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THE CONCENTRATION OF LACTOFERRIN AND ITS RELATIONSHIP WITH MINERALS AND AMINO ACIDS IN COWS MILK

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Abstract

Lactoferrin is a biologically active glycoprotein of the transferrin family found mainly in milk and to a lesser extent in other biological fluids as well as the secondary granules of neutrophils. As an iron-binding molecule, Lactoferrin is involved in the transport and excretion of iron but also known to bind to proteins such as IgA, casein, lysozyme and to DNA. This study aimed at establishing the relationships between Lactoferrin in cow milk and the concentration of minerals and amino acids. Ten Holstein Frisian cows in their first lactation were used in this study. Colostrum sample were collected immediately after parturition and milk samples were collected weekly for the first 60 days of lactation. Concentrations of minerals and amino acids in colostrum and milk samples were determined and used to construct the relationships between these variable and Lactoferrin concentration. The concentration of Lactoferrin in the colostrum was higher (P<0.05) than in milk (732.8 vs 350.3mg /L). The concentration of Lactoferrin in milk declines with advancing lactation. The concentrations of some minerals (calcium, phosphorus, magnesium and iron) in colestrum were 130 mg / L, 0.82%, 76.00 mg / L and 16.52 ppm respectively. The correlation coefficient between the lactoferrin and calcium, phosphorus, magnesium were positive and signifecant namely, 0.665, 0.268 and 0.289 respectively. The correlation coefficient between lactoferrin and iron was negative and significant (- 0.614) . Moreover, the correlation coefficient between Lactoferrin and the methionine and Lucien were positive and highly significant (0.18125 and 0.33908 respectively), While with the non-essential amino acids was being positive and significant. It conclusion, lactoferrin has a tight relationship with main milk components, being has an important role for birth calves through its high percentages in colostrum. Also, the repeatability between lactoferrin and minerals has a pronounced importance for birth calves growth.

Key words: *Lactoferrin, component minerals, amino acid, cow milk.*

INTRODUCTION

Both milk and colostrum provide a complete food source for newborn calves. The colostrum is considered as the only source of primary acquired immunity for the newborn. The immune secretions in the breast, will be eliminated by specialized receptors, both colostrum and milk contain viable cells, which include cytokines, proteins and antimicrobial peptides such as lactoferrin, defenses, cathelicidins. The immune system in the colostrum is associated with the ability of the new born's intestine, allowing the passage of large molecules such as alklopeulenat molecules, as the concentrated immunity in the colostrum and intestinal permeability decreases rapidly and gradually in the first 48 hours of the baby's life (Moore et al., 2005), so it is necessary for new born calves to address an adequate amount of the colostrum during the period of his life to gain negative immunity and to be able to survive until the autoimmune system is fully develop. The immune factors, in milk and colostrum play an important role in defending the protected newborn from pathogenic organisms (Boyso – Oviedo et al., 2007.

The innate immune system is the essential line is to protect the body from infectious pathogens before the immune system in the saliva is initiated, as it represents a complex interaction between the cellular and molecular processes, which aims to discover the causes of harmful diseases and eliminating them at a later time, having the innate immune system evolution of the cow's udder to a highly effective defense mechanism in the host (Rainard and Riolled, 2006), it has also been assumed that the udder itself may be an extension for the innate immune system (Vorbach et al., 2006).

One of the most significant immune factors is the lactoferrin protein which is found in colostrum and milk in a number of mammals, as well as physiological fluids, but in different concentrations, so their concentrations in colostrum and milk is higher than the rest of the fluid as well as the concentration of lactoferrin protein, depending on the type of animal, also the different concentrations of immune factors in cow's milk during sickness or healthy cases. In one of the studies milk samples were taken from healthy cows (189 cows), the results showed the presence of more than 20-folds of concentrated lactoferrin. Similarly, many differences occurred in the concentrations of individual Lactoperoxidase in goats and cow's milk (Fonteh et al., 2002). Such a large variation in animals suggests an opportunity to determine where the milk's immune factors would be higher so that we can create herds for commercial exploitation.

