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*Original article*

# Blood plasma protein and lipid profile changes in calves during the first week of life

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## Abstract

The present study was undertaken to determine blood plasma protein and lipid profile changes in healthy Polish Holstein-Fresian calves of Black-and-White variety. Blood was drawn immediately after birth, before first colostrum intake and at the 3rd, 6th, 12th, 24th, 36th, 48th and 72nd hour of life. Subsequent four blood samples were collected at 24 hour intervals until the 7th day of life. Plasma proteins within the isoelectric point ranging from 3.0 to 10.0 were separated using high resolution two-dimensional electrophoresis. Among the 74 protein spots detected and analyzed, 16 were significantly altered during the first week of life. Differentially expressed spots were excised from the gels and subjected to peptide mass fingerprinting using MALDI-TOF MS. In total, 12 spots were successfully identified, which correspond to three proteins, namely: apolipoprotein A-I, apolipoprotein A-IV and fibrinogen gamma-B chain. A gradual increase in plasma triglyceride, total cholesterol, HDL and LDL cholesterol values was shown during the first seven days of calves life. The lowest concentration of these indicators were observed at birth and was followed by a rapid increase during the first week of postnatal life. These changes appear to be related to the transition in energy sources, from a maternal nutrient supply comprising mainly carbohydrates and amino acids to a diet which was rich in fat – colostrum and milk. This was reflected by the intense up-regulation of plasma proteins related with lipid transport and lipoprotein metabolism during the first week of life.

**Key words**: 2-DE, proteome, blood plasma, neonatal calves, lipid profile

## Introduction

Many recent studies indicate, that the most intense adaptive changes to the new environment in calves occur during the first week of life. In spite of the fact that the gastrointestinal tract (GIT) of the newborn calves is relatively mature, its dynamic morphological growth and functional maturation is observed during the early postnatal period. These changes are essential for rapid digestion and absorption of food, which is highly variable in either a quantitative or qualitative manner during the first days of life (Blum and Hammon 2000).

Colostrum, the first food for the newborn calves is not only a valuable source of nutrients but also abounds in bioactive compounds such as: immuno-

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globulins, peptides, hormones, growth factors, nucleotides and enzymes. Intestinal absorption of these molecules and its further passage to the circulation is possible only within the first 24 hours of life (Uruakpa et al. 2002). Thus, first colostrum intake is crucial for the newborn calf as it initiates a number of physiological processes, resulting in different metabolic changes, e.g. increased synthesis of endogenous proteins. The epitheliochorial placenta of the bovine species prevents from effective transfer of maternal plasma proteins to the conceptus, thus newborn calves are considered as a suitable model for the study of dynamic plasma protein profile changes during the early postnatal period (Skrzypczak et al. 2011, Herosimczyk et al. 2012).

Colostrum and milk intake also provokes changes in nutritional status of the neonatal calves, including dynamic qualitative and quantitative changes in plasma lipoprotein composition and lipid profile values (Blum 2006, Jankowiak et al. 2010). Numerous studies on newborn calves have shown that colostrum and milk ingestion, both considered as a rich source of fat, causes gradual increase in plasma total cholesterol, HDL and LDL cholesterol and triglyceride concentrations during the first week of calves life (Hammon and Blum 1998, Kuhne et al. 2000, Jankowiak et al. 2010). According to Blum and Hammon (2000) the main reasons responsible for the observed phenomenon are both high amount of ingested fat and increased intestinal fat absorption in order to maintain positive energy balance status.

Proteomics enables the simultaneous and comprehensive analysis of thousands of proteins present in any biological compartments e.g. blood plasma (Herosimczyk et al. 2006). However, the studies focused on proteomic analysis of calves blood plasma proteins in the early postnatal period are sparse. Previously, Talukder et al. (2002) investigated transfer of the bovine colostral macromolecules from the gastrointestinal tract into the circulation of calves at 6 and 12 h after colostrum administration. The authors observed significant changes in the expression of lactoferrin (Lf), transferrin (Tf), immunoglobulin G (IgG) and epidermal growth factor (EGF). Proteomic tools have also been employed to reveal molecular serum/plasma biomarkers for the detection of growth promoting substances that are illegally used in the veal calves. Recently, two-dimensional electrophoresis (2-DE), matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF-MS) and liquid chromatography-hybrid ion trap time of flight mass spectrometry (μLC-IT-TOF/MS) have been successfully applied to the global analysis of plasma from calves treated orally with the androgenic steroids such as boldenone and boldione (Draisci et al. 2007). Draisci et al. (2007) showed that single dose of the boldenone resulted in the overexpression of the N-terminal truncated form of apolipoprotein A-I and thus may be considered as a candidate biomarker for the anabolic androgens treatment. Nevertheless, in the available literature there is a lack of information concerning other age-dependent and diet-dependent plasma proteome changes that occur in response to dynamic adaptation processes, during the early postnatal period.

