Lactoferrin Supplementation Induced Awassi Lambs Production with High Immunity and Iron Levels

Emad Gh.AL-Abbasy [1*], Adnan Gh. Jalal [1*]

Abstract

Background: Formation evaluation plays a pivotal role in identifying lithology and its depth of occurrence in gas fields. This study focuses on the Bengal Basin, aiming to achieve lithology identification and shale volume estimation in a gas well. Method: The gamma ray (GR) log is utilized to measure natural radioactivity, with spectral gamma ray (SGR) employed to capture concentrations of potassium, uranium, and thorium in clastic sedimentary formations. Lithology identification is conducted using resistivity, SGR, and GR logs, while shale volume estimation utilizes standard models including GR and true resistivity approaches. Results: The lithology of the studied well predominantly comprises clastic sedimentary rocks, specifically sand and shale. The reservoir rock type is primarily sandstone, with sand being the dominant fraction and shales appearing laminated. Concentrations of thorium, uranium, potassium, and gamma ray are approximately 12.46 ppm, 2.28 ppm, 1.73%, and 100 API, respectively. The shale volume of the gas reservoir ranges from 12% to 29%. Conclusion: The estimated shale volume provides valuable insights for assessing effective porosity and hydrocarbon saturation in shaly sand reservoirs, facilitating gas resource estimation and reservoir characterization in the sedimentary basin. This study underscores the significance of comprehensive formation evaluation techniques in optimizing reservoir management and resource extraction strategies.

Keywords: Lactoferrin, Immunoglobulins, Iron concentration, Transferrin, Awassi lambs

Significance | Lactoferrin supplementation might increase immune function and iron levels in Awassi lambs, improving health and productivity.

*Correspondence. Emad Gh.AL-Abbasy, Adnan Gh. Jalal, Department of Animal Production, Faculty of Agriculture, University of Tikrit
E-mail: dr.emadghaib@tu.edu.iq, adnan.g.jalal001@st.tu.edu.iq

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Introduction

The demand for animal products in the Near East and North Africa, including all Arab countries, is projected to rise significantly, surpassing the current production rates. This trend, as reported by the FAO, necessitates a substantial increase in animal production. Special attention must be given to sheep breeding, as sheep and goats are the most prevalent livestock in this region. Consequently, feed production must also escalate to match the growing number of animals.

To meet these demands and improve the productivity of each animal in diverse environments, it is crucial to address the increase in morbidity and mortality among newborn lambs. This issue poses a significant economic challenge for sheep farms (Gokce & Erdogan, 2013). The neonatal period is critical in the life of a lamb, marked by a deficiency in immunoglobulins and a reliance on colostrum as the primary source of these essential antibodies. This phase, often referred to as the period of negative immunity, is characterized by a heightened vulnerability to diseases. Hence, preventing infections, promoting growth performance, and strengthening the immune system in newborn lambs are paramount (Gokce, 2013; Brujeni et al., 2010).

Feeding newborn lambs, a sufficient amount of colostrum is vital, as it provides all the necessary nutrients and biological elements to combat bacteria and viruses. Colostrum's significance lies in its high content of immunoglobulins and antibodies, which are essential

Author Affiliation.
[1] Department of Animal Production, Faculty of Agriculture, University of Tikrit.

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for building immunity and fostering the growth and development of newborns (Simonia et al., 2020). Colostrum's role extends beyond nutrition to vital biological functions, contributing to microbial balance and enhancing intestinal immune resistance (Tacoma et al., 2016; Al-Obaidi, 2018; Ochoa et al., 2015).

Lactoferrin, a component of colostrum, has been identified as a key player in this process. Studies have shown that lactoferrin, along with immunoglobulins, is part of the innate immune system, promoting microbial balance and enhancing intestinal immunity in both infants and animals. This study aims to explore the effects of administering two levels of lactoferrin powder on the concentration of immunoglobulins (IgG, IgM, IgA), iron levels, transferrin, and transferrin saturation percentage in the blood of Awassi lambs before weaning.

