



# IMPACT OF MASTITIS ON LIVER ENZYMES AND MILK COMPOSITION IN IRAQ DAIRY CATTLES

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## Abstract

Mastitis is the most prevalent dairy herds, this study was conducted to investigate the difference in liver enzymes activity in infected and healthy cows for this purpose 120 hybrid dairy cows were used 35<sup>th</sup> of them were infected with mastitis, while the others were healthy. The results showed significant ( $P \leq 0.05$ ) increase in the number of somatic cells in the milk of infected cows compared to healthy one, also the bacterial isolation and identification recorded significant increase in the number of samples infected Str. Spp. compared with other spp. of bacteria. Liver enzymes activity measurement recorded significant ( $P \leq 0.01$ ) increase in the activity of (AST) of infected cows compared with that of healthy, while other enzyme activity recorded significant differences among experimental samples. Mineral concentration measurement showed significant ( $P \leq 0.01$ ) increase in the Na and CL concentration and decrease significant ( $P \geq 0.01$ ) in the K and Ca concentration. Fat, total protein and lactose concentration measurement recorded in significant differences among experimental samples. Blood parameters WBCs, Hb and HCT were significantly higher in the infected cows, RBCs, MCH and MCHC were recorded significantly differences. It can concluded that Staphylococcus is the mean cause of mastitis in dairy cows, which lead to increase the number of somatic cells in the milk, activity of AST, WBC, number Hb, HCT, Na, CL and decrease K and Ca levels in the infected cows.

**Key words :** Somatic cell count, Blood parameter, Mineral concentration, Enzyme activity.

## Introduction

Mastitis is the most common disease in dairy cattle, have an economic impact through the lack of milk production or lack of treatment, this disease comes as a result of several factors: Environmental, nourishment and Immunological response. Subclinical Symptoms: a cow doesn't show any visible sign of infection or abnormalities of the udder and Clinical Symptoms: the disease can be identified by abnormalities in udder such as pain, swelling, heat, redness and hardness. Other indication of mastitis such as, watery appearance of milk and florin clots reduction in milk yield, increase in body temperature and lack of appetite milk composition differ depend upon, species, age, udder health, milk yield season, nutrition management and season (Ogola *et al.*, 2007). Somatic cells increase in milk after birth in the production of colostrum's before the cow settle down at the end of seasonal period of milk. This done by many factors: 1.

Seasonal period 2. Farm management (EL feki, 2008). The end of the nipple changes against the pathogens is the first factor, where the physiological and histological characteristics of nipple channel sealed with Keratin, which prevent invasion of pathogenic where the control of pathogens from the end of the nipple stimulate the early leukocytes from entering the end of infection (Audis, 1995). The increase can concentration of fat in milk indicates a decrease in the lactose by the cells which leads to a small volume of milk and decreased in the manufactured of fat.

The lipid membrane of milk are subjected to the action of lipase enzyme that produced by the leukocytes, which invade the mammary glands just response to inflammation and result triglyceride decomposition this is due to a slight increase in milk. Lactose decrease may be due to damage of the epithelial tissues of the alveoli of mammary gland, infected of mammary gland by bacteria which has ability to fermented lactose at the infected udder that due to the action of bacteria (Ogola *et al.*, 2007). Aim of the research:

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The aim of this study was to investigate the effects of mastitis in Iraqi dairy cattle on liver enzymes, milk components and blood parameters.

### Materials and Methods

The research as carried out on a herds of Iraqi crossbred from Frisian at Diwaniya cattle station in Qadisiyah governorate, the study started from 1<sup>st</sup> of March till to 10<sup>th</sup> of April 2017. 120 cows were examined, 35 were infected with mastitis and samples of blood were taken from the jugular vein of infected cows and samples of milk taken of infected teats. other samples were taken from healthy cows for comparison. The results were analyzed in the laboratory of the Department of Food Science at the Faculty of Agriculture, AL-Qasim Green University and the blood analyzes in the civil laboratories in Diwaniyah. All cattle were subjected to clinical examination through visual examination, oedema and redness. Milk samples were collected from each quarter of the cows udder before morning milking after cleaning the end of the teat with 70% ethanol ignore the first drops of milk and then collect 10 ml. of sterile milk from each teat in a sterile tube stored in the refrigerator at 4°C until the samples are analyzed. Blood collect from jugular vein from all cows with sterile test tubes with (EDTA) and without (EDTA). Milk tests were conducted through the bacteriological examination. 34.2% were diagnosed with *Staphylococcus aureus* 17.1%, *E. coli*, 49.7% mixed bacteria. All samples were transferred to the laboratory using sterile tubes. Samples were planted on culture media to determine the type of bacteria, causing the infection, where the cultivation of samples on the culture media (Blood agar, mannitol salt agar, nutrient agar and macconkey agar). All cultural petri dishes were incubated at 37°C for 24-72 hours, the developing colonies were isolated and identified by biochemical test and gram stain.

