



## EFFECT OF SOME NATURAL ANTIMICROBIAL SYSTEMS IN NORTH SINAI GOAT MILK ON DIFFERENT TYPES OF PATHOGENIC BACTERIA

[31]

El-Gendy, Marwa H.H.<sup>1</sup>; Y.M. Kamel<sup>1</sup>; M.A. El-Nawawy<sup>2</sup> and M.N.I. Magdoub<sup>2</sup>

1- Animal Production Division, Desert Research Center, Ministry of Agriculture, Cairo, Egypt

2- Food Science Department, Faculty of Agriculture, Ain Shams University, Shobra El-Kheima, Cairo, Egypt

**Keywords:** Lactoperoxidase system, Lysozyme, Goat Milk, Pathogenic bacteria

**Abbreviation key:** LPs = lactoperoxidase system, Lz = lysozyme

### ABSTRACT

The objective of this study was to investigate the effect of activation of lactoperoxidase system (LPs) and lysozyme (Lz) as antimicrobial natural systems in North Sinai goat milk on six pathogenic bacteria (*E.coli* O157:H7, *S. typhimurium*, *Y. enterocolitica*, *B. cereus*, *List. monocytogenes* and *Staph. aureus*). Samples of goat's milk were collected under complete aseptic conditions during 2004. Three combination groups from sodium thiocyanate with sodium percarbonate were tested for LPs activation as follows: Group 1 (G<sub>1</sub>) (14mg + 30mg), Group 2 (G<sub>2</sub>) (15mg + 10mg) and Group 3 (G<sub>3</sub>) (20mg + 25mg), respectively. The counts of these bacteria were recorded after 6, 12 and 18 hr inoculation. In general, both of LPs groups and Lz concentrations have positive impact to reduce the gram positive and negative bacteria in goat milk. The effect of LPs and Lz was more effective on *L. monocytogenes* than other gram positive bacteria and on *S. typhimurium* than other gram negative bacteria after 6, 12 and 18 hr incubation. Also, the bacterial count (log cfu/ml) was increased linearly along the studied times in all treatments of both LPs and Lz. The combination of 14mg sodium thiocyanate + 30mg sodium percarbonate, which

used to activate LPs, was the highest significantly positive affected. Moreover, the positive effect of Lz increased by increase its concentrate.

### INTRODUCTION

Goats constitute an import animal resource under arid and semi-arid conditions. The total number of goats in Egypt is about 5 millions heads. Average daily milk yield of goat in Egypt is varied and ranged from 0.2 to 1.2 kg /head/day according to different location, breeds and stage of lactation. Moreover, the average lactation period ranged from 120 to 180 days. Goats constitute the majority of animal population in Sinai. They account for an average of about 61% of the total number of the animals' population (MoALR, 2005).

Milk is considered as the best environment to activation and growth of bacteria. Therefore it is subjected to contaminate by bacteria and (or) yeast. The major natural antimicrobial proteins of milk are Lactoperoxidase system, Lysozyme, Lactoferrin and Immunoglobulins (Naidu, 2000).

The lactoperoxidase enzyme (EC 1.11.1.7) is present at concentration of 10-30 µg/ml in cow milk, while, it is ranged from 0.1 to 0.7 µg /ml in goat milk (Fonteh *et al* 2002). But the enzyme requires extra different concentrates of hydrogen peroxide and thiocyanate to activate it; in this case it is called lactoperoxidase system (LPs). The LPs has been recommended for preservation of raw milk in areas where it is not possible to use me-

chanical refrigeration for technical and/or economic reasons (IDF, 1988; FAO, 1999).

Lysozyme enzyme (EC 3.2.1.17) is responsible for the hydrolysis of  $\beta$ -1,4-glycosidic linkage of the peptidoglycan in the cell wall of bacteria. Gram positive bacteria have a very thick peptidoglycan layer, while gram negative bacteria have a thin peptidoglycan layer. Therefore the Lz action is more effected on the gram-negative bacteria than the gram-positive bacteria (Jollès and Jollès 1984).

The objective of this study was to determine the effect of activation of lactoperoxidase system and lysozyme on six different pathogenic bacteria.

## MATERIALS AND METHODS

### Milk Samples

Samples of goats milk were collected from El-Arish city (North Sinai governorate) about 320 km North East of Cairo during 2004. Data were collected as a part of the project sponsored by MERC, USA titled "Multinational approaches to enhance goat production in the Middle East". The milk was collected under complete aseptic conditions during the middle stage of lactation season and subjected individually to analysis by California mastitis test (CMT) to avoid the mastitic samples. The first three squirts of milk were discarded from each teat and samples were collected into sterile bottles and transmitted to the laboratory for bacteriological examination at 8°C.

