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Research article

LIPID AND LIPOPROTEIN PROFILE CHANGES IN NEWBORN CALVES IN RESPONSE TO THE PERINATAL PERIOD

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The aim of this study was to evaluate the dynamic changes of serum lipid and lipoprotein profiles in 6 newborn calves during the first five days of life. From each calve blood sampling was performed daily starting from day 1 (after colostrum intake) until day 5 of life. Blood samples collected from each animal were tested for serum total lipids, phospholipids, non-esterified fatty acids (NEFAs), triglycerides, very low density lipoproteins (VLDLs), total cholesterol (Total-Chol), high density lipoproteins (HDLs) and low density lipoproteins (LDLs). One-way repeated measures analysis of variance (ANOVA) was applied to determine the effect of days of life on the studied parameters in calves. A statistically significant effect of days of life was found on all serum lipid and lipoprotein indices measured in calves with the exception of NEFAs that showed unchanged values throughout the monitoring period. The changes observed in calves during the early postnatal period are most likely due to the transition in energy sources, from a maternal nutrient supply comprising mainly carbohydrates and amino acids to the colostrum and milk diet rich in fat.

Key words: calves, lipid profile, lipoprotein profile, perinatal period

INTRODUCTION

The neonatal period represents a critical stage in the development of physiological functions. During this phase the newborn has to face several adjustments to adapt the body systems to extrauterine life [1-3]. Newborns are in metabolically unstable conditions, which make these subjects particularly sensitive to perinatal diseases resulting in high mortality rates [4,5]. Diseases of the newborn and neonatal mortality are a major cause of economic loss in livestock production [6,7]. Gastrointestinal disorders such as diarrhea are particularly frequent and are often followed by fatal systemic diseases [8]. In spite of the fact that the gastrointestinal tract of the newborn calves is relatively mature, its dynamic morphological growth and functional maturation

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is observed during the early postnatal period and depends by the transition from placental nutrition to alimentation of the newborn. At birth, the oral nutrient intake becomes the only source of nutrition. Colostrum is not only a valuable source of nutrients but also abounds in bioactive compounds such as: immunoglobulins, peptides, hormones, growth factors, nucleotides, and enzymes. It is stated that failure to ingest colostrum after birth in calves leads to complex metabolic and endocrine alterations [9]. The intake of colostrum in the first hours after birth is important for the normal morphological and functional maturation of the gastrointestinal tract and thus, for the adequate systemic adaptation to the new environment after birth, including feeding [3]. Thus, the first colostrum intake is crucial for the newborn calf as it initiates a number of physiological processes, resulting in different metabolic changes, including increased synthesis of endogenous proteins and dynamic qualitative and quantitative changes in blood lipid profile values and lipoprotein composition [9-11]. Lipids play a crucial role in mammals' metabolism, in fact these biological molecules function as the storage form of energy (triglycerides), vital component of cell membranes and as precursor of all steroid hormones (cholesterol). Lipids are insoluble in plasma and are transported bound to carrier proteins called lipoproteins including high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL).

Because lipid metabolism is a key aspect of the metabolic adaptations occurring during the neonatal period in the offspring, the aim of this study was to investigate the dynamic changes of serum lipid and lipoprotein profiles in calves during the first five days of life.

MATERIALS AND METHODS

Farm condition and animals

Six Holstein newborn calves (3 females and 3 males, mean body weight at birth 40.39 ± 0.63 kg) selected from a farm located in Sicily, Italy (36°42'38"N 14°47'15"E, 51 m above sea level) were used for this study. This area is characterized by: minimum and maximum mean annual temperature between 15.2°C and 21.9°C, mean annual relative humidity of 67.64 % and mean annual rainfall of 35.6 mm. The farms implements a dry period of 60 days and a period of steaming-up of 15 days before calving. The farm had a milk production of about 10000 kg /year and good milk quality (3.7% milk-fat, 3.4% milk protein). All calves were born full term and they underwent a clinical examination (evaluation of body temperature, heart rate, respiratory rate, auscultation of lungs and heart, external palpation of the umbilicus) at birth and throughout the study (data not shown). All animals were kept with their mothers in indoor pens under natural photoperiod and ambient temperature.

