j.Egypt.vet.med.Assoc 82, no 4. 229 - 245 (2022)

EFFECT OF LACTOFERRIN SUPPLEMENTATION ON BLOOD PROFILE, IMMUNITY AND GROWTH PERFORMANCE IN NEWLY BORN CALVES

By

Nashwa A. Omar*, Abdel-Aziz. A. M.*, Wafa . W. M. and El-Nagar H. A.****

* Pharmacology,* Clinical Pathology Animal Health Research Institute, Tanta Lab.** Cattle Breeding Department, Animal Production Research Institute and ** Biotechnology Researches Department, Animal Production Research Institute, Agricultural Research Center (ARC).

ABSTRACT

A total number of 15 Friesian calves at 3 days of age with 33.47±0.42 kg live body weight were used in this study. Calves were divided into 3 equal groups. During the experimental period

(28 days), calves in all groups were given a similar amount of their dam milk. All calves appeared in healthy state and free of any diseases. The first group was fed cow milk in two meals at 7:00 a.m. and 7:00 p.m. and they were given a starter with berseem hay (BH) and were kept as normal control group (G 1), the second group was fed on the control diet with1 mg/calve supplementation of lactoferrin (G 2) and the 3rd group was fed on the control group`s diet with 2 mg lactoferrin/calve (G 3). All calves were clinically observed for 28 days; during the experimental period, live body weight (LBW) and dry matter intake (Milk, Starter, Berseem hay, concentrate feed mixture and rice straw) were recorded at weekly intervals, then total body gain was calculated every 15 days with fecal scores and respiratory scores were recorded for each calf to monitor their health throughout the trial. Finally two blood samples were collected from each calf of all groups after 2 weeks and 4 weeks post treatment for hematological and biochemical examinations. Calves received lactoferrin showed expressive elevation in serum total protein, globulin, glucose levels, IgG and IgA concentrations while expressed significant decrease in serum total lipids, cholesterol, and ALT, AST, creatinine and blood urea nitrogen levels. Body weight gain, average body weight gain and total body weight gain were significantly increased. In this study it could be concluded that lactoferrin could be a beneficial supplement in the neonatal calves` diets prior to weaning.

Key words:

Lactoferrin, Immunity, Growth, Calves.

INTRODUCTION

Lactoferrin is an iron binding glycoprotein which is found in milk, whey and is found in high concentrations in colostrum, endocrine and exocrine excretions **(Steijns and Hooijdonk, 2000,Pan *et al.*,2007).**Colostral LF is involved in intestinal and immunological development, improved their grain intake and average daily gain so it plays a vital role in calves` health **(Connelly and Erickson, 2016).**

Lactoferrin (LF) is an important part of immuno-globulins; it`s a member of transferrin proteins family and is the first innate immune system produced from neutrophils, it is found in mammalian colostrum and milk. Moreover it is a specialized immune protein which is characterized by high bioactivity through its ability to bind iron, so it was called red milk protein when firstly discovered. LF concentration varies according to animal type and offspring. Pathogens control lactoferrin amount, as LF concentration increases during inflammation and viral infection. LF primary function is to protect mammary gland after birth **(Al-kudsi and Khalid, 2019).**

Lactoferrin is involved in a broad spectrum of biological actions involving antimicrobial, antiviral, antimycotic and anti-inflammatory activities **(Simonia *et al.*, 2020)**. Lactoferrin plays an important role in Ferro kinetics as it binds with free iron at high affinity limiting available irons amount for metabolism of microorganisms. Its vital role in host defense mechanisms represented at bactericidal and bacteriostatic effects; where it prohibits the proliferation of other microbes such as viruses and fungi. Lactoferrin is also involved in the immune system modulation and late studies suggested that lactoferrin directly reshuffles both function and production of monocytes and neutrophils **(Cavestro *et al.*, 2002).**

The current work was designed to declare the effect of LF supplementation on live body weight (LBW), total body gain, hematological and biochemical parameters of calves.

MATERIAL AND METHODS

Drugs:

Lactoferrin: (Pravotin-100 mg - Medizen Pharmaceutical Industries, Borg Al Arab, Egypt) was offered to calves in different groups by mixing in morning milk meals just before feeding, at a doses of 1 mg/Calf (G 2) and 2 mg/Calf (G 3) once a day for 28 days **(Connelly and Erickson, 2016).**

Cow Milk was collected from El-Gemmeza Animal production station, and the starter was given to calves from Marg Fodder Factory - Ministry of Agriculture and Land Reclamation. The berseem hay (BH; Trifolium alexandrinum) was pliable at experimental farm of El-Gemmeza Animal Production Experimental Station belong to Agriculture Research Center.

Experimental design and treatment:

This study was carried in El-Gemmeza Animal Production Experimental Station in the middle Delta of Egypt belonged to Animal Production Research Institute (APRI), incorporation with Animal Health Research Institute (AHRI) Tanta branch, belonged to Agricultural Research Center, Ministry of Agriculture and Land Reclamation.

Fifteen Friesian calves at 3 days of age with 33.47±0.42 kg live body weight were individually protected in pens (1.0 × 1.5 m) with rice straw bed with free access to clean drinking water. Calves in the 1st group were fed cow milk in two meals at 7.0 a.m. and 7.0 p.m. and they were given a starter with berseem hay and kept as control group (G1). Calves in G2 fed on the control diet with 1mg LF/calve, calves in G3 were fed 2mg LF/calve. Lactoferrin were offered to calves in different groups by mixing in morning milk meals just before feeding**.** After 2 hours, a starter was allowed to calves with free choice. The calves were shaved over jugular vein to ease the collection of blood samples.

All calves were clinically observed for 28 days; during the experimental period (3-30 days of age), live body weight (LBW) and dry matter intake (milk, starter, berseem hay (BH), concentrate feed mixture (CFM) and rice straw (RS) of calves were on record at weekly intervals, then total body gain was calculated every 15 days with fecal scores and respiratory scores were recorded for each calf to monitor their health throughout the trial. Finally two blood samples were collected from each calf of all groups 2 weeks and 4 weeks post treatment for hematological and biochemical examinations.