### Chemicals

Lactoperoxidase bovine milk: was obtained from Sigma Co., St Louis, Mo 63178, USA.

Lysozyme chicken egg white: Biochemika, dialyzed (Fluka Chemie AGCH - 9470, Buchs).

Sodium percarbonate ( $\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$ ): was obtained from BDH chemicals Ltd. Poole England.

Sodium thiocyanate (Na SCN): LOBA chemie PVT.LTD was used as a source of SCN<sup>-</sup>.

### Bacterial and Cultures

Six strains of pathogenic bacteria as slant were obtained from Egyptian Microbial Collection [EMCC] at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University. The bacteria cultures were used in this study to test the 6 strains in different 6 media as presented in Table (1).

Table 1. Origin of various bacterial strains and media used for investigation

Strain	ATCC*	Media
<i>Escherichia coli</i> O157: H7	35150	Macconkkey agar
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>typhimurium</i>	2556	Tryptic Soya agar
<i>Yersinia enterocolitica</i>	23715	CIN agar
<i>Bacillus cereus</i>	49064	<i>Bacillus cereus</i> selective agar and supplement
<i>Listeria monocytogenes</i>	19116	Tryptic Soya agar plus 0.6 % yeast extract
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	29740	Manittol Salt agar

\*ATCC = American Type Culture Collection.

### Preparation of Bacterial Suspensions

The suspensions of each pathogen were prepared by transferring fresh colonies grown on selective medium into sterile physiological saline solution using sterile loops. The respective suspensions were thoroughly mixed using whirl mixer and adjusted by either adding more saline or more bacteria to the turbidity of McFarland Standard 1.0 (1%  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ; 1%  $\text{H}_2\text{SO}_4$ ).

### Microbiological Analysis

Milk samples were first brought to room temperature (20-25°C) before inoculation with the specific pathogens. The total 2400 ml of milk was equally divided into 6 samples and inoculated with 1 ml of the appropriate suspensions of the 6 pathogen bacteria. Then each milk sample was aseptically divided into 10 portions (40 ml). After a period of 1 h, to enable the bacteria to adapt, milk samples, which have been activated for LPs and Lz. After inoculation of the milk with the specific pathogen bacteria, 1 ml sample was drawn from inoculated milk and transferred into 9 ml of sterile quarter strength Ringer's solution. Serial dilutions were made to determine the initial number of each pathogen in the milk samples. The strains were plated in the pervious specific media and incubated at 37°C for 24 hours. The bacteria

counted as cfu/ml after 6, 12 and 18 hours from the beginning of inoculation.

### Activation of Lactoperoxidase System

Sodium thiocyanate (Na SCN) and sodium percarbonate ( $\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$ ) were used to activate LPs, three combination groups were tested. Group 1 ( $G_1$ ) was 14mg Na SCN + 30mg  $\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$ , Group 2 ( $G_2$ ) was 15mg Na SCN+ 10mg  $\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$ , and Group 3 ( $G_3$ ) was 20mg Na SCN+ 25mg  $\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$ .

### Lysozyme Concentration

Three different concentrates of Lz chicken egg white [50 (C1), 150 (C2) and 200 (C3)  $\mu\text{g}/\text{ml}$ ] in addition to control (C) were used to determined the effect of them on pathogenic bacteria in milk

### Statistical Analysis

Data included the count (cfu/ml) of 6 different studied bacteria strains were analyzed after transformed using log transformation by the General Linear Model (GLM) procedure of SAS (1998), according to the following model:

$$Y_{ijk} = \mu + G_i + T_j + e_{ijk}$$

Where,

- $Y_{ijk}$  = any observation,  
 $\mu$  = overall mean,  
 $G_i$  = the effect of  $i^{\text{th}}$  LPs groups or  $i^{\text{th}}$  Lz concentrations  $i=1-4$ ,

- $T_j$  = the effect of  $j^{\text{th}}$  times,  $j=1-3$ ,  
 $e_{ijk}$  = the residual assumed to be normally and independently distributed with mean 0 and variance  $\sigma^2_e$

## RESULTS AND DISCUSSION

### Lactoperoxidase system (LPs)

The results presented in Table (2) show that, there were highly significant ( $p < 0.01$ ) differences between the LPs studied groups and between the three studied times on the all six studied bacteria. These differences could occurred due to the effects of the different combinations from sodium thiocyanate with sodium percarbonate which let to decrease the count of the different studied six bacteria strains than control after 6, 12 and 18 hours incubation. These results were in agreement with (Zapico *et al* 1993; Touch *et al* 2004 and Seifu *et al* 2004) which found that, LPs inhibited the growth of the same six studied pathogenic bacteria in goat milk after 12 hours.

