Effect of Lactoferrin on some Hematological Parameters in Karadi Male Lambs after Weaning

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Abstract

This study presents the multifunctional roles and specific beneficial effect of giving three levels of lactoferrin protein on hematological parameters in karadi male lambs after weaning, 16 Karadi male lamb were utilized, 3 months old, with an average live weight of (21±0.41 kg), Male lamb were partitioned haphazardly and similarly to four groups (control, Treatment 1 were given (500 mg LF per week)Treatment 2(1000 mg LF per week) Treatment 3(1500 mg LF per week)for each lamb, dosing capsules by 500 mg oral dose until the end of the experiment. Some blood parameters, proteins, and enzymes in the serum were analyzed results showed a significant increase (P>0.05) in Hb concentration on the period 3 of the study in the third treatment group (13.550 gm/dl) comparing to the lambs of the control group (11.650 gm/dl). lambs of the third treatment group on the period 3 showed a significant (P>0.05) increase in RBC (3.890 $\times 10^{12}$/L) as compared to the control group lamb(2.670$\times 10^{12}$/L), the first treatment group on the period 2 showed a significant (P>0.05) increase in WBC (11.890×106 cells/ml) as compared to the control group lamb (9.125×106 cells/ml).

Keywords: Lamb, Lactoferrin (LF), Blood parameters

تأثيرلاكتوفييرين في بعض صفات الدم لذكورالحملان الكرادية بعد الفطام

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المستخلص

هذه الدراسة اوضحت الدور مناعي والتأثير الخاصة لبروتين اللاكتوفييرين في بعض صفات الدم لذكور الحملان الكرادية بعدالفطام، استخدم 16 حملا من ذكور الكرادي تراوحت اعمارها بحدود (3) اشهر وبمتوسط وزن(0.41±21كغم). تم تقسيمها الى اربع معاملات متساوية، معاملة السيطرة، المعاملة الاولى (جرعت ب 500 ملغم لاكتوفيرين اسبوعيا) والمعاملة الثانية (

جرعت ب 1000 ملغم لاكتوفيريين اسبوعيا) والمعاملة الثالثة (جرعت ب 1500ملغم اسبوعيا). التجربة استغرقت 60 يوم. اجريت بعض التحاليل الدموية، بروتين الدم وانزيمات البلازما. اوضحت النتائج زيادة معنوية(P>0.05)في تركيز الهيموكلوبين لحملان المعاملة الثالثة في المرحلة الثالثة (13.550 غم/ دسي مل) مقارنة بحملان معاملة السيطرة (11.650 غم/ دسي مل). حملان المعاملة الثالثة في المرحلة الثالثة اظهرت زيادة معنوية (P<0.05) في اعداد كريات الدم حمراء (3.890 ×10 **12** /لتر) عند مقارنتها مع حملان معاملة السيطرة (2.670 ×10 **12** /لتر)، وحصول زيادة معنوية (P>0.05) في حملان المعاملة الأولى في المرحلة الثانية في اعداد كريات الدم البيضاء (11.890 ×10 **6** خلية /مل) بالمقارنة مع حملان معاملة السيطرة (9.125 ×10 **6** خلية /مل).

Introduction

Lactoferrin (it also defined lactotransferrin) consists of glycoprotein, and an individual from a transferrin family, this is a result of fit for authoritative and exchanging Fe3+ ions (Metz-Boutigue, et al. 1984). LF is additionally called a multifunctional glycoprotein that is found for the most part in milk and colostrum(Walker 2010).just as in tears and salivation emissions (Van der Strate, et al. 2001). Scientist discovered LF in neutrophil content especially neutrophil granules and different discharges of body (Iyer and Lonnerdal 1993). The convergence of Lactoferrin is expanded while the body contaminated by infection aggravation. Its focus increments in all body liquids yet in the nidus of irritation the largest amounts have been recorded (Birgens 1985). Lactoferrin has a significant function in most organic capacities, LF partook in the improvement of a neonatal calf, restraining bacterial cells digestion (Teraguchi, et al. 1994), and expanding detention of glucose (Ogata, et al. 1998).LF has a major function on the versatile insusceptible framework by quickening the development of T-cell antecedents into skilled aide cells and furthermore assumes a huge role in the separation of youthful B-cells into antigen-introducing cells(Actor, et al. 2009). the safe reaction improved by LF that can prompt B-and T-cell capacities (Yen, et al. 2011).LF connect with microorganisms straightforwardly and through its insusceptible modulatory impact shield the digestive system of the infant from disease, LF may build retention of IgG and supplements by the digestive tract this is invigorated by expanding intestinal cell development (Robblee, et al. 2003). the purpose of this experiment is to find the lactoferrin effect on some hematological parameters in Karadi male lamb after weaning.

