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Effects of *Hippophae rhamnoides* L. Leaf and Marc Extract with Reduced Tannin Concentration on the Health and Growth Parameters of Newborn Calves

L. Liepa[1*], E. Zolnere[2], I. Dūrītis[1], I. Krasnova[3] and D. Segliņa[3]

[1]Faculty of Veterinary Medicine, Latvia University of Agriculture, Jelgava, Latvia.
[2]Private Practising Veterinarian, Jelgava Region, Latvia.
[3]Institute of Horticulture, Latvia University of Agriculture, Latvia.

Authors' contributions

This work was carried out in collaboration between all authors. Author LL designed the study, managed the literature searches, wrote the protocol and the first draft of the manuscript. Author EZ managed the experiment and analyses of the study part with animals. Author ID performed the statistical analysis. Authors IK and DS conducted the analyses of the study part with plant extracts. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the effects of orally administered mixture of *Hippophae rhamnoides* leaf and marc extracts (HLM) on health parameters of newborn calves.
Study Design: Starting from birth day 0 (D0) till day 15 (D15) the extract of HLM was given orally to newborn calves before feeding them milk at an increased dosage from 5 to 8 ml/calf/ two times a day for prophylaxis of nutritional diarrhoea. The calves were clinically examined and weighed. Biochemical and morphological analyses of blood were determined.
Place and Duration of Study: Clinical institute, Faculty of Veterinary Medicine and the Institute of Horticulture at Latvia University of Agriculture, Jelgava, Latvia; within one year.
Methodology: HLM was prepared and chemically analysed.The control (C) and experimental (E) group - each consisted of 10 newborn calves. Clinical examination of calves was performed every

**Corresponding author: E-mail: laima.liepa@llu.lv;*

day. Calves were weighed on D0, D15, D30, venous blood samples for biochemical and haematological analyses were collected on D1, D10, D15, D30. Data were analysed using software program SPSS 17.5.

Results: Tannin content decreased by 33.14% in HLM after addition of polyethylene glycol. In E, there was a better thermoregulation on D1. Diarrhoea incidence was fewer in E group (3 calves) than in C group (5 calves) and diarrhoea started later in E group (D6) compared to C group (D4). Daily weight gain on D0-D15 was significantly higher in E (473.1+/-35.0 g/day) than in C (386.6+/-36.9 g/day) (P= .05) and it correlated (r =- .625) with serum haptoglobin (Hpt) concentration. In E, Hpt significantly decreased on D10 and D15, compared with C. In E there was induced reduction of lymphocytes' count in blood- on D30 it was 27.9±6.5 $x10^9$/μL (P< .01), lower than in C- 64±2.1 $x10^9$/μL.

Conclusion: HLM as feed additive can reduce diarrhoea incidences in calves, promote growth rate, reduce Hpt concentration and lymphocytes' count in blood.

Keywords: Hippophae rhamnoides L. extracts; tannins; calf; weight gain; haptoglobin.

1. INTRODUCTION

In recent years the antibacterial resistance problem has become more topical in dairy medicine. In newborn calves intestinal bacteria can become resistant at an early stage if antibiotics are started to be fed in sub-therapeutic doses as growth promoters or disease preventers. Broad spectrum antibiotics in therapeutic doses are often used for the treatment of calf diarrhoea without previous pathogen diagnostics and making an antibiogramm [1,2]. Nowadays different plant extracts are being used instead of antibiotics as safe animal feed additives against pathogens and without development of antibacterial resistance [3]. The most active plant extract compounds with antimicrobial, anti-inflammatory and anti-oxidative activity are phenolics (tannins), glicosides, terpenoids, alkaloids [4,5]. Phytochemical tannic acid or tannin is effective in modulating or inhibiting the efflux activity of Methicillin resistant *Staphylococcus aureus* (MRSA) [6,7]. *Hippophaë rhamnoides* L. (Elaeagnaceae), commonly known as sea buckthorn, is a Eurasian nitrogen-fixing actinomycetes plant species, producing yellow-orange to red berries at the end of summer. *Hippophaë rhamnoides* L. is a deciduous, dioecious, branched, spiny shrub or a small tree 3–4 m in height. Leaves are alternate, narrow and lanceolate, with a silver-gray color.

