Research Article

Analysis of apolipoprotein A-IV expression and the levels of blood plasma main lipid fractions of calves during the first week of life before and after the administration of milk replacer supplemented with lactose

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Abstract: The current study presents an analysis of main lipid fractions and expression of apolipoprotein A-IV (apoA-IV) of the blood plasma of calves during the first week of life, before and after administration of a milk replacer with additional quantity of lactose. The experiment was performed on 7 male Polish-Friesian calves, var. Black-and-White. Blood samples were collected before the morning feeding from the 6th to 8th day of life. In the evening on the 6th day of the experiment and in the morning on the 7th day of the experiment, monohydrate lactose in an amount of 2 g per 1 kg of body weight was added to the milk replacer. In all tested calves, after the administration of the additional quantity of lactose, there was a significant increase observed in expression of apoA-IV in the blood plasma. At the same time, no significant differences in the concentrations of triglycerides, total cholesterol, and low-density lipoprotein cholesterol were recorded, both before and after the administration of an additional dose of lactose. A gradual low increase observed in the plasma high-density lipoprotein cholesterol of calves allows us to conclude that the increase of this lipid fraction after the supply of an additional lactose is associated with elevated expression of plasma apoA-IV.

Key words: Newborn calves, dietary lactose, lipids, apolipoprotein A-IV

1. Introduction

The greatest economic losses in large-scale cattle breeding occur while rearing the calves. According to statistical data from a number of countries, from 12.5% up to 30% of the animals die during this period (1). Environmental and dietary factors should be listed among many reasons of high neonatal mortality of calves. Poor farm hygienic conditions and improper feeding of young animals significantly contribute to an increase in the incidence of diarrhea and pneumonia, which are considered the main causes of deaths of calves in the first 3 weeks of life (2). The method of feeding in large-scale breeding prevents individual treatment, and thus the feeds, including milk replacers, should meet the highest standards. In recent years, interest in improving nutrition has greatly increased, with special emphasis on precise ration balancing at the level of basic ingredients as well as the use of different kinds of feed additives. Modern nutrition of young animals and monitoring of their health status can provide further satisfactory production and economic effects.

One of the most important elements of newborn calves' adaptation to extrauterine life is the ability of self-feeding and changes in the metabolism, including replacing the main energy source, which in utero was carbohydrates, to fatty acids, in which colostrum is particularly enriched (3). Therefore, knowledge and monitoring of selected indicators of lipid metabolism seem to be important in assessing the health status of calves. Apolipoproteins (apo), a class of polypeptides present on the surface of the plasma lipoproteins, play an important role in lipid metabolism and transport (4). Apolipoproteins differ in terms of structure and function, and thus they are divided into a number of classes ranging from apoA to apoJ. These main classes are divided into subclasses, among which apoA-I, apoA-II, apoA-IV, apoB-48, apoB-100, apoC-I, apoC-II, and apoC-III should be listed (5). Of particular interest is apoA-IV, due to its wide biological roles in lipid metabolism. Since the discovery of this apolipoprotein in 1974, it has been attributed with various important functions (6). Numerous studies suggested that apoA-IV participates in several steps of the reverse cholesterol transport pathway, which removes and transports cholesterol from peripheral cells to the liver or steroid organs (7). ApoA-IV is necessary for effective activation of the enzyme

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lecithin cholesterol acyltransferase (8,9). ApoA-IV also modulates the activation of lipoprotein lipase and the cholesteryl ester transfer protein, which mediates transfer of cholesteryl esters from high-density lipoprotein (HDL) to low-density lipoprotein (LDL) (10,11). Moreover, this apolipoprotein can affect the regulation of gastric motility and gastric acid secretion (12). ApoA-IV also exerts an antiatherosclerotic and antioxidant effect (13,14). ApoA-IV in mammals is synthesized mainly in the enterocytes of the small intestine and its plasma level increase is observed after ingestion of a fatty meal (15). According to Stan et al. (16), one of the factors that may increase the expression of apoA-IV in plasma is the spectrum of carbohydrates in the diet, such as glucose, fructose, or lactose. Considering these facts, we conducted a study aimed at determining whether after the administration of milk replacer to calves with an additional quantity of lactose the expression of plasma apoA-IV would be increased. Furthermore, in order to assess whether any changes in the expression of ApoA-IV would be reflected in changes in the plasma concentration of the major lipid fractions, we measured the concentrations of total cholesterol (TC), HDL cholesterol (HDLC), triglycerides (TG), and LDL cholesterol (LDLC) both before and after the administration of an additional dose of lactose with milk replacer formulation.

