

## RELATIONSHIP BETWEEN LACTOFERRIN CONCENTRATION, MILK PRODUCTION, MASTITIS, SCC AND CONTAGIOUS BACTERIAL PATHOGENS

El-Awady<sup>1</sup>, H.G., Salem<sup>1</sup>, A. Y., Tag El- Dein<sup>2</sup>, M.A., Mostafa<sup>2</sup>, A.M.I. and Abu EL-Naser<sup>3</sup>, I.A.M.

<sup>1</sup> Animal Production Dept. Faculty of Agriculture Kafrelsheikh Uni, Egypt, <sup>2</sup> Animal Production Research Institute, Egypt

<sup>3</sup> Department of Animal Production, Faculty of Agriculture Damietta University, Egypt

E-mail –hassanelawady63@yahoo.com

### ABSTRACT

During the period from 2012 to 2014, total of 794 normal lactation records of Friesian cows were raised on El-Karada Animal Production Research Station, Animal Production Research Institute (APRI), Ministry of Agriculture situated at Kafrelsheikh Governorate, Egypt. The initial numbers of normal lactation records included were 49 Friesian cows to estimate effect of somatic cell counts (SCC) and lactoferrin concentration (LFC) levels on traits studied, total milk yield (TMY), mastitis (MAST) and bacterial counts of Staphylococcus sp (Staph), Streptococcus sp (Strep), Escherichia coli (E. coli) and Bacillus sp (Baci), effect of mastitic and nonmastitic cases on same traits, determine the effect of using Lf and EEP (Ethanol extract of propolis) as a natural antibiotic either alone or mixing with certain industrial antibiotics on udder health and bacterial counts. Data were analyzed using SAS, (2004). Unadjusted means of TMY, SCC, and LFC were 3557.5Kg, 463.4x10<sup>3</sup> cells/ml and 308.6µg/ml and bacterial count of Staph, Strep, E.coli and Baci were 512.2, 529.1, 250.6 and 254.3x10<sup>3</sup> cell/ml, respectively. SCC levels did not have any effect on TMY till 200 x10<sup>3</sup> cell/ml of milk, then milk yield beginning slightly decrease till 1000 x10<sup>3</sup> cell/ml of milk ratio of incidence clinical mastitis, LFC, Staph, Strep, E. coli and Baci, the same trend with SCC which decrease with the lower levels of SCC and increase with higher levels of SCC, thus, an increase in the SCC in milk is an indicator of inflammation in the udder.

With decreasing milk yield, ratio of incidence clinical mastitis, SCC, Staph, Strep, E. coli, Baci and LFC in milk increases, estimates decreased SCC, different bacterial counts investigation (Staph, Strep, E.coli and Baci) and MAST in all groups treatments comparison control group, and decreasing in average of SCC, Staph, Strep, E.coli and Baci were clear approximately, the highest decline rate between zero time and after 8h in all groups, the highest drop in SCC, four type bacteria (Staph, Strep, E.coli and Baci) at different time and MAST in (T6) compare with all other treatments. Correlation between TMY and udder health traits (SCC, MAST, LFC, Staph, Strep, E.coli and Baci) were low and negative ranged from (-0.23 to -0.07). While the correlation among udder health traits had positive ranged from (0.27 to 0.88), the estimate of correlation among LFC, SCC and MAST had strong ranged from (0.79 to 0.88) and the correlation among Staph, Strep, E.coli and Baci

were positive ranged from (0.27 to 0.55). Lf could be used as a complementary test to SCC and possibly a diagnostic test of subclinical mastitis in dairy cattle and used Lf combination with gentamicin to treat mastitis more efficiently from used alone.

**Key words:** *lactoferrin, mastitis, Staphylococcus aureus, Streptococcus uberis*

## INTRODUCTION

Mastitis is a disease of major economic importance in the dairy industry worldwide due to loss of milk production and reduction of milk quality as well as an increased usage of drugs and veterinary services. Piepers et al., (2007); Malinowski and Klossowska (2010) and Smulski et al., (2011) the mastitis caused by *S. aureus* is characterized by significantly lower cure rates compared with infections caused by other microorganisms. Melchior et al., (2006) reported that the dramatic spreading of antibiotic-resistant staphylococci and also other groups of microorganisms is caused by unreasonable usage of chemotherapeutics, especially during long-term therapy with the same group of antibiotics and their usage without a prior susceptibility. New antibacterial substances are sought for and trials with known compounds of different origins are being performed. Wakabayashi et al., (2006) and Leclercq et al., (2013) indicated that involving the use of natural substances with antibacterial activity, lactoferrin (Lf) is an iron-binding glycoprotein of the transferrin family present in milk as well as other secretions and neutrophil granules in mammals. And considered to be an important host defense molecule; lactoferrin concentration (LFC) shown to be higher in milk of subclinical and clinical mastitic cows. The main objectives of this study were to determine the effect of SCC levels on TMY, MAST, LFC and bacterial counts, effect of LFC levels on TMY, SCC, MAST and bacterial counts, effect of MAST on TMY, SCC, LFC and bacterial counts and determine effect of using Lf and EEP (Ethanol Extract of Propolis) on udder health.

