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Colostrum-supplemented transition milk positively affects serum biochemical parameters, humoral immunity indicators and the growth performance of calves

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ABSTRACT

The aim of this study was to determine the effect of colostrum-supplemented transition milk on the growth rate of newborn calves. Thirty-six day-old female Holstein calves with a birth weight of 43.4 ± 5.5 kg were randomly assigned to a treatment group and a control group. On day 1, all calves received one portion (3 kg) of colostrum within 2 h after birth. Between the second feeding and the age of 5 days, control calves received only transition milk that had been collected from dams shortly before feeding, and the treatment group received 1.5 kg of colostrum collected from dams on day 1 and 1.5 kg of transition milk from subsequent milkings. Monthly body weight and average daily gain were used to measure growth performance. Blood samples were collected from the jugular vein at multiple time points to evaluate the biochemical and immune status of calves. Colostrum-supplemented transition milk increased the concentrations of serum immunoglobulins, triacylglycerols, cholesterol and total protein. The activity of lactate-dehydrogenase and ceruloplasmin decreased, whereas lysozyme activity increased with time in the control group. It can be concluded that additional five days of colostrum feeding positively influences non-specific humoral immunity indicators and serum biochemical parameters in dairy calves.

1. Introduction

High morbidity and mortality rates of calves can cause significant economic loss and reduce the profitability of milk production (Raboisson et al., 2016). Thus, proper calf management and rearing are important considerations in livestock production. Newborn calves are agammaglobulinemic and susceptible to infection. To prevent health problems, calves acquire immunity from colostrum which is a natural source of immune factors and nutrients (Godden, 2008; Raboisson et al., 2016; Torsein et al., 2011). In this paper, colostrum will be defined as a secretion collected from the first milking, while milk from subsequent milkings is referred to as “transition milk”.

It has been suggested that calves should be fed only high-quality colostrum containing at least 50 g/L of immunoglobulins (IgG) (McGuirk and Collins, 2004). However, IgG concentrations in bovine colostrum can vary. Several factors have been shown to affect IgG concentrations in colostrum, including parity, breed, age, the metabolic status of dam, and the length of the dry period (Gomez and Chamorro, 2017; Morin et al., 2010; Quigley et al., 2013; Weaver et al., 2000). Inadequate colostrum intake and its poor quality can result in the failure of passive transfer (FPT) which is a commonly encountered problem whose causes and consequences have been well documented (Beam et al., 2009; Chigerwe et al., 2008; Furman-Frątczak et al., 2011). FPT is diagnosed when IgG serum concentrations drop below 10 g/L during the first 24 h after birth. Serum IgG levels higher than 10 g/L point to successful passive transfer of immunity. In calves, morbidity and mortality are minimized when IgG serum levels exceed 16 g/L (Godden, 2008; Güngör et al., 2004). Immunoglobulins are absorbed most effectively during the first 24 h postpartum; therefore, calves should receive 2 to 4 L of colostrum during the first feeding within the first 6 h postpartum. Importantly, the volume offered to the calf should depend on the quality of colostrum. Ideally, the portion should provide at least 200 g of IgG, which is regarded as sufficient for reaching IgG serum concentration higher than 10 g/L (Raboisson et al., 2016; Vasseur et al., 2010). Additionally, colostrum contains more vital ingredients such as maternal leukocytes, growth factors, hormones,

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cytokines and nonspecific antimicrobial factors (Ontsouka et al., 2016). These immunological and biochemical components also protect agammaglobulinemic calves and support the establishment and development of the gut microbiome (Barrington and Parrish, 2001; Godden, 2008; Gomez and Chamorro, 2017). Despite the benefits of colostrum, artificial feeding (for example, with the use of an oroesophageal tube) is the method of choice in calves that experience problems with colostrum drinking to prevent FPT (Godden, 2008). Therefore, in this study, we hypothesized that the supplementation of transition milk with colostrum could improve the immune status and blood biochemical parameters of calves and, consequently, their growth performance.

2. Materials and methods

2.1. Dairy farm and experimental design

The study was conducted on a commercial free-stall dairy farm located in the north-eastern region of Poland (Warmian-Masurian Voivodeship), where approximately 370 Polish Holstein-Fresian lactating cows were housed and fed according to the recommendations of the German Agricultural Society (Deutsche Landwirtschafts-Gesellschaft, DLG). During the dry period, all cows were vaccinated against colibacillosis and rota- and coronaviruses. Cows in maternity pens were monitored until calving. After birth, female calves born within two months (mean birth weight 43.4 ± 5.5 kg) to primiparous and multiparous dams [(the average parity of the dams was 2.47 ± 0.47, Mean ± SEM for control group (CR) and 2.39 ± 0.36, Mean ± SEM for treatment group, (TRT)] were assigned to a CR (n = 17) or a TRT (n = 18). After parturition calves were separated from dams and kept in individual pens until transfer (at the age of 3 months) to another building within the farm where they were placed in group pens. Additionally, after parturition, colostrum was harvested from each dam within 1 h post partum. In the TRT group, all colostrum collected on the first day (two milkings) was pooled in 1 kg bottles separately for each calf, and kept in the freezer (−20°C). Calves were fed only from colostrum and/or transition milk milked from their dams. Before feeding to calves, colostrum was thawed in a warm water bath (not exceeding 40°C). Colostrum temperature was checked before feeding. The target temperature of colostrum was approximately 39°C.

