Original Paper

Effect of Feed Microbiology on the Growth of Holstein Calves

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Diarrhea is a common problem on dairy farms, which in turn leads to reduced development of dairy calves. The aim of the study was to assess the influence of colostrum period quality on the health status and development of dairy calves during the first fifteen days of life. The study was carried out under the conditions of 1. zemědělská a. s. Chorušice. 30 calves were included in the study. After birth, female Holstein cattle were randomly divided into three groups with different feeding management practices. During the entire monitoring period, zootechnical parameters were recorded and health status was evaluated. Twelve samples of different colostrum were taken. These samples were subjected to microbiological analysis and the presumed causative agents of diarrheal diseases were identified on the farm. The results showed the influence of colostrum period management on calf development. The relationship between microbiology and length of colostrum intake in calves was investigated. With the right approach to the colostrum period, we can raise healthy and prosperous calves.

Keywords: Holstein calves, colostrum microbiological, growth intensity

1 Introduction

Understanding the intricate interplay between colostrum microbiology, calf health, and prevention of calf diarrhoea during the colostrum period is fundamental to ensuring the welfare and future productivity of the dairy herd. At birth, the gastrointestinal tract (GIT) of calves is relatively mature. The intake of colostrum is essential for the survival of calves in the newborn period; it induces transient systemic metabolic and endocrine phenomena and has longer-term effects, especially in terms of immunoprotection and nutritional status. It is widely known that colostrum provides essential nutrients to newborns, but colostrum is increasingly being shown to affect newborns through non-nutritive substances. Thus, newborn calves need to absorb colostral Ig; high mortality results from *Escherichia coli* and other infections if IgG_1 concentrations are low. Poor digestive function caused by insufficient passage during this period is accompanied by aberrant GIT disorders and is a major cause of morbidity and mortality in newborn calves (Blum, 2006). One of the most important factors for the immune function of newborn cattle is the first feeding of colostrum. Colony-forming units are a very important parameter that indicates the quality of many products, colostrum is no exception. One of the quality criteria for bovine colostrum is the total number of microorganisms according to the Commission regulation, which sets the same hygienic requirements for the quality of cow colostrum as for raw bovine milk; TPC does not exceed 100,000 $CFU.ml^{-1}$ (>5 log $CFU.ml^{-1}$) (European Commission, 2006). High levels of colostral bacteria, especially coliform bacteria, can interfere with immunoglobulin absorption in several ways, as confirmed in studies by Cummins et al. (2017) and Godden et al. (2012). Colostrum has health and nutritional value for calves but is also a potential source of exposure to microbial pathogens such as *Escherichia coli*, *paratuberculosis*, and other bacterial species. These pathogens can cause diseases such as diarrhoea and respiratory diseases and can block the passive absorption of antibodies from the gut into the circulatory system (Gelsinger et al., 2015). In this context, the aim of our study is to explain the complex relationship between colostrum microbiology, calf health outcomes, and the incidence of diarrhoea during the colostrum

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period. By examining microbial composition, assessing calf growth parameters, and evaluating the incidence of diarrhoea, we aim to provide insights that will contribute to informed strategies to improve the health of dairy calves during this critical phase of their lives.

2 Material and methods

Thirty newborn female Holstein cattle were divided into three groups with different feeding regimens. After birth, all groups received pasteurized colostrum with a quality of at least 24% Brix. Calves were housed and fed individually. The monitored feeding period was five days. Calves from the second and third groups were fed the same milk substitute as the calves from the first group on the sixth day. The first group of calves (n = 10) was fed milk substitute from the second day of life. The second group of calves (n = 10) was fed mixed fresh transit colostrum from calving dairy cows. The third group of calves (n = 10) was fed with preserved transit colostrum from one dairy cow. Calves were fed twice a day in a volume of 6–8 litres. We used potassium sorbate (E 202) for preservation. We used the method of diluting and preserving colostrum according to the instructions of Anexa Veterinary Services. Canned transit colostrum was kept in the cold at a temperature of 3–5 °C. Twelve samples were analysed (colostrum, n = 4; mixed fresh transit colostrum, n = 4; preserved transit colostrum from one dairy cow, n = 4). Samples were collected in sterile 20 mL resealable vials and stored frozen at -20 °C. The total number of microorganisms, lactobacilli and coliform bacteria was monitored in individual samples. The microbial population of the samples was also investigated. These analyses were carried out in cooperation with the Biotechnology Institute of the Faculty of Biotechnology and Food Science of the Slovak University of Agriculture. The influence of feed contamination with microorganisms on the health status of all calves was monitored. The milk substitute has not been microbially tested. The health status of the calves was monitored for fifteen days. Calves were checked twice a day. Reactions to the environment, appetite, amount, and consistency of feces were monitored. Calves were weighed at birth, on the sixth and fifteenth day of life. Calves were measured at birth and on the fifteenth day of life.

