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SERUM LEVELS OF INSULIN LIKE GROWTH FACTOR - I AND TOTAL PROTEIN IN NEWBORN CALVES OFFERED DIFFERENT AMOUNTS OF COLOSTRUM

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The aim of this investigation was to determine the concentrations of insulin like growth factor-I (IGF-I) and total protein in blood serum from calves consuming different amounts of colostrum during the first 32 h of postnatal life, as well as at 7 days old. The experiment involved two groups of calves. The first group was offered the standard amount of colostrum while the second group received half the recommended amount.

At birth serum concentration of IGF-I was in the range from 5 to 13 nmol/L. Compared to the initial level there was a marked decrease of mean IGF-I concentration in both groups of calves at 2 h after the first intake of colostrum. Thereafter, the level gradually increased until 20 h of age, more markedly in the group which received the full ration of colostrum. The highest mean IGF-I concentrations were recorded at 20 h in both groups (11.81 nmol/L for group one and 8.96 nmol/L for group two), and were significantly higher than values recorded 2h after the first intake of colostrum. Two hours after the third intake of colostrum, serum concentration of IGF-I dropped in both groups, compared with the value recorded at 20 h.

During the time period from 4 h to 32 h after birth, the calves that received the standard amount of colostrum had a significantly higher mean concentration of serum IGF-I than the calves which received an insufficient amount.

Calves were born with serum concentrations of total proteins, which were lower than those found for adult animals. Serum protein concentrations in the calves that consumed normal amounts of colostrum increased more rapidly than in the calves that received insufficient amounts of colostrum. Presumably, total protein concentrations increased as a consequence of colostral immunoglobulin absorption.

Thus our results indicate that the amounts of colostrum received during the first 32 h of postnatal life had strong effects on the serum concentrations of IGF-I and total proteins.

The differences in IGF-I concentrations between the two groups of calves may have been a consequence of greater colostral IGF-I

absorption and/or the result of greater absorption of other colostral components, which stimulate endogenous synthesis of this bioactive substance in the tissues of neonatal calves.

Key words: neonatal calves, colostrum, insulin like growth factor-I, total protein, immunoglobulins.

INTRODUCTION

Colostrum is the only and sufficient source of nutrients (proteins, fat and carbohydrates) for newborn calves. In addition to the provision of nutrients, colostrum intake is essential for normal growth and health protection of newborns. Namely, it is an extremely rich source of immunoglobulins, which can be absorbed intact by neonatal intestine only for a limited time after birth (approximately 38 hours). Thus, colostrum intake provides passive immunity essential for the calf's survival. Colostrum also contains bioactive and growth-promoting substances (enzymes, hormones and growth factors) which are not essential but are very important for normal growth and development of newborns. Insulin-like growth factor I (IGF-I) is present in cow colostrum in high concentrations (Oda *et al.*, 1989, Schams, 1994).

Colostral IGF-I ingestion has a strong influence on overall systematic and intestinal growth and development. It reduces intestinal enzyme activity in the early-postnatal period when the calves are colostrum fed (Read *et al.*, 1994).

Examination of colostral IGF-I absorption from neonatal intestine has been the subject of many investigations (Hammon *et al.*, 1997; Lee *et al.*, 1995; Odle *et al.*, 1996; Vacher *et al.*, 1995). In addition, the local effects of colostral IGF-I on neonatal intestine has been studied (Baumrucker *et al.*, 1994; Buhler *et al.*, 1998; Guilloteau *et al.*, 1995). Hammon *et al.* (1997) demonstrated that IGF-I concentrations in blood serum of calves depend markedly on the nutritional status in the neonatal period of life.

The objective of many experiments was to study the quantitative and qualitative changes of serum total protein concentrations in neonatal calves (Vukotić, 1967; Kiryama *et al.*, 1989; Mao *et al.*, 1994). It has been established that enterocytes, having membrane receptors for the Fc fragment (Bush *et al.*, 1971; Staley *et al.*, 1985), are capable of transfering intact immunoglobulin molecules from the intestinal lumen into the vascular system. Passage is most intensive during the first six hours of life (Marx *et al.* 1979). New enterocytes, 38 h after birth, are no longer able to take up intact colostral immunoglobulins because of the absence of Ig receptors on their membranes. Thus, changes in neonatal calf proteinemia are a consequence of abundant absorption of colostral proteins, specially immunoglobulins in the first hours of life.