All cows were fed a balanced diet in accordance to the nutritional requirements for the peripartum period. Table 1 shows the chemical composition of the diets used during

the dry period, steaming-up and subsequent early lactation. Water was available *ad libitum*. Calves had access to cows' breast milk *ad libitum*. The compositional analysis of colostrum and milk content of samples collected from cows is shown in Table 2.

Table 1. Feed ingredients (%) and chemical composition (%) of dairy cows diets during the dry period, final part of the dry period (steaming-up) and early lactation

Feed ingredients (%)	Period		
	Dry	Steaming-up	Early lactation
Alfalfa silage	54.0	23.0	32.5
Corn gluten feed	6.4	-	-
Corn	5.4	39.1	26.8
Straw	25.0	12.5	-
Corn silage	8.0	4.0	16.4
Soybean meal	-	9.8	6.8
Starch	-	10.0	6.5
Whole cottonseed	-	-	8.2
Mineral and vitamins*	1.2	1.6	2.8
Composition (%)			
Crude Protein	12.43	13.87	16.66
Ethereal Extract	4.18	4.55	5.32
Ash	7.55	7.83	7.34
Ndf	50.45	43.99	33.41
Nfc	25.49	33.56	38.27
Dry Metter Degradable	52.38	59.68	68.22
Adf	24.66	24.46	20.12
Starch	11.71	16.49	25.94
Calcium	0.39	0.41	0.98
Phosphorus	0.24	0.24	0.43
Magnesium	0.30	0.29	0.31
Sodium	0.10	0.11	0.62
Potassium	0.43	0.51	1.39
Chlorine	0.20	0.19	0.28
Sulfur	0.16	0.16	0.21

*Mineral and vitamin mixture contained 5 g/kg of DiCa P, 8 g/kg of limestone flour, 5 g/kg of salt, 2.5 g/kg of Cal–Mag, 80 g of manganous oxide, 200 g of copper sulfate, 125 g of zinc oxide, 18 g of potassium iodate, 20 g of sodium selenite (4.6%), 10 g of cobalt sulfate, 8 MIU/t of vitamin A, 2 MIU/t of vitamin D3, 15,000 IU/t of vitamin E

Table 2. Compositional analysis of colostrum and milk content of samples collected from cows during the monitoring period

Analyte	Colostrum		Milk		
	Day 1	Day 2	Day 3	Day 4	Day 5
Fat (%)	6.70±0.28	5.44±0.16	3.89±0.22	3.90±0.36	3.58±0.21
Protein (%)	14.92±0.47	8.32±0.64	5.12±0.36	4.11±0.42	3.60±0.29
Lactose (%)	2.49±0.04	3.95±0.16	4.45±0.27	4.73±0.38	5.00±0.31
Total solids (%)	27.64±0.35	17.49±0.22	14.13±0.47	13.32±0.52	12.94±0.36
Ash (%)	1.11±0.05	0.92±0.04	0.87±0.03	0.83±0.04	0.82±0.05

Blood sampling and analysis

The same operator performed blood sampling from each animal in the morning (7:00 AM), by jugular venipuncture in vacutainer tubes containing a cloth activator agent (Terumo Co., Tokyo, Japan) in order to perform serum analysis. From each calve blood sampling was performed every day starting from day 1 (after colostrum intake) until day 5 of life.

Blood samples were left at room temperature for 20 min and then they were centrifuged at 3.000 rpm for 10 min and the obtained sera were stored at -20°C until analysis. Only the non-haemolysed sera were analyzed to estimate the concentration of total lipids, phospholipids, non-esterified fatty acids (NEFAs), triglycerides, total cholesterol (Total-Chol), high density lipoproteins (HDLs) and low density lipoproteins (LDLs) using commercially available kits by means of an automated UV spectrophotometer (model Slim SEAC, Florence, Italy). The very low density lipoprotein fraction (VLDLs) was estimated as one-fifth of the concentration of triglycerides [12].

All treatments, housing and animal care reported below were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments [13].