Therefore the lactoferrin protein (Lf) is one of the specialized immune proteins and outstanding high energetic effectively through its ability to bind iron, as it is considered one of the proteins sugary basal (Basic glycol-proteins) and a member of a family of proteins Transferrin type. (Jenssen and Hancock, 2009), also this protein has attracted the attention of researchers to study and try to increase the effectiveness of vital during digestion, it emerged as an increase in the effectiveness of the dissolved lactoferrin protein, either by physical or enzymatically by 8-10 times more compared with the natural protein lactoferrin protein (Van der Kraan et al., 2004; Gifford et al., 2005; Flores-Villasenor et al., 2010). This study aims to find the relationship between the concentration of the lactoferrin protein and the minerals, amino acids in colostrum and milk for the usage in increasing the immunity of calves and its better growth.

MATERIALS AND METHODS

This study was conducted at Al-Naser Dairy Cattle Station / Aswera (50 km South of Baghdad) during the period from 15/1/2013 to 1/4/2013. The heifers were inseminated at the age of 16-18 month and not less than 370 kg body weight. Ten cows were randomly selected (at first lactation) to determine the milk lactoferrin concentrations and its contents of amino acid also some mineral in milk (Ca, P, Mg and Fe). Sample of milk were collected fortnightly (after one day of birth) for two month. The calcium, phosphorus and magnesium in milk were estimated according to Richards (1954). Cations were determined according the following equation:

$$\text{ml} - \text{eqw. Ca/L} = \frac{\text{Fercent volume x titer}}{\text{Volume of filtrate}} \times 1000$$

The magnesium was not t estimated directly from the filtrate, so it firstly estimated the calcium concentration, and then calcium and magnesium collectively according the equation:

$$\text{ml} - \text{eqw. Ca} + \text{mg/L} = \frac{\text{Fercent volume x titer}}{\text{Volume of filtrate}} \times 1000$$

Then from the deference get mg concentration.

P: concentration was calculated according to Olsone et al. (1961) method. The Fe was calculated using atomic absorption from the following method:

Fe	Wave length	Fuel ratio	Slit nm	mA
	248.3	2.0A-Ac	0.5	4.0

Amino acids concentration was determined using HPLC according to the Feng et.al.(2004) and calculated from the following equation:

$$\text{Acid conc. } \mu\text{g/ml} = \frac{\text{F Bundle area of the sample}}{\text{St bundle area}} \text{st. conc. XNo of dilution}$$

St.bundle area

HPLC were used to determined lactoferrin concentration according to the following equation :

$$\text{Lactoferrin conc. } \mu\text{g/ml} = \frac{\text{Bundle area of the sample}}{\text{St bundle area}} \text{st. conc. XNo of dilution}$$

Statistical analysis:

SAS (2010) were used to detect the effect of week and month effects of lactoferrin on some production, traits Duncan test (1955) was used to compare among means.

The correlation coefficient between lactoferrin and each of amino acids and some minerals were calculated under 0.05-0.01 probability.

RESULTS AND DISCUSSIONS

Milk production: daily milk production was recorded rather than monthly milk production. Daily milk production means were 16.95±0.84; 18.50±84 kg respectively while monthly milk production means were 508.50±25.31; 555.0±25.39, respectively for two month of study.