From the results presented for serum IGF-I concentrations during the neonatal period, it is apparent that there are inconsistencies about whether the rise of its concentration is a consequence of greater colostral IGF-I absorption or due to endogenous synthesis in the various tissues of the calves.

The aim of our investigation was to determine if two levels of colostrum feeding affect concentrations of IGF-I and total protein in blood serum from newborn calves.

MATERIAL AND METHODS

Animals

The experiment involved two groups of eight calves of the Holstein-Friesian breed at Jabučki Rit farm (Belgrade Agricultural Corporation). The mean body mass of the calves at birth was 39.5±5.3 kg for group one and 36.5±5.1 kg for group two. All calves were born within 2 days and immediately placed in individual boxes in a byre where the temperature ranged from 15 to 20°C.

Colostral intake

The calves were suckled on their dam's colostrum 2 h, 12 h and 24 h after birth through artificial teats. Calves in the first group were offered 1.5 L for the first two meals followed by 2 L colostrum for the third. Calves in the second group received 0.75 L for the first two meals and 1 L for the third.

Blood sampling

Blood samples were collected by v. jugularis puncture with a sterilised needle into tubes. After coagulation and centrifugation the serum was aspirated and stored at - 20°C for later analysis. Blood samples were taken at birth (0h) and 4h, 6h, 8h, 16h, 18h, 20h, 28h, 32h and 7 days later.

Colostrum sampling

Colostrum samples were collected from mothers of newborn calves immediately before suckling at 2h, 12h and 24h postpartum. Colostrum was stored at - 20°C for later analysis.

Analyses

Commercial ^{125}I-RIA kits validated for bovine serum and colostrum were used to determine IGF I as described earlier (Nikolić *et al.* 1996; 1998).

Total protein concentration in colostrum was determined by the biuret reaction (Gornall *et al.*, 1949).

Statistical analysis

The results are expressed as mean (M), standard deviation (SD), standard error (SE) and CV for each group of calves. Probability and the statistical significance of differences between mean values were calculated using Student's t-test. Simple correlation coefficient and the regression of IGF-I concentrations in relation to body weights were also calculated and analyzed.

RESULTS

The IGF-I and total protein concentrations found in colostrum of mothers of calves in both groups involved in our experiment are presented in Table 1.

It can be seen that the mean concentration of IGF-I decreased from the 1st to 3rd milking postpartum ($p < 0.001$). The mean concentration of total protein in colostrum also decreased between 1st milking and that at 12h ($p < 0.01$) and 24h postpartum ($p < 0.001$).

There was no difference in mean IGF-I and total protein concentrations between colostrum ingested by calves in group one and group two.

Mean values for IGF-I concentrations in blood serum samples in group one are given in Table 2.

Table 1. The mean serum levels of IGF-I(nmol/L) and total protein (g/L) in colostrum offered to each group of calves

Group n = 8	IGF-I			TOTAL PROTEIN		
	Milking 1	Milking 2	Milking 3	Milking 1	Milking 2	Milking 3
I						
M	56.0	31.1	19.1	136.9	58.1	31.0
SD	20.8	19.0	13.2	51.9	30.8	10.2
SE	7.4	6.7	4.7	18.3	10.9	3.6
CV(%)	37.2	61.0	69.3	37.9	53.0	33.0
II						
M	60.6	35.2	17.9	134.3	62.8	27.5
SD	24.5	14.5	10.5	51.9	25.3	7.5
SE	8.7	5.1	3.7	18.4	9.0	2.7
CV(%)	40.4	41.2	58.5	38.7	40.3	27.4

At birth mean serum IGF-I concentration was 9.98 nmol/L The lowest mean IGF-I concentration (8.97 nmol/L) was recorded at 4h after birth, followed by a gradual increase until 20h of age. The highest IGF-I concentration was recorded at 20 h of age (11.81 nmol/L), and was significantly higher than the value found 2 h after the first intake of colostrum ($p < 0.05$). Thereafter, a general negative trend in concentration was seen down to 10.68 nmol/L at 32 h of age.