Statistical analysis

All results are expressed as mean ± standard deviation (SD).

All data were tested for normality of distribution using the Shapiro-Wilks test. All data were normally distributed ($P>0.05$) and statistical analysis was performed. One-way repeated measures analysis of variance (ANOVA), followed by Bonferroni post hoc comparison test, was applied to determine the statistical effect of day of life on BW, serum total lipids, phospholipids, NEFA, triglycerides, Total-Chol, HDLs, LDLs and VLDLs values measured in calves. P values < 0.05 were considered statistically significant. Data were analyzed using the software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003).

RESULTS

None of the animals included in the study showed clinical signs of disease during the monitoring period. A statistically significant effect of days of life was found on all serum lipid and lipoprotein indices in calves during the first five days of life with the exception of NEFAs (*P>0.05*) that showed unchanged values throughout the monitoring period. Serum total lipids showed higher values 4 and 5 days after birth respect to days 1, 2 and 3 (*P < 0.001*), whereas serum phospholipids values were higher 3, 4 and 5 days after birth respect to days 1 and 2, and 5 days after birth respect to days 3 and 4 (*P<0.01*). Triglycerides and VLDLs showed increased levels 4 and 5 days after birth respect to days 1 and 2 (*P<0.05*). Total-Chol and HDLs showed higher values 4 and 5 days after birth compared to day 1 (*P<0.05*); whereas, LDLs values were higher 2 and 3 days after birth compared to day 1 (*P<0.05*) (Figure 1).

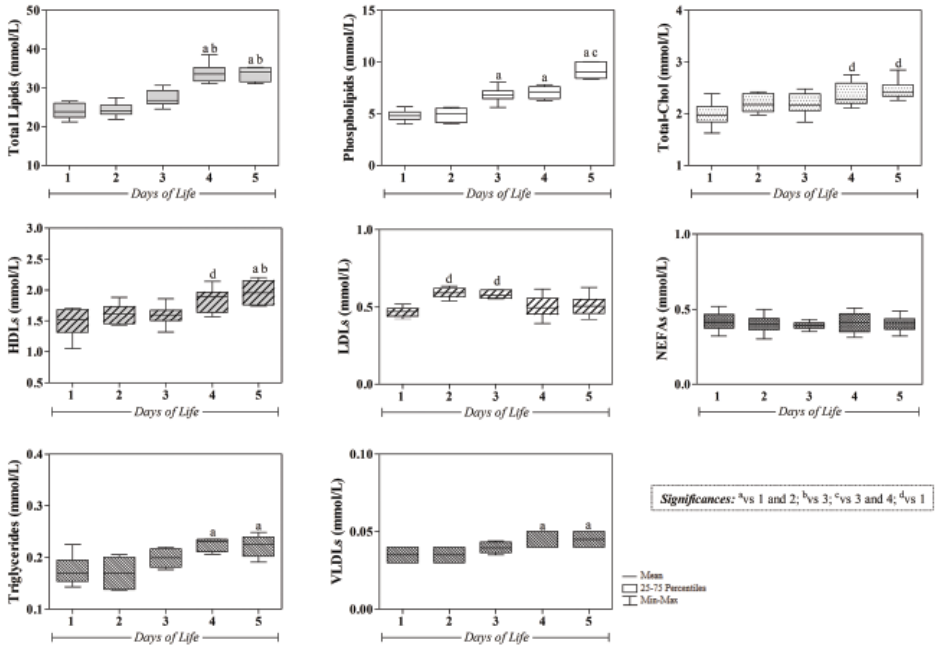

Figure 1. Trends and related statistical significances of serum total lipids, phospholipids, total cholesterol (Total-Chol), high density lipoproteins (HDLs), low density lipoproteins (LDLs), non-esterified fatty acids (NEFAs), triglycerides and very low density lipoproteins (VLDLs) values observed in calves during the first five days of life