## MATERIALS AND METHODS

### Data and management

The present investigation were collected from 794 normal lactation records of 49 Friesian cows during the period from 2012 to 2014 raised in El-Karada Animal Production Research Station, Animal Production Research Institute (APRI), Ministry of Agriculture situated at Kafrelsheikh Governorate in the Northern Part of Nile Delta of Egypt. Cows were kept under a similar system of feeding and management practiced on the farm applied by APRI. All cows were fed on good quality concentrate ration. During winter and spring months (December

to May), animals were supplied with Egyptian clover (*Trifolium alexandrinum*), while during summer and autumn (June to November), animals were fed on dry ration, mainly either Egyptian cover hay or green sweet sorghum. Also, rice straw was available around the year. Feeds were supplied to cows according to their live body weight, milk production and pregnancy status. Portable water and mineral mixture were available freely. Cows were milked twice daily in a parallel by DeLaval Alpha milking machine. Milk yield was recorded daily to the nearest 100 g at each milking.

### **Experimental animals**

Normal cows (without mastitis) were divided into seven groups (7 cows in each group), 1<sup>st</sup> group without treatment (control), 2<sup>nd</sup> group injected propolis (10ml), 3<sup>rd</sup> group injected Lf (10ml), 4<sup>th</sup> group injected combination of propolis (5ml) and ampicillin (5ml), 5<sup>th</sup> group injected combination of propolis (5ml) and gentamicin (5ml), 6<sup>th</sup> group injected combination of Lf (5ml) and ampicillin (5ml), 7<sup>th</sup> group injected combination of Lf (5ml) and gentamicin (5ml) from teats. Samples were taken from all groups treated on zero time, 4, 6 and 8 h, after treatments and determine number of SCC/ml<sup>3</sup>, LFC/ml<sup>3</sup> and four types of bacteria in milk for different times of treatment.

### **Udder health traits**

The somatic cell counts was measured by [Fossomatic 5000 (Foss Electric A/S 69, Stangerupade DK 3400 Hilleroed, Denmark Company)] from a sample of milk collected during the morning milking Cheng et al., (2008). The determinations of the somatic cell counts were performed in Dairy Services Unit which belongs to the (APRI). Somatic cell counts classified into ten levels. A case of clinical mastitis was that the veterinary treated case either with or without teat injury at any time between calving and the end of lactation or until culling (0 = normal cow and 1= mastitis cow). Lactoferrin analyzed by HPLC (D-7000Merck-Hitachi, Germany) equipped with a pump (model L-6200, Merck-Hitachi, Germany) and a diode array detector (L-3000, Merck-Hitachi, Germany), detection of the Lactoferrin was monitored by standard compound (Marcucci et al., 2001). The LFC was classified into 10 levels. Estimated the type of bacteria (*Streptococcus* sp, *Staphylococcus* sp, *E.coli* sp and *Bacillus* sp ) in milk samples by using Petri plat with a specific media on different time ( zero time and after 4, 6 and 8h) to determine the type of pathogen and bacterial count every individual type, bacterial types were identified by colonial and microscopic morphology examination activity.

### **Statistical analysis**

Data were analyzed using SAS, (2004) in order to determine the fixed effects to be included in the model SCC levels and LFC on TMY, MAST, type of bacteria (*Strep. sp*, *Staph. sp*, *E.coli* and *Baci sp*).

The following fixed model (1) was used:

$$Y_{ijk} = \mu + S_i + L_j + e_{ijk} \quad (1)$$

Where:

$Y_{ijk}$ = observed values,

$\mu$ = overall mean,

$S_i$ = fixed effect of  $i^{\text{th}}$  SCC levels,

$L_j$ = fixed effect of  $j^{\text{th}}$  LFC levels and

$e_{ijk}$ = random error term.

Treatments (6) and mastitis (0 = normal cow and 1= mastitis cow) were analyzed to determine the effect of treatment and mastitis on the TMY, SCC and four types of bacteria.

The following fixed model (2) was used

$$Y_{ijk} = \mu + T_i + \text{MAST}_j + e_{ijk} \quad (2)$$

Where:

$Y_{ijk}$ = observed values,

$\mu$ = overall mean,

$T_i$ = fixed effect of  $i^{\text{th}}$  treatment,

$\text{MAST}_j$  = fixed effect of  $j^{\text{th}}$  mastitis and

$e_{ijk}$  = random error term.

## RESULTS AND DISCUSSION

### Means and variation

**Table (1): Overall mean, standard deviation (S.D) and coefficient variations (C.V) for different traits studied**

Traits	Mean	S.D	C.V %
TMY	3557.5	1210.1	34
SCC	463.4	167.7	36
LFC	308.6	106.6	34
Staph	512.2	202.1	39
Strep	529.1	247.5	46
E.coli	250.6	80.9	32
Baci	254.3	127.2	50

Unadjusted means, standard deviation (S.D) and coefficient variations (C.V) for different traits studied are presented in Table (1). The present values for means of TMY (3557.5 Kg) was lower than estimated by Haile-Mariam et al., (2003) (5558 Kg) and Tag El-Dein, (1997) (5465 Kg) while higher than estimated by Hussein, (2000) (3181 Kg). While the average of SCC ( $463.47 \times 10^3$  cells/ml) nearest the value estimated by Van den Borne et al., (2001) ( $461 \times 10^3$  cell/ml), El-Arian and El-Awdy, (2008) ( $426 \times 10^3$  cell/ml) on Friesian cattle in Egypt and El-Awdy and Oudah, (2011) ( $453 \times 10^3$  cell/ml). The ratio of clinical mastitis in present results 12% was lower than estimated by Lam et al., (2013) were 33% in

year (2004) and (25%) in year (2009) and Green et al., (2004) found that the mean of mastitis from 722 case was (16%). The present results for mean of LFC (308.6µg/ml), highest than, estimated by Wojdak et al., (2006) conformed that the LFC in normal bovine to be between (20 and 200µg/ml).