Sampling:

Blood samples:

At the 14^{th} and the 28^{th} day of the experiment before morning feeding, two types of blood samples were collected from each of the fifteen calves in all groups from jugular vein using vacuum tubes, collection of the first blood samples were done in vacutainer tubes (Venoject, Terumo) have Ethylene diamine tetra acetic acid (EDTA) as an anticoagulant for hematological examination (RBCs count, Hb concentration, Packed Cell Volume %, total and differential leukocytic counts). Determination of hematological parameters was performed by

using a veterinary haematology analyzer (Exigo, Boule medical AB., and Sweden). In a plain centrifuge tube the second blood sample (5 ml) was collected and left to clot, after that was centrifuged at 3000 r.p.m. for 20 minutes for separation of serum. At -20°C the sera were then stored to be frozen until the biochemical analysis (Determination of AST, ALT, creatinine, uric acid, total protein, albumin, globulin, glucose, total lipids, and cholesterol).

Experimental protocol:

Clinical Examination:

Within the experimental period live body weight (LBW) and dry matter intake (Milk,starter, CFM,BH and RS) of all calves were recorded at weekly intervals, then total body gain was calculated. Daily recording of body temperature and respiratory rate was done.

Biochemical analysis:

Serum biomarkers were pinpointed using a commercial kit as directed by manufacturers. All parameters were measured spectrophotometrically by using standardized test-kits.

Serum analysis for total proteins concentration **(Henry, 1964),** Albumin **(Doumas *et al.*, 1997),** while, globulin concentration calculation was dobe by subtraction of albumin from total proteins concentration, total lipids **(Zöllner and Kirsch, 1962),** total cholesterol **(Richmond, 1973),** glucose **(Trinder, 1969)** by using commercial kits (Nanjing Jiancheng Biochemical Reagent Co., China).

Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were assessed according to **Reitman and Frankel (1957)** technique.

Uric acid and creatinine determination according to **Newman and Price (1999)** using kits of Biodignostic, Cairo, Egypt.

Serum Immunoglobulins (IgG, IgM and IgA) concentrations were determined using quantitative ELISA (Bovine IgG, IgM and IgA ELISA quantitative kit, Bethyl laboratories, UK) according to **Killingsworth and Savory (1972).**

Recording of fecal scores and respiratory scores for each calf to monitor their health throughout the trial every 15 days.

Fecal scores were determined according to **Larson *et al.* (1977),** where fecal fluidity was 1 if normal and 4 if liquid state, fecal thickness was 1 if normal and 5 if viscous and fecal smell was 1 if normal and 3 at very bad smile state.

Respiratory scores were assigned on a 1 to 3 scale according to **Bascom *et al.* (2002)** where 1 in normal case, 2 at runny nose/eyes, and 3 where there was mucus discharge from nose/eyes

and fever. Total health score was calculated by feces fluidity, thickness, smell summation and respiratory scores.

Statistical Analysis:

The obtained data were registered using Excel software and analyzed statistically using one-way analysis of variance for evaluation of the treatment effect by using os **IBM SPSS (2017)** statistical program version 25 in a totally random design. Checking the significant differences by Duncan's multiple range test **(Duncan, 1955).**

RESULTS AND DISCUSSION

Table (1) revealed that G2 and G3 calves which had been supplemented with LF showed signi ficant increase in body weight gain, average body weight gain (ABWG) and total body weight gain (TBWG) greater than control group calves (G1) which had not been supplemented with LF this result agreed with those of **Prenner *et al.* (2007) and Khalid and Al-Kudsi (2018)**who recorded that LF addition to the feeding of Holstein calves caused elevated weight gain and ABWG explaining that ,this increase may be due to lactoferrin effect on the calves` general health and improvement of feed intake amount and the low incidence of diseases.

The increase in body weight may be due to ameliorated health of treated calves **(Joslin *et al.*, 2002)** and this had been accomplished through three actions of LF: growth factor activity **(Zhang *et al.*, 2001),** antibacterial activity**(Teraguchi *et al.*,1994)** and LF capability to spur glucose absorption**(Teraguchi *et al.*,1998)** so elevated growth responses may be related to LF competence to enhance alimentary growth, nutrients absorption and consequently feed adequacy **(Robblee *et al.*, 2003).**

Moreover**, Prgomet *et al.* (2007)** reported LF effect to improve animal performance via gastr ointestinal tract morphology modulation and peyer's patches enlarged size in calves that had been fed lactoferrin.

Table (1): Effect of lactoferrin treatment on calves' body weight.

	G1	G2	G3
Initial BW	**33.60±1.03^{a}**	**33.40±0.75^{a}**	**33.40±0.51^{a}**
BW after 15/d	**45.80±0.86^{c}**	**49.60±1.03^{b}**	**53.20±1.28^{a}**
BW after 30/d	**61.00±1.14^{c}**	**66.80±2.54^{b}**	**75.20±1.36^{a}**
TBWG initial to 15/d	**12.20±0.37^{c}**	**16.20±0.37^{b}**	**19.80±0.80^{a}**
ABWG initial to 15/d	**0.81±0.02^{c}**	**1.08±0.02^{b}**	**1.32±0.05^{a}**
TBWG 15/d to 30/d	**15.20±0.49^{b}**	**17.20±1.66^{b}**	**22.00±0.84^{a}**

ABWG 15/d to 30/d	**1.01±0.03[b]**	**1.15±0.11[b]**	**1.47±0.06[a]**

Table (2) declared that Calves received LF (Group 2 and 3) showed expressive elevation in serum total protein and globulin levels allover the experimental period when compared to con trol group in a dose-dependent manner; the increase in serum total protein in LF supplemente d groups was mainly due to the significant increase in serum globulin which reflect the immu ne-modulatory effect of LF, this outcome is in accordance with **Husain and Aref (2020).**