2. Materials and methods

2.1. Experimental animals
The experiment was carried out on seven Polish-Holstein Friesian male calves, var. Black-and-White. They were fed colostrum until 3 days of age at the dairy farm, then transported to the Faculty of Biotechnology and Animal Husbandry at the West Pomeranian University of Technology in Szczecin (Poland) at 3 days of age. The animals were held in single pens and were fed with commercial milk replacer Mlekovit Imupro, manufactured by Polmass, Poland. The milk replacer contained 23% crude protein, 16% crude fat, 0.1% crude fiber, 45% lactose, 7.5% crude ash, 1.7% lysine, 0.42% methionine 0.9% calcium, and 0.7% phosphorus. The calves were fed milk replacer at 10% of their body weight twice a day. During the experiment the calves did not have access to water. Blood samples were collected before the morning feeding from the 6th to 8th day of life. In the morning of the 6th day of the experiment and in the evening of the 7th day of the experiment, monohydrate lactose (Pharma Cosmetic) in the amount of 2 g per 1 kg of body weight was added to the milk replacer. The use and handing of animals for this experiment was approved by the Local Commission of Ethics for the Care and Use of Laboratory Animals (No. 3/2010 of 14.01.2010).

2.2. SDS-PAGE and western blot
Expression of apoA-IV in the blood plasma was measured using the western blot technique. First, in the blood plasma, the total protein concentration was determined using the Bradford method (Protein Assay Dye Reagent Concentrate, Bio-Rad). The samples were diluted in SDS buffer, which contained a final concentration of 62 mM Tris, 0.1 M SDS, 8.7% glycerol, 0.09 mM bromophenol blue, and 355 mM 2-mercaptoethanol. The samples, warmed to 37 °C, were loaded on 12% polyacrylamide gels (10 µg denatured protein dissolved in SDS buffer per sample well) and run for 120 min at 110 V. The proteins of studied gels were then electrotransferred (10 V, 15 min) to PVDF membranes. The membranes were blocked with 5% nonfat milk in PBS-T (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, and 0.1% Tween 20, pH 7.5) for 1 h and incubated overnight at 4 °C with rabbit polyclonal antibodies anti-apoA-IV NBP 1-86164 (Novus Biologicals) diluted 1:400, followed by incubation with secondary antirabbit (STAR 124P AbD Serotec) horseradish peroxidase-conjugated antibodies. The labeling was visualized by an enhanced chemiluminescence (ECL Plus) system and exposure to a CCD camera (Versadoc 4000MP, Bio-Rad). Optical density (OD) results obtained by densitometer are presented as bands, evaluated by the use of Quantity One software. For apoA-IV the OD values of 42-kDa bands were determined and use to estimate and compare expression of this protein in the blood plasma samples.

2.3. Analyses of lipid concentrations in blood plasma
The concentrations of TC, HDLC, and TG were determined spectrophotometrically using colorimetric test kits (Biomaxima) according to the manufacturer's specifications. The concentration of LDLC was calculated on the basis of the Friedewald formula (LDL = TC – HDL – [TG/2.2]) (17).

2.4. Statistics
Mean values and standard deviations were calculated. The resulting data were analyzed by ANOVA with repeated measurements and the Duncan multiple range post hoc test (Statistica 10.0) in order to test significance of differences.