Methods And Materials

1.1 Location of the experiment This study was conducted in the Animal science farm, College of Agricultural engineering Sciences, University of Sulaimani, from the August of 2018 to November of 2018.

1.2 Animals and experimental design A total of sixteen Karadi male lambs, 3 months old, with an average live weight of (21±0.41 kg) were used in this study. four equal groups were separated by dividing Male lambs randomly. The control group were not given the LF protein. the first group treated by lactoferrin protein (500 mg for every week)for each lamb until the finish of the experience amid two months. While (1000 mg)lactoferrin protein per week was given to the second treatment group for each lamb, dosing capsules by 500 mg oral dose twice a week until the end of the experience. And the third treatment group were given(1500 mg per week) for each lamb, dosing capsules by 500 mg oral dose third a week until the end of the experience.

1.3 Preparing lactoferrin in gelatin capsules In order to give the exact dose of lactoferrin for each lamb. The exact quantity of lactoferrin was weighed on the required dose, lactoferrin dose (500 mg per week), lactoferrin dose (1000 mg per week) and (1500 mg per week) empty gelatin capsules were used to LF and was orally given to the lambs daily according to their treatments.

1.4 Experimental diets and feeding A different pen was utilized for every male lamb amid the trial time frame. The concentrate diet used consisting of wheat, barley, yellow corn, soybean meal, salt, and minerals.While wheat straw was available ad libitum as basal diet. offering food was once at about 9:00 a.m daily.in quantities calculated by (3%) percent of live body weight to support maintenance and daily gain.

1.5 Health control at the start of the trial, lambs were soaked orally against inside worms, ascarids, lungworms, and tapeworms and rehashed 14 days after the fact. Against outer and inside parasites, lambs were additionally treated toward the beginning of the analysis and after 14 days by means of subcutaneous of lamb. Lambs were also vaccinated (Vaccine) polyvalent inactivated vaccine against Clostridial infections in ruminants.

1.6 Blood parameters and analysis Auto Hematology Analyzer (Medtronic Colter), It is mainly used to detect the parameters of the series of red blood cells, white blood cells, hemoglobin in the blood.The blood samples 5-7 mL were collected from each male lamb via jugular vein puncture using disposable needles(18 gauge)and vacutainer tubes before treatment

and every four weeks during the experimental period (2months), hematological examination(RBC count, WBC count, and Hemoglobin content (Hb), Albumin, Globulin ,GOT were decided from the entire blood tests. After clotting the blood in room temperature serum was separated for each sample and centrifugated at (4000 rpm) for (15 min) in special Eppendorf tubes for biochemical measurements in Auto Analyzer (Cobas).

1.7 Albumin determination Method Principle: Bromocresol green (BCG) forms with albumin, in succinate buffer (acid medium) a colored complex. The colored complex power estimated at 630 nm is relative to egg whites focus on the example. Concentration in the test:

1. Succinate buffer 100 mmol/L
2. Bromocresol green (BCG) 0.27 mmol/L

$$\text{Albumin concentration (gm/dl)} = \frac{\text{Samples}}{\text{Standard Solution}} \text{X concentration of ST.}$$

1.8 Globulin determination The determination of globulin concentration in blood serum was done through a simplified mathematical method by subtraction of the albumin concentration that previously estimated from total protein concentration.

Globulin concentration (gm/dl) = Total protein concentration – Albumin concentration.

