



RESEARCH ARTICLE

Effect of Lactoferrin Treatment on Growth and E-Coli Measurement Rate in Feces with Some Blood in Awassi Lambs during Lactation

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ARTICLE INFO	ABSTRACT
Received: May 27, 2024 Accepted: Jul 1, 2024	This study was conducted in the animal field, affiliated to the Department of Animal Production at the Faculty of Agriculture - University of Tikrit for the period from 1/11/2023 to 1/2/2024 by (90) days, to study the effect of lactoferrin administration on growth (weight gain), the rate of measuring E-Coli in the stool, and some physical blood parameters (hemoglobin, the number of white blood cells and the number of red blood cells) among suckling float lambs by using (15) Awassi infant lambs, aged between 3-6 days and with an average weight of 3.30 kg, the lambs were divided The infant into three randomly distributed treatments, the first treatment (control) without the dose of lactoferrin, the second treatment dose 1 g of lactoferrin and the third group 3 g of lactoferrin The results of the study showed a high significant difference ($P<0.01$) for the growth trait of the third treatment lambs that were given 3 g lactoferrin compared to the control group (without lactoferine) and the second treatment that was given 1 g lactoferrin at the age of 30 days, as the average weight of the third group was 15.45 ± 0.63 kg compared to the average body weight of lambs for the control group and the second group 13.23 ± 1.0 and 13.30 ± 1.2 kg respectively, while body weight at the age of 60 days found high significant superiority ($P<0.01$) in favor of the lambs of the second and third groups, as it reached 19.82 ± 0.54 , 21.16 ± 0.66 and 23.24 ± 0.62 kg for the control treatment and the second group and the third group respectively, this result was withdrawn to body weights within 90 days of the start of the experiment The results of the study showed a high significant difference ($P<0.01$) for the growth trait of the third treatment lambs given 3 g lactoferrin compared to the control group (without lactoverine) and the second treatment given 1 g lactoferrin at 30 days of age. The average weight of the third group was 15.45 ± 0.63 kg compared to the average body weight of the lambs of the control group and the second group 13.23 ± 1.0 and 13.30 ± 1.2 kg respectively, while the body weight at the age of 60 days found a high moral superiority ($P<0.01$) in favor of the lambs of the second and third groups, as it reached 19.82 ± 0.54 , 21.16 ± 0.66 and 23.24 ± 0.62 kg for the control treatment and the second group and the third group respectively, this result was withdrawn to body weights within 90 days of the start of Experiment.
Keywords Lactoferrin Awassi lambs E. coli Growth rate Blood parameters	
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INTRODUCTION

Sheep occupy an important rank of livestock, which constitutes an important element in the agricultural sector (Odeh, 2010) The success of such projects depends on the care of lambs before and after, especially newborns, and lambs are considered one of the widely spread animals and number more than 970 million lambs around the world and the economic value of these animals

varies from one country to another and is determined by the efficiency of productivity and reproduction (Cheng et al., 2020) and (Barzanji, 2019). Infant lambs suffer from a deficiency in the blood Kama clobin and needs to consume colostrum as the main source of immune clobulin and this period is associated with the occurrence of diseases so you need to prevent infection and enhance growth performance and strengthen the immune system in the newborn (Gokcet, 2013 and Brujeni et al. 2010) by paying attention to feeding newborn lambs on a sufficient amount of colostrum to contain all the nutrients and biological against bacteria and viruses important for gaining immunity and improving the growth and development of newborns (Simonia et al., 2020) The importance of colostrum lies in the fact that it contains immunoglobulins and antibodies that are a key immune component in colostrum Tacoma et al., 2016), Ochoa et al. (2015) found that lactoferrin with clobulins is part of the innate microbial balance system that enhances intestinal immune resistance in infants and animals, while Liao et al. (2019) noted that lactoferrin is an active iron-bound glycoprotein that has many roles and film events, including rapid response against infections and activation of the immune system, as well as its important role as an anti-pathogen to bacteria viruses and other organisms. Microstructure Al-Qudsi and Khalid (2018) pointed out that lactoferrin is important in protecting the mammary glands after childbirth as lactoferin is a multifunctional protein and affects economically important features, such as milk composition and skeleton in lambs (Guo et al., 2020). (AL-Abbasy et al., 2020) indicated that giving lactoferrin 4 g and 8 g respectively to buffalo calves at the age of 60 days recorded significant superiority in body growth, weight gain, body measurements and concentration of immune cholbulins on calves of the same age that did not take lactoferrin, from here this study was conducted, which aimed to find out the effect of lactoferrin treatment on growth and the rate of measuring E-Coli in the feces of Awassi lambs \ and some blood parameters before weaning down to conclusions and recommendations necessary for the extent of benefit from the effect of lactoferrin on newborn lambs.