3 Results and discussion

The study was aimed at evaluating the health status weight and height of calves from birth to the fifteenth day of life. Thirty Holstein heifers were included in the study. Between the first and third groups (<0.05), we found a significant difference in weight (table 1) and height rump – Tail Head (table 2). A significant difference in weight between groups persisted on days six and fifteen. The difference in height was observed on the fifteenth day. No difference in weight and height (>0.05) was found between the first and second groups, nor between the second and third groups. A difference in the subjective assessment of health status was observed between all groups. In the first group, 90% of the calves got diarrhea and in the second group, 80%, which is more frequent compared to the third group, where the frequency of diarrhea was lower. It occurred in only 30% of cases. Diarrhea occurred in calves between the seventh and fifteenth day. Calves in the first and second groups had diarrhea for a longer period compared to the third group. Calves in the first group had a waterier consistency of feces compared to the second and third groups. The second group often had foul-smelling diarrhea. Calves from all groups reduced their milk intake and showed apathy during diarrhoea. Pharmacological treatment was required in the first group (n = 4), the second group (n = 5) and the third group (n = 2). The determination of therapy and treatment was under the supervision of a veterinarian.

Based on the results, it is believed that calves receiving transit preserved colostrum are healthier and grow faster. Conversely, calves with a reduced period of colostrum feeding are more susceptible to diseases and lag in growth. Calves fed mixed transit colostrum receive an adequate amount of nutrients but are exposed to various pathogenic microorganisms daily. From the analysis of the collected samples (table 3), the most frequently occurring microorganisms in calf feed are evaluated. Many of them are a common cause of diarrhea and gastrointestinal disorders in calves. The immune system

Table 1 Methods of microbiological analysis used

Group of microorganisms	Degree of dilution	Culture medium	Method of media application	Cultivation temperature (°C)	Cultivation time (h)
Total number of microorganisms	10^{-2}–10^{-5}	Plate Count Agar	by pouring 1 ml	30	48–72
Lactobacilli	10^{-1}–10^{-5}	M. R. S.	by pouring 1 ml	30	48–72
Coliform bacteria	100–10^{-3}	VRBL	on the surface 1 ml	37	24–48

Basic dilution (10^{-1}): 5 ml + 45 ml physiological solution’

Table 2 Weight of calves during the experiment

Weight of calves at birth (kilograms)					
Groups	N	mean	standard deviation	minimum	maximum
First group	10	36·70	4·968	29	43
Second group	10	37·40	4·695	30	43
Third group	10	40·60	4·477	33	47
Weight of calves on the sixth day (kilograms)					
First group	10	40·70[a]	4·111	33	46
Second group	10	42·00[ab]	4·619	35	47
Third group	10	46·60[b]	3·806	38	52
Weight of calves on the fifteenth day (kg)					
First group	10	42·00[a]	5·598	32	48
Second group	10	45·30[ab]	5·208	37	52
Third group	10	50·40[b]	2·275	46	56

Table 3 Calves height rump – Tail Head

Height calves at birth (cm)					
Groups	N	mean	standard deviation	minimum	maximum
first group	10	83·80	3·795	77	91
second group	10	84·60	2·591	82	90
third group	10	85·70	2·627	82	90
Height calves on the fifteenth day (cm)					
first group	10	90·30[a]	2·869	86	95
second group	10	91·10[ab]	3·315	87	95
third group	10	94·10[b]	2·234	91	98

Table 4 Microbiological analysis of samples (CFU·ml^{-1})

Groups	Sample	Total number of microorganisms	Lactobacilli	Coliform bacteria	Isolated genera and species of bacteria
Colostrum	Primipar 1	$2.5·10^3$	$1.5·10^2$	$8.4·10^1$	*Staphylococcus simulans*, *Citrobacter koseri*
	Primipar 2	$1.4·10^5$	$9.9·10^4$	$7.2·10^2$	*Staphylococcus* sp·, *Serratia ureilytica*
	Multipar 1	$4.9·10^3$	$2.5·10^2$	$3.9·10^1$	*Pantoea agglomerans*
	Multipar 2	$1.2·10^6$	$1.0·10^6$	$4.0·10^1$	*Acinetobacter gyllenbergii*, *Hafnia alvei*
Canned transit colostrum	Primipar 1	$2.3·10^5$	$1.9·10^4$	$1.6·10^2$	*Escherichia coli*, *Enterobacter asburiae*
	Primipar 2	$1.1·10^6$	$9.9·10^5$	$7.2·10^1$	*Staphylococcus* sp·, *Serratia ureilytica*
	Multipar 1	$1.4·10^6$	$1.6·10^4$	$4.1·10^1$	*Pseudomonas* sp·,
	Multipar 2	*	*	$1.7·10^1$	*Acidovorax*
Transit mixed colostrum	3	$2.4·10^4$	$1.1·10^4$	$2.3·10^1$	*Staphylococcus* sp·, *Corynebacterium* sp·,
	4	$1.3·10^5$	$1.3·10^5$	$1.2·10^2$	*Staphylococcus warneri*, *Pichia kudriavzevii*, *Magnusiomyces* sp·,
	5	*	*	$1.5·10^4$	*Acinetobacter baumannii*, *Escherichia coli*
	6	$1.2·10^7$	$2.5·10^5$	$1.8·10^4$	–

