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Acta Veterinaria-Beograd 2015, 65 (2), 260-270
UDK: 636.2.084.1
DOI: 10.1515/acve-2015-0022

Research article

CHANGES IN THE CONCENTRATIONS OF ACUTE PHASE PROTEINS IN CALVES DURING THE FIRST MONTH OF LIFE

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(Received 25th August 2014; Accepted 25th March 2015)

The objective of this study was to describe the physiological changes in the concentrations of acute phase proteins (APPs) in calves during the first month of life, including pre-, postcolostral and milk feeding period. Seven clinically healthy calves were used in this study. Calves received colostrum and whole milk from their dams. The first blood sampling was performed before colostrum intake (day 0) and then at 1, 2, 7, 14 and 30 days of age. Blood serum was analyzed for the concentrations of haptoglobin (Hp), serum amyloid A (SAA), α1-acid glycoprotein (AGP), ceruloplasmin (Cp), and albumin (Alb). The results showed significant changes in the serum concentrations of Hp, SAA and Cp ($P<0.001$, $P<0.01$, $P<0.01$). Their lowest concentrations were found after birth, and a gradual increase was observed after colostrum intake until day 7 of life. Another trend was observed in the concentrations of albumin with a more marked decrease of values 1 day after colostrum intake and subsequent significant increase of values until the end of the first month of age ($P<0.001$). Sampling time had no significant effect on the concentrations of AGP. The values observed at birth and on day 1 of life were relatively stable. The concentrations of AGP increased slightly from day 2 until the end of the first month of age. These results suggest that the concentrations of APPs in the neonatal period are influenced by colostrum intake and age. This should be taken into consideration for the precise interpretation of these analytes in young animals.

Key words: acute phase proteins, age, calves, colostrum, neonatal period.

INTRODUCTION

Adaptation of neonatal animals to the extra-uterine life is a complicated and critical process in the life of domestic animals, in which intense structural and functional changes take place [1]. The placenta of bovine species prevents the effective transfer of maternal plasma proteins to the conceptus, thus the immune system of newborn calves is naive to the wide variety of pathogens present in the environment and depend on their dam to provide passive imunity through the colostrum [2]. Colostrum is the first source of nutrition in neonatal ruminants, supplying not only nutritional substances essential for proper growth and development of the organism, but having

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also a fundamental biological function, promoting immunoglobulin transfer from the dam to the newborn prior to the cessation of macromolecular transport [3]. It also abounds in a wide variety of bioactive components such as major milk proteins, hormones, growth factors, cytokines, lactoferrin, lactoperoxidase or lysozymes, responsible for initiating, controlling and supporting many biological processes [4,5]. This complex of proteins plays an essential role in passive immune transfer and consequently actively participates in the protection of the neonate against pathogens and other environmental challenges [6,7].

The immune system of the neonate is functional, but immature [8]. Functional immaturity of neonatal lymphocytes was reported during the first weeks of life [9]. Thus, colostral transfer of immune components to obtain passive immunity and an effective non-specific immune response may be important for the adaptation to the extra-uterine life [10]. The acute phase response is part of the innate immune response comprising a series of biochemical defence mechanisms in response to disturbances in the homeostasis caused by infection, tissue injury, trauma or surgery, neoplastic growth or immunological disorders [11]. One of the most important metabolic changes during the acute phase response is the strongly increased synthesis of a group of plasma proteins, namely acute phase proteins [12]. The profile of acute phase proteins differentiates between animal species. In cattle, serum proteins that have been recognized as important acute phase proteins include haptoglobin, serum amyloid A, fibrinogen, ceruloplasmin and α_1-acid glycoprotein [13]. Acute phase proteins are involved in many reactions during the acute phase response, and are thought to be beneficial to the organism by preventing microbial growth and further tissue damage, and by helping to restore the homeostasis [14]. Additionally, acute phase protein enhance the non-specific immune response through increased activation of leukocytes and the complement system [15]. In neonates, they represent the first line of defence against potential pathogens [16]. Thus, characterization of changes in the concentrations of acute phase proteins after birth could elucidate the role of the inflammatory response in the adaptational mechanisms of the newborn [17]. Structural, functional and metabolic alterations occuring in calves during the early postnatal period are reflected by changes in haematological, mineral, enzymatic, energy, and other profiles, including the blood serum protein profile [18]. Acute phase proteins may show also relevant changes in concentrations in the blood serum of calves during postnatal development. The objective of this study was to evaluate the changes in the concentrations of selected acute phase proteins in clinically healthy calves during the first month of life, and to study these changes with regard to the process of neonatal adaptation to postnatal life including precolostral, colostral and milk feeding period.

