EFFECT OF SOME IMMUNOSTIMULANTS ON QUALITY OF COW'S MILK

By

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ABSTRACT

Two hundred cow's milk samples collected from apparently healthy cows at Alexandria Governorate, were examined microbiologically. The obtained results indicated that the mean values of total bacterial count, Coliforms, Staphylococci, yeasts and molds were $4.2 \times 10^7 \pm 0.92 \times 10^7$, $3.4 \times 10^5 \pm 1.2 \times 10^5$, $5.9 \times 10^4 \pm 0.61 \times 10^4$, $7.1 \times 10^2 \pm 1.87 \times 10^2$ and $1.1 \times 10^2 \pm 0.99 \times 10^2$, respectively. *Coliforms, Staphylococcus auerus* and *Staphylococcus epidermidis* could be isolated at varying percentages.Moreover, this study was carried out to evaluate the effect of immunotherapy on quality of cow's milk. Levamisole was administered to cows in private farm as an unspecific immunopotentiating agent. Levamisole was chosen because it is considered a safe drug that is ordinarily used in veterinary medicine as an anthelmintic agent. Levamisole was given subcutaneously in repeated doses of 2.5 mg/kg b.wt. weekly for 6 weeks during the dry period. Also a product containing Vit. E 100 mg and sodium selenite 1 mg/ml was given to cows in the same farm as an unspecific immunopotentiating agent. It was given in a group of cows in repeated doses of 1 ml / 50 kg b.wt. intramuscularly at weekly intervals 6 times during the dry period, and during lactation in an another group in repeated doses of 1 ml / 50 kg b.wt. intramuscularly day after day for six times. Milk samples were collected from treated groups as well as from untreated control ones. The samples were subjected to count somatic cell, neutrophils, lymphocytes and total colony count as well as isolation of microorganisms in addition to determination of immunoglobulins.The results indicated that levamisole and Vit. E and sodium selsnite as immunostimulants play an important role in reduction of elevated high somatic cell counts, and total bacterial counts as well as reducing the incidence of subclinical mastitis, consequently immunostimulants could be successfully used to improve the milk quality.

INTRODUCTION

Milk is a perfect food stuff, it furnishes the human body, but it is also considered an excellent medium for bacterial growth as it contains proteins, fat and carbohydrates and an average pH value of 6.6 which is nearly ideal for most organisms. Several types of microorganisms including *Coliform, Staphylococcal* bacteria, yeast and molds may gain entrance through different sources as environmental, intramammary and normal udder flora and multiply rapidly when the conditions become favourable for their growth **(Pankey, 1989)**. Consequently, consumption of raw milk from animals infected with pathogenic microorganisms leads to outbreaks of food poisoning. Production of high keeping quality milk in dairy farms depends essentially on minimizing bacteria and excluding chemical contaminants. It also requires healthy dairy animals which are the result of many management factors including mastitis control and herd health programs **(Bodman *et al.*, 1988)** Nowadays, there is a great interest directed towards the immune system and the methods that enhance mammary gland resistance to microbial infection to reduce the effects of existing infections without use of antibiotics or antimicrobial agents. Such approaches are becoming more attractive because of the increasing pressure from the consumers or regulatory agencies to decrease the risk of drug residues in milk, secondly because of the difficulty of obtaining satisfactory results with the existing antibiotic formulations **(Hemingway, 1999)**.

The present investigation was designed to spotlight on the hygienic status of Friesian cow's milk collected from different dairy farms at Alexandria Governorate and to determine the effect of levamisole and Vit. E. & sodium selenite as immunopotentiating agents on quality of cow's milk.

MATERIAL AND METHODS

Sampling:

Two hundreds freshly drawn cow's milk samples were collected in sterile bottles from apparently healthy cows after cleaning the teats with soaked soap and water and disinfection with 70% ethyl alcohol. Samples were transferred to the laboratory without delay for microbiological examination. Enumeration of total bacteria, *Coliforms, Staphylococci,* yeasts and molds were carried out according to the procedures described by

APHA (1985). The suspected colonies of Coliforms and Staphylococci were isolated, purified and identified according to **Krieg and Holt (1984)**.

Experimental design:

One hundred Friesian dairy cows of about 6 years old belonging to a private farm at Alexandria governorate suffering from subclinical mastitis were used in these studies. The animals were clinically normal and free from internal parasites.