of calves relies heavily on the passive transfer of immunity from colostrum. In the first days of life, the intestinal wall of calves is colonized by microorganisms from colostrum, which prevents colonization by pathogenic microflora of the environment.

Rearing calves is a crucial period for initiating the development of the animal. Any mistake made during this time cannot be qualitatively replaced. If we neglect the rearing of calves during the milk feeding period, and heifers do not double their weight and achieve optimal height at the end of the second month, according to the growth curve, we will incur significant economic losses (Šimko et al., 2014). The pre-weaning period remains one of the most demanding phases in the lives of calves, reflected in high morbidity and mortality rates. Calves are highly susceptible to diseases during this period due to various factors, including unsuccessful passive transfer of immunity, social group changes, and inadequate welfare (van Niekerk et al., 2021). Feeding with transition milk not only meets nutritional requirements but also improves the growth, health, and subsequent productivity of born heifers (Xu et al., 2021). This confirms our findings that calves fed with preserved transition milk are healthier compared to calves on milk replacer and mixed transition milk. Bovine milk provides a high amount of nutrients and significant factors that support the immune system and intestinal maturation. The maturation and function of the neonatal intestine allow the calf to digest and absorb the nutrients provided by the milk and transition milk. Therefore, milk intake promotes the initiation of anabolic processes in various tissues, stimulates postnatal body growth, and organ development (Hammon et al., 2020). The local gastrointestinal effects of quality milk include the probiotic contribution of oligosaccharides, providing a substrate for the growth of beneficial microbes such as *Bifidobacterium* (Xu, Mann, & Curone et al., 2020). From these statements, we can confirm that calves receiving preserved transition milk are healthier and have a lower frequency of diarrhea (Morrill et al., 2012). In their study, Morrill et al. (2012) reference the work of Gulliksen et al. (2008), who found that mastitis often occurs during the dry period and can affect the quality of milk. They state that somatic cell counts greater than 50,000 cells.ml^{-1} affect milk, and the average immunoglobulin content was less than 30 $mg.ml^{-1}$. Maintaining a clean environment, appropriate bedding, and dry cow management are procedures that can help reduce not only somatic cell counts but also the number of coliform bacteria in milk. The most common bacteria in milk include *Staphylococcus*, *Mycoplasma*, Lachnospiraceae,

Table 5 Cross tabulation of the genus of identified microorganisms

Genus	Samples					Total
	canned transit colostrum – multipar	canned transit colostrum – primipar	transit mixed colostrum	colostrum – primipar	colostrum – multipar	
No identification	0	0	1	0	0	1
Acidovorax	1	0	0	0	0	1
Acinetobacter baumannii	0	0	1	0	0	1
Acinetobacter gyllenbergii	0	0	0	0	1	1
Citrobacter koseri	0	0	0	1	0	1
Corynebacterium sp.	0	0	1	0	0	1
Enterobacter asburiae	0	1	0	0	0	1
Escherichia coli	0	1	1	0	0	2
Hafnia alvei	0	0	0	0	1	1
Magnusiomyces sp.	0	0	1	0	0	1
Pantoea agglomerans	0	0	0	0	1	1
Pichia kudriavzevii	0	0	1	0	0	1
Pseudomonas sp.	1	0	0	0	0	1
Serratia ureilytica	0	1	0	1	0	2
Staphylococcus simulans	0	0	0	1	0	1
Staphylococcus sp.	0	1	1	1	0	3
Staphylococcus warneri	0	0	1	0	0	1
Total	2	4	8	4	3	21

