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Original Research

# Association of lactoferrin with some immunological and blood traits of Holstein calves in the middle of Iraq

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**ABSTRACT:**

This study was carried out at the Al-Salam dairy farm, at Latifiya, 25 km south of Baghdad. The laboratory experiments on blood analysis were conducted for a period from 3 September 2017 to 11 December 2017, for investigating the effect of adding different levels of lactoferrin (0, 3 and 6 g Lf/day) to colostrum and milk in immunity and a number of blood traits in 18 Holstein calves from birth to 60 days. The results of the study showed significant differences (P<0.05) in the concentration of IgG in calves' blood, with increased lactoferrin concentration level at the age of 30 days in calves' blood of the control group, second and third treatment, 10.13±0.52, 11.90±0.72 and 12.67±0.63 mg/m respectively. IgM and IgA concentrations were not affected by the treatments at the age of 30 days. At the age of 60 days, the differences were significant (P<0.05) in IgG and IgM for the third treatment calves (6 g Lf), then for the second treatment calves (3 g Lf), while the lowest concentrations for the control group. The results of the this study showed significant differences (P<0.05) in the transferrin concentration of the calves blood at 30 and 60 days for the control group (without Lf) compared with the calves of the second treatment groups and the third. The iron level in the blood at the age of 60 days were also significant (P<0.01), where the concentration increased by increasing the level of lactoferrin and reached maximum (140.00±0.67 mcg/ml) for the third treatment of the calves and below (141.70±1.14 mcg/ml) in the control group. The Total Iron Binding Capacity (TIBC) and transferrin saturation ratio were not significantly affected by the addition of lactoferrin. There seen a significant difference (P<0.05) in the logarithm of *E. coli* bacteria in the feces of calves according to the treatment given.

**Keywords:**
Lactoferrin, Immunity globulins, Holstein calves.

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## INTRODUCTION

The purpose of obtaining high production efficiency in milk and meat needs more attention on the infant calves. It is the nucleus of the herd through which optimum production can be accessed. Intestinal diseases and inefficiency of the immune system in the born calves are major causes of high losses (Sangild *et al*., 2000). Colostrum and milk are the integrated food of newly born calves, as colostrum is the only food source of initial acquired immunity (Barrington and Parish, 2001), because, it contains high protein and minerals content compared with milk (Foley and Otterby, 1978). The colostrum proteins include immunoglobulin, which the newborn calf can take advantage of it in the first three days after borne for intestinal containment of the receptors, or so-called sites of the absorption of immunoglobulin. Both the concentration of immunity in colostrum and intestinal permeability decreases rapidly and gradually within the first 24 hours after birth (Moore *et al*., 2005). It is therefore necessary in this short period to feed the calf with sufficient amount of colostrum to gain immunity until it develops its own immune system (Wheeler *et al*., 2007), Colostrum components also develop the digestive system (Odle *et al*., 1996; Blum and Hammon, 2000), and the absorptive capacity of the intestines (Rauprich *et al*., 2000). Lactoferrin (LF) is an important part of the immunoglobulins, which are found in colostrum and milk of many mammals. It is present as well as of different concentrations in the body fluids and external secretions, but its concentration in colostrum and milk is higher than the another liquids (Masson and Heremans, 1971; Brock, 1980). The concentration of lactoferrin varies according to the type of animal and its offspring. For example, in cow's milk, it varies depending on the type of cow and the amount of milk produced (Fonteh *et al*., 2002). Lactoferrin is the first innate immune system produced from white blood cells (specifically granular cells neutrophils). It is the pathogens that control the amount of lactoferrin, as it is observed to increase its concentration with inflammation or virus infection (Kanyshkova *et al*., 2001). The primary function of it is to protect the mammary gland after birth (Legrand *et al*., 2005). In addition, lactoferrin is a specialized immune protein characterized by high bioactivity through its ability to bind iron and a member of the family of transferrin proteins (Jenssen and Hancock, 2009).

Due to the wide biologic importance of lactoferrin, it has attracted the attention of a large number of researchers to benefit from its effectiveness. The first international conference of lactoferrin was held in the United States in 1992, followed by periodic international conferences, the second in the United States of America in 2005, France in 2007, China in 2009, Mexico in 2011, Italy in 2013, Japan in 2015, The latest of which was the Thirteenth Congress, held in Rome, Italy, in 2017, which included a review of the latest research and development of lactoferrin in terms of structural structure and functions (Shimazaki and Kawai, 2017). The importance of this protein and its uses are not much researched in Iraq; to indicate its effect of adding in the feeding of Holstein calves, this study was carried out.

