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Low doses of lactoferrin supplementation in weaning calves

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Different trials demonstrated lactoferrin (LF) to possess antimicrobial, antiviral, antimycotic and anti-inflammatory activity. This molecule is an iron-binding protein that could have preventive effects on calf diseases. Several authors studied the effects of LF at doses between 1 and 10 g/calf/day as a supplement in milk administrated to weaning calves. The results are variable and not always consistent. Twenty-two female replacement calves divided into 2 groups (Control-C and Treated-LF) during a 56-d experimental period were employed to investigate the effect of the use of 0.1 g/d of LF during weaning on growth performances, feed efficiency and health status. The field trial was conducted employing an early weaning protocol (49-d of length, excluding the colostral phase). After parturition, density and immunoglobulin G (IgG) content of dam colostrum were measured as a colostrum quality indicator. Only colostrum with at least 50 mg/mL of IgG was bottle-fed to the calf. Morphometric measurements and feedstuff intake were recorded weekly. Health status and milk consumption were evaluated daily. Calves receiving low doses of LF had numerically less incidence of diarrhoea than the C group ($P > 0.05$). From a statistical point of view, any significant difference was observed between groups both on growth performances and feed efficiency. A trend for an increase of the FCR was found for LF group at weaning ($P = 0.099$). More researches are needed to define the optimal dose and the real action of LF in weaning calves.

Keywords: calf, lactoferrin, pre-weaned, performances, health status

1 Introduction

Mortality and morbility due to enteric diseases in calves is cause of financial losses for farmers, and is one of the most common reason leading to the administration of antibiotic therapies in pre-weaned calves. The incidence rate of diarrhoea in calves up to 30-d of age ranged from 10 to 20% (Svensson et al., 2003). The latter is a multifactorial disease dependent either on infective and viral agents, which cause severe intestinal lesions, alterations in enzyme activity and nutrient transport mechanism, or a combination of these effects. This disease is favored by specific environmental conditions, farm management, nutrition and immune status (Cho & Yoon, 2014; Cardoso et al, 2020). Some biosecurity practices are able to reduce the environmental risk of diarrhoea including calf separation from the dam after birth and the cleanliness of the environment for the newborn calf (Maunsell & Donovan, 2008). Moreover, the management and nutritional condition of the dam, as well as the correct colostrum and milk administration to the newborn calf can prevent above mentioned diseases (Bielmann et al., 2010; Heinrichs et al., 2020; Shah et al., 2019). Particularly, the nutritional composition of the colostrum together with its content of immunoglobulins (Ig), maternal leucocytes, growth factors, hormones and

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cytokines and non-specific antimicrobial factors (lysozyme, lactoferrin and lactoperoxidase) enhance the calf health status (Godden, 2008; Morrill et al., 2012).

Lactoferrin (LF) is an iron-binding gycoprotein naturally present in various biological fluids including colostrum, bronchial secretions and tears (García-Montoya et al., 2012; Pempek et al., 2018). Its synthesis undergoes control of mucosal epithelial cells and, in case of inflammation, the level of this glycoprotein rise considerably, from 0.4 - 2 up to 200 mg/L in plasma when septicemia occurs (Legrand et al., 2008). LF functions include antiviral, antimycotic, antimicrobial, anti-inflammatory, immunostimulant and anti-oxidant activities, as well as the enhancement of the intestinal iron absorption. Over time many functions have been recognized to LF even though the most studied and known is certainly its antibacterial action. The antimicrobial activity occurs through two systems: its iron-binding capacity and its strong interaction with other molecules and the cell surface (Pan et al., 2007; García-Montoya et al., 2012). Furthermore, the pepsinic digestion of bovine LF produces a series of peptides called lactoferricines which have strong antimicrobial activity against both Gram+ and Gram-.