2.2. Diets

Until 5 d of age, all calves were fed twice a day (3 kg/feeding; 0800 h and 1600 h) according to the described regimen. On d 1, all calves received one portion (3 kg) of colostrum within 2 h after birth. Between the second feeding and the age of 5 days, CR calves received only transition milk that had been collected from dams shortly before feeding, whereas TRT calves received 1.5 kg of colostrum collected from dams on day 1 and 1.5 kg of transition milk from subsequent milkings (6 kg/d in total). Beginning on d 6, all calves were placed on an identical feeding regimen. All calves received milk replacer (MR; All Milk Protein; 21% of CP and 18% of fat on a DM basis; Polmass SA, Bydgoszcz, Poland) between d 6 and d 90, that was formulated to obtain 100 g DM/L. MR volume was decreased on subsequent days from 6.0 L/calf/day (between d 6 to d 10) to 4.0 L/calf/day (between d 11 and 30) and to 3.0 L/calf/day (between d 31 and d 90). Calves had unlimited access to fresh water from d 1.

Between d 11 and d 30, calves were fed solid feed (textured starter (CS); ground triticale and straw) ad libitum. From d 31, corn silage was introduced to the diet at 0.5 kg/d, whereas CS and triticale meal were limited to 1.0 kg/d. Between d 61 and d 90, calves received 2.0 kg of CS, 1.0 kg of ground triticale and 1.5 kg of corn silage. At 91 d of age, all calves were fed a diet that met their nutrient requirements according to DLG recommendations and contained soybean meal (0.5 kg/d), rapeseed meal (0.3 kg/d), triticale (1.5 kg/d), vitamin premix (0.2 kg/d) and molasses pulp (0.5 kg/d). The MR and CS used in this study contained no antibiotics, and the quality of feedstuffs was controlled by a licensed nutritionist. The trial lasted until 180 d of age, and it was conducted from September 2012 to April 2013 (Table 1).

Table 1
Ingredients and composition of the basal diets of Holstein calves at 1 to 180 days of age, kg unless noted otherwise.

Feed	Days of age					
	1–5	6–10	11–30	31–60	61–90	91–180
Milk from dam, liter	6.0	–	–	–	–	–
Milk replacer[1], liter	–	6.0	4.0	3.0	3.0	–
Calf starter[2]	–	–	Ad lib.*	1.0	2.0	–
Triticale meal	–	–	Ad lib.	1.0	1.0	–
Straw	–	–	Ad lib.	Ad lib.	Ad lib.	1.0
Corn silage (30–35% dry matter)	–	–	–	0.5	1.5	5.0
Haylage	–	–	–	–	–	3.0
Soybean meal	–	–	–	–	–	0.5
Rapeseed meal	–	–	–	–	–	0.3
Triticale	–	–	–	–	–	1.5
Vitamin premix	–	–	–	–	–	0.2
Molasses pulp	–	–	–	–	–	0.5

* Ad lib. - Ad libitum.

[1] Composition: all milk protein, 21%; vegetable oil, 18%; ash, 9%; fiber, 0.40%; lactose, 45%, lysine, 1.74%; methionine, 0.38%; calcium, 1.00%; phosphorus, 0.65%; sodium, 0.60%; manganese, 80 mg/kg; zinc, 70 mg/kg; copper, 15 mg/kg; iron, 100 mg/kg; cobalt, 2.0 mg/kg; iodine, 1.2 mg/kg; selenium, 0.3 mg/kg; α-tocopherol, 182 mg/kg; immunoglobulins, 1.0 g/L.

[2] Composition: crude protein, 34%; fat, 4%; ash 7.8%; crude fiber 4.7%; calcium, 1.3%; propionic acid, 0.1%; phosphorus, 0.5%; sodium, 0.2%; magnesium, 0.8%; sucrose, 0.8%; vitamin A, 30,000 IU/kg; vitamin D3, 3100 IU/kg; vitamin E, 40 mg/kg; α-tocopherol, 36.4 mg/kg; manganese, 135 mg/kg; zinc, 280 mg/kg; iron, 220 mg/kg; copper 48 mg/kg; cobalt, 95 mg/kg; iodine, 2.5 mg/kg; selenium, 21 mg/kg.

2.3. Sample collection and analysis

Blood was collected from the jugular vein of each calf on d 1, 3, 5, 15, 30 and 60 of age, into 9 mL Vacuette® Serum Separation probes. Sampling was done 2 h after morning feeding. Blood was left to clot at 36°C and subsequently centrifuged (10 min × 3000 rpm; MPW 223e centrifuge, MPW Med. Instruments, Warsaw, Poland). The serum was collected and frozen at