### Effect of LPs on growth of gram negative bacteria

According to Bjorck *et al* (1975); Reiter *et al* (1976) and Wolfson & Sumner (1994), bactericidal activity in milk after LPs activation has been demonstrate against many gram negative bacteria including *Y. enterocolitica*, *S. typhimurium* and *E. coli* O175:H7.

Table 2. Analysis of variances of the main effects using LPs activation on different six strains pathogenic bacteria in goat milk.

S.O.V	df	MS					
		<i>B. cerues</i>	<i>Staph. aureus</i> subsp <i>aureus</i>	<i>List.</i> <i>monocytogenes</i>	<i>Y. entero-</i> <i>colitica</i>	<i>S. typhim-</i> <i>urium</i>	<i>E. coli</i> O175:H7
Total	11						
Time	2	16.18**	13.06**	17.76**	13.26**	20.34**	12.27**
Groups	3	0.34**	1.02**	0.63**	1.25**	0.61**	0.91**
Error	6	0.02	0.05	0.05	0.09	0.02	0.03

\*\* $P < 0.01$ , df = degrees of freedoms, S.O.V= Source Of Variation, MS = Mean Squares.

The results of the effect of LPs on growth of gram negative bacteria are present in Table (3). *Y. enterocolitica* was increased in G1, G2, G3 (LPs activation groups) and in the control milk samples by 0.6, 1.2, 2 and 2.4 log, respectively after 6 hours from initial value (6.4). Also, it was increased by 2.1, 3.3, 3.6 and 3.9 log after 12 hours and by 4.8, 5, 5.4 and 5.6 log after 18 hours.

*S. typhimurium* was decreased by 0.01 log units from initial value (6.6) after 6 hours from inoculated, while, it was increased in G 2, G3 and in the control by 0.4, 0.7 and 0.8 log, respectively after 6 hours of incubation. Also, it was increased by 1.4, 1.9, 2.4 and 2.8 log after 12 hours, and by 4.4, 4.9, 5.1 and 5.2 log after 18 hours in G1, G2, G3 and in the control, respectively.

*E. coli* was increased in G1, G2, G3 and in the control by 0.8, 1, 1.7 and 2.2 log, respectively after 6 hours from initial value (7.2). Also, it was increased by 2.3, 2.9, 3.5 and 3.8 log after 12 hours and by 4.5, 4.8, 5.1 and 5.4 log after 18 hours. These results were agreement with the results obtained by Zapico *et al* (1995) which found that *E. coli* counts was not growing on raw goat milk at 4°C and influence of LPs activation at this temperature and at 8°C *E. coli* was able to grow in control milk and observed in activated LPs after 2 days. Also, Vannini *et al* (2004) found that the *E. coli* was decreased when treated the skim milk by LPs activation. These results could be explained by Seifu *et al* (2004) which showed that the LPs was bacteriostatic against *E. coli*, in study to investigate the effects of the LPs on the growth and survival of *E. coli* in Saanen and South African Indigenous goat milk kept for 6 hours at 30°C. In this case, the LPs may be a promising alternative in controlling the growth of foodborne pathogens in goat milk.

#### Effect of LPs on growth of gram positive bacteria

The results of the effect of LPs on growth of gram positive bacteria are presented in Table (4). *B. cereus* count increased both in G1, G2, G3 and in the control milk samples by 0.8, 1.1, 1.3 and 1.9 log, respectively after 6 hours from initial value (6). Also, it was increased by 2.8, 3.1, 3.2 and 3.5 log after 12 hours and by 5, 5.2, 5.4 and 5.6 log after 18 hours. These results were disagreement with the results obtained by Marks *et al* 2001, who found that activation of LPs was not effected *B. cereus* spores in milk. Also, the LPs treated

milk, rather than heat-shocking of spores, is responsible for the greater keeping quality of milk pasteurized at 72°C/15 s compared with 80°C/15 s.

*Staph. aureus subsp aureus* count increased both in G1, G2, G3 and in the control milk samples by 0.5, 0.5, 1.3 and 2 log, respectively after 6 hours from initial value (6.5).

Also, it was increased by 1.8, 2, 2.8 and 3.4 log after 12 hours and by 4.3, 4.5, 4.8 and 5 log after 18 hours. In case of compared the results of *Staph. aureus* with the results obtained by Seifu *et al* (2004) in Saanen goat milk, he found that the mean of *Staph. aureus* count decreased by 0.1 log in the LP-treated milk and increased by 0.14 log in the control milk at the end of the incubation period.

