

MICROBIOLOGICAL QUALITY OF GOAT MILK IN AWASSA, ETHIOPIA

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ABSTRACT: The microbiological quality of raw milk obtained from Borana goat breed was assessed under two storage temperatures. Milk samples obtained from Borana goats were examined for standard plate (SPC), coliform (CC), lactic acid bacteria (LABC), yeast and mould (YMC), and aerobic spore-forming bacteria (ASBC) counts at 4°C and 25°C over a storage period of 96 h. The general microflora and types of lactic acid bacteria were also determined in raw goat milk kept at 25°C. Raw goat milk stored at 4°C had a mean SPC, CC, LABC, YMC and ASBC of 2.3×10^8 , 1.4×10^7 , 1.6×10^8 , 1.6×10^3 , and 2.3×10^5 cfu/ml, respectively; whereas the corresponding counts in raw goat milk stored at 25°C were 2.1×10^9 , 4.4×10^8 , 1.2×10^9 , 1.4×10^3 , and 4.5×10^5 cfu/ml, respectively. The organisms isolated from raw goat milk samples held at 25°C were *Staphylococcus* spp. (36%), Enterobacteriaceae (29%), *Bacillus* spp. (22%), *Micrococcus* spp. (13%), and *Streptococcus* spp. (< 1%). The LAB isolates were *Lactococcus* spp. (57%), *Lactobacillus* spp. (23%) and *Leuconostoc* spp. (21%). Raw goat milk kept at 4°C had a shelf life of 24 h; however, raw goat milk kept at 25°C had a shelf life of only 12 h. The isolation of pathogenic bacterial genera from the goat milk samples examined in this study suggests that consumption of raw goat milk can pose public health problems. Thus, strict hygienic measures need to be taken during the production and handling of goat milk at the study area.

Key words/phrases: Borana goat, Ethiopia, goat milk, microbiological quality

INTRODUCTION

Goat milk and goat milk products are important sources of protein for humans in many developing countries (Devendra, 1999; Haenlein, 2004). The demand for goat milk and goat milk products is increasing in many countries due to the image of health foods attached to goat milk and goat milk products (Klinger and Rosenthal, 1997). However, the production and handling of goat milk remains to be a major problem limiting its consumption. The dispersed nature of production across the diversity of small farms, problems of collection, poor handling systems, inadequate transport and refrigeration facilities all create a considerable challenge to goat milk production in several developing countries (Klinger and Rosenthal, 1997; Devendra, 1999).

Milk in general and goat milk in particular is a highly nutritious food suited for the growth of both spoilage and pathogenic organisms. Various bacterial infections have been linked to consumption of raw goat milk (Chubb *et al.*, 1985; Vasavada, 1988; Darnton-Hill *et al.*, 1987). It has been reported that pathogenic bacteria such as *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*,

Salmonella typhimurium and *Yersinia enterocolitica* can survive and multiply in goat milk and can be transmitted to humans (Roberts, 1985; Darnton-Hill *et al.*, 1987; Little and Louvois, 1999). Most reports of processing of goat milk do not include pasteurisation (Loewenstein *et al.*, 1980; Klinger and Rosenthal, 1997). Consumption of cheese made from unpasteurized goat milk has been identified as the cause of epidemics of brucellosis (Thapar and Young, 1986; Wallach *et al.*, 1994), listeriosis and food poisoning due to enterotoxin production by staphylococci (De Buyser *et al.*, 2001).

The bacteriological quality of goat milk has been reported in some countries. Goat milk produced in Australia (Jensen and Hughes, 1980; Cox and MacRae, 1989) and Greece (Anifantakis, 1993) was reported to be of poor microbiological quality. However, goat milk produced in the UK (Roberts, 1985) and Northern Ireland (Espie and Mullan, 1987) was of satisfactory microbiological quality.