−20°C until analysis. Immunological parameters (total IgG (IgG) content, activity of lysozyme (lys) and ceruloplasmin (cp)) and biochemical parameters (liver enzymes: aspartate transaminase and alanine transaminase (AST, ALT), cholesterol (chol), glucose (gluc), urea (urea), alkaline phosphatase (AP), lactate dehydrogenase (LDH), triglycerides (TAG), creatinine (crea) and total protein (TP) content) were analyzed. The concentrations of IgG and TP were measured according to the colorimetric micromethod (Sigma, Diagnostic Kits) proposed by Lowry et al. (1951) and modified by Siwicki and Anderson (1993). Lysozyme activity was determined by the turbidimetric method developed by Parry et al. (1965) and modified by Siwicki and Anderson (1986), and cp activity was evaluated according to the method of Siwicki and Studnicka (1986). The activity (U/L) of ALT, AST, AP and LDH was determined by the kinetic method proposed by the International Federation of Clinical Chemistry (IFCC). The concentrations (mg/dL) of crea, urea, TAG, gluc, and chol were measured by the modified method of Jaffe (crea), the urease-glutamate dehydrogenase UV method (urea), the glucose oxidase-peroxidase method (gluc), the glycerokinase peroxidase-peroxidase method (TAG), and the cholesterol oxidase-peroxidase method (chol). All biochemical parameters were determined using the MINDRAY BS-120 automatic biochemistry analyzer (Mindray Medical International Ltd., Shenzhen, China) and diagnostic kits (Alpha Diagnostics, Warsaw, Poland).

Additionally, IgG concentration was estimated with commercial

Bovine IgG ELISA test (Cat. No. E11-118; Bethyl Laboratories Inc., Montgomery, TX, USA) according to the manufacturer protocol. Briefly, 100 µL of standards and samples were loaded onto a 96-well plate and left for

1 h. The plate was then washed four times, followed by addition of detection antibodies into each well and incubated for 1 h. After the incubation plate was washed, streptavidin-conjugated horseradish peroxidase (SA-HRP) was loaded to each well. After 30 min of incubation substrate was loaded into the wells and left for 30 min. Next, the reaction was stopped with addition of sulfuric acid, and the absorbance was read at 450 nm with Tecan Sunrise microplate reader (Tecan Trading AG, Switzerland). Samples were analyzed in duplicate and all steps were performed at room temperature.

2.4. Clinical records

Each calf was examined daily by one of the team members who was at the farm during the experimental period. All records were collected after morning feeding. Diarrhea was diagnosed according to the protocol given by Larson et al. (1977). Scoring included fecal fluidity (from 1 to 4; 1 = normal, 4 = watery) and consistency (from 1 to 5; 1 = normal, 5 = constipated). Diarrhea was diagnosed if calf scored at least 3 in fluidity but no more than 3 in consistency for two days in a row. Respiratory disease was diagnosed if at least two of given symptoms occurred simultaneously (coughing, serious nasal and lachrymal discharge, increased respiratory rate, increased breath sounds, and pyrexia), as described by Pedersen et al. (2009). Additionally, the effect of colostrum-supplemented transition milk on the growth performance of calves was determined based on changes in body weight (BW) and average daily gain (ADG). Starting on d 1, all animals used in the experiment were weighed on a monthly basis until 6 months of age.

2.5. Statistical analysis

The data were analyzed using the MIXED procedure developed by the SAS Institute (2018), using repeated measures with a first-order autoregressive covariance structure in time. The model included the fixed effects of treatment, time (d or week), and the interaction between treatment and time. The Bonferroni correction method was used to adjust for multiple comparisons. The results are presented as least squares means and SEM. The threshold of significance was set at $P \leq 0.05$.

3. Results

The mean concentration of colostral IgG in dams was 82.7 g/L in the CR group and 71.7 g/L in the TRT group ($P < 0.05$). Twenty-four h after calving, only one out of 35 calves enrolled to the study failed to reach the 10 g/L serum IgG threshold. In contrast, 35% of CR calves (6/17) and 22% of TRT calves (4/18) had IgG below 16 g/L. In TRT calves, average serum IgG levels were higher than in the CR group (Fig. 1a) throughout the experiment. Despite lower average IgG concentrations in colostrum, one day-old TRT calves had 12% higher serum IgG concentrations relative to CR calves. It should be noted that serum IgG concentrations on d 1 exceeded 16 g/L in both groups. At the end of the transition milk feeding period (d 5), serum IgG levels were 57% higher in TRT calves than in CR animals ($P < 0.05$). However, on d 60 the difference decreased to 18% in favor of TRT calves ($P < 0.05$). Of note, in TRT group compared with CR, we recorded lower incidence of diarrhea (6 vs 10 cases, respectively) and respiratory disorders (2 vs 3 cases, respectively).

Colostrum-supplemented transition milk decreased lys activity in TRT calves ($P < 0.05$) compared with CR calves (Fig. 1b). Although on d 1, lys activity was 51% lower in TRT calves than in CR calves, the difference between groups was minimized to 34% on d 5 ($P < 0.05$). At the end of the experiment, lys activity was 27% higher in CR calves