All vegetative parts of *H. rhamnoides* (in particular, berries and leaves) are considered to be a good source of a large number of bioactive substances like vitamins, carotenoids, phytosterols, organic acids, polyunsaturated fatty acids and some essential amino acids [8]. *H. rhamnoides* leaves (HL) are by-products during harvesting, when branches together with fruits are cut, and then frozen and cleaned. Depending on harvesting time leaves contain nutrients and bioactive substances which mainly include flavonoids, carotenoids, free and esterified sterols, triterpenols and isoprenols. The leaves are an equally rich source of important antioxidants including β-carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid and significant values of microelements. The principal components of tannin fraction present in leaves are hydrolysable gallo- and ellagi-tannins of monomeric type [6]. The major phenolic compounds in HL are: hydrolysable tannins I-III, catechin, kaempherol, quercetin, epigallocatechin, kampherol-3-O-glycoside and procianidin dimer aglycone [9]. Amongst the phenolic compounds in HL, those in highest concentrations are hydrolysable (HT) and condensed (CT) (proanthocyanidins) tannins, the concentrations of which depend on cultivars and season [9]. The HL extracts are rich in medically active compounds - flavonoids, tannins, quercetine derivates and triterpenes [10,11]. The aqueous extract of HL (AHL) and hydroalcoholic extract of HL (HHL) are rich in flavonoids and phenolics: the total flavonoid concentration in HHL is 20.76±1.35 mg rutin equivalents/g dry leaf, and in AHL- 14.90±1.10 mg rutin equivalents/g dry leaf; the total amount of phenols in HHL is 56.28±2.30 mg gallic acid equivalents/g dry leaf, and in AHL- 40.49±2.10 mg gallic acid equivalents/g dry leaf. From the phenols of AHL, in higher concentrations there are quercetin-3 galactoside, quercetin-3 glycoside, but in HHL- more of kaempferol and isorhamnetin content [11]. Both extracts have high antioxidant and reducing potential, it correlates with the amount of extract, but for HHL the reducing potential is higher compared to that

of AHL. Both extracts have cytoprotective effect against H_20_2 and HX-XO toxic action, and in vitro they have produced antibacterial effect against *S. aureus, E. faecealis, P. aeruginosa, B. cereus.* SBL extracts can be used in stress management, for treating inflammatory and free radicals mediated diseases [11,12].

Condensed tannins in a moderate concentration of 20-55 g CT/kg of dry matter (DM) in the feed of sheep improve protein absorption from the intestines, mitigate emission of CH_4, improve immunity and enhance resistance of gastrointestinal (GI) pathogens, but in high concentration CT above 60 g/kg DM – reduce the absorption of protein and reduce the immunity of animals [13]. There are problems with quantifying CT in plant substances due to the lack of suitable analytical methods for it [14]. HT are potentially toxic to ruminants. Metabolites (like pyrogallol) produced as a result of the degradation of HT by ruminal microbes are hepatotoxic and nephrotoxic [12,15]. Such effects are observed when rats receive AHL in doses of 1g/kg body weight or more. In rats the maximal effective adaptogenic dose of AHL is 100mg/kg body weight [12]. The astringent taste of tannins is associated with the formation of tannin-glycoproteins complexes in the saliva. They stimulate salivation and make feed unpalatable [14]. Animals do not like to eat tannin-rich feed, they lose live weight and productivity [14,15]. In non-ruminants, tannins reduce absorption of essential amino acids (especially methionine), lipids in intestines and reduce body weight gain [14,16,17]. The reduction of tannins with polyethylene glycol (PEG) in animals' feedstuff improves the growth of lambs, piglets and kids, thus improving the quality of lambs' meat [17].

In the newborn calves' stomach digestive processes occur like in monogastric animals, but rumination functions of forestomach develop in about a six month period. In Latvia, the statistically highest incidence of diarrhoea in calves is observed in the first month of life**.** On many farms there are problems with colostrum management, correct feeding strategy or hygiene. In such circumstances problems regarding malnutrition or diarrhoea of calves, reduced weight gain of calves often develop.