MATERIAL AND METHODS

Animals and blood sample collection

Seven clinically healthy calves (4 males and 3 females) of a low-land black spotted breed and its crossbreeds were included into this study. The calves were born at the Clinic for

Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice with an average birth weight of 36.1 ± 2.3 kg and an average body weight of 64.8 ± 5.5 kg at the end of the 1st month. Calves were born as singles from 3 cows and 4 heifers with pregnancies of normal lenght and uncomplicated births. After birth they were not separated from their dams and were kept individually in pens. Animals were stabled on straw litter. Calves were allowed to suck their dams and the first colostrum they received within 2 hours after birth. Subsequently until the end of the study the calves received colostrum and whole milk according to their appetite without restriction of the sucking frequency and volume during the day. The calves had free access to water and hay during the time under study. The health status of the calves was evaluated daily. Clinical examination included behaviour, appetite, rectal temperature, heart and respiratory rates, pulmonary sounds, nasal discharge, eye discharge, navel infection and fecal consistency. All the animals were clinically healthy and in good general health condition without any obvious clinical signs of diseases during the time of the study.

The first blood sampling was performed before colostrum intake (precolostral sample, d 0) within 30 minutes after birth and then at 1, 2, 7, 14 and 30 days of age. Blood was collected by direct puncture of *v. jugularis* into serum gel separator tubes without anticoagulant. Blood samples were allowed to clot at room temperature, and then centrifuged at 3000 g for 30 minutes to separate the serum. The harvested blood serum was dispensed into plastic tubes, and stored at -20 °C until analysed.

Laboratory analyses

Blood serum was analysed for the concentrations of haptoglobin (Hp, mg/ml), serum amyloid A (SAA, μg/ml), α_1-acid glycoprotein (AGP, μg/ml), ceruloplasmin (Cp, μg/ml), and albumin (Alb, g/l). Haptoglobin was assessed using commercial colorimetric kits (Tridelta Development, Ireland) in microplates, based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. Serum amyloid A was analysed by sandwich enzyme linked immunosorbent assay (ELISA) using commercial kits (Tridelta Development, Ireland). The concentrations of AGP and Cp were analysed by ELISA method using commercially available diagnostic kits (Cusabio, China). The reading of absorbancies and the consecutive calculation of final concentrations of these variables were performed on automatic microplate reader Epoch (BioTek, USA). The values of albumin were determined using commercial diagnostic kits (Randox Laboratories, United Kingdom) on automatic biochemical analyser ALIZE (Lisabio, France).

Statistical analysis

Arithmetic means (x) and standard deviations (SD) for each evaluated acute phase protein and sample collection were calculated using descriptive statistical procedures. Kolmogorov-Smirnov Test for normality was used to determine whether sample data were normally distributed. The significance of the effect of age on the concentrations of the analyzed variables during the whole monitored period was examined by non-parametric Friedman's rank sum test (P-value). The significances of the differences in the results between the sample collection points were evaluated by Dunn's Multiple Comparisons Test. All statistical analyses were carried out using the programme GraphPad Prism V5.02 (GraphPad Software Inc., California, USA).

RESULTS

The data referring to the concentrations of evaluated acute phase proteins are presented in Table 1 and are shown in Figures 1a-e.

Table 1. Changes of the concentrations of evaluated acute phase proteins in calves during the first month after birth

Variables		Age of calves (days)						P value
		0	1	2	7	14	30	
Hp (mg/ml)	x	$0.11^{a,x}$	0.14^{b}	0.22^{a}	$0.34^{x,b}$	0.23	0.27	< 0.001
	±SD	0.03	0.03	0.04	0.15	0.12	0.19	
SAA (μg/ml)	x	$20.5^{A,a}$	132.2	138.5	155.6^{A}	147.5^{a}	96.2	< 0.01
	±SD	14.5	40.4	50.2	34.4	37.4	57.4	
AGP (μg/ml)	x	312.2	320.6	236.6	285.5	385.8	422.6	n. s.
	±SD	257.4	193.4	130.0	143.1	198.6	104.8	
Cp (μg/ml)	x	26.0^{a}	37.4	38.2	73.7	71.0^{a}	55.2	< 0.01
	±SD	15.3	19.1	15.5	47.1	39.9	17.1	
Alb (g/l)	x	30.5	$24.0^{A,x}$	25.9^{B}	29.5	33.3^{A}	$35.5^{x,B}$	< 0.001
	±SD	2.9	1.5	1.8	2.1	3.0	1.2	

Means with the same superscripts in rows differ significantly: a,b – P < 0.05; A,B – P < 0.01; x – P < 0.001

During the first month of life of the calves significant changes were found in the concentrations of Hp (Table 1, P<0.001). The lowest concentrations were observed at birth, which was followed by a gradual significant increase up to day 7 of life (P<0.001, Fig. 1a). Until the end of the evaluated period the mean values were higher than on the first days after birth. Significant changes during the evaluated time were found also in the concentrations of SAA (P<0.01). The lowest mean value was found at birth. From the day 1 after birth, more marked increase of values was recorded with a further gradual increase of values being the significantly highest at the age of 7 days (P<0.01). From day 14 of life, a decrease of concentrations was observed, with a marked decrease on the 30th day of life (Fig. 1b).