The level of *Staph. aureus* in the LP-activated Saanen goat milk after 6 h of incubation was 41% lower than its level in the control milk.

Table 3. LSM ± SE of LPs groups and three different times effect on gram negative pathogenic bacteria in goat milk.

Items	Log cfu / ml		
	<i>Y. enterocolitica</i>	<i>S. typhimurium</i>	<i>E. coli</i> O175:H7
Overall mean	9.70±0.123	0.09±0.075	10.35±0.145
groups			
C	10.34 <sup>a</sup> ±0.170	9.53 <sup>a</sup> ±0.080	10.97 <sup>a</sup> ±0.102
G1	8.87 <sup>b</sup> ±0.170	8.51 <sup>b</sup> ±0.080	9.72 <sup>b</sup> ±0.102
G2	9.56 <sup>c</sup> ±0.170	8.99 <sup>c</sup> ±0.080	10.10 <sup>c</sup> ±0.102
G3	10.05 <sup>d</sup> ±0.170	9.34 <sup>d</sup> ±0.080	10.61 <sup>d</sup> ±0.102
Time (hours)			
6	7.95 <sup>a</sup> ±0.076	7.06 <sup>a</sup> ±0.070	8.61 <sup>a</sup> ±0.088
12	9.58 <sup>b</sup> ±0.076	8.71 <sup>b</sup> ±0.070	10.33 <sup>b</sup> ±0.088
18	11.58 <sup>c</sup> ±0.076	11.52 <sup>c</sup> ±0.070	12.11 <sup>c</sup> ±0.088

<sup>a, b, c, d</sup> mean values with the different superscript letters within the same item are significantly different (p<0.05).

LSM = LS Mean, SE = Stander Error.

C = Control group.

G1 = Group1 (14 mg Sodium thiocyanate + 30 mg Sodium percarbonate).

G2 = Group2 (15 mg Sodium thiocyanate + 10 mg Sodium percarbonate).

G3 = Group3 (20 mg Sodium thiocyanate + 25 mg Sodium percarbonate).

Table 4. LSM  $\pm$  SE of LPs groups and three different times effect on gram positive pathogenic bacteria in goat milk

Items	Log cfu/ml					
	B. cereus		Staph. aureus subsp aureus		List. monocytogenes	
	LSM	SE	LSM	SE	LSM	SE
Overall mean	9.16 $\pm$ 0.060		9.29 $\pm$ 0.085		9.03 $\pm$ 0.113	
Groups						
C	9.59 <sup>a</sup> $\pm$ 0.077		10.00 <sup>a</sup> $\pm$ 0.135		9.57 <sup>a</sup> $\pm$ 0.126	
G1	8.80 <sup>b</sup> $\pm$ 0.077		8.75 <sup>b</sup> $\pm$ 0.135		8.50 <sup>b</sup> $\pm$ 0.126	
G2	9.02 <sup>c</sup> $\pm$ 0.077		8.89 <sup>b</sup> $\pm$ 0.135		8.86 <sup>c</sup> $\pm$ 0.126	
G3	9.22 <sup>c</sup> $\pm$ 0.077		9.53 <sup>c</sup> $\pm$ 0.135		9.19 <sup>c</sup> $\pm$ 0.126	
Time (Hours)						
6	7.20 <sup>a</sup> $\pm$ 0.067		7.61 <sup>a</sup> $\pm$ 0.054		7.13 <sup>a</sup> $\pm$ 0.110	
12	9.05 <sup>b</sup> $\pm$ 0.067		9.06 <sup>b</sup> $\pm$ 0.054		8.66 <sup>b</sup> $\pm$ 0.110	
18	11.22 <sup>c</sup> $\pm$ 0.067		11.20 <sup>c</sup> $\pm$ 0.054		11.30 <sup>c</sup> $\pm$ 0.110	

<sup>a, b, c, d</sup> mean values with the different superscript letters within the same item are significantly different ( $p < 0.05$ ), LSM = LSMean, SE = Stander Error.

(i) for details of studied groups, see Table 3.

*List. monocytogenes* count increased both in G1, G2, G3 and in the control milk samples by 0.6, 0.9, 1.1 and 2 log, respectively after 6 hours from initial value (6). Also, it was increased by 2, 2.8, 3 and 3.2 log after 12 hours and by 5, 5.2, 5.5 and 5.6 log after 18 hours. These results were in the same trend found by EL-Nawawy (1993) who shows the *List. monocytogenes* was sensitive in camel milk with activated LPs and stored at different temperature up to 7 days.