This study's objective was to determine the impact of lactoferrin supplementation on the immune function and iron levels of Awassi lambs. By analyzing the concentrations of immunoglobulins (IgG, IgM, IgA), iron, transferrin, and transferrin saturation, the study aims to draw conclusions and provide recommendations on the benefits of lactoferrin for improving the health and productivity of newborn lambs.

Materials and 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 the breeding cages, as the lambs were placed in cages with an area of 15 m 2 and dimensions of 3×5 m in a semi-closed barn, the experimental transactions were divided into three randomly distributed transactions On three treatments, the first treatment (control) without the dose of lactofurin, the second treatment dose of 1 g of lactoferrin, and the third group 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.

Blood models

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 placed in tubes without anticoagulants and left at room temperature for at least 15 minutes to allow serum separation, then placing the tubes in the centrifuge for five minutes at a speed of 20.000 and collecting the serum. In special IPNDORF tubes are used to measure biochemical standards including IgG, IgA and IgM immunoglobulins, and to measure iron concentration, transferrin and transferrin saturation ratio using Cobas C6000 automatic analyzer. Serum separation was carried out in the laboratory of the Department of Animal Production, Faculty of Agriculture, Tikrit University, and blood tests were conducted in Ashti Laboratory – Kirkuk.

Measurement of the concentration of immunoglobulins IgG, IgM, IgA

The concentration of IgG immunoglobulins was measured using the method of radial immunodiffusion plate RID, as a Kit produced by the Italian company LTA was used, which contains a panel consisting of 15 foci, each focus contains the Agarose gel containing (Goat antiserum IgG), and the method of work was done according to what was stated in the manufacturer's instruction leaflet attached to the kit (Kit) for this examination

Iron concentration measurement

The analysis of iron concentration measurement was conducted according to the method of the French company BIOLABO and the steps attached to its kit (Kit), where three solutions were prepared (sample model Assas, Standard, Blank), and 200 microliters of lamb serum sample were placed in the sample model and then the chemical analyzer KENZA 240 TX was used of French origin and produced by the French company BIOLABO.

Transfer concentration measurement

The transfer concentration measurement analysis was performed using the Alisa (Stat Fax 2400) device of American origin, according to the method of the American company K-ASSAY and with the steps attached to its kit.

Measuring the saturation ratio of transferrin

calculated by following the following equation:

Transferrin saturation ratio = (Iron concentration)/TIBC x 100

Mathematical Model

$Yij = \mu + Ti + eij$

Whereas:

Yij : Viewing value j of the transaction i.

μ : the general average of the trait studied.

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

eij = experimental error that is distributed normally and independently with an average of zero and an equal variance of 2 σe.

Results and Discussion

Effect of lactoferrin on the concentration of immunoglobulins 30 days old

The results of the statistical analysis indicate a significant superiority ($P<0.01$) in the concentration of IgG in the blood of lambs for the second treatment groups (1 g lactoferrin) and the third treatment (3 g lactoverine) on the lambs of the control group (without lactoferrine) at the age of 30 days, as the average concentration of IgG in the blood of lambs of the third and second treatments was 13.20 ± 0.1 and 10.40 ± 0.3 mg/ml respectively. The median concentration of IgG in the blood of the control group lambs was 9.30 ± 2.1 mg/ml (Table 1). This increase may be attributed to the effect of lactoferrin in improving and increasing the intestinal absorption of IgG immunoglobulins, as well as the fact that lactoferrin regulates the body's immune system and stimulates and activates the production of B plasma cells, through which Ig molecules are synthesized (Hurley, 2003). On the other hand, the results of the study indicated that there were no significant differences at the age of 30 days in the concentration of IgM in the blood of lambs of the three treatment groups (control group, second treatment group and third treatment group), as the average concentration of IgM in the blood of their lambs was 0.58±0.1, 0.62±0.1 and 00.78±0.1 mg/ml respectively (Table 1). The results of the study also showed no significant differences in the concentration of IgA in the blood between the lambs of the three treatment groups, the control group, the second treatment group and the third treatment at the age of 30 days, for which the average concentration of IgA was 0.084 ± 0.001, 0.086 ± 0.002 and 0.085 ± 0.001 mg/ml respectively. This result may be consistent with the results of the study of (Prgomet et al. 2007), which was conducted on a group of newborn Friesian calves, where lactoferrin protein was added in the nutrition of those calves and a significant increase in the concentration of IgG in the blood was observed, in addition to that, this result is consistent with the results of the study of ALkudsy and Waleed (2018), Shea et al. (2009) and Comstock et al. (2014). They pointed out that the addition of lactoferrin in the nutrition of newborns may lead to an increase in the efficiency of IgG absorption and thus increase its concentration in the blood.