Different in vivo studies reported either positive (Habing et al., 2017; Joslin et al., 2002; Pempek et al., 2018; Robblee et al., 2003) or no effect (Cowles et al., 2006; English et al., 2007) of the LF supplement in calves. Lower mortality at six weeks after arrival of the veal calves was reported by Pempek et al. (2018) and in calves diagnosed with diarrhoea by Habing et al. (2017). On the contrary, no effects on body weight (BW), average daily gain (ADG) or prevention of disease were reported by Cowles et al. (2006), Pempek et al. (2018) and English et al. (2007) feeding 0.5 or 1 g/day of LF. Interestingly the gain to feed ratio decreases linearly with the increase of the LF supplementation moving from 1 to 3 g/d in a study performed by Robblee et al. (2003). Overall, studies tested doses of LF between 1 and 10 g/day and as suggested by Pempek et al. (2018) different doses need to be investigated. Moreover, each preventive treatment should not excessively impact on calf growth costs, increasing the need for testing low doses of active molecules. Antimicrobials alternatives with a proven efficacy and reducing the need for antibiotic treatments are demanded due to the increasing public and regulatory pressure. For this reasons and for the promising effects of LF, the purpose of this study was to evaluate the effects of the administration of low doses of LF during the pre-weaning period, on the occurrence of gastrointestinal diseases and on growth performances.

2 Material and methods

This study was conducted following the guidelines of EU Directive 2010/63/EU relative to the care and use of experimental animals.

2.1 Calves management and treatment

The experiment was performed as a complete randomized block design in a dairy farm located in North of Italy (N 45.223, E 9.873) during spring period on a total of 22 Italian Holstein female calves. After birth, the animals were separated from their dams within 6 hours from parturition. Colostrum was collected from each calving cows and tested for IgG content using a hydrometer (colostrometer™, ©Biogenics, Mapleton, Oregon) as described by Mechor et al. (1992) and for quality using a refractometer (automatic thermal compensator; Tecnolatte S.r.l). These parameters were evaluated to exclude the failure of passive transfer-effects on health status. Only colostrum containing at least 50 mg/mL of immunoglobulin G (IgG) was fed to the respective calf while a good quality colostrum previously stored was administered in the other cases. Newborn calves were bottle-fed 2 L of colostrum within 2 hours after birth and further 2 liters during the following 12 hours. Each calf was housed in an individual pen previously cleaned, sanitised and bedded with straw during the whole trial. After the colostral phase (lasting 2 days) each calf was assigned to one of two groups, control (C) and treated (LF), and starting from day 3 calves received 5 liters of pasteurised whole milk from cattle. Milk was administered at the temperature of 40°C, in two meals with 12 h interval (6.00 am, 6.00 pm). Milk of the LF group was supplemented with 0.1 g/calf/day of LF once a day for a 56-d interval. Starting from 3-d calves had also *ad libitum* access to a starter feedstuff and fresh water. The semi-texturized starter feedstuff consisted of a mixture of soybean meal (25.90%), cracked barley (18.09%), flacked maize (17.68%), sugarcane molasses (15.33%), soybean hulls (9.10%), wheat bran (6.23%), mineral-vitamin premix (4.97%), roasted soybean (0.94%), maize meal (0.90%) and carobs pulp (0.87%). The chemical composition is reported in Table 1. Calves were weaned at the end of the seventh experimental week (49 days of trial), when they reached a starter feedstuff intake close to 800 g/d. At the same time the LF administration was stopped. Subsequently, the calves were monitored for another week (up to 56 days).

Table 1 Chemical composition (± standard deviation) of pasteurised whole milk and calf starter feedstuff

Item[1]	Milk	Starter feedstuff[2]
DM (%)	12.50 ± 0.07	87.92 ± 0.32
EE (% DM)	3.76 ± 0.05	2.10 ± 0.09
CP (% DM)	3.48 ± 0.05	20.17 ± 0.31
CF (% DM)	-	6.94 ± 0.47
aNDF (% DM)	-	15.78 ± 0.54
Starch (% DM)	-	26.97 ± 0.72
Ash (% DM)	-	9.31 ± 0.29
Urea mg/ml	23.03 ± 2.36	-
Lactose %	4.96 ± 0.03	-
Casein %	2.71 ± 0.03	-

[1] DM: dry matter; EE: ether extracts; CP: crude protein; CF: crude fibre; aNDF: amylase treated neutral detergent fibre.