Materials and Research Methods

This study was conducted in the animal field of the Department of Animal Production at the Faculty of Agriculture - University of Tikrit for the period from 1/11/2023 to 1/2/2024, the lambs were distributed into three groups of five lambs for each group, and the lambs were numbered with plastic numbers in addition to placing the numbers of the lambs for each treatment on each cage of breeding, as the lambs were placed in cages with an area of 15 m² and dimensions of 3×5 m in a semi-closed barn, the experimental transactions were divided into three Randomly distributed treatments on three treatments, the first treatment (control) without the dose of lactoferrin, the second treatment was a dose of 1 g of lactoferrin, and the third group was 3 g of lactoferrin, and each group included 4 lambs with their mothers in order for the infant to obtain breast milk continuously as food for growth.

Feeding System

Concentrated protein was used in feeding mothers and manufactured in the form of a belt by Wafi Al-Nadia Company - Erbil in addition to wheat straw to protect ewes from swelling or fermentation provided food to ewes such as 9 am and 6 pm with the provision of water sources and ewes went out every day to theaters for sports while lambs relied on feeding on breast milk and lambs received an oral dose of lactoferrin in the form of liquid after mixing with water every day Continuously, the lambs were weighed each week during the experiment using a special 100 kg reader scale, where lambs are isolated from mothers at 10 p.m. and the empty weight is measured at 9 a.m. and adopted as the weekly growth weight. The process of giving the exact dose of lactoferrin to each pregnancy was weighed the amount of lactoferrin minutes using a sensitive balance with the required dose 1 g and 3 g of lactoferrin and the oral dose of lambs was dosed daily after mixing the substance used with water using the oral dose syringe and received the first treatment 1 g of the dose of lactofrin while the second treatment received 3 g of the dose of lactofrin.

Blood Samples

Blood samples were collected in (30, 60 and 90) of the experiment, as samples were drawn from the blood after isolating lambs for 12 hours from the mothers, as blood samples were drawn from the jugular vein by a 10 ml wine syringe after collecting blood and placing an amount in anticoagulant tubes to be used for CPC analysis that requires complete blood without clotting, blood tests were conducted in the laboratory of Ashti – Kirkuk.

Measurement of the numbers of E-Coli bacteria in the stool samples were collected from lamb feces at the age of 30, 60 and 90 days and transferred directly to the laboratory of Ashti - Kirkuk for the purpose of detecting the numbers of E-Coli bacteria, the numbers of bacteria as stated in APHA ((1978) were estimated using MacConkey ager medium and incubation for 48 hours at a temperature of 37 ° C, and then the colony numbers of dark brown and fermented E-Coli bacteria for lactose sugar were calculated using the medical equipment india of Indian origin. The culture medium was prepared by dissolving 51.5 g of MacConkey ager in a liter of distilled water and in a special glass flask and then subjecting it to sterilization at 120 ° C for 15 minutes and then leaving it to cool in the atmosphere of the room, and finally poured into sterile plastic dishes (Petri dish).

