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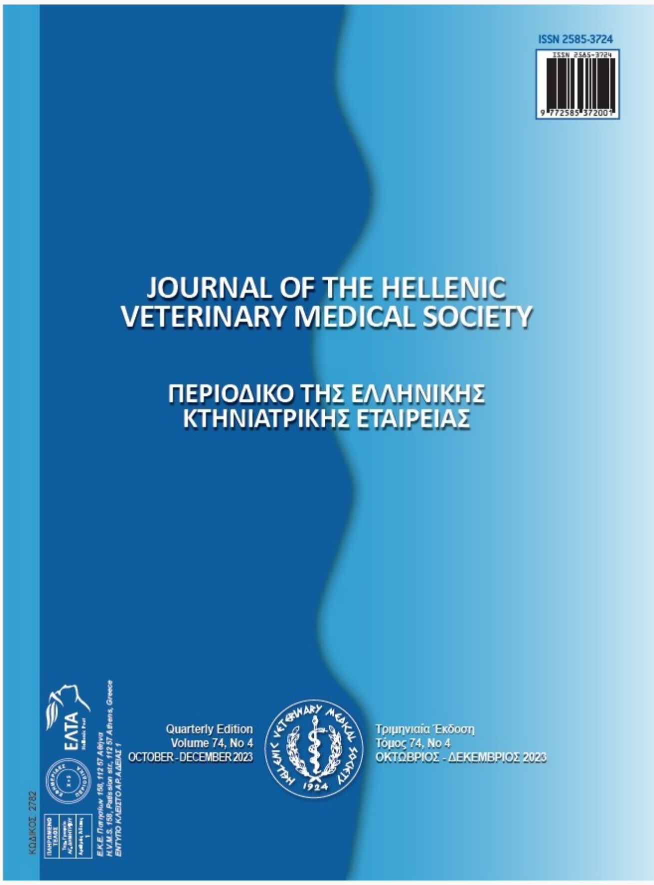

Effect of flaxseed oil on colostrum composition, immunoglobulin concentration and immune response in neonatal friesian calves

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Effect of flaxseed oil on colostrum composition, immunoglobulin concentration and immune response in neonatal friesian calves

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ABSTRACT: The current study aimed to investigate how flaxseed oil supplementation affects immunological response of new born calves in terms of change in colostrum's composition and immunoglobulin concentrations. A total of 28 Friesian cows during the late pregnant (8 months pregnant) were divided into two groups (14 cows/ group). The first group (G1) consider as a control group, and the second group (G2) was supplemented with 2.5% DMI flaxseed oil for 4-6 weeks before calving. Results showed that the age (days) had lower significant effects on immunoglobulin (IgG, IgA, IgM and total Ig) concentrations of colostrum, however, colostrum at 3 days of age showed a significant lower Igs concentration than that at the first day. Immunoglobulin concentrations of colostrum in G2 were significantly higher in IgG, IgA, and total Ig compared with the control (G1). In addition, colostrum composition in G2 was significantly higher in protein, fat and total solids values compared with colostrum composition in G1. Besides, the higher significant plasma values of IgG, IgA, IgM and total Ig were detected on the 7th day, while the lowest values were detected on the first day in newborn calves. Overall means concentrations of immunoglobulin IgG, IgM and Ig in plasma were significantly higher in newborn calves which received flaxseed oil (G2) compared with calves in G1. Treatment with flaxseed oil has an immune-stimulant effect on suckling newly born.

Keywords: Friesian cows; Flaxseed oil; Colostrums composition; Immunoglobulin;

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INTRODUCTION

Flaxseed oil is rich (55%) in linolenic acid, which belongs to omega-3 fatty acids (Mustafa *et al*., 2002; Pilar *et al*., 2017). Omega-3 fatty acid decreases the chance of cardiovascular disease, hypertension, and arthritis, as well as has a significant impact on nervous system development (Sinclair *et al*., 2002). Flaxseed oil has a high amount of polyunsaturated fatty acids (PUFAs), particularly alpha-linolenic acid (ALA), therefore, it is the major component of various enzymes involved in protein metabolism and organ growth, so that, it could help for growth and immune response (Abu El-Hamd *et al*., 2019). Flaxseed oil contains various health-promoting chemicals, such as phytosterols, which have an anti-sclerotic action, carotenoids and phenolic acids, which have an antioxidant activity (Di Nunzio *et al*., 2016). Alpha linolenic acid is an omega-3 fatty acid that is transformed into eicosapentaenoic acid (EPA), which is a precursor of eicosanoids. The latter are hormone-like molecules necessary for immunological response. Furthermore, flaxseed contains another omega-3 fatty acid which is the docosahexanoic acid (DHA) that has a critical role in cell membrane integrity and the function of brain and eye (Conners, 2000).