Dry period treatment:

Group (1): 20 cows were Injected subcutaneously with levamisole as Ucimisol (Amon Pharmaceutical Industrial Company) at a dose rate of 2.5 mg/1 kg bwt weekly for 6 weeks. The last injection was at least one month before calving according to **Flesh *et al.,* (1982)**.

Group (2): 20 cows received Vit. E 100 mg and sodium selenite 1mg / ml / 50 kg b.wt. (Medico-ERPBV.,) intramuscularly during the last two months of pregnancy 6 times at weekly interval and an additional dose was given just before calving according to the advice of **Anderson (1984)**.

Group (3): 20 cows served as a control during the dry period treatment.

Milk samples were collected before treatment and 1, 2 and 3 months after calving.

Lactation period treatment:

Group (4): 20 lactating cows in the same farm received Vit. E 100 mg and sodium selenite 1mg / ml / 50 kg b.wt. (Medico-ERPBV.,) for six times day after day according to **Hidiroglou (1989)**. Milk samples were collected before injection and 1, 2 and 3 months after treatment.

Group (5): 20 lactating cows used as a control.

Milk samples were collected according to **Mark (1993)**. All samples were subjected to the following examinations: Somatic cell count, was performed according to **IDF (1981)**; enumeration of microorganisms according to **APHA (1985)**. The suspected colonies were isolated and identified according to **Krieg and Holt (1984)**. Immunoglobulin concentration in milk was determined by the sodium sulphide turbidity test according to **Stone and Gitter (1969)** and by using the spectrophotometric determination as described by **Khalil (1975)**.

Statistical analysis:

The obtained results were statistically analyzed according to **Snedecor and Cochran (1980).**

RESULTS AND DISCUSSION

The results summarized in Table (1) pointed out that the minimum total bacterial count/ml of raw cow's milk samples was 3.9×10^5, the maximum was 2.8×10^8, with a mean value of $4.2 \times 10^7 \pm 0.92 \times 10^7$. The high microbial count in milk rendering it of poor quality which generally arises from subclinical mastitic infection and the surfaces of the teats.

The Coliform count/ml was ranged from 3.4×10^3 to 2.2×10^6, with a mean value of $3.4 \times 10^5 \pm 1.2 \times 10^5$ (Table 1). The isolated *Coliforms* were *escherichia coli* (53.5%), *Enterobacter cloacae* (9.3%), *Enterobacter aerogenes* (11.5%), *Klebsiella oxytoca* (9%), *Klebsiella pneumoniae* (12%) and *Citrobacter freundii* (4.5%), Table (2). The presence of Coliforms in raw cow's milk samples may induce objectionable changes in milk or it may, at time, constitutes a public health hazard **(Foster *et al.*, 1983).**

Staphylococci were detected in 70% of samples with a mean value of 5.9×10^4/ml (a range of $2.9 \times 10^2 - 1.9 \times 10^5$), Table (1). The isolated *Staphylococci* could be identified as *Staph. aureus* (26%) and *Staph. epidermidis* (44%), Table (2). *Staphylococcus* bacteria are wide spread in nature, they are members of the normal bacterial flora of skin and mucous membranes of man and animals. It is worth to mention that the presence of *Staph. aureus* in milk even at low numbers must be regarded as a public health hazard as it has been established that although *Staph. aureus* may lose its viability in food by heat and chemical treatment, its enterotoxins still exist and cause food poisoning **(Ahmed *et al.*, 1988).** Yeasts could be detected in 17.5% of samples with a mean value of $7.1 \times 10^2 \pm 1.87 \times 10^2$/ml. While, molds were present in 10% of samples with a mean count of $1.1 \times 10^2 \pm 0.99 \times 10^2$/ml. From the public health point of view, many of these organisms are mycotoxin producers and often incriminated as causative agents of many infections in man and animals **(Staton, 1977).**

Sanitary restriction and hygienic measures should be applied to improve the quality of raw cow's milk and to safeguard consumers.

