Acta Veterinaria (Beograd), Vol. 57, No. 2-3, 191-198, 2007.

DOI: 10.2298/AVB0703191R UDK 619:612.12

CONCENTRATION OF CORTISOL, INSULIN, GLUCOSE AND LIPIDS IN THE BLOOD OF CALVES AT VARIOUS AGES

RADOJIČIĆ BILJANA, ŠAMANC H and PEJIN IVANA

Faculty of veterinary medicine, Beograd

(Received, 15 September, 2006)

Clinically healthy calves (n=30) of the Holstein-Friesian breed, at different ontogenic periods (15 days, 2 and 4 months of age), were investigated in this work.

Blood samples were obtained by jugular venepuncture, always in the morning, 1 hour after feeding. The concentration of glucose was determined in the blood. Cortisol, insulin, lipids and cholesterol concentrations were determined in the blood sera.

After statistical analysis of the obtained results, we found that the mean values for serum cortisol concentrations in calves at 15 days and 2 months of age were very similar. At 4 months of age, cortisol level was slightly lower, but the difference was not statistically significant. The mean values of insulin concentration was higher in calves at 2 months of age when forage and concentrated feed were offered besides milk, but in 4 months old calves the values were significantly lower ($p<0.05$).

The mean blood glucose concentrations were similar and higher than the physiological values in calves at 15 days and 2 months of age, but values at 4 months of age were significantly lower ($p<0.01$).

Rapid absorption of glucose in the calves' digestive system at 15 days and 2 months of age, contributed to the high level of glycaemia 1 hour after milk intake.

Key words: cortisol, insulin, glucose, lipids, blood, calves

INTRODUCTION

Glucocorticoids play an important role in many metabolic pathways concerned with development and control, and their serum concentrations reflect the overall balance between synthesis, secretion and elimination. Differences in basal circulating concentrations have been reported to vary from 1.0 to 50 nmol/L, dependent of breed, age, nutrition, and other various stresses (Gončarova, 1985; Hristov *et al*., 1994; Nikolić *et al*., 1998). Secretion of cortisol in the organism is increased during foetal development, neonatal growth, the periparturient period, especially during delivery, and in the adaptation of organisms to various stresses. Glucocorticoids have an important influence on sexual features and in many

enzymatic procesess, such as intermediary metabolism of carbohydrates, protein and lipids.

Many investigators have observed the functional activity of suprarenal glands cells (adrenocortical cells) at various ages (in different ontogenic periods) as well as during adaptation of the organism to different stresses, especially in young animals (calves and lambs). The results gave rather different basal physiological values (1.0-25.4 nmol/L), similar results to other authors (Gončarova, 1985; Richet, 1986; Silke, 1989; Radojičić, 1995; Nikolić *et al*., 1998; Šamanc H., 1999; Radojičič and Šamanc, 2000; Stojić *et al*., 2002). However, in the available published literature there is little information about the relationship of basal physiological cortisol concentrations to other homeostatic hormones and the metabolites they control, together with other parameters responsible for neuroendocrine regulation of homeostasis.

One of these hormones is insulin, a polipeptide produced in the B cells of pancreatic islets. Insulin is very important in the overall control of anabolic processes involving lipids and proteins, although some results indicate that insulin is not a key hormone for the regulation of the physiological level of glycaemia in ruminants.

It is well known that carbohydrates in the feed for ruminants are degraded to lower fatty acids, mainly acetic, butyric and propinic acid in the rumen. Acetic and butiryc acid provide around 70% of the energy required for synthetic reactions in the organism, while propionate is a key precursor in gluconeogenesis. In ruminants glucocorticoids have a much greater influence on this process than insulin (Gröhn, 1985; Radojičić, 1995). There are few studies in the published literature about the role of lower fatty acids in the functional activity of endocrine glands, such as the adrenal cortex and endocrine pancreas, in young ruminants (Hristov *et al*., 1994). Cleary they have an important influence but there is not enough information about their effect on balance and regulation in intermediary metabolism, which is especially important in ruminants.

In the early neonatal period calves are exclusively fed on milk, so regulation of glycaemia and gluconeogenesis is controlled in a similar way to that in monogastric animals. Earlier investigations of several hormones with homeostatic and homeorhetic roles showed changes in their concentrations with time levels the responsibility of many hormones with a homeostatic and homeorhetic role, changing their concentrations of daily exposition (circadian and diurnal rhyhtms), as well as alterations after exogenous adminstration of substances like ACTH or propionate (Radojičić, 1995; Radojičić and Šamanc, 2000; Radojičić *et al*., 2003).