[2] Feedstuff ingredients (DM basis): cracked barley 33.59%; soybean meal (CP 47%) 21.21%; sugarcane molasses (sugar 57%) 17.68%; soybean hulls 7.07%; wheat bran 6.19%; peas 6.18%; mineral-vitamin premix 4.62%; maize meal 2.65%; roasted soybean 0.81%.

2.2 Calf starter and milk analysis

Starter and milk were sampled weekly. Starter samples were processed as described by Righi et al. (2017), Simoni et al. (2020) and Comino et al. (2014). In brief, dried samples were ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm screen and analysed for crude protein (CP) and ether extract (EE), according to AOAC (2005). The ash content was determined by ignition to 550°C while aNDF, ADF and lignin contents were determined using the methods described by Van Soest et al. (1991). Milk samples were analyzed as described in Righi et al. (2016). Briefly, fat, protein and lactose content were determined by infrared analysis using a Milko-Scan FT6000 (Foss Electric, Denmark); casein content with the same instrument by Fourier Transformed Infrared (IR) Spectroscopy; and urea titration was performed by enzyme reaction catalysed by urease, using Bun Analyzer 2 apparatus, by means of P/N 667510 kit Bun reagent.

2.3 Treatments in case of disease

The health status of the calves was monitored daily. In case of pathological conditions, the duration and the type of pharmacological intervention adopted was recorded. The fecal score (0: normal; 3: watery, sifting through bedding) was evaluated according to the calf health scoring chart of the school of the veterinary medicine of the University of Wisconsin-Madison. In case of high faecal scores (more than 2) the calf was registered as a “sick” animal. Serious cases of diarrhoea, in presence of fever, were treated for 4 days with Marbofloxacin (Marbox® CEVA, Turkey) at a dose of 1 ml/50 Kg BW. The total number of treatment days per group was then calculated.

2.4 Measurements

Within the first two days of life each calf was weighed and subjected to morphometric measurement including height at withers and at rump, rump width and heart girth circumference. Measures were repeated weekly.

Milk and feedstuff intake were determined and recorded weekly on the base of the feed administered and orts. The feed conversion ratio (FCR) was calculated on a daily base as ratio between daily feedstuff and milk DMI and average daily gain (ADG). Crude protein intake (CPI) was calculated as sum of feedstuff CP (DM) and milk CP (DM) intake. Metabolisable Energy (ME) intake was calculated as sum of the energy from feedstuff and milk based on the NRC (2001) equations for feedstuff and milk energy content estimation. The CP efficiency was calculated as ADG/CPI.

2.5 Statistical analysis

The incidence of diarrhea was compared between group using the z-test. Days of antibiotic treatment, as well as colostrum density and quality were compared between groups through the Students t-test for independent samples. Weekly measures of each group (morphometric measures, BW, intake, ADG, FCR and CP efficiency) were analysed using the repeated measure procedure of the General Linear Model of the SPSS for Windows software package (version 26.0; SPSS Inc., Chicago, IL). Group, interval and their interaction were used as fixed factors. BW, wither height, rump height, rump width and hearth girth at each interval were covaried for their value at birth, while ADG was covaried for BW at birth. Data were reported as least squared means and statistical significance was set at $P \leq 0.05$.

3 Results and discussion

The IgG of the colostrum as well as the Brix values were similar in the two groups (72.8 vs 73.7 mg/ml and 23.8 vs 23.3 brix for C and LF, respectively). These values indicate that both groups received a good quality colostrum, allowing newborn calves to acquire passive immunity through the consumption of colostral IgG (Bartier et al., 2015; Lokke et al., 2016).

