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SERUM HORMONES AND INSULIN-LIKE GROWTH FACTOR-I IN MALE AND FEMALE CALVES AND THEIR POSSIBLE RELATION TO GROWTH

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Precolostral and preprandial concentrations of insulin, cortisol, triiodothyronine (T3) and insulin-like growth factor-I (IGF-I) were determined in the blood serum of male and female Holstein calves at 0 and 4.5 months, of age. The values obtained were similar for both sexes except for slightly but statistically sigificantly higher mean neonatal cortisol, higher IGF-I at 4.5 months and lower T3 at 4.5 months in the male (n=21) than in the female group (n=22). Insulin, cortisol and T3 concentrations fell and IGF-I increased with age. Moreover, statistically significant correlation coefficients were found between neonatal T3 and cortisol levels and between final IGF-I concentrations and both daily gain and final body weight in each group. Multiple regression analysis indicated that only two of the nine parameters examined (neonatal T3 and IGF-I concentrations at 4.5 months) were associated independently with rate of gain of the female calves (R^2 = 0.495). However, seven of the nine variables together accounted for 83% of the variance associated with daily gain in the male calves (R^2 = 0.831). Nevertheless, the contribution of measurements obtained at birth alone was not sufficiently large to allow prediction of subsequent growth because, even in the male calves, they only described 60% of the variance associated with daily gain in the period examined.

Key words: IGF-I, T3, cortisol, calves, growth.

INTRODUCTION

According to Anderson and coworkers (Anderson, Bergen, Merkel, Enright, Zinn, Refsal and Hawkins, 1988) improvements in the efficiency of animal production will come with an increased understanding of the nature of the influence of the endocrine system on growth. The complexity of hormonal interactions and their final effects on the rate of deposition of muscle, bone and adipose tissue in cattle are gradually being elucidated (Trenkle, 1981; Buttery, 1983; Spencer, 1985; Simmen, 1991).

While the somatotropin/insulin-like growth factor-I (IGF-I) axis was found to be most related to composition of gain in growing beef bulls (Anderson *et al.*, 1988; Arthur, Makarediran, Salmon and Price, 1990), the importance of its connection with thyroid status has recently been recognized (Elsasser, Rumsey and Kahl, 1993).

Moreover, sex steroids and insulin may also be involved in IGF-I production in cattle (Plouzek and Trenkle, 1991). The central role of IGF-I in mediating the anabolic effects of somatotropin in young bulls was noted some time ago (Lund-Larsen, Sundby, Kruse and Velle, 1977) but difficulties in accurate determination of circulating concentrations of IGF-I (Holly annd Hughes, 1994), together with lack of knowledge about the roles of specific binding proteinns have delayed proper assessment of its action in comparison with research on steroid, thyroid and peptide hormones such as insulin.

There is still much controversy concerning the influences of age, breed, sex and nutrition on peripheral circulating concentrations of hormones in cattle and the consequent effects they may have on performance. As a contribution to this field of study and with the final aim of using metabolic parameters obtained in calves to predict their future performance, total IGF-I, triiodothyronine (T3), insulin and cortisol were determined in calves kept under normal production conditions on a large farm. Possible associations of serum concentrations of these substances with growth rate were sought.

MATERIALS AND METHODS

Animals.

The experiment included all calves (Holstein-Friesian) born on 13 May Cattle Farm, Zemun between 11 and 31 May from which it was possible to take a blood sample before intake of colostrum. The group originally consisted of 47 animals (22 males and 25 females). They were fed colostrum twice and then 4 to 5 litres liquid milk daily for 15 days in individual boxes. A concentrate mixture (0.18 crude protein) based on ground maize meal and sunflower oilmeal was offered ad libitum. The calves were then kept in groups of 18 to 20 on straw and offered hay as well as concetrate.

Procedure

Blood samples were taken from the jugular vein on the day of birth and about 4.5 months later between 10.30 and 11.25 in early October when the calves were weighed and sent to the feedlots. The blood was allowed to coagulate and the serum separated by centrifugation. One animal of each sex died from natural causes (pneumonia) before reaching 4.5 months of age, one female calf was being treated for pneumonia at the time of the second collection and another female calf could not be identified, so four fewer sera were available at the second time interval. The remaining animals were clinically healthy.

Analyses

The hormones T3 and cortisol were determined by radioimmunoassay (RIA) using commercial kits in accordance with the instructions (INEP Diagnostics, Zemun). Insulin was measured using a heterologous RIA system which included ^{125}I-porcine insulin, rabbit antibodies to porcine insulin and standard solutions of bovine insulin (Nikolić, Ivanoska, Krainčanić, Marinković and Kostić, 1989).

IGF-I was determined after separation of binding proteins by the method of Daughaday and coworkers (Daughaday, Parker, Borowsky, Trivedi and Kapadia, 1982) with cryoprecipitation (Breier, Gallaher and Gluckman, 1991).

Since bovine IGF-I has the same aminoacid composition as human IGF-I (Simmen, 1991), recombinant human IGF-I (Serva Feinbiochemica, Germany) was used as the working standard and label. Applying the modification of the chloramine-T method described for insulin (Nikolić et al., 1989), IGF-I was labelled with radioactive iodine (^{125}I) to a specific activity of around 125 μCi/μg, i. e. an average of less than one labelled tyrosine per molecule. Using rabbit anti-IGF-I (UCB-Bioproducts, Belgium) as the reagent and polyethyleneglycol assisted ovine anti-rabbit immunoglobulin-G antibodies to separate bound and free fractions, a RIA system was set up and standardised against a reference preparation of IGF-I (WHO 87/518). This gave 101.2 (1.3)% mean (s.e.) of the expected values for IGF-I within the range of 0.081 to 1.625 nmol/L in direct analyses, 95.2 (3.3)% when passed through the extraction procedure alone and 97.3 (0.7)% when passed through the extraction procedure in the presence of calf serum (n = 3; one newborn, two at 4 months). Recovery in the presence of serum from the newborn calf with the lowest apparent concentration of IGF-I was 71%. Apparent values were used in all calculations. Extraction of a 1:1 mixture of calf serum and zero standard solution gave 98.8 (3.9)% of the expected value for IGF-I indicating parallelism.