Table (2) explicated that Calves received LF (Group 2 and 3) showed significant increase in glucose level allover the experimental period when compared to control group; this result is in accordance with **Mallaki *et al.*, (2021) and Aoyama *et al.*, (2022)** who revealed that LF elevated glucose level as LF improved energy balance; which was previously recorded by **Muri *et al.* (2005) and Cowles *et al.* (2006)** who declared LF roles in augmenting and expanding intestinal epithelial size and function leading to more glucose absorption. Our results proclaimed that calves received LF (G2 and G3) expressed significant decrease in serum total lipid and cholesterol levels allover the experiment when compared to control group; this result agreed with **Takeuchi *et al.*, (2004), Morishita *et al.*, (2013), Li and Hsieh, (2014), Morishita *et al.*, (2016) and Nozari *et al.*, (2018).** LF has a beneficial effect on serum total lipid and cholesterol levels and this is could be interpreted by LF interaction electrostatically with bile acids so inhibiting intestinal cholesterol absorption and thus leads to raised cholesterol excretion as assumed by **Nakamura *et al.*, (2016).** Our results revealed that calves received LF (G2 and G3) expressed significant decrease in serum ALT and AST levels allover the experiment when compared to control group in a dose-dependent manner; this result agreed with **Li, and Hsieh, (2014), Aoyama *et al.*, (2022) and Elazab *et al.*, (2022)** who reported that LF decreased serum ALT and AST levels as LF provokes hepato-protective cytokine production (Interlukin-11) **(Oda *et al.*, (2020).** Table (2) showed that calves received LF (G2 and G3) expressed significant decrease in creatinine and blood urea nitrogen (BUN) levels when equate to control group; this result agreed with **Okazaki *et al.*, (2012), Kimoto *et al.*, (2013), Hsu *et al.*, (2020) and Zahan *et al.*, (2022)** who declared that lactoferrin ameliorated elevated creatinine and BUN levels; this may be due to repressed oxidative stress and boosted renal antioxidant armory (GSH, SOD, GPx, TAC) with encore of NOX-1, Nrf-2 and HO-1 levels **(Arab *et al.*, 2018).**

Table (2): Effect of lactoferrin treatment on blood biochemical parameters.

Item	Treatment Period	G1	G2	G3
Total protein (mg/dl)	Pre-Treatment	7.06±0.12^{a}	7.20±0.14^{a}	7.32±0.16^{a}
	Post 14/d Treatment	6.27±0.20^{b}	6.85±0.21^{a}	7.26±0.13^{a}
	Post 28/d Treatment	6.43±0.18^{b}	7.14±0.21^{a}	7.47±0.13^{a}
Albumin (mg/dl)	Pre-Treatment	4.16±0.14^{a}	4.24±0.17^{a}	4.32±0.11^{a}
	Post 14/d Treatment	3.63±0.17^{a}	3.50±0.11^{a}	3.56±0.19^{a}
	Post 28/d Treatment	3.83±0.05^{a}	4.11±0.22^{a}	4.19±0.13^{a}
Globulin (mg/dl)	Pre-Treatment	2.91±0.06^{a}	2.96±0.08^{a}	3.00±0.17^{a}
	Post 14/d Treatment	2.63±0.18^{c}	3.35±0.13^{b}	4.01±0.25^{a}
	Post 28/d Treatment	2.60±0.19^{c}	3.03±0.04^{b}	3.28±0.11^{a}
Glucose (mg/dl)	Pre-Treatment	108.03±3.64^{a}	109.23±4.36^{a}	108.36±3.80^{a}
	Post 14/d Treatment	87.91±3.43^{b}	99.42±3.99^{a}	101.83±2.58^{a}
	Post 28/d Treatment	85.88±3.14^{b}	92.26±3.76^{a}	95.70±2.84^{a}
Total lipids (mg/dl)	Pre-Treatment	73.34±3.02^{a}	73.82±3.86^{a}	73.50±3.57^{a}
	Post 14/d Treatment	78.18±2.80^{a}	72.64±3.43^{b}	69.92±3.84^{b}
	Post 28/d Treatment	72.24±4.03^{a}	69.18±2.69^{ab}	69.09±3.20^{b}
Cholesterol (mg/dl)	Pre-Treatment	61.36±1.81^{a}	60.92±2.17^{a}	61.10±1.70^{a}
	Post 14/d Treatment	79.12±2.99^{a}	75.20±3.91^{ab}	70.56±3.19^{b}
	Post 28/d Treatment	95.48±3.18^{a}	90.78±4.04^{ab}	88.46±3.14^{b}
ALT (IU/l)	Pre-Treatment	17.82±2.18^{a}	17.58±1.77^{a}	17.64±1.46^{a}
	Post 14/d Treatment	19.66±2.37^{a}	17.08±2.52^{ab}	14.74±2.68^{b}
	Post 28/d Treatment	19.82±2.62^{a}	16.74±2.29^{ab}	15.16±2.46^{b}
AST (IU/l)	Pre-Treatment	68.00±2.45^{a}	68.40±2.30^{a}	68.20±3.11^{a}
	Post 14/d Treatment	74.60±6.47^{a}	65.20±5.31^{b}	53.20±5.85^{c}
	Post 28/d Treatment	62.80±4.97^{a}	56.20±5.26^{ab}	51.80±4.38^{b}
Creatinine (mg/dl)	Pre-Treatment	1.58±0.64^{a}	1.52±0.54^{a}	1.55±0.61^{a}
	Post 14/d Treatment	2.01±0.55^{a}	1.39±0.64^{ab}	1.01±0.41^{b}
	Post 28/d Treatment	1.83±0.63^{a}	1.23±0.73^{ab}	0.88±0.47^{b}
Urea (mg/dl)	Pre-Treatment	8.13±1.14a	8.15±1.00a	8.10±0.90a
	Post 14/d Treatment	8.07±0.63a	7.00±0.43ab	6.01±0.53b
	Post 28/d Treatment	7.52±0.80a	6.15±0.54ab	5.61±0.75b