The prevalence of diarrhea was only numerically different between groups with values of 18.9% in the LF group and 27.3% in the C group. Such pathological events generated an average of 0.72 days of treatment/calf in the LF group and 1.09 days of treatment/calf in the C group during the pre-weaning period. Our results suggest that LF can exert its antimicrobial, antiviral and immunomodulatory effect reducing the diarrhea incidence even if statistical differences were not found also concerning days of treatment, probably due to the initial good health status and to the low number of animals involved in the present study. This is similar to the result of a study conducted by Habing et al. (2017) on calves with severe diarrhoea. In the cited trial, calves were administered 30 ml of water containing 3 g of LF or 30 mL of extract equivalent to 10 g of whole garlic, or just water (control) once a day for 3 days as a therapeutic anti-diarrhea treatment. In the cited study, in fact, the mortality and culling rate was reduced by 50% after 120 days following diagnosis in calves treated with LF compared with both control and garlic treated calves. Based on the current knowledge of the LF activities, we can speculate that the phenomenon observed depends on a greater sensitivity of the intestinal mucosa of calves treated with regard to pathogens. Particularly, LF enhances local mucosal and systemic immune responses, usually generated by the gut associated lymphatic organs, activating macrophages and promoting the NK cell cytotoxicity (Legrand et al., 2008). The treatment with bovine LF strongly inhibited the hemagglutination activity of type 1 fimbriated E. coli (Teraguchi et al., 1996), and in vitro decrease the count of E. coli O1 and O26 at a concentration of 10-20 mg/ml (Taha et al., 2019). Moreover, LF interferes with the synthesis and yield of the rotavirus antigene (Superti et al., 1997).

Table 2 reports the values of BW and morphometric evaluations of the studied calves measured during the whole experimental period. No differences were found for either BW, height at wither and rump, rump width and heart girth circumference. No interaction was found between treatment and interval and the latter factor was significant indicating a physiological growth of the calves. In analogy, no differences in morphometric evaluations were reported by English et al. (2007), except for rump height which was lower in calves fed 0.5 g/d than in animals receiving 1 g/d of LF and control calves. On the other side, Joslin et al. (2002) and Robblee et al. (2003) found positive effects of LF administration especially on pre-weaning heart girth gain and ADG, respectively. However, it should be noted that Robblee et al. (2003) tested a dose of LF at least 10 times higher in comparison to the one tested in the present study and for a shorter period. Moreover, the LF administration was performed during the pre-weaning period which took place in an earlier stage of life. In our trial, starting from the same initial weight (38.3 vs 38.4 kg for C and LF groups, respectively), no differences between groups were observed on BW and all the morphometric traits as well as the ADG at all the intervals considered. The BW at the beginning of the experiment was similar to that recorded by Habing et al. (2017) and slightly lower than the value reported by Joslin et al. (2002), Robblee et al. (2003) and Pempek et al. (2018). This is probably related to the calves gender (Abdel fattah et al., 2019) and to genetic selection for calving ease (Jamrozik & Miller 2014). No differences were found with regard to the ADG differently to the results of Robblee et al. (2003), who reported a linear increase of the ADG with the dose of LF during the pre-weaning period. Despite a general lower BW at birth, the ADG at 6 weeks was similar to the one reported by Pempek et al. (2018). In our study, the maximum value of ADG was achieved during the final week of the weaning period (7th week) for calves in the C group (0.18 kg/d higher than the LF group in the same week).

Table 2 Morphometric measurements of the calves in the control (C) and treated (LF) groups. The LF group received 0.1 g/calf/day once a day in the milk up to 42 days of age metabolic energy (ME) supplied with the whole diet and feed conversion ratio (FCR).