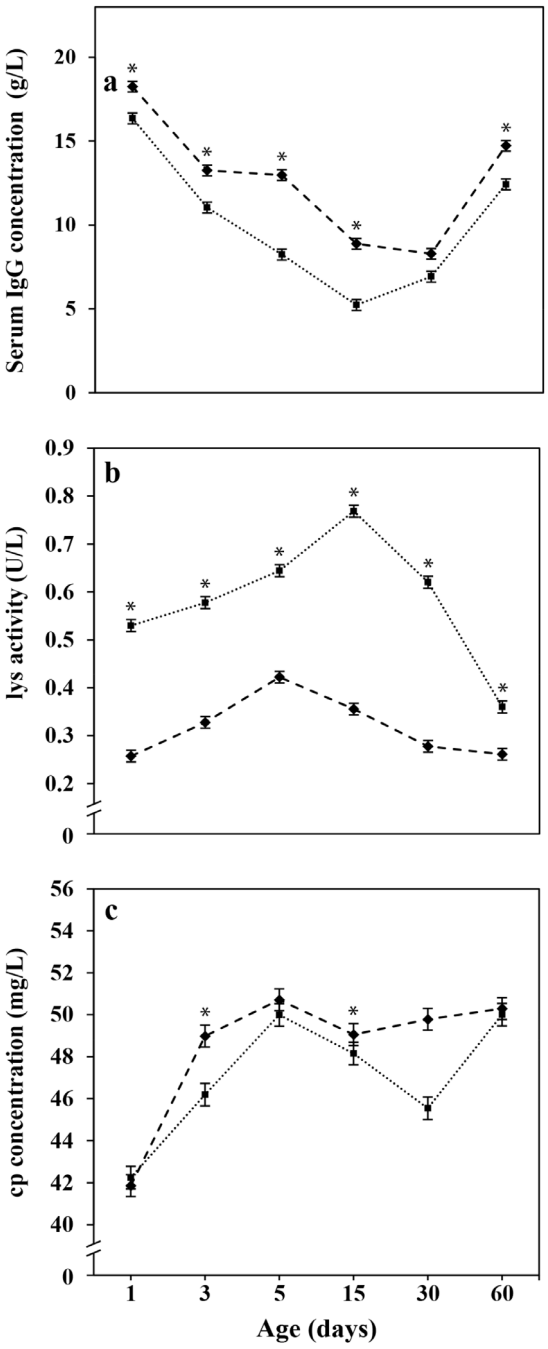

Fig. 1. Serum concentrations of immunoglobulin G (IgG) (a), the activity of lysozyme (lys) (b) and concentration of ceruloplasmin (cp) (c) in Polish Holstein-Fresian calves at 1, 3, 5, 15, 30 and 60 days of age. CR (■; dotted line) and TRT calves fed with a mixture of colostrum and transition milk (1:1 (v/v)) from d 2 to d 5 (◆; dashed line). Asterisks indicate differences between groups at the respective time points ($P < 0.05$).

than in TRT calves. Most importantly, TRT calves had lower overall lys activity throughout the experiment ($P < 0.05$). Although cp concentration was higher in TRT calves than in CR calves, significant differences were observed only at 3 and 30 d of age (6% and 9%, respectively).

The effect of colostrum-supplemented transition milk on selected serum biochemical variables (ALT, AST, AP, LDH, gluc, chol, TAG, urea, crea and TP) is presented in Table 2. The treatment influenced LDH activity and the blood concentrations of chol, TAG, TP and urea ($P < 0.05$), but not the activity of ALT, AST and AP, or gluc and crea

Table 2
The effect of the applied treatment on the serum concentrations of selected blood variables[1] in Holstein calves.

Item[1]	Group[2] CR	Mean	SEM	TRT Mean	SEM	*P*-value
ALT	IU	12.7	0.29	12.6	0.28	0.78
AST	IU	41.4	0.37	40.8	0.36	0.20
AP	IU	212	7.71	233	7.50	0.06
LDH	IU	692[a]	14.2	748[b]	13.8	< 0.05
gluc	mg/dL	101	2.14	105	2.09	0.19
chol	mg/dL	62.6[a]	1.15	80.5[b]	1.12	< 0.05
TAG	mg/dL	25.2[a]	0.75	29.3[b]	0.73	< 0.05
urea	mg/dL	51.3[a]	0.61	55.5[b]	0.59	< 0.05
crea	mg/dL	1.01	0.02	0.98	0.01	0.33
TP	g/L	51.3[a]	0.62	55.5[b]	0.61	< 0.05

[1] ALT – alanine aminotransferase, AST – aspartate aminotransferase, AP – alkaline phosphatase, LDH – lactate dehydrogenase, gluc – glucose, chol – cholesterol, TAG – triglycerides, urea – BUN urea, crea – creatinine, TP – total protein.

[2] CR – control group; TRT – treatment group fed with a mixture of colostrum and transition milk (1:1 (v/v)) from d 2 to d 5. a-b Least square means with different superscripts differ significantly at $P < 0.05$ within rows denoting measurement dates.

levels. All analyzed biochemical parameters were affected by sampling time ($P < 0.05$). The time × treatment effect was observed for AST, AP, chol, TAG, urea and crea ($P < 0.05$).

In comparison with calves from the CR group, calves from the TRT group had lower ALT activity between d 1 and d 15, but the noted difference was not significant (Fig. 2a). AST activity was 15% higher in TRT calves on d 1, but it decreased on d 15 and d 30 relative to CR calves ($P < 0.05$) (by 10% and 17%, respectively) (Fig. 2b). On d 60, average AST activity was similar in both groups. AP activity varied throughout the experiment. On d 1, AP activity was 31% higher in TRT calves, whereas on d 3 and d 5 it decreased by 13% and 10%, respectively ($P < 0.05$). On subsequent days, AP activity increased in TRT calves to reach 142% of the average value determined in the CR group, but on d 60, the difference fell to 14% in favor of TRT calves ($P < 0.05$). Although LDH activity was lower in the CR group throughout the experiment, differences between groups were observed only on d 1 and d 60 (Fig. 2d) ($P < 0.05$).