### Objectives

- To study the effect of lactoferrin in the immunoglobulin (IgG, IgM and IgA) of serum for calves.
- To study the effect of lactoferrin in the concentration of iron and transferrin and transferase saturation ratio and (total iron binding capacity) in the blood of calves.
- To study the effect of lactoferrin in the numbers of *E. coli* bacteria in the feces calves.

## MATERIALS AND METHODS

The study was carried out at Al-Salam National Dairy farms in Latifiya (25 km south of Baghdad governorate), which includes a herd of Holstein cows. 18 calves (10 females + 8 males) at one day old weighing 35±2 kg were used for the experiment for the period

from 3 September to 11 December 2017. The calves were transformed from the birth barn to a closed barn, they remained there until weaning age. Artificial feeding was adopted to feed calves, using a bucket for each calf. The calves were fed on the colostrum during the first three days at the rate of 4 liters/calf/d. divided for two time, as 2 liters for morning and evening. With the provision of clean water continuously, after the end of the colostrum, the newborn was fed raw milk, which is taken from the cows with the amount of 4 l/calf divided into two halves, two liter at morning and two liter at evening/calf until 60 days of age. In the second week of age, the concentrate and the hay were given in small quantities for calves to eat early. Calves were under veterinary care throughout the experiment. The calves were divided into three equal and random groups. Each group had 6 calves, the first group was the control group (without adding lactoferrin in feeding), the 2nd and 3rd groups, were supplemented with lactoferrin powder at the concentration of 3 and 6 g/day/calf respectively with milk at morning meal, from 1 to 60 days. Lactoferrin powder is dissolved in colostrum or milk which is used for nutrition. (Connelly and Erickson, 2016)

Blood sample were collected from jugular vein (10 ml) of the calves in the experimental period at 30 and 60 days. Samples were collected in 10 ml tubes, each tube were marked with calf number and date of the blood collection. After the completion of the blood collection, the tubes were left for 15-20 minutes, then the serum was separated from the blood by centrifugation at a speed of 3500 cycles/m for 15 minutes. The tubes were then transferred to a laboratory by cooled box with a temperature 3-4°C for laboratory analysis. (Carcanglu and Pazzola, 2002).

**Measuring the concentration of IgG, IgM and IgA**

IgG immunoglobulin concentration was measured using the radiocarbon plate method (Radial immune diffusion plate RID), A kit from the Italian company LTA, which contains a 15-point board and each locus contains the agarose gel, which contains the Goat antiserum IgG. The operation was done in accordance with the attached leaflet for the kit manufacturer of this test. The diameter of the loop was compared with the concentration in the calibration table attached to the kit to determine the IgG concentration for each sample. The measurement of IgM concentration was followed by the same method of action recorded in the leaflet attached to the kit for this test. An IgM kit containing the goat antisera IgM gel was used from the same company (Italy LTA).

A special kit from the same company was used to measure the concentration of IgA. The tablet contains an agarose gel but consists of Goat antiserum IgA. The method of work was followed in accordance with the Kit attached and similar to the method used in the measurement of IgG ANOVA except for leaving the dish after the samples were placed for 96 hours at room temperature. (Mancini *et al*., 1965; Fahey and McKelvey,1965)

**Measurement of iron concentration**

The analysis of the concentration of iron was done according to the method of BIOLABO kit using, the steps prescribed in the kit which has been prepared from three solutions (Sample Assas, Standard, Blank).

200 microliters of the calf serum sample were placed in the sample and then was used as a chemical analyzer KENZA 240 TX, French origin and is produced by a French company- BIOLABO. (Tietz, 1999).

**Transferrin concentration measurement**

Transferrin concentration was measured in a national laboratory using the US Stat-2400 device, and according to the K-ASSAY method and the steps associated with its kit. **(**Lizana and Hellina, 1974; Stremberg, 1977)

**Total iron binding capacity measurement**

The TIBC analysis was carried out according to the BIOLABO method and the steps attached to its kit, using the French KENZA MAX Biochemistry produced

by the French company, BIOLABO (Tietz, 1999, 2006).

**Transferrin saturation measurement:**

The following equation is used

$$\text{Transferrin saturation ratio} = \frac{\text{Fe concentration}}{\text{TIBC}} \times 100$$

**Statistical Analysis**

SAS 2012 was used to analysis the data to study the effect of different transactions in the studied characteristics according to a Complete Random Design (CRD), and compared the differences between the averages by Duncan Multiple range test 1955.