Table (2) showed that somatic cell count levels and lactoferrin concentration levels non-significant effect on TMY, but had significant effect ( $P \leq 0.05$ ) on mastitis and had significant effect ( $P \leq 0.05$ ) ( $P \leq 0.01$ ) on counts of types bacteria (Staph, Strep, E.coli and Baci).

**Table (2): Analysis of variance for total milk yield, mastitis and four types of bacteria.**

S.O.V	d.f	Traits											
		TMY		Staph		Strep		E.coli		Baci		MAST	
		M.S	F	M.S	F	M.S	F	M.S	F	M.S	F	M.S	F
SCC	9	1472289	0.99 <sup>NS</sup>	584385	37.4 <sup>**</sup>	471933	11.0 <sup>**</sup>	48208	9.7 <sup>**</sup>	161718	17.1 <sup>**</sup>	25.9	0.09 <sup>*</sup>
LFC	9	1085395	0.73 <sup>NS</sup>	81631	5.2 <sup>**</sup>	78697	1.8 <sup>*</sup>	9890	2.0 <sup>*</sup>	19108	2.0 <sup>*</sup>	20.1	0.07 <sup>*</sup>
Error	775	1473488	---	15642	---	42784.4	---	4966.1	---	9420.3	---	288.1	---

\* Significant at ( $P \leq 0.05$ )

\*\*Significant at ( $P \leq 0.01$ )

**Effect of SCC levels on traits studied.**

The present results showed that the SCC levels did not have any effect on TMY till 200 cell/ml of milk, then milk yield beginning slightly decrease till 1000.000 cell/ml of milk Table 3. The results obtained are similar to findings reported by Roger and Peter (1995) every increase in cell count of 1000.000 cell/ml there will be reduction of 2.5% in milk yield. ALCL., (2004) reported that cows with 800.000 cell/ml suffer from dropping in milk production 11% and added that SCC for individual cow is a better indicator of milk production and loss due mastitis. El-Awady and Oudah, (2011) found that milk yield losses increase with increasing SCC and therefore decreasing in profitability. Results Table (3) cleared that ratio of incidence clinical mastitis, LFC, Staph, Strep, E. coli and Baci, the same trend with SCC were decrease in the lower levels of SCC and increase with higher levels of SCC, thus, an increase in the SCC in milk is an indicator of inflammation in the udder. Agreement with these results Cheng et al., (2008) indicated that milk LFC tended to be high when SCC increased.

Smith et al., (2001); El-Awady and Oudah, (2011) and Helal, (2013) noticed that SCC in milk increase with increasing number of infected quarter. The highest average of bacterial counts at level  $1000 \times 10^3$  cell/ml for Staph, Strep, Baci and E.coli, while the lowest average at level  $0-100 \times 10^3$  cell/ml.

**Table (3) Effect of somatic cell count levels on TMY, MAST, LFC, Staph, Strep, E. coli and Baci**

Level of SCC x10 <sup>3</sup>	No	Traits						
		TMY	MAST	LFC	Staph	Strep	E.coli	Baci
		Mean±S.E	Ratio	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
Overall mean	794	3525±50	18%	376±18	501±22	533±16	259±15	256±18
0-100	154	3557±43	00%	68±24	315±27	349±38	204±22	202±14
>100-200	144	3708±274	00%	75±27	329±13	444±19	206±29	207±12
>200-300	190	3656±93	01%	118±13	394±27	432±28	226±24	234±15
>300-400	122	3634±86	3%	181±21	445±35	489±28	234±23	225±24
>400-500	50	3565±76	7%	241±14	511±27	547±10	255±35	253±25
>500-600	35	3426±116	17%	290±10	656±24	664±24	293±32	336±36
>600-700	43	3432±197	22%	424±19	724±28	835±79	319±30	404±31
>700-800	27	3432±237	27%	578±22	901±47	958±116	310±28	418±32
>800-900	17	3493±326	33%	692±32	943±48	933±118	347±25	467±43
>900-1000	12	3255±739	38%	898±41	1372±50	1231±156	378±67	622±61

**Effect of LFC levels on traits studied.**

Table (4) showed that LFC could be used as a complementary test to SCC and possibly a diagnostic test of subclinical mastitis in dairy cattle, which elevated Lf and was accompanied by decreasing milk yield. Increase ratio of incidence clinical mastitis, SCC, Staph, Strep, E. coli and Baci, associated with increased LFC in milk. Results obtained similar to those reported by Hogeveen et al., (2011) increasing levels of LFC declining average of productive traits because LFC increase in the case of microbial attack and udder infection. Kawai et al., (1999) found that the mean of LFC in milk from dairy cows incidence clinical mastitis was 849 µg/ml. Kutilla, (2004) increasing numbers of bacterial attacking the udder LFC increases in milk, it has the characteristics of immune to reduce bacterial species that cause mastitis. Fang et al., (1999) indicated that increasing number of E. coli, Staph. aureus, Bacillus subtilis and Strep. mutans increasingly LFC in milk.