Local goats are kept and managed to serve relatively resource poor farmers by providing essential nutrients in the form of milk and cash compared with large ruminants in southern and southeastern parts of Ethiopia (Fekadu Beyene,

1994). In these parts of the country, goat milk is either consumed fresh or mixed with cow milk to be fermented for churning into butter or consumed as fermented milk. The annual goat milk production, from an estimated 9.6 million goats in the country, is 17,000 metric tons (FAO, 2002). The production of milk and milk products in Ethiopia is generally a household process that takes place under unsanitary conditions and poor handling practices (Mogessie Ashenafi and Fekadu Beyene, 1994). Milk produced under such conditions may serve as a vehicle for the transmission of pathogens to humans. Production, processing and/or marketing of goat milk could be improved if the microbial quality of raw goat milk is better understood. Such information could be used while setting quality standards for dairy products from goats in the country and may also contribute to the database on microbiological quality of goat milk from a relatively uninvestigated geographical area. Not much is known nor published about the microbiological quality of goat milk in Ethiopia. The objective of this study was, therefore, to assess the microbiological quality of raw goat milk under two storage temperatures.

MATERIALS AND METHODS

Study area and experimental animals

This study was conducted at Awassa College of Agriculture, which is located in the Rift Valley at an altitude of 1,700 m above sea level at 7° N latitude and 38° E longitude, 275 km south of the capital, Addis Ababa. The mean annual precipitation, mean monthly relative humidity, mean monthly minimum and maximum temperatures of the study area were 894.8 mm, 67.2%, 12.2°C and 27.1°C, respectively. Ten indigenous Borana goats which delivered kids within one week were selected. The goats grazed on natural pasture and were given hay supplement (Rhodes grass, Desmodium, Vetch mixture) and 300 g of concentrate (linseed cake 33% (w/w), wheat bran 33% (w/w), corn 33% (w/w), salt 0.5% (w/w), bole 0.5% (w/w)) per head daily (Eyassu Seifu, 1998). The composition of "bole" (mineral lick) was 34 g/kg calcium, 0.3 g/kg phosphorous, 7.8 g/kg of potassium, 54.1 g/kg of sodium, 807.4 mg/kg iron, 451.5 mg/kg manganese, 11.5 mg/kg copper, and 53.2 mg/kg zinc. The crude protein, crude fiber, total digestible nutrient and ash contents of corn were 10% (w/w), 2.6% (w/w), 85% (w/w) and 1.6%, respectively; while that of

wheat bran were 17.1% (w/w), 11.3% (w/w), 70% (w/w) and 6.9% (w/w), respectively; and that of vetch were 20.8% (w/w), 30.6% (w/w), 57% (w/w) and 9.1% (w/w), respectively.

Sample collection and treatment

Milk samples were collected from the does six times (two samples each at early, mid and late lactations) during a lactation period of 120 days. The evening prior to milking, kids were separated from their dams until the does were hand-milked in their pen early in the morning. Milkers washed their hands, the udder and teats of the does with clean water and dried them with a clean towel before milking. Three litres of milk obtained from the goats were bulked in a sterile container and transported to the laboratory under cold storage within one hour of milking. Upon arrival at the laboratory, the milk sample was thoroughly mixed and its temperature and pH were measured. A portion of the milk sample (500 ml) was divided into separate bottles for the determination of the chemical composition. The remaining milk sample was used for the microbiological analyses. The milk sample intended for the microbiological analyses was equally divided into two sterile universal bottles and labeled as A1 and A2. Sample A1 was kept at 4°C in a refrigerator and A2 was kept at 25°C in an incubator.

Since the goats considered in this study were obtained from the Awassa College research farm and were kept under relatively better management conditions, the observations on these goats may not reflect the exact situation of goats kept by rural farmers and hence the results of this study should be interpreted cautiously.

Chemical composition

The fat content of the milk samples was determined by the Gerber method (Bradley *et al.*, 1993), the protein content by the Kjeldahl method using Kjeltac System I according to the International Dairy Federation standard (IDF, 1993), the total solids (TS) content by using a forced draft oven at 100 ± 2°C for 3 h (Bradley *et al.*, 1993) and the solids-not-fat (SNF) content was calculated by difference (*i.e.*, %TS-%fat). The pH of the milk samples was determined by using a digital pH meter (Orion FA 210, Massachusetts, USA). The experiment was repeated six times at different time intervals and each analysis was done in duplicate.