Generally, the goats' milk can be kept safe for 6 h at 30 – 37 °C through the activation of LPs before the pasteurization and manufacture.

#### Lysozyme (Lz) concentrations

The results presented in Table (5) show that, there were highly significant ( $p < 0.01$ ) differences between different concentrations of Lz and between the three studied times on the all six studied bacteria. It could be these differences occurred due to the effects of the different concentrations of Lz which let to decreased the count of the different studied six bacteria strains than control along at 6, 12 and 18 hours incubation. These results were in

agreement with Hughey and Johnson (1987) who found that Lz inhibited the growth of the same six studied pathogenic bacteria. Also, the results were in agreement with Abdou *et al* (2005) which found that the Lz derived from hen egg has inhibitory effect against pathogenic bacteria specially *Bacillus* species.

Table 5. Analysis of variances of the main effects using different concentrations of Lz on different six pathogenic bacteria in goat milk.

S.O.V	df	MS					
		B. cereus	Staph. aureus subsp aureus	List. monocytogenes	Y. enterocolitica	S. typhimurium	E. coli O175:H7
		Total	11				
Time	2	16.29**	16.41**	18.94**	15.62**	23.46**	15.63**
Conc.	3	0.41**	0.38**	0.49**	0.49**	0.06**	0.31**
Error	6	0.03	0.04	0.05	0.11	0.01	0.5

\*\*  $P < 0.01$ , df = degrees of freedoms, MS = Mean Squares., conc. = concentrations, S.O.V= Source Of Variation

#### Effect of Lz on growth of gram negative bacteria

The results of the effect of Lz on growth of gram negative bacteria are present in Table (6). *Y. enterocolitica* was increased in different concentrations of Lz (C1, C2, C3) and in the control (C) milk samples by 2, 1.6, 1 and 2.8 log, respectively after 6 hours from the initial value (6). Also, it was increased by 4, 3.7, 3.4 and 4 log, respectively after 12 hours and by 5.9, 5.8, 5.5 and 5.9 log, respectively after 18 hours incubation. The results were confirm the results obtained by Hughey and Johnson (1987) who found that Lz inhibited the growth of *Y. enterocolitica* bacteria when used in concentration of 20 and 200  $\mu$ g/ml.

*S. typhimurium* was increased in C1, C2, C3 and C by 0.9, 0.8, 0.8 and 1 log, respectively after 6 hours incubation from the initial value (6.2). Also, it was increased by 3.3; 2.9, 2.8 and 3.4 log, respectively after 12 hours and by 5.8, 5.7, 5.6 and 5.8 log, respectively after 18 hours. These results were higher than 0.18 log cfu/ml was found by Vannini *et al* (2004) in skim milk after Lz treatment at 37°C for 24 hours. In addition, Masschalck *et al* (2001) tested the sensitization of *S.*

*typhimurium* suspensions by adding Lz under pressure. They found that adding 100 µg/ml even had a protective effect against pressure caused an inactivation of *S. typhimurium* bacteria.

Table 6. LSM ± SE of Lz concentrations and three different times effect on gram negative pathogenic bacteria in goat milk.

Items	Log cfu/ml					
	<i>Y. enterocoli-</i>		<i>S. typhi-</i>		<i>E. coli</i>	
	<i>tica</i>		<i>murium</i>		O175:H7	
	LS	SE	LS	SE	LSM	SE
Overall						
mean	9.70 ± 0.123		9.09 ± 0.075		10.35 ± 0.145	
Concentrations						
C	10.21 <sup>a</sup> ± 0.188		9.51 <sup>a</sup> ± 0.069		10.80 <sup>a</sup> ± 0.135	
C1	9.93 <sup>b</sup> ± 0.188		9.43 <sup>b</sup> ± 0.069		10.69 <sup>b</sup> ± 0.135	
C2	9.67 <sup>b</sup> ± 0.188		9.27 <sup>b</sup> ± 0.069		10.36 <sup>b</sup> ± 0.135	
C3	9.26 <sup>b</sup> ± 0.188		9.20 <sup>b</sup> ± 0.069		10.09 <sup>b</sup> ± 0.135	
Time (Hours)						
6	7.80 <sup>a</sup> ± 0.162		7.00 <sup>a</sup> ± 0.060		8.39 <sup>a</sup> ± 0.117	
12	9.47 <sup>b</sup> ± 0.162		9.22 <sup>b</sup> ± 0.060		10.75 <sup>b</sup> ± 0.117	
18	11.76 <sup>c</sup> ± 0.162		11.83 <sup>c</sup> ± 0.060		12.32 <sup>c</sup> ± 0.117	

<sup>a, b, c, d</sup> mean values with the different superscript letters within the same item are significantly different (p<0.05)., LSM =LSMean, SE = Stander Error.