This study was aimed at determining plasma protein changes in healthy Polish Holstein-Fresian calves of Black-and-White variety before first colostrum administration ("zero" blood sample) and in the $6^{th}$, $12^{th}$, $24^{th}$, $48^{th}$, $72^{nd}$, $168^{th}$ hour of life. The second objective was to analyse changes in the plasma lipid profiles including: total cholesterol, triglycerides, HDL and LDL cholesterol during the first seven days of postnatal life. Lipid profiles were measured at the following time points: 0, $3^{rd}$, $6^{th}$, $12^{th}$, $24^{th}$, $36^{th}$ $48^{th}$, $72^{nd}$, $96^{th}$, $120^{th}$, $144^{th}$, $168^{th}$ hours of life.

## Materials and Methods

### Animals

A total of seven healthy male Polish Holstein-Fresian calves of Black-and-White variety were used. Calves were born from multiparous cows aged between 4 to 6 years with pregnancies of normal length. Nutrient composition of the forage fed to lactating dairy cows is presented in Table 1. Immediately after birth calves were separated from their dams and were kept in the individual pens under the same environmental conditions. During the first three days of life, calves were fed colostrum (37°C) from their dams in the amount of 4 l/calf/day and then the mature milk of dams were fed in the amount of 6 l/calf/day until the seventh day of postnatal life. The calves were fed three times daily, at 6 a.m., noon, and 6 p.m. The use and handling of animals for this experiment was approved by the Local Commission of Ethics for the Care and Use of Laboratory Animals (No. 4/2008 of 24.01.2008).

### Sample collection

Blood was drawn via jugular venipuncture into tubes pre-coated with $K_3$EDTA immediately after birth, before first colostrum feeding ("zero" blood sample) – and at the $3^{rd}$, $6^{th}$, $12^{th}$, $24^{th}$, $36^{th}$, $48^{th}$ and $72^{nd}$ hour of calves life. Subsequent 4 blood samples were collected at 24 hour intervals until the $7^{th}$ day of

Table 1. Nutrient composition of the forage fed to lactating dairy cows.

| Ingredients | In daily dose [kg] | DM [kg] |
|---|---|---|
| Alfalfa silage | 6.0 | 2.34 |
| Maize silage | 19.0 | 7.95 |
| Straw | 1.0 | 0.92 |
| Total roughage | 26.0 | 11.22 |
| Concentrate mixture* | 8.0 | 7.08 |
| Corn cob silage | 2.0 | 1.15 |
| Dried sugar beet pulps | 0.5 | 0.45 |
| Salt | 0.07 | 0.07 |
| Molasses | 0.8 | 0.63 |
| Brewer's spent grains | 3.0 | 0.79 |
| Total concentrate | 14.37 | 10.16 |
| Total forage | 40.37 | 21.38 |
| Nutrients | Forage [kg] | In concentrate |
| Crude protein | 3.862 kg | 18.065% |
| Neutral deterg fiber | 6.586 kg | 30.808% |
| Non fiber carbohydrate | 8.436 kg | 39.460% |
| Fat | 0.780 kg | 3.648% |
| Ne lact 3X | 35.547 mcal | 75.421 mcal/cwt |
| Sol prot, % of CP | 30.489 | 30.489 |
| RDP, % of CP | 66.620 | 66.620 |
| RUP, % of CP | 33.374 | 33.374 |
| Calcium | 0.198 kg | 0.928% |
| Phosphorus | 0.101 kg | 0.473% |
| Potassium | 0.274 kg | 1.283% |

* concentrate mixture contains: barley meal, triticale meal, corn meal, extruded soy meal, extruded canola meal

life always three hours after morning feeding. Samples were then centrifuged at 3000 rpm for 10 min at 4°C. Next, plasma samples were stored at (-80)°C until use. Duplicate analysis were performed for each plasma sample both for electrophoretic and biochemical studies.

## Two-dimensional electrophoresis (2-DE)

High-abundant proteins were depleted with ProteoExtract® Albumin/IgG Removal Kit (Calbiochem). Proteins were then dissolved in the lysis buffer (5 M urea, 2 M thiourea, 4% w/v CHAPS, 40 mM Tris, 0,2% w/v 3-10 ampholytes and 2 mM TBP). For analytical gels 70 µg of proteins and for preparative gels 1500 µg of proteins were loaded. Analytical gels were used for image analysis and preparative gels for spot extraction and MS identification. The first dimension was run (Protean® IEF Cell, Bio-Rad) using 3-10, 17 cm NL (nonlinear) ReadyStrip™ IPG Strips (Bio-Rad) in total 97500 Vh. Prior to the second dimension SDS-PAGE (sodium dodecyl sulfate – polyacrylamide gel electrophoresis), focused IPG strips were reduced with DTT and then alkylated with iodoacetamide. After that strips were placed on the top of 12% SDS polyacrylamide gels. The gels were run in the second dimension in Protean Plus™ Dodeca Cell™ electrophoretic chamber (Bio-Rad) at 40V for 1 h and then at 120V for 15 h at 10°C. After 2-DE separation, analytical gels were visualized with silver stain (Chevallet et al. 2006) and preparative gels with colloidal Coomassie Brilliant Blue G-250 (Candiano et al. 2004).

## Image analysis

Gels were scanned using GS-800™ Calibrated Densitometer (Bio-Rad). The 2-D image computer analysis was performed using PDQuest Analysis software version 8.0.1 Advanced (Bio-Rad). Analytical

procedures performed on each gel included: spot background substraction, spot detection and matching. Normalization of each individual spot was performed using local regression model (LOESS). Coefficient of variation (CV) was calculated for replicate groups and then significance of protein expression changes was measured.