At 7 days of age mean IGF-I concentration was 9.11 nmol/L which was not significantly different from the initial level ($p > 0.05$).

Serum levels for IGF-I found in the second group of calves are shown in Table 3.

Mean serum concentration of IGF-I at birth was 8.41 nmol/L. A gradual increase in serum IGF-I concentration occurred after the second intake of colostrum up to 20 h of age when the highest level occurred (8.96 nmol/L). Mean IGF-I concentration then decreased significantly ($p < 0.05$) to 7.24 nmol/L at 28 h after birth and remained at that level until 32 h of age.

At 7 days of age mean serum concentration of IGF-I was 8.16 nmol/L and there was no statistically significant difference compared with the initial level ($p < 0.05$).

During the time period from 4 h to 32 h after birth, the calves, which received the standard amount of colostrum had significantly higher serum IGF-I concentrations than the calves which received an insufficient amount (Fig. 1).

Table 2. The mean serum levels of IGF-I (nmol/L) in the group of calves offered the full ration of colostrum

	Age of calves (hours/days)										
n=8	0h	4h	6h	8h	16h	18h	20h	28h	30h	32h	7 days
M	9.98	8.97	9.43	10.58	11.12	11.26	11.81	11.16	11.38	10.68	9.11
SD	1.69	2.51	1.66	2.02	2.61	2.64	2.69	2.59	2.63	2.53	2.58
SE	0.59	0.89	0.58	0.71	0.92	0.93	0.95	0.91	0.93	0.89	0.91
CV(%)	16.93	27.98	17.60	19.09	23.47	23.44	22.78	23.21	23.11	23.69	28.32

↓ - colostral intake time

Table 3. The mean serum levels of IGF-I (nmol/L) in the group of calves ofered half recommended amount of colostrum

	Age of calves (hours/days)										
n=6	0h	4h	6h	8h	16h	18h	20h	28h	30h	32h	7. day
M	8.41	7.19	7.35	7.07	8.00	8.72	8.96	7.24	7.13	6.88	8.16
SD	2.55	1.46	0.95	1.02	1.11	1.29	0.40	1.33	1.63	1.73	2.47
SE	0.90	0.52	0.33	0.36	0.39	0.46	0.14	0.47	0.58	0.61	0.87
CV(%)	30.32	20.30	12.92	14.43	13.87	14.79	4.46	18.37	23.58	25.14	30.27