Gay and Besser, (1991) reported that failure of passive immunity transmission immediately after delivery is associated with both the dam's characteristics (low colostrum production and poor quality) and the characteristics of neonatal calves (insufficient food intake due to insufficient ingestion or intestinal absorption). To achieve proper passive immunity transfer, 4 kg of colostrum should be ingested during the first 12 hours after delivery (Gay and Besser 1991; Feitosa *et al*., 2003; Radostits *et al*., 2007). Immunoglobulins (Ig), particularly IgG, are plentiful in bovine colostrum. During colostrogenesis, Igs are moved from the animal body to the udder (Barrington and Parrish, 2001). Breed, number of post-calving milking, parity, duration of the dry period, the season of calving and body condition score all influence Ig content in colostrum (Quigley *et al*., 1994; Jaster, 2005; Kehoe *et al*., 2007). Concentration of the total Ig in colostrum ranged from 2719 to 8850 mg/dL on Holstein cows (Vaz *et al*., 2004). IgG concentration of 1540 mg/dL in cow colostrum at the first day of lactation (Soares Filho *et al*., 2001). IgG has a pivotal role in maximizing the passive immunity transmission during colostrogenesis (Fischer-Tlustos *et al*., 2020). There are two subtypes of bovine IgG: IgG1 and IgG2 (Larson *et al*., 1979). The IgG1 and IgG2 transfer inside colostrums which start at five weeks before calving and it reached the peak at one to three days before delivery (Sasaki *et al*., 1976). The IgG1 transport is aided by binding to receptors on IgG1 binding breast leukocytes and mammary epithelial cells (Barrington *et al*., 1997).

Feeding the newborn animals with colostrum is important because of their great nutritional and immunological values (Cross and Gill, 2000; He *et al*., 2001). Cattle's synepitheliochorial placenta prevents maternal antibodies from reaching the foetus during pregnancy. Colostrum plays a critical role in local and systemic immunity transmission in ruminants (Salmon 1999). Colostrum can help to prevent infections including bronchopneumonia, umbilical problems, and septicemia on the first few days after birth (Radostits *et al*., 2007).

Therefore, the main objective of this study is to investigate how flaxseed oil supplementation affects immunological response of new born calves in terms of change in colostrum's composition and immunoglobulin concentrations.

MATERIALS AND METHODS

The current work was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center. This study was carried out after an agreement from the Animal Care and Ethics Committee of Kafrelsheikh University, Egypt (license number, KFS1345/10).

Animals and management

A total of 28 late pregnant Friesian cows (8 months of pregnancy) were divided into two groups according to their body weight (BW) and parity (14 cows/group). The first group (G1) was considered as a control and feed on diet without any supplementation, while the diet of the second group (G2) was supplemented with 2.5% dry matter intake (DMI) flaxseed oil (Tanta Flax and Oil Company, Egypt) and given to cows 4-6 weeks before calving. The flaxseed oil was extracted using mechanical pressing. Flaxseed oil was mixed with concentrated feed. All cows were fed a basal ration consisted of concentration feed mixture (CFM), fresh Berseem (FB) and rice straw (RS) to cover their requirements according to NRC (2001). Water was available throughout the day. The chemical composition of the experimental feedstuffs was analyzed according to A.O.A.C. official method (2012) which presented in Table (1). After calving, the new-

Table 1: Chemical analysis of feed stuffs (% on dry matter basis).

Item (%)	CFM*		Rice straw	Fresh Berseem
	Control	Treatment		
Dry matter, DM	91.28	91.24	88.74	16.58
Organic matter, OM	90.56	89.86	82.83	88.19
Crude protein, CP	16.43	16.37	1.61	14.32
Crude fiber, CF	10.96	10.62	37.36	24.67
Ether extract, EE	4.91	8.52	1.51	6.04
Nitrogen free extract	57.38	53.13	42.35	43.16
Ash	9.32	10.36	17.18	11.81

*CFM concentrates feed mixture.

born calves in the two groups suckled individually their dam's colostrum for three days.

