were randomly assigned to 4 treatment groups each was made up of 6 goats per groups. Lyophilized lactobacillus cells were reconstituted by dissolving 1 gr in 10ml of normal saline (approximately 10¹⁰ CFU/ml). The first group was kept on basal diet alone and considered as control group. Second group fed on the basal diet

and were also dosed with 0.3ml of *L.acidophilus*. Third group were fed on the basal diet, dosed with 0.3 ml of *L.acidophilus* and infected with 0.3 ml of 10^5 CFU/ml of enterotoxogenic *E.coli*. Fourth group was fed on basal diet and infected with 0.3 ml of 10^5 CFU/ml of enterotoxogenic *E.coli*. The treatment above was repeated on the second day. A post ingestion period of 18 day was observed after administration of culture and blood sample were collected for serum biochemical analysis for the following parameter using kits from Boehringer-Mannheim Company – Germany.

- 1- Sorbitol dehydrogenase
- 2- Lactate dehydrogenase
- 3- Aspartate amino transferase

4- Alkaline phosphates

5- Cholesterol

Freshly voided fecal materials were collected and pooled from each goat (1gr/goat) at days zero and 3. The faeces were homogenized in normal saline and serially diluted and plated on MRS agar for the enumeration of lactobacilli and on MacConkey's agar for enumeration of enterobacteriaceae especially *E.coli*. The plates were incubated at 37 C° for 24 hours and colony forming units on the plates were recorded (19). The data gathered from toxicologic assay and faecal flora were processed using one way analysis of variance (ANOVA). SPSS. 12. The level of significance was set at ($P < 0.05$). Means were compared by Duncan test.

Result and Discussion

The aspartate amino transferase (AST) activity in animals of third group was highest and significantly different ($P < 0.05$) from the control (First group) (Table 1). AST is an enzyme that increased in activity in diseases such as severe bacterial infection and tumors of organ such as heart and muscle (8). Lactobacilli can translocate and survive in the spleen, liver and lungs (4,6). In the course of their translocation they can cause cellular injury that may increase AST level in the serum. This may account for increase in AST observed for goats of third group compared with the control group. The higher AST level in third group may be due to the combine activities of Lactobacillus and *E.coli* in the GIT. In their study (27) reported that, to obtain protective effect in animals, treatment with probiotic agents had to be initiated 10 days before challenge with pathogens but in this report oral dosing with Lactobacillus and challenge with *E.coli* was simultaneous. The results of lactate and sorbitol dehydrogenases (LDH and SDH) activities in the serum revealed that, The third group were significantly higher ($P < 0.05$) than the control. LDH and SDH is principally found in the liver and together is regarded as being more specific than AST alone for detecting liver cell damaged (8,18). The

implication of this result is that, there is a pronounced toxicologic effect in goats of the third and fourth groups. The lower LDH and SDH in goats treated with lactobacillus alone (Second group) compared with the control indicate liver function improvement brought about by the lactobacillus. Hepatocytes play a major role in absorbing and metabolizing many toxic chemicals (12). They are therefore liable to injury by various chemicals including food. The alkaline phosphatase (ALP) activity of goats treated with lactobacillus and *E.coli* (Third group) and those treated with *E.coli* alone (fourth group) were significantly higher than those treated with lactobacillus alone (Second group). A rise of ALP activity has been linked with an increased osteoblastic activity (3) and lack of bile flow (Cholestasis). Only slight anti cholesterolemic effect was also observed in goats treated with lactobacillus. Lactobacilli has been found to have direct effect on cholesterol level by assimilation and removal from the growth medium. This has been demonstrated in pigs (15) and rats (5). Serum ALP levels has been reported to increase with increase in the serum cholesterol (18). The ability of isolates to protect the GIT against pathogens can be confirmed by monitoring

the count of enterobacteria especially *E.coli* and beneficial bacteria especially lactobacilli in goats faeces (7). There was an increase in fecal lactobacilli count in goats treated with lactobacilli (Table2). A slight decrease in enteric bacteria count was also observed in most of the goats. There was increase enterobacteria and lactobacilli count from day zero to day 3 in both control groups (First and fourth groups). In a similar study (9) reported an increase of lactobacilli count in faeces of rats that was basal diet devoid of probiotic agents. The high lactobacilli count in goats

treated with lactobacilli and *E. coli* (Third group) may be responsible for the partial protection of the GIT of goats in this group. Earlier report showed that a selected probiotic strain *L.reuteri* and *L.acidophilus* showed an increasing effect in numbers of enterobacteria in piglets (23). The ability of lactobacilli to produce toxic metabolites such as lactic acid, hydrogen peroxide (H_2O_2) and bacteriocins has been suggested as being responsible for their ability to inhibit other bacteria (19). Other factors such as host immunomodulation also play a prominent role. (13).

Table (1): Serum biochemical markers in different groups

Group	AST /IU/L	LDH /IU/L	SDH /IU/L	ALP /IU/L	Cholesterol Mg/d/
1 St group	182.612 ± 32.3 a	128.47 ± 22.91 a	19.43 ± 2.01 a	102.9 ± 1.223 a	93.5 ± 7.216 a
2nd group	233.623 ± 12.621 b	80.32 ± 7.81 b	14. 26 ± 1.75 a	104.263 ± 1.26 a	29.63 ± 6.22 b
3ed group	635.264 ± 33.3 c	520.83 ± 22.6 c	68. 67 ± 14.22 b	1560.12 ± 31.12 b	165.12 ± 3.61 c
4th group	422. 621 ± 12.25 d	160.4 ± 11.3 d	45.02 ± 4.22 c	520.62 ± 11.12 c	92. 63 ± 6.22 a

Table (2): Total count of faecal bacteria X 10⁶/ml

Group	<i>Enterobacteriaceae</i>		<i>Lactobacillus</i>	
	<i>Day zero</i>	<i>Day 3</i>	<i>Day zero</i>	<i>Day 3</i>
1 St group	5.72 ± 0.82 a	6.61 ± 1.23 b	6.12 ± 0.51 a	7.22 ± 0.21 b
2nd group	5.52 ± 0.863 a	5.32 ± 0.36 b	5.32 ± 0.621 a	8.36 ± 0.261 b
3ed group	5.92 ± 0.29 a	5.71 ± 0.42 b	5.39 ± 0.52 a	7.38 ± 0.76 b
4th group	5.56 ± 0.36 a	6.99 ± 0.42 b	5.22 ± 0.59 a	6.21 ± 0.30 b

■different litters means significant differences.

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التأثير الوقائي والأمين للعصيات اللبنية المحبة للحموضة المستخدمة كمعزز حيوي في الماعز

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الخلاصة

استهدفت الدراسة معرفة التأثير الوقائي للعصيات اللبنية المحبة للحموضة *Lactobacillus acidophilus* المعزولة من حليب الأغنام الطازج كمعزز حيوي Probiotic في الماعز . وقد أظهرت نتائج الدراسة ان هذه الجراثيم أدت الى تحسين وظائف الكبد حيث أظهرت فعالية إنزيمي اللاكتيك ديهيدروجينيز والسوربيتول ديهيدروجينيز Lactate & Sorbitol dehydrogenase انخفاضاً في مجموعة الحيوانات التي جرعت بالعصيات اللبنية المحبة للحموضة فقط مقارنة مع مجموعة السيطرة كما أظهرت الدراسة حصول اختزال في عدد الجراثيم المعوية في الحيوانات التي جرعت العصيات اللبنية بعد ثلاثة أيام من التجريع وقد تمت ملاحظة التأثير الوقائي لهذه الجراثيم على القناة الهضمية .