Effect of Lactoferrin on the Concentration of Immunoglobulins 60 Days

The results indicated significant differences (0.05>P) in the concentration of IgG in the blood at the age of 60 days in favor of lambs of the third treatment group (3 g lactofrin) and lambs of the second treatment (1 g lactofrin) compared to the lambs of the control treatment (without lactoferrin) as the concentration of IgG in the blood of lambs of the third treatment was 21. 20.90 ± 0.70 mg/ml, second (19.70 ± 0.50 mg/ml) while the IgG concentration in the blood of lambs treated control was 20.90 ± 0.70 mg/ml. On the other hand, the results of the study showed a significant superiority (0.05>P) in the concentration of IgM at the age of 60 days in favor of lambs of the second and third treatment groups (1.83 ± 0.10 and 1.95 ± 0.13 mg/ml respectively) at the expense of the group of control treatment lambs (1.75± 0.12 mg/ml), while no significant differences were recorded in the concentration of IgM between the second and third treatment lambs at the same age. . On the other hand, the results showed that there were no significant differences at the age of 60 days in the concentration of IgA in the blood between the lambs of the three treatment groups (control and the second and third group), as their average IgA concentration was 0.24± 0.04, 0.27± 0.04 and 0.26± 0.05 mg/ml respectively (Table 2). These results are consistent with the results of the study of (Comstock et al. 2014), whose results indicated an increase in the concentration of both IgG and IgM in the blood of newborns when lactoferrin is added in their nutrition, and the reason for this increase may be due to the positive effect of lactoferrin on the regulation of the body's immune system and the stimulation and activation of plasma cell production, through which Ig molecules are synthesized (Hurley,2003), as well as the effect of lactoferrin in activating and improving the intestinal absorption of immunoglobulins found in colostrum and milk used in feeding those newborns (Prenner et al. 2007).

Effect of Lactoferrin on the Concentration of Immunoglobulins 90 Days

The results indicated significant differences (P<0.01) in the concentration of IgG, IgM and IgA in the blood at the age of 90 days in favor of lambs of the third treatment group (3 g lactoferrine) and the second group (1 g lactoferrine) compared to the control group (without lactofrin), as the third treatment recorded an increase in the concentration of immunoglobulins (IgG, IgM and IgA) at a rate of 21.20 ± 0.30, 2.10 ± 0.18 and 0.28 ± 0.07 mg/ml respectively While the values of the second group were 20.11 ± 0.40 and 1.90 ± 0.24 and 0.30 ± 0.07 mg/ml, respectively, compared to the control group 17.40 ± 0.75, 1.65± 0.16, 0.21 ± 0.05 mg/ml, respectively, and this increase may be due to the positive effect of lactoferrin on the regulation of the body's immune system and the stimulation and activation of plasma cell production, through which Ig molecules (Hurley, 2003), as well as the effect of lactoferrin in activating and improving the intestinal absorption of immunoglobulins found in colostrum and milk used in feeding those newborns (Prenner et al. 2007).