It is evident from the results recorded in Table (3) that treatment of subclinical mastitic cows during the dry off period with levamisole led to a significant reduction of

milk somatic cell count in comparison with somatic cell count of pretreatment or non-treated group within the same period. These findings agree to a certain extent with those reported by **Ishikawa *et al.,* (1982).**

Vit. E and sodium selenite treated dry cows resulted in reduction of somatic cell count post calving. In the same time, Vit. E and sodium selenite treated lactating cows with subclinical mastitis, the somatic cell count decreased significantly post-treatment (Table 3). Our results agree with those of **Smith *et al.,* (1985), Smith (1986), Weiss *et al.,* (1990)** and it is worthy to mention that, despite significant reduction in mean values of somatic cell counts in Vit. E and sodium selenite and levamisole treated groups, it is still higher than the normal level of less than 300,000 cells/ml milk as reported by **Amal (1986)** and **Beaudeau *et al.,* (1998)**, however, **Jacquet *et al.,* (1975)** and **King (1978)** recorded that the normal total somatic cell count per ml milk of cows ranged between 49,000 and 637,000 with an average of 500,000.

Table (4) showed that the milk neutrophil count was significantly decreased in cows treated with levamisole during the dry period. Also, cows treated with Vit. E and sodium selenite during dry period or during lactation resulted in a significant decrease in neutrophil count. On the other hand, lymphocyte count was significantly increased following levamisole or Vit. E and sodium selenite treatment. These findings are in accordance with that have been reported by **Ishikawa and Shimizu (1983)**. The increase in the lymphocyte count leads to increasing phagocytic power of the mammary gland to engulf any infectious agent **(Tizard, 1983).**

Table (5) revealed a significant elevation in the level of milk immunoglobulins following levamisole treatment during dry period or Vit. E and sodium selenite during dry period or lactation period. These results substantiate what have been reported by **Ishikawa *et al.,* (1982)**. Also, these results agree with **Nickerson (1989)** who stated that milk immunoglobulin concentration increased after levamisole treatment and it was suggested that treatment served to aid udder health.

The results illustrated in Table (6) indicated the decrease in total colony count, with levamisole or Vit. E and sodium selenite treatment during dry period or treatment with Vit. E and sodium selenite during lactation period. These results reflect a good immune status of the udder and agree with **Zehner *et al.,* (1986)** and **Ishikawa *et al.,* (1982)**.

The incidence of subclinical mastitis decreased in cows received levamisole or Vit. E and sodium selenite during dry period or Vit. E and sodium selenite during lactation

(Table 7). These results coincided with those reported by **Flesh** *et al.,* **(1982), Ishikawa** *et al.,* **(1982), Smith** *et al.,* **(1985)** and **Ndiweni and Finch (1991)**.

It could be concluded that the immunostimulant effect of levamisole and Vit. Et. al, & sodium selenite aid in udder health through enhancement of production of milk lymphocytes and immunoglobulins which resulted in reduction of elevated somatic cell counts, total bacterial counts and incidence of subclinical mastitis, hence, immunostimulants could be used to improve milk quality.

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Table (1) : Statistical analytical results for microbial counts/ml of raw cow's milk samples.

Counts	Positive samples		Count/m		
	No.	%	Min.	Max.	Mean ± SEM
Total bacterial	200	100	3.9×10^5	2.8×10^8	$4.2 \times 10^7 \pm 0.92 \times 10^7$
Coliform	200	100	3.4×10^3	2.2×10^6	$3.4 \times 10^5 \pm 1.20 \times 10^5$
Staphylococci	140	70	2.9×10^2	1.9×10^5	$5.9 \times 10^4 \pm 0.61 \times 10^4$
Yeast	35	17.5	1.0×10^2	8.0×10^2	$7.1 \times 10^2 \pm 1.87 \times 10^2$
Mold	20	10	80	3.4×10^2	$1.1 \times 10^2 \pm 0.99 \times 10^2$

Min. = minimum; Max. = maximum; SEM = standard error of mean.

Table (2) : Incidence of isolated Coliforms and Staphylococci in raw cow's milk samples.

Isolates	No. of samples	%
1. Coliforms:		
- *Escherichia coli*	107	53.5
- *Enterobacter cloacae*	19	9.5
- *Enterobacter aerogenes*	23	11.5
- *Klebsiella oxytoca*	18	9.0
- *Klebsiella pneumoniae*	24	12.0
- *Citrobacter freundii*	9	4.5
2. Staphylococci:		
- *Staphylococcus aureus*	52	26.0
- *Staphylococcus epidermidis*	88	44.0

Table (3) : The effect of immunotherapy on the levels of somatic cell count (SCC) of milk.