Colostrum sampling

Colostrum samples were taken once daily for three days, on the 1st, 2ed and 3rd days postpartum to measure the IgG, IgA and IgM using radio immunodiffusion kits.

Blood sampling

Blood samples (5ml) were collected from all calves at the morning from the jugular vein using heparinized vacutainer tubes. Blood was collected before feeding and drinking at 1-, 2- and 3-days post calving and at 1, 2, 3 and **7** days of age in newborn calves. The blood samples were centrifuged at 4000 rpm for 15 minutes. The plasma was collected and then stored at -20°C until analysis.

Bovine IgG ELISA kits were used to determine the amounts of immunoglobulins (IgG) in blood plasma, following the manufacturer's instructions (Alpha Diagnostic International is based in Texas, while Kamiya Biomedical Company is based in Seattle, Washington, USA) according to the producer protocol. The serum samples were diluted at 1:1000 and a standard curve was generated for each set of samples. The absorbance was recorded by an EPOCH microplate reader at 450 nm wavelengths. Intra- and inter-assay coefficients of variation (CV) were both <10%.

The concentration of total proteins and albumin in blood plasma were determined by using a commercial kit (Diagnostic System Laboratories, Inc., USA). Plasma globulin was calculated by subtracting concentration of albumin from total proteins.

Statistical analysis:

The factorial design was used for statistical analyses of colostrum composition and immunoglobulin concentrations in colostrum and blood. The following models were used to analyze the experiment data using the SAS (2004) package:

$$Y_{ijk} = U + H_i + S_j + HS_{ij} + e_{ijk}$$

Where:

Yijk = Observed values.

U = Overall mean.

Hi = treatments.

Sj = age (days).

HSij = Interaction due treatments x age (day).

eijk = Random error.

Duncan Multiple Ranges Test was used to see if there were any significant variations between the means at a level of P< 0.5 (Duncan, 1955).

RESULTS

Colostrum composition

Data in Table 2 shows that the colostrum composition during the first three days after calving, treatment and the interactions on dams. The effects of days were significantly low (P<0.001) in protein, fat and total solids. The high percentages of protein, fat and total solids were observed to reduce by days of age after calving where, the highest values were in the first day, (14.15, 6.65 and 23.22%, respectively), lowered in the second day (8.44, 4.5 and 17.69, respectively) and the third day (5.17, 3.37 and 14.32%, respectively), respectively. While, lactose was lower in the first day 2.85% and higher in the third day 4.25%.

Colostrum composition in dams that received flaxseed oil (G2) has significantly higher percentage of protein and fat compared with the control group (G1). However, total solids percentages were not significant.

Immunoglobulin concentrations in colostrum

Data in Table 3 shows the immunoglobulin concentrations during the first three days after calving, treatment, and the interactions on dams. The effect of days was significant (P<0.001) in immunoglobulin concentrations (IgG, IgA, IgM and total Ig), where the highest concentrations were in the first day and the lowest concentrations were in the third day. In the first day, the concentrations of IgG, IgA, IgM and total Ig increased by 273.7, 187.3, 67.06 and 162.2% compared with the third day.

Immunoglobulin concentrations of colostrum in dams received flaxseed oil were significantly (P<0.01) higher (12.35 vs 14.26 g/L IgG, 4.2 vs 4.78 g/L IgA and 17.95 vs 21.14g/L IgM) than in the control.

Total immunoglobulin in the blood plasma of calf

The results in Figures 1-4 showed that immunoglobulin concentrations during the first seventh days after calving, treatment, and interactions between them. In newborn calves, the effect of days was higher significantly (P<0.001) in IgG, IgA, IgM and total Ig, where the highest concentrations were in the seventh day and the lowest values were in the first day. In the third day, immunoglobulin concentrations IgG,

Table 2: Effect of flaxseed oil on colostrum composition of Friesian dams in treatment and control groups.