The aim of our study was to find out the effect of orally administered HLM extracts with reduced tannins concentration on the health and growth parameters in newborn calves. Various scientific studies conducted on *H. rhamnoides* during the last decade confirm its medicinal and nutritional values [18], but there are no results found in scientific literature about clinical experiments with calves using HLM as growth promoters and for the prophylaxis or treatment of calf diarrhoea. No information was found about the concentration of tannins in Latvia cultivated *H. rhamnoides* leaf and marc extracts, as well as about safe and effective doses of HLM extracts in feeding of newborn calves.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

Leaves with shoots of *Hippophaë rhamnoides* L. were collected at Baltplant Ltd., Latvia, GPS location: N: 56°36′39″; E: 23°17′50″, in the middle of June. Samples were collected from ten randomly selected 7-year-old male and female trees, grown at a 2 × 4 m spacing without fertilization and irrigation. Two female cultivars 'Botanicheskaya Lubitelskaya' and 'Prozrachnaya' (selected by T. T. Trofimov, the Moscow Botanical Garden) and male plants (open pollinated seedlings of unknown origin) were used in the experiment.

2.2 Preparation of Leaf/Mark Extracts and Determination of Tannins Concentration

Leaves with shoots were dried using the equipment "ORAKAS" (Finland) on sieves with forced air circulation at +40 ±2 ºC; duration of drying on average was 12 hours. The dried sample of *H. rhamnoides* was pulverised in the Blixers® mill (Blixer 3, Robot Coupe USA).

Extraction was performed in several steps. First, dry ethanol/water extract was prepared: dry (moisture content 10.6%), powdered *H. rhamnoides* leaves with shoots were mixed with 40% ethanol in a ratio of 1:10 and placed in the ultrasonic bath (Sonorex RK 510 H, Bandelin electronic GmbH & Co. KG, Germany) for 1h at 40ºC. Then the extract was decanted and the remaining mass was pressed through a lavender fabric filter. The press cake was dried using the equipment "ORAKAS" on sieves with a forced air circulation at +43 ±2ºC till the moisture content was 9.7%. The obtained ethanol/water extract was evaporated in a water bath at +50ºC till a creamy consistency (yielding 3.5-4% of the extract).

The second step was performed by taking the dried press cake of leaves with shoots and berries for oil extract preparation. Both pressed cakes were separately mixed with refined rapeseed oil in ratio 1:10, placed in the ultrasonic bath (Sonorex RK 510 H, Bandelin electronic GmbH & Co. KG, Germany) for 1h at 40ºC. Then the extracts were decanted, the remaining mass was pressed through a lavender fabric filter. Both extracts (from leaves with shoots and from berries) were mixed in a ratio of 1:1.

Thirdly, when all of the extracts were prepared, they were mixed together and polyethylene glycol (PEG) 5 mg per 10 ml was added.

The total tannin concentration was determined by a method described by Paaver [19]. The analyses were carried out in three replicates.

2.3 Experimental Animals

The experiment was performed in a dairy herd with 280 dairy cows, in April-July, 2015. On this farm calves had problems with diarrhoea of nutritional reasons. The causes of nutritional diarrhoea were the following: too late and too little doses of colostrum in the night time, sometimes poor nutritional hygiene and keeping of calves. A month before starting the experiment, calves' morbidity of diarrhoea was 31%, but mortality 4%. The control (C) and experimental group (E) each consisted of 10 female gender calves with a mean living weight at birth in C - 45.2±4.5 kg and in E - 42.3±3.1 kg. Each E and C group's calf received 2 litres of colostrum in two hours after birth and they were kept in individual cages. The barn temperature was 20-25°C. All experimental animals in the first month of life received mother's colostrum for three days, after that till D30 - whole milk at an increasing volume of 2-4 litres (depending on the age and body weight) two times per day; from the fourth day of age all calves received a starter at an increasing dosage of 100-800 g/day (depending on the age and body weight); but clover hay and fresh drinking water were available *ad libitum* all the time.