The effect of colostrum-supplemented transition milk on the serum concentrations of gluc, chol, TAG and TP at different time points is presented in Fig. 3a–d. TRT calves had higher average concentrations of chol and TP compared with CR calves (by 28% and 8%, respectively; $P < 0.05$) at each time point, but colostrum-supplemented transition milk had a significant effect only on chol levels. In TRT calves, average TAG concentrations were 49% higher between d 1 and d 15, and 16% lower on d 30 and d 60 relative to CR calves ($P < 0.05$). Colostrum-supplemented transition milk had no significant effect on serum gluc concentrations in calves.

In contrast, urea and crea levels were affected by colostrum-supplemented transition milk (Fig. 4). On average, the treatment led to a 10% decrease in serum urea concentrations in the TRT group ($P < 0.05$). The treatment also lowered crea concentrations (Fig. 4b) in TRT calves between d 3 and d 60, but a significant treatment effect was reported only on d 1, 3 and 5.

Colostrum-supplemented transition milk influenced BW and ADG ($P < 0.05$) (Fig. 5a-b). The average birth weight was 43 kg (42 kg in the CR group and 44 kg in the TRT group), without significant differences between groups. Between weaning and 3 months of age, CR calves were characterized by higher ADG than TRT calves. However, after weaning, ADG was 30% higher in the TRT group between 3 and 6 months of age ($P < 0.05$). Differences in BW were not observed between groups until 5 months of age. However, at 6 months of age, TRT calves were 9% heavier than CR calves (205 kg vs. 188 kg; $P < 0.05$).

4. Discussion

Blood IgG concentrations in newborn calves are closely related to the quality of colostrum. According to the current industrial recommendations, colostrum should contain at least 50 g/L of immunoglobulins to prevent FPT (Morrill et al., 2012; Vasseur et al., 2009). In the present study, average IgG concentration in colostrum determined for the entire herd was 77.2 g/L, which points to its high quality. The calculated average concentration of IgG was higher than that noted by Furman-Frątczak et al. (2011) (65.5 g/L) but lower than that reported by Kinal et al. (2004) (79.5 g/L). According to several authors (Faber et al., 2005; Swan et al., 2007; Weaver et al., 2000), FPT is diagnosed if serum IgG falls below 10 g/L within 24–32 h after birth. In our experiment, only one CR calf failed to reach the recommended threshold within 24 h after birth. Moreover, despite lower colostrum quality, colostrum-supplemented transition milk increased serum IgG concentrations in calves receiving the treatment. In this study, colostrum-supplemented transition milk seem to offer more effective immunological protection due to increased serum IgG concentrations on d 60. In the CR group, 60% of calves were treated for diarrhea. Additionally, the incidence of respiratory disorders in this group reached 18%. Our results correspond with those reported by Żychlińska-Buczek et al. (2015), but are higher than those noted by Svensson et al. (2006) and Gulliksen et al. (2009). In contrast, the incidence of diarrhea and upper respiratory tract infections in TRT calves was 40% and 33%, respectively, supporting a positive effect of colostrum-supplemented transition milk on the health status of calves. Although IgG are absorbed most effectively within the first 20 h postpartum, the efficiency of this process drops rapidly 12 h after delivery (Ontsouka et al., 2016; Suh et al., 2003), it seems that the applied treatment enhanced acquiring immunity in supplemented calves.

In our study, calves from the TRT group had lower lys activity than calves from the CR group. Lys is an antimicrobial enzyme that is found in cells and tissues and is involved in non-specific innate immunity; therefore, increased lys activity in early stages of life might be considered beneficial (Firth et al., 2005; Gueorguiev et al., 1996). Calves from the CR group were characterized by lower values of growth parameters than calves from the TRT group. The absence of clinical disease symptoms and improved growth in the TRT group point to reduced absorption of lys from colostrum, but are not indicative of subclinical infections (Piantedosi et al., 2010). Previous research (Li et al., 1995) indicates that glycation, a posttranslational protein modification which requires the presence of proteins and sugars in the same matrix, significantly decreases lys activity. However, the above conclusions should be formulated with caution due to limited data on changes in lys activity in newborn calves.

The activity of cp, a non-specific humoral immunity indicator, is yet another health status parameter that was analyzed in this study. According to Calamari et al. (1982), an increase in cp activity during the first days of life is linked with liver function after birth. Thus, our observations of changes in cp activity, accompanied by higher IgG concentrations and lower lys activity, indicate that alterations in cp levels are associated with Cu release from the liver (Abeni et al., 2012). The above phenomenon as well as the lower incidence of clinical symptoms of infection and a fast growth rate of calves can be attributed to the healthy development of the gastrointestinal tract (Zwierzchowski et al., 2016).

Multiple blood variables were measured during the experiment. The effect of aging on changes in biochemical parameters has been widely discussed (Knowles et al., 2000; Mohri et al., 2007; Pavlik et al., 2010; Zwierzchowski et al., 2016). However, the aim of this study was to determine alterations in selected parameters associated with gastrointestinal function as a natural consequence of experimental diet manipulation. ALT, AST, and AP are non-specific enzymatic disease markers associated with liver function, growth and development of skeletal muscles and bone mineralization (Mohri et al., 2007). In our study,