Table 1. Daily and monthly milk production (kg) of cow during experiment

Milk production	Milk amount ±**se**
Daily milk (1)	16.95 ± 0.84
Daily milk (2)	18.50±0.84
Milk production in 1st month	508.50±25.31
Milk production in 2nd month	555.00±25.39

(1) and (2) daily milk in 1st and 2nd month

Milk lactoferrin concentrations:

Table 2 showed that the colostrum lactoferrin concentration is higher than its concentrations in the milk. The results also observed that the lactoferrin concentration decreased with increasing milk production, and this is consistent with those reported by Hiss et al. (2009), Piccinini et al. (2007) that the lactoferrin is higher in the early days of breastfeeding. This increasing may be due to the several reasons, including its contribution in transferring mineral elements, especially iron to the new born calves through the milk, as well as its participating in the growth of bacteria symbiotic in the intestines of the calves, along with its role in the inhibition of the pathogenic bacteria proliferation in the intestine, as well as serving as a counter and others antioxidant the anticlotting through susceptibility of lactoferrin to connect iron ion (Huang et al., 1999) as well as that of the immune proteins specialized to play an important role in the incidence of various infections, it also helps the blood to compose in the spinal cord and helps to reduce the level of iron level in the blood (Wong et al., 2009; Wang and Hurly, 1998) its concentration also increases during infection. It is noted that there is a highly significant relationship ($P <0.01$) between the lactoferrin and weeks of production**.**

Table 2 shows the amount of lactoferrin in cow's milk.

Table 2. The amount of lactoferrin in cow's milk

period	Lactoferrin	Sig.
colostrum	732.78±28.03a	**
2 week	604.60±23.94b	**
4 week	541.11± 19.20 c	**
6 week	402.67±17.87d	**
8 week	350.31± 16.24 d	*

Means in the same column with different superscripts were significantly different ($P < 0.05$).

Minerals in milk: as shown in Table 3 the colostrum content of calcium was higher than this content of calcium in normal milk being 130±11.70 mg/L and then this amount was gradually decreased as calcium is very important element for all organism bodies, for example calcium is major constituent of bones and teeth in addition to its role is most of metabolic activities inside the body. However calcium have important role is transformation of neural signs and natural activity of muscle, blood clotting and the activation of certain enzyme and hormones. On other hands there was a decrease in calcium level in coincidence with the increase of milk production.

The content of magnesium in milk has similar trend of calcium (Table 3) but the level of magnesium in colostrum was higher than its content in normal milk being (76.00±3.03 mg/L), and then the level of magnesium in colostrum gradually decreased in coincidence with the increase of the amount of milk production. The level of magnesium at parturition is a reflexion of fetal response to dame status during pregnancy. However the effect of magnesium is very big during first week of life as it reverse. The reverse of independent life of intent in the absence of direct factors that transported by dam blood. Certain studies indicated that magnesium affect on neuron excitation and response it has main role in activating of some enzyme , And that the magnesium element role in protein synthesis digest vitamin As well as it immune function and this explains the high value Magnesium element in the colostrum in the first few weeks of suckling (Spear and Weiss, 2008).

As for the iron element was accounted in colostrum, it is less than the rate of milk as the percentage (16.52±0.72). The reason may be due to the low percentage of iron in the milk that it is one of the encouraging factors on oxidative stress (Huang et al., 1999). And it is one of the factors that help fat oxidation to be the roots of ion ferrous accelerates oxidation by breaking down peroxides it decomposition of hydrogen peroxide into an effective free radicals (Miller et al., 1996). Also show in the table to iron ratio began increase with the increase in milk production and because it is an important element being included in the composition of and because it is an important element being included in the hemoglobin which enters in the composition of the blood protein also enters in the composition of respiratory enzymes which present in mitochondria. As well as Table 3 shows that the percentage of phosphorus element in colostrum (0.80±0.01) compared with milk, we note that the ratio of phosphorus have declined after two weeks coincides with the increase in the quantity of milk product. It may be due to the high percentage of phosphorus element in colostrum to the importance of this element in the body It is important as a vital element enters with calcium in bone formation, proteins and fats, and enters in the composition of some co-enzymes. There are also some with and some types of sources of the energy stored in the compound energy adenosine tri-phosphate (ATP). Each of calcium, phosphorus plays a vital role in most tissues of the body with roles with structural roles in cell membranes, also participating in cellular processes, bones and teeth (McDowell and Arthington, 2005). Underwood and Suttle (1999) showed the possibilities DNA synthesis, contact between the cells, the cell membrane fluidity, tracks metabolic.