Variables[2]	Treatment	Weeks								overall	SEM	p-value[1]		
		1	2	3	4	5	6	7	8			T	W	T*W
BW (Kg)	C	38.5	39.1	40.9	44.6	48.7	53.9	60.1	64.8	48.8	0.76	0.403	0.057	0.689
	LF	38.8	39.7	41.4	45.6	50.1	55.9	60.8	65.5	49.7				
	SEM	0.42	0.48	0.61	0.76	0.83	1.06	1.15	0.96					
	P-value	0.184	0.236	0.537	0.349	0.297	0.341	0.765	0.563					
HW (cm)	C	78.28	79.00	80.31	82.04	83.50	85.36	86.90	88.20	82.95	0.307	0.125	0.012	0.742
	LF	78.63	79.00	81.33	83.05	84.41	86.00	87.28	88.94	83.71				
	SEM	0.587	0.545	0.529	0.487	0.477	0.408	0.405	0.427					
	P-value	0.568	0.132	0.216	0.138	0.153	0.255	0.520	0.279					
HR (cm)	C	81.68	82.63	83.77	85.00	86.73	88.32	90.06	91.16	86.17	0.312	0.285	≤0.001	0.658
	LF	81.78	83.10	84.23	86.09	87.54	89.14	90.62	92.12	86.83				
	SEM	0.675	0.597	0.497	0.508	0.556	0.493	0.433	0.439					
	P-value	0.850	0.527	0.547	0.198	0.313	0.259	0.443	0.175					
RW (cm)	C	17.77	18.23	18.36	18.59	19.27	19.77	20.23	20.64	19.11	0.093	0.611	0.063	0.761
	LF	17.68	18.27	18.55	18.82	19.32	19.82	20.55	20.86	19.23				
	SEM	0.130	0.150	0.154	0.157	0.153	0.173	0.164	0.146					
	P-value	0.657	0.883	0.549	0.467	0.883	0.896	0.344	0.459					
CRF (cm)	C	78.34	79.79	81.20	83.51	86.78	89.91	93.29	96.02	86.10	0.501	0.589	0.047	0.860
	LF	79.02	80.35	81.57	84.45	86.95	90.46	93.80	95.89	86.56				
	SEM	0.529	0.514	0.614	0.745	0.651	0.663	0.627	0.548					
	P-value	0.287	0.367	0.700	0.477	0.874	0.665	0.657	0.896					
ADG (Kg/d)	C	0.05	0.09	0.26	0.52	0.59	0.75	0.88	0.61	0.47	0.027	0.540	0.060	0.499
	LF	0.11	0.12	0.24	0.61	0.64	0.82	0.70	0.68	0.49				
	SEM	0.025	0.025	0.033	0.039	0.044	0.064	0.064	0.076					
	P-value	0.238	0.524	0.791	0.291	0.528	0.602	0.170	0.624					

[1]T=treatment, W=week, T*W= interaction treatment per week

[2] BW: body weight; HW: height at withers; HR: height at rump; RW: rump width; CRF: heart girth circumference; ADG: average daily gain.

Table 3 Starter feedstuff intake, crude protein intake (CPI), CP efficiency, total energy supplied with the whole diet (milk and feedstuff) and feed efficiency (DMI/ADG)

Variables[2]	Treatment	Weeks								overall	SEM	p-value[1]		
		1	2	3	4	5	6	7	8			T	W	T*W
Starter feedstuff DMI (g/d)	C	8.47	70.62	211.85	381.33	567.76	593.18	824.81	1285.23	492.96	34.38557	0.796	≤0.001	0.821
	LF	11.30	81.92	228.80	440.65	621.43	629.90	773.96	1316.30	513.03				
	SEM	4.745	14.703	46.481	52.173	54.258	74.868	66.882	111.265					
	P-value	0.774	0.711	0.769	0.537	0.619	0.744	0.743	0.823					
CPI (g/d)	C	15.35	36.24	64.34	98.66	136.48	140.87	188.15	259.23	117.42	6.674	0.796	≤0.001	0.822
	LF	16.21	38.52	67.76	110.63	147.30	148.28	177.90	265.50	121.51				
	SEM	1.870	2.966	5.607	9.375	10.522	10.944	15.401	13.490					
	P-value	0.826	0.711	0.769	0.537	0.619	0.744	0.743	0.823					
ME (Mcal/d)	C	2.17	3.68	4.12	4.59	5.15	5.19	5.87	3.76	4.32	0.098	0.800	≤0.001	0.940
	LF	2.23	3.71	4.17	4.76	5.30	5.30	5.72	3.85	4.38				
	SEM	0.198	0.043	0.081	0.136	0.152	0.158	0.219	0.196					
	P-value	0.897	0.712	0.769	0.537	0.618	0.744	0.743	0.824					
FCR (DMI/ADG)	C	nd[3]	12.00	4.28	2.17	2.66	2.00	1.80	2.55	3.72	0.406	0.764	≤0.001	0.710
	LF	nd	11.00	6.31	1.83	2.08	1.51	2.48	2.37	3.94				
	SEM	nd	1.956	0.837	0.148	0.329	0.198	0.249	0.336					
	P-value	nd	0.928	0.304	0.214	0.450	0.354	0.099	0.798					
CP efficiency	C	4.65	2.25	4.1	6.11	4.99	6.13	5.46	2.64	4.54	4.589	0.608	0.122	0.659
	LF	7.53	3.62	4.26	6.48	4.88	5.88	4.54	2.77	4.99				
	SEM	2.11	0.828	0.63	0.682	0.56	0.713	0.588	0.4					
	P-value	0.508	0.421	0.903	0.792	0.928	0.864	0.449	0.87					