In this study we investigated the relation between two homeostatic hormones responsible for the regulation of many metabolic processes and some metabolites: glucose, proteins and lipids in calves of different ages. They included various ontogenic periods, from entirely milk feeding, through the introduction of forage and feed concentrates, to the completion of development of microfloral function in the rumen (Radojičić, 1995; Šamanc *et al*., 1999).

MATERIAL AND METHODS

Animals. The investigations involved clinically healthy female calves (n=30), Holstein-Friesian breed, in various ontogenic periods (15 days of age; 2 and 4 months of age).

Procedures. Blood samples were obtained in the morning 1 hour after feeding, by puncture of v. jugularis with a sterilised needle and collected in heparinised tubes, as well as non-heaprinised tubes for serum separation. After decantation serum samples were stored at -18 ºC.

Analyses. Blood glucose concentration was determined using Dextrostix tracks, which were read on an Eyeton Refraktans colorimeter. Cortisol was determined in blood serum by liquid phase radioimmunoassay (RIA) adapted to cover the range of concentrations from 6.9 to 690 nmol/L (Nikolić *et al*., 1998). Insulin was measured using a hetrologous RIA which included standard solution of bovine insulin. Lipid and cholesterol concentrations were determined using commercial Radonja-Sisak test and spectrphotometric measurement (Unicam-1000).

Statistical procedures. The obtained results are expressed mean values, with standard deviation, standard error, coefficient of variation and interval of variation for each investigated group of calves (x, CD, SE, Cv % and Iv). After analysis of variance the Tucky-Snedecor test, was used to evaluate the statistical significance of differences between mean values, for the different ontogenic periods.

RESULTS

The obtained results showed that mean concentrations of serum cortisol, were very similar in calves at 15 days and 2 months of age. At 4 months of age the mean value was somewhat lower, but the difference between investigated groups, was not statistically significant, owing to large individual variations (Cv=34.2 % to 61.0 %; Table 1).

Table 1. Mean values of serum cortisol concentrations (nmol/L) in calevs at various ages

Statistical parameters	15 days	2 months	4 months
n	30	28	26
x	8.12	8.10	5.70
SD	4.96	3.23	1.95
SE	0.90	0.61	0.38
Cv %	61.0	39.8	34.2
Min.	1.8	4.5	3.3
Max.	25.1	19.9	10.7

The mean serum insulin concentrations was highest in calves at 2 months of age. Values at 4 months were significantly lower ($p<0.05$) despite large individaul variations (Table 2).

Table 2. Mean values for serum insulin concentrations (mIU/L) in calves at various ages

Statistical parameters	15 days	2 months	4 months
n	30	28	26
x	47.7	69.9	28.4
SD	43.9	55.2	13.6
SE	8.0	10.3	2.7
Cv %	92.0	78.9	48.1
Min.	10.7	12.1	14.7
Max.	195.6	234.5	87.1

$p<0.05$

The mean levels of glycaemia in the investigated calves was highest at 15 days of age and then decreased. The value at 4 months of age was significantly lower than for the other groups ($p<0.01$; Table 3).

Table 3. Mean values for blood glucose concentrations (mmol/L) in calves at various ages

Statistical parameters	15 days	2 months	4 months
n	30	28	26
x	8.70	7.32	4.30
SD	2.29	1.65	0.90
SE	0.41	0.31	0.17
Cv %	26.3	22.5	20.9
Min.	4.40	4.44	2.66
Max.	15.50	11.63	7.21

$p<0.01$

The mean values for lipids and cholesterol concentrations in the blood serum of the calves, were similar and stable at each investigated period, as well as being within the physiological range for these metabolites. Thus, no significant changes were found (Table 4 and 5).

Table 4. Mean values for blood serum lipids concentrations (g/L) in calves at variuos ages

Statistical parameters	15 days	2 months	4 months
n	30	28	26
x	3.28	3.60	2.99
SD	0.97	0.79	0.77
SE	0.17	0.14	0.15
Cv %	29.5	21.9	25.7
Min.	1.31	1.85	1.48
Max.	5.54	5.44	3.88

Table 5. Mean values for blood serum cholesterol concentrations (mmol/L) in calves at various ages

Statistical parameters	15 days	2 months	4 months
n	30	28	26
x	2.71	3.02	2.54
SD	0.74	0.85	0.68
SE	0.13	0.16	0,13
Cv %	27.3	28.1	26.7
Min.	1.19	0.90	1.50
Max.	4.32	4.35	4.41

DISCUSSION

Similarly to other mammals, the ruminant suprarenal glands, synthetise and secrete glucocorticoids, mineralocorticoids and several androgenic steroids. In calves the relationship between peripheral cortisol and corticosterone concentrations (7:1) is very stable and it is not necessary to separate them before determination of their serum concentrations (Gončarova, 1985; Radojičić, 1995; Šamanc *et al*., 1999; Radojičić and Šamanc, 2000; Stojić *et al*., 2002).