Essential amino acids concentrations in milk

Table 4 explains the absence of significant differences observed for the histidine acid, which shows its concentration in colostrum to be higher than its concentration in milk (239.96 ±62.67). As for the threonine acid, its concentration in colostrum is also higher than the normal milk, but there were no significant differences observed, the results of both (tryptophan, tyrosine) acids show that their concentrations in the colostrums is higher than the normal concentration in milk, reaching proportions in colostrum to (251.80±61.92; 258.80±42.65). Significant differences were observed between the averages of the acid concentrations observed during periods of the study. The valine acid' concentration in colostrum is less than the concentration in breast milk, its concentration in the colostrum is (149.80 ±39.21), this is also observed for the methionine acid which contains a concentration of (223.49± 49.48), with no significant differences observed. The results also showed that the amino acids (isoleucine, leucine, lysine and valine), their concentrations in the colostrum was higher than the normal concentration in milk, reaching their concentration in the colostrum to 451.50+119.04; 551.50+156.20; 591+117.31; 939.80+153.65), respectively.

Non-essential amino acids concentrations in the milk:

The results showed that the concentration of amino acids in cow's milk, varies from period to another during the experiment, due to the nutrition, also the aspartic acid observed to be high at ($P < 0.01$) colostrum (233.04± 33.96), also the results showed no significant differences in the presence of glutamic acid. As for the glycine acid, its amount in the colostrum was higher than the rate in the natural milk, reaching per colostrum to 320.76± 118.84.

It was noted that the presence of high significant differences for this acid in milk, when compared to the averages during the experiment, as for the arginine acid, its concentration in the colostrum was higher than the rate in the normal milk as well as for alanine reaching their percentage in the colostrum to 173.50+34.04; 367.76+86.40, respectively.

The results showed highly significant differences in amino acids concentration in milk. It is possible that the reason behind the fluctuation in the concentration of amino acids, is the nutrition, as well as to the viability of microorganisms to manufacture amino acids and the proportion of acids that reaches the mammary gland, and that the high concentration of certain amino acids in the colostrum of that in breast milk could have caused due to the importance of these acids in growth as interference in the formation of the protein. The presence of the amino acids, arginine and lysine at a high concentration in the colostrum, may be due to their importance, they are considered one of the significant acids of the lactoferrin polypeptide protein which give it a positive charge of these acids that play an important role in giving adjective positive for peptides and easily chemically linked (Dionysius & Milne chemically bacteria cell wall, 1997), where the amino acids, containing multi-negatively charged fatty sugars such as (aspartic, histidine and tyrosine) in linking iron ion in comprising a strong complex The arginine forms a strong component of a complex CO3-2 linking carbonate ions (Baker, 1994).

The correlations between lactoferrin and some productivity the qualities

The results showed that there is a negative relationship between the lactoferrin and daily as well as monthly milk yield, the value of the correlation coefficient between the total lactoferrin and daily as well as monthly milk yield were 0.065 and 0.652 respectively (Table 6) there were significant differences between lactoferrin and milk yield. The results also showed the presence of correlation between the lactoferrin ratios milk components as well as in relation to the minerals (calcium, phosphorus, magnesium) and there is a correlation between lactoferrin and the iron has been related to the negative as a decline in the concentration of lactoferrin accompanied by a rise in the proportion of iron in the milk, which is linked to the production of milk.

The correlation coefficient between lacto-ferrin and essential amino acids:

The results in the Table 7 show a significant correlation between the protein lactoferrin and some Essential amino acids found in milk, Informed the correlation coefficient between the lactoferrin and essential amino acids (His, Thre, Trp, Tyro, Val 0.24, 0.006, 0.30, 0.31, and 0.25) respectively.