Blood tests include:

1. Total W.B.C. counts (leukocytes)
2. differential leukocyte counts and calculate the proportion of the neutrophil cells to lymphocytes
3. Red blood cells count (erythrocyte)
4. Haemoglobin
5. P.C.V. (hematocrit)
6. Liver enzymes activity
7. Total protein
8. Lipid profile

Somatic cells :calculated the number of somatic cells in the milk done by (porta Scc cow test). In this test, the

number of somatic cells is calculated and reveals subclinical mastitis before the onset of clinical signs.

This test is done by three steps:

1. Add one drop of milk to the test strip.
2. Add (3) drops of activator solution. 3-Read the results after 45 minutes by observing the color of the tape and electronic reading, if the number of somatic cells is too high the color of the tape begins to develop in a few minutes.

### California Mastitis test (CMT)

When there is a marked change in the new milk appears water or milk fluffy and sticky, this indicates that the animal is infected with mastitis and this situation is late as the milk may appear normal while the animal is infected with mastitis. In order to obtain an examination by which the signs of mastitis infection can be shown in first stage (CMT) is considered the best and gives the best test.

### The method of test

1. Pull the first drop of milk from each teat and place in each plastic dish with four cavities and each cavity with one teat.
2. The dish is tilted slightly to estimate the amount of milk in each cavity (about half teaspoon).
3. Add an equal amount of the chemical reagent to the milk.
4. Move the dish in order to mix the milk with the reagent and notice the changes on the milk as the natural milk and cow healthy to be liquid and easily shed. When a lot or small part of the milk are formed this indicates that the animal is infected with mastitis disease.

### Results and Discussion

A few hours after udder infection by pathogenic micro organism, the number of somatic cells in the milk increases in response to the activation of inflammatory processes. The international dairy products association has recommended the classification of milk for cows infected with subclinical mastitis using the Scc thresholds its 500,000 cell/ml. In Europe there is a trend that cannot be used for milk containing somatic cells more 400,000 cell/ml, for human consumption , while the limits are 750,000 cells/ml in USA and 500,000 in Canada. From this recommendation, we have milk samples classified in two categories:

Normal (value less than 500,000 cells/ml) and subclinical mastitis (value above 500,000 cells/ml)

**Table 1 :** The following table shows the changes on milk and the evaluation of the examination.

Evaluation of the test	Milk changes	cc <sup>3</sup> /W.B.C.
Negative	Liquid and lack of precipitation.	Less than 200,000
1-	A little sediment and fading with string.	500,000– 200,000
2-	Apparent precipitation and lack of conglomerate.	1,500,000– 500,000
3-	Consistence clot.	4 million– 1,500,000
4-	A clear conglomerate stick to the dish.	More than 44 million

Statistical analysis was performed using the (Anova one way ) group pad prism –version (group pad) software.inc. California USA.

**Table 2 :** The number of somatic cells in healthy and mastitis cows.

Infected MilkNormal Milk				Treatment
Av.	SE±ME	Av.	SE±ME	
500-1500	54.241±0.345	120– 270	9.919±0.675	SCC x10 <sup>3</sup>

**Table 3 :** Number of bacteria that caused mastitis in hybrid cattle.

Bacterial isolates	No.	%
<i>Staphylococci</i>	12	34.2
<i>E. coli</i>	6	17.1
<i>Streptococcus agalactia</i>	4	11.4
<i>Streptococcus dysagalactia</i>	4	11.4
<i>Streptococcus ubreis</i>	3	8.5
<i>Pseudomonas aerogenosa</i>	3	11.4
<i>Actinomyces pyogenes</i>	2	5.7
Total	35	100%

After tests on 80 cows, 20 of them were found to have subclinical mastitis, a counting for 25% of the total cows. In these 20 cows increase in the number of somatic cells was observed. The values obtained where between 500, 000 – 1500,000 million cells/ml. In healthy cows the number of somatic cells doesn't exceed 270,000 cells/ml. and the number of somatic cells was higher in mastitis milk.

After milk samples were taken from infected cows and in plant on culture media, a number of bacteria that caused mastitis were obtained in table 3.

The porta Scc cow test results depend upon the color, if the color is light, the number of somatic cells become a little, or if the color more opaque, the cells number is too high.

Mastitis is a common disease of diary cows, where the disease is transmitted by contaminated mechanical milking tool, hands, mattresses and tools. Milk stopped of infected cattle is often cause change in blood flow, which leads to determination the amount of fluid fill out the udder and this contributes for reducing udder size and tumor

growth. The study deal with comparison between the number of somatic cells infected and healthy cows, in tables 2 and 4 found significant differences ( $P < 0.01$ ) that indicates the somatic cells increase in number due to mastitis, the study agree with researcher Bahaji and Saravanan (2016). There is an increase in the number of somatic cells, chlorine and acidity of milk. deal with the types of isolated bacteria from infected cows with each species in the table (3), staphylococci occupied 34.2% more than of the rest species, this study agree with researcher Asadi *et al.* (2009). The isolated and predominant bacteria in clinical and subclinical infected samples were *Staphylococcus aureus*.