2.4 Feeding Strategy of the Extract

In E group, the HLM was given to calves orally with a syringe before milk feeding at an increasing dose of 5 to 8 mL/two times a day. It is 0.11 to 0.15 mL/kg body weight starting from the second time of feeding on D0 till D15. The extract was not administered within D 15 to D 30. In group C distilled water was given in the same way and doses. The ill calves with signs of diarrhoea received the same doses of HLM as the clinically healthy calves.

2.5 Health and Performance of Calves

2.5.1 Measure of body weight gain and clinical examination

The body weight of calves was measured three times with digital animal scales (with the precision of ±5 g): before receiving the first colostrum dose - on D0, at the end of feeding extract – on D15 and at the end of experiment - on D30. Every calf's body weight gain was calculated individually in the following periods: D0-D15, D15-D30 and D0-D30. Clinical examination of calves was performed every day. The rectal temperature was measured with a digital thermometer (°C). Appetite (amount of milk sucked), sucking reflex (strong; weak; none), temperament (lively or depressed; standing or compulsive lying) hydration level (%) - skin tent test in cervical region, faeces consistency (1 - 4 points score: 1 - normal; 2 - semi-formed, pasty; 3 - loose, but sits on the top of bedding; 4 - watery); discharge from nostrils, eyes (1 - none; 2 - small amount; 3 - moderate amount; 4 - heavy amount) were also determined. Every ill animal was examined in a more detailed way, especially cardiovascular and respiratory system, and in fresh faeces the pathogen of diarrhoea was diagnosed with quick ELISA test strips (BIO K 156, Bio-X Diagnostics, Ltd., Belgium). Diagnostics of four pathogens: *Cryptosporidium parvum, Escherichia coli* strain F5 (K99), *Coronavirus* and *Rotavirus* was carried out. None of the ill calves in E and C groups tested positive to these pathogens, and none of these calves received medical treatment in the period of diarrhoea.

2.5.2 Haematological analyses

The venous blood samples for hematological analyses were obtained from *vena jugulare* (just before feeding the calves) with G18 needle in a vacuum tube with EDTA stabilizer four times- on D1 (11-12 hours after administering the first HLM dose), D10, D15 and D30. The blood haematological analyses were performed in a 24-hour-period after the collection of blood samples using the equipment Exigo EOS VET (Boule Medical AB, Ltd., Sweden) and using an optical microscope Nicon Eclipse 80i (top ocular CFI 10x22, samples were investigated in an oil

immersion at 100x magnification) at the Faculty of Veterinary Medicine, Latvia University of Agriculture.

2.5.3 Venous blood biochemical analyses

The venous blood samples for biochemical analyses were obtained at the same time with a hematological sample - in two vacuum tubes with a clot activator. Blood lactate and glucose concentration were analysed by manual digital quick test analysers Lactate Scout (EKF Diagnostics Holdings Plc., UK) and FreeStyl Optium (Abbott, Ltd., UK) in 5 minutes after venipuncture on D10 and D15. After allowing the specimen in the vacuum tubes to clot for 20-40 minutes, the serum was decanted with a single centrifugation - five minutes at a speed of 1500 times/min. Serum samples (obtained on D1, D15 and D30) were stored at -30°C till the end of the experiment. The biochemical analyses of the serum - total protein, albumin, urea, creatinine, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) - were performed using the equipment Mindray B 380 (Mindray Bio-Medical Electronics Co., Ltd., China) at the Faculty of Veterinary Medicine, Latvia University of Agriculture. The haptoglobin (Hpt) concentration in calves' serum was analysed by using enzyme-linked immunosorbent Assay Kit for Hpt, kit SSEA817Bo (Cloud-Clone corp., USA) according to the instruction manual of Cloud-Clone corp., USA, at the Institute of Food Safety, Animal Health and Environment BIOR, Ltd., Latvia.

2.6 Statistical Analysis

The data obtained in the study were statistically processed with the SPSS 17.5 software program. The arithmetic mean value and the standard error (SEM) and standard deviation (SD) for each parameter were calculated. To compare the mean parameters between groups, the one-way analysis of variance (ANOVA) was applied for comparing the mean values of several unrelated samples [20].