The table shows a positive and highly significant link between lactoferrin and methionine acid. The same applies to the Lucien and methionine acids the correlation coefficient with lactoferrin, 0.18, 0.33 respectively. It was also shown that the amino acids are associated positive and significant with Lactoferrin, This means that any increase in the concentration of lactoferrin, accompanied by an increase in the concentration of essential amino acids in milk.

The correlation coefficient between lacto-ferrin and non-essential amino acids in milk:

The results in Table 8 shows that the correlation coefficient between lactoferrin and non-essential amino acids (Glycine, Glutamic acid) found in milk was positive and high significant, the correlation coefficient was 0.36 and 0.35 respectively. The link between lactoferrin and the other non-essential amino acid has been positive and significant as shown in the Table 8. This may be due to the importance of amino acids in the body-building protein to the fact that amino acids are the structural units of protein.

Table 3. Some of the minerals in cow milk during the study

Period	Ca mg/L	Mg mg/L	Fe ppm	K %
Colostrum	130.0±11.70a	76.00±3.05a	16.52±0.72a	0.82±0.10a
2 weeks	78.50±8.88b	58.00±7.27b	18.74±0.27b	0.39±0.01b
4 weeks	64.50±6.43bc	40.50±5.84c	17.70±0.33bc	0.39±0.06c
6 weeks	58.00±3.51c	44.00±7.29cd	19.69±0.53c	0.38±0.06c
8 weeks	49.50±4.5 c	47.00±3.00d	22.44±0.95d	0.32±0.02c

Means in the same row with different superscripts were significantly different (P < 0.05).

Table 4. Essential amino acids concentration of (mg \ liter) in cow's milk during the period of study.

acid	colostrum	2 weeks	4week	6 weeks	8weeks	Sig.
HIS	367.80±86.40^{a}	157.50±28.59a	191.20±44.59a	174.35±35.31a	115.10±16.81a	*
Thre	173.50±34.04^{a}	90.80±14.95ab	114.20±35.96a	148.00±35.96	105.10±18.37b	*
Trp	251.80±61.92^{a}	175.70±39.83^{ab}	118.70±32.83^{b}	130.80 ± 21.79^{b}	121.17±17.13^{b}	*
Tyro	149.80±39.21^{a}	125.00±23.00^{ab}	166.70±46.65^{b}	130.80 ±20.79a	119.00±12.09a	*
Val	223.49±49.48^{a}	133.90±34.05a	111.00±25.19a	311.30±133.83a	221.50±47.10a	*
Met	982.90±233.24^{a}	277.00±117.30a	184.30±47.78a	158.40±34.26a	141.20±27.10a	*
Iso	592.10±117.31^{a}	818.60±76.77^{ab}	297.20±45.10^{b}	806.10±123.49^{b}	224.00±40.93^{b}	*
Leu	551.50±156.20^{a}	395.10±64.17^{ab}	209.40±49.87^{b}	402.60±138.66^{b}	141.10±23.61^{b}	*
Ph	451.50±119.14^{a}	689.30±159.14^{ab}	293.20±29.89^{b}	712.50±152.66^{b}	195.10±26.95^{b}	*
Ly	113.26±21.62a	457±30119.04^{ab}	408.80±63.71^{b}	318.90±111.81^{b}	169.90±26.95^{b}	*

Means in the same row with different superscripts were significantly different (P < 0.05).

