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Research Article
Variation of Microbial Load of Sudanese Nubian Goat' Milk as Affected by Lactoperoxidase Enzyme System, Stage of Lactation and Storage Temperature

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Abstract
This study was carried out to improve the keeping quality and to prolong the shelf life of milk of Sudanese Nubian goat at different stages of lactation and storage temperatures (5±2, 13±2, 25±2 and 37±2°C) by using Lactoperoxidase Enzyme System (LPS). Morning milk samples (36 samples) were collected from Nubian goat unit of the Faculty of Animal Production University of Khartoum during February to November, 2009. The result showed that the treated LPS milk samples were significantly ($p \leq 0.05$) affected by the addition of the enzyme, stages of lactation and storage temperature. Moreover, the study showed reduction in total viable bacterial count, coliform, psychrotrophic bacteria and yeast and mould content of treated samples with the reducing of degrees of temperature compared with the control samples kept under the same condition. Samples kept at 37±2°C showed higher microbial load and shorter shelf life compared with that stored at 5±2°C. It was concluded that application of the LPS inhibited the growth and multiplication of microorganisms and prolonged the shelf life of goat's milk under Sudan condition.

Key words: Nubian goat, stage condition, microbial load, milk shelf life

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cannas *et al.* (2008) reported that goat are widespread worldwide due to their nutritional and environmental adaptability and are reared in wide range of breeding and feeding systems. The use of goat's milk becomes an opportunity to diversity the dairy market since it allows to develop added value of fermented products with particular characteristics in comparing to cow's milk (Vargas *et al.*, 2008). Haenlein (2004) illustrated that goat's milk fatty acids have become established medical treatments for an array of clinical disorders. Goat's milk fats have a unique metabolic ability to limit cholesterol deposits in arteries (Alferez *et al.*, 2001).

Nubian goat was found in North Sudan, Egypt, Ethiopian highland and Eritrea (French, 1970; Steele, 1996). The Nubian goat is predominantly distributed in the Northern river Nile and Khartoum state, where they play a substantial role in the livelihood of many families, it have small size, low maintenance and valuable in small farm situation where limited land and resources existed (El-Basheir, 2008). Similarly Abdalla and El-Zubeir (2007) concluded that establishment of specialised farms of dairy goats in order to ensure the food security in rural areas can fill the gap of domestic production of milk supply. However, Abd El Gadir and El Zubeir (2005) suggested that some conditions must be taken into consideration such as feed intake and the proper selection of Nubian goats for crossing with Saanen in order to get good performing goats. Hamid and Musa (2013) reported that activated milk samples by the LPS extended the shelf life of cow milk by at least 8 h at incubation temperature (37°C). El Zubeir (2012) reported 9 days and 3 days at refrigeration and room temperature, respectively, for camel milk. Rasbawati *et al.* (2014) reported that lactoperoxidase system decreasing total bacteria from 8-5 log CFU mL^{-1}. Boulares *et al.* (2011) reported that by activation of the LPS in raw milk, it is possible to store ovine, bovine and caprine milk at 4°C for several days. Similarly, Saad *et al.* (2013) reported that the LPS treated milk samples showed non significant differences in acidity, TBC, coliform and psychrotrophic counts compared to milk samples kept at refrigerator.

Zapico *et al.* (1995) reported that activation of LPS might be a useful procedure in preserving raw goat milk quality by addition of low levels of thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2). Nigussie and Seifu (2008) reported that on farm activation of the LPS combined with container smoking can significantly improve the keeping quality of goat milk during storage and transportation at an ambient temperature. Further, Seifu *et al.* (2004) reported that preservation of goat cheese milk by the LP system can be used to improve the microbiological quality and flavour of Gouda cheese without any detrimental effect on the gross chemical composition of the cheese. In the present study, the objectives are to study the effect of LPS from on milk of Nubian goat in different stages of lactation and to correlate the preservation of LPS on the shelf life of goat's milk in relation to storage temperature.