**Effect of treatments on traits studied**

The present results showed that decreased SCC, different bacterial counts investigation (Staph, Strep, E.coli and Baci) and MAST in all groups treatments comparison group control, and decreasing in average of SCC, Staph, Strep, E.coli and Baci were clear approximately, the highest decline rate between zero time and after 8h in all groups, the highest drop in SCC, four type bacteria (Staph, Strep, E.coli and Baci) at different time and MAST in (T6) compare all other treatments Table 5. That indicated improve in the health status of the animal, which usage Lf with gentamicin had a

**Table 4: Effect of LFC levels on TMY, SCC, MAST, Staph, Strep, E.coli and Baci**

Level µg/ml	N	Traits						
		TMY	SCC	MAST	Staph	Strep	E.coli	Baci
		Mean±S.E	Mean±S.E	Ratio	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
Overall mean	794	3525±50	466±8	18%	501±22	533±16	259±15	256±14
0-100	154	3825±94	377±27	00%	426±28	492±11	229±17	212±15
>100-200	144	3887±72	382±16	00%	442±17	522±10	231±15	230±15
>200-300	190	3868±66	399±25	01%	441±26	509±19	235±19	229±14
>300-400	122	3834±111	457±27	3%	499±13	500±14	241±16	248±18
>400-500	50	3766±168	536±13	7%	637±25	593±26	269±12	258±16
>500-600	35	3463±29	568±17	17%	653±30	609±31	282±22	308±26
>600-700	43	3308±173	679±19	22%	835±42	775±62	315±14	436±33
>700- 800	27	3296±217	759±22	27%	855±46	935±119	382±24	441±25
>800- 900	17	2602±284	817±19	33%	946±42	957±119	385±17	478±45
>900-1000	12	2033±202	909±32	38%	986±52	977±40	402±32	536±38

significant impact on improving the health status of the animal. Similar results were reported by Klungland at al., (2001) and Detilleux, (2002) and Leclercq et al., (2013) Lf may be active in modulation and regulation of macrophages, lymphocytes and neutrophil function .Due to its properties, Lf is one of the more important factors that prevent and control mastitis in dairy cows. Diarra et al., (2002) and Kutila, (2004) Lf with its specific antimicrobial effect would be a good contender for a non-antibiotic treatment of infections. Lf infusion could potentially be useful in the treatment of bovine mastitis, and could partly replace the use of antimicrobials. Hong et al., (2006) and Sakwinska et al., (2011) studied the inhibitory activity of gentamicin on Staphylococcus aureus that could cause mastitis after 8h of incubation a significant reduction in viable counts was reported.

**Table (5) Effect of treatments on traits studied (SCC, Staph, Strep, E.coli and Baci at (0, 4, 6 and 8h) and mastitis**

Time	Traits	Overall mean	Treatments						
			Control	T1	T2	T3	T4	T5	T6
Zero time	SCC0	466±12	568±14	528±15	484±12	476±16	446±17	417±15	402±11
	Staph0	501±22	597±25	538±17	495±15	497±16	490±18	508±18	465±16
	Strep0	533±16	668±27	710±40	603±22	544±15	487±11	447±11	434±12
	E.coli0	259±15	280±16	261±17	252±15	272±14	258±11	249±26	198±6
	Baci0	256±18	353±16	317±19	270±18	292±15	256±25	249±26	204±4
At 4 hour	SCC4	422±29	533±11	485±12	476±10	409±13	404±15	384±13	348±9
	Staph4	452±14	577±25	490±15	421±12	466±13	433±12	398±29	374±10
	Strep4	473±11	642±30	584±24	502±14	466±10	391±28	388±29	357±10
	E.coli4	211±18	277±26	268±18	225±15	219±24	226±27	193±15	140±4
	Baci4	219±19	337±13	244±17	221±27	241±14	203±25	149±13	117±3
At 6 hour	SCC6	320±10	501±10	464±12	449±11	354±11	343±10	339±10	270±7
	Staph6	370±18	565±25	313±11	349±37	377±19	377±29	362±19	270±9
	Strep6	368±11	648±32	461±15	394±10	370±29	327±29	322±27	286±7
	E.coli6	178±29	243±27	155±15	166±14	159±23	160±24	150±13	114±3
	Baci6	161±15	325±15	182±11	168±25	165±23	150±31	109±12	85±2
At 8 hour	SCC8	299±11	540±12	470±12	385±10	308±18	259±28	255±19	170±6
	Staph8	278±27	573±25	219±27	224±26	256±27	222±27	224±17	161±5
	Strep8	300±12	675±26	255±29	179±16	271±28	193±15	193±25	171±5
	E.coli8	140±28	248±26	162±26	133±19	125±13	139±14	103±13	82±2
	Baci8	158±7	354±15	223±28	184±14	206±12	184±13	99±12	44±1
	MAST	12%	22%	18%	16%	10%	9%	7%	4%

T1 (10ml propolis)-T2 (10ml lactoferrin)-T3(5ml propolis+5ml ampiclin)-T4(5ml propolis+5ml gentamicin)-T5(5ml lactoferrin+5ml ampiclin)-T6(5ml lactoferrin+5ml gentamicin)

Hillerton and Kliem, (2002) found that in case of the use of the antibiotic gentamicin that about 22% of Streptococcus in the study had a resistance to the antibiotic, as well as about 56% of this type of bacteria has a resistance to the antibiotic ampicillin confirmed that industry antibiotic with adding lactoferrin more effective of used alone.