### Mass spectrometry (MS)

Protein spots, with altered expression, were manually excised from coomassie stained gels and decolorized (25 mM $NH_4HCO_3$ in 5% v/v ACN) then dehydrated with 100% ACN and vacuum dried (Concentrator 5301, Eppendorf). Next, samples were incubated with trypsin (20 µl/spot of 12.5 µg/ml in 25 mM $NH_4HCO_3$; Sigma-Aldrich, St. Louis, MO) for 16 h at 37°C. Resulted peptides were extracted with 100% ACN, combined with an equal volume of matrix solution (5 mg/ml CHCA, 0,1% v/v TFA, 50% v/v ACN) and loaded onto a MALDI-MSP AnchorChip™ 600/96 plate (Bruker Daltonics, Germany). For calibrating mass scale Peptide mass standard II (Bruker Daltonics, Germany within mass range 700-3200 Da) was used. Mass spectra were acquired in the positive-ion reflector mode using Microflex™ MALDI TOF mass spectrometer (Bruker Daltonics, Germany). The PMF (peptide mass fingerprinting) data were compared to mammalian databases (SWISS-PROT; http://us.expasy.org/uniprot/) by the aid of MASCOT search engine (http://www.matrixscience.com/). The database search in MASCOT was conducted using a mass accuracy of 150 ppm, one missing peptide cleavage site, partial carbamidomethylation of cysteine and partial methionine oxidation. A protein was successfully identified when a significant MASCOT score was achieved ($p \leq 0.05$).

### Analyses of lipid concentration in blood plasma

The concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and triglycerides (TG) were determined spectrophotometrically (PowerWave™ XA, BioTek) using colorimetric test kits (Biolabo, Aqua-Med, BioMaxima), accordingly to manufacturer's specifications. The concentration of low-density lipoprotein cholesterol (LDLC) was calculated on the basis of the Friedewald's formula (LDL = TC – HDL – [TG/2.2]).

### Statistics

Mean values and standard deviations were calculated. The resulting data were analysed by ANOVA with repeated measurements and Tukey's multiple range post hoc test (software: Statistica 8.0™) in order to test the significance of difference.

## Results

### Blood plasma proteome changes

Results of 2-DE pattern representative for all analysed gels, are shown in Fig. 1. Among 74 analysed protein spots, 16 were significantly altered during the first week of life. In total, 12 spots were successfully identified, that corresponded to 3 proteins, namely: apolipoprotein A-I, apolipoprotein A-IV and fibrinogen gamma-B chain. Four spots were unidentified despite repeated analysis. Unidentified spots were marked with SSP numbers (SSP: 5101, 5106, 5301, 5302), which were created during the analysis with the PDQuest 8.0. Advanced software.

The expression profile of apo A-I (Fig. 2A) and apo A-IV (Fig. 2B) followed the same pattern of changes during the first week of life. Expression of protein spots were low immediately after birth (0 h) and was followed by a significant ($p \leq 0.01$) increase from the 6$^{th}$ until the 168$^{th}$ hour of life. Similar changes were also observed in the case of fibrinogen gamma-B chain (Fig. 2C), which expression was relatively low at 0 h and statistically increased ($p \leq 0.01$) from the 48$^{th}$ h of life until the end of the experimental period. Unidentified spots: SSP 5101 (Fig. 3A), SSP 5106 (Fig. 3B), SSP 5301 (Fig. 3C) and SSP 5302 (Fig. 3D) were up-regulated in plasma immediately after birth (0 h). From the 6$^{th}$ until the 168$^{th}$ hour of life a significant ($p \leq 0.05$) decrease in their expression was observed.

### Blood plasma lipid changes

Mean plasma concentrations of TG, TC, HDLC and LDLC are shown in Table 2. At birth, mean plasma triglyceride concentration was 0.42 mmol/l and decreased until the 3$^{rd}$ hour of life (0.32 mmol/l). From the 3$^{rd}$ hour until the 144$^{th}$ hour significant ($p \leq 0.01$) increase in the concentration of TG was observed. The concentration of TC during the first week of life ranged between 0.85 and 3.21 mmol/l. The lowest value of this variable was demonstrated at birth which was followed by a significant ($p \leq 0.01$) increase until the end of the experimental period. Immediately after birth, mean HDLC concentration was 0.37 mmol/l and was followed by a slight decrease to the value of 0.34 mmol/l. From the 3$^{rd}$ until the 24$^{th}$ hour of life an increase in HDL cholesterol was demonstrated. However, the changes observed were not