There was a significant difference  $P < 0.01$  between AST enzyme in infected and healthy cows and that disease lead to increase the enzymes of myocardial infection and skeletal muscle disease, while there are no significant differences with the rest of the enzymes, in the table 5. This study is agree with Asadi *et al.* (2009). There is a high concentration of liver enzymes and trace minerals in 30 cows with mastitis. The effect of mastitis on milk components of mineral salts, where the elements of Sodium is found in high quantities in the blood as it leaks in to milk leading to an increase in the concentration of milk above the normal in case of disease, where the increase was significant difference  $P < 0.01$  the concentration of chlorine increase in milk as a result of the supply of blood to milk (table 6).

Potassium is the most abundant mineral in milk however a leak of milk during intra cellular pathway lead to leak of concentration. It is also observed that the level of calcium and phosphorus was affected by mastitis. Its reduction in calcium and phosphorus write down by Bogin *et al.* (1973) and Coulan (2002). The study deal with the affected of mastitis on an organic milk components. As increased concentration of fat in milk, indicates a decrease in the manufacture of lactose by the cells and this lead to a lack of milk volume, which contributes to a slight reduction in the manufacture of Fat (table 7). Brack Malar *et al.* (2004), the reduction of Fat from a quarter of the udder is likely to occur due to lack of glands secretion.

**Table 4 :** The number of somatic cells in cows' milk examined by the (porta Scc cow test).

Porta SCC cow test (1000x)	Quarter of the udder	Number of the cow
750	RR	1276
500	FR	
500	FL	
500	LR	
1500	RR	1465
750	FR	
500	FL	
250	LR	
<100	RR	1765
<100	FR	
<100	FL	
<100	LR	
3000	RR	1654
1500	FR	
1500	FL	
750	LR	
<100	RR	1564
<100	FR	
<100	FL	
<100	LR	
500	RR	1657
750	FR	
500	FL	
500	LR	
750	RR	1455
500	FR	
500	FL	
500	LR	
<100	RR	1334
250	FR	
<100	FL	
250	LR	
750	RR	1243
500	FR	
750	FL	
500	LR	
<100	RR	1767
<100	FR	
<100	FL	
<100	LR	
250	RR	1878
<100	FR	
<100	FL	
250	LR	

FR = front right  
RR = Rear Right

LR = left Rear  
FL = Front Left

**Table 5 :** Effects of mastitis disease on liver enzymes.

Parameter	Control	Infected
ALT (U/L)	41.00±0.36	43.83±2.85
AST (U/L)	37.00±0.44 B	115.16±15.74 A
LDH (U/L)	35.00±0.57	31.50±3.14

**Table 6 :** Effects of mastitis on milk components just for mineral salt.

Parameter	Control	Infected
Sodium	52.93±1.200 B	91.97±5.553 A
Chloride (mg/dl)	0.09±0.005 B	0.187±0.446 A
Potassium	167.74±2.603 A	151,56±2.445 B
Calcium	126.29±0.882 A	90.45±1.913 B

**Table 7 :** Effect of mastitis on milk components for organic materials.

Parameter	Control	Infected
Fat	3.53±0.08	3.93±0.79
Total protein	3.58±0.04	3.0517±0.05
Lactose	4.90±0.03	3.46±0.15

**Table 8 :** Effect of mastitis on different blood characteristic tests.

Parameter	UNIT	Control	Infected
W.B.C.	c./100cc	6.7483±0.11B	9.42±0.18A
R.B.C	c./ 100 cc	6.36±0.16	6.70±0.12
Hb	g/dl	8.42 ±0.24 B	9.44±0.27A
H.C.T. (p.c.v.)	%	27.38±0.15B	33.20±1.65A
M.C.V	Pg/dl	43.30±0.20A	49.28±2.86B
M.C.H.	Pg/dl	13.26±0.14	14.08±0.62
M.C.H.C.	Pg/dl	30.52±0.23	28.68±0.72

The lipid membranes are subject to the lipase enzyme action and are produced by white blood cells (leukocytes) which invade the mammary glands in response to infection. The result hydrolysis of triglyceride, which is a result of slight increase in milk fat, low lactose in milk was observed due to damage of epithelial cells of alveoli. Mastitis caused by micro organism has ability to ferment milk lactose in infected udder, Audist *et al.* (1995). Effect of mastitis on different blood tests compared to healthy cows in the table 8. There was an increase in the proportions of white blood cells (leukocytes), hemoglobin (Hb), Pocket cell volume (P.C.V.) and media circular volume (M. C. V.). The high number of W.B.C. in infected cow was significantly different ( $P<0.05$ ).

### Conclusion

From this study, we concluded that *Staphylococcus aureus* is the main cause of number of somatic cells in infected milk, increase AST enzyme, sodium above normal

W.B.C., Hb, MCV and decrease K, Ca levels for infected cows.

### Recommendation

1. To observe the real mastitis, approximately 1% of mastitis causes non infections, may be physical injury. The vast majority of causes are infections with staphylococcus or others.
2. Use the pesticide to disinfectant the field, insects populations can increase the incidence of mastitis, infection rate may be exceed 30%.
3. The insects carry disease that moving bacteria from the skin into the surface of the teats.

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