Type of treatment	Mean SCC before treatment	Mean SCC after treatment (after calving)
Dry period treatment:		
- Levamisole (group 1)	$13.22 \times 10^5 \pm 3.20 \times 10^5$	$7.83 \times 10^5 \pm 2.30 \times 10^{5*b}$
- Vit. E and sodium selenite (group 2)	$14.88 \times 10^5 \pm 4.22 \times 10^5$	$8.40 \times 10^5 \pm 3.20 \times 10^{5*b}$
- Control (group 3)	$14.55 \times 10^5 \pm 4.33 \times 10^5$	$13.86 \times 10^5 \pm 2.20 \times 10^{5\ a}$
Treatment during lactation:		
- Vit. E and sodium selenite (group 4)	$14.96 \times 10^5 \pm 4.80 \times 10^5$	$5.33 \times 10^5 \pm 1.90 \times 10^{5*b}$
- Control (group 5)	$13.19 \times 10^5 \pm 3.10 \times 10^5$	$13.66 \times 10^5 \pm 4.20 \times 10^{5\ a}$

* = Values before and after treatment differ significantly (P<0.05).

Means with different letters in the same column and period differ significantly (P<0.05).

Table (4) : The effect of immunotherapy on the neutrophils and lymphocytes of milk.

Type of treatment	Mean values before treatment	Mean values after treatment (after calving)
Dry period treatment		
<u>Neutrophils</u>		
Levamisole (group 1)	$80.22 \times 10^4 \pm 3.2 \times 10^4$	$55.2 \times 10^4 \pm 4.3 \times 10^{4*b}$
Vit. E and sodium selenite (group 2)	$82.11 \times 10^4 \pm 3.6 \times 10^4$	$61.1 \times 10^4 \pm 8.3 \times 10^{3*b}$
Control (group 3)	$77.60 \times 10^4 \pm 3.6 \times 10^4$	$71.8 \times 10^4 \pm 6.1 \times 10^{4\ a}$
<u>Lymphocytes</u>		
Levamisole (group 1)	$10.33 \times 10^3 \pm 3.2 \times 10^3$	$34.8 \times 10^3 \pm 2.3 \times 10^{3*a}$
Vit. E and sodium selenite (group 2)	$11.10 \times 10^3 \pm 1.1 \times 10^3$	$15.8 \times 10^3 \pm 0.6 \times 10^{3*b}$
Control (group 3)	$11.20 \times 10^3 \pm 1.1 \times 10^3$	$8.9 \times 10^4 \pm 3.2 \times 10^{4\ c}$
Treatment during lactation		
<u>Neutrophils</u>		
Vit. E and sodium selenite (group 4)	$78.20 \times 10^4 \pm 2.3 \times 10^4$	$64.3 \times 10^4 \pm 1.6 \times 10^{4\ *b}$
Control (group 5)	$75.10 \times 10^4 \pm 1.1 \times 10^4$	$77.3 \times 10^4 \pm 3.3 \times 10^{4\ a}$
<u>Lymphocytes</u>		
Vit. E and sodium selenite (group 4)	$9.10 \times 10^3 \pm 1.1 \times 10^3$	$13.0 \times 10^3 \pm 1.1 \times 10^{3*a}$
Control (group 5)	$10.50 \times 10^3 \pm 1.3 \times 10^3$	$9.8 \times 10^3 \pm 1.9 \times 10^{3\ b}$

* = Values before and after treatment differ significantly (P<0.05).

Means with different letters in the same column, period and parameter differ significantly (P<0.05).

Table (5) : The effect of immunotherapy on immunoglobulin (gram %) of milk.

Type of treatment	Mean immunoglobulin before treatment	Mean immunoglobulin af ter treatment (after calving)
Dry period treatment:		
- Levamisole (group 1)	0.296 ± 0.009	$0.993 \pm 0.036*a$
- Vit. E and sodium selenite (group 2)	0.289 ± 0.003	$0.891 \pm 0.061*a$
- Control (group 3)	0.277 ± 0.018	$0.279 \pm 0.013\ b$
Treatment during lactation:		
- Vit. E and sodium selenite (group 4)	0.286 ± 0.007	$0.889 \pm 0.007*a$
- Control (group 5)	0.269 ± 0.006	$0.267 \pm 0.008\ b$

* = Values before and after treatment differ significantly (P<0.05).

Means with different letters in the same column and parameter differ significantly (P<0.05).

Table (6): The effect of immunotherapy on total colony count (TCC) of milk.

