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THYROID GLAND HORMONES IN NEWBORN CALVES TREATED WITH CLINOPTILOLITE RECEIVING DIFFERENT AMOUNTS OF COLOSTRUM

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The aim of this study was to investigate the influence of the natural mineral adsorber clinoptilolite on serum levels of thyroid hormones in newborn calves in the first 48 hours postpartum. A total number of 68 newborn Holstein calves divided in four groups were used in the present study. Calves were randomly assigned to one of the following treatments: 1) 0.75 L of colostrum in 12h intervals, with 5 g/L of clinoptilolite immediately after birth (0h), at 12h and 24h after birth (0.75+); 2) 0.75L of colostrum in 12h intervals (0.75-), which represents the first control group; 3) 1.5L of colostrum in 12h intervals, with 5 g/L of clinoptilolite immediately after birth (0h), at 12h and 24h after birth (1.5+), and 4) 1.5 L of colostrum in 12h intervals (1.5-), which represents the second control group.

The calves were born with high blood serum thyroid hormones concentrations (9.7-13.5 nmol/L for T_3 and 201-235 nmol/L for T_4). At 6. hours after birth serum thyroid hormone levels increased in all groups, but become significantly lower at 48. hours after birth. Clinoptilolite treatment could influence the rise in blood serum thyroid hormones concentration during the early postnatal period. This was most evident in the treated group of calves that received 1.5 L of colostrum (T_3, 6h, $11.7\pm3.4:19.4\pm7.4$, $p<0.01$; 1.5+ vs. 1.5-). Results indicate that there is a possible effect of clinoptilolite treatment on blood serum thyroid hormones concentration in newborn calves during the first 48 hours of life.

Key words: Thyroxine, triiodothironine, colostrum, clinoptilolite, newborn calves.

INTRODUCTION

Thyroid gland and somatotropic axis hormones probably have a key role in the distribution of nutrients from the gastrointestinal tract to the various tissues and organs during growth. Development and growth rate of neonatal calves depend on sufficient colostrum supply, which provides high amounts of nutrients and biologically active non-nutrient substances, such as immunoglobulins, hormones, and growth factors (Campana *et al*., 1995). Quantifying the relationship between nutrient intake and hormone production is an important step needed to advance the field of hormonal regulation of growth. There is evidence that in newborn Holstein-Friesian calves the diet could significantly influence plasma thyroxine concentrations (Grongnet *et al*., 1985). Previous work has indicated that different amounts of ingested colostrum could have only minor effects on plasma levels of thyroid hormones (Stojić *et al*., 2002). At the same time, the amount of colostrum ingested during the first 32 hours of life had a strong effect on serum concentrations of IGF-I and total proteins (Kirovski *et al*., 2002). Clinoptilolite is a naturally occurring mineral adsorbent with a special porous crystalline structure that can be adapted for a variety of uses. It is reported that clinoptilolite can increase the rate of colostral immunoglobulin G resorption in newborn calves and piglets (Stojić *et al*., 1995; Stojić *et al*., 1998). We have hypothesized that clinoptilolite treatment will increase the rate of resorption of nutrients from colostrum, providing newborn animals with sufficient energy, thus lowering thyroid hormone concentrations. The aim of this work was to determine the possible influence of clinoptilolite treatment on the blood serum concentrations of thyroid hormones in newborn calves offered different amounts of colostrum during the first 48 hours of life.

MATERIAL AND METHODS

Experimental design. A total number of 68 newborn Holstein-Friesian calves was used in the present study. The calves were randomly assigned to one of the following treatments: 1) 0.75L of colostrum in 12h intervals, with 5 g/L of clinoptilolite immediately after birth (0h), at 12h and 24h after birth (0.75+); 2) 0.75L of colostrum in 12h intervals (0.75-), which represents the first control group; 3) 1.5L of colostrum in 12h intervals, with 5g/L of clinoptilolite immediately after birth (0h), at 12h and 24h after birth (1.5+), and 4) 1.5L of colostrum in 12h intervals (1.5-), which represents the second control group. Number of serum samples analyzed for thyroid hormones is indicated in parenthesis in tables 3. and 4.

Blood serum collection. The blood samples were obtained from the jugular vein four times: at birth (0h), 6, 24. and 48. hours after birth. After coagulation and centrifugation the blood serum was separated and stored at -20°C for subsequent analyses.

Preparation of clinoptilolite suspension. Clinoptilolite (Minazel-S, ITNMS, Belgrade, Serbia and Montenegro) suspension was prepared in accordance with the producer's instructions. The chemical composition of Minazel-S is given in table 1, as determined on ARL 94000 X-ray Spectrometer.