↓ - colostral intake time

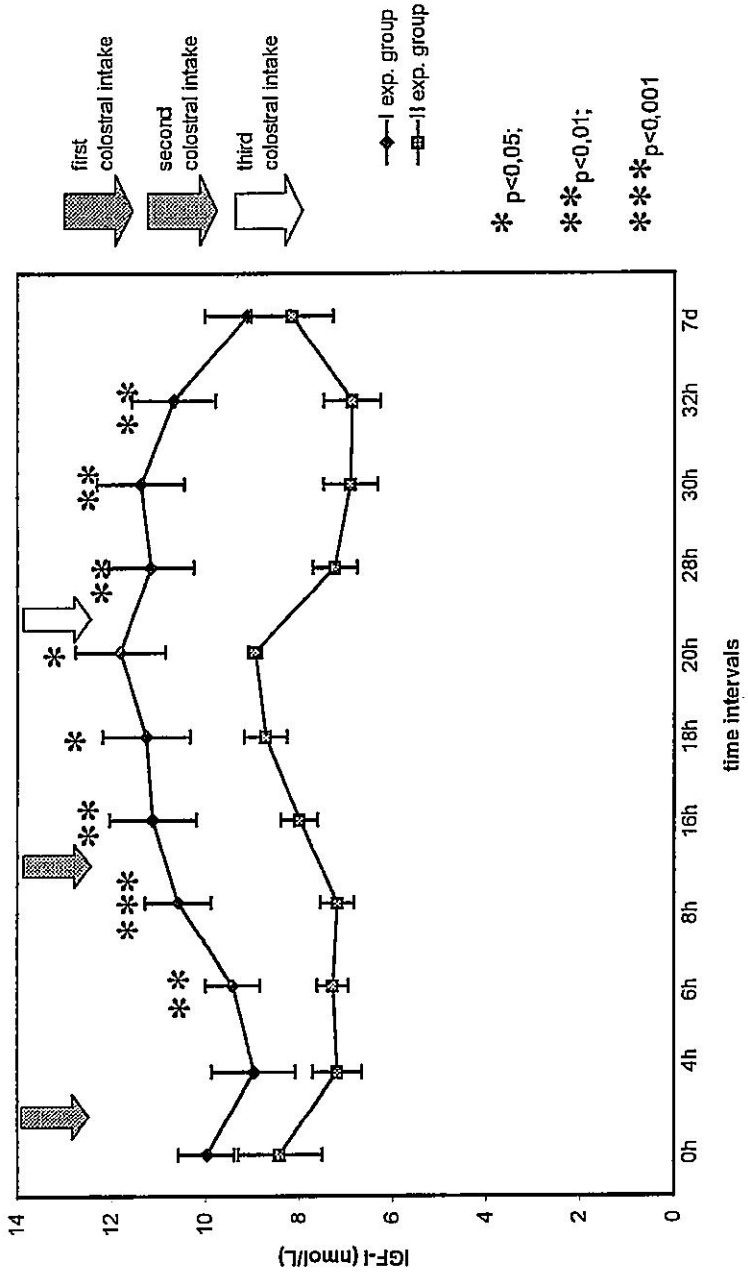

Figure 1. Mean serum IGF-I concentration (M SE nmol/L) in both experimental groups of calves

Before first colostrum intake serum IGF-I concentrations were positively correlated with birth weight (r=0.702, p<0.01). (Fig 2).

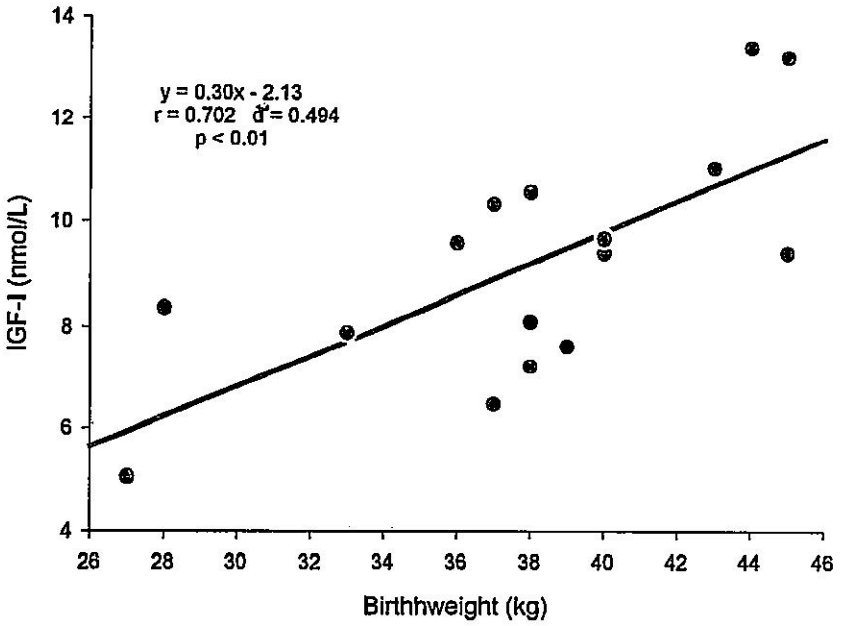

Figure 2. Correlation between IGF-I levels and birth weight

The results for total protein concentrations in each group of calves are shown in Table 4 and Table 5.

Calves were born with total serum protein concentrations, which were lower than those found for adult animals (45.4 g/L for group one and 44.0 g/L for group two). Thereafter, mean values increased in both groups, more markedly in the group which received the full ration of colostrum. In group one mean protein concentrations were in the range from 54.7 g/L (4 h of age) to 70.3 g/L (32 h of age). In comparison with the initial level the difference became statistically significant 4h after birth ($p < 0.05$). In the group of calves which received half the recommended amount (group two) protein concentrations were in the range from 42.7 g/L (4 h of age) to 62.4 g/L (32 h of age). In comparison with the initial level the difference became statistically significant 16h after birth ($p < 0.05$).

At 7 days of age serum concentrations of total protein in both groups of calves were within the range found for adult animals (69.5 g/L for group one and 60.0 g/L for group two).