Item	Colostrum composition (%)			
	Protein	Fat	Lactose	Total solids
Effect of days:				
Day 1	14.15±1.5[a]	6.65±0.61[a]	2.85±0.42[b]	23.22±1.6[a]
Day 2	8.44±1.3[b]	4.50±0.70[ab]	3.65±0.36[ab]	17.69±1.7[b]
Day 3	5.17±1.2[c]	3.55±0.57[b]	4.25±0.41[a]	14.32±1.1[c]
Effect of treatment:				
Control (C)	8.87±0.12[b]	4.73±0.09[b]	3.52±0.35	17.76±1.2
Treatment (T)	9.44±0.15[a]	5.13±0.11[a]	3.65±0.27	19.06±1.1
Effect of interaction:				
Control × day1	13.45±1.6	6.4±0.58	2.8±0.37	22.12±1.4
Control × day 2	8.14±1.4	4.3±0.75	3.6±0.34	17.14±1.8
Control × day 3	5.02±1.2	3.5±0.11	4.15±0.43	14.02±1.2
Treatment × day 1	14.85±1.5	6.9±0.67	2.9±0.39	24.32±1.7
Treatment × day 2	8.14±1.2	4.7±0.67	3.7±0.37	18.24±1.8
Treatment × day 3	5.32±1.2	3.8±0.12	4.35±0.41	14.62±1.3

[a, b and c:] Within the same column, the means are considerably different at (P<0.05).

Table 3: Effect of flaxseed oil on immunoglobulin in colostrum of Friesian dams in treatment and control groups.

Item	Immunoglobulin concentrations (g/L)			
	IgG	IgA	IgM	Total Ig
Effect of days:				
Day 1	18.08±2.1[a]	3.53±0.41[a]	7.98±1.2[a]	29.06±2.7[a]
Day 2	13.58±1.9[b]	2.08±0.32[b]	4.19±0.9[b]	20.13±1.9[b]
Day 3	7.28±1.7[c]	0.89±0.33[c]	1.35±0.5[c]	9.44±1.5[c]
Effect of treatment:				
Control	12.35±0.57[b]	2.03±0.12	4.20±0.19[b]	17.95±0.6[b]
Treatment	14.26±0.59[a]	2.24±0.21	4.78±0.17[a]	21.14±0.6[a]
Effect of interaction:				
Control × day1	16.56±2.3	3.24±0.32	7.64±1.1	27.34±3.1
Control × day 2	12.54±1.6	2.01±0.24	3.72±0.8	18.48±2.6
Control × day 3	6.00±1.7	0.83±0.11	1.24±0.7	8.02±1.9
Treatment × day 1	19.59±2.3	3.62±0.27	8.25±1.3	30.78±3.4
Treatment × day 2	14.62±1.8	2.15±0.14	4.65±0.8	21.78±2.6
Treatment × day 3	8.58±0.9	0.95±0.10	1.45±0.6	10.86±1.2

[a, b and c:] Within the same column, the means are considerably different at (P<0.05).

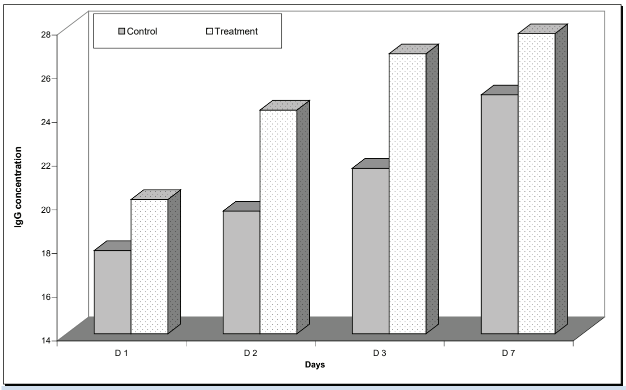

Figure 1. Effect of flaxseed oil on IgG concentrations (g/L) in blood plasma of Friesian newborn in different ages.

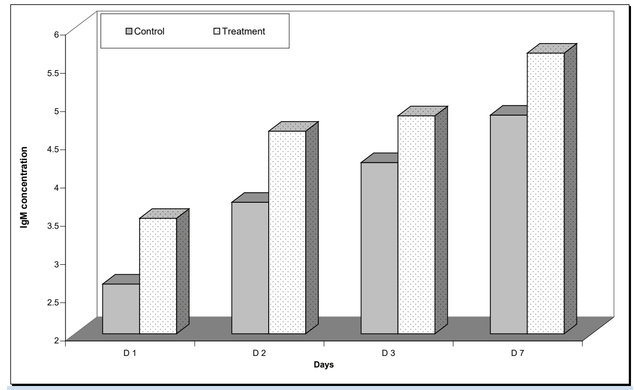

Figure 3. Effect of flaxseed oil on IgM concentrations (g/L) in blood plasma of Friesian newborn in different ages.