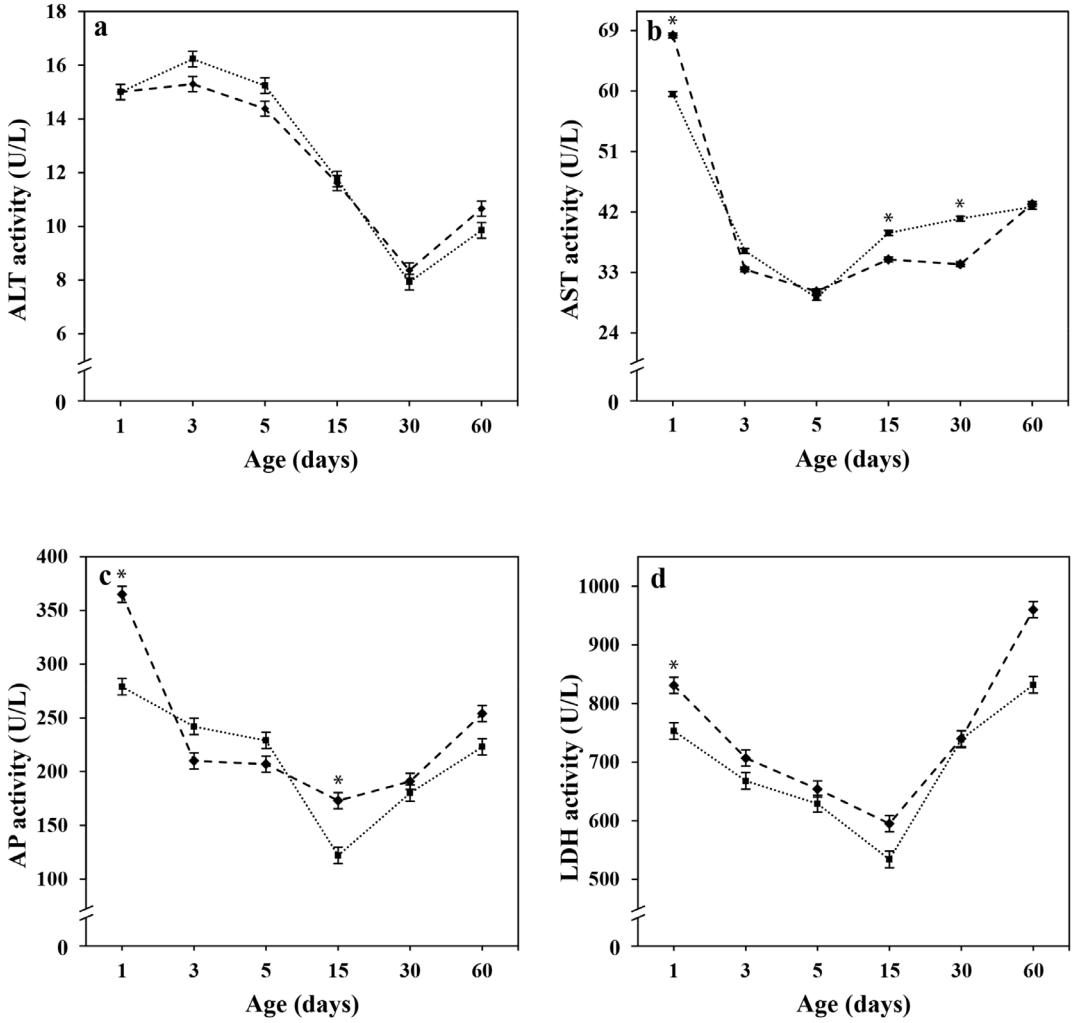

Fig. 2. Activity of serum enzymes (alanine aminotransferase - ALT (a), aspartate aminotransferase – AST (b), alkaline phosphatase - AP (c), lactate dehydrogenase - LDH (d)) in Polish Holstein-Fresian calves at 1, 3, 5, 15, 30 and 60 days of age. CR (■; dotted line) and TRT calves fed with a mixture of colostrum and transition milk (1:1 (v/v)) from d 2 to d 5 (◆; dashed line). Asterisks indicate differences between groups at the respective time points ($P < 0.05$).

colostrum-supplemented transition milk had no effect on ALT and AST activity (Table 2), which can be considered desirable. Any changes in the activity of those enzymes should be treated with caution and approached as a potential marker of liver dysfunction. It should be noted that increased AST activity in ruminants was associated with liver damage caused by high-grain diets (Castillo et al., 2012). According to Egli and Blum (1998), increased AP and LDH activity in the first stage of life is related to colostrum ingestion. Our findings confirm this hypothesis. The average activity of AP and LDH was higher in TRT calves that gained weight at a faster rate. Rapid weight gain requires better bone mineralization which stimulates AP release. LDH is also considered as a non-specific marker of disease since there are many factors contributing to its activity variations. Additionally, LDH activity differ between tissues, cells, fluids and those differences can be enhanced depending on the disease the animal is suffering from (Mohri et al., 2007; Nagy et al., 2013). For instance, some authors (Żarczyńska et al., 2012) link increased LDH activity with muscular dystrophy, but it was not the case in our study since we did not observe any symptoms of this disease. On the contrary, there are reports suggesting LDH fluctuations during respiratory diseases (Nagy et al., 2013). However, we did not analyze each isoform separately, therefore any conclusion coming from our study should be drawn with caution.