C = control.

C1 = 50 µg /ml of Lz chicken egg white.

C2 = 150 µg /ml of Lz chicken egg white.

C3 = 200 µg /ml of Lz chicken egg white.

*E. coli* was increased in C1, C2, C3 and C by 2.6, 1.9, 1.7 and 2.9 log, respectively after 6 hours incubation from the initial value (6.1). Also, it was increased by 4.7, 4.7, 4.4 and 4.9 log, respectively after 12 hours incubation and by 4.5, 4.8, 5.1 and 5.4 log, respectively after 18 hours incubation. These results were in agreement with Branen and Davidson (2004) who observed that Lz inhibited growth of *E. coli* O104: H21 strain in UHT milk at 25°C for 24 hours, which consistently more resistant than the studied *E. coli* O157:H7 strain.

#### Effect of Lz on growth of gram positive bacteria

The results of the effect of Lz on growth of gram positive bacteria are presented in Table (7).

*B. cereus* count increased in C1, C2, C3 and C by 0.9, 0.8, 0.5 and 1.7 log, respectively after 6 hours from the initial value (6.1). Also, it was increased by 2.9, 2.7, 2.3 and 3.2 log, respectively after 12 hours incubation and by 5, 4.9, 4.8 and 5.4 log, respectively after 18 hours. These results were in the same trend found by Hughey and Johnson (1987) on *B. cereus*. While, the Lz effect was slightly inhibited the strain growth.

*Staph. aureus* count increased in C1, C2, C3 and C by 1, 0.7, 0.7 and 1.8 log units, respectively after 6 hours from the initial value (6.2). Also, it was increased by

Table 7. LSM ± SE of Lz concentrations and three different times effect on gram positive of pathogenic bacteria in goat milk.

Items	Log cfu/ml					
	<i>B. cereus</i>		<i>Staph. aureus</i>		<i>List. monocy-</i>	
			subsp <i>aureus</i>		<i>togenes</i>	
	LSM	SE	LSM	SE	LSM	SE
Overall						
mean	9.16 ± 0.089		9.29 ± 0.085		9.03 ± 0.113	
Concentrations						
C	9.51 <sup>a</sup> ± 0.095		9.77 <sup>a</sup> ± 0.112		9.47 <sup>a</sup> ± 0.135	
C1	9.00 <sup>b</sup> ± 0.095		9.38 <sup>b</sup> ± 0.112		9.12 <sup>b</sup> ± 0.135	
C2	8.90 <sup>b</sup> ± 0.095		9.18 <sup>b</sup> ± 0.112		8.89 ± 0.135	
C3	8.62 <sup>c</sup> ± 0.095		8.93 <sup>c</sup> ± 0.112		8.51 ± 0.135	
Time (Hours)						
6	7.07 <sup>a</sup> ± 0.082		7.22 <sup>a</sup> ± 0.098		7.02 <sup>a</sup> ± 0.117	
12	8.85 <sup>b</sup> ± 0.082		9.47 <sup>b</sup> ± 0.098		8.64 <sup>b</sup> ± 0.117	
18	11.10 <sup>c</sup> ± 0.082		11.26 <sup>c</sup> ± 0.098		11.33 <sup>c</sup> ± 0.117	

Means with the different superscript letter within the same item are significantly (P<0.05) different.

(i) for details of studied concentrations, see Table 6.

3.5, 3.2, 2.8 and 3.7 log, respectively after 12 hours incubation and by 5.2, 5.1, 4.8 and 5.3 log, respectively after 18 hours incubation. These results confirmed Maga *et al* (1998) who observed that Lz was able to make the growth of *Staph. aureus* in milk slowly. Moreover, the Lz at average concentration of 0.38 mg/ml have bacteriostatic against *Staph. aureus*.

*List. monocytogenes* count increased in C1, C2, C3 and C by 1, 0.8, 0.4 and 1.9 log, respectively after 6 hours from the initial value (6). Also, it was increased by 2.9, 2.6, 2.1 and 3 log, respectively after 12 hours incubation and by 5.5, 5.3, 5

and 5.6 Log, respectively after 18 hours incubation. These results confirmed that Lz inhibited the growth of *List. monocytogenes* Scott A, obtained by Branen and Davidson (2004). Also, these results agreement with Hughey and Johnson (1987) who suggest that Lz could have positive effective in foods as a safety factor to assist in the inhibition of *List. monocytogenes*. Also, the values were disagreement with Kihm *et al* (1994) who found that the *List. monocytogenes* was completely resistant to inactivation by Lz in whole or skim milk at 4°C for 6 days.

