



Effect of Lactation Periods and Mastitis of Mactoferin Level in Sheep, Goat and Cow Milk



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Abstract

THE aim of study was effect of postpartum duration and mastitis on lactoferrin level in colostrum and milk of cows, sheep and goats. Milk samples were taken from farm animals in the Al-Alam area, east of Tikrit. 24 animals were used in the experiment, 8 animals each from sheep, cows, and goats, also 24 cases suffering from mastitis were subject in current study. Milk was taken from these animals at different intervals, namely the first day of birth, one week, two weeks, and 3 weeks after birth. The percentage of lactoferrin in the milk was measured using an HPLC test, and the percentage of protein in the colostrum was higher than its percentage in milk, The average percentage of protein in sheep's milk was higher than that in goat's milk, while the percentage in cow's milk was lower. The average concentration of lactoferrin protein on the third week after birth in cow's milk was 33.5 parts per million, followed by goat milk with a higher concentration of 86 parts per million, then sheep's milk, which is the highest, with a lactoferrin concentration of 99.2 parts per million. While the average percentage of lactoferrin protein in cow's milk a second week after birth was 36.9, and in goat milk was 97.8, while in sheep's milk the percentage of lactoferrin protein was 124.5. The percentage of lactoferrin present in the milk first weeks after birth was higher, and the results were as follows: 40.5 in cow's milk, 120.6 in goat's milk, and 139.8 in sheep's milk. As for the results of the percentage of lactoferrin in milk first day of birth, it was the highest compared to the rest of the results, and it is as follows : the lowest percentage in cow's milk is 43.6 , followed by goat's milk with a percentage of 131.5 , while sheep's milk is the richest in lactoferrin with a percentage of 144.9. At the end of the study, it can be said that the percentage of lactoferrin protein in milk is affected by the stages of milk production, as its concentration at the beginning differs from its concentration at the end. also so the lactoferrin concentration is high all mastitis cases in compare with healthy cases.

Keywords: lactoferrin, milk, HPLC.

Introduction

Milk is one of the most important types of food that is considered a major source in the human diet at various age levels, especially for newborns, because it contains many nutrients and proteins that play an important role in maintaining human health, and the protein lactoferrin is one of these proteins found in milk [1]. Lactoferrin It is an iron-binding glycoprotein from the transferrin family with a molecular weight of 80 kilodaltons consisting of 703 amino acids [2,3]. It is mainly found in milk, in addition to its presence in fluids and external secretions of the body of various mammals, such as tears, saliva, cervical mucus, and cell granules. Neutrophils in a form devoid of iron [4,5,6]. The lactoferrin protein was isolated for the first time in 1939 and was called the red protein when it was discovered as a result of its association with iron [7,

8]. Lactoferrin is considered a protein with multiple physiological functions, as studies have shown 20 different functions that lactoferrin performs in mammals, for example regulating iron absorption in the intestine, antioxidant, anti-cancer, and anti-microbial and anti-inflammatory [9]. Lactoferrin plays a role in autoimmunity and exerts a variety of biological activities, including antimicrobial, antiviral, antioxidant activities, immunomodulation, modulation of cell growth, and inhibition of many biologically active compounds such as lipopolysaccharide and glucosaminoglycans [10]. Due to the nutritional and health importance of lactoferrin and the fact that milk is rich in lactoferrin, this study was conducted to estimate its concentrations in the milk of cows, sheep and goats using HPLC technology.

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Material and Methods

Animals groups: The current study conducted in the Al-Alam area -Salhaldeen province. two groups of animals were subjected in current study. First early birthing animals (8cow, 8ewes and 8goats) all the animals appear healthy clinically by clinically examined before birth and throughout the study period. Excluded animals were those that showed signs of disease before birth until the end of the study period. The second group consist from 24 lactation animals suffering from mastitis according to the California test(CMT which conducted by use of DeLaval CMT Kit- India and according to Producing company instructions, physical changes that occurred in the milk, such as changes in color and texture [11,12].