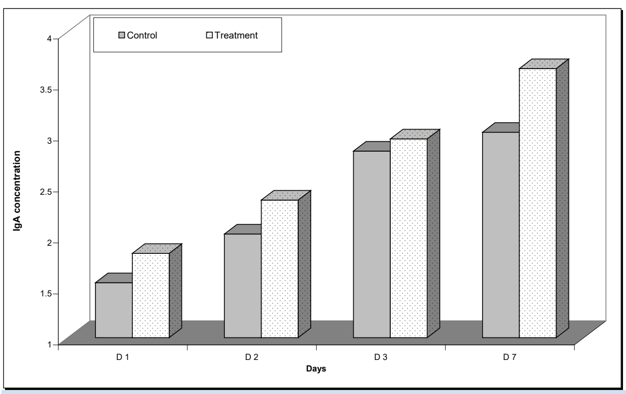

Figure 2. Effect of flaxseed oil on IgA concentrations (g/L) in blood plasma of Friesian newborn in different ages.

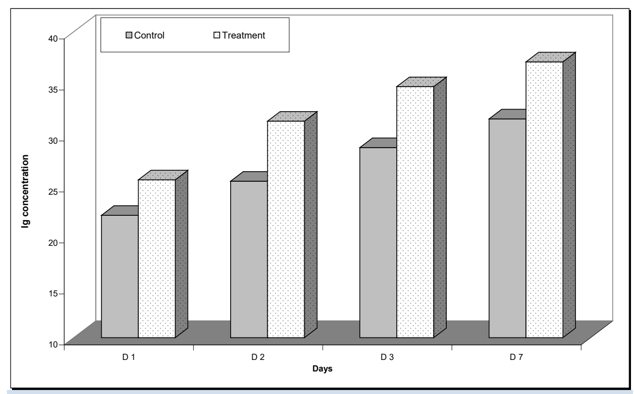

Figure 4. Effect of flaxseed oil on Ig concentrations (g/L) in blood plasma of Friesian newborn in different ages.

IgA, IgM and total Ig were increasing by 273.7, 187.3, 67.06 and 162.2% compared with the first day. The improvement in the immunoglobulin concentrations in seventh day may be due to the increase of immunity response of the calf. Age had significant ($P<0.001$) effect on the concentrations of IgG, IgA, IgM and total Ig, where the concentrations of IgG, IgA, IgM and total Ig increased after birth up to the seventh day of age (Figure 5 and 6). Treatment of flaxseed oil was significantly higher ($P<0.01$) in concentrations of immunoglobulin IgG, IgM and total Ig in blood plasma for all days compared with the control group (Table 4).

Health status of calves

The results showed that there were no mortality and morbidity rates in treatment. While, the control

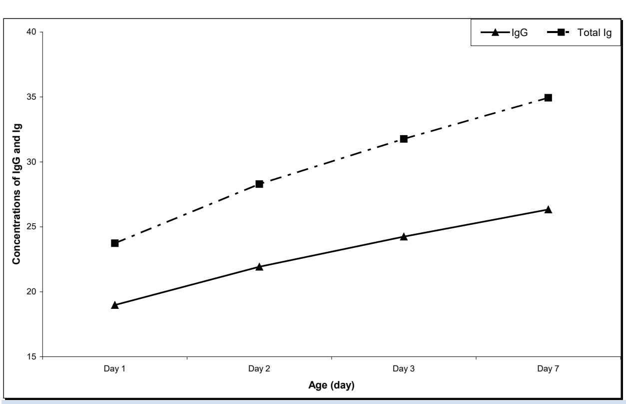

Figure 5. Effect of days on IgG and Ig concentrations (g/L) in blood plasma of Friesian newborn in different ages.