1.9 GOT (Glutamate Oxaloacetic transaminase or Aspartate aminotransaminase) This method developed by(Karmen 1955) and optimize by (Henry, et al. 1960) (following modified IFCC recommendations, 1986), reaction scheme is as follow: ASAT

L-Aspartate + 2-Oxoglutarate ↔ Oxaloacetate+L- Glutamate

MDH

Oxaloacetate + NADH + H^+→ Malate + NAD^+

The reduction in absorbance because of the change of NADH into NAD+ is relative to AST action in the example, is estimated at (340 nm). Reagents:

Working reagents which are composed of 5 mmol/L EDTA, 12 mmol/L 2-Oxoglutarate, 200 mmol/L L-Aspartate, 495 UI/L MDH, 820 UI/L LDH, ≤ 0.18 mmol/L NADH, 80 mmol/L Tris Buffer, 7.80±0.1 pH at 30 ℃.

Assay procedure:

The GOT concentration procedure

Pipette into 1cm path length thermostated cuvette:	
Reagent	1 mL
Bring to 37 C then add:	
Serum samples	100 µL
All tubes were mixed start a timer.	

1- Records initial absorbance after 1 minute at 340 nm. Record the absorbance again consistently amid 3 minutes.

2-. Calculated absorbance change per minute (ΔOD /min), which calculate according to the following equation:

AST concentration (IU/L) = (ΔOD plasma sam/min) X 1746

1.10 Statistical Analysis Statistical investigation framework – XLSTAT 2016 program was utilized for information investigation, In this examination for finding the impact of three concentration of lactoferrin (500 mg and 1000 mg and 1500 mg) Complete Randomized Design (CRD) was used.and to decide the important contrasts between methods was utilized Duncan various range test. The factual model for the examination of fluctuation was:

$Yij = \mu + Ai + eij$

Where:-
Yij = observation j in level i of factor A (j = 1,...., 6)
μ = the overall mean
Ai = the effect of level i of factor A (i = 1, 2, 3)
eij = random error associated with means = 0 and variance δ2e

Results and Discussion

Table 1: Effect of different orally level of Lactoferrin on the red blood cell count (Mean±SE)

Treatments	RBC gm/dl Periods (four weeks)			Means
	1	**2**	**3**	
Control group (C)	**1.975±0.098 a**	**2.253±0.162 a**	**2.670±0.192 c**	**2.299±0.11 a**
First orally level of lactoferrin (T1)	**1.818±0.251 a**	**2.538±0.365 a**	**2.930±0.155 bc**	**2.428±0.19 a**
Second, orally level of lactoferrin (T2)	**2.303±0.126 a**	**2.853±0.241 a**	**3.205±0.158 b**	**2.787±0.14 a**
Third orally level of lactoferrin (T3)	**2.188±0.407 a**	**2.573±0.221 a**	**3.890±0.088 a**	**2.883±0.26 a**

A significant difference (P<0.05) was controlled by a varied letter (a, b, c) in the similar column. Nonsignificant differences (P<0.05) were found between the four experimental groups. Lambs of the third treatment group had the highest value (2.883±0.262gm/dl) followed by lambs of the second treatment group(2.787±0.147gm/dl) and then the lambs in the first and control group(2.428±0.198,2.428±0.198, gm/dl) respectively. Moreover, RBC numbers between control and

treated groups are significantly different in the period three (P<0.05) of the experiment. this result probably belongs to using of LF. Similarly, some studies have shown the effect of lactoferrin on increasing red blood cells, it also has shown that lactoferrin protects human RBCs from oxidative worry in its monoferric

structure (Maneva, et al. 2003).Another study detected that lactoferrin potentially have a stimulatory role in erythropoiesis, Absorbed lactoferrin from intestinal lumen due to increasing hepatic protein synthesis it also due to the stimulation of hematopoietic cells within the liver (Calhoun and Brown 1975).

Treatments	WBC gm/dl Periods (four weeks)			Means
	1	2	3	
Control group (C)	**10.925±1.654 a**	**9.125±1.062 b**	**11.425±1.135 a**	**10.492±0.74 a**
First orally level of lactoferrin (T1)	**10.575±1.093 a**	**11.825±0.576 a**	**8.750±0.260 a**	**10.383±0.53 a**
Second orally level of lactoferrin (T2)	**11.075±0.848 a**	**9.275±0.528 b**	**11.850±1.457 a**	**10.733±0.62 a**
Third orally level of lactoferrin (T3)	**10.475 ±0.841 a**	**11.000±0.316 ab**	**11.833±0.403 a**	**11.103±0.34 a**