Glucocorticoids have very different functions in the organism during development, growth and cell differentiation. They play a role in the induction of delivery, foetal development and in the adaptation of the organism to various stress factors. Thus, an increased concentration of cortisol in the foetal plasma is an initial sign for delivery (Thun, 1987), while increased cortisol levels after delivery indicate stress (Hunter *et al*., 1977).

However, it is not clear why changes in the concentration of insulin are very different in the early period of calf development and how these two hormones

interact, depending on the type of nutrition, in the regulation of glucose metabolism, as indicated by the level of glycaemia. Mitin and Mijić (1981) suggested that insuline concentrations in very young calves were lower than in older calves, which is confirmed by our results for postprandial values at 15 days and 2 months of age. Later on the insulin concentrations decreased to approach the values found for cows (about x =14.6 mUI/L).

The mean values of glycaemia, confirm those found by other authors. The high values for glucose concentration in the calves at 15 days and 2 months of age, reflect the rapid absorption of available nutrients. Thus, 1 hour after feeding milk, physiological hyperglycaemia was evident. The range of 3.2-6.2 mmol/L is similar in the early neonatal period for both calves and monogastric animals. In our study the mean glucose concentration was highest at 15 days of age, and significantly different from the value at 4 months of age, ($p<0.01$), when glycaemia is mainly regulated by glycocorticoids and before development of microfloral function in the rumen is finished (Stamatović *et al*., 1984; Johnson, 1986; Radojičić, 1995; Radojičić *et al*., 2003).

The mean values for cholesterol concentrations in the blood serum of each group of calves in our examination, were stable and within the physiological range (2.2-5.5 g/L : 1.3-6.0 mmol/L) similar to cows (Damnjanović, 1990). Our results indicate that blood serum lipid and cholesterol concentrations in calves, are not dependent on age or type of nutrition, as there were no significant differences between the groups.

ACKNOWLEDGEMENT
The authors like to thank Dr J. Anna Nikolić, for overseering to hormone analyses and editing the language of the manuscript.

Address for correspondence
Biljana Radojičić, DVM, PhD
Bulevar Oslobođenja 18
11000 Belgrade
Serbia
e-mail: birafvm@Eunet. yu

REFERENCES

1. *Damnjanović Z,* 1990, Koncentracija lipida i lipoproteina u krvnom serumu zdravih i ketoznih krava*, Magistarski rad*, FVM Beograd.
2. *Dvorak M,* 1972, Adrenocortical function in foetal, neonatal and young pigs, *J Endocrinol*, 54, 473-81.
3. *Gončarova J,* 1985, Nespecifični faktori na imuniteta pri teleta v zavisimost od s držanieto na kortikosteroidni hormoni, *Veterinarnomed nauki*, 8, 33-46.
4. *Gröhn J,* 1985, Liver function and morphology associated with fatty liver and ketosis in dairy cows, Disertation, College of veterinary medicine, Helsinki, Finland.
5. *Hunter JT, Fairclough RJ, Peterson AJ, Welch RSA,* 1977, Foetal and maternal hormonal changes proceeeding normal bovine parturition, *Acta endocrinolog,* 84, 653-66.
6. *Hristov S, Đurđević Đ, Grubić G, Bogdanović V, Vidić R, Bovan LJ,* 1994, Koncentracija kortizola u krvnom serumu goveda, *Vet. glasnik,* 48, 853-9.
7. *Johnson D, Mitchell G, Tucker R, Muntifering R*, 1986, Pancreatic amylase plasma glucose and insulin response to propionate and monensin in sheep, *J Dairy Sci.,* 69, 52-7.