[1] T=treatment, W=week, T*W= interaction treatment per week

[2] Starter feedstuff DMI: Dry matter intake of the feedstuff fed to the calves; CP intake: crude protein intake calculated as feedstuff CP (DM)+ milk CP (DM); ME: metabolic energy: energy from feedstuff and milk; FCR: feed conversion ratio, calculated as a ratio between daily DMI from feedstuff and milk and average daily gain; CP efficiency: crude protein efficiency.

[3] nd: not determined.

The ADG was at the maximum level one week earlier for calves of the LF group. Similarly to our results, other authors did not report differences in LF group compared to a control group on BW and ADG of calves fed either 0.5 or 1 g/d of LF for 56 days (English et al., 2007), 7 days (Pempek et al., 2018) or 49 days (Cowless et al., 2006).

The consumption of the starter feedstuff and total CP and ME are shown in Table 3. Similarly to the results on the morphometric evaluation, no differences were found between the considered groups. They had similar intake of feedstuff DM both on a daily and weekly base, as well as the same CPI and ME from both feedstuff and milk. Also the FCR and the crude protein efficiency were similar between the two groups indicating the null effect of the low doses long-term administration of LF.

The overall starter DMI was lower in comparison to that reported in the literature by Robblee et al. (2003) and Joslin et al. (2002), probably as a result of a general lower BW at birth of our calves in comparison with those enrolled in the cited studies, which induced a lower DMI especially in the first 3 weeks of experiment.

Regarding the effects of LF, results of the present study agree with those of Cowles et al. (2006) which underlines how the administration of LF during a 49-d weaning in milk does not induce significant differences in both milk replacer or starter intake and in total DMI, BW, ADG, gain/DMI, fecal scores, days medicated and skeletal measurements between the control group and LF group (receiving 1g/d of LF). Conversely, Robblee et al. (2003) and Joslin et al. (2002) observed how the administration of LF for a shorter period was able to improve the ingestion of feed and induced better FCR than untreated calves. A tendency for better FCR was found for LF group only at weaning in our study ($P = 0.099$).

Nonetheless, our trial evidenced only a decreased incidence of calves diarrhoea of the LF treated calves. This agrees with the results of Prenner et al. (2007), who demonstrated that the administration of LF for 3 days did not lead to significant differences in relation to the ingestion of feed starter and ADG, but was able to increase the levels of IgG in the serum of treated calves, with a positive impact on the health of the animals.

4 Conclusions

Results obtained in this study indicate that the low doses long-term administration of LF during the pre-weaning period numerically reduce the diarrhoea incidence. With regards to the other traits (growth and morphological) considered in the study, administration of LF did not lead to any difference. Further tests both *in vitro* and *in vivo* may be conducted to investigate the effects of different doses and more prolonged administration of LF.

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References

Abdel fattah, A., Mohammed, H., Youssef, M., Saleem, A.-S., & Youniss, I. (2019). Assessment the Calf's Welfare Due to The Gender, Number of Offspring and Calving Status in Holstein Calves. SVU-International Journal of Veterinary Sciences, 2(1), 119–130. https://doi.org/10.21608/svu.2019.6656.1002

Bartier, A. L., Windeyer, M. C., & Doepel, L. (2015). Evaluation of on-farm tools for colostrum quality measurement. Journal of Dairy Science, 98(3), 1878–1884. https://doi.org/10.3168/jds.2014-8415

Bielmann, V., Gillan, J., Perkins, N. R., Skidmore, A. L., Godden, S., & Leslie, K. E. (2010). An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. Journal of Dairy Science, 93(8), 3713–3721. https://doi.org/10.3168/jds.2009-2943

Cardoso, C.L., King, A., Chapwanya, A., & Esposito, G. (2020). Ante-natal and post-natal influences on neonatal immunity and growth of dairy calves – A review. Animal Husbandry, Dairy and Veterinary Science, In press. https://doi.org/10.15761/AHDVS.1000182