Calves from the TRT group were characterized by higher values of Glu, chol, TAG, and TP. According to Reynolds et al. (2003), in the first stage of life, glu levels are associated with colostrum intake, whereas in later stages, an increase in glu concentrations is linked with up-regulated corticosteroid secretion due to intensified growth. The latter increases chol and TAG levels (Cavestany et al., 2005). The concentrations of glu, chol, and TAG can be altered by infection or diet (Bozukluhan et al., 2017). This observation is consistent with our results because the incidence of diarrhea was higher in the CR group than in the TRT group (10 vs. 6 diarrhea cases, respectively). Calloway et al. (2002) suggested that TP is a potential FPT diagnostic tool. In the proposed approach, serum TP levels should not decrease below 52 g/L 72 h postpartum. In our study, the average TP concentration in CR calves at 3 d of age was 51.3 g/L, which indicates that the suggested safety threshold was not reached. Indeed TP was correlated with IgG concentration ($P < 0.001$), however correlation coefficient indicated moderate link between both parameters ($r = 0.437$; Figure S1). In 8 out of 17 calves in the CR group TP was below the recommended level of 52 g/L. In contrast, only one TRT calf failed to reach the TP concentration of 52 g/L. These results corroborate previous research findings (Cuttance et al., 2017; Ježek et al., 2006; Knowles et al., 2000; Mohri et al., 2007; Zwierzchowski et al., 2016).

Urea and crea were monitored as the biochemical indicators of hydration (Stockham and Scott, 2008). An increase in urea levels that is not accompanied by changes in crea levels is associated with diarrhea incidence rates (Pekcan et al., 2012). This observation is consistent with

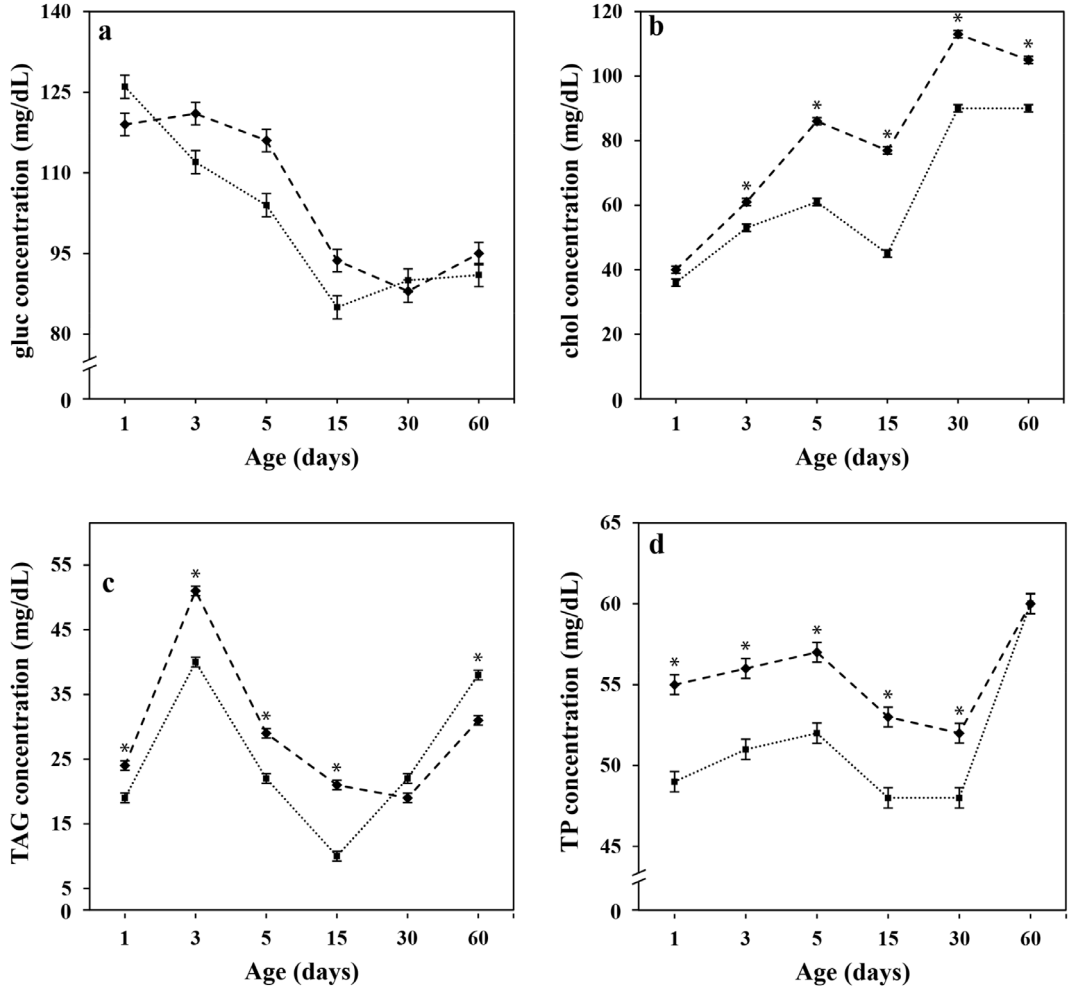

Fig. 3. Serum variables (gluc - glucose (a), chol - cholesterol (b), TAG - triglycerides (c), TP - total protein (d)) in newborn Polish Holstein-Fresian calves on d 1, 3, 5, 15, 30 and 60 day of age. CR (■; dotted line) and TRT calves fed with a mixture of colostrum and transition milk (1:1 (v/v)) from d 2 to d 5 (◆; dashed line). Asterisks indicate differences between groups at the respective time points ($P < 0.05$).