Statistical Model :

$Yij = \mu + Ti + eij$

## RESULTS AND DISCUSSION

**Effect of studied treatments on the concentration of globulins in the blood of calves at the age of 30 days**

The results of the study showed a significant superiority ($P < 0.05$) in the concentration of IgG in the blood of the calves of the 2nd (3 g Lf) and 3rd groups (6 g Lf) on compared to the control group (without Lf) at the age of 30 days. The average IgG concentration in the second and third groups were 11.90±0.72 and 12.67±0.63 mg/ml, respectively, while the average IgG concentration in the calves blood for control group was 10.13±0.52 mg/ml (Table 1). On the other hand, the results of the study showed no significant differences at the age of 30 days in the concentration of IgM in the blood of the calves of the three groups, a control and , 2nd and the 3rd group. The average concentration of IgM in the blood of the calves was 0.68±0.02, 0.62±0.04 and 0.65±0.02 mg/ml, respectively (Table 1).

The results showed no significant differences in the concentration of IgA in the blood between the calves of the three groups, the control group, the 2nd and the 3rd group at the age of 30 days, which showed an IgA concentration of 0.089±0.001, 0.081±0.002 and 0.083±0.001 mg/ml, respectively (Table 1). This is compatible with the results of Prgomet *et al*. (2007) who conducted study on a group of Friesian calves. There seen a significant increase in the concentration of IgG in the blood when lactoferrin was added to the diet of those calves. As the researcher noted, this increase may be due to the effect of lactoferrin in improving and increasing the intestinal absorption of IgG, also lactoferrin regulates the immune system and stimulates to produce plasma B cells through which Ig molecules are synthesized (Hurley, 2003). This finding is consistent with the results of Prenner *et al*. (2007), Shea *et al*. (2009) and Comstock *et al*. (2014). The results of their study showed that the addition of lactoferrin in the nutrition of newborns may lead to increase efficiency of absorption of IgG and then increase its concentration in the blood.

**Effect of treatments on the concentration of the immunoglobulin of the calves at the age of 60 days**

Table 2 shows significant differences ($P<0.05$) in IgG blood concentration at the age of 60 days in favour of calves of the 3rd group (6 g Lf) compared to control calves (without Lf). The concentration of IgG in third-calves treated blood was 21.06±0.94 mg/ml, while the concentration of IgG in the calves blood of the control group was 19.23±0.81 mg/ml. In the same contex, the second treated calves (3 g Lf) recorded an average of 20.09±0.72 mg/ml of IgG concentration in their calves' blood. On the other hand, the results of the study

**Table 1. Effect of treatments on the concentration of immunoglobulin in the blood of calves at the age of 30 days**

| S. No | Treatment | Mean±SE (mg/ml) | | |
|---|---|---|---|---|
| | | IgG | IgM | IgA |
| 1 | Control (without Lf) | 10.13±0.52[b] | 0.68±0.02[a] | 0.089±0.001[a] |
| 2 | Lactoferrin (3gm/day) | 11.90±0.72[a] | 0.62±0.04[a] | 0.081±0.002[a] |
| 3 | Lactoferrin (6gm/day) | 12.76±0.63[a] | 0.65±0.02[a] | 0.083±0.001[a] |
| | | * | NS | NS |

NS: non significant; *($P<0.05$): The averages with different letters within the same column vary significantly between them.

**Table 2. Effect of treatments on the concentration of blood immunoglobulin in calves at the age of 60 days**

| S. No | Treatment | Mean±SE (mg/ml) | | |
|---|---|---|---|---|
| | | IgG | IgM | IgA |
| 1 | Control (without Lf) | 19.23±0.81[b] | 1.63±0.14[b] | 0.25±0.04[a] |
| 2 | Lactoferrin (3gm/day) | 20.09±0.72[ab] | 1.76±0.09[a] | 0.23±0.04[a] |
| 3 | Lactoferrin (6gm/day) | 21.06±0.94[a] | 1.81±0.09[a] | 0.27±0.05[a] |
| | | * | NS | NS |

NS: non significant; *(P<0.05): The averages with different letters within the same column vary significantly between them.

showed a significant difference (P<0.05) in the concentration of IgM at age of 60 day for the calves of the 2nd and 3rd groups (1.76±0.09 and 1.81±0.09 mg/ml, respectively) and on the calves treated at the control group (1.63±0.14 mg/ml).

No significant differences in IgM concentration were observed between the calves of the 2nd and 3rd treatment groups at the same age (Table 2).

On the other hand, the results of the study showed no significant differences at the age of 60 day in the blood IgA concentration between the calves of the three treatment groups *viz*., control; 2nd and 3rd (table 2).