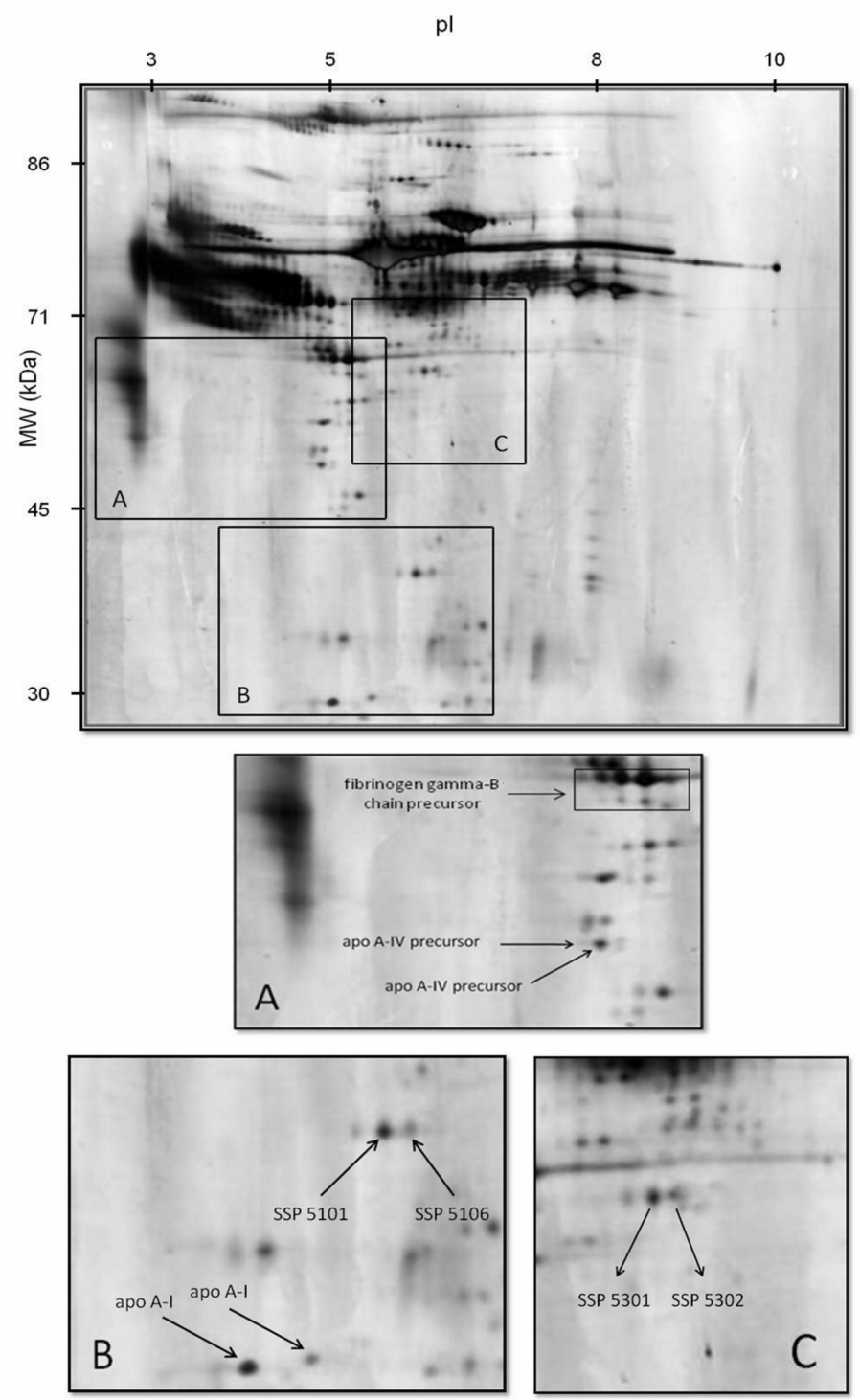


Fig. 1. Silver-stained representative 2-D protein map of the calves blood plasma. The insert: A, B and C correspond to framed gel fragments. The enlarged panels present the location of significantly altered protein spots on the 2-D gel.