MATERIALS AND METHODS

Animals and experimental procedure: Seven Nubian goats, under semi traditional farming system were used as experimental animals during the period of February to November, 2009. Samples of milk were obtained at morning from Nubian goat unit of the Faculty of Animal Production. The milk samples from each animal was divided into two equal portions, one portion was preserved with the lactoperoxidase enzyme system (obtained from Ministry of Animal Resources, offered by the FAO for field trail of the LPS in Sudan) while the second was kept as control. Each portion was further subdivided into four equal groups, the first was kept at 37°C, the second was kept at 25°C, the third was kept at 13°C and the fourth was kept at refrigerator (6±2°C).

Analysis of the samples: Microbiological examinations such as preparation of serial dilutions, preparation of the media were carried out by Houghtby *et al.* (1992) while sterilization of equipments was done as described by Wehr and Frank (1992). The milk samples were subjected to determine the total viable bacterial count according to Houghtby *et al.* (1992). The coliform count was done as described by Harrigan and McCance (1976) and yeast count and psychotropic bacterial count were estimated according to Frank *et al.* (1992). Acidity and clot–on-boiling test were carried out daily until the spoilage of samples, according to APHA (1976) and IDF (1990), respectively.

Statistical analysis: The data were analyzed by using factorial arrangement. The test was used to evaluate the mean differences among different treatments at $p \leq 0.05$ significance level (SAS., 1988; Dowdly and Wearden, 1991).

RESULTS AND DISCUSSION

Table 1 showed higher log total bacterial count of control goat's milk samples that kept at different degrees of temperature compared to the LPS treated samples. While the LPS treated samples contain lower psychrotrophic bacterial count than the control samples under the same conditions (Table 2).

Table 1: Effect of LPS enzymes, temperature and lactation stages on log total bacterial count of goat's milk

	Lactation stages					
	Control milk			LPS milk		
Temperature (°C)	1st	2nd	3rd	1st	2nd	3rd
5±2	4.87±0.87^{de}	4.85±0.50^{e}	4.88±0.79^{de}	4.50±0.12^{h}	4.52±0.55^{h}	4.53±0.79^{h}
13±2	5.02±0.08^{c}	5.04±0.32^{c}	5.03±0.15^{c}	4.72±0.07^{g}	4.70±1.01^{g}	4.71±1.02^{g}
25±2	5.10±0.07^{bc}	5.13±0.18^{b}	5.15±0.12^{b}	4.81±0.08^{f}	4.82±0.32^{f}	4.80±0.14^{f}
37±2	5.23±0.05^{a}	5.22±1.02^{a}	5.23±0.14^{a}	4.90±0.08^{d}	4.89±0.26^{d}	4.91±0.14^{d}
p-value	0.0438*					
SE±	0.0075					

Means sharing the same superscript letters are not significantly (p>0.05) different according to DMRT, SE±: Experimental standard error, p-value: Level of significance (probability)

Table 2: Effect of LPS enzymes, temperature and lactation stages on log psychrotrophic bacterial count of goat's milk

	Lactation stages					
	Control milk			LPS milk		
Temperature (°C)	1st	2nd	3rd	1st	2nd	3rd
5±2	0.00±0.00^{m}	2.88±2.43^{kl}	2.90±2.45^{k}	0.00±0.00^{m}	2.40±2.77^{l}	2.39±2.76^{l}
13±2	0.00±0.00^{m}	4.01±1.72^{h}	4.07±1.77^{g}	0.00±0.00^{m}	3.28±2.24^{j}	3.26±2.22^{ij}
25±2	0.00±0.00^{m}	4.74±0.30^{b}	4.71±0.37^{bc}	0.00±0.00^{m}	4.53±0.31^{c}	4.51±0.29^{cd}
37±2	0.00±0.00^{m}	4.84±0.30^{a}	4.82±0.28^{a}	0.00±0.00^{m}	4.44±0.48^{ef}	4.46±0s.50^{e}
p-value	0.0283*					
SE±	0.0671					

Table 3: Effect of LPS enzymes, temperature and lactation stages on coliform count of goat's milk