Aguila and Brock, (2001) and Plaffl et al., (2003) observed that Lf has antimicrobial activity, especially against coliform bacteria, such as Escherichia coli, which cause severe mastitis in dairy cows. Salomao et al., (2008) McDougall et al., (2009) and Nickerson, (2009) and Raghukumar et al., (2010) EEP showed in vitro and in vivo antimicrobial activity mainly against, Gram positive Staph. sp and Strep. sp, Gram-negative bacteria E. coli. In contrast, Pyörälä and Pyörälä (1998) reported that Staphylococcus aureus infection resistant to antimicrobial treatment, especially when the isolates are penicillin-resistant.

#### **Incidence of clinical mastitis**

Milk production decreased with udder infection the average of TMY in clinical mastitis cows was 3364.3Kg, while in normal cows were 3785.9Kg Table 6. This result agreement with those found by



Geary et al., (2012) indicated that in clinical mastitis cows the average of TMY decreased 682Kg in comparison with healthy cows. Grohn et al., (2004) cows with clinical mastitis the animal stop for milk production in single quarter or in all quarters. The present means of SCC and LFC in clinical mastitis cows were  $578.6 \times 10^3$  cell/ml and  $600.0 \mu\text{g/ml}$ , while in normal (without mastitis) cows were  $325.1 \times 10^3$  cell/ml and  $177.9 \mu\text{g/ml}$  Table 6. Mastitis was increased gradually with the increase of SCC and LFC in milk from case clinical mastitis cows, so SCC and LFC are the best indicators to sub-clinical mastitis. Results agreement with Barkema et al., (1998) and Caraviello et al., (2005) reported that clinical mastitis is characterized by abnormal milk or visible abnormalities of the udder such as hot and swollen udders, the SCC in milk samples have been used for the detection of clinical and sub-clinical mastitis in cows. Kawai et al., (1999) and Wojdak et al., (2006) the range for LFC in milk from dairy cows with clinical mastitis was higher than, that for normal. The present result indicated that four type of bacteria (Staph, Strep, E.coli and Baci) the most common pathogens causes' mastitis. The means of Staph, Strep, E.coli and Baci in clinical mastitis cows were 902.9, 900.3, 352.8 and  $481.6 \times 10^3$  cell/ ml respectively, compared with healthy cows were 458.4, 497.8, 240.2 and  $230.5 \times 10^3$  cell/ml respectively table 6. These result agreement with Benfield et al., (2007); Piepers et al., (2007) and Kurlenda and Grinholc, (2012) reported that Staph. aureus is a common pathogen responsible for infections.

**Table (6) Effect of incidence clinical mastitis on traits under investigation**

Variable	N	Traits						
		TMY	SCC	LFC	Staph	Strep	E.coli	Baci
		Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
Overall mean	794	3525±101	466±18	376±24	501±22	533±16	259±25	256±28
Clinical Mastitis	102	3364±115	578±33	600±19	902±42	900±45	352±30	481±37
Normal	692	3785±46	325±14	177±15	458±24	497±25	240±25	230±222

Hirvonen et al., (1999) and Burvenich et al., (2003) the severity of mastitis has been demonstrated to be significantly related to bacterial count in milk. Levison et al., (2016) the most mastitis pathogens were Baci. sp, Strep. sp, Staph. sp and E. coli. Van den Borne et al., (2011) found that higher correlation between clinical mastitis (CM) and increasing SCC in cows and the risk for CM increased with increasing SCC.

### Correlations

The estimate of correlation between TMY and udder health traits (SCC, MAST, LFC, Staph, Strep, E.coli and Baci) were low and negative ranged from (-0.23 to -0.07). While the correlation among udder health traits had positive ranged from (0.27 to 0.88), which the estimate of correlation among LFC, SCC and MAST had strong ranged from (0.79 to 0.88) and the correlation among Staph, Strep, E.coli and Baci were positive ranged from (0.27 to 0.55) Table7. The estimate on present results similarly found by, Ikonen et al., (2004) the correlation between TMY and SCC was high and negative. Cheng et al., (2008) Lf was significantly associated with milk production -0.47 and shown the LFC was correlated with SCC 0.37. Banos and Shook, (1990) correlation between SCC and the mastitis traits were greatest 0.71. The same previous author's showed that correlation between mastitis and Strep. dysgalactiae, Strep. uberis, and E. coli ranged from 0.54 to 0.69 and were lowest for Staph. aureus mastitis 0.44.

**Table (7) Different correlations among traits under investigation.**

Traits	TMY	SCC	MAST	LFC	Staph	Strep	E.coli
TMY							
SCC	-0.12						
MAST	-0.16	0.88					
LFC	-0.08	0.79	0.81				
Staph	-0.23	0.76	0.84	0.68			
Strep	-0.19	0.77	0.78	0.61	0.55		
E.coli	-0.09	0.71	0.51	0.56	0.47	0.41	
Baci	-0.07	0.67	0.41	0.46	0.47	0.38	0.27

Olde Riekerink et al., (2007), Schukken et al., (2009) and Oliveira et al., (2013) the correlation between MAST and Staph ranged from 0.55-0.62 and between Mast and Strep ranged from 0.54-0.59. Banos and Shook (1990) Phenotypically, Staph. aureus is known to be associated with high SCC.