8. *Nikolić JA, Šamanc H, Begović J, Krsmanović J, Aleksić S, Mišćević B et al.,* 1998, Basal serum cortisol concentrations in cattle, *Acta veterinaria* 48, 5-6, 265-76.
9. *Nikolić JA, Šamanc H, Damnjanović Z, Ratkovič M, Radojičić B, Begović J*, 1996, Concentrations of serum cortisol, insulin and glucagon in young calves before and after receiving propionate orally, *Proc Nutr Soc*, 55, 98.
10. *Mitin V, Mijić B,* 1981, Razine insulina u krvnom serumu goveda, *Veterinarski arhiv*, 51, 5, 189-96.
11. *Richet E, Safwete A, Davico Marie-Jeanne, Dalle M, Barlet JP*, 1987, Fonction corticosurrenalienne shez le jeune veau, Physiologie et pathologie perinatales chez les animo de farmes, 247-56.
12. *Radojičić B,* 1995, Ispitivanje uticaja propionata na nivo kortizola, insulina, glukoze i lipida u krvi goveda, *Doktorska disertacija*, Beograd.
13. *Radojičić B, Šamanc H,* 2000, Koncentracija kortizola u krvi teladi različitog uzrasta nakon aplikacije ACTH, *Veterinarski glasnik*, 54, 3-4, 117-23.
14. *Radojičić B, Šamanc H, Ivanov I,* 2003, Uticaj peroralnog davanja propionata na koncentraciju kortizola, insulina, glukoze i lipida u krvi teladi različitog uzrasta. 3. Simpozijum "Ishrana zdravstvena zaštita i reprodukcija u govedarstvu", Vrbas, *Vet glasnik, Suppl,* 57, 5-6, 375.
15. *Stamatović S, Šamanc H, Mandić L,*1984, Prilog izučavanju glikemije novorođene teladi, *Vet. glasnik,* 38, 12, 1039-43.
16. *Silke S,* 1989, Die nebeunierenrindenfunktionsprufung beim neugeborenen Kalb mit dem ACTH-Stimulierungstest, *Inaugural Disseratation*, Hannover.
17. *Stojić V , Nikolić JA, Huszenicza GY, Šamanc H, Gvozdić D, Kirovski D,* 2002, Plasma levels of triidothyronine, thyroxine and cortisol in newborn calves, *Acta veterinaria,* 52, 2-3, 85-96.
18. *Šamanc H, Nikolić JA, Bugarski D, Kulcsar M, Ivanov I, Huszenicza GY,* 1999, Glycaemia, glucocorticoids and adrenocortical reserve in postpartal dairy cows, *Acta veterinaria*, 49, 5-6, 281-8.
19. *Thun C,* 1987, Untercshungen Uber die Tagesrhythmik von cortisol beim Rind*, Verlag Fischer,* Stuttgart.

KONCENTRACIJA KORTIZOLA, INSULINA, GLUKOZE I LIPIDA U KRVI TELADI RAZLIČITOG UZRASTA

RADOJIČIĆ BILJANA, ŠAMANC H i PEJIN IVANA

SADRŽAJ

U ovom radu su izneti rezultati ispitivanja vršenih na zdravim teladima (n=30), Holštajn-Frizijske rase, u različitim periodima ontogeneze (od 15 dana, 2 i 4 meseca uzrasta).

Krv za analize je uzimana punkcijom iz vene jugularis, uvek ujutro, 1 čas nakon obroka.

U krvi je određivana koncentracija glukoze, a u krvnom serumu koncentracija kortizola, insulina, holesterola i lipida.

Nakon statističke obrade podataka, dobijeni rezultati su ukazali da su prosečne koncentracije kortizola u krvnom serumu ispitivane teladi bile približno iste, u periodu od 15 dana i 2 meseca, dok je kod teladi u uzrastu od 4 meseca

koncentracija kortizola bila nešto niža (ta razlika nije bila statistički značajna). Prosečna koncentracija insulina u krvnom serumu ispitivane teladi je bila najviša kod teladi uzrasta od 2 meseca, onda, kada pored mlečnog obroka počinje i prihranjivanje kabastom i koncentrovanom hranom, dok je kod teladi u uzrastu od 4 meseca, koncentracija insulina bila značajno niža ($p<0,05$). Prosečne koncentracije glukoze u krvi teladi su iznad fizioloških vrednosti (i približno iste) u 15 dana i sa 2 meseca uzrasta, dok je u uzrastu teladi od 4 meseca, ova vrednost značajno niža ($p<0,01$).

Kod teladi uzrasta od 15 dana i 2 meseca, 1 čas nakon mlečnog obroka, dolazi do značajnog povećanja nivoa glikemije.