our findings. All cases of diarrhea were recorded until 21 d of age, thus reflecting changes in urea and crea. Interestingly, calves from the TRT group were diagnosed with diarrhea at an earlier stage of life, but the supplementation of transition milk with colostrum delivered health benefits at later stages. In previous studies (Adams et al., 1993; Thrall et al., 2012), elevated crea levels were noted in calves when skeletal muscles were used as a source of energy. This is a highly undesirable situation which negatively affects the growth and

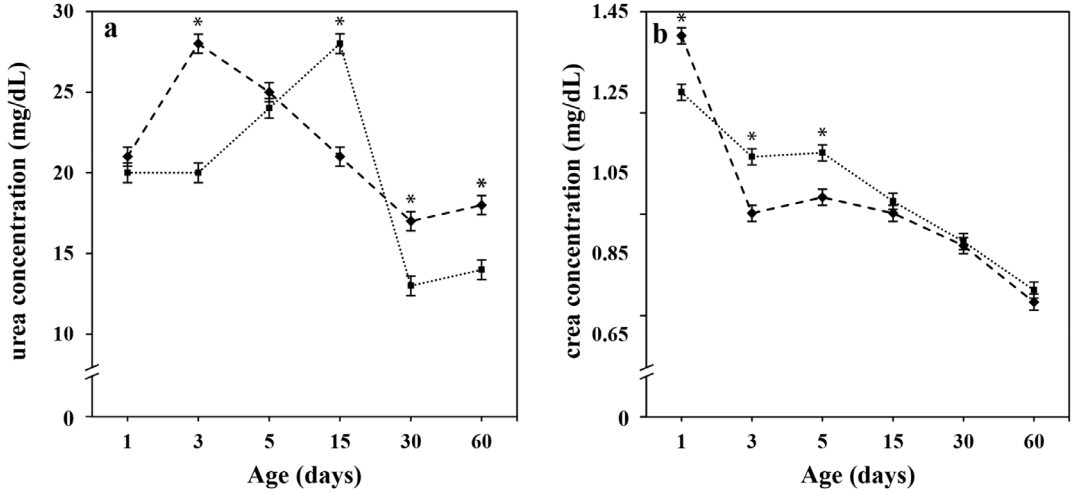

Fig. 4. Serum variables (urea - BUN urea (a), crea - creatinine (b)) concentrations in newborn Polish Holstein-Fresian calves on d 1, 3, 5, 15 and 60 of age. CR (■; dotted line) and TRT calves fed with a mixture of colostrum and transition milk (1:1 (v/v)) from d 2 to d 5 (◆; dashed line). Asterisks indicate differences between groups at the respective time points ($P < 0.05$).

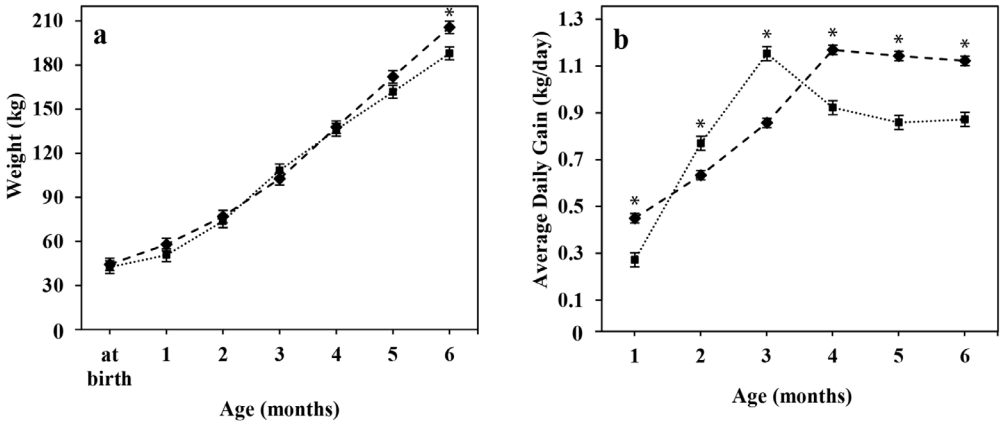

Fig. 5. Average weight (a) and daily gain (b) of Polish Holstein-Fresian calves at birth and at the age of 1, 2, 3, 4, 5 and 6 months. CR (■; dotted line) and TRT calves fed with a mixture of colostrum and transition milk (1:1 (v/v)) from d 2 to d 5 (◆; dashed line). Asterisks indicate differences between groups at the respective time points ($P < 0.05$).

development of calves. A decline in urea concentrations was also reported as a potential indicator of accelerated growth in calves (Otto et al., 2000).

5. Conclusions

In conclusion, this study revealed the benefits of transition milk supplementation with colostrum. Additional volumes of colostrum in the diet positively influenced non-specific humoral immunity indicators and serum biochemical parameters in Holstein calves. Colostrum-supplemented transition milk decreased the prevalence of upper respiratory tract infections and diarrhea incidence. An alternative strategy of calf feeding when high-quality colostrum is unavailable was also proposed in the study.

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Author declaration

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of us.

I confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing I confirm that we have followed the regulations of our institutions concerning intellectual property.

I further confirm that any aspect of the work covered in this manuscript that has involved experimental animals has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

I understand that as the Corresponding Author I am the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). I am responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

CRediT authorship contribution statement

Grzegorz Zwierzchowski: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing. **Jan Miciński:** Resources, Methodology. **Roman Wójcik:** Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Jacek Nowakowski:** Validation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2020.103976.

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