3. RESULTS AND DISCUSSION

3.1 Tannins Concentration in HLM

The tannins content in different raw materials of *H. rhamnoides* berries processing by-products and different extracts obtained from them are shown in Table 1. The tannin content decreased by 33.14% in the final extract (HLM) after the addition of PEG.

3.2 Health and Daily Weight Gain of Calves

3.2.1 Body weight gain and clinical examination

All of the most important results of the calves' growth performance and clinical examination are shown in Table 2. Healthy calves with similar mean body weight on D0 in E group- 42.3±4.1 kg and C - 45.2±5.5 kg were used in the experiment. The daily weight gain of calves in both groups was low (below 500 g/day) due to the feeding of dosed milk two times a day and due to a few incidences of nonspeciffic diarrhoea. In a 30-day- period E calves grew better, the total live weight increased by 12.4 kg in E and 10.1 kg in C. In SBL receiving period, the E calves had significantly (P = .05) better body weight gain 473.1±35.0 g/day than in C - 386.6±36.9 g/day, and this effect continued till D30 - 418.9±45.0 g/day and 358.2±45.3 g/day, respectively.

In E group, diarrhoea incidences were less frequent (3 cases) than in C (5 cases). In E group, diarrhoea started a little later - on D6-8, than in C group - on D4-7. Diarrhoea of nutritional causes was mild - mostly 2 points – of pasty consistency, but in E group, the duration of diarrhoea was shorter - on average 3.7 days compared to that in C – on average 4.4 days. The mean body temperature in D1-D15 period of E calves +39.0±0.1^{0}C was insignificantly (P = .09) higher than in C group +38.8±0.1^{0}C, but in E group two calves with diarrhoea had elevated temperature above +39.5°C 3 days, whereas in C group - only one calf had it higher than +39.5°C for one day. It means that E calves in total had more septicaemia days than C animals [21]. In C group, on D1 in four calves the body temperature was below +38.3°C (a mild hypothermia sign) and temperament for 1-3 days corresponded to 2 points, in E group - only one calf had such a low body temperature (+38.0°C), but not one calf had a reduced temperament. As all calves in both groups consumed equal amounts of colostrum in the first feeding time, better thermoregulation of E calves could be associated with more energy or nutrient absorption from the intestinal content, with more active temperament of E animals (more shivering or earlier standing up) and that can improve the thermoregulation of newborns [21].

Table 1. Total tannin content in the raw material and extracts

Samples	mg/g FW**	
	Average	SEM
Powdered leaves with shoots	4681	53.9
Oil extract from *H. rhamnoides* marc	703.6	2.7
*The final HLM (without PEG)	606.9	0.8
The final HLM (together with PEG)	405.8	6.7
Refined rapeseed oil	6.3	0.2

** Final extract (leaves with shoots and marc ethanol/water/oil)*
*** FW- Fresh weight*

Table 2. The most important clinical results of C and E calves in D0-D30 period

Indices	Control	Experiment
Diarrhoea incidence (D1-D15)	5	3
Diarrhoea severity (points)	2	2
Beginning day of diarrhoea	D4-D7	D6-D8
Mean duration of diarrhoea (days)	4.4	3.7
Mean body temperature D1-D15 (°C)	+38.8±0.1	+39.0±0.1
Calves with mean body temperature on D1 below +38.3°C	4	1
Days of mean body temperature above +39.5°C (in total)	1	3
Calves with reduced temperament on D1	4	0
Body weight on D0 (kg)	45.2±5.5	42.3±4.1
Body weight on D30 (kg)	55.3±1.9	54.7±2.9
Body weight gain D0-D15 (g/day)	386.6*±36.9	473.1*±35.0
Body weight gain D15-D30 (g/day)	358.2±45.3	418.9±45.0
Body weight gain D0-D30 (g/day)	369.9*±33.9	453.3*±31.9

**Asterisks indicate statistically significant values from control, P = .05*
Values are reported as mean ± SEM for group of ten animals (n = 10)