Table 2: Effect of different orally level of Lactoferrin on the white blood cell count (Mean±SE)

A significant difference (P<0.05) was controlled by a fluctuated letter (a, b) in a similar section. Between all gatherings, non-significant expanding were seen for the white blood cells, but in the period two it was significantly increased (P<0.05) in lambs of the first and third treatment groups (11.825±0.576, 11±0.316 gm/dl) respectively as compared to the lambs of the second(9.275±0.528gm/dl) and control groups(9.125±1.062 gm/dl). The reason may due to the use of LF because LF has a big role in increasing and developing white blood cells. Researches revealed that LF including caused an expansion in the white blood cell checks, Lactoferrin can support the immune system as an antioxidant .and in the processes of immunity especially white blood cells functions LF has a significant role. In vitro studies have demonstrated that lymphocytes contain specific lactoferrin receptors and are responsive to lactoferrin(Bennett and Davis 1981, Hashizume, et al. 1983). Lactoferrin have an effective role in the maturation and function of immune system cells. Significantly LF exerts its effect on cells involved in the commitment of pathogens (antigens) and can direct the development of adaptive immunity, The

ability of LF to promote antigen-specific delayed-type hypersensitivity (DTH) responses and to activate bacillus Calmette-Guerin (Mycobacterium strain) (BCG)-specific T cells suggests that LF plays a role in the initiation of T-cell activation through the modulation of dendritic cell function(Hwang, et al. 2005).

Treatments	hemoglobin gm/dl Periods (four weeks)			Means
	1	2	3	
Control group (C)	**9.300±0.204 a**	**10.600±0.334 a**	**11.650±0.620 b**	**10.517±0.36 a**
First orally level of lactoferrin (T1)	**10.375±0.256 a**	**11.200±0.238 a**	**12.100±0.308 b**	**11.225±0.22 a**
Second orally level of lactoferrin (T2)	**10.300±0.549 a**	**10.725±0.347 a**	**11.075±0.144 b**	**10.700±0.22 a**
Third orally level of lactoferrin (T3)	**9.800±0.889 a**	**10.725±0.375 a**	**13.550±0.419 a**	**11.358±0.57 a**

Table 3: Effect of different orally level of Lactoferrin on the hemoglobin (Mean±SE)

Treatments	Albumin gm/dl Periods (four weeks)			Means
	1	2	3	
Control group (C)	**3.500±0.071 a**	**3.875±0.085 a**	**3.725±0.063 a**	**3.700±0.06 a**
First orally level of lactoferrin (T1)	**3.475±0.063 a**	**4.025±0.293 a**	**3.300±0.135 a**	**3.600±0.13 a**
Second orally level of lactoferrin (T2)	**3.325±0.155 a**	**3.700±0.041 a**	**3.500±0.178 a**	**3.508±0.08 a**
Third orally level of lactoferrin (T3)	**3.550 ±0.096 a**	**3.700±0.108 a**	**3.650±0.250 a**	**3.633±0.08 a**

Various letters (a,b) in a similar column means significantly different (P<0.05).

Measurable investigation (Table 3) uncovered noteworthy contrasts in the hemoglobin concentration between treatments during the third period, the third treatment group (13.550±0.419 gm/dl) compared to other groups (11.075±0.144,12.100±0.308,11.650±0.620 gm/dl) respectively. results show hemoglobin concentration different during the periods of the experiment.studies pointed out that there are positive correlation between the concentration of lactoferrin in serum and the concentration of hemoglobin has resulted in the availability of lactoferrin to raise the proportion of Hb as well as some blood characters lactoferrin enhanced iron metabolism and increase the percentage of blood hemoglobin (Doornenbal, et al. 1988), the

concentration of hemoglobin it also increased with age progress.(Davidsson, et al. 1994) reported that Lactoferrin from animal milk seems to affect intestinal iron absorption, Also affect increasing hemoglobin.