In the concentrations of AGP in relation to the age of the calves no significant changes were found. The values observed shortly after birth (d 0) and on day 1 of life were roughly uniform. A slight decrease of AGP concentrations was recorded on day 2 with a subsequent gradual increase until the end of the 1st month (Fig. 1c). The AGP concentrations 30 days after birth were higher than the values found before the intake of colostrum. Sampling time had a significant effect on the concentrations of ceruloplasmin (P<0.01). The lowest mean value was found at birth. From the day 1 after birth, a more marked gradual increase of its values was recorded up to day 7 of life (Fig. 1d). A subsequent slight decrease of mean concentrations was observed from day 14 until the end of the evaluated period. The age of calves had a significant effect also on the concentrations of albumin (P<0.001). Its concentrations decreased more markedly 1 day after colostrum intake (Fig. 1e). From the 2nd day till the end of the first month of life a gradual increase of the values was observed, with the highest mean concentration at the end of the evaluated period.

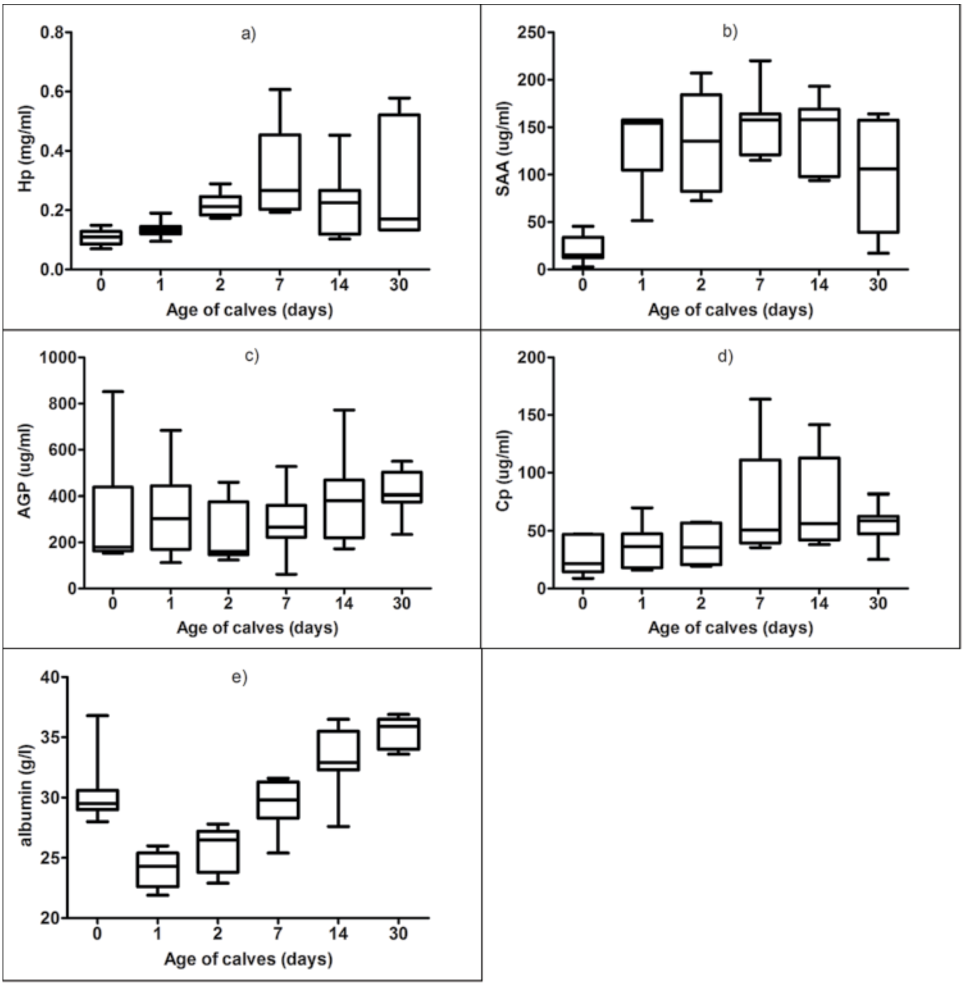

Figure 1a-e. Distribution of the concentrations of Hp (a), SAA (b), AGP (c), Cp (d) and albumin (e) in calves during the first month after birth. The plots show the median (line within box), 25th and 75th percentiles (box), minimal and maximal values (whiskers)