Table 1. Chemical composition of the mineral adsorbent (%)

Component	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	Na_2O	K_2O	L.I.
Content	66.46	12.77	2.66	0.12	3.22	1.11	0.78	1.21	9.15

The cation exchange capacity (CAC) and type of exchanging cations were determined by the ammonium acetate method (Table 2).

Table 2. CEC and exchanging cations of the mineral adsorbent

Exchanging cation	Ca^{++}	Mg^{++}	Na^{++}	K^{+}	Total
CEC mmol/100g	121	25	25	2	173

Determination of serum thyroid hormones concentrations. Blood serum thyroid hormones concentrations were determined by radioimmunoassay using commercial kits in accordance with the instructions (INEP Diagnostics, Zemun).

Statistical analysis. The results are expressed as mean values (M) and standard deviation (SD), for each group of calves. Probability and statistical significance of differences between mean values were calculated using Student's t-test.

RESULTS

Blood serum triiodothyronine (T_3) concentrations. The results of blood serum triiodothyronine concentrations are presented in Table 3.

Table 3. Blood serum triiodothyronine (T_3) concentration (Mean±SD nmol/L) in newborn calves

Time	Treated and control groups of calves			
	0.75+	0.75–	1.5+	1.5–
0h	9.7 ± 3.9^{ab} (17*)	13.2 ± 6.2^{a} (15)	10 ± 4.2^{ab} (16)	13.5 ± 7.1^{b} (15)
6h	11.4 ± 2.4^{a} (17)	12.7 ± 5.2^{a} (15)	11.7 ± 3.4^{a} (16)	19.4 ± 7.4^{a} (13)
24h	8.6 ± 2.4^{b} (17)	8.7 ± 4.2^{b} (15)	8.7 ± 2.2^{b} (16)	9.6 ± 4.9^{b} (13)
48h	6.9 ± 1.4^{c} (17)	6.1 ± 2.2^{c} (15)	6.6 ± 1.5^{c} (16)	5.4 ± 3.0^{c} (12)

Legend: *number of blood samples; ^{a,b,c}Means in a same column not sharing a common superscript are significantly different ($p<0.05$).

The blood serum T_3 concentrations were high in newborn calves, and have increased even more 6h after birth. This increase was most evident in the control group receiving 1.5L of colostrums (from 13.5±7.1 at birth to 19.4±7.4 nmol/L at 6. hours after birth). This initial increase in the blood serum T_3 concentrations was followed by a decrease to intermediate levels at 24h after birth. The T_3 concentrations becoming significantly lower in all experimental groups at 48h after birth (5.4-6.9 nmol/L).

Blood serum T_3 concentration was significantly different at 6h after birth between the control and treated groups receiving 1.5L of colostrum (Fig. 1). At 6h after birth blood serum T_3 concentration in the treated calves was significantly lower compared to the second control group (11.7±3.4:19.4±7.4 nmol/L, $p<0.01$, 1.5+ vs. 1.5-). A similar situation was observed between the first control and the treated groups of calves that received 0.75L of colostrum, although the differences between the mean T_3 values at 6h after birth were not significant (11.4±2.4: 12.7±5.2, $p>0.05$, 0.75+ vs. 0.75-).

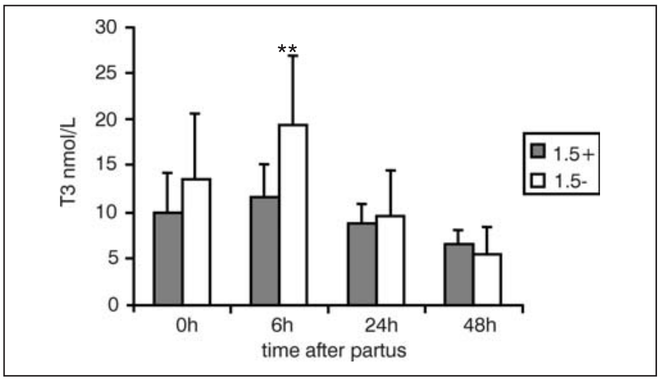

Figure 1. Blood serum T_3 concentration (Mean ± SD nmol) in the control (1.5–) and clinoptilolite treated (1.5+) groups of calves receiving 1.5L of colostrum (**p<0.01)

Blood serum thyroxine (T_4) concentrations. The results of blood serum thyroxine (T_4) concentrations are presented in Table 4.