### Conclusion

The present results had showed that the estimates of correlations among SCC, LFC and mastitis were high and positive, so these indicate that LFC could be used as a complementary test to SCC and possibly a diagnostic test of subclinical mastitis in dairy cattle. Using lactoferrin with gentamicin or ampicillin and using EEP mixed with

gentamicin more effective of decreasing bacterial counts in milk and improving udder health.

### References

- Aguila, A. and J.H. Brock (2001) Lactoferrin: antimicrobial and diagnostic properties. *Biotechnol. Apl.* 18:76-83.
- ALCL, (2004) Somatic cell count display Agricultural instruments Canada :Ltd.
- Banos, G. and G. E .Shook (1990) Genotype by environment interaction and genetic correlations among parities for somatic cell count and milk yield. *J. Dairy Sci.* 73: 2563 - 2573.
- Barkema, H.W., Y.H. Schukken, T.J.G.M. Lam, M.L. Beiboer, H. Wilmink, G. Benedictus and A. Brand (1998) Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *J. Dairy Sci.* 81: 411-419.
- Benfield, T., F. Espersen, N. Frimodt-Moller, A.G. Jensen, A.R. Larsen , L.V. Pallesen, R. Skov, H. Westh and P.Skinhoj (2007) Increasing incidence but decreasing in-hospital mortality of adult *Staphylococcus aureus* between 1981 and 2000. *Clin. Microbiol Infect.* 13: 257–263.
- Burvenich, C., V. Van Merris, J. Mehrzad, A. Diez-Fraile and L. Duchateau (2003) Severity of *E. coli* mastitis is mainly determined by cow factors. *Vet. Res.* 34: 521-564.
- Caraviello, D.Z., K.A. Weigel, G.E. Shook and P.L. Ruegg (2005) Assessment of the impact of somatic cell count on functional longevity in Holstein and Jersey cattle using survival analysis methodology. *J. Dairy Sci.* 88: 804-811.
- Cheng,J.B., J.Q. Wang, D.P. Bu, G.L.Liu , C.G. Zhang, H.Y.Wei, L.Y.Zhou and J.Z. Wang (2008) Factors affecting the lactoferrin concentration in bovine milk. *J. Dairy Sci.* 91: 970-976.
- Detilleux, J.C. (2002) Genetic factors affecting susceptibility of dairy cows to udder pathogens. *Vet. Immunology and Immunopathology.* 88:103-110.
- Diarra,M.S., D.Petitclerc and P.Lacasse (2002) Effect of lactoferrin in combination with penicillin on the morphology and the physiology of *Staphylococcus aureus* isolated from bovine mastitis .*J.Dairy Sci.* 85: 1141–1149.
- El-Arian, M. N. and H. G. El-Awady (2008) Assessment of the genetic relationships between udder health and milk production traits in relation to selection for improving resistance to mastitis in Friesian cows in Egypt. *J. Agric. Sci. Mansoura Univ.* 33:181-192.
- El-Awady, H. G. and E. Z. M. Oudah (2011) Genetic and Economic Analysis for the Relationship between Udder Health and Milk Production Traits in Friesian Cows.*Asian-Aust. J. Anim. Sci.*Vol. 24, No.11: 1514 - 1524.
- Fang, W., R.A. Almeida and S.P. Oliver (1999) Lactoferrin is involved in increased adherence of *Streptococcus uberis* to bovine mammary epithelial cells. *Nat. Mast. Coun. Ann. Meet. Proc.* 174-175.

- Geary, U., N. Lopez-Villalobos, N. Begley, F. McCoy, B. O. Brien, L. O.Grady and L. Shalloo (2012) Estimating the effect of mastitis on the profitability of Irish dairy farms. *J. Dairy Sci.* 95:3662-3673.
- Green M.J., L.E. Green, Y.H. Schukken, A.J. Bradley, E.J. Peeler, H.W. Barkema, Y. de Haas, V.J. Collis and G.F .Medley (2004) Somatic cell count distributions during lactation predict clinical mastitis. *J. Dairy Sci.* 87: 1256-1264.
- Grohn, Y. T., D.J. Wilson, R. N. Gonzlez, J. A. Hertl, H. Schulte, G. Bennett, and Y. H. Schukken (2004) Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* 87:3358-3374.
- Haile-Mariam, M., P.J.Bowman and M.Goddard (2003) Genetic and environmental relationship among calving interval survival, persistency of milk yield and somatic cell count in dairy cattle *Live.Prod.Sci.* 80:189-200.
- Helal, A.I.K ( 2013) The influence of somatic cell count in milk on reproductive performance of Frisian dairy cattle, M.Sc.Thesis, Fac. agric. Kafrelsheikh Univ. Egypt.
- Hirvonen, J., K.Eklund, A.M. Teppo, G. Huszenicza, M. Kulcsar, H. Saloniemi, S.Pyorala (1999) Acute phase response in dairy cows with experimentally induced *Escherichia coli* mastitis. *Acta., Vet. Scand.,* 40: 35-46.
- Hillerton, J.E. and K.E. Kliem (2002) Effective treatment of *Streptococcus uberis* clinical mastitis to minimize the use of antibiotics. *J. Dairy Sci.* 85: 1009-1014.
- Hogeveen, H., K. Huijps, and T. J. G. Lam. (2011) Motivating producers to improve udder health: Impact of economics. *N. Z. Vet. J.* 59:16-23.
- Hong, H., M.R.Landauer, M.A. Foriska and G.D. Ledney (2006) Antibacterial activity of the soy isoflavone genistein. *J .Basic. Microbiol* 46: 329-335.
- Hussein, K. (2000) Environmental and genetical factors affecting milk production of Friesian breed. PhD.Thesis, Fac. Agric. Mansoura Univ. Egypt.
- Ikonen, T., S. Morri, A.M. Tyriseva, O. Ruottinen and M. Ojala (2004). Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. *J Dairy Sci.* 87:458-467.
- Kawai , K., S. Hagiwara, A. Anri and H. Nagahata (1999) Lactoferrin concentration in milk of bovine clinical mastitis. *Vet. Res. communications,* 23:391-398.
- Klungland, H., A.Sabry, B.Heringstad, H.G.Olsen, L. Gomez- Raya, D.I.Vage, I. Olsaker, J.Odegard, G. Klemetsdal, N. Schulman, J. Vilkki, J. Ruane,M. Aasland, K.Ronnin-gen and S.Lien (2001) Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. *Mammalian Genome,* 12:837-842.
- Kurlenda, J. and M. Grinholc (2012) Alternative therapies *Staphylococcus aureus* diseases. *Drug Res.* 59: 171-184