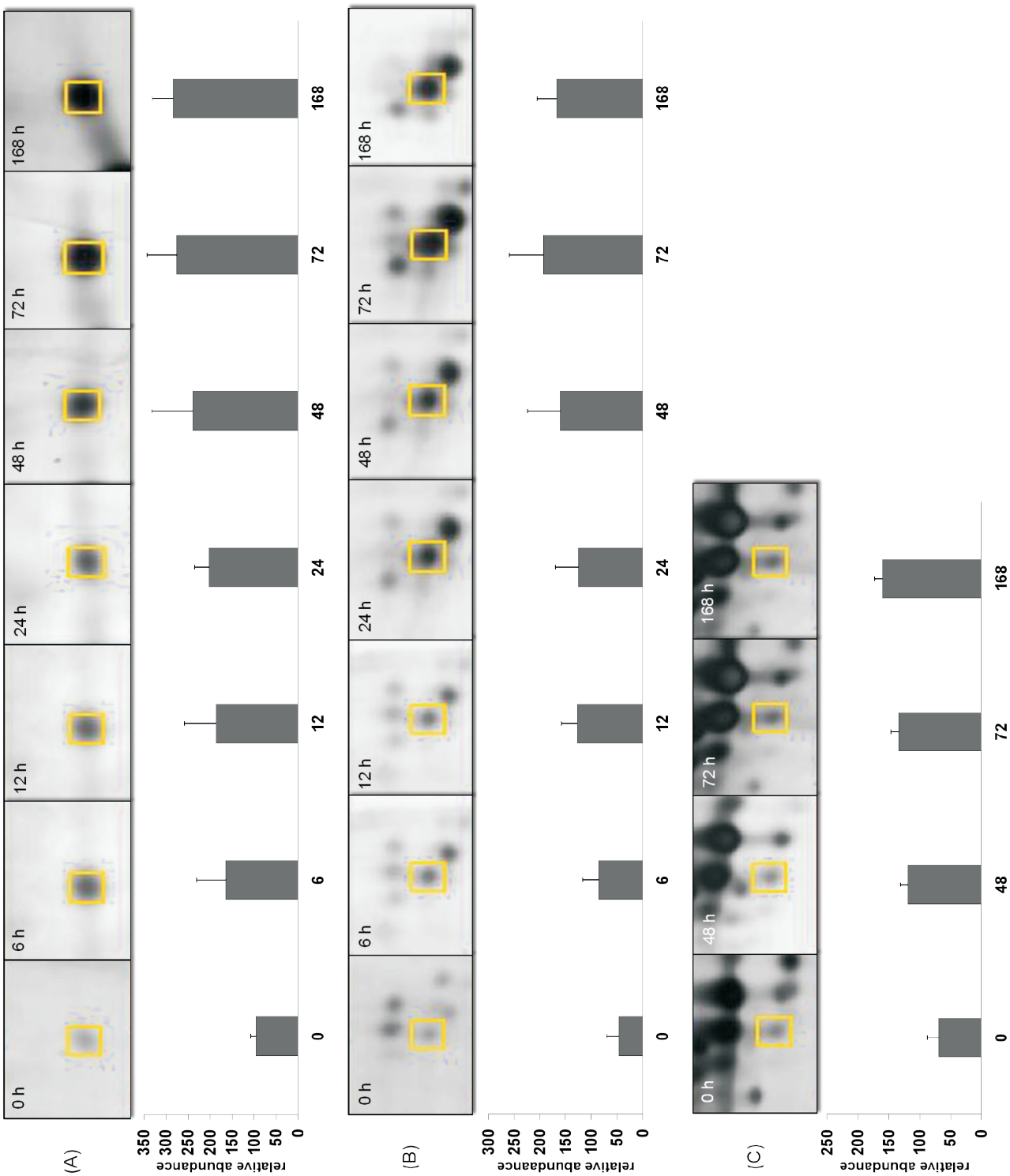


Fig. 2. Protein spots exhibiting significant expression changes in the blood plasma of neonatal calves: (A) apolipoprotein A-I, (B) apolipoprotein A-IV, (C) fibrinogen gamma-B chain. Sampling times: before colostrum administration (0 h) and at the 6$^{th}$, 12$^{th}$, 24$^{th}$, 48$^{th}$, 72$^{nd}$ and 168$^{th}$ hour of life. Graphs, placed under each image of spot expression profile, present its average relative abundance changes.