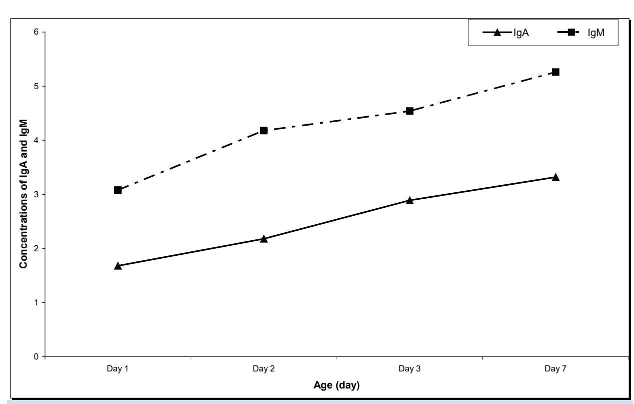

Figure 6. Effect of days on IgA and IgM concentrations (g/L) in blood plasma of Friesian newborn in different ages.

Table 4: Effect of flaxseed oil on immunoglobulin concentrations (g/L) in blood plasma of Friesian newborn in treatment and control groups.

Item	Immunoglobulin concentrations (g/L)			
	IgG	IgA	IgM	Total Ig
Control	20.99±0.57[b]	2.13±0.12	3.54±0.19[b]	17.95±0.6[b]
Treatment	24.74±0.59[a]	2.37±0.21	4.20±0.17[a]	21.14±0.6[a]

[a, b and c:] Within the same column, the means are considerably different at (P<0.05).

group had appeared morbidity rates in terms of diarrhea (2 calves), pneumonia (1 calf) and cough (1 calf) during the first week of the calves' life.

DISCUSSION

Colostrum composition is formed from protein, lipids, carbohydrates, hormones for growth, immunoreactive cells are all examples of water and fat soluble vitamins (Kehoe and Heinrichs, 2007) and cytokines (Hagiwara *et al*., 2000). Immunoglobulins, lactoferrin, transferrin, -lactalbumin and -lactoglobulin, and albumin are the primary proteins found in colostrum (Kehoe and Heinrichs, 2007). The IgG is the most common kind of immunoglobulin (85-90%) in cow colostrum (Larson *et al*., 1979). Colostrum composition in dams received flaxseed oil was significantly higher of protein, fat and total solids percentages compared with the control group. Treatment with flaxseed oil was improved the colostrum composition, particularly protein and fat percentages being 6.4 and 8.5%, respectively compared the control group (Abu El-Hamd *et al*., 2015). The same trend was also reported by El-Diahy *et al*. (2016) who found that supplementing diets of lactating Friesian cows with flaxseed oil showed significantly higher (P<0.05) percentage of fat and protein in milk cows than that in control. However, the percentage of lactose was not affected by treatment. Flax-fed cows generated greater protein milk than cows fed casein-protected linola, according to Goodridge *et al*. (2001). Larsen *et al*. (2012) found that claim that of up to 6.8% oilseed supplementation can be fed without causing production issues and, in many cases, with favorable outcomes such as enhanced milk fatty acid profiles.

The IgG is divided into two subclasses, IgG1 and IgG2, which is interesting to note (Larson *et al*., 1979). IgG1 is the most common IgG subclass, and it can fix complement (Ber, 1973). Blood immunoglobulin G1 is transmitted to colostrum (Barrington *et al*., 1997). IgG1 and IgG2 transport into colostrum starts 5 weeks prior to postpartum, and 1 to 3 days prior to postpartum, respectively (Sasaki *et al*., 1976). The IgG1 transport is aided by binding to receptors on IgG1 binding breast leukocytes and mammary epithelial cells (Barrington *et al*., 1997). Quigley *et al*. (1994) reported 6580 mg/dL IgG, 240 mg/dL IgM, and 170 mg/dL IgA in the colostrum of Jersey cows before the first milking, and during the second milking, Oyeniyi and Hunter, (1978) detected a concentration of IgG of 3240 mg/dL and 2540 mg/dL in the colostrum during the first and second milking, respectively of Holstein cows. At the same time, in the colostrum of Holstein cows, Pritchett *et al*. (1991) found mean levels of 4,800 mg/dL IgG1, whereas, Kehoe *et al*. (2007) reported values of 3500 mg/dL IgG1, 600 mg/dL IgG2, 170 mg/dL IgA, and 430 mg/dL IgM. In this study, immunoglobulin concentrations of colostrum were significantly (P<0.01) higher of IgG, IgA and total Ig being 12.35 vs 14.26 g/L IgG, 4.2 vs 4.78 g/L IgA and 17.95 vs 21.14 g/L total Ig in dams received flaxseed oil compared with the control. The improvement in the immunoglobulin concentrations in first day may be due to the increase of protein in colostrum. Results of this study agree with Mohammadi *et al*. (2014) who, found that cows given extruded linseed exhibited greater IgM and IgG levels. The Ig absorption from colostrum is time-dependent, and delays in reaching the small intestine could have a deleterious impact on absorption rate and amount (Desjardins-Morrissette *et al*., 2018).