Table 4: Effect of different orally level of lactoferrin on the Albumin concentration (Mean±SE)

Treatments	Globulin gm/dl Periods (four weeks)			Means
	1	**2**	**3**	
Control group (C)	**2.975±0.075 a**	**2.475±0.232 a**	**2.650±0.087 b**	**2.700±0.10 a**
First orally level of lactoferrin (T1)	**2.950±0.290 a**	**2.025±0.118 a**	**2.850±0.050 a**	**2.608±0.15 a**
Second orally level of lactoferrin (T2)	**3.250±0.096 a**	**2.650±0.218 a**	**2.650±0.029 b**	**2.850±0.11 a**
Third orally level of lactoferrin (T3)	**2.950±0.166 a**	**2.550±0.359 a**	**2.575±0.063 b**	**2.692±0.13 a**

Including LF was not influenced fundamentally on the centralization of albumin in the serum Table (4). It is similar to another study results that reported LF is binding to albumin molecules in milk (Roseanu and Brock 2006), The association between lactoferrin and the major bovine whey proteins, α-lactalbumin and albumin has been studied by immunochemical techniques. Bovine lactoferrin is able to form non-covalent complexes with albumin (Lampreave, et al. 1990).The albumin main function in the body is to keep up the osmotic weight of blood and furthermore is in charge of the connection of numerous materials and exchange inside the body to play out its capacities (Prosser 1991). Therefore, giving a high concentration of LF was an indicator to improve the animal's health.

Table 5: Effect of different orally level of Lactoferrin on the globulin concentration (Mean±SE)

Different letters (a, b) in the same column means significantly difference (P<0.05).

Karadi lambs received first orally level of LF had a significantly higher (P<0.05) serum globulin concentration than the other groups in the period three. Their averages being (2.650±0.087, 2.850±0.050, 2.650±0.029, and 2.575±0.063) gm/dl for the control, first, second and third gatherings respectively (Table 5).The purpose behind the expansion in serum globulin centralization of lambs in the principal treatment from the period three of the test is that uncovered state that there was an upgrade of LF in the body by rehashed treatment for the times of the investigation . The liver produces most of the globulins, as well as the immune system, are made others. Certain globulins bind with hemoglobin. Other globulins work as a metal transporter, such as iron, in the blood and help fight against infection (Prosser 1991).

Table 6: Effect of different orally level of Lactoferrin on the (GOT) (Mean±SE)

Treatments	GOT IU/L Periods (four weeks)			Means
	1	**2**	**3**	
Control group (C)	**84.500±7.500 a**	**106.500±18.897 a**	**102.500±10.650 a**	**97.833±7.497 a**
First orally level of lactoferrin (T1)	**91.500±5.252 a**	**142.025±32.354 a**	**131.000 ±11.867 a**	**121.508±12.379 a**
Second orally level of lactoferrin (T2)	**88.500±2.872 a**	**108.175±5.392 a**	**103.600±8.651 a**	**100.092±4.077 a**
Third orally level of lactoferrin (T3)	**96.325±6.182 a**	**103.175±5.702 a**	**162.775±7.373 b**	**120.758±9.609 a**

Various letters (a, b) in the similar column imply significantly various (P<0.05).

GOT table outcomes recognized that lambs of the control and treatment groups were altogether unique (P<0.05). The third treatment group demonstrates an abnormal states, while the control group indicates lower concentration of GOT. Also, results discovered that in the period three of the experiment GOT level was significantly different among the groups, the third treatment group was significantly increased (162.775±7.373 IU/L) compared to the first, second and control groups (131.000 ±11.867, 103.600±8.651, 102.500±10.650 IU/L) respectively.. A GOT protein is found in the distinctive tissue cells of the body in the common state, but when a damage of the cells in a specific tissue is happening this enzyme will enters into the bloodstream and there by increase its level and this is evidence of a defect either because of disease or the

occurrence of bruising or others (Bicek, et al. 2005). So, in general, the decrease of GOT level in blood serum is a sign of health point of the tissue. Lactoferrin cause an increase in the level of GOT in the serum, in some human subjects, injection of 5 mg of LF increased liver enzymes.

Conclusions

In this study high level of LF improves the blood picture and immunological status of the lambs through the significant increase in **RBC**, **Hb**, **WBC, GOT,** Also lactoferrin at first orally level had a significant effect on serum protein **Globulin**.This study detected the efficacy of LF doses more than 1500 mg/wk and the efficacy of supplementing LF in the diet after weaning. More investigation required for detecting LF effect on other biochemical parameters in various periods. Other consecutive studies are required to find the effect of lactoferrin on productive parameters.

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