Samples collection

Milk samples were taken from 24 animals, eight each from cows, sheep, and goats, at specific time periods, namely the first day of birth, one week, two weeks, and three weeks after birth, to measure the percentage of lactoferrin in the milk.

Sample preparation

The fat was removed from milk by centrifugation at 3000 g of 15 min. In order to separate the whey, the samples were precipitated with 1 mol/l HCl to pH 4.6 and centrifuged at 3000 g at 15 min again. The samples of whey were stored at -18°C till the analysis.

Lactoferrin estimation

HPLC model SYKAM (Germany) It was used to analyses add detection of Lactoferrin. The mobile phase was water/acetonitrile/Trifluoroacetic acid (95:5:0.1) flow rate at 1.0 mL/min , column was C18- ODS (25cm * 4.6 mm) and the detector UV-Vis at = 205nm [13].

Statistical analysis

Were done by SPSS version 23.0, One-way ANOVA, between animal groups was analyzed using MANOVA, LSD. P value < 0.05 was considered statistically significant.

Results and Discussion

The tables 1,2,3 showed that lactoferrin concentration decrease with progress of lactation period this agreed with study of [15,16]. In study shows that Lactoferrin concentration in sheep milk , goat milk and cow milk are 0.002- 0.2 mg/ml [17,18]. Lactoferrin-induced bacteriostasis of gram-

negative bacteria also involves its interaction with lipid A of lipopolysaccharide (LPS) and with poreforming proteins (porins) of the outer membrane, altering the integrity and permeability of the bacterial wall. In gram positive pathogens, the antibacterial mechanisms of lactoferrin are still not well understood [19,20]. Fluctuations in lactoferrin concentrations in milk during lactation were observed as far back as the 1970s. Under normal conditions, the lactoferrin concentration of colostrum milk is up to about 2 mg/ml and decreases gradually to baseline levels during lactation period (0.02 to 0.5 mg/ml) [21].

The table (4) shows significant increase in the lactoferrin concentration in the milk of cows, ewes, and goats with mastitis in compare with healthy animals. This agreed with study of [22,23].

The mammary gland react with bacterial through a complex network of specific and nonspecific defense mechanisms. peptidoglycan, LPS, and lipoteichoic acid cause change in anatomical, cellular, and soluble Factors of mammary gland that act in a tightly coordinated fashion. Soluble factors involved in innate immunity are mainly peptides and proteins including, among others, lysozyme, lactoperoxidase, and lactoferrin [24].

Conclusion

In conclusion it can be said that the percentage of lactoferrin protein in milk is affected by the stages of milk production, as its concentration at the beginning differs from its concentration at the end. also so the lactoferrin concentration is high all mastitis cases in compare with healthy cases.

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Conflict of interest

There are no conflicts of interest to be declared.

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Author contributions

Conceptualization, study design and sample collection Muna H. Muna H. Shabeeb.

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TABLE 1. It shows the levels of lactoferrin in goat milk at different milking periods.

Goat	Day1	Week1	Week2	Week3
1	131.5	120.6	97.8	86.0
2	128.3	118.1	95.9	85.0
3	129.8	121.7	97.1	86.4
4	133.5	122.2	98.3	86.6
5	133.7	123.4	100.1	87.3
6	127.9	118.3	95.9	84.3
7	130.8	119.7	97.0	85.7
8	131.2	120.0	97.6	86.1
Total	130.8A	120.5 B	97.3 C	85.9 D

TABLE 2. It shows the levels of lactoferrin in Cow's milk at different milking periods.

Cow	Day1	Week1	Week2	Week3
1	43.6	40.5	36.9	33.5
2	40.9	38.7	33.4	29.3
3	42.7	39.3	35.9	32.5
4	45.2	42.1	39.5	36.7
5	44.3	41.8	37.0	34.2
6	44.0	40.9	36.2	31.8
7	43.1	41.0	36.1	33.9
8	45.4	41.2	40.3	36.3
Total	43.66A	40.6a	36.9 B	33.5 b

TABLE 3. It shows the levels of lactoferrin in Sheep milk at different milking periods.