Microbial counts

Goat milk samples were diluted in quarter strength Ringer's solution and volumes of one ml of appropriate dilutions were pour-plated separately for the following tests. Standard plate count (SPC) was determined on plate count agar (Oxoid, Hampshire, England) after incubation at 30°C for 48 h; coliform count (CC) on violet red bile agar (Oxoid) after incubation at 30°C for 24 h; and lactic acid bacteria count (LABC) on MRS agar (Oxoid) after incubating anaerobically at 30°C for 48 h (Bradley *et al.*, 1993). Mesophilic aerobic spore-forming bacteria count (ASBC) was determined in the same way as the SPC except that the milk samples were heat treated in a water bath at 80°C for 10 minutes prior to plating and colonies were counted after incubation at 30°C for 3 days. Yeast and mould counts (YMC) were determined by using chloramphenicol bromophenol blue agar consisting of (g/litre in distilled water) 5.0 yeast extract, 20.0 glucose, 0.1 chloramphenicol, 0.01 bromophenol blue, 15.0 agar. The pH of the medium was 6.0–6.4. Colonies were counted after incubating the plates at 25°C for 5 days. Counts were made on samples of raw goat milk stored at 4°C or 25°C for 0 (initial count at the time of plating), 12, 24, 48, 72, and 96 h. All experiments were done in duplicate.

Assessment of the microflora of raw goat milk and isolation of lactic acid bacteria

The types of bacteria present in raw goat milk held at 25°C were determined by plating raw goat milk samples on SPC agar. Colonies grown on MRS agar were used for the isolation of lactic acid bacteria (LAB). Ten percent of the colonies (24 h old) grown on SPC agar or MRS agar were picked at random and a total of 111 isolates for the general microflora and 53 isolates for the LAB were further examined microscopically, purified by repeated plating and differentiated by various cultural and biochemical tests to genus level following standard methods (Harrigan and McCance, 1976; Kiss, 1984; Holt *et al.*, 1994). Morphological evaluation using a phase contrast microscope, Gram reaction, oxidative/fermentative test, catalase test, oxidase test and sugar fermentation profiles were used among others to determine the microflora of raw goat milk samples.

Statistical analysis

The result of microbial counts was first transformed to logarithmic values. The mean

values at the different storage periods for each count type at each storage temperature were compared using the analysis of variance technique. Means were compared at 5% significance level, when F values were significant, using the Turkey's Honestly Significant Difference test as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of raw Borana goat milk used in this experiment is indicated in Table 1. The composition of Borana goat milk observed in this study is similar to the composition of some tropical goat breeds milk reported by Agnihotri and Prasad (1993); however, these values are much higher than that reported for temperate goat breeds milk such as Saanen (Boyazoglu and Morand-Fehr, 2001). Goat milk with higher contents of TS, SNF, fat and protein results in higher cheese yield and it coagulates quickly and forms firmer curd than milk containing low levels of these components (Clark and Sherbon, 2000). The higher protein, fat, TS and SNF content of Borana goat milk observed in this study suggest that Borana goat milk could be of significant importance for cheese production.

Table 1. Chemical composition and pH of Borana goat milk (n = 6).

Parameter	Mean ± SD
Fat (% w/w)	7.8 ± 1.9
Protein (% w/w)	6.2 ± 0.4
TS (% w/w)	20.3 ± 2.6
SNF (% w/w)	12.9 ± 0.9
pH	6.67 ± 0.1

n, number of samples; SD, standard deviation; TS, total solids; SNF, solids-not-fat.

Microbial counts of raw goat milk

During storage of raw goat milk at 4°C, SPC, CC and ASBC decreased at 24 h of storage (Table 2). However, after 24 h, SPC, CC and ASBC showed significant ($p < 0.05$) increase to reach maximum level at 72 h for SPC and ASBC and at 96 h for CC. On the other hand, LABC increased with time and reached its maximum level at 72 h in raw goat milk stored at 4°C (Table 2). Yeasts and moulds showed a rather parallel pattern of growth with LAB in raw goat milk kept at 4°C (Table 2).

The number and types of microorganisms present in milk and milk products at any particular period depend on the microbiological quality of the raw material, the conditions under which the products were produced and also on the temperatures and duration of storage (Burgess *et al.*, 1994). The initial (0 h) SPC and CC of raw goat milk observed in this study (Table 2) are higher than those reported by other authors (Espie and Mullan, 1987; Tirard-Collet *et al.*, 1991). The suggested standard by the Department of Agriculture and Fisheries for Scotland (1984) indicates SPC of 50,000 bacteria/ml and CC of <1000/ml in raw goat milk.