Total protein concentrations were significantly higher in group one than in group two where the calves consumed less colostrum. Even at day 7 the difference in protein concentrations between group one and group two remained significant $p = 0.0502$. (Fig. 3)

Table 4. The mean serum levels of total protein (g/L) in the group of calves offered the full ration of colostrum

	Age of calves (hours/days)										
n=6	0h	4h	6h	8h	16h	18h	20h	28h	30h	32h	7 days
M	45.4	54.7	58.4	61.0	61.2	63.8	68.3	68.4	70.1	70.3	69.5
SD	6.7	10.2	9.2	6.3	8.7	10.1	7.5	5.5	8.0	8.0	10.1
SE	2.4	3.6	3.2	2.2	3.1	3.6	2.7	2.0	2.8	2.8	3.6
CV(%)	14.7	18.6	15.7	10.4	14.3	15.8	11.0	8.1	11.4	11.4	14.5

- colostral intake time

Table 5. The mean serum levels of total protein (g/L) in the group of calves ofered half recommended amount of colostrum

	Age of calves (hours/days)										
n=6	0h	4h	6h	8h	16h	18h	20h	28h	30h	32h	7 days
M	44.0	42.7	47.2	49.8	55.5	57.7	59.8	61.1	59.8	62.4	60.0
SD	14.0	10.6	10.4	10.0	2.8	4.7	4.7	6.0	8.6	12.4	8.6
SE	5.0	3.7	3.7	3.5	1.0	1.7	1.7	2.1	3.0	4.4	3.0
CV(%)	31.9	24.8	22.0	20.1	5.1	8.1	7.9	9.8	14.4	19.3	14.2