Concentrations of immunoglobulin IgG, IgM and total Ig in blood plasma were higher significantly (P<0.01) in newborn calving received flaxseed oil is 12.8% compared with the newborn in the control group. The results agree with Abu El-Hamd *et al*. (2015) who found that total immunoglobulins concentrations in plasma of calves received flaxseed oil increased at a range from 10.22 to 18.30% as compared with control. In addition, plasma immunoglobulins concentrations increased gradually with advancing age from birth up to weaning in both groups. Jezek *et al*. (2009) found that age increased on the concentrations of IgG of 12 to 20 week of age, and Mohammadi *et al*. (2014) reported that cows fed extruded linseed showed greater IgG levels. IgG levels were also increasing as postpartum days progressed, reaching 8.57, 9.20 and 9.78 g/ml on days 1, 7 and 14, respectively. At the beginning of life, the immune system of calves is not able to respond at the level of adult animals and they are more susceptible to infection in this period. They must get colostrum soon after birth to effectively absorb colostral immunoglobulin IgG (Besser and Gay, 1994).

To promote efficient passive immunity transmission, the neonatal calf is born with a naive immune system and must be fed enough amounts (3-4 L) of high-quality colostrum containing 50 g of IgG/L within the first 2 hours of life (Godden et al. 2019). Transfer of passive immunity in beef calves, on the other hand, is dependent on prompt voluntary ingestion of colostrum. Beef calves typically voluntary consume approximately half of what a dairy calf would consume at first colostrum feeding (McGee and Earley, 2019). On the other hand, greater IgG concentration in beef colostrums may be required to achieve a successful transfer of passive immunity. The IgG level in calves' serum between 24 and 48 hours of age should be at least 10 g/L for successful passive immunity transfer (Raboisson *et al*., 2016; McGee and Earley, 2019). According to previous studies, the IgG half-life in calves' serum is influenced by the quality of their mothers' nutrition before parturition (Price *et al*., 2017).

Fontanillas *et al*. (1998) showed an increase in the amounts of ALA and longer-chain omega-3 fatty acids in subcutaneous fat samples when linseed oil was given at 4% of the diet compared to two monounsaturated fatty acid sources. Animals fed flax were found to have greater levels of omega-3 fatty acids (Maddock *et al*., 2003). According to Eddie *et al*., (2004) dietary changes to milk's fatty acid composition might increase the potentially beneficial supply of -linolenic and conjugated linoleic acids, as well as total MUFAs and PUFAs, to the human diet. When compared to control steers, Farren *et al*. (2002) discovered that flax-fed steers had considerably greater levels of haptoglobin blood, a favorable signal of immunological response. Milk is almost devoid of omega-3 fatty acids. As a result, supplementing milk with omega-3 PUFAs may help septicemic calves have a more suitable inflammatory response (Ballou, 2012). Lombard *et al*. (2019) found that for calves to acquire IgG and other immune components, they must consume colostrum. The process of consuming colostrum and transferring immunoglobulins into the blood of a calf is known as immunoglobulin transfer (passive transfer of immunity). Passive immunity transfer is critical for dairy calves' short- and long-term health (Saldana *et al*., 2019).

Based on the foregoing results, improving the chemical composition of colostrum during the 1st three days of age with increasing its quality in terms of enhancement in concentration of immunoglobulins in colostrum of dams and blood serum of calves was reflected in improving health status of calves by reducing mortality and morbidity rates in terms of diarrhea, Pneumonia and Cough. These finding may suggest the economic value of treatment during the sucking period.

CONCLUSION

Dietary treatment with 2.5% DMI flaxseed oil for Friesian cows at late pregnancy (8 month of pregnancy) had positive effects on colostrum composition and its immunoglobulin classes (IgG, IgA and IgM). Treatment with flaxseed oil has a positive impact on immune response on suckling newborn.

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CONFLICT OF INTEREST

None declared.

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