Halomonas, *Bacteroides*, *Acinetobacter*, *Actinomycetales*, Enterobacteriaceae, *Fusobacterium*, *Pseudomonas*, *Clostridiales*, *Bacteroidales*, *Prevotella*, Ruminococcaceae. Microbial differences between samples were observed only in the right front and rear quarters of the mammary gland. The *Staphylococcus* genus was most prevalent in milk samples regardless of quarter condition, parity, and mastitis status. Additionally, *Staphylococcus*, *Fusobacterium*, *Acinetobacter*, and *Bacteroides* genera were confirmed in milk samples from older cows compared to primiparous cows. It has been shown that bovine milk has a very diverse and rich microbial composition (Lima et al., 2017). We confirmed the same bacterial genera in our samples. Some bacterial genera confirmed in our samples may not directly cause diarrhea in calves. However, they weaken the immunity of calves, occupy space in the intestine for the settlement of beneficial bacteria, and utilize their substrate. They create favorable conditions for the colonization of the most common causes of diarrhea. The most common pathogens include Coronavirus, *E. coli,* Rotavirus, *Cryptosporidia*, *Salmonella typhimurium*, *Salmonella dublin*, Clostridia – types A and C (Nociar, 2012). Fischer et al. (2019) state that phagocytes in milk help calves with a high infectious pressure and provide protection to the digestive tract against disease-causing substances. They write that lymphocytes, along with immunoglobulins A, G, and M, provide information to the immune system of calves about the formation of the most important antibodies against disease-causing microorganisms and agents in their environment.

Staněk and Guspan (2019) state in their article that calves are fully dependent on colostrum immunity until the 6th week of age, but they begin to develop their own antibodies from the 2nd day of life. Their quantity is negligible and gradually increases. Feeding with colostrum still offers benefits after the closure of the gut, even though the absorption of immunoglobulins no longer occurs. One of the advantages may be that bioactive compounds such as hormones or oligosaccharides can stimulate the development of the gastrointestinal tract (Godden et al., 2019). Shaw et al. (2020) found in their study that a group of calves that overcame prolonged diarrhea was 34 kg lighter than the clinically healthy group (276 kg versus 310 kg). This difference is statistically significant and translates into higher rearing costs. This confirms our findings that calves fed with preserved transition milk grew better in the first days of life, which

Table 6 Statistical analysis of samples (CFU.ml^{-1})

	Sample	N	Mean (CFU.ml^{-1})	Standard deviation (CFU.ml^{-1})	Minimum (CFU.ml^{-1})	Maximum (CFU.ml^{-1})
Total number of microorganisms (CFU.ml^{-1})	canned transit colostrum – multipar	1	1,400,000.00	–	1,400,000.00	1,400,000.00
	canned transit colostrum – primipar	2	665,000.00	61,5182.90	230,000.00	1,100,000.00
	transit mixed colostrum	3	4,051,333.33	688,3951.29	24,000.00	12,000,000.00
	colostrum – primipar	2	71,250.00	97,227.18	2,500.00	140,000.00
	colostrum – multipar	2	602,450.00	845,063.31	4,900.00	120,000.00
Lactobacilli (CFU.ml^{-1})	canned transit colostrum – multipar	1	16,000.00	–	16000.00	16000.00
	canned transit colostrum – primipar	2	504,500.00	686,600.68	19,000.00	990,000.00
	transit mixed colostrum	3	130,333.33	119,500.35	11,000.00	250,000.00
	colostrum – primipar	2	49,575.00	69,897.51	150.00	99,000.00
	colostrum – multipar	2	500,125.00	706,930.00	250.00	1,000,000.00
Coliform bacteria (CFU.ml^{-1})	canned transit colostrum – multipar	2	29.00	16.97	17.00	41.00
	canned transit colostrum – primipar	2	116.00	62.23	72.00	160.00
	transit mixed colostrum	4	8,285.75	9,563.83	23.00	18,000.00
	colostrum –primipar	2	402.00	449.72	84.00	720.00
	colostrum – multipar	2	39.50	0.71	39.00	40.00

is a prerequisite for faster growth in the future. Our claims are also supported by Bouška et al. (2007) and Hu et al. (2020), who found that calves that did not experience diarrhea for fourteen days grew faster, were healthier, and reached breeding maturity sooner, allowing them to be inseminated. Furman-Fratczak (2011) found that morbidity and the intensity of the course of the disease were lowest in calves receiving quality milk with a lower number of colony-forming units. As a result, they grew better and exhibited better health.

The study's findings require further validation as the research was conducted on a small number of individuals and at a single location. The listed values are only part of a larger study that is being carried out.

4 Conclusions

Calves fed transit transition colostrum had a lower incidence of diarrhea compared to calves fed milk replacer and transit mixed colostrum. Providing intermediate milk with the same microbial contamination had a positive effect on calves in the first days of life, leading to higher daily gains and good health status. Calves exposed to various microbial contaminations have a weakened immune system and are more susceptible to the causative agents of diarrheal diseases. Healthier heifers are predicted to reach first insemination weight faster and lower age at first calving. The findings of the study may contribute to the proper adjustment of the transition period of calves on the farm or serve as a basis for further research.

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