In general, both of LPs and Lz have positive impact to reduce the gram positive and negative bacteria in goat milk collected from North Sinai of Egypt. The effect of LPs and Lz was more effective on *List. monocytogenes* than other gram positive bacteria and on *S. typhimurium* than other gram negative bacteria at 6, 12 and 18 hours incubation.

### CONCLUSIONS

About the all six studied pathogenic bacteria could be concluded that, both of LPs and Lz have less log cfu/ml count than control. The combination of 14mg sodium thiocyanate + 30mg sodium percarbonate, which used to activate LPs, was the highest significantly positive affected. Moreover, the positive effect of Lz was increased by increase its concentrate. It could be recommended that, our results were obtained on goat milk only and further studies for application in various types of milk produced through different desert animals i.e. sheep and camel are required to give insight into what may happen natural antimicrobial system(s) in milk.

### REFERENCES

- Abdou, A.M.; S. Higashiguchi; A.M. Aboueleinin; M. Kim and H.R. Ibrahim (2005). Antimicrobial peptides derived from hen egg lysozyme with inhibitory effect against *Bacillus* species. *Food control*. Available online. [www.sciencedirect.com](http://www.sciencedirect.com)
- Bjorck L.; C.G. Rosen; V. Marshall and B. Reiter (1975). Antimicrobial activity of lactoperoxidase system against pseudomonads and other Gram negative bacteria, *Appl. Microbiol.* 30: 199-204.
- Branen, K.J. and P.M. Davidson (2004). Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *Inter. J. Food Microbiol.* 90 (1): 63-74.
- EL- Nawawy, M.A. (1993). Lactoperoxidase in camel milk and effect on *Listeria monocytogenes*. *Annals of Agric. Sc. Moshtohor.* 31(3): 1535-1542.
- FAO (1999). Manual on the use of the LP system in milk handling and preservation. *Food and Agriculture Organization of the United Nations.* p 30, FAO, Rome.
- Fonteh, F.A.; A.S. Grandison and M.J. Lewis (2002). Variations of lactoperoxidase activity and thiocyanate content in cows' and goats' milk throughout lactation. *J. Dairy Res.* 69(3): 401-409, IAC, Rome.
- Hughey, V.L. and E.A. Johnson (1987). Antimicrobial activity of lysozyme against bacteria involved in food spoilage and food-borne disease. *Appl. Environ. Microbiol.* 53(9): 2165-2170.
- IDF (1988). Code of practices for the preservation of raw milk by the lactoperoxidase system. *Bulletin 234 of the International Dairy Federation, Brussels: International Dairy Federation: 5-15.*
- Jollès, P. and J. Jollès (1984). What's new in lysozyme research? *Molecular and Cellular Biochemistry*, 63: 165-189.
- Kihm, D.J.; Leyer, G.J.; G.H. An and Johnson E.A. (1994). Sensitization of heat-treated *Listeria monocytogenes* to added lysozyme in milk. *Appl. Environ. Microbiol.* 60, (10): 3854-3861.
- Maga, E. A.; G. B. Anderson; J. S. Cullor; W. Smith and J. D. Murray (1998). Antimicrobial properties of human lysozyme transgenic mouse milk. *J. Food Prot.* 61 (1): 52-56.
- Marks, N. E.; A. S. Grandison and M. J. Lewis (2001). Challenge testing of the lactoperoxidase system in pasteurized milk. *J. Appl. Microbiol.* 91(4): 735-741.
- Masschalck, B.; R. Van Houdt; E.G.R. Van Haver and C.W. Michiels (2001). Inactivation of gram-negative bacteria by lysozyme, denatured lysozyme, and lysozyme-derived peptides under high hydrostatic pressure. *Appl. Environ. Microbiol.* 67(1): 339-344.
- MoALR (2005). *Agricultural Statistics, Economic Affairs Sector.* p 223. Ministry of Agriculture and Land Reclamation, Cairo, Egypt.
- Naidu, A.S. (2000). Overview In: *Food Antimicrobial Systems*, pp. 1-14. CRC Press, Boca Raton, FL.