Standard plate count is a useful indicator for monitoring the sanitary conditions present during the production, collection, and handling of raw milk (Chambers, 2002). In most cases, an SPC increase correlates well with unsanitary conditions existing within the milk collection and handling system in the milk house (Chambers, 2002). The higher SPC of raw goat milk observed in this study may probably be attributed to poor production and handling conditions of the goat milk at the study area. Higher bacterial counts contribute to poor quality of milk and inferior milk products.

Coliforms could contaminate milk from manure, bedding material and possibly also from contaminated water, soil and inadequately cleaned milking machines (Kalogridou-Vassiliadou, 1991). Thus, separate milking parlours for the goats and hygienic milking procedure might help to alleviate

this problem. Coliforms can also contaminate milk from body surfaces of animals and milk handlers (Ray, 2001). Despite the fact that the milkers washed their hands before milking the goats, the goat milk samples examined had higher coliform count. This may be attributed to inadequate personal hygiene and improper sanitation of the milking environment. The goats used in this experiment were hand-milked at their pen, which might partly contribute to the increased coliform counts.

Mesophilic aerobic spore-forming bacteria count of raw goat milk kept at 4°C ranged from an initial count of 1.62×10^3 cfu/ml to a maximum of 1.2×10^6 cfu/ml at 72 h of storage (Table 2). The initial count of aerobic spore-formers observed in this study is higher than that reported for raw goat milk elsewhere (Espie and Mullan, 1987; Anifantakis, 1993). High numbers of spore-forming bacteria in milk may indicate unsanitary production practices and may cause product defects (Frank *et al.*, 1993). Aerobic spore-forming bacteria such as *Bacillus* spp. cause sweet curdling defect in pasteurised milk, and coagulation of canned evaporated milk (Frank *et al.*, 1993). The fact that 22% of the organisms isolated from the goat milk samples examined were spore-forming *Bacillus* spp. (Table 3) implies that heat treated goat milk and goat milk products can easily be spoiled due to growth of *Bacillus* spp. unless special precautions are taken during post-pasteurization handling.

Table 2. Mean (\pm SD) microbial counts (\log_{10} cfu/ml) of raw Borana goat milk during storage at 4°C and 25°C (n = 6).

4°C	SPC	CC	LABC	YMC	ASBC
0 h	5.68 ^a \pm 0.69	2.64 ^a \pm 0.78	5.86 ^a \pm 0.53	2.46 ^a \pm 1.01	3.20 ^a \pm 0.62
12 h	6.08 ^a \pm 1.34	3.52 ^a \pm 1.36	6.11 ^a \pm 1.12	2.78 ^a \pm 1.17	4.23 ^{a*} \pm 0.92
24 h	6.00 ^a \pm 0.79	3.46 ^a \pm 0.77	6.23 ^a \pm 1.30	2.92 ^a \pm 0.87	4.20 ^{a*} \pm 0.85
48 h	7.98 ^{bc} \pm 0.98	5.45 ^b \pm 1.04	7.46 ^b \pm 0.83	3.04 ^a \pm 0.89	4.72 ^{bc} \pm 1.02
72 h	8.92 ^c \pm 0.89	6.93 ^{bc} \pm 1.10	8.82 ^b \pm 0.83	3.59 ^a \pm 0.81	6.08 ^d \pm 0.62
96 h	8.68 ^c \pm 0.84	7.89 ^c \pm 0.54	8.43 ^b \pm 0.74	3.41 ^a \pm 0.84	5.04 ^{dc} \pm 0.93
25°C					
0 h	5.68 ^a \pm 0.69	2.64 ^a \pm 0.78	5.86 ^a \pm 0.53	2.46 ^a \pm 1.01	3.20 ^a \pm 0.62
12 h	8.00 ^b \pm 0.83	4.28 ^b \pm 0.59	7.00 ^b \pm 1.15	2.73 ^a \pm 0.85	4.18 ^b \pm 1.20
24 h	8.46 ^b \pm 0.68	7.32 ^c \pm 0.73	7.69 ^b \pm 0.91	3.04 ^a \pm 0.87	4.72 ^{bc} \pm 1.07
48 h	9.18 ^{bc} \pm 0.75	8.82 ^d \pm 0.42	9.00 ^c \pm 0.49	3.08 ^a \pm 0.59	5.85 ^{cd} \pm 0.96
72 h	9.85 ^c \pm 0.79	9.18 ^d \pm 0.48	9.60 ^c \pm 0.63	3.46 ^a \pm 0.92	6.23 ^d \pm 0.65
96 h	9.52 ^c \pm 0.59	8.64 ^d \pm 0.78	9.30 ^c \pm 0.62	3.34 ^a \pm 1.03	5.38 ^{cd} \pm 0.80