Table 4. Blood serum thyroxine (T_4) concentration (Mean±SD nmol/L) in newborn calves

Time	Treated and control groups of calves			
	0.75+	0.75–	1.5+	1.5-–
0h	$201±57^{b}$ (13)	$209±44^{b}$ (13)	$201±44^{b}$ (13)	$235±100^{ab}$ (13)
6h	$265±59^{a}$ (13)	$293±106^{a}$ (13)	$264±86^{a}$ (13)	$312±84^{a}$ (13)
24h	$214±44^{b}$ (12)	$215±96^{ab}$ (12)	$196±62^{b}$ (12)	$223±75^{b}$ (12)
48h	$162±27^{c}$ (12)	$171±56^{b}$ (12)	$140±35^{c}$ (12)	$144±55^{c}$ (12)

Legend: *number of samples; ^{a,b,c}Means in a same column not sharing a common superscript are significantly different ($p<0.05$).

Mean values of blood serum T_4 concentrations in all four groups of calves are given in Table 4. Our results indicate that the calves were born with high blood serum T_4 concentrations (201-235 nmol/L), having an increase in the levels of T_4 at 6h after birth, that was more evident in the control groups of calves (209=>293 nmol/L in 0.75- group, 235=>312 nmol/L in 1.5- group of calves). This initial rise in blood serum T_4 concentration was followed by a decrease at 24h after birth. The mean values of T_4 in all four groups of calves becoming significantly lower at 48h after birth compared to the previously observed values.

Mean blood serum T_4 concentration between the control and treated groups of calves were not significantly different during all four periods of examination. However, the mean values of blood serum T_4 were lower at most periods in both groups of treated calves compared to the control, especially at 6h after birth in calves that received 1.5L of colostrum (Fig. 2).

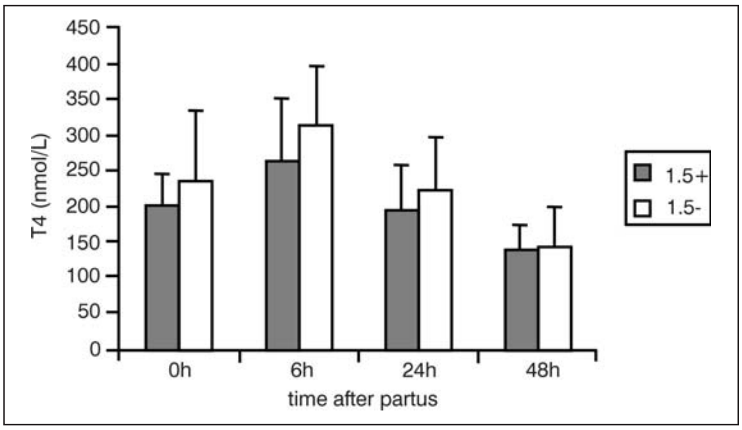

Figure 2. Blood serum T_4 concentration (Mean ± SD nmol) in the control (1.5-) and clinoptilolite treated (1.5+) groups of calves receiving 1.5L of colostrum

DISCUSSION

Thyroid gland function status in newborn calves has been examined in many studies (Kahl *et al*. 1977; Davicco *et al*., 1982; Jovanović *et al*., 1982; Ronge *et al.*, 1988; Hadorn *et al*. 1997), including our recent study concerning the intake of different amounts of colostrum (Stojić *et al*., 2002). These results indicate that there is a neonatal reserve of thyroid hormones that is readily available during the critical time after birth. At the same time the reducion of the amount of colostrum intake did have only minimal effects on plasma thyroid hormones concentrations (Stojić et al., 2002). Our present results confirmed the high level of blood serum thyroid hormones in newborn calves immediately after birth. Concentrations of T_4 and T_3 are very high at birth and decrease on day 7 after birth (Jovanović *et al*., 1982; Vermoreal *et al*., 1989; Stojić *et al*., 2002). Extrauterine transition of newborn ruminants after parturition induces a substantial rise in plasma T_3 and T_4 concentrations (Davicco *et al*., 1982, Polk, 1995). The thermal balance in calves during the neonatal period is supported by the well developed thermogenic mechanisms including shivering thermogenesis in muscle tissue and non-shivering thermogenesis in brown adipose tissue (BAT) (Alexander *et al*., 1975). Brown adipose tissue is a specialized tissue capable of generating heat by uncoupling oxidative phosphorilation from mitochondrial respiration. Thyroid hormones play an essential role in regulating BAT thermogenesis in newborn ruminants (Schermer *et al*., 1996). It is estimated that about 60% of plasma T_3 concentrations in newborn lambs is derived from the conversion of T_4 to T_3 by iodothyronine 5'-deiodinase (5'-DI) in peripheral tissues (Klein *et al*., 1980), and since BAT contains a high concentration of 5'-DI (Nicol *et al*., 1994) it could be a significant source of circulating T_3.