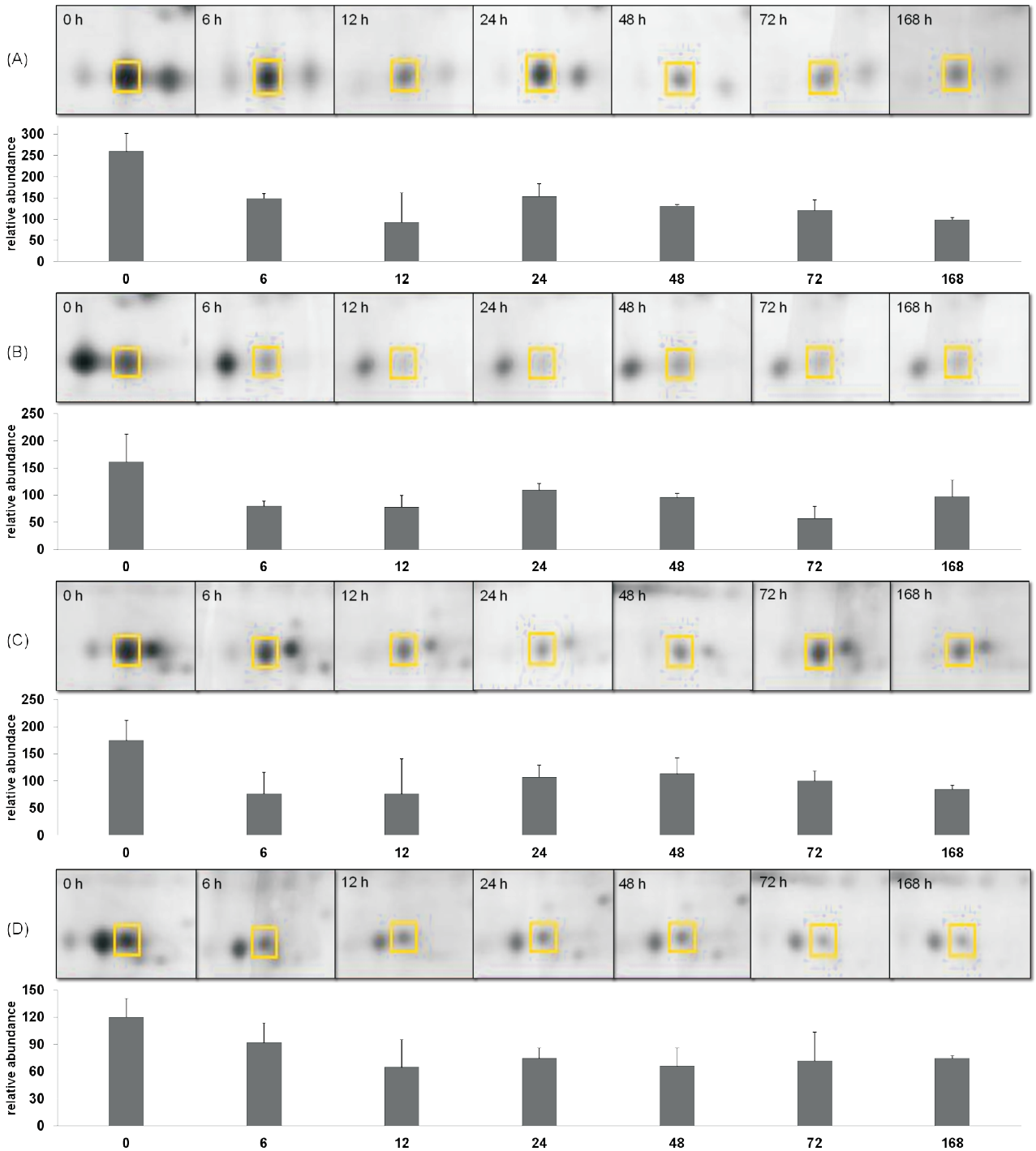


Fig. 3. Unidentified protein spots exhibiting significant expression changes in the blood plasma of neonatal calves: (A) SSP 5101, (B) SSP 5106, (C) SSP 5301, (D) SSP 5302. Sampling times: before colostrum administration (0 h) and at the 6$^{th}$, 12$^{th}$, 24$^{th}$, 48$^{th}$, 72$^{nd}$ and 168$^{th}$ hour of life. Graphs, placed under each image of spot expression profile, present its average relative abundance changes.

statistically significant. Mean plasma HDLC levels from the 36$^{th}$ hour increased significantly ($p \leq 0.01$) towards the end of the experimental period. The lowest value of LDLC was noted at birth (0.28 mmol/l), which was followed by an increase to the level of 0.41 mmol/l. These changes were not significant. The average plasma LDL cholesterol concentration was comparatively stable during the first week of life. Significant differences ($p \leq 0.01$) in LDLC were observed only between zero time sampling and the 72$^{nd}$ and 168$^{th}$ hour of life.

Table 2. The average calves plasma triglyceride, total cholesterol, HDL and LDL cholesterol values in the following hours after birth.

| | Triglycerides [mmol/l] | Total cholesterol [mmol/l] | HDL cholesterol [mmol/l | LDL cholesterol [mmol/l] | Hour of Life |
|---|---|---|---|---|---|
| $\bar{x}$ | **0.42$^{a}$** | **0.85$^{A}$** | **0.37$^{A}$** | **0.28$^{A}$** | **0** |
| SD | 0.20 | 0.15 | 0.10 | 0.14 | |
| $\bar{x}$ | **0.32$^{A}$** | **0.92$^{B}$** | **0.34$^{B}$** | **0.41** | **3** |
| SD | 0.17 | 0.19 | 0.08 | 0.16 | |
| $\bar{x}$ | **0.39$^{b}$** | **0.94$^{Ca}$** | **0.39$^{C}$** | **0.37** | **6** |
| SD | 0.27 | 0.18 | 0.10 | 0.13 | |
| $\bar{x}$ | **0.36B$^{c}$** | **0.98$^{Db}$** | **0.43$^{Da}$** | **0.39** | **12** |
| SD | 0.30 | 0.22 | 0.11 | 0.13 | |
| $\bar{x}$ | **0.42$^{d}$** | **1.14$^{E}$** | **0.57$^{Eb}$** | **0.36** | **24** |
| SD | 0.25 | 0.30 | 0.17 | 0.20 | |
| $\bar{x}$ | **0.40$^{e}$** | **1.47$^{ABFabc}$** | **0.82$^{ABCFa}$** | **0.42** | **36** |
| SD | 0.22 | 0.23 | 0.16 | 0.20 | |
| $\bar{x}$ | **0.41$^{f}$** | **1.58$^{ABCDG}$** | **0.98$^{ABCDGb}$** | **0.35** | **48** |
| SD | 0.19 | 0.20 | 0.10 | 0.18 | |
| $\bar{x}$ | **0.58** | **2.00$^{ABCDEHc}$** | **1.17$^{ABCDEH}$** | **0.62$^{A}$** | **72** |
| SD | 0.17 | 0.34 | 0.17 | 0.11 | |
| $\bar{x}$ | **0.63** | **2.40$^{ABCDEFGId}$** | **1.66$^{ABCDEFGHI}$** | **0.46** | **96** |
| SD | 0.26 | 0.33 | 0.18 | 0.25 | |
| $\bar{x}$ | **0.71$^{Ac}$** | **2.80$^{ABCDEFGH}$** | **1.99$^{ABCDEFGHc}$** | **0.47** | **120** |
| SD | 0.36 | 0.44 | 0.33 | 0.14 | |
| $\bar{x}$ | **0.75$^{ABabdef}$** | **2.91$^{ABCDEFGHd}$** | **1.92$^{ABCDEFGHJ}$** | **0.46** | **144** |
| SD | 0.35 | 0.36 | 0.57 | 0.06 | |
| $\bar{x}$ | **0.62** | **3.21$^{ABCDEFGHI}$** | **2.39$^{ABCDEFGHIJc}$** | **0.62$^{A}$** | **168** |
| SD | 0.46 | 0.43 | 0.15 | 0.19 | |

The capital letters are used to show the statistical significance level at P≤0.01 and the small letters are used to show the statistical significance level at P≤0.05.