3. Results
The results for the identification and analysis of the expression of apoA-IV in the blood plasma of calves tested are shown in the Figure. Western blot of blood plasma samples of all the tested calves revealed strong bands with a mass of 42 kDa. Based on the analysis of average OD values of these bands, it was found that before lactose addition to milk replacer, the average OD of apoA-IV in the blood plasma was 2861.85 ± 355.11. After the first administration of milk replacer with lactose, apoA-IV expression was 3058.00 ± 318.29. After the second dose of
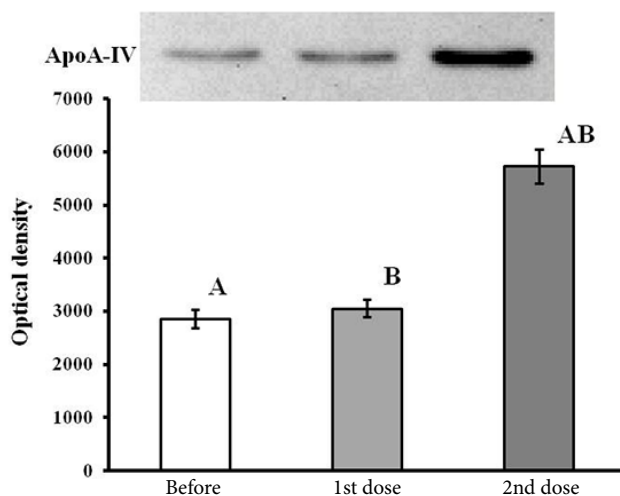
Figure. Expression of apoA-IV in the blood plasma of calves during their first week of life before and after the administration of milk replacer supplemented with lactose. The same capital letters are used to show significant differences.

lactose, a statistically significant increase in the expression of apoA-IV was recorded (P < 0.0001). The average OD of this apolipoprotein after two additional doses of lactose was 5731.18 ± 637.72.

The average values of the concentration of individual lipid fractions in the plasma of calves obtained in the experiments are shown in the Table. The concentrations of TC and TG, both before and after two administrations of lactose to the milk replacer, were relatively stable and averaged 0.68 mmol/L and 2.11 mmol/L, respectively. After the supply of milk replacer containing lactose, a slight increase was found in HDLC and a slight decrease in LDLC in the blood plasma of calves. However, these changes were not statistically significant.

4. Discussion
It is known that in mammals during intestinal fat absorption the synthesis, secretion, and plasma levels of apoA-IV are markedly increased (16,18). It was demonstrated in humans, rats, pigs, and calves that the expression of apoA-IV is low directly after birth and increases during the first days of life (19–23). Many authors observed increased expression of this apolipoprotein in the neonatal period, which was associated with elevated intestinal lipid absorption in that period caused by a high fat intake with milk and energy needs (16). In neonatal rats, the expression of apoA-IV gradually increases and reaches values typical for adults after 14 days of life (22). In addition to lipids, other dietary factors have also been identified, capable of stimulating apoA-IV gene expression or plasma concentration. According to Nunez et al. (24), in preterm newborn infants, dietary nucleotides known to promote intestinal growth and maturation are likely to cause an increase in apoA-IV in plasma and elevate the activity of lecithin cholesterol acyl transferase. Sato et al. (25) found in rat pups that a diet composed of casein and lactose increased intestinal apoA-IV levels, but without alterations of its plasma concentration. In turn, Stan et al. (16) reported that carbohydrates, including lactose, caused a rapid rise in serum apoA-IV. In adult rats after the supply of a diet rich in sucrose, an increase was found in cellular and nuclear apoA-IV mRNA levels in the liver. However, there was no simultaneous increase in the intestinal mRNA level and plasma concentration of apoA-IV (26,27). The results of the present experiment may indicate that the double administration of additional quantities of lactose with milk replacer resulted in a statistically significant increase in the expression of plasma apoA-IV in the calves analyzed. Thus, the results obtained appear to confirm the findings of the aforementioned authors, who reported that apart from the fat intake, carbohydrates, including lactose and its breakdown products, such as glucose and galactose in the diet, can also increase the synthesis and secretion of intestinal apoA-IV, which is reflected in the observed increase of this apolipoprotein in blood plasma. The explanation of this process, however, requires further and more detailed research.

In comparison to monogastric animals, ruminants, including cattle, have a characteristic plasma lipid profile.

Table. The concentration of triglycerides, total cholesterol, and HDL and LDL cholesterol in the blood plasma of calves during their first week of life before and after the administration of milk replacer supplemented with lactose.