Type of treatment	Mean TCC before treatment	Mean TCC after treatment (after calving)
Dry period treatment		
- Levamisole (group 1)	$36.3 \times 10^5 \pm 3.2 \times 10^5$	$16.3 \times 10^5 \pm 3.4 \times 10^{5*b}$
- Vit. E and sodium selenite (group 2)	$35.9 \times 10^5 \pm 3.2 \times 10^5$	$22.9 \times 10^5 \pm 1.2 \times 10^{5\ *b}$
- Control (group 3)	$37.6 \times 10^5 \pm 2.6 \times 10^5$	$36.9 \times 10^5 \pm 3.3 \times 10^{5\ a}$
Treatment during lactation		
- Vit. E and sodium selenite (group 4)	$39.9 \times 10^5 \pm 2.9 \times 10^5$	$21.2 \times 10^5 \pm 3.2 \times 10^{5*b}$
- Control (group 5)	$38.5 \times 10^5 \pm 4.2 \times 10^5$	$34.9 \times 10^5 \pm 2.3 \times 10^{5\ a}$

$*$ = Values before and after treatment differ significantly (P<0.05).

Means with different letters in the same column and period parameter differ significantly (P<0.05).

Table (7): The effect of immunotherapy on incidence of subclinical mastitis in dairy cows.

Type of treatment	Before treatment		After Treatment		
			Mastitic		Cured
	No.	%	No.	%	%
Dry period treatment					
- Levamisole (group 1)	20	100	11	55	45
- Vit.E and sodium selenite (group 2)	20	100	10	50	50
- Control (group 3)	20	100	18	90	10
Treatment during lactation					
- Vit. E and sodium selenite (group 4)	20	100	13	65	35
- Control (group 5)	20	100	19	95	5

الملخص العربي

تأثير بعض منشطات المناعة على جودة لبن الأبقار

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تم فحص عدد ٢٠٠ عينة من لبن الأبقار ميكروبيولوجياً و قد وجد أن متوسط العدد الكلى للميكروبات، ميكروبات الكوليفورم، الميكروبات العنقودية و الخمائر و الفطريات هو كالتالى ٤,٢ × ١٠^٧ ± ٠,٩٢ × ١٠^٧، ٣,٤ × ١٠^٥ ± ١,٢ × ١٠^٥، ٥,٩ × ١٠^٥ ± ٠,٦١ × ١٠^٤، ٧,١ × ١٠^٢ ± ١,٨٧ × ١٠^٢ و ١,١ × ١٠^٢ ± ٠,٩٩ × ١٠^٢ فى المللى الواحد على التوالى. كمـا أمكن عزل كل من الكوليفورم و الميكروبات العنقودية بنسب مختلفة.

و قد تنـاول البحـث أيضاً دراسـة تقييـم تـأثير بعض منشطات المناعـة مثل الليفـاميزول و فيتامين هـ والسيلينيوم على جودة لبن الأبقار، و قد أختير الليفـاميزول لأنه عقار آمـن يستخدم كمضاد للديدان و قد تم حقنه تحت الجلد فى مجموعة من الأبقار خـلال فـترة الجفاف بجرعة قدرها ٢,٥ مجم/كيلو جرام من وزن الحيوان مرة أسبوعياً لمدة ستة أسابيع. أما فيتامين هـ ١٠٠ مجم و سيلينات الصوديوم ١ مجم/ملل فقد تـم حقنـه بجرعة ١ مـل/٥٠ كجم فـى العضل أسبوعياً لمـدة ٦ مرات فى مجموعة من الأبقار خلال فترة الجفاف، كما تم حقن هذا العقار ٦ مرات يوم بعـد يـوم فـى مجموعة من الأبقار أثناء فترة الحليب بجرعات ١ ملل/٥٠ كجم فى العضل.

و قد جمعت عينات اللبن مـن الحيوانـات المعاملة قبل العلاج و بعد العلاج بالإضافة إلـى المجموعـات الضابطـة و قـد تـم تحليـل عينـات اللبن و تقديـر العدد الكلى للخلايا الجسمية و عـدد النيتروفيل و الخلايا الليمفاوية و العدد الكلى للبكتريـا بالإضافة إلى تقديـر الأميونوجلوبيولين و قد إتضح من الدراسة أن الليفاميزول و فيتانين هـ وسيلينات الصوديوم كمنشطات للمناعة لـها دور كبير فى اختزال عدد الخلايا الجسمية و العـدد الكلى للبكتريـا و بالإضافة إلى ذلك أنها تقلل نسبة إلتهاب الضرع تحت الإكلينيكى و بالتالى من الممكن استخدام منشطات المناعة فى تحسين جودة اللبن.