Results of Grongnet *et al*. (1985), who showed that the thyroid status is dependent on the intensity of colostrum feeding, were not entirely confirmed by our study. Thyroid hormones blood serum concentrations were not significantly different in newborn calves receiving full (1.5L) or half ration (0.75) of colostrum. However, Hamon and Blum (1998) reported T_3 levels that were twice as high in the group M (receiving milk) as in groups C_6 (colostrum, 6 times) or C_1 (colostrum, one time) at day 2 of age, although this difference was not significant. They concluded that the T_3 decline in plasma after birth is influenced by colostrum intake, but it is speculative and needs further investigation.

Triiodothyronine is present in cow's milk in considerably higher amounts than T_4 (Ronge *et al*., 1988), However, the direct effect of increased milk intake on the blood plasma thyroid hormones is less probable, relating to the results of Hamon *et al*., (2002), who didn't find any changes in serum thyroid hormones concentrations in calves offered ad libitum vs. restricted colostrum and milk diet.

There is evidence that T_4 levels decrease during energy deficiency in newborn calves (Grongnet *et al*., 1985; Kinsbergen *et al*., 1994), but 24 hours of food restriction did not have significant effects on thyroid hormones concentration in newborn calves (Hadorn *et al*., 1997). Our results indicate that at 6. hours after birth blood serum T_3 concentration was significantly lower in the group of calves fed 1.5L of colostrum treated with clinoptilolite, compared to the control group of calves (Fig. 1). Furthemore, T_3 and T_4 blood serum concentration is consistently lower in the treated group of calves receiving 1.5L of colostrum during all periods of the experiment (Fig. 1. and 2.). Clinoptilolite treatment seems to effectively increase intestinal resorption of biologically active substances from the colostrum in newborn calves and pigs (Stojić et al, 1995, 1998, 2003). Present results indicate the possible influence of clinoptilolite treatment on thyroid hormones blood serum concentrations in newborn calves. The effect of the mineral adsorbent clinoptilolite could be based on the increase of resorption of nutrients from the colostrum, providing the newborn calves with sufficent energy precursors, but this hypothesis needs further investigation.

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KONCENTRACIJA HORMONA TIREOIDNE ŽLEZDE U KRVNOM SERUMU NOVOROĐENE TELADI TRETIRANE KLINOPTILOLITOM I NAPAJANE RAZLIČITIM KOLIČINAMA KOLOSTRUMA

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SADRŽAJ

Cilj ovog rada bio je da se odrede koncentracije hormona tireoidne žlezde u toku prvih 48 sati života u krvnom serumu novorođene teladi tretirane klinoptilolitom i napajane različitim količinama kolostruma. Ogled je izveden na ukupno 68 teladi, podeljene u četiri grupe: 1) grupa teladi napajanih sa 0.75L kolostruma u intervalima od 12 sati i tretiranih klinoptilolitom odmah posle rođenja (0h), 12h i 24h (0.75+), 2) prva kontrolna grupa teladi napajana sa 0.75L kolostruma u intervalima od 12 sati (0.75–), 3) grupa teladi napajanih sa 1.5L kolostruma i tretiranih klinopilolitom odmah posle rođenja (0h), 12h i 24h (1.5+), i 4) druga kontrolna grupa teladi napajanih sa 1.5L kolostruma u intervalima od 12 sati (1.5–). Uzorci krvi su uzimani neposredno nakon rođenja, 6-og, 24-og i 48-og sata nakon rođenja.

Telad se rađaju sa visokom koncentracijom hormona tireoidne žlezde u krvnom serumu (0. sat, koncentracija T_3 : 9.7-13.5 nmol/L; koncentracija T_4: 201-235 nmol/L). Nakon 6 sati od rođenja koncentracije tireoidnih hormona u krvnom serumu teladi se povećava, a posle 48 sati od rođenja zapaža se statistički značajno sniženje njihove koncentracija u odnosu na sve prethodne periode ispitivanja. Tretman klinoptilolitom utiče na stepen porasta koncentracije tireoidnih hormona kod novorođene teladi u toku prvih 6 sata ispitivanja. Novorođena telad napajana sa 1.5L kolostruma koja su tretirana klinoptilolitom (11.7±3.4 nmol/L) 6. sata imala su statistički značajno nižu ($p<0.01$) koncentraciju T3 u krvnom serumu u odnosu na kontrolnu grupu životinja (19.4±7.4 nmol/L). Ovi rezultati ukazuju na moguće postojanje značajnog uticaja tretmana klinoptilolitom na promene u koncentraciji hormona tireoidne žlezde u krvnom serumu teladi u toku prvih 48 sati nakon rođenja.