Parameter	Before lactose addition	After lactose addition	
		1st dose	2nd dose
Triglycerides (mmol/L)	0.72 ± 0.38	0.69 ± 0.45	0.63 ± 0.16
Total cholesterol (mmol/L)	2.04 ± 0.27	2.09 ± 0.56	2.21 ± 0.55
HDL cholesterol (mmol/L)	1.05 ± 0.48	1.19 ± 0.43	1.23 ± 0.15
LDL cholesterol (mmol/L)	0.61 ± 0.32	0.55 ± 0.12	0.67 ± 0.23

Fermentation of the rumen content, associated with intensive lipid hydrolysis and biodegradation of the bulk of fatty acids by bacteria and protozoa, causes the fatty acids absorbed from the small intestine to be primarily of the saturated type. The continuous flow of the chyme to the small intestine with a high content of unsaturated fatty acids promotes the formation of mainly the very-low-density lipoprotein (VLDL) fraction in the enterocytes. Under natural conditions, approximately 73% of lipids present in the lymph flowing from the gastrointestinal tract are of the VLDL fraction, and only about 23% are of the chylomicron fraction (3,28). Cattle and other ruminants are characterized by a low plasma concentration of TG and TG-rich lipoproteins (29,30). In contrast to monogastric animals, the major fraction of plasma lipoproteins in ruminants is HDL, which in adult cattle can constitute up to 80% of all lipoproteins (31). Low concentration of the VLDL fraction in the blood plasma of ruminants is related to its low biosynthesis and secretion in the liver (3,32). Moreover, unlike monogastric animals, TG formed by the esterification of free fatty acids in ruminants does not undergo hydrolysis in the hepatocytes, which together with a low capacity of VLDL synthesis promotes accumulation of TG, which can lead to so-called fatty liver (3). In newborn calves up to 1–2 months of age, the reticulorumen is poorly developed, and hence the lack in this period of fermentation processes and numerous microbial modifications of the chemical structure of dietary lipids. Therefore, calves, often referred to as preruminants, can be functionally considered monogastric (28). However, serum lipoproteins of newborns calves are characterized by and compared with those of adult animals, while the quantitative composition in the perinatal period is closely associated with dietary intake.

In the present study, TG concentration in the first week of life of the calves was relatively stable and ranged from 0.63 to 0.72 mmol/L; double application of a milk replacer to calves with the addition of lactose did not result in significant changes in the concentration of plasma TG. Similar results were obtained by Herosimczyk et al. (19). Those authors found that the level of plasma TG in 7-day-old calves amounted on average to 0.62 mmol/L. Jankowiak et al. (3) indicated that the concentration of TG in the blood plasma of calves from day 5 to 8 of age ranged from 0.47 to 0.68 mmol/L. The main factor conditioning the relatively high, compared to adults, TG concentration in the first days of life is the supply of high fat content in the colostrum and milk. In calves in which the supply of colostrum was restricted directly after birth, TG increase

was not observed even up to 7 days of age (33). The average concentration of total cholesterol in the animals studied was lower than that observed by other authors in calves fed mother's milk (19). Low TC concentration was probably due to the fact that calves from 4 days of age were fed only milk replacer formulation. According to Hammon and Blum (33), a significant impact on the concentration of cholesterol in the blood plasma is had by the total contents of lipids and unsaturated and saturated fatty acids in the diet, and the administration of milk replacers to calves during the first days of life significantly reduces the increase in plasma TC level. As compared to calves fed mother's milk, the current experiments also found lower HDLC concentration values. However, the LDLC concentration was similar (3,19). Lower HDLC fraction is probably associated with a lower level of cholesterol in the studied calves, because, as mentioned earlier, the vast majority of ruminant blood plasma TC is located in HDL. In the experimental calves, a slight increase in HDLC was observed after the supply of additional doses of lactose. The elevated expression of apoA-IV in plasma accompanying these changes allowed us to assume that an increase in the HDLC fraction was associated with a rise of the plasma concentration of this apolipoprotein. It is known that apoA-IV circulates in the blood plasma predominantly in a form not bound with lipoproteins or bound with the mentioned HDL fraction (34).