Calves received LF (G3) showed significant increase in RBCs count, Hb concentration and PCV %, allover the experiment when compared to control normal group throughout the whole experiment; this may be due to lactoferrin stimulation of erythropoiesis and raising of hepatic

protein synthesis, LF also stimulates hematopoietic cells **(Calhoun and Brown 1975**)**;** also LF intensified iron metabolism and elevate hemoglobin percentage **(Doornenbal, *et al.* 1988)**, also **Davidsson, *et al.*, (1994)** reported that LF appears to affect intestinal iron absorption, thus increase hemoglobin concentration. These findings agreed with **Kume and Tanabe (1996), Husain and Arif (2020) and Mallaki *et al.*, (2021),** while disagreed with **Muri *et al.* (2005)** who reported no difference in RBCs count and Hb concentration in calves received LF and this difference may be due to the difference in duration of LF supplementation, doses and molecular structure of the drug**.**

Table (3) represented that total leukocytic count (T.L.C.) showed significant decrease in group 2 and 3 allover the experimental period when compared to control group and these findings were supported by the results recorded by **Reznikov, (2014) and Mallaki *et al.*, (2021)** which could be due to lactoferin reducing effect on pathogens levels in the alimentary tract **(Weinberg and Des, 2007).**

Neutrophils count showed significant decrease in groups 2 and 3 allover the experimental period when compared to control group and these findings were supported by the results recorded by **(Kurz and Willett, 1991, Egli and Blum, 1998, Muri *et al.* 2005 and Mallaki *et al.*, 2021)** such decrease could be due to haemodilution as assumed by **Muri *et al.* (2005) and Legr and, (2016)** who proposed that lactoferrin is a constituent of secondary neutrophil granules. These results disagree with **Prgomet *et al.* (2007*)*** who stated that LF cause elevated number of peripheral blood leucocytes and **Morshedi *et al.*, (2021)** who reported that adding 800 mg/Kg diet lactoferrin stimulates Asian sea bass` non-specific immune response; this difference may be due to species and dosage difference.

Lymphocytes count was significantly increased in group 2 and 3 at 28^{th} day of the experiment when compared to control group and these findings were supported by the results recorded by **Mallaki *et al.*, (2021)** who reported that LF elevated lymphocyte percentage.

Monocytes count showed significant decrease in group 2 and 3 allover the experimental period when compared to control group and these findings were supported by the results recorded by **Reznikov, (2014)** who recorded that LF decreased monocytes percent. While disagreed with **Hellweg *et al.*, (2008)** who recorded that lactoferrin elevated the number of monocytes.

Table (3): Effect of lactoferrin treatment on blood hematological parameters.

Item		Treatment Period	G1	G2	G3
RBC ($x10^6$)		Pre-Treatment	7.32±1.58[a]	7.36±1.92[a]	7.34±1.72[a]
		Post 14/d Treatment	7.84±1.87[b]	9.06±1.68[ab]	11.10±2.17[a]
		Post 28/d Treatment	9.18±1.40[b]	10.40±1.30[b]	12.64±1.66[a]
Hb (g/dl)		Pre-Treatment	9.82±1.93[a]	9.90±2.03[a]	9.66±1.34[a]
		Post 14/d Treatment	8.98±2.41[b]	10.26±2.49[ab]	13.02±2.86[a]
		Post 28/d Treatment	10.14±2.27[b]	10.76±2.35[b]	14.28±2.44[a]
PCV%		Pre-Treatment	31.00±8.25a	30.60±7.83a	30.80±8.35a
		Post 14/d Treatment	30.20±5.14b	33.10±6.02ab	40.20±5.21[a]
		Post 28/d Treatment	33.20±5.15b	34.80±4.99ab	43.50±5.62[a]
T.L.C. ($x10^3$)		Pre-Treatment	8.42±1.58[a]	8.50±1.21[a]	8.48±1.40[a]
		Post 14/d Treatment	9.14±0.85[a]	8.32±0.96[ab]	7.58±1.14[b]
		Post 28/d Treatment	10.10±1.07[a]	7.84±1.21[b]	6.52±0.91[b]
D.L.C.	Neutrophils (%)	Pre-Treatment	3.62±0.31[a]	3.82±0.54[a]	3.48±0.71[a]
		Post 14/d Treatment	4.04±0.38[a]	3.58±0.41[ab]	3.15±0.47[b]
		Post 28/d Treatment	4.65±0.49[a]	3.58±0.55[b]	3.20±0.45[b]
	Lymphocytes (%)	Pre-Treatment	4.46±1.24[a]	4.36±0.59[a]	4.62±0.76[a]
		Post 14/d Treatment	4.20±0.63a	4.40±0.51[a]	4.73±0.44[a]
		Post 28/d Treatment	3.06±0.43c	3.95±0.61b	5.05±0.54[a]
	Basophils (%)	Pre-Treatment	0.04±0.02a	0.04±0.02[a]	0.05±0.02[a]
		Post 14/d Treatment	0.03±0.02a	0.05±0.02[a]	0.04±0.02[a]
		Post 28/d Treatment	0.04±0.02a	0.03±0.01[a]	0.05±0.01[a]
	Monocytes (%)	Pre-Treatment	0.15±0.03[a]	0.17±0.08[a]	0.14±0.04[a]
		Post 14/d Treatment	0.19±0.02[a]	0.16±0.02[b]	0.14±0.02[b]
		Post 28/d Treatment	0.20±0.02[a]	0.14±0.02[b]	0.12±0.02[b]

Table (4) revealed that G2 calves showed significant rise in IgG concentration 14 days post treatment, while G3 calves showed significant elevation in IgG concentration allover the experiment when emulate to control group calves. This result agreed with that of **Prgomet *et al.*, (2007**) who recorded that LF increased serum IgG levels, while disagreed with **Connelly and Erickson, (2016)** who reported that LF did not improve serum IgG level during the first 24 hours; this different may be due to difference in experiment duration.