Sheep	Day 1	Week1	Week2	Week3
1	144.9	139.8	124.5	99.2
2	145.4	140.4	124.8	99.7
3	146.2	141.0	125.6	100.1
4	147.1	142.2	126.9	101.4
5	142.8	139.5	122.4	97.6
6	143.3	141.3	123.7	98.5
7	143.1	140.5	122.9	97.9
8	144.5	142.7	126.0	98.8
Total	145.4 ^A	141.1 ^a	124.6 B	99.1 ^C

TABLE 4. The concentration of lactoferrin in the milk of cows, ewes, and goats with mastitis.

Milk from animal suffering from mastitis		Milk from healthy animals
Number of samples	Milk lactoferrin concentration Mean & standard deviation mg/ml	lactoferrin concentration
8 cows	90.14 \pm 0.22 A	33.5 B
8 ewes	220.98 \pm 3.30A	99.1B
8 goats	530 \pm 1.20A	98.9B

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تأثير فترة الرضاعة والتهاب الضرع على مستوى اللاكتوفيرين في حليب الأغنام والماعز والابقار

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الخلاصة

تم اخذ عينات الحليب من حيوانات المزارع الحيوانية في منطقة العلم شرق تكريت ، تم استخدام ٢٤ حيوان في التجربة، ٨ حيوانات من كل من الأغنام والابقار والماعز ، كما تمت دراسة ٢٤ حالة مصابة بالتهاب الضرع. تم اخذ الحليب من هذه الحيوانات وعلى فترات مختلفة وهي اول يوم ولادة وبعد اسبوع واسبوعين و ٣ أسابيع بعد الولادة. تم قياس نسبة اللاكتوفيرين في الحليب بواسطة فحص (HPLC) وكانت نسبة البروتين في اللبأ اعلى من نسبته في الحليب. وكذلك كان معدل نسبة البروتين في حليب الأغنام اعلى من نسبته في حليب الماعز اما في حليب الابقار فكانت نسبته الأقل . بلغ معدل تركيز بروتين اللاكتوفيرين في الأسبوع الثالث الابقار ٣٣,٥ جزء في المليون ويليها حليب الماعز بمعدل تركيز اعلى وهو ٨٦ جزء في المليون ثم حليب الأغنام وهي الأعلى معدل ويبلغ تركيز اللاكتوفيرين فيها ٩٩,٢ جزء في المليون . بينما كان معدل نسبة بروتين اللاكتوفيرين في حليب الابقار في الأسبوع الثاني من الولادة ٣٦,٩ وفي حليب الماعز ٩٧,٨ اما في حليب الأغنام فبلغت نسبة بروتين اللاكتوفيرين ١٢٤,٥ . وكانت نسبة اللاكتوفيرين الموجود في الحليب في الأسبوع الاول من الولادة اعلى والنتائج كالتالي في حليب الابقار ٤٠,٥ وفي حليب الماعز ١٢٠,٦ اما في حليب الأغنام ١٣٩,٨ . اما نتائج نسبة اللاكتوفيرين في الحليب في اليوم الاول من الولادة كانت الأعلى مقارنة مع بقية النتائج وهي كالتالي اقل نسبة في حليب الابقار وهي ٤٣,٦ ويليها حليب الماعز بنسبة ١٣١,٥ اما حليب الأغنام فهو الأغنى باللاكتوفيرين بنسبة ١٤٤,٩ . وفي نهاية الدراسة يمكن القول ان نسبة بروتين اللاكتوفيرين في الحليب تتأثر بمراحل انتاج الحليب إذ ان تركيزه في بدايتها يختلف عن تركيزه في نهايتها . كما أظهرت الدراسة ارتفاع واضح لنسبة اللاكتوفيرين عند الحيوانات المصابة بالتهاب الضرع مقارنة مع الحيوانات السليمة.

الكلمات المفتاحية : لاکتوفیرین ، حليب ، HPLC.