- Kuttila, T. (2004) Role of Lactoferrin in Treatment of Bovine Mastitis. M.Sc. Thesis, Clin. Vet. Sci. Fac. Vet. Medi. Univ. Helsinki.
- Lam, T. J. G. M., G. van Schaik, and H. Hogeveen (2013) Improving bovine udder health: A national mastitis control program in the Netherlands *J. Dairy Sci.* 96 :1301-1311
- Leclercq, G., N. Gengler, H. Soyeurt, C. Bastin (2013) Genetic variability of the mid-infrared prediction of lactoferrin content in milk for Walloon Holstein first-parity cows. *Live. Sci.* 151:158-162.
- Levison, L.J., E.K. Miller-Cushon, A.L. Tucker, R. Bergeron, K.E. Leslie, H.W. Barkema and T.J. DeVries (2016) Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms, *J. Dairy Sci.* 99:1341-1350.
- Malinowska, E. and A. Kłossowska (2010) Mastitis caused by coagulase-negative staphylococci in cows. *Med. Weter.* 66: 89-92.
- Marcucci, M.C., F. Ferreres, C. Garcia-Viguera, V.S. Bankova, S.L. De Castro, A.P. Dantas (2001) Phenolic compounds from Brazilian propolis with pharmacological activities. *J. Ethnopharma*, 74:105-12.
- McDougall, S., K. Parker, C. Heuer and C. Compton (2009) A review of prevention and control of heifer mastitis via non-antibiotic strategies. *Vet. Microbiol.* 134:177-185.
- Melchior, M. B., H. Vaarkamp and J. Fink-Gremmels (2006) Biofilms: A role in recurrent mastitis infections, *Vet. J.* 171: 398-407.
- Nickerson, S. (2009) Control of heifer mastitis: antimicrobial treatment—an overview *Vet. Microbiol.* 134:128-135.
- Olde Riekerink, R. G. M., H. W. Barkema, and H. Stryhn (2007) The effect of season on somatic cell count and the incidence of clinical mastitis. *J. Dairy Sci.* 90:1704-1715.
- Oliveira, L., C. Hulland, and P. L. Rugg (2013) Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. *J. Dairy Sci.* 96:7538-7549
- Piepers, S., L. De Meulemeester, A. de Kruif, G. Opsomer, H.W. Barkema, and S. De Vliegher (2007) Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. *J. Dairy Res.* 74: 478-483.
- Plaffl, M., S.L. Wittmann, H.H. Meyer, and R.M. Bruckmaier (2003) Gene expression of immunologically important factors in blood cells, milk cells, and mammary tissue of cows. *J. Dairy Sci.* 86: 538-545.
- Pyörälä, S.H.K. and E.O. Pyörälä (1998) Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows. *J. Am. Vet. Med. Assoc.* 212: 407-412.
- Raghukumar, R., L. Vali, D. Watson, J. Fearnley and V. Seidel (2010) Antimethicillin resistant *Staphylococcus aureus* activity of Pacific propolis and isolated prenyl flavanones. *Phyto. Res.* 24:1181-1187.
- Roger, B. and E. Peter (1995) Mastitis control in dairy herds an illustrated and practical guide 1<sup>st</sup> Edition Farming Press Publications, UK.

- Sakwinska, O., D. Morisset, J. Madec, A. Waldvogel, P. Moreillon and M. Haenni (2011) Link between Genotype and Antimicrobial Resistance in Bovine Mastitis-Related Staphylococcus aureus Strains, Determined by Comparing Swiss and French Isolates from the Rhoane Valley, applied and environmental microbial.77:3428-3432.
- Salomao, K., P. Pereira, L. Campos, C. Borba, P. Cabello, M. Marcucci and S. De Castro (2008) Brazilian propolis: correlation between chemical composition and antimicrobial activity. Evid Based Complement Alternat Med. 5:317-324.
- Schukken, Y.H., D.J. Wilson, F.Welcome, L. Garrison-Tikofsky and R.N Gonzalez (2009) Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34: 579-596.
- Smith, K. L., J. E. Hillerton, and R. J. Harmon (2001) National Mastitis Council guidelines on normal and abnormal raw milk based on somatic cell counts and signs of clinical mastitis.
- SAS (2004) Statal analysis system User Guide SAS Inst. University of Massachusetts site license for the SAS System
- Smulski, S., E.Malinowski, M. Kaczmarowski and H. Lassa (2011) Occurrence, forms and etiologic agents of mastitis in Poland depending on size of farm. Med. Weter, 67:190-193.
- Tag El-Dein, M.A. (1997) Studies on cattle, pheotypic and genetic parameters of some performance traits in Friesian cattle. PhD. Thesis, Fac. Agric. Alex. Univ. Egypt.
- Van den Borne, B.H.P, J.C.M. Vernooij, A.M. Lupindu, G. van Schaik, K. Frankena, T.J.G.M. Lam and M. Nielen (2011) Relationship between somatic cell count status and subsequent clinical mastitis in Dutch dairy cows. Preventive Vet. Medi. 102:265- 273
- Wakabayashi, H., Y.Koji and T.Mitsunori (2006) Lactoferrin research, technology and applications. Inter. Dairy J. 16: 1241-1251
- Wojdak, K., M. Kmiec and J. Ziemak (2006) Associations between bovine lactoferrin gene polymorphism and somatic cell count in milk. Vet. Med., 51, (1): 4-20.