These results were consistent with the results of the Prenner *et al*. (2007) study, which showed an increase in the concentration of IgG in calves' blood when lactoferrin supplementation was added. In addition to this, results of the Comstock *et al*. (2014), showed an increased in both IgG and IgM concentrations in the newborn blood when lactoferrin were supplemented through the feed. The increase may be due to the effect of positive lactoferrin on regulating the body's immune system and stimulating the production of plasma B cells through which Ig molecules are produced (Hurley, 2003). It is also due to the effect of lactoferrin in the activation and improvement of intestinal absorption of immunoglobulin in the colostrum and milk which were fed to those calves. In addition, Prgomet *et al*. (2007) noted that the addition of lactoferrin in calf feeds may promote an increased level of serum immunoglobulin (Ig).

**Effect of treatments on the concentration of transferrin, iron, TIBC and transferrin saturation at 30 days of age**

The results of the present study showed that there was a significant different (P<0.05) in the transferrin concentration of the blood at the age of 30 days in the calves of the control groups (without Lf) and the 2nd group (3 g Lf). Transferrin concentration was 3.00±0.18 and 2.90±0.13 mg/ml respectively compared with the 3rd group calves (6 g Lf) (2.50±0.11 mg/ml). In the same context, there was no significant difference between the calves of the 2nd and 3rd treatments group in the transferrin concentration at the age of 30 days (Table 3). Transferrin concentration in the three calves groups was within the normal limits. Moser *et al*. (1994) indicated that transferrin concentration in the blood of healthy calves and cows was 2.0-6.6 mg/ml. In small animals it is little lower. On the other hand, the results of the study showed no significant differences at the age of 30 days in the concentration of iron in the blood of calves between the three groups of transactions, control group, 2nd and 3rd, with the mean iron concentration in the blood of their calves, with the values of 137.60±0.94, 138.40±1.03 and 140.00±0.67 µg/dL

**Table 3. Effect of transferrin concentration, iron, TIBC, and transferrin saturation at the age of 30 days**

| S. No | Treatment | Mean±SE | | | Transferrin saturation% |
|---|---|---|---|---|---|
| | | Transferrin (mg/ml) | Fe (µg/dL) | TIBC[1] (µg/dL) | |
| 1 | Control (without Lf) | 3.00±0.18[a] | 137.60±0.94[a] | 425.07±10.17[a] | 32.52±1.11a |
| 2 | Lactoferrin (3gm/day) | 2.90±0.13[a] | 138.40±1.03[a] | 421.52±9.54[a] | 32.12±0.82a |
| 3 | Lactoferrin (6gm/day) | 2.50±0.11[b] | 140.00±0.67[a] | 416.30±10.72[a] | 33.21±0.93a |
| | | * | NS | NS | NS |

NS: non significant; *(P<0.05): The averages with different letters within the same column vary significantly between them.

**Table 4. Effect of transferrin concentration, iron, TIBC, and transferrin saturation at the age of 60 days**

| S. No | Treatment | Mean±SE | | | Transferrin saturation% |
|---|---|---|---|---|---|
| | | Transferrin (mg/ml) | Fe (µg/dL) | TIBC[1] (µg/dL) | |
| 1 | Control (without Lf) | 3.62±0.13[a] | 141.70±0.94[a] | 433.20±15.09[a] | 32.39±1.49[a] |
| 2 | Lactoferrin (3gm/day) | 3.11±0.12[a] | 153.00±1.03[a] | 425.10±12.68[a] | 35.47±1.12[a] |
| 3 | Lactoferrin (6gm/day) | 3.07±0.13[b] | 155.70±0.67[a] | 422.60±15.51[a] | 36.80±1.21[a] |
| | | * | NS | NS | NS |

NS: non significant; *(P<0.05): The averages with different letters within the same column vary significantly between them.

respectively (Table 3). Studies showed that the normal level of iron concentration in cows is 57-162 µg/dL (Radostits *et al*., 2000). While the results of the Osman and Al-Busadah (2003) showed that iron concentration in healthy cows was 168±13.9 µg/dL. On the other hand, the results of the present study showed no significant differences in Total Iron Binding Capacity (TIBC) between the calves of the control group, the 2nd group and the 3rd treatment group at the age of 30 days, The average concentration of TIBC was 425.07±10.17, 421.52±9.54 and 416.30±10.72 µg/dL respectively (Table 3), Studies have indicated that the normal level of TIBC concentration in cattle ranges from 334.50 - 671.00 µg/dL (Hussein *et al*., 2007). Table 3 shows no significant difference in transferrin saturation at the age of 30 days among the calves at the three groups. The control treatment is 32.52±1.11%. The 2nd group is 32.12±0.82% and the 3rd group is 33.21±0.93%. The lower concentration of transferrin in the calves blood, at the 3rd treatment significantly, may be due to the insignificant increase in the concentration of iron in the calves of that group, Killip *et al*. (2007) and Macedo and Sousa (2008) noted that low iron concentration in blood leads to an increase in transferrin concentration in blood.