## Discussion

The results of the present study indicate that blood plasma proteins in calves undergoes dynamic changes during the first week of life. Especially, first colostrum intake triggers variety of physiological mechanisms, which finds its reflection in changes in plasma protein composition. Among the 16 significantly altered spots 4 were corresponding to apolipoprotein A-I and apolipoprotein A-IV. Among numerous biological roles, which are described to these proteins, lipid transport and lipoprotein metabolism seem to have the most important functions. In cattle, apo A-I comprises the major protein component in HDL and it is mainly synthesized by liver parenchymal and small intestine cells (Miyamoto and Katoh 1997). This protein acts as a cofactor for lecithin-cholesterol acyltransferase (LCAT), an enzyme responsible for cholesteryl esters production, which are then transported into the core of the HDL particles. This contributes to functional maturation of high density lipoproteins (Miyamoto and Katoh 1997). In the present study a significant (p≤0.01) increase was observed in the expression of apo A-I in the plasma of calves during the first week of life. Marcos et al. (1991) reported, that concentration of apo A-I in the bovine fetal plasma was maintained on a steady level during the whole gestation period and it was considerably lower when compared with plasma apo A-I concentration in calves during the early postnatal period. The authors postulated that the observed phenomenon was primarily related to the increase in the HDL particle size.

Hormones and nutrients, particularly zinc and copper, are the main factors which influence apolipoprotein A-I expression (Hargrove et al. 1999, Herosimczyk et al. 2011). Most of these factors exert its influence directly through gene activation. *In vitro* and *in vivo* studies unambiguously indicate that insulin, thyroxine, triiodothyronine and glucocorticoids are responsible for simultaneous increase in both: apo A-I mRNA level in hepatocytes and apo A-I plasma

concentration (Masumoto et al. 1988, Nakamura et al. 1999). The concentrations of the above mentioned hormones are very high in blood plasma of calves from the first hours after birth until the seventh day of life and this probably contributes to increased hepatic synthesis of apo A-I and its release into the bloodstream.

A significant ($p \leq 0.01$) increase was also demonstrated in plasma apo A-IV expression during the first week of life. This protein is known to play a role in the regulation of the metabolism of both triglyceride-rich lipoproteins (TRL) and high density lipoproteins (HDL) (Huang et al. 2012). The expression changes of apo A-IV described in the present study were in accordance with the report of Huang et al. (2012). The author observed intense up-regulation of this protein in the blood plasma of piglets during the first 24 hours after colostrum administration. Huang et al. (2012) showed that active lipid absorption from ingested food (colostrum) was the main factor that stimulated the increase in apo A-IV synthesis in the small intestine and its further secretion into the bloodstream. Lu et al. (2003) reported that triglyceride absorption resulted in an increased induction of apo A-IV gene transcription in the enterocytes of the newborn piglets. The authors demonstrated that the activity of these mechanisms were the highest during the early postnatal period and apo A-IV gene expression was lower in 14-day old piglets when compared with the newborns.

The above mentioned up-regulation of apo A-I and apo A-IV was in accordance with the blood plasma lipid changes. The low concentration of plasma total cholesterol observed in the calves at birth followed by a rapid increase during the first week of life is consistent with the previous report of Rauprich et al. (2000). Undoubtedly the main reason for the demonstrated effect was the ingestion of rich in fat diet (colostrum and milk). Hammon and Blum (1998) reported that plasma TC values were very low or even decreased when calves were fed with either water with glucose or milk-replacer. LDL and HDL lipoproteins are characterised by the highest cholesterol content. According to Bauchart et al. (1993) LDL is the major lipoprotein in fetal calf plasma and subsequent shift to HDL as the predominant class is observed after colostrum and milk ingestion. In the present study the concentration of both plasma HDL and LDL lipoproteins increased in calves during the first seven days of postnatal life. Moreover, these results unambiguously indicated that cholesterol was progressively transferred from LDL to HDL during the early postnatal period.

A gradual increase in plasma triglyceride (TG) level during the first seven postnatal days of life was found in the present study. A similar pattern of changes in plasma TG values in calves was described by Rauprich et al. (2000). Chylomicrons and very low density lipoproteins (VLDL) are considered as the main transporters of TG in all animal species (Bauchart et al. 1993). Plasma triglyceride levels were influenced by the diet, in this case by colostrum and mature milk. Both feeds provided rich sources of fatty acids (Hammon and Blum 1998). It has been previously shown that plasma TG level increases gradually in calves fed colostrum and mature milk during the first seven days of postnatal life (Blum and Hammon 2000).

An increased expression of fibrinogen gamma-B chain was observed in plasma of calves during the first week of life. However, there are no data available regarding plasma concentration of this protein in calves during the early postnatal period.

The main reasons of failed identification of 4 protein spots were probably: inadequate protein content and limited number of bovine sequences in the available databases.