Cho, Y. il, & Yoon, K. J. (2014). An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. Journal of Veterinary Science, 15(1), 1–17. https://doi.org/10.4142/jvs.2014.15.1.1

Comino, L., Tabacco, E., Righi, F., Revello-Chion, A., Quarantelli, A., & Borreani, G. (2014). Effects of an inoculant containing a Lactobacillus buchneri that produces ferulate-esterase on fermentation products, aerobic stability, and fibre digestibility of maize silage harvested at different stages of maturity. Animal Feed Science and Technology, 198, 94–106. https://doi.org/10.1016/j.anifeedsci.2014.10.001

Cowles, K. E., White, R. A., Whitehouse, N. L., & 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. https://doi.org/10.3168/jds.S0022-0302(06)72532-2

English, E. A., Hopkins, B. A., Stroud, J. S., Davidson, S., Smith, G., Brownie, C., & Whitlow, L. W. (2007). Lactoferrin supplementation to holstein calves during the preweaning and postweaning phases. Journal of Dairy Science, 90(11), 5276–5281. https://doi.org/10.3168/jds.2007-0361

García-Montoya, I. A., Cendón, T. S., Arévalo-Gallegos, S., & Rascón-Cruz, Q. (2012). Lactoferrin a multiple bioactive protein: An overview. Biochimica et Biophysica Acta - General Subjects, 1820(3), 226–236. https://doi.org/10.1016/j.bbagen.2011.06.018

Godden, S. (2008). Colostrum Management for Dairy Calves. Veterinary Clinics of North America - Food Animal Practice, 24(1), 19–39. https://doi.org/10.1016/j.cvfa.2007.10.005

Habing, G., Harris, K., Schuenemann, G. M., Piñeiro, J. M., Lakritz, J., & Clavijo, X. A. (2017). Lactoferrin reduces mortality in preweaned calves with diarrhea. Journal of Dairy Science, 100(5), 3940–3948. https://doi.org/10.3168/jds.2016-11969

Heinrichs, A. J., Jones, C. M., Erickson, P. S., Chester-Jones, H., & Anderson, J. L. (2020). Symposium review: Colostrum management and calf nutrition for profitable and sustainable dairy farms. Journal of Dairy Science, 103(6), 5694–5699. https://doi.org/10.3168/jds.2019-17408

Jamrozik, J., & Miller, S. P. (2014). Genetic evaluation of calving ease in Canadian Simmentals using birth weight and gestation length as correlated traits. Livestock Science, 162(1), 42–49. https://doi.org/10.1016/j.livsci.2014.01.027

Joslin, R. S., Erickson, P. S., Santoro, H. M., Whitehouse, N. L., Schwab, C. G., & Rejman, J. J. (2002). Lactoferrin supplementation to dairy calves. Journal of Dairy Science, 85(5), 1237–1242. https://doi.org/10.3168/jds.S0022-0302(02)74187-8

Legrand, D., Pierce, A., Elass, E., Carpentier, M., Mariller, C., & Mazurier, J. (2008). Lactoferrin structure and functions. In Bioactive components of milk (pp. 163–194). Springer, New York, NY. https://doi.org/10.1007/BF02018076

Lokke, M. M., Engelbrecht, R., & Wiking, L. (2016). Covariance structures of fat and protein influence the estimation of IgG in bovine colostrum. Journal of Dairy Research, 83(1), 58–66. https://doi.org/10.1017/S0022029915000734

Maunsell, F., & Donovan, G. A. (2008). Biosecurity and Risk Management for Dairy Replacements. Veterinary Clinics of North America - Food Animal Practice, 24(1), 155–190. https://doi.org/10.1016/j.cvfa.2007.10.007

Mechor, G. D., Gröhn, Y. T., McDowell, L. R., & Van Saun, R. J. (1992). Specific Gravity of Bovine Colostrum Immunoglobulins as Affected by Temperature and Colostrum Components. Journal of Dairy Science, 75(11), 3131–3135. https://doi.org/10.3168/jds.S0022-0302(92)78076-X

Morrill, K. M., Conrad, E., Lago, A., Campbell, J., Quigley, J., & Tyler, H. (2012). Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. Journal of Dairy Science, 95(7), 3997–4005. https://doi.org/10.3168/jds.2011-5174

NRC. (2001). Nutrient requirements of dairy cattle. National Research Council. (N. A. Press, Ed.) (Nutrition,). National Academies.