	Lactation stages					
	Control milk			LPS milk		
Temperature (°C)	1st	2nd	3rd	1st	2nd	3rd
5±2	0.00±0.00^{l}	4.30±0.45^{jk}	4.32±0.24^{j}	0.00±0.00^{l}	4.17±0.44^{l}	4.18±0.22^{k}
13±2	0.00±0.00^{l}	4.67±0.23^{gh}	4.69±0.12^{g}	0.00±0.00^{l}	4.43±0.35^{i}	4.49±0.15^{h}
25±2	0.00±0.00^{l}	4.96±0.26^{c}	5.04±0.07^{b}	0.00±0.00^{l}	4.73±0.40^{f}	4.88±0.09^{d}
37±2	0.00±0.00^{l}	5.13±1.19^{ab}	5.15±0.11^{a}	0.00±0.00^{l}	4.85±0.36^{e}	4.94±0.06^{cd}
p-value	0.0281*					
SE±	0.0059					

Coliform count of goat's milk samples at 5±2, 13±2, 25±2 and 37±2°C) degrees of temperature were higher in control samples compared with LPS treated samples as illustrated in Table 3. The highest log yeast count was estimated in the control milk samples (3.92±1.81) compared to LPS treated samples (3.55±1.70) stored at 37±2°C (Table 4).

From the results of this study, addition of lactoperoxidase enzyme system, degrees of temperature and stages of lactation were reducing the viable bacterial count and yeast and mould content of goat's milk samples (Table 1 and 4). Those findings agreed with that obtained by Seifu *et al.* (2005) who stated that LPS in goat's milk inhibit the growth and proliferation of many fungal species. Onneile (2006) and Saad *et al.* (2013) also reported that the inhibitory effect of lactoperoxidase enzyme system (LPS) is depending on the storage temperature of LPS treated milk. This was proven previously by Gaya *et al.* (1991) who reported that the lactoperoxidase-thiocyanate-hydrogen peroxide system has been reported to be a feasible method for the temporary preservation of raw milk. The application of the LPS for milk preservation aimed at raising regional awareness to safe, cheap and effective alternative milk preservation method (El Zubeir *et al.*, 2006). However, Abdalla and El-Zubeir (2007) suggested the initiation of standards and grades of raw goat milk to get good quality milk from goats.

The LPS treatment has resulted in increased milk shelf life (Table 5), which was effective at all degrees of temperature (5±2, 13±2, 25±2 and 37±2°C) and tend to be more efficient as storage temperature decreases (Table 5). These results are in conformity with those obtained by other authors who also demonstrated the effectiveness of the

Table 4: Effect of LPS enzymes, temperature and lactation stages on yeast count of goat's milk

	Lactation stages					
	Control milk			LPS milk		
Temperature (°C)	1st	2nd	3rd	1st	2nd	3rd
5±2	0.00±0.00^{m}	2.36±2.03^{j}	2.70±2.10^{i}	0.00±0.00^{m}	1.54±1.97^{l}	1.52±1.95^{lm}
13±2	0.00±0.00^{m}	3.10±1.06^{f}	3.07±1.03^{g}	0.00±0.00^{m}	2.06±1.95^{k}	2.04±1.91^{kl}
25±2	0.00±0.00^{m}	3.36±1.50^{d}	3.21±2.14^{e}	0.00±0.00^{m}	3.05±1.91^{gh}	2.94±2.02^{h}
37±2	0.00±0.00^{m}	3.90±1.79^{ab}	3.92±1.81^{a}	0.00±0.00^{m}	3..51±1.39^{c}	3.55±1.70^{b}
p-value	0.0272*					
SE±	0.0059					

Table 5: Mean±SD of shelf-life (days) of goat's milk as affected by addition of LPS enzymes

		Lactation stages					
		Control milk			LPS milk		
Temperature (°C)		1st	2nd	3rd	1st	2nd	3rd
5±2	Range (days)	5-6	5-6	5-6	7-8	7-8	7-8
	Mean±SD	6.49±4.34	6.67±4.36	6.83±4.43	7.76±4.08	7.87±4.19	7.82±4.14
13±2	Range (days)	4-5	4-5	4-5	6-7	6-7	6-7
	Mean±SD	5.91±3.95	6.00±4.07	6.03±4.20	7.42±4.13	7.46±4.17	7.39±4.10
25±2	Range (days)	1-2	1-2	1-2	2-3	2-3	2-3
	Mean±SD	1.34±0.7	1.27±0.5	1.39±0.8	2.41±0.06	2.36±0.04	2.30±0.30
37±2	Range (days)	0.42-0.46	0.42-0.46	0.42-0.46	1.04-1.13	1.04-1.13	1.04-1.13
	Mean± SD	0.42±0.08	0.39±0.05	0.42±0.02	1.08±0.02	1.10±0.03	1.10±0.02