Table 5. Non-essential amino acid concentrations(mg/liter) in cow's milk during the study.

acids	colostrum	2 weeks	4 weeks	6 weeks	8 weeks	Sign.
ASP	233.04 ± 33.96^{a}	278.30 ± 26.89^{a}	166.70 ± 42.82^{ab}	198.20 ± 36.79^{ab}	145.00 ± 21.31^{b}	*
GLU	113.26 ± 21.26^{a}	162.10 ± 20.02^{b}	97.20 ± 15.57^{bc}	100.70 ± 26.12^{bc}	76.40 ± 10.35^{c}	*
SER	320.76 ± 118.84^{a}	106.40 ± 17.56^{a}	73.70 ± 26.93^{a}	92.20 ± 24.12^{a}	81.00 ± 13.74^{a}	NS
GLY	239.96±62.67a	$109 \pm 20 \pm 13.38^{ab}$	160.70 ± 36.05^{b}	100.90 ± 24.26^{b}	83.50 ± 31.44^{b}	*
Arg	148.60±33.46a	139.50 ± 25.44^{ab}	222.80 ± 44.54^{b}	313.60 ± 62.03^{b}	206.60 ± 17.93^{a}	*
Pro	285.80±42.65a	106.30 ± 18.31^{ab}	104.20 ± 27.90^{b}	179.80 ± 33.80^{bc}	915.91 ± 90.90^{c}	*
Cyst	939.80 ± 153.65^{a}	517.80 ± 218.11^{a}	513.90 ± 123.91^{a}	517.80 ± 218.11^{a}	410.90 ± 51.20^{a}	NS
Ala	204.00 ± 39.94^{a}	160.60 ± 22.34^{b}	111.70 ± 32.35^{b}	160.60 ± 22.34^{b}	79.30 ± 13.86^{b}	*

Means in the same row with different superscripts were significantly different ($P < 0.05$).

Table 6. Coloration coefficient between lactoferrin, milk production and some minerals

The adjectives	Corrol. coef.	Sign.level
Lactoferrin &daily milk production	-- 0.065	*
Lactoferrin &monthly milk production	-- 0.652	*
Lactoferrin & calcium	0.665	*
Lactoferrin & phosphorus	0.268	*
Lactoferrin &magnesium	0.289	*
Lactoferrin & ferrous	– 0.614	*

** Sign < 0.01 * Sign. P< 0.05

Table 7. Correlation coefficient between lactoferrin and essential amino acids

The adjectives	Correlation coefficient	Sign. level
Lactoferrin & His	0.24694	*
Lactoferrin &Thre.	0.00679	*
Lactoferrin & Trp.	0.30260	*
Lactoferrin &Tyro.	0.31887	*
Lactoferrin &Val.	0.25665	*
Lactoferrin &Met.	0.18125	**
Lactoferrin & EL. acid	0.39654	*
Lactoferrin & Leu.	0.33908	**
Lactoferrin & Phenil.	0.23851	*
Lactoferrin & Lys.	0.35479	*

** Sign < 0.01 * Sign. P< 0.05

Table 8. Coloration coefficient between lactoferrin and non-essential amino acids

The adjectives	Corre. Coeff.	Sign. level
Lactoferrin & ASP acid	0.17	*
Lactoferrin &Glu acid	0.36	**
Lactoferrin &Ser acid	0.17	*
Lactoferrin &Gly acid	0.35	**
Lactoferrin &Arg acid	0.08	*
Lactoferrin &Ala acid	0.17	*
Lactoferrin &Pro acid	0.17	*
Lactoferrin &Cyst acid	0.29	*

** Sign < 0.01 * Sign. P< 0.05

CONCLUSIONS

The results showed that the concentration of amino acids in cow's milk, varies from period to another during the experiment, due to the nutrition, also the aspartic acid observed to be high at (P <0.01) colostrum (233.04± 33.96), also the results showed no significant differences in the presence of glutamic acid. There is a correlation between the lactoferrin ratios milk components as well as in relation to the minerals (calcium, phosphorus, magnesium). It was also shown that the amino acids are associated positive and significant with Lactoferrin. This means that any increase in the concentration of lactoferrin, accompanied by an increase in the concentration of essential amino acids in milk . The link between lactoferrin and the other non-essential amino acid has been positive and significant.

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