The effect of lactoferrin on iron and transferrin concentration and saturation ratio at the age of 30 days

The results of the study showed that there were no significant differences at the age of 30 days in the concentration of iron in the blood of lambs between the three treatments (control group, second treatment group and third treatment group), as the average iron concentration in the blood of their lambs was 129.80±0.70, 133.22± 1.30, 134.50 ± 0.89 μg/dL respectively (Table 4). On the other hand, the results of the current study indicated a significant decrease (0.05>P) in the concentration of transferrin in the serum of lambs of the third treatment (2.10 ± 0.30 mg/ml) compared to the lambs of the control treatment (2.90± 0.12 mg/ml) and the lambs of the second treatment (2.80 ± 0.16 mg/ml) at the age of 30 days. In the same vein, no significant differences were found between the second and third treatment lambs in the transfer concentration at 30 days of age (Table 4). The concentration of transferrin in the lambs of the three groups was within normal limits, as Evan et al. (1956) pointed out that the concentration of transferrin in the blood

Table 1. Effect of lactoferrin on the concentration of immunoglobulins 30 days old (Mean± standard error) (mg/mL)

IgA	IgM	IgG	Transaction
0.084±0.001 a	0.58±0.1 a	9.30±2.1 c	Control (no lactoferrin)
0.086±0.002 a	0.62±0.1 a	10.40±0.3 b	Lactoferrin 1 gm/day
0.085±0.001 a	0.78±0.1 a	13.20±0.1 a	Lactoferrin 3 gm/day
NS	NS	**	Moral level

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

Table 2. Effect of lactoferrin on the concentration of immunoglobulins age 60 days (Mean± standard error) (mg/mL)

IgA	IgM	IgG	Transaction
0.24±0.04 a	1.75±0.12 b	18.80±0.78 b	Control (no lactoferrin)
0.27±0.04 a	1.83±0.10 a	19.70±0.50 a	Lactoferrin1 gm/day
0.26±0.05 a	1.95±0.13 a	20.90±0.70 a	Lactoferrin 3 gm/day
NS	*	*	Moral level

Averages with different letters within the same column differ significantly among themselves.
*NS: non-significant, * (P<0.05).*

Table 3. Effect of lactoferrin on the concentration of immunoglobulins 90 days old (Mean± standard error) (mg/mL)

IgA	IgM	IgG	Transaction
0.21 + 0.05 b	1.65 + 0.16 b	17.40 + 0.75 b	Control (no lactoferrin)
0.30 +0.07 a	1.90 + 0.24 a	20.11 + 0.40 A	Lactoferrin 1 gm/day
0.28 + 0.07 a	2.10+0.18a	21.20 + 0.30 A	Lactoferrin 3 gm/day
**	**	**	Moral level

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

Table 4. Effect of lactoferrin on iron and transferrin cocentration and saturation at 30 days of age (Mean± standard error)

Transferrin saturation (%)	Transferrin (mg/mL)	Iron (µg/dL))	Transaction
32.52±1.11 a	2.90±0.12 a	129.80±0.70 a	Control (no lactoferrin)
32.12±0.82 a	2.80±0.16 a	133.22±1.30 a	Lactoferrin 1 gm/day
33.21±0.93 a	2.10±0.30 b	134.50±0.89 a	Lactoferrin 3 gm/day
NS	*	N.S	Morale level

Averages with different letters within the same column differ significantly among themselves.
*NS: non-significant, * (P<0.05).*

Table 5. Effect of lactoferrin on iron and transferrin concentration and saturation at 60 days of age (Mean± standard error)

Transferrin saturation (%)	Transferrin (mg/mL)	Iron (µg/dL))	Transaction
31.20±1.90 a	2.90±0.12 a	137.40±1.90b	Control (no lactoferrin)
33.30±1.20 a	2.70±0.15 b	144.17±3.30 a	Lactoferrin 1 gm/day
34.40±1.70a	2.30± 0.13 b	149.90±2.20 a	Lactoferrin 3 gm/day
NS	*	**	Morale level

Averages with different letters within one column differ significantly from each other.
NS: Not significant(P<0.05) . (p<0.01) ***

Table 6. Effect of lactoferrin on iron and transferrin concentration and saturation at 90 days of age (Mean± standard error)

Transferrin saturation (%)	Transferrin (mg/mL)	Iron (µg/dL))	Transaction
33.12±1.70 a	3.00±0.13 a	138.22±2.00 b	Control (no lactoferrin)
34.10±1.50 a	1.98±0.18 b	141.22±2.30 a	Lactoferrin 1 gm/day
33.16±1.90a	1.90±0.13 b	150.28±1.80 a	Lactoferrin 3 gm/day
N.S	**	**	Morale level