3.2.2 Haematological analyses

The most important findings in the blood morphological analyses are summarized in Table 3. In all calves of E and C groups in D1-D30 period the hydration status was similar, the fluctuations of haematocrit (Hct) were on average within the limit of 20.3 to 24.5%. The number of red blood cells (RBC) in E tended to be higher than in C starting from D1 – 6.4±0.3 $x10^3$/μL and 4.7±0.7 $x10^3$/μL (*P* = .06), on D10 - 6.5±0.3 $x10^3$/μL and 5.6±0.7 $x10^3$/μL (*P* = .05), till D30 – 7.8±0.3 $x10^3$/μL and 6.6±0.6 $x10^3$/μL (*P* = .05), respectively. On D1, in C the RBC concentration was below the normal physiological range level (5 to 10 $x10^3$/μL) [22]. The concentration of haemoglobin increased gradually with insignificant differences between both groups, and it is a normal observation in calves in the first 56 days of life that the haemoglobin level is below 8 g/dL [23]. The influence of inflammation was observed in both experimental groups, but on D10 it was significantly higher in E group than in C group: the number of white blood cells (WBC) was 15.1±3.9 $x10^9$/μL and 9.5±0.6 $x10^9$/μL (*P* = .05), segmented neutrophils - 5.9±1.7 $x10^9$/μL and 2.6±0.4 $x10^9$/μL (*P* = .05) and band neutrophils – 0.6±0.1 $x10^9$/μL and 0.3±0.1 $x10^9$/μL (*P* = .05), respectively. It could be associated with completion of the diarrhoeal period in C earlier (on average before D10), then in E (D10 was only the middle of diarrhoeal period). However, in E group the number of white blood cells tended to be higher on D15 (*P* = .08) and on D30 (*P* = .07) compared to group C. There was no established reason of elevated number of band neutrophils and metamyelocytes on D1 - it could be associated with an undiagnosed infection in the newborn calves or with the digestion of an undiagnosed *E. coli* strain before colostrum which could produce an endotoxin. In such a situation a “left shift” could develop in 4 to 6 hours with the appearance of immature granulocytes in the blood stream [24].

But on D1 and on D15 the number of metamyelocytes in E calves was significantly lower than in C calves: on D0 – 0.01±0.01 $x10^9$/μL and 0.04±0.01 $x10^9$/μL (P = .01), and on D15 – 0 $x10^9$/μL and 0.04±0.01 $x10^9$/μL (P = .05), respectively. It means that E calves had a better defence against pathogens or toxins on D1 than C animals. At the same time the inflammation reaction in E calves on D30 was higher than in C calves: WBC – 12.2±1.8 $x10^9$/μL and 8.5±0.6 $x10^9$/μL (P = .07), segmented neutrophils - 4.1±1.1 $x10^9$/μL and 2.0±0.2 $x10^9$/μL (P < .01) and lymphocytes – 3.4±0.9 $x10^9$/μL and 5.4±0.4 $x10^9$/μL (P = .05). The reduction of lymphocytes' count in the blood of E calves over the period from D15-D30 is associated with reduced formation of B-cells and CD4-positive T-cells, it means, lowered antibody-producing activity and reduced defence against pathogens at this time [25]. We could speculate that in E calves decreased lymphocyte proliferation could be associated with HLM tannins' milk nutrients binding activity or with anti - bacterial and anti – inflammatory action of tannins [11,12] in gastrointestinal tract. In milk are several active compounds which can influence intestinal mucosal immunity; for example, casomorphin is specific immunologically relevant substance contained in milk, which regulates T lymphocytes' natural killers activity, mononuclear cells and neutrophyls locomotion [26]. In both situations, reducing absorption of immunologically active compounds of milk and reducing bacterial content in intestines, could be delayed development of active immunity in newborn calves.