Serum IgM findings in lactoferrin-treated groups (G2-G3) revealed non-significant effect on IgM concentration allover the experiment when compared to normal control group.

These findings agreed with the previous study of **Eslamloo *et al.*, (2012) and Khuyen *et al.*, (2017)** who recorded that LF did not affect serum IgM.

Our results revealed that G3 calves which had been supplemented with LF exhibited significant elevation in IgA concentration allover the experiment by comparison to control group calves which had not been supplemented with LF, this result agreed with that of **(Jang *et al.*, 2015).**

Table (4): Effect of lactoferrin treatment on immunoglobulin G, M and A.

Item	Treatment Period	G1	G2	G3
IgG (g/l)	Pre-Treatment	23.68±0.72^{a}	23.80±0.58^{a}	23.50±1.08^{a}
	Post 14/d Treatment	17.12±1.18^{c}	18.62±0.76^{b}	21.80±1.00^{a}
	Post 28/d Treatment	15.56±0.67^{b}	16.80±0.86^{b}	20.18±1.13^{a}
IgM (g/l)	Pre-Treatment	1.80±0.15^{a}	1.82±0.18^{a}	1.76±0.10^{a}
	Post 14/d Treatment	1.44±0.09^{a}	1.51±0.11^{a}	1.68±0.07^{a}
	Post 28/d Treatment	1.64±0.18^{a}	1.70±0.06^{a}	1.82±0.47^{a}
IgA (g/l)	Pre-Treatment	0.30±0.04^{a}	0.30±0.04^{a}	0.30±0.05^{a}
	Post 14/d Treatment	0.31±0.07^{b}	0.41±0.09^{b}	0.51±0.05^{a}
	Post 28/d Treatment	0.44±0.06^{b}	0.52±0.06^{b}	0.62±0.07^{a}

The present results of feces scores as fluidly and smell showed lower values in G2 and G3 when compared to G1, but did not differ significantly. The respiratory score showed non-significant low value in G2 and G3 than in G1, also, the score of fecal thickness was improved as influenced by lactoferrin treatments in G2 and G3 but without significant differences.

These outcomes reflected that calves` total health score in G3 was low and followed by G2,

but G1 showed high score (Table 5).

The observed amelioration of immunity, liver (ALT and AST) and kidney functions (Creatinine and urea) of G3 calves declared the decrease in respiratory score, feces fluidly, fecal smell and total health scores, which may reflect clear refinement in calve health status in association with reduction of diarrheal incidence.

Generally, lactoferrin had antimicrobial and antiviral effects **(Ishikawa *et al.,* 2013).** Also, it had immune stimulatory effect by increasing type I interferon (IFN) production, which suppresses viral replication in the small intestine, which activate the natural killer cells **(Kuhara *et al.,* 2006).** Lactoferrin cause isotype switching of B cells that increase the production of secretory immunoglobulin A in small intestine, which prevents the pathogens attachment to intestinal mucosa **(Jang *et al.,* 2015).** Also, lactoferrin can activate CD4+ and CD8+ T cells in alimentary lymphoid tissues **(Wang *et al.,* 2000).**

Table (5): Effect of lactoferrin treatment on health status.

Item	Treatment Period	G1	G2	G3
Respiratory Score	**Pre-Treatment**	**1.00±0.00^{a}**	**1.00±0.00^{a}**	**1.00±0.00^{a}**
	Post 14/d Treatment	**1.40±0.89^{a}**	**1.00±0.00^{a}**	**1.00±0.00^{a}**
	Post 28/d Treatment	**1.40±0.89^{a}**	**1.00±0.00^{a}**	**1.00±0.00^{a}**
Fecal Fluidity	**Pre-Treatment**	**1.00±0.00^{a}**	**1.00±0.00^{a}**	**1.00±0.00^{a}**
	Post 14/d Treatment	**1.40±0.55^{a}**	**1.20±0.45^{a}**	**1.00±0.00^{a}**
	Post 28/d Treatment	**1.40±0.55^{a}**	**1.60±0.55^{a}**	**1.00±0.00^{a}**
Fecal Thickness	**Pre-Treatment**	**1.00±0.00^{a}**	**1.00±0.00^{a}**	**1.00±0.00^{a}**
	Post 14/d Treatment	**2.00±1.41^{a}**	**1.40±0.89^{a}**	**1.00±0.00^{a}**
	Post 28/d Treatment	**2.00±1.41^{a}**	**2.20±1.10^{a}**	**1.00±0.00^{a}**
Fecal Smell	**Pre-Treatment**	**1.00±0.00^{a}**	**1.00±0.00^{a}**	**1.00±0.00^{a}**
	Post 14/d Treatment	**1.60±0.89^{a}**	**1.20±0.45^{a}**	**1.00±0.00^{a}**
	Post 28/d Treatment	**1.40±0.55^{a}**	**1.80±0.84^{a}**	**1.00±0.00^{a}**
Health Score	**Pre-Treatment**	**4.00±0.00^{a}**	**4.00±0.00^{a}**	**4.00±0.00^{a}**
	Post 14/d Treatment	**6.40±2.51^{a}**	**4.80±1.79^{a}**	**4.00±0.00^{a}**
	Post 28/d Treatment	**6.20±2.28^{a}**	**6.60±2.41^{a}**	**4.00±0.00^{a}**

CONCLUSIONS

It could be concluded that Lactoferrin addition to calves diet in precocious ages after birth had improved influence on body weight gain, average daily gain and is beneficial for performance; these restraint may be due to ameliorated health in treated calves. Further research is necessary to determine the optimum amounts of LF to be supplemented to milk or milk replacer and if LF would be beneficial as a precautionary supplement or as a therapy for diarrhea.