Table 6: Effect of LPS enzymes and storage temperature on acidity of goat's milk

Temperature (°C)	Control milk	LPS milk
52	0.50±0.58^{e}	0.22±0.05^{h}
132	0.63±2.09^{d}	0.35±0.14^{g}
252	0.77±5.47^{b}	0.45±0.19^{f}
372	1.00±8.04^{a}	0.73±0.02^{c}
p-value	0.0482*	
SE±	0.0059	

LPS in preserving milk especially in rural areas where refrigeration facilities are absent to farmers (Fonteh *et al*., 2005; Ndambi *et al*., 2008; El Zubeir, 2012). Comparisons were made between treated milk samples stored under (37±2°C) and under refrigeration (5±2°C) as shown in Table 1-4. It was observed that the LPS treated milk stored at the refrigerator had a lower microbial load than the same milk stored under 37±2°C. This supported the previous finding of Saad *et al*. (2013) who reported that the total bacterial count of sheep milk treated by lactoperoxidase system were increased gradually with increasing temperature. Table 5 showed long shelf life of all treated goat's milk samples at the three stages of lactation compared with the control ones that affected significantly ($p \leq 0.05$) by LPS, storage temperature and stages of lactation. These results are in conformity with that obtained by Zapico *et al*. (1995), who demonstrated that activation of the LPS might be a useful procedure in preserving raw goat milk quality by addition of low levels of thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2). Similarly, Saad *et al*. (2013) found that the shelf life of sheep milk samples were affected significantly ($p \leq 0.001$) by addition of LPS, storage temperature and the interaction between temperature and LPS.

The addition of the LPS has extended shelf life of goat's milk to 1.04-1.13, 2- 3, 6- 7 and 7- 8 days compared to 0.42-0.46, 1-2, 4-5 and 6-7 day for untreated samples at storage temperature of 37±2, 25±2, 13±2 and 5±2°C, respectively (Table 5). This supported by Saad *et al*. (2013), who reported that the shelf life of sheep milk samples were affected significantly ($p \leq 0.001$) by LPS, storage temperature and interaction between temperature and LPS. The results of Nubian goat's milk samples trails were monitored using the acidity and clot on boiling test to detect the milk deterioration for both LPS treated and untreated milk samples. The means titratable acidity and standard errors of LPS treated goat's milk samples kept at 5±2, 13±2, 25±2 and 37±2°C were 0.22±0.05, 0.35±0.14, 0.45±0.19 and 0.73±0.02%, respectively, as shown in Table 6. On the other hand, the titratable acidity of the control milk samples kept at the same degrees of temperature were 0.50±0.58, 0.63±2.29,

0.77±5.47 and 1.00±8.04%, respectively (Table 6). From Table 6, the data showed that there was significant ($p \leq 0.05$) effect on titratable acidity of goat's samples treated by LPS in comparison with the control samples during the same storage temperature (5±2, 13±2, 25±2 and 37±2°C). However, an increase was observed in titratable acidity at increasing the degrees of temperature in both LPS treated and control goat's milk samples. The LPS treated goat's milk samples kept at 5±2°C showed reduction in acidity compared to control samples at the same degree of temperature (Table 6). The acidity of LPS treated and control goat's milk samples kept at 13±2 and 25±2°C were found to increase gradually with increasing the degrees of temperature as shown in Table 6. It is clear that LPS can be applied to extend the shelf life of milk, which might enable smallholder dairy farmers who lack the cooling facilities to deliver it in the collection centers or consumption areas (El Zubeir, 2012; Saad *et al*., 2013).

CONCLUSION

The LPS is effective in prolonging the shelf life of goat milk. Moreover, its activity was affected by the stage of lactation and storage temperature. The prolongation of the shelf life would improve and facilitate adequate marketing for better utilization of goat milk in tropical areas.

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