Measurement of the Numbers of E-Coli Bacteria in the Stool Samples

were collected from lamb feces at the age of 30, 60 and 90 days and transferred directly to the laboratory of Ashti - Kirkuk for the purpose of detecting the numbers of E-Coli bacteria, the numbers of bacteria as stated in APHA ((1978) were estimated using MacConkey ager medium and incubation for 48 hours at a temperature of 37 ° C, and then the colony numbers of dark brown and fermented E-Coli bacteria for lactose sugar were calculated using the medical equipment india of Indian origin. The culture medium was prepared by dissolving 51.5 g of MacConkey ager in a liter of distilled water and in a special glass flask and then subjecting it to sterilization at 120 ° C for 15 minutes and then leaving it to cool in the atmosphere of the room, and finally poured into sterile plastic dishes (Petri dish).

Calculation of Red Blood Cells

Red blood cells were calculated using the Hemocytometer counting chip, according to the method of Hughes and his group (2004), where blood is drawn by its pipette (attached with the counting chip) to the limit of the mark 0.5 and completes the volume to the mark 101 with Hyme's solution, then mixed inside the pipette for ten seconds, to prepare the counting chip neglect the first three drops and use the fourth on the edge of the connection of the chip with its cover, and leave for two minutes for the purpose of stability and stability of cells and examined with light microscopy The magnification power of 40x counts the number of cells in five large squares and each large square contains 16 small squares (Hughes et al., 2004).

The total number of red blood cells is extracted as in the following equation:

The number of red blood cells in 1 mm³ of blood = $n \times 200 \times 50$,

n = number of red blood cells withdrawn.

200 = blood dilution ratio (1: 200).

50 = blood volume diluted in 80 small squares.

Calculation of White Blood Cells White Blood Cells

were calculated using the Hemocytometer counting chip by drawing blood by its pipette (attached with the counting slide) to the mark 0.5 and then complete the size to mark 11 with a Turkish solution Turkey's Solution, and after mixing the contents of the pipette by shaking by hand for ten seconds, the sample is left for three minutes for the purpose of pigmenting the nuclei of white blood cells, and

to prepare the counting slide, neglect the first three drops, The fourth is used by placing it on the edge of the connection of the chip with its cover, and left for two minutes for the purpose of stability and stability of the cells and then examined with optical microscopy at a magnification of 40X by calculating its number inside the large square (Hean, 1995).

The total number of white blood cells is extracted as in the following equation:

The number of leukocytes in 1 mm³ of blood = $n \times 20 \times 10$

n = number of leukocytes withdrawn.

200 = blood dilution ratio (1:20).

10 = diluted blood volume

Hemoglobin

The method of estimating hemoglobin relied on converting it into a complex compound Cyanomethemoglobin using the Drabkins reagent, as 0.02 ml of blood was withdrawn and mixed with 5 ml of this reagent and left for 5 minutes and then placed in a centrifuge at a speed of 3000 cycles / minute and for 15 minutes for the purpose of getting rid of the nuclei and sheaths of red blood cells and reading it using a spectrophotometer and

Statistical Analysis System (SAS (2012) was used in data analysis to study the effect of different coefficients on the traits studied according to a complete random design (CRD), and the significant differences between the averages were compared with the Duncan (1955) multi-range test.

Mathematical model of design: (Statistical I Model).

$$Y_{ij} = \mu + T_i + e_{ij}$$

if : Y_{ij} : viewing value j of the transaction i.

μ : the general average of the trait studied.

T_i : effect of treatment i (0, 1 and 3 g/day).

e_{ij} : random error that is distributed normally with an average of zero and a variance of σ^2e .