In summary, the present research found that in the first week of life, after the administration to calves of an additional quantity of lactose with milk replacer formulation, there was a significant increase observed in expression of apoA-IV in the blood plasma. At the same time, no significant differences in the concentrations of TG, TC, and LDLC were recorded, both before and after the administration of an additional dose of lactose. A gradual low increase observed in the plasma HDLC of calves allows us to conclude that the increase of this lipid fraction after the supply of an additional lactose is associated with elevated expression of plasma apoA-IV. The present study demonstrated that the use of a milk replacer supplemented with lactose, in addition to increasing the levels of apoA-IV, did not seem to affect the changes in individual lipid fractions in the blood plasma of calves during the first week of life

References

1. Singh DD, Kumar M, Choudhary PK, Singh HN. Neonatal calf mortality – an overview. Intas Polivet 2009; 10: 165–169.

2. Al-Shami SA. Comparative study of heamatological and blood biochemical components in milk-fed and conventionally-reared Hassawi breed calves. Scientific Journal of King Faisal University 2007; 8: 99–106.

3. Jankowiak D, Kochel A, Drozd R, Kowalska J, Muszczyński Z. The changes of concentration of the triglicerydies, total cholesterol, HDL and LDL cholesterol in the blood plasma of the limousine breed calves in the first ten days of the postnatal life. Acta Sci Pol Zootechnica 2010; 9: 107–118 (in Polish with English abstract).

4. Baroukh N, Bauge E, Akiyama J, Chang J, Afzal V, Fruchart JC, Rubin EM, Fruchart-Najib J, Pennacchio LA. Analysis of apolipoprotein A5, C3 and plasma triglyceride concentrations in genetically engineered mice. Arterioscler Tromb Vasc Biol 2004; 24: 1297–1302.

5. Irshad M, Dubey R. Apolipoproteins and their role in different clinical conditions: an overview. Indian J Biochem Biophys 2005; 42: 73–80.

6. Swaney JB, Rese H, Eder HA. Polypeptide composition of rat high density lipoprotein: characterization by SDS-gel electrophoresis. Biochem Biophys Res Commun 1974; 59: 513–519.

7. Haiman M, Salvenmoser W, Scheiber K, Lingenhel A, Rudolph C, Schmitz G, Kronenberg F, Dieplinger H. Immunohistochemical localization of apolipoprotein A-IV in human kidney. Kidney Int 2005; 68: 1130–1136.

8. Steinmetz A, Barbaras R, Ghalim N, Clavey V, Fruchart JC, Ailhaud G. Human apolipoprotein A-IV binds to apolipoprotein A-I/A-II receptor sites and promotes cholesterol efflux from adipose cells. J Biol Chem 1990; 265: 7859–7863.

9. Steinmetz A, Utermann G. Activation of lecithin: cholesterol acyltransferase by human apolipoprotein A-IV. J Biol Chem 1985; 260: 2258–2264.

10. Guyard-Dangremont V, Lagrost L, Gambert P. Comparative effects of purified apolipoproteins A-I, A-II, and A-IV on cholesteryl ester transfer protein activity. J Lipid Res 1994; 35: 982–992.

11. Main LA, Ohnishi T, Yokoyama S. Activation of human plasma cholesteryl ester transfer protein by human apolipoprotein A-IV. Biochim Biophys Acta 1996; 1300: 17–24.

12. Whited KL, Lu D, Tso P, Kent Lloyd KC, Raybould HE. Apolipoprotein A-IV is involved in detection of lipid in the rat intestine. J Physiol 2005; 569: 949–958.

13. Simon T, Cook VR, Rao A, Weinberg RB. Impact of murine intestinal apolipoprotein A-IV expression on regional lipid absorption, gene expression, and growth. J Lipid Res 2011; 52: 1984–1994.

14. Kohan AB, Wang F, Xiaoming Li, Bradshaw S, Yang Q, Caldwell JL, Bullock TM, Tso P. Apolipoprotein A-IV regulates chylomicron metabolism-mechanism and function. Am J Physiol Gastrointest Liver Physiol 2012; 302: G628–G636.