REFERENCES

Al-kudsi, N. H. and Khalid, W.A. (2019): Association of lactoferrin with some immunological and blood traits of Holstein calves in the middle of Iraq. Journal of Research in Ecology. 6 (2): 1778-1787.

Arab, H.H., Salama, S.A. and Maghrabi, I.A. (2018): Camel Milk Ameliorates 5-Fluorouracil-Induced Renal Injury in Rats: Targeting MAPKs, NF-κB and PI3K/Akt/eNOS Pathways. Cell Physiol Biochem. 46 (4):1628-1642. doi: 10.1159/000489210. Epub 2018 Apr 20.

Aoyama, Y., Naiki-Ito, A., Xiaochen, K., Komura, M., Kato, H., Nagayasu, Y., Inaguma, S., Tsuda, H., Tomita, M., Matsuo, Y., Takiguchi, S. and Takahashi, S. (2022): Lactoferrin Prevents Hepatic Injury and Fibrosis via the Inhibition of NF-κB signaling in a Rat Non-Alcoholic Steatohepatitis Model. Nutrients. 14, 42. https:// doi.org/10.3390/nu14010042.

Bascom, S.S.; James, R.E.; McGilliard, M.L. and Hovingh, E.P. (2002): Performance of Jersey bull calves fed whole milk or milk replacers with varying fat/protein ratios. J. Dairy Sci., 80 (1):79.

Berlutti, F., Pantanella, F. Natalizi, T. Frioni, A. Paesano, R. Plowmen, A. and Valenti, P. (2011): Antiviral properties of lactoferrin-Anatural immunity molecule. Molecules 16:6992-7018.

Calhoun, M. L. and Brown, E. (1975): Hematology and hematopoietic organs." Diseases of Swine. Iowa State University Press, Ames, IA: 38-71.

Cavestro, G., Ingegnoli, A.V., Aragona, G., Loris Borghi, L., Nicola Mantovani, N., Alta villa, N., Dal Bò, N., Pilotto, A., Bertelè, A., Franzè, A., Di Mario, F., Borghi, L. (2002): Lactoferrin: Mechanism of action, clinical significance and therapeutic relevance. Acta bio-medica: Atenei Parmensis 73(5 - 6):71-3.

Connelly, R. A. and Erickson, P. S. (2016): Lactoferrin supplementation of the neonatal calf has no impact on immunoglobulin G absorption and intestinal development in the first days of life. J. Anim. Sci. 94:196–200.

Constable, P. D. (2004): Antimicrobial use in the treatment of calf diarrhea. J. Vet. Intern. Med. 18:8-17.

Cowles, K.E., White, R. A., Whitehouse, N. L. and Erickson, P. S. (2006): Growth Characteristics of Calves Fed an Intensified Milk Replacer Regimen with Additional Lactoferrin. Journal of Dairy Science. 89 (12): 4835 - 4845.

Davidsson, L., Kastenmayer, P., Yuen, M., Lönnerdal, B. and Hurrell, R. F. (1994): Influence of lactoferrin on iron absorption from human milk in infants." Pediatric Research 35 (1): 117-124. 7.

Doornenbal, H., Tong, A. and Murray, N. (1988): Reference values of blood parameters in beef cattle of different ages and stages of lactation." Canadian Journal of Veterinary Research 52 (1): 99.

Doumas, B.T., Watson, W.A. and Biggs, H.A. (1997): Albumin standards and the measurements of serum albumin with bromocresol green. Clin. Chem. Acta, 31: 87-96. https://doi.org/10.1016/S0009-8981 (96)06447-9.

Duncan, D.B. (1955): "Multiple range and multiple F tests". Biometrics., 11: 1-42. https://doi.org/10.2307/3001478.

Egli, C.P., and Blum, J.W. (1998): Clinical, haematological, metabolic and endocrine traits during the first three months of life of suckling Simmentaler calves held in a cow-calf operation. J.Vet.Med. A 45:99 -118.

Elazab, M.F.A., Elbaiomy, A.E.A., Ahmed, M.S., Alsharif, K.F., Dahran, N., Elmahallawy, E.K. and Mokhbatly, A.A. (2022): Ameliorative Effects of Bovine Lactoferrin on Benzene-Induced Hematotoxicity in Albino Rats. Front. Vet. Sci. 9:907580. doi: 10.3389/fvets.2022.907580.

Eslamloo, K., Falahatkar, B. and Yokoyama, S. (2012): Effects of dietary bovine lactoferrin on growth, physiological performance, iron metabolism and non-specific immune responses of Siberian sturgeon *Acipenser baeri*. Fish and Shellfish Immunology. 32 (6): 976-985.

Hellweg, P., Krammer-Lukas, S., Strasser, A. and Zentek, J. (2008): Effects of bovine lactoferrin on the immune system and the intestinal microflora of adult dogs. Archives of Animal Nutrition. 62(2): 152-161. DOI: 10.1080/17450390801892575.

Henry, R. J. (1964): Clinical Chemistry, Principles and Techniques, Hoeber Medical, Harper-Row, 190.

Hsu, Y.H., Chiu, I.J., Lin, Y.F., Chen, Y.J., Lee, Y.H. and Chiu, H.W. (2020): Lactoferrin Contributes a Renoprotective Effect in Acute Kidney Injury and Early Renal Fibrosis. Pharmaceutics. 2020 May; 12 (5): 434. doi: 10.3390/pharmaceutics12050434.

Husain, R.A. and Arif, M.K**. (2020):** Effect of Lactoferrin on some Hematological Parameters in Karadi Male Lambs after Weaning. J. of Kirkuk Univ. for Agri. Sci. (11) No. (2),

IBM Corp. Released (2017): IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.(https://www.ibm.com/support/pages/how-cite-ibm-spss-statistics-or-earlier-versions-spss).