RESULTS AND DISCUSSION:

The Effect of Lactoferrin on Growth

The results of the study showed a high significant difference ($P < 0.01$) for the growth trait of lambs of the third treatment (3 g lactoferrin) compared to the control group (without lactoferrin) and the second treatment (1 g lactoferrin) at the age of 30 days, as the average weight of the third group at the age of 30 days was 15.45 ± 0.63 kg, while the average body weight of lambs for the control group and the second group was 13.23 ± 1.0 and 13.30 ± 1.2 kg respectively. On the other hand, the results showed a high moral superiority ($P < 0.01$) in favor of the lambs of the second and third groups for the characteristic of body weight at the age of 60 days compared to the lambs of the control group, and the average weight of the lambs at the age of 60 days for the three groups of control treatment, the second group and the third group 19.82 ± 0.54 , 21.16 ± 0.66 and 23.24 ± 0.62 kg respectively, and at the same time there were no significant differences between the second and third groups, This result was withdrawn to body weights within 90 days of the start of the experiment, as the third and second groups repeatedly outperformed the first group. (Table 1), and the reason may be that this increase is due to the effect of lactoferrin on the health of infant lambs, an improvement in the amount of diet intake, and a decrease in the incidence of diseases, especially diarrhea (Prenner et al., 2007), or the increase in weight rate may be due to the effect of lactoferrin as a growth factor by stimulating the growth of the mucous membrane of the small intestine and then increasing its absorption of feed

nutrients or increasing its efficiency in nutritional conversion (AL-Abbasy et al., 2020) and this is consistent with the results of the study of Joslin et al. (2002) and Robblee et al. (2003), as they found that lactoferrin supplementation improved diet intake, growth and health of newborns.

Table 1: Effect of lactoferrin on average body weight at 30, 60 and 90 days of age

Average weight \pm standard error (kg)			
Transaction	At 30 days of age	At 60 days of age	At 90 days of age
Control (no lactoferrin)	13.23 \pm 1.0 b	19.82 \pm 0.54 b	23.63 \pm 0.62 b
Lactoferrin 1 gm/day	13.30 \pm 1.2 b	21.16 \pm 0.66 a	25.01 \pm 0.66 a
Lactoferrin 3 gm/day	15.45 \pm 0.63 a	23.24 \pm 0.62 a	26.41 \pm 0.54 a
Moral level	**	**	**

Averages with different letters within the same column differ significantly among themselves. ** (P<0.01).

Effect of Lactoferrin on the Measurement Rate of E-Coli in Feces

The results of the study showed that there were no significant differences in the number of E-Coli bacteria in the stool between the lambs of the three treatment groups at the age of 30 days, represented by the control group (without lactoferrin), the second treatment group (1 g lactoferrin) and the third treatment group (3 g lactoferrin), as the number of colonies of E-Coli bacteria in the feces of their lambs was 1.60 \pm 0.03, 1.80 \pm 0.01, 1.78 \pm 0.02 log CFU¹/ml respectively. On the other hand, the results of the study showed a significant decrease (0.05>P) at the age of 60 and 90 days in the number of colonies of E-Coli bacteria in lamb feces for the third treatment (1.55 \pm 0.02) and (1.50 \pm 0.02 log CFU¹/ml) respectively compared to the control treatment lambs (2.00 \pm 0.04) and (2.30 \pm 0.01 log CFU¹/ml) and the second treatment lambs (2.10 \pm 0.02) and (2.20 \pm 0.02 log CFU¹/ml). According to (Table 2), this shows the ability of lactoferrin to reduce and eliminate the presence of E. coli and therefore lactoferrin can be used as an immune treatment against Escherichia coli infection that affects the intestines of infant lambs and reduce a high level of Escherichia coli in manure because lactoferrin has properties against pathogenic bacteria such as E. coli bacteria and a decrease in the presence of pathogenic microorganisms in the digestive system leading to a decrease in the level of diarrhea and intestinal damage (Jang et al., 2008) through Pulling iron ions and preventing microorganisms to benefit from iron and thus inhibit and prevent their growth as well as the ability of lactoferrin to adhere to the outer surface of the cell bacteria works to break down the outer membrane of bacteria (Gonzalez et al., 2009)

Table 2: Effect of Lactoferrin on E-Coli Measurement Rate in Feces

Average \pm standard error (log CFU ¹ /ml)

Transaction	At 30 days of age	At 60 days of age	At 90 days of age
Control (no lactoferrin)	1.60±0.03 a	2.00±0.04 B	2.30±0.01 b
Lactoferrin 1 gm/day	1.80±0.01 a	2.10±0.02 B	2.20±0.02 b
Lactoferrin 3 gm/day	1.78±0.02 a	1.55±0.02 A	150±0.02a
Moral level	NS	*	*

Averages with different letters within one column differ significantly from each other.