DISCUSSION

The most intense adaptive changes to the new environment in newborn animals occur during the first week of life when the type of feeding switches from parenteral during the intrauterine development to entirely enteral after birth [1,3,6,14]. Colostrum

and milk intake initiates a number of physiological processes resulting in metabolic and nutritional status changes [1,3,5,6,11]. The results obtained in the present study showed that the serum lipid and lipoprotein indices undergo dynamic changes throughout the monitored period in newborn calves. In particular, the serum values of total lipids, phospholipids, triglycerides, VLDLs, Total-Chol and HDLs showed a gradual increase throughout the first five days of life, whereas, LDLs values showed an increase until day 3 followed by a slight decrease in days 4 and 5. These results agree with previous studies carried out on newborn calves [10,15,16] and could be related to colostrum and milk intake. In particular, the main reasons responsible for the trend observed in the studied parameters are both a high amount of ingested fat and increased intestinal fat absorption in order to maintain positive energy balance status [1]. In the present study the concentration of both serum HDLs and LDLs increased in calves during the first five days of life. Moreover, these results indicate that cholesterol is progressively transferred from LDLs to HDLs during the early postnatal period. LDL is the major lipoprotein fraction in fetal calve serum [16] and the subsequent shift to HDL as the predominant class is observed after colostrum and milk ingestion [3]. Several authors demonstrated that if colostrum was withheld for the first 24 h after birth, serum NEFAs values increased, whereas serum triglycerides, phospholipids, Total-Chol and cholesterol ester fractions remained lower than in calves fed with colostrum immediately after birth [9,18]. Other authors showed that serum triglycerides, phospholipids and cholesterol concentrations are higher in calves fed colostrum for 3 days than in those fed only milk replacer [15,16]. The mechanisms of colostrum effects on lipid status on newborn calves are not known. It has been speculated that bioactive components, such as insulin-like growth factor I and insulin, modify both the digestion and absorption of lipids, by possibly altering lipase activity or fatty acids binding proteins [1].

CONCLUSION

The results obtained in the present study revealed serum lipid and lipoproteins dynamic modifications in calves during the first five days and the main changes observed are most likely due to the transition in energy sources, from a maternal nutrient supply comprising mainly carbohydrates and amino acids to a diet rich in fat (colostrum and milk). Moreover, the present findings indicate that transport of cholesterol is progressively transferred from LDL to HDL during the early postnatal period. The present study underlines the importance of improving the understanding of metabolic demands during the perinatal period in order to promote offspring growth and well-being.

Authors' contributions

The idea for the paper was conceived by GP. The experiments were designed by FA and CG. The experiments were performed by FF, MP and EG. The data were analyzed by EG and SDP, and the paper was written by FA. The manuscript was critically revises for important intellectual content by GP. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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PROMENE U LIPIDNOM I LIPOPROTEINSKOM PROFILU KOD NOVOROĐENIH TELADI KAO ODGOVOR NA PERINATALNI PERIOD

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Cilj studije je bio da se obavi evaluacija dinamičkih promena u profilima lipida i lipoproteina seruma kod 6 novorođenih teladi tokom prvih pet dana života. Od svakog teleta, uzorak krvi je uzman svakoga dana, počevši od prvog dana života (posle uzimanja kolostruma), i sve do starosti od 5 dana. Uzorci krvi, uzeti od svake životinje, ispitivani su na ukupne serumske lipide, fosfolipide, neesterifikovane masne kiseline (NEFA), trigliceride, lipoproteine veoma male gustine (VLDL), ukupni holesterol (Total-Chol), lipoproteina velike gustine (HDL) kao i lipoproteine male gustine (LDL). Za statističku obradu rezultata, upotrebljena je analiza (ANOVA), u cilju određivanja uticaja broja dana života na ispitivane parametre. Statističkii značajan efekat dana bio je ustanovljen u slučaju svih serumskih lipida i lipoproteina sa izuzetkom NEFA kada su uočene nepromenjene vrednosti tokom celog perioda posmatranja. Promene koje su mogle da se uoče kod teladi tokom ranog postnatalnog perioda su najverovatnije uslovljene tranzicijom izvora energije i to od hranljivih materija poreklom od majke koje su se uglavnom sastojale od ugljenih hidrata i aminokiselina u odnosu na isranu kolostrumom i mlekom koja je bogata mastima.