Ishikawa, H., Awano, N., Fukui, T., Sasaki, H. and Kyuwa, S. (2013): The protective effects of lactoferrin against murine norovirus infection through inhibition of both viral attachment and replication. Biochem. Biophys. Res. Commun., 434, 791-796.

Jang, Y.S., Seo, G.Y., Lee, J.M., Seo, H.Y., Han, H.J., Kim, S.J., Jin, B.R., Kim, H.J., Park, S.R. and Rhee, K.J (2015): Lactoferrin causes IgA and IgG2b isotype switching through betaglycan binding and activation of canonical TGF-β signaling. Mucosal. Immunol. 2015, 8, 906-917.

Joslin, R. S., Erickson, P. S., Santoro, H. M., Whitehouse, N. L., Schwab, C. G. and Rejman, J. J. (2002): Lactoferrin Supplementation to Dairy Calves. J. Dairy Sci. 85 (5): 1237-1242.

Khalid, W.A. and Al-Kudsi, N.H. (2018): Effect of lactoferrin on growth of Holstein calves in the m iddle of Iraq Journal of Research in Ecology. 6 (2): 1788-1793.

Killingsworth LM. and Savory, J. (1972): Manual Nephelometric Methods for Immunochemical determination of immunoglobulins IgG, IgA and IgM. Clin. Chem., 18 (4): 335-339. https://doi.org/10.1093/clinchem/18.4.335.

Kimoto, Y., Nishinohara, M., Sugiyama, A., Haruna, A. and Takeuchi, T. (2013): Protective effect of lactoferrin on Cisplatin-induced nephrotoxicity in rats. J. Vet. Med. Sci. 2013, 75, 159-164.

Kuhara, T., Yamauchi, K., Tamura, Y. and Okamura, H. (2006): Oral administration of lactoferrin increases NK cell activity in mice via increased production of IL-18 and type I IFN in the small intestine. J. Interferon Cytokine Res., 26, 489-499.

Kume, S., and Tanabe, S. (1996): Effect of supplemental lactoferrin with ferrous iron on iron status of newborn calves. J. Dairy Sci. 79:459-464.

Kurz, M. M., and Willett, L. B. (1991): Carbohydrate, enzyme and hematology dynamics in newborn calves. J. Dairy Sci. 74:2109 -2118.

Khuyen, T.D., Syaghalirwa, N.M., Cornet, V., Douxfils, J., Betoulle, S., Bossier, P., Felipe, E. R., Tort, L. and Kestemont, P. (2017): Physiological and immune response of juvenile rainbow trout to dietary bovine lactoferrin. Fish and Shellfish Immunology. (71): 359-371. https://doi.org/10.1016/j.fsi.2017.10.027.

Larson, L.L.; Owen, F.G.; Albright, J.L.; Appleman, R.D.; Lamb, R.C. and Muller, L.D. (1977): Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy. Sci., 60: 989-991.

Legrand, D. (2016): Overview of lactoferrin as a natural immune modulator. J. Pediatr. 173, 10-15.

Li, Y.C. and Hsieh, C.C. (2014): Lactoferrin Dampens High-Fructose Corn Syrup-Induced Hepatic Manifestations of the Metabolic Syndrome in a Murine Model. PLoS One. 9 (5): e97341. doi: 10.1371/journal.pone.0097341.

Lombard, J. E., Fossler, C. P. Adams, A. E. and Kopral, C. A. (2015): Use of antibiotics on U.S. dairy operations. Pages 298-299 in Proceedings of the 2015 Conference of the American Association of Bovine Practitioners.

Mallaki, M., Hosseinkhani, A., Taghizadeh, A., Hamidian, G., and Paya, H. (2021): The Effect of Bovine Lactoferrin and Probiotic on Performance and Health Status of Ghezel Lambs in Preweaning Phase. Iranian Journal of Applied Animal Science. 11 (1): 101-110.

McGowan, M.W.; Artiss, J.D.; Strandbergh, D.R. and Zak, B. (1983): A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem., 29 (3): 538-542. PMID: 6825269.

Morishita, S., Kawaguchi, H., Ono,T., Miura, N., Murakoshi, M., Sugiyama, K., Kato, H., Tanimoto, A. and Nishino, H. (2016): Enteric lactoferrin attenuates the development of high-fat and high-cholesterol diet-induced hypercholesterolemia and atherosclerosis in Microminipigs, Bioscience, Biotechnology, and Biochemistry, 80:2, 295-303, DOI: 10.1080/09168451.2015.1091713

Morishita, S. Ono, T., Fujisaki, C., Ishihara, Y, Murakoshi, M., Kato, H., Hosokawa, M., Miyashita, K., Sugiyama, K. and Nishino, H. (2013): Bovine lactoferrin reduces visceral fat and liver triglycerides in ICR mice. Journal of Oleo Science. 62 (2): 97-103.

Morshedi, V., Bojarski, B., Hamedi, S., Torahi, H., Hashemi, G. and Faggio, C. **(2021):** Effects of Dietary Bovine Lactoferrin on Growth Performance and Immuno-physiological Responses of Asian Sea Bass (Lates calcarifer) Fingerlings. Probiotics and Antimicro. Prot. (13), 1790–1797. https://doi.org/10.1007/s12602-021-09805-4

Muri, C., Schottstedt, T., Hammon, H. M., Meyer, E. and Blum, J. W. (2005): Hematological, Metabolic, and Endocrine Effects of Feeding Vitamin A and Lactoferrin in Neonatal Calves, J. Dairy Sci. 88:1062-1077 © American Dairy Science Association.

Nakamura, K., Morisihta, S., Ono, T., Murakoshi, M., Sugiyama, K., Kato, H., Ikeda, I. and Nishino, H. (2016): Lactoferrin interacts with bile acids and increases fecal cholesterol excretion in rats. Biochemistry and Cell Biology 95(1). DOI:10.1139/bcb-2016-0052.