DISCUSSION

The results of this study showed a significant effect of colostrum intake and age on the concentrations of the most of evaluated acute phase proteins. Highly significant changes were observed in the concentrations of SAA, with the lowest values found at birth. The most marked changes occured between the time before colostrum intake and 24 hours after the colostrum feeding, when its low concentrations in calves increased rapidly after colostrum intake. The mean serum concentration of SAA increased by more than 6-fold from 20.5 μg/ml on day 0 to 132.2 μg/ml on day 1, with a further slight increase until the age of 7 days. Elevation of SAA concentrations immediately after birth has been described in pigs and horses [21,22]. In newborn calves, very low SAA concentrations in samples collected 10 minutes after parturition were reported [23]. Similarly to our results, an earlier study demonstrated that the concentration of SAA in the blood plasma of calves was relatively low within few hours after birth (0.06 mg/ml) and significantly increased on the third day of life (0.09 mg/ml) [20]. Colostrum contains high quantities of inflammatory mediators, including cytokines, which are the main inducers of the production of acute phase proteins by the liver [24]. Therefore, one of the possible factors responsible for higher concentrations of SAA in newborn calves is the absorption of colostral inflammatory inducers such as cytokines. In newborn calves, interleukin-6 in the blood plasma is not present immediately after birth [25]. After colostrum intake, the concentrations of this cytokine in calves increase, with the highest values occuring within 24 hours of life. Thus, pro-inflammatory cytokines absorbed from the colostrum may directly stimulate the production of acute phase proteins or activate the circulating lymphocytes and neutrophils [26]. Another possible factor contributing to higher SAA concentrations in newborn calves is direct transport of acute phase proteins from colostrum to the calf, as colostrum of healthy cows contains high concentrations of mammary-associated amyloid A [27]. Human mammary-associated SAA was shown to have a primarily protective effect on the gastrointestinal tract of neonates by stimulating mucin production and reducing the adherence of pathogens [28]. A possible role for mammary SAA in supporting the welfare of calves was suggested, but the direct transfer of SAA from colostrum to the circulation of newborns has not been completely investigated [27].

Significant age related variations were seen for the concentrations of haptoglobin with a gradual increase of the values up to day 7 of life. Similarly, very low Hp values at birth and an increase during the first week of life were reported by other authors [20,23]. High concentrations of Hp during the first two weeks of life were also demonstrated in a group of 14 calves [29]. On the other hand, an earlier study described that the concentrations of Hp in newborn calves did not differ between samples obtained at birth, at 1 day of age and at 10 days of age [30]. Low Hp concentrations after birth may be related to the immaturity of the neonatal liver to produce Hp in a situation where Hp consumption is increased because of haemolysis of fetal erythrocytes [31].

The transfer of maternal Hp to newborn piglets via colostrum was demonstrated [32]. The increasing levels of Hp in suckling piglets may have protective functions and play a role in the intrinsic defence mechanisms of newborn piglets if Hp plays a role in altering the T helper cells 1-2 balance to achieve immune homeostasis [33, 41]. However, the direct transfer of Hp from colostrum to newborn calves was not demonstrated, which may be related to the different hepatic regulation of Hp production between calves and piglets [34]. Similarly to haptoglobin, the lowest values of ceruloplasmin were found at birth, which was followed by a gradual increase up to the 7th day of life. Low concentrations of Cp were reported at birth, which increased in the first days of life, influenced by response to birth or related to the activity of the liver [35]. Hepatic production of acute phase proteins is one of the mechanisms involved in the compensation of a functionally immature immunological system during the early neonatal period, which facilitate adaptation to extrauterine life [9]. Higher concentrations of acute phase proteins in calves can be explained also by the influence of other stressors, infections or various inflammatory diseases (e.g. respiratory, gastrointestinal, navel diseases) [19]. Another study suggested that the changes in acute phase protein concentrations in newborn animals may reflect the adaptation mechanisms necessary for extrauterine life [36].