- colostral intake time

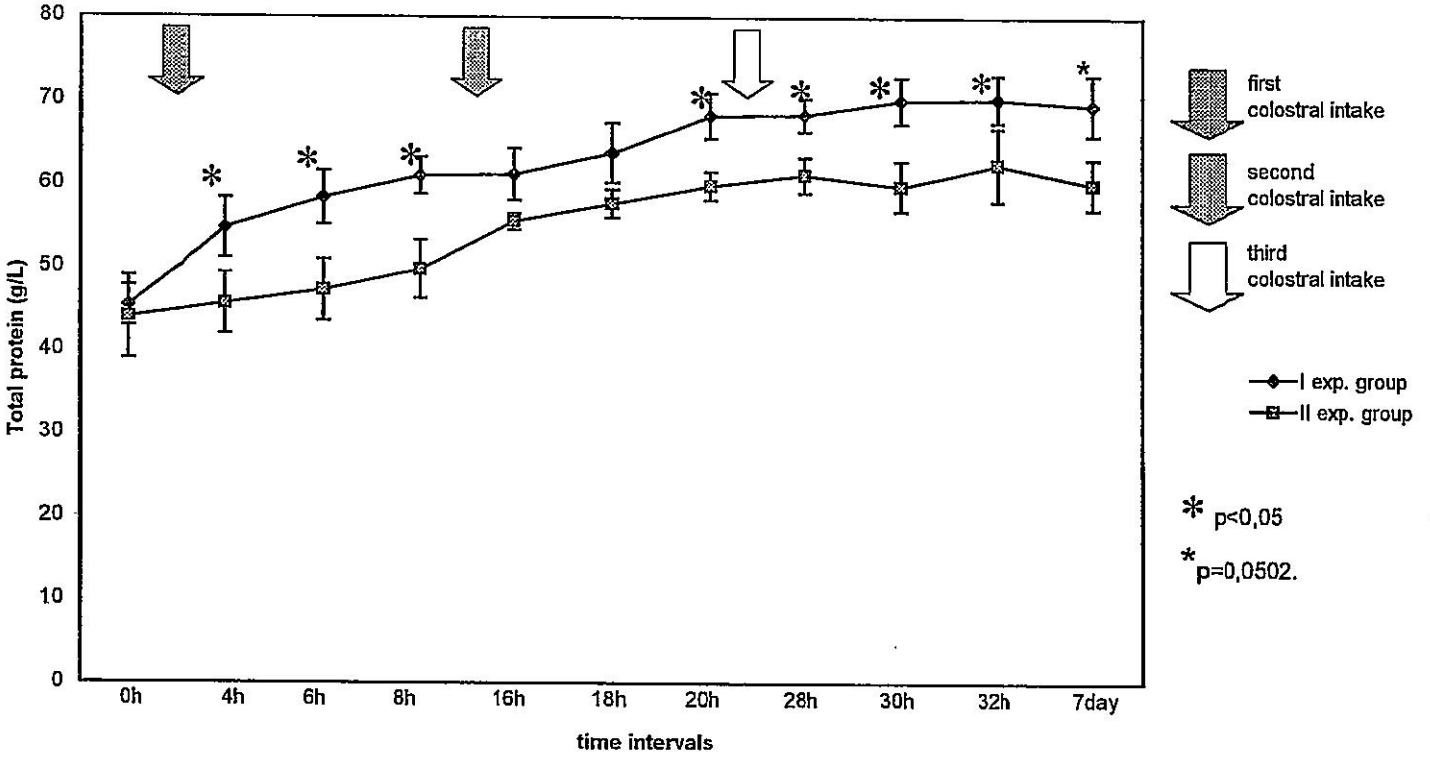

Figure 3. Mean plasma total protein concentration (M ± SE g/L) in both experimental groups of calves

DISCUSSION

The results obtained here for colostral levels of IGF-I in Holstein dairy cows on the day of parturition correspond to those obtained by other authors, which ranged from around 18.8 nmol/L (Hammon *et al.*, 2000) to 76 nmol/L (Hadsell, 1999). In agreement with our data, other investigators, too, found a decrease of IGF-I levels from 1st to 3rd milking.

As expected, the typical changes in total protein concentration in colostrum were exhibited by our cows. Our data for protein concentrations were similar to those reported by many other authors (Oyeniyi *et al.*, 1978, Grutter *et al.*, 1991). Namely, there was a significant decrease of total protein levels in colostrum within two days following parturition.

Calves were born with total serum protein concentrations lower than those found for adult animals (Vukotić, 1967; Kurz *et al.*, 1991). In both groups of calves serum protein concentrations sharply increased within first two hours after the first meal, more markedly in group one, and then remained elevated until the end of the experimental period. Presumably, total protein concentrations increased as a consequence of colostral immunoglobulin absorption.

The significantly lower serum protein concentrations in the group which consumed restricted amounts of colostrum indicate that the quantity of colostrum consumed during the first 32 h of postnatal life had a prolonged effect on the concentration of serum proteins, probably as a consequence of the long half-life of circulating immunoglobulins. Thus, higher concentrations of total protein in calves fed normal amounts of colostrum remained on day 7 which confirms the results of other studies (Hammon *et al.*, 1997).

IGF-I at birth originates from endogenous synthesis in various fetal tissues. Our data for initial concentrations of serum IGF-I were in accordance with previous studies (Grutter *et al.*, 1991; Nikolic *et al.*, 1996). Groenewegen and coworkers (1990) indicated that serum IGF-I levels appear to reflect growth rate in young cattle. Breier and coworkers (1988) and D'Ercole and coworkers (1980) found a high positive correlation between birth weight and IGF-I concentrations. Our results confirm these findings. Moreover, there was no immediate increase of serum IGF-I concentrations after the first meal. This result agrees the findings of other authors (Baumrucker *et al.*, 1994; Eglia *et al.*, 1998; Hammon *et al.*, 1997). In addition, we recorded a slight decrease in serum IGF-I concentrations at 4 h of age (group one) and at 4 h, 6 h and 8 h of age (group two) compared with initial levels. Hadorn and coworkers (1997) found that omission of colostrum intake provoked a rapid fall of blood IGF-I in neonatal calves. This was also the case in neonatal calves, when milk replacer, containing less nutrients and energy than colostrum, or water were fed instead of colostrum (Hammon *et al.*, 1998, Grutter *et al.*, 1991). Thus feeding first colostrum appears to have a particularly great influence on IGF-I concentrations.