NS: Not significant, * (P<0.05).

Effect of Lactoferrin on Some Physical Blood Traits at 30, 60 and 90 Days

The results of the study showed that there were no significant differences at the age of 30 and 60 days in hemoglobin, the number of white blood cells and the number of red blood cells in the blood of lambs between the three treatments (control group, second treatment group and third treatment group) Table 3 and 4 While the results of the study showed a significant superiority (P<0.05) for white blood cells, erythrocytes and hemoglobin at 90 days of age for the third treatment (3 g lactoverine) on the control treatment (without lactoferrin) and the second treatment (and 1 g lactoferrin) where the average white blood cells for the third treatment was 9.25±1.18 compared with the control treatment and the second treatment 6.78±1.75 and 7.90±2.35 103/ml respectively, in the erythrocyte rate for the third treatment was 12.75±0.12 compared to the control treatment of 9.70± 0.20 and the second treatment 10.80±0.21 106/ml , while the hemoglobin for the third treatment was 13.89± 0.30 compared to the second treatment 10.00± 0.34 and the first (control) 11.33± 0.40 g/dL It seems that the positive effect of lactoferrin in the immune response had a positive effect on the rate of leukocytes (Rosa.,et al.2008) and with regard to erythrocytes and hemoglobin, the administration of lactoferrin may have a positive effect on the immune system, which enhanced the production of hemoglobin and worked to improve iron absorption, which leads to an increase in the concentration of hemoglobin in the blood and this improves the ability of the blood to transport oxygen to various organs of the body, which supports vital functions and improves overall health. or that lactoferrin has enhanced reactive oxygen production and stimulated blood cell receptor expression (Anand et al., 2015).

Table 3: The effect of lactoferrin on some physical blood characteristics at 30 days of age

Average ± standard error			
Transaction	White blood cells 103/ml	Red blood cells 106/ml	Hemoglobin in the blood gm/dl

Control (no lactoferrin)	5.087 ± a 0.04	10.330±a 0.07	10.40±0.36 a
Lactoferrin1 gm/day	5.097±0.07 a	10.265±0.14 a	9.82±0.34 a
Lactoferrin 3 gm/day	5.072±0.07 a	10.400±0.36 a	10.07±0.19 a
Moral level	N.S	N.S	N.S

Averages with different letters within the same column differ significantly among themselves.

NS: Non-significant.

Table 4: The effect of lactoferrin on some physical blood characteristics at 60 days of age

Average ± standard error			
Transaction	White blood cells 103/ml	Red blood cells106/ml	Hemoglobin in the blood gm/dl
Control (no lactoferrin)	6.75±0.75 a	10.50±0.20 a	11.33± 0.40 a
Lactoferrin1 gm/day	6.67±0.35 a	10.50±0.21 a	10.77± 0.34 a
Lactoferrin 3 gm/day	5.25±0.18 a	10.37±0.12 a	10.66± 0.30 a
Moral level	NS	NS	NS

Averages with different letters within one column differ significantly from each other.

NS: Not significant.

Table 5: The effect of lactoferrin on some physical blood characteristics at 60 days of age

Average ± standard error			
Transaction	White blood cells 103/ml	Red blood cells106/ml	Hemoglobin in the blood gm/dl
Control (no lactoferrin)	6.78±1.75 b	9.70±0.20 b	11.33±0.40 b

Lactoferrin1 gm/day	7.90±2.35 b	10.80±0.21 b	10.00±0.34 b
Lactoferrin 3 gm/day	9.25±1.18 a	12.75±0.12 a	13.89±0.30 a
Moral level	*	*	*

Averages with different letters within one column differ significantly from each other.

NS: not significant, * (P<0.05).

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