## Conclusion

In conclusion, blood plasma protein profile changes in calves during the first week of life. These changes are most likely due to transition in energy sources, from a maternal nutrient supply comprising mainly carbohydrates and amino acids to a diet rich in fat (colostrum and milk). This is reflected by the intense up-regulation of blood plasma proteins related to lipid transport and lipoprotein metabolism, namely apo A-I and apo A-IV during the first week of life in the calves. The up-regulation of above-mentioned proteins is consistent with the blood plasma lipid changes. Moreover, the present findings indicate that transport of cholesterol is progressively transferred from LDL to HDL during the early postnatal period. However, it should be emphasised that the analysis of neonatal calves plasma still represents a great challenge for 2-DE and MALDI TOF MS techniques, which finds its reflection in failed identification of significantly expressed protein spots reported in the present paper. Therefore, the present results may be considered as preliminary data and await for further refinement.

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## References

Bauchart D (**1993**) Lipid absorption and transport in ruminants. J Dairy Sci 76: 3864-3881.

Blum JW (**2006**) Nutritional physiology of neonatal calves. J Anim Physiol Anim Nutr (Berl) 90: 1-11.

Blum JW, Hammon H (**2000**) Colostrum effects on the gastrointestinal tract and on nutritional, endocrine and metabolic parameters in neonatal calves. Livest Prod Sci 66: 151-159.

Candiano G, Bruschi M, Musante L, Santucci L, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L, Righetti PG (**2004**) Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis 25: 1327-1333.

Chevallet M, Luche S, Rabilloud T (**2006**) Silver staining of proteins in polyacrylamide gels. Nat Protoc 1: 1852-1858.

Draisci R, Montesissa C, Santamaria B, D'Ambrosio C, Ferretti G, Merlanti R, Ferranti C, De Liguoro M, Cartoni C, Pistarino E, Ferrara L, Tiso M, Scaloni A, Cosulich ME (**2007**) Integrated analytical approach in veal calves administered the anabolic androgenic steroids boldenone and boldione: urine and plasma kinetic profile and changes in plasma protein expression. Proteomics 7: 3184-3193.

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

Hargrove GM, Junco A, Wong NCW (**1999**) Hormonal regulation of apolipoprotein A-I. J Mol Endocrinol 22: 103-111.

Herosimczyk A, Dejeans N, Sayd T, Ożgo M, Skrzypczak WF, Mazur A (**2006**) Plasma proteome analysis: 2D gels and chips. J Physiol Pharmacol (Suppl 7) 57: 81-93.

Herosimczyk A, Lepczyński A, Dratwa-Chałupnik A, Kurpińska A, Klonowska A, Skrzypczak WF (**2011**) Age-related changes of selected blood biochemical indicators in dairy calves during their first week of life. Folia Biol (Krakow) 59: 25-30.

Herosimczyk A, Lepczyński A, Ożgo M, Skrzypczak WF (**2012**) Effect of age and food intake on the selected blood plasma/serum proteins in calves during the early postnatal period. Med Weter 68: 265-268.

Huang Y, Olson DJ, Gordon JR, Middleton DM, Simko E (**2012**) Plasma protein profiles of neonatal pigs before and after suckling. Can J Vet Res 76: 1-7.

Jankowiak D, Kochel A, Drozd R, Kowalska J, Muszczyński Z (**2010**) The changes of concentration of the triglycerides, 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 9: 107-118.

Kuhne S., Hammon HM, Bruckmaier RM, Morel C, Zbinden Y, Blum JW (**2000**) Growth performance, metabolic and endocrine traits and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. J Anim Sci 78: 609-620.

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

Marcos E, Mazur A, Cardot P, Coxam V, Rayssiguier Y (**1991**) Developmental changes in plasma apolipoproteins B and A-I in fetal bovines. Biol Neonate 59: 22-29.

Masumoto A, Koga S, Uchida E, Ibayashi H (**1988**) Effects of insulin, dexamethasone and glucagon on the production of apolipoprotein A-I in cultured rat hepatocytes. Atherosclerosis 70: 217-223.

Miyamoto T, Katoh N (**1997**) Secretion of apolipoprotein A-I by calf liver parenchymal cells. Am J Vet Res 58: 811-815.

Nakamura T, Fox-Robichaud A, Kikkawa R, Kashiwagi A, Kojima H, Fujimiya M, Wong NC (**1999**) Transcription factors and age-related decline in apolipoprotein A-I expression. J Lipid Res 40: 1709-1718.

Rauprich AB, Hammon HM, Blum JW (**2000**) Influence of feeding different amounts of first colostrum on metabolic, endocrine and health status and on growth performance in neonatal calves. J Anim Sci 78: 896-908.

Skrzypczak WF, Ożgo M, Lepczyński A, Herosimczyk A (**2011**) Defining the blood plasma protein repertoire of seven day old dairy calves – a preliminary study. J Physiol Pharmacol 62: 313-319.

Talukder MJ, Takeuchi T, Harada E (**2002**) Transport of colostral macromolecules into the cerebrospinal fluid via plasma in newborn calves. J Dairy Sci 85: 514-524.

Uruakpa FO, Ismond MAH, Akobundu ENT (**2002**) Colostrum and its benefits: a review. Nutr Res 22: 755-767.