Our results show that serum IGF-I concentrations were higher in group one than in group two from 4 h to 32 h of age and indicate that the amounts of colostrum received during the first 32 h of postnatal life had strong effects on the concentrations of IGF-I. Differences in IGF-I concentrations between the two groups of calves involved in our investigation may be a consequence of greater colostral IGF-I absorption and/or a result of greater endogenous synthesis in various tissues of the neonatal calves. Greater synthesis may be due to the twofold

greater intake of colostral nutrients in group one. Namely, the group of calves which received the full ration of colostrum consumed double the amount of colostral proteins, as well as energy and other colostral components which might stimulate endogenous synthesis of IGF-I in neonatal tissues. Muira and coworkers (1992) demonstrated that amounts of ingested colostral proteins appear to play a major role in the regulation of hepatic IGFI-I production. These authors suggest that colostral IGF-I does not enter the calf circulation in measurable quantities and that it mainly has local effects on neonatal intestine.

Two hours after the third intake of colostrum IGF-I serum concentration decreased in both groups, compared with value recorded at 20 h. The established rapid fall in concentration is a characteristic of all low molecular weight proteins (Kiriyama *et al.*, 1989).

At 7 days of age there were no differences in serum IGF-I levels between the groups of calves. These data indicate that the amounts of ingested colostrum had no influence on the development of mechanisms involved in the maintenance of blood IGF-I level.

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KONCENTRACIJA IGF-I I UKUPNIH PROTEINA U KRVNOM SERUMU NOVOROĐENE TELADI NAPAJANE RAZLIČITOM KOLIČINOM KOLOSTRUMA

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SADRŽAJ

Cilj ovog rada bio je da se odrede koncentracije insulinu sličnog faktora rasta-I (IGF-I) i ukupnih proteina u krvnom serumu teladi koja su napajana različitom količinom kolostruma tokom prvih 32 sata postnatalnog života kao i 7. dana života. Ogled je izveden na dve grupe od po osam teladi: prva grupa teladi je napajana sa normalnom količinom kolostruma, dok je druga grupa bila napajana sa duplo manjom količinom kolostruma. Koncentracija IGF-I u krvnom serumu teladi pri rođenju bila je u rasponu od 5 do 13 nmol/L. Nakon napajanja prvim kolostrumom kod obe grupe je došlo do značajnog pada koncentracije IGF-I u odnosu na koncentraciju određenu nultog sata života. Posle toga koncentracija je kod obe grupe postepeno rasla do 20. sata života pri čemu je porast bio izraženiji kod grupe koja je napajana normalnom količinom kolostruma. 20. sata je kod obe grupe utvrđena najviša koncentracija IGF-I (11.81 nmol/L u krvnom serumu prve grupe i 8.96 nmol/L u krvnom serumu druge grupe teladi) i ona je bila značajno viša od vrednosti utvrđene 4. sata života, a dva sata posle prvog kolostralnog napoja. Dva sata nakon napajanja trećim kolostrumom došlo je do pada koncentracije IGF-I u krvnom serumu obe grupe teladi, pri čemu je pad bio statistički značajan u odnosu na 20. sat života samo kod druge grupe.

U vremenskom periodu od 4. do 32. sata života telad prve grupe koja su napajana normalnom količinom kolostruma imala su statistički značajno višu koncentraciju IGF-I u krvnom serumu u odnosu na telad koja su napajana manjom količinom kolostruma.

Telad obe ogledne grupe su rođena sa koncentracijom proteina koja je značajno niža nego kod odraslih jedinki. Porast koncentracije proteina u krvnom serumu teladi napajane sa duplo manjom količinom kolostruma u ispitivanom periodu je bio znatno sporiji u poređenju sa rastom proteinemije teladi prve ogledne grupe.

Naši rezultati ukazuju da nivo popijenog kolostruma u prva 32h neonatalnog života ima veliki uticaj na koncentraciju insulinu sličnog faktora rasta I (IGF-I) i ukupnih proteina.

Za porast proteinemije možemo sa sigurnošću tvrditi da je rezultat resorpcije kolostralnih imunoglobulina.

Različiti nivoi IGF-I utvrđeni u našem ogledu na dve grupe teladi mogu biti posledica veće resorpcije IGF-I prisutnog u kolostrumu i/ili veće resorpcije nekih od još neidentifikovanih sastojaka kolostruma koji indukuju endogenu sintezu ovog biološki aktivnog jedinjenja u mnogim tkivima novorođene jedinke.