Newman, D.J and Price, C.P. (1999): Renal Function and Nitrogen Metabolites. In: Tietz Textbook of Clinical Chemistry, Burtis, C.A. and E.R. Ashwood (Eds.). 3rd Edn., W.B. Saunders Co., Philadelphia, ISBN-13: 9780721656106, 1204 - 1270.

Nozari, S., Maroufi, N.F., Nouri, M., Oskouei, M.P., Shiralizade, J., Yekani, F., Mamipour, M. and Faridvand, Y. (2018): Decreasing serum homocysteine and hypocholesterolemic

effects of Bovine lactoferrin in male rat fed with high-cholesterol diet. J Cardiovasc Thorac Res. 10 (4): 203–208. doi: 10.15171/jcvtr.2018.35.

Oda, H., Tanaka, M., Yamauchi, K. and Abe, F. (2020): Effect of oral administration of bovine lactoferrin on alcohol-induced liver injury model rats. Milk Science (69)1:8-16. DOI:10.11465/milk.69.3

Okazaki, Y., Kono, I., Kuriki, T., Funahashi, S., Fushimi, S., Iqbal, M., Okada, S. and Toyokuni, S. (2012): Bovine lactoferrin ameliorates ferric nitrilotriacetate-induced renal oxidative damage in rats**.** J Clin Biochem Nutr. 51(2):84-90.doi: 10.3164/jcbn.11-100. Epub 2012 Aug 4.

Pan, Y., Rowney, M., Guo, P. and Hobman, P. (2007): Biological properties of lactoferrin: An overview. Australian Journal of Dairy Technology 39 (1):97-101.

Prenner, M.L., Prgomet, C., Sauerwein, H., Pfaffl, M.W., Broz, J. and Schwarz, F. (2007): Effects of lactoferrin feeding on growth, feed intake and health of calves. Archives of Animal Nutrition, 61(1): 20-30.

Prgomet, C., Prenner, M., Schwarz, F. and Pfaffl, M. (2007): Effect of lactoferrin on selected immune system parameters and the gastrointestinal morphology in growing calves. J. Anim. Physiol. Anim. Nutr. 91 (3), 109 -119.

Reitman, S. and Frankel, S. (1957): A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28: 56-63. https://doi.org/10.1093/ajcp/28.1.56.

Reznikov, E. (2014): Lactoferrin in neonatal development: Effects on intestinal structure and intestinal function and immune response in a piglet model of systemic Staphylococcus aureus infection. Ph D. Thesis. University of Illinois at Urbana Champaign, Champaign, Illinois.

Richmond, W. (1973): Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem., 19 (12): 1350-1356. https://doi.org/.

Robblee, E. D., Erickson, P. S., Whitehouse, N. L., McLaughlin, A. M., Schwab, C. G., Rejman, J. J. and Rompala, R. E. (2003): Supplemental Lactoferrin Improves Health and Growth of Holstein Calves during the Preweaning Phase. J. Dairy Sci. 86 (4):1458-1464.

Simonia, M., Baldrighia, N., Degolab, F., Marchic, L., Marsegliaa, A. and Righia, F. (2020): Low doses of lactoferrin supplementation in weaning calves. Acta fytotechn zootechn, 58-66. (Monothematic Issue: Future Perspectives in Animal Production), 58-66. http://www.acta.fapz.uniag.sk .https://doi.org/10.15414/afz.2020.23.mi-fpap.58-66.

Steijns, J. M., and Hooijdonk, A. C. M. (2000): Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. Br. J. Nutr. 84:S11–S17.

Takeuchi, T., Shimizu, H., Ando, K. and Harada, E. (2004): Bovine lactoferrin reduces plasma triacylglycerol and NEFA accompanied by decreased hepatic cholesterol and triacylglycerol contents in rodents. Br J Nutr. 91(4):533-8. doi: 10.1079/BJN20041090.

Teraguchi, S., Ozawa, K., Yasuda, S., Shin, K., Fukuwatari, Y. and Shimamura, S. (1994): The bacteriostatic effects of orally administered bovine lactoferrin on intestinal Enterobacteriaceae of SPF mice fed bovine milk. Biosci. Biotech. Biochem. 58:482-487.

Teraguchi, S., Ogata, T., Shin, K., Kingaku, M., Fukuwatari, Y., Kawase, K., Hayasawa, H. and Tomita, M. (1998): The mechanism of in vivo bacteriostasis of bovine lactoferrin. Adv. Exp. Med. Biol. 443:239 - 246.

Trinder, P. (1969): Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Ann. Clin. Biochem, 6: 24-27. https://doi.org/10.1177/000456326900600108.

Wang, W.P., Iigo, M., Sato, J., Sekine, K., Adachi, I. and Tsuda, H. (2000): Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. Jpn. J. Cancer Res., 91, 1022-1027.

Weinberg, E.D. and Des, C.P. (2007): Antibiotic properties and applications of lactoferrin. Curr. Pharm. Des. 13 (8), 801-811.

Zahan, S., Ahmed, K.A., Moni, A., Sinopoli, A., Ha, H. and Uddin, J. (2022): Kidney protective potential of lactoferrin: pharmacological insights and therapeutic advances. Korean J Physiol Pharmacol. 26 (1): 1-13. doi: 10.4196/kjpp.2022.26.1.1

Zhang, P., Sawicki, V., Lewis, A., Hanson, L., Nuijens, J. H. and Neville, M. C. (2001): Human lactoferrin in the milk of transgenic mice increases intestinal growth in10-day-old suckling neonates. Adv. Exp. Biol. 501:107–113.

Zöllner, N. and Kirsch, K. (1962): Colorimetric Method for Determination of Total Lipids. Journal of Experimental Medicine, 135, 545-550. https://doi.org/10.1007/BF02045455.