3.2.3 Blood biochemical analyses

The blood biochemical analyses of E and C calves characterising absorption of colostrum nutrients, metabolic status of liver and kidneys are shown in Table 4. On D1, E calves had insignificantly (P = .09) higher serum GGT 361.7±104.6 U/L and total protein 64±3.4 g/L concentration than C calves - 161±41.5 U/L and 59.3±1.4 g/L, respectively. These indices characterize absorption of colostrum nutrients, especially, immunoglobulins. A better thermoregulation on D1 and later start of diarrhoea of E calves could be associated with higher absorption of colostrum compounds under the influence of HLM. On D1, the significantly (P < .05) higher AST level (in physiologically normal level) of E calves could be associated with very slight increase in permeability of hepatocytes membranes due to HLM action or with the activation of muscle cells' enzyme due to the compression of calves in the parturition process. At the same time, during experiment D0-D15 and 15 days after finishing oral administration of HLM hepatotoxic and nephrotoxic action of HLM were not observed [2,19], because the renal filtration ability characterizing indices (urea and creatinine) and hepatocytes damage characterizing enzymes (AST and GGT) were in physiologically normal level [22].

The blood biochemical analyses characterising inflammatory processes, bacterial toxaemia and lactic acidosis level of E and C calves are shown in Table 5. On D1, in the serum of both groups were elevated the levels of inflammation acute phase protein Hpt. Like in haematological analyses, there were no clear reasons for the inflammation processes in calves just after birth. Regarding the influence of HLM consumption in E calves a reduction of serum Hpt concentration was detected after D10, and on D15 it was 0.2±0 ng/L – significantly (P < .01) lower than in C - 2.3±0.5 ng/L. In C the Hpt concentration increased constantly - on D10 it was 1.6±0.1 ng/L - significantly (P = .01) higher than in E, but on D15 the Hpt concentration reached 2.6±0.1 ng/L (P < .01). Reduction of serum Hpt concentration in E calves could be explained with antioxidative, cytoprotective and thereby non - specific anti - inflammatory action of HLM tannins in the animals' organism [8]. The Hpt level has an inverse relation to the average daily weight gain of animals [27], and in our experiment a negative correlation (r = - .626; P < .01) was detected between Hpt concentration and body weight of calves.

On D10 the glucose concentration of E calves (7±0.5 mmol/L) was significantly (P = .05) lower than in C (8.5±0.4 mmol/L) due to higher bacterial toxaemia of E calves at the diarrhoea time (higher mean body temperature in the diarrhoea period and more band neutrophils in blood) [28]. At the same time, no difference was found in lactate concentration of E and C animals. However, at the end of receiving HLM on D15, in E (1.7±0.2 mmol/L) a reduction tendency (P = .08) of lactic acidosis level was observed compared with C (2.8±0.5 mmol/L).

Table 3. The most important results of E and C calves blood morphological analyses

Indices	D1		D10		D15		D30	
	C	E	C	E	C	E	C	E
WBC (x10^9/μL)	8.1±1.1	10.1±1.5	9.5*±0.6	15.1*±3.9	8.0±0.8	11.1±1.4	8.5±0.6	12.2±1.8
RBC (x10^3/μL)	4.7±0.7	6.4±0.3	5.6*±0.3	6.5±0.3*	6.1±0.3	6.6±0.3	6.6*±0.6	7.8*±0.3
Hct (%)	20.5±1.8	24.5±1.4	20.3±1.7	22.5±1.4	20.7±1.5	22.0±1.5	21.1±1.7	24.0±1.4
Haemoglobin (g/dL)	7.5±0.6	7.9±0.5	7.1±0.5	7.9±0.5	7.7±0.5	8.2±0.5	8.2±0.4	9±0.4
Band neutrophils (%)	4.5±0.6	3.8±0.3	4.1±0.5	4.0±0.4	3.2±0.6	3.0±0.2	2.8±0.4	1.6±0.4
Band neutrophils (abs) (x10^9/μL)	0.3±0.0	0.4±0.1	0.3*±0.1	0.6*±0.1	0.2**±0.01	0.3**±0.04	0.2±0.03	0.2±0,1
Segmented neutrophils (%)	26.5±3.1	28.9±2.7	29.7±4.1	35.1±3.4	31.8±4.6	36.2±3.7	24.3**±2.3	34.1**±7.8
Segmented neutrophils (abs) (x10^9/μL)	2,3±0,4	3±0,6	2.6*±0.4	5.9±1.7*	2.7±0.6	3.6±0.7	2.0**±0.2	4.1**±1.1
Lymphocytes (%)	58,7±4,4	56,8±3,3	56.2±3.9	49.4±3.9	56.2±3.8	50.2±3.5	64.0**±2.1	27.9**±6.5
Lymphocytes (abs) (x10^9/μL)	4,8±0,5	5,5±0,7	4.8±0.3	6.5±1.6	4.3±0.4	5.2±0.7	5.4*±0.4	3.4*±0.9
Metamyelocytes (%)	0.4**±0,2	0.1**±0.1	0.4±0.2	0.2±0.1	0.17*±0.17	0*	0.17±0.17	0.25±0.25
Metamyelocytes (abs) (x10^9/μL)	0.04**±0.01	0.01**±0.01	0.03±0.01	0.03±0.02	0.01*±0.01	0*	0.02±0.02	0.03±0.03