Superscripts in the same column within a storage temperature with different letters indicate significant difference ($p < 0.05$); cfu, colony forming units; SPC, standard plate count, CC, coliform count, LABC, Lactic acid bacteria count, YMC, yeast and mould count, ASBC, mesophilic aerobic spore-forming bacteria count, n, number of samples, SD, standard deviation.

The aerobic spore-forming bacteria seemed to have almost a similar growth rate at 4°C and 25°C (Table 2). This might be associated with the presence of high numbers of psychrotrophic spore-forming bacteria such as *Bacillus* spp. in the goat milk samples. Psychrotrophs are microorganisms that grow at refrigeration temperature (0–5°C) irrespective of their optimum growth temperature (Ray, 2001). They usually grow rapidly between 10 and 30°C (Ray, 2001). Some species of *Bacillus* are psychrotrophs and their spores can germinate and outgrow even at refrigeration temperature (4.5°C); they can as well grow at a temperature range of 10 and 30°C (Ray, 2001). Chambers (2002) reported that *Bacillus* spp. were the predominant microflora in refrigerated laboratory pasteurized milk even under situations where no post-pasteurization contamination took place.

The mean YMC of raw goat milk (Table 2) observed in this study is within the range for YMC reported by Espie and Mullan (1987). They reported that raw goat milk from Northern Ireland had a mean YMC of 8.5×10^1 cfu/ml ranging from 4.0 to 2.2×10^5 cfu/ml. On the other hand, Barbosa and Miranda (1986) reported a mean YMC of 2.0×10^3 cfu/ml, in raw goat milk from Portugal. Fresh milk only rarely contains yeasts and moulds (Burgess *et al.*, 1994); however, spores of moulds are widely distributed in the atmosphere, particularly in the air and dust and spoil a number of dairy products (Kosikowski and Mistry, 1999). Thus, the high YMC observed in the present study suggests that yeasts and moulds might have got access to the goat milk samples during milking of the goats in their pen.

Yeasts and moulds showed almost a similar growth rate at 4°C and 25°C (Table 2). This might be attributed to the presence in higher numbers of psychrotrophic yeast and mould species in the goat milk samples. Moulds, yeasts, many Gram-negative bacteria and Gram-positive bacteria such as *Bacillus* spp. are psychrotrophs (Ray, 2001).

In raw goat milk stored at 4°C, no significant increase in count was observed for all the microbes until 24 h of storage (Table 2); however, during storage of raw goat milk at 25°C, most of the microbial counts at and after 12 h of storage were significantly ($p < 0.05$) higher than the initial count (Table 2).

Tirard-Collet *et al.* (1991) reported that a significant increase in SPC, CC, and YMC in raw goat milk kept at 3°C from Quebec, Canada, was observed after 3 days of storage. During storage at 4°C, the raw goat milk samples used in this study

kept unspoiled only for 24 h. This period is much less than that reported by others for raw goat milk stored at a refrigeration temperature. This could have been attributed to the high initial microbial load in the raw goat milk. Thus, when initial contamination level is high, cold storage of raw goat milk is of little value as it does not arrest the growth of certain microorganisms. Hence, good manufacturing practice need to be implemented to obtain goat milk with a reasonable microbial load. In raw goat milk samples kept at 25°C, most of the microbial counts at and after 12 h of storage were significantly higher than the initial count and microbial counts increased with length of storage period in most cases. Thus, at 25°C, raw goat milk may hardly be kept fresh for more than 12 h.