- Reiter, B.; V.M.E. Marshall; L. Bjorck and C.G. Rosen (1976). Nonspecific Bactericidal Activity of the Lactoperoxidase-Thiocyanate-Hydrogen Peroxide System of Milk against *Escherichia coli* and Some Gram-Negative Pathogens. *Infect. Immu.* 13 (3):800-807.
- SAS (1998), *User's Guide. 6.12 Ed*, Statistical Analysis Systems Institute Inc. Cary NC 27511-8000, USA.
- Seifu, E.; E.M. Buys; E.F. Donkin and I.M. Petzer (2004). Antibacterial activity of the lactoperoxidase system against food-borne pathogens in Saanen and South African indigenous goat milk. *Food Control.* 15(6): 447-452.
- Touch, V.; S. Hayakawa; S. Yamada and S. Kaneko (2004). Effects of a lactoperoxidase-thiocyanate-hydrogen peroxide system on *Salmonella enteritidis* in animal or vegetable foods. *Inter. J. Food Sci.* 93: 175-183.
- Vannini, L.; R. Lanciotti; D. Baldi and M.E. Guerzoni (2004). Interactions between high pressure homogenization and antimicrobial activity of lysozyme and lactoperoxidase. *Inter. J. Food Microbiol.* 94 (2):123-135.
- Wolfson, L.M. and S.S. Sumner (1994). Antimicrobial activity of the lactoperoxidase system against *Salmonella typhimurium* in tryptic soya broth in the presence and absence of heat treatment. *J. Food Prot.* 57: 365-368.
- Zapico, P.; P. Gaya; M. Nunez and M. Medina (1993). Goats' milk lactoperoxidase system against *Listeria monocytogenes*. *J. Food Prot.* 56(11): 988-990.
- Zapico, P.; P. Gaya, M. Nunez and M. Medina (1995). Activity of goats' milk lactoperoxidase system on *Pseudomonas fluorescens* and *Escherichia coli* at refrigeration temperatures. *J. Food Prot.* 58: 1136-1138.





## تأثير بعض النظم الطبيعية المضادة للميكروبات فى لبن الماعز بشمال سيناء على أنواع مختلفة من البكتريا المرضية

[٣١]

مروه حاتم حسن عبدالمنعم الجندى<sup>١</sup> - ياسر محمود كامل<sup>١</sup> - محمد عبدالرازق النواوى<sup>٢</sup> -  
محمد نبيل المجدوب<sup>٢</sup>

١. شعبة الإنتاج الحيوانى- مركز بحوث الصحراء- وزارة الزراعة- القاهرة- مصر  
٢. قسم علوم الأغذية- كلية الزراعة- جامعة عين شمس- شبر الخيمة- القاهرة- مصر

ذات تأثير ايجابي على تقليل أعداد البكتريا الموجبة والسالبة لجرام فى لبن الماعز. وقد كان لكلام نظام اللاكتوبيريوكسيديز و الليسوزيم تأثير أعلى على *List. monocytogenes* بالمقارنة بباقي انواع البكتريا الموجبة لجرام وكذلك على *S. typhimurium* بالمقارنة بباقي البكتريا السالبة لجرام محل الدراسة بعد ٦، ١٢، ١٨ ساعة من التحضين. وقد أشارت النتائج إلى أن هناك زيادة خطية لاعداد البكتريا مترامنة مع أوقات الدراسة فى المعاملات لكلام نظام اللاكتوبيريوكسيديز و الليسوزيم. وقد كان أعلى تأثير ايجابي لنظام اللاكتوبيريوكسيديز عند تنشيطه باستخدام ١٤ ملجرام من ثيوسيانات الصوديوم + ٣٠ ملجرام من فوق أكسيد الصوديوم. وكذلك كان تأثير الليسوزيم يزداد ايجابيا بزيادة التركيزات منه.

تهدف هذه الدراسة الى تحديد تأثير تنشيط بعض النظم الطبيعية كنظام اللاكتوبيريوكسيديز و الليسوزيم فى لبن الماعز بشمال سيناء على ٦ انواع من البكتريا المرضية ( *E.coli O157:H7*, *S. typhimurium*, *Y. enterocolitica*, *B. cereus*, *List. monocytogenes* and *Staph. aureus* ). وقد تم جمع عينات من لبن الماعز طبقا للشروط الصحية خلال عام ٢٠٠٤. استخدمت ثلاث توليفات من ثيو سيانات الصوديوم وفوق أكسيد الصوديوم لتنشيط نظام اللاكتوبيريوكسيديز على النحو التالي: المجموعة الأولى (١٤ ملجرام + ٣٠ ملجرام) والمجموعة الثانية (١٥ ملجرام + ١٠ ملجرام) والمجموعة الثالثة (٢٠ ملجرام + ٢٥ ملجرام) على الترتيب. وقد تم تسجيل نتائج عد البكتريا بعد ٦، ١٢، ١٨ ساعة من التحضين. عموما، كانت كلامن مجاميع نظام اللاكتوبيريوكسيديز وتركيزات الليسوزيم