**Effect of the treatments in the concentration of transferrin, iron, TIBC and transferrin saturation ratio at the age of 60 days**

The results showed significant differences (P<0.05) between the three groups in the transferrin concentration of calves' blood at the age of 60 days. The control group calves (without Lf) achieved the highest concentration of transferrin (3.62±0.13 mg/ml) compared with the calves of the second (3 g Lf) and the third groups (6 g Lf).

The concentration of transferrin in the blood of their calves were 3.11±0.12 and 3.07±0.13 mg/ml respectively. In the same vein, there were no significant difference between the calves of the second and third treatments in the transferrin concentration at the same age (Table 4).

On the other hand, the results showed a high mental superiority (0.01% P) in the concentration of iron in the blood at the age of 60 days in the calves of the second and third treatments, with an average iron concentration of 153.00±1.03 and 155.70±0.67 µg/dL, respectively, while mean iron concentration in calves were 141.70±1.14 µg/dL at the same age (Table 4). Atyabi *et al*. (2006) showed that the average iron concentration of healthy calves at 60 days was 141.52 µg/dL. The results of the present study indicated that there were no significant differences at the age of 60 days in the TIBC concentration among calves between the three groups, the control, the second and the third

**Table 5. Effect of the coefficients in the *E. coli* measurement rate in the feces**

| S. No | Treatment | Mean±SE (log CFU[1]/ml) | |
|---|---|---|---|
| | | At 30 day of age | At 60 day of age |
| 1 | Control (without Lf) | 2.28±0.01[a] | 2.23±2.02 |
| 2 | Lactoferrin (3gm/day) | 2.21±0.01[a] | 2.06±0.03 |
| 3 | Lactoferrin (6gm/day) | 2.29±0.03[a] | 2.25±0.02 |
| | | NS | * |

NS: non significant; *(P<0.05): The averages with different letters within the same column vary significantly between them.

group, with the values of 433.20±15.09 and 425.10±12.68 and 422.60±15.51 µg/dL respectively (Table 4). The results of Atyabi *et al*. (2006) showed that the average TIBC concentration in healthy calves at 60 days was 494.45 µg/dL. On the other hand, the results of the study did not show significant differences in transferrin saturation in the blood of calves at the age of 60 days between the three groups viz., control group (32.39±1.49%), the 2nd group (35.47±1.12%) and the 3rd group (36.80±1.21%) (Table 4). The increase in iron concentration may be due to the metabolic processes that occur on the lactoferrin within the body, release of lactoferrin-related iron and thus increase its concentration in the blood (Nakanishi *et al*., 2010). In addition, the increase in iron concentration in blood may be due to the positive role of lactoferrin in improving the amount of food intake and promote the absorption of iron in the body, lactoferrin is effective for the prevention of iron deficiency resulting from metabolic processes and metabolism of oral bovine anemia (Paesano *et al*., 2010; Rezk *et al*., 2015).

**Effect of studied treatments on *E. coli* measurement rate in feces**

Table 5 shows no significant differences in the number of *E. coli* bacteria in the feces between the calves of the three treatment groups at 30 days in control group (without Lf), the second treatment group (3 g Lf) and the third treatment group (6 g Lf), The colonies of *E. coli* in the calf feces were 2.28±0.01 and 2.21±0.01 and 2.26±0.1 l/ml, respectively. On the other hand, the results of the study showed a significant decrease ($P<0.05$) at the age of 60 days in the preparation of *E. coli* colonies in the calves for the second treatment (2.06±0.03 l/ml) compared to the control group calves (2.23±2.02 l/ml). and calves of the third group (2.29±0.03 l/ml) (Table 5). The decrease in the number of *E. coli* bacteria in the stool may be due to the effectiveness of the lactoferrin protein against the activity of the microbes in the digestive system, including the gram -negative *E. coli* bacteria (Tomita *et al*., 1991; Teraguchi *et al*., 1994; Robblee *et al*., 2003; Gonzalez-Chavez *et al*., 2009).

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