Pan, Y., Rowney, M., Guo, P., & Hobman, P. (2007). Biological properties of lactoferrin: An overview. Australian Journal of Dairy Technology, 62(1), 31–42.

Pempek, J. A., Holder, E., Proudfoot, K. L., Masterson, M., & Habing, G. (2018). Short communication: Investigation of antibiotic alternatives to improve health and growth of veal calves. Journal of Dairy Science, 101(5), 4473–4478. https://doi.org/10.3168/jds.2017-14055

Prenner, M. L., Prgomet, C., Sauerwein, H., Pfaffl, M. W., Broz, J., & Schwarz, F. J. (2007). Effects of lactoferrin feeding on growth, feed intake and health of calves. Archives of Animal Nutrition, 61(1), 20–30. https://doi.org/10.1080/17450390600973675

Righi, F., Simoni, M., Foskolos, A., Beretti, V., Sabbioni, A., & Quarantelli, A. (2017). In vitro ruminal dry matter and neutral detergent fibre digestibility of common feedstuffs as affected by the addition of essential oils and their active compounds. Journal of Animal and Feed Sciences, 26(3), 204–212. https://doi.org/10.22358/jafs/76754/2017

Righi, F., Simoni, M., Malacarne, M., Summer, A., Costantini, E., & Quarantelli, A. (2016). Feeding a free choice energetic mineral-vitamin supplement to dry and transition cows: Effects on health and early lactation performance. Large Animal Review, 22(4), 161–170.

Robblee, E. D., Erickson, P. S., Whitehouse, N. L., McLaughlin, A. M., Schwab, C. G., Rejman, J. J., & Rompala, R. E. (2003). Supplemental lactoferrin improves health and growth of holstein calves during the preweaning phase. Journal of Dairy Science, 86(4), 1458–1464. https://doi.org/10.3168/jds.S0022-0302(03)73729-1

Shah, A. M., Shah, A. R., Hassan, M. F., Yousif, M., & Wang, Z. (2019). Colostrum composition and its importance to the health ofanimals - A review. Punjab University Journal of Zoology, 34(2), 197–206. https://doi.org/10.17582/journal.pujz/2019.34.2.197.206

Simoni, M., Temmar, R., Bignamini, D. A., Foskolos, A., Sabbioni, A., Ablondi, M., Quarantelli, A., & Righi, F. (2020). Effects of the combination between selected phytochemicals and the carriers silica and Tween 80 on dry matter and neutral detergent fibre digestibility of common feeds. Italian Journal of Animal Science, 19(1), 723-738. https://doi.org/10.1080/1828051X.2020.1787882

Superti, F., Ammendolia, M. G., Valenti, P., & Seganti, L. (1997). Antirotaviral activity of milk proteins: Lactoferrin prevents rotavirus infection in the enterocyte like cell line HT-29. Medical Microbiology and Immunology, 186(2–3), 83–91. https://doi.org/10.1007/s004300050049

Svensson, C., Lundborg, K., Emanuelson, U., & Olsson, S. O. (2003). Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Preventive Veterinary Medicine, 58(3–4), 179–197. https://doi.org/10.1016/S0167-5877(03)00046-1

Taha, N., El barbary, H., Ibrahim, E., Mohammed, H., & Wahba, N. (2019). Application of lactoferrin as a trial to control E.Coli O1and O26 in pasteurized milk. Benha Veterinary Medical Journal, 36(2), 360–366. https://doi.org/10.21608/bvmj.2019.15172.1054

Teraguchi, S., Shin, K., Fukuwatari, Y., & Shimamura, S. (1996). Glycans of bovine lactoferrin function as receptors for the type 1 fimbrial lectin of Escherichia coli. Infection and Immunity, 64(3), 1075–1077. https://doi.org/10.1128/iai.64.3.1075-1077.1996

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science, 74(10), 3583–3597. https://doi.org/10.3168/jds.s0022-0302(91)78551-2