The results observed in this study showed in calves roughly uniform AGP concentrations shortly after birth with an increase of values from day 2 of life until the end of the first month of age. Rocha et al. [35] demonstrated also that the concentrations of AGP were relatively stable in the first days after birth, increasing gradually until 30 days of life. On the other hand, other researchers recorded in calves the highest plasma AGP levels immediately after birth (1368 μg/ml), which gradually decreased to 249 ± 100 μg/ml during the first three days of life, similar to the normal adult bovine range [37]. Similarly, Orro et al. [20] obtained the highest plasma concentration of AGP at birth, followed by a decrease during the first 3 weeks of life to adult levels. A rise of AGP values already in the fetal stages may be related to the synthesis of AGP in the embryonic liver [37]. A different AGP isoform was described in neonatal calves compared with the isoform present in adults [20]. These studies indicate that the production of AGP in the neonatal period is fetally regulated and its high serum concentrations after birth are not necessarily a sign of the activation of the acute phase response by external stimuli. The increase of the concentrations of AGP from the 2nd day of life until the end of the first month, observed in calves in our study, may be associated with the normal process of growth, and may be related to the exposure of animals to changing environmental conditions and nutritional factors.

The normal process of growth in calves was accompanied by significant changes also in the values of albumin, which is the major negative acute phase protein. Various studies showed in calves a progressive increase of albumin concentrations from birth until the age of 80 days [29,38]. The increase of albumin together with the age is a physiological phenomenon and higher concentrations of albumin in young and growing animals help to maintain the metabolic balance [39]. Our results showed in calves initially a decrease of the concentrations of albumin on the first day after birth probably due to the effect of dilution after colostrums intake. This was followed by gradual increase of the values until the end of the first month of life. These changes in albumin concentrations may reflect the albumin's medium half-life that ranges from 14 to 16 days in ruminants, after which period the liver is responsible for albumin synthesis [40].

In conclusion, our results showed significant changes in the most of evaluated proteins. They reflect the response of the calves to changes in nutrition and adaptation of calves to extrauterine life. Seeing that acute phase proteins play an important role in the innate immune response of the host, the changes observed in their concentrations after birth indicate the importance of acute phase proteins in the defence mechanisms of newborn calves. Thus, the changes in their concentrations described in newborn animals are not necessarily caused by some disease-related factors, but may reflect the physiological adaptation of newborns to the extrauterine life. These changes in young animals should be taken into consideration when interpreting the concentrations of some acute phase proteins. Thus, presented results contribute to the knowledge about the pattern of acute phase proteins in newborn and young calves. The results confirm the need for age specific reference values also for these variables when considering precise interpretation of laboratory results. The obtained data would be useful for clinicians in the evaluation of health state and various pathological conditions, including neonatal diseases in calves.

Acknowledgements

this work was supported by the Slovak Research and Development Agency under contract No. APVV-0475-10 and by Scientific Grant Agency of Ministry of Education SR No. 1/0447/14 and 1/0154/15.

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PROMENE KONCENTRACIJE PROTEINA AKUTNE FAZE TOKOM PRVOG MESECA ŽIVOTA TELADI

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Cilj ovog istraživanja je bio da se opišu fiziološke promene u koncentraciji proteina akutne faze (APP) kod teladi u toku prvog meseca života, uključujući i prekolostralni, postkolostralni i mlečni period ishrane. Ogled je izvršen na sedam klinički zdravih teladi. Telad su dobijala kolostrum i puno mleko od svojih majki. Prvo uzorkovanje krvi izvršeno je pre uzimanja kolostruma (dan 0) i 1., 2., 7., 14. i 30. dana starosti. Krvni serum je ispitivan na sadržaj haptoglobina (Hp), serum amiloida A (SAA), α1-kiselog glikoproteina (AGP), ceruloplazmina (Cp) i albumina (Alb). Rezultati su pokazali signifikantne promene koncentracije Hp, SAA i Cp ($P<0,001$; $P<0,01$; $P<0,01$) u krvnom serumu. Najniže koncentracije su zabeležene nakon rođenja i postepeno su rasle nakon unosa kolostruma do 7 dana starosti. Drugi trend je primećen u koncentracijama albumina sa izraženijim padom vrednosti 1 dan posle uzimanja kolostruma i kasnijim značajnim povećanjem do kraja prvog meseca starosti ($p<0,001$). Vreme uzorkovanja nije imalo značajan efekat na koncentraciju AGP . Vrednosti uočene na rođenju i prvog dana života bile su relativno stabilne. Koncentracije AGP su se neznatno povećavale počev od 2. dana pa do kraja prvog meseca starosti. Ovi rezultati ukazuju na to da uzimanje kolostruma i starost imaju uticaj na koncentraciju APP kod teladi u neonatalnom periodu. Navedeno treba uzeti u obzir za precizno tumačenje ovih analita kod mladih životinja.