Averages with different letters within the same column differ significantly among themselves.
*NS: Non-significant, * (p<0.05). (p<0.01) ***

of healthy sheep is 2.4-2.8 mg / ml, that the decrease in the concentration of transferrin in the blood of lambs of the third treatment significantly may be due to the non-significant increase in iron concentration in the lambs of that group, as the decrease in the concentration of iron in the blood leads to an increase in the concentration of transferrin in the blood, The measurement of iron concentration in the blood represents iron bound to transferrin, and the measurement of transferrin concentration represents non-iron bound transferrin, and the higher the iron concentration, the lower the concentration of transferrin (Killip et al., 2007) and (Macedo and Sousa, 2008).

Effect of lactoferrin on iron and transferrin concentration and saturation ratio at 60 days

The results showed a high significant superiority (0.01>P) in the characteristic of iron concentration in the blood at the age of 60 days in lambs of the second and third treatments, as the average iron concentration was 144.17± 3.30 and 149.90± 2.20 μg/dl respectively, while the average iron concentration in the control treatment lambs was 137.40 ± 1.90 μg/dl at the same age. There were no significant differences in the concentration of iron in the blood of lambs between the second and third treatments, on the other hand, the results of the current study showed significant differences (0.05>P)) among the three treatment groups in the blood transfer concentration in lambs at 60 days of age, the control treatment group (without lactoferrin) achieved the highest transfer concentration rate (2.90 ± 0.12 mg/ml) compared to calves of the second treatment groups (1 g lactoferrin) and the third treatment group (3 g lactoferrine) where the transfer concentration in the blood of their lambs was 2.70 ± 0.15 and 2.30± 0.13 mg/ml, respectively. In the same context, there were no significant differences between the lambs of the second and third treatments in the concentration of transferrin at the same age (Table 5), and on the other hand, the results of the study did not show significant differences in the percentage of transferrin saturation in the blood of lambs at the age of 60 days between the groups of the three treatments, the control group (31.20 ± 1.90%), the second treatment group (33.30 ± 1.20%) and the third treatment group (34.40 ± 1.70%). The reason for the significant increase in iron concentration may be due to the metabolic processes that occur on the lactoferrin protein inside the body and the release of iron associated with lactoferrin and then increase its concentration in the blood, as well as the presence of lactoferrin protein receptors in mucosal cells, which help to absorb lactoferrin and thus increase iron concentration (Nakanishi et al., 2010), in addition to that the increase in iron concentration in the blood may be the result of the positive role of lactoferrin in improving the amount of food intake and enhancing iron absorption in the resulting body Oral administration of lactoferrin is effective for the prevention of iron deficiency anemia (Paesano et al., 2010, Rezk et al., 2015).

Effect of lactoferrin on iron and transferrin concentration and saturation ratio at age 90 days

The results did not differ much in the characteristic of iron concentration and transferrin concentration in the blood at the age of 90 days in lambs of the third treatment and lambs of the second treatment, as it recorded a high significant superiority (0.01>P) on the lambs of the first treatment (control), which strengthened the results of the previous 60 days and there were no significant differences in the percentage of transferrin saturation in the blood of lambs at the age of 60 days between the totals of the three treatments and the table of 6 Illustrates this.

Conclusion

The study concludes that lactoferrin supplementation significantly enhances the immune function and iron levels in newborn Awassi lambs, with a notable increase in IgG, IgM, and IgA concentrations in the blood. Lambs receiving 3 g/day of lactoferrin showed the most significant improvements, particularly in IgG levels, compared to the control group. Although transferrin levels were lower in the lactoferrin-treated groups, this decrease correlates with increased iron concentrations, suggesting enhanced iron absorption and metabolism. These findings highlight the potential of lactoferrin as a dietary supplement to improve the health and productivity of lambs, reducing neonatal morbidity and mortality. Further research may explore optimal dosing and long-term effects on lamb growth and immunity.

Author contributions

All authors contributed equally.

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Competing financial interests

The authors have no conflict of interest.

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