Microflora of raw goat milk

Out of the total 111 isolates of raw goat milk, *Staphylococcus* spp. accounted for 36% followed by Enterobacteriaceae (29%), *Bacillus* spp. (22%), *Micrococcus* spp. (13%) and *Streptococcus* spp. (<1%) (Table 3). Among the 53 isolates of LAB, *Lactococcus* spp. accounted for 57%, *Lactobacillus* spp. accounted for 23% and *Leuconostoc* spp. accounted for 21% (Table 3).

Table 3. The microflora and lactic acid bacteria isolated from Borana goat milk kept at 25°C.

Types of bacteria	No. of isolates	Percent of the total isolates
General microflora (n=111)		
<i>Staphylococcus</i> spp.	40	36
Enterobacteriaceae	32	29
<i>Bacillus</i> spp.	24	22
<i>Micrococcus</i> spp.	14	13
<i>Streptococcus</i> spp.	1	0.9
Lactic acid bacteria (n = 53)		
<i>Lactococcus</i> spp.	30	57
<i>Lactobacillus</i> spp.	12	23
<i>Leuconostoc</i> spp.	11	21

n = total number of isolates.

The organisms isolated from raw goat milk in this study are similar to those isolated from raw goat milk in Australia (Jensen and Hughes, 1980) and in the UK (Roberts, 1985). Since the family Enterobacteriaceae includes many genera and species that are enteric pathogens, enumeration of the whole group could be used as a better indicator of the level of sanitation, possible fecal contamination and possible presence of enteric pathogens (Forsythe and Hayes, 1998; Ray, 2001). The increase to large numbers of Enterobacteriaceae

in the goat milk observed in this study indicates that it is possible for pathogens to multiply under the present conditions of production and handling of goat milk.

Staphylococci are the most serious causes of caprine and bovine mastitis (Hunter, 1984). In the present study, *Staphylococcus* spp. were the most abundant (36%) organisms in raw goat milk. This is in line with earlier reports (Harvey and Gilmour, 1988; Kalogridou-Vassiliadou, 1991; Contreras *et al.*, 1993; Boscós *et al.*, 1996). Chubb *et al.* (1985) reported that the main organism isolated from goat milk samples from New South Wales, Australia, was *Staphylococcus* (87%). Similarly, White and Hinckley (1999) reported that the most prevalent mastitis agent in goat milk from the USA was non-hemolytic *Staphylococcus* (38.2%) followed by *Staphylococcus aureus* (11%) and *Streptococcus* spp. (4.1%). The abundance of *Staphylococcus* spp. in the goat milk samples examined in the present study suggests the probable presence of mastitis in the goat herd at the time of the experiment. Staphylococci are generally present in the nose, throat, skin and hair of healthy humans and animals (Ray, 2001). Contamination of the goat milk samples used in this study by staphylococci might have occurred from these sources. Thus, sanitation of the production environment and proper personal hygiene among milk handlers is important.

In the present study microorganisms found in raw goat milk were identified only to genus level. Due to the fact that some of the bacterial genera isolated consist strains and/or species that are pathogenic, it is likely that the raw goat milk examined in this study had organisms that can cause illnesses in human beings. Thus, further study is needed to isolate species that might pose public health problems due to consumption of raw goat milk produced in the study area.

The dominant LAB isolated from Borana goat milk in this study were *Lactococcus* spp. followed by *Lactobacillus* spp. and *Leuconostoc* spp. This is in line with the report by Almaz Gonfa *et al.* (2001) who reported that the dominant LAB isolated from a traditional fermented cows' milk ("Ergo") belonged to the genera *Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus*. Further characterization and identification of LAB from goat milk is essential to develop suitable indigenous starter cultures for use in large-scale manufacture of traditional fermented goat milk products.

In conclusion, raw milk obtained from Borana goat during the current study was poor in microbial quality. The high microbial count and the presence of pathogenic bacterial genera in the goat milk indicate that consumption of raw goat milk may pose public health hazards. Since the goat milk samples used in this study were obtained from goats kept at the Awassa College research farm which is produced and managed under relatively better hygienic conditions as compared to those produced at the farmer's level, it is highly likely that goat milk produced by individual farmers under a relatively poor hygienic conditions could contain as high bacterial load or even worse. Thus, scrupulous hygienic measures need to be taken during production and handling of goat milk.

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