*Asterisks indicate statistically significant values from control, * P = .05; ** P = 0.01; Values are reported as mean ± SEM for group of ten animals (n = 10)*

Table 4. The most important results of blood biochemical analyses of C and E calves

Indices	D1		D15		D30	
	C	E	C	E	C	E
Total Protein (g/L)	59.3±1.4	64±3.4	55.9±1.5	54.6±1.1	56.2±1.3	47.8±7.0
Albumins (g/L)	13.4±1.33	15.0±1.44	15.2±1.61	15.3±1.49	25.8±5.22	23.1±4.36
Globulins (g/L)	46.6±1.7	48.9±3.7	40.7±1.9	39.3±1.7	33.8±4.5	22.7±5.8
Urea (mmol/L)	1.7±0.2	2.1±0.2	1.8±0.1	1.8±0.1	2.6±0.4	2.2±0.2
Creatinine (μmol/L)	51.9±4.5	64.2±4.7	54.2±4.9	51±2.3	74.9±11.8	64.8±12.3
AST (U/L)	31.6*±2.3	43.0*±4.7	17.7±2.5	20.7±3.2	34.1±7.1	27.9±5.7
GGT (U/L)	161±41.5	361.7±104.6	25.7±4.5	34.3±10.3	21.7±4.2	20.8±6.5

*Asterisks indicate statistically significant values from control, * P < .05; ** P < 0.01; Values are reported as mean ± SEM for group of (ten animals (n = 10)*

Table 5. The blood biochemical analyses characterising inflammatory processes, bacterial toxaemia and lactic acidosis level of e and c calves

Indices	D1		D10		D15		D30	
	C	E	C	E	C	E	C	E
Glucose (mmol/L)	10.2±0.5	10.2±0.6	8.5*±0.4	7.0*±0.5	7.9±0.4	7.1±0.2	6.6±0.3	7.2±0.8
Lactate (mmol/L)	-	-	2.1±0.2	2.2±0.3	2.8±0.5	1.7±0.2	-	-
Hpt (ng/L)	1.1±0.2	1.0±0.1	1.6*±0.1	1.0*±0.1	2.3**±0.5	0.2**±0.0	-	-

*Asterisks indicate statistically significant values from control, * P < .05; ** P < 0.01;*
Values are reported as mean ± SEM for group of (ten animals (n = 10)

4. CONCLUSION

The mixture of the *Hippophaë rhamnoides* L. leaf and marc extracts with reduced concentration of tannins, as feed additives in doses of 5-8 mL/calf/ two times a day can improve thermoregulation of newborn calves and delay the beginning of non-specific diarrhoea for a few days due to the improvement of absorption of colostrum nutrients and immunoglobulins in the intestines in the 24-hour- period after the birth. Using HLM for 15 days induce reduction of inflammatory proceses in newborn animals, lower the defence mechanism against pathogens, but calves have a better daily weight gain and this growth promoting effect lasted for at least 15 days after the completion of receiving this extract. More experiments are necessary with different doses of HLM in older calves when the lymphocytes count increases and the differentiation is finished.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental procedures with calves were performed in accordance with guidelines of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and the experiment has been examined and approved by the Ethics Council of Animal Protection at University of Agriculture, Jelgava, Latvia.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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