### الملخص العربي

العلاقة بين تركيز اللاكتوفريين و انتاج اللبن و التهاب الضرع و عدد الخلايا الجسدية و البكتيريا المعدية الممرضة

حسن غازي العوضي<sup>1</sup> عاطف يوسف سالم<sup>1</sup> محمد عبد الحليم تاج الدين<sup>2</sup> احمد محمد

ابراهيم مصطفى<sup>2</sup> ابراهيم عطا محمد ابوالنصر<sup>3</sup>

<sup>1</sup> قسم الانتاج الحيواني - كلية الزراعة جامعة كفر الشيخ. <sup>2</sup> معهد بحوث الانتاج الحيواني الجيزة- مصر. <sup>3</sup> قسم الانتاج الحيواني- كلية الزراعة جامعة دمياط

تم تحليل البيانات باستخدام برنامج SAS (2004) لتقدير تأثير مستويات الخلايا الجسدية ومستويات اللاكتوفريين علي انتاج اللبن و التهاب الضرع و الانواع البكتيرية, E.coli, Staph, Baci Strep وذلك لعدد 794 سجل حليب لابقار الفريزيان المرباه في مصر خلال الفتره من 2012 حتي 2014 بمحطة بحوث الانتاج الحيواني بالقرضا التابعة لمعهد بحوث الانتاج الحيواني. كان متوسط الصفات المدروسة كالتالي انتاج اللبن الكلي 3557.7 كجم و الخلايا الجسدية 10x363<sup>3</sup> خلية /مل و تركيز اللاكتوفريين 308 ميكروجرام /مل و نسبه الاصابة بالتهاب الضرع (12%) وكانت الاعداد البكتيرية للانواع المدروسة E.coli, Strep

, Staph, Baci 512 و529 و250 و $10 \times 254$  خلية<sup>3</sup>/مل علي الترتيب . لم يكن لمستويات الخلايا الجسدية تأثير علي انتاج اللبن حتي مستوي  $10 \times 200$  خلية<sup>3</sup>/مل وبزيادة مستويات الخلايا الجسدية ينخفض انتاج اللبن وقد وجد ان اعلي متوسط لإنتاج اللبن عند مستوي الخلايا الجسدية <  $10 \times 200 - 100$  خلية<sup>3</sup>/مل بينما كان اقل انتاج للبن عن اعلي مستوي للخلايا الجسدية <  $10 \times 1000 - 900$  خلية<sup>3</sup>/مل وايضا عند هذا المستوي من الخلايا الجسدية اعلي متوسط من الاصابة بالتهاب الضرع وتركيز اللاكتوفريين و العدد البكتيري بمعنى انه بزيادة العدد البكتيري يزداد مستوي الخلايا الجسدية وبذلك تعتبر زيادة اعداد الخلايا الجسدية في اللبن دليل علي حدوث التهاب للضرع وبدراسة تأثير المستويات المختلفة من تركيز اللاكتوفريين في اللبن علي الصفات المدروسة وجد ان انتاج اللبن ينخفض بزيادة مستوي تركيز اللاكتوفريين ويزداد مستوي الاصابة بالتهاب الضرع وتزداد اعداد الخلايا الجسدية وكذلك الاعداد البكتيرية في اللبن. المعاملة (6) (لاكتوفريين +جنتاميسين) كانت افضل المعاملات حيث كان فيها اقل متوسط لعدد الخلايا الجسدية والتهاب الضرع والعدد البكتيري بينما كان اعلي متوسط من هذه الصفات في مجموعة المقارنة. كان الارتباط بين إنتاج اللبن والصفات الصحية للضرع منخفض وسليبي بينما كان الارتباط بين الصفات الصحية للضرع ارتباط ايجابي. والخلاصة انه يمكن استخدام تركيز اللاكتوفريين في اللبن كدليل علي ارتفاع اعداد الخلايا الجسدية وعلي الحالة الصحية للضرع ووجد ان حقن اللاكتوفريين مع الجنتاميسين 10مل في كل حلقة يؤدي الي قتل البكتريا الموجودة في اللبن او تحفيز الجهاز المناعي لمواجهتها وقد تبين ان استخدام اللاكتوفريين مع الجنتاميسين له فاعلية كبيرة في تقليل العدد البكتيري بنسبه اكبر من استخدام اللاكتوفريين بمفرده.