15. Weinberg RB, Gallagher JW, Fabritius MA, Shelness GS. ApoA-IV modulates the secretory trafficking of apoB and the size of triglyceride-rich lipoproteins. J Lipid Res 2012; 53: 736–743.

16. Stan S, Delvin E, Lambert M, Seidman E, Levy E. ApoA-IV: An update on regulation and physiologic functions. Biochim Biophys Acta 2003; 1631: 177–187.

17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clin Chem 1972; 18: 499–502.

18. Apfelbaum TFN, Davidson O, Glickman RM. Apolipoprotein A-IV synthesis in rat intestine: regulation by dietary triglyceride. Am J Physiol 1987; 252: G662–G666.

19. Herosimczyk A, Lepczyński A, Ożgo M, Dratwa-Chałupnik A, Michałek K, Skrzypczak WF. Blood plasma protein and lipid profile changes in calves during the first week of life. Pol J Vet Sci 2013; 16: 425–434.

20. Huang Y, Olson DJ, Gordon JR, Middelton DM, Simko E. Plasma protein profiles of neonatal pigs before and after suckling. Can J Vet Res 2012; 76: 1–7.

21. Lu S, Yao Y, Wang H, Meng S, Cheng X, Black DD. Regulation of apoA-IV transcription by lipid in newborn swine is associated with a promoter DNA-binding protein. Am J Physiol Gastrointest Liver Physiol 2003; 284: 248–254.

22. Elshourbagy NA, Boguski MS, Liao WS, Jefferson LS, Gordon JM. Expression of rat apolipoprotein A-IV and A-I genes: mRNA induction during development and in response to glucocorticoids and insulin. P Natl Acad Sci USA 1985; 82: 8242–8246.

23. Steinmetz A, Czekelius P, Thiemann E, Motzny S, Kaffarnik H. Changes of apolipoprotein A-IV in the human neonate: evidence for different inductions of apolipoproteins A-IV and A-I in the postpartum period. Atherosclerosis 1988; 69: 21–27.

24. Nunez MC, Ayudarte MV, Morales D, Suarez MD, Gil A. Effect of dietary nucleotides on intestinal repair in rats with experimental chronic diarrhea. J Parenter Enteral Nutr 1990; 14: 598–604.

25. Sato M, Imaizumi H, Mori H, Sugano M. Regulation of intestinal apo A-IV mRNA abundance in rat pups during fasting and refeeding. Biochim Biophys Acta 1992; 1165: 93–101.

26. Radosavljevic M, Lin-Lee YC, Soyal SM, Srobl W, Seelos C, Gotto AM, Patsh W. Effect of sucrose diet on expression of apolipoprotein genes A-I, C-III and A-IV in rat liver. Atherosclerosis 1992; 95: 147–156.

27. Strobl W, Knerer R, Gratzl R, Arbeiter K, Lin-Lee YC, Patsh W. Altered regulation of apolipoprotein A-IV gene expression in the liver of the genetically obese Zucker rat. J Clin Invest 1993; 92: 1776–1773.

28. Bauchart D, Durand D, Laplaud PM, Forgez P, Goulinet S, Chapman MJ. Plasma lipoproteins and apolipoproteins in the preruminant calf, *Bos* spp: density distribution, physicochemical properties, and the in vivo evaluation of the contribution of the liver to lipoprotein homeostasis. J Lipid Res 1989; 30: 1499–1514.

29. Mazur A, Ozgo M, Rayssiguier Y. Altered plasma triglyceride-rich lipoproteins and triglyceride secretion in feed-restricted pregnant ewes. Vet Med Czech 2009; 54: 412–418.

30. Christie WW. Lipid Metabolism in Ruminant Animals. 1st ed. New York, NY, USA: Pergamon Press; 1981.

31. Bauchart D. Lipid absorption and transport in ruminants. J Dairy Sci 1993; 76: 3864–3881.

32. Grummer RR. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J Dairy Sci 1993; 76: 3882–3889.

33. Hammon HM, Blum JW. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. J Nutr 1998; 128: 624–632.

34. Seishima M, Torizawa H, Muto Y, Noma A. Increased serum apoA-IV concentrations in experimental uremic rats. J Lipid Res 1992; 33: 1441–1447.