Statistical analysis

Split plot two factor (age, sex) analysis of variance was carried out. Values for missing data at 4.5 months were calculated for one male calf and the degrees of freedom used in estimating the statistical significance of differences between means reduced accordingly. Simple correlation coefficients and multiple regression of hormone concentrations in relation to daily gain and final body weights were also calculated and analysed for the whole group (n = 43) for which complete data were available, as well as for males and females separately (n = 21; n = 22).

RESULTS

The mean rate of gain of the male calves was 50 g more than that of the female calves, but the difference was not statistically significant due to large differences between individual animals. Thus, daily weight gain ranged from 0.346 kg to 0.836 kg.

Mean values and ranges for body weight and hormone concentrations at both ages examined are given in Table 1. Mean neonatal levels of T3, cortisol, insulin and IGF-I were similar in male and female calves, the females tending to exhibit slightly higher insulin and IGF-I concentrations and slightly lower cortisol concentrations, the last difference being statistically significant (P = 0.05). At 4.5 months of age (range 4.3 to 4.9 months), T3, insulin and cortisol concentrations were lower, while total IGF-I levels were slightly greater than just after birth. The increase was statistically significant for the group of male calves, which achieved a higher mean value for IGF-I at this age than the group of females. Jovanović, Đurđević and Stojić (1982) found that T3 concentrations of calves at birth are twice the adult level, the decline to adult levels starting at 4 days and finishing at 4 weeks of age.

Table 1. Body weights and serum hormone levels in male and female Holstein calves just after birth and at 4.5 months of age

Sex	Age (mo)		Body weight (kg)	T3	Cortisol	Insulin	IGF-1
				(nmol/litre)			
F	0	Mean	38.1^{a}	3.05^{c}	24.8^{b}	0.225^{c}	8.8^{ab}
	4.5	Mean	116.9^{c}	2.18^{b}	21.1^{a}	0.098^{ab}	9.3^{b}
M	0	Mean	39.9^{b}	3.12^{c}	32.7^{c}	0.198^{bc}	8.2^{a}
	4.5	Mean	123.9^{d}	1.81^{a}	20.2^{a}	0.095^{a}	10.1^{c}
		s.e.	2.3	0.19	3.5	0.028	0.9

a, b, c, d
Mean values with different superscripts are significantly differentn ($P < 0.05$; error d. f. = 41)

Overall, no statistically significant individual correlations were found between neonatal concentrations of hormones and either daily gain or body weight reached at 4. 5. months of age. However, both body weight at this age an daily gain over the previous period were significantly correlated with concurrent IGF-I, T3 and insulin concentrations ($r=0.592$ $P < 0.01$; $r = 0.384$ and $r = 0.315$ $P < 0.05$ respectively for body weight); $r=0.554$ $P < 0.01$; $r = 0.364$ and $r = 0.343$ $P < 0.05$ respectively for daily gain).

The correlation coefficient matrices for male and female calves separately are given in Table 2. Statistically significant coefficients were found between neonatal T3 and cortisol levels and between final IGF-I concentrations and both daily gain and final body weight in each group. In addition, many other variables were significantly correlated in the male calves (Table 2), whereas only the association between insulin and IGF-I concentrations at 4.5 months old was statistically significant in the female group. Moreover, multiple regression analyses of final body weight and mean daily gain on all other nine parameters measured uncovered various statistically significant relations which differed markedly between the sexes (Table 3). Thus, in the 21 male calves for which complete data were available, standard partial regression coefficients of all the variables except T3 concentration at 4.5 months of age and postnatal cortisol concentration exhibited Studentn t-values of 1.82 and above, indicating independent effects. Combined together, these seven parameters accounted for 83% and 82% of the variance observed in the daily gain and final body weights of the male calves respectively ($R^2 = 0.831$ and 0.821). Thus, the apparently significant correlations between daily gain and both initial serum cortisol and final T3 levels in the male calves (Table 2), are not independent effects but related to the close correlations of these parameters with initial T3 and final IGF-I concentrations respectively.

Table 2. Correlation coefficiennt (r) matrix for the eleven parameters determined in male (below diagonal; n = 21) and female (above diagonal; n = 22) Holstein-Friesian calves

	$T3_0^+$	$T3_4^+$	Cor_0^+	Cor_4^+	Ins_0	Ins_4	IGF_0	IGF_4	Bwt_0	Bwt_4	Gain
$T3_0$		−0.30	0.63	−0.16	0.16	0.16	−0.42	0.15	0.32	−0.06	−0.24
$T3_4$	0.07		−0.15	0.20	−0.01	−0.16	−0.23	0.36	0.19	0.47	0.39
Cor_0	0.61	−0.12		0.11	−0.13	0.11	−0.29	0.33	0.19	0.15	0.15
Cor_4	−0.11	−0.09	0.17		0.33	−0.21	0.00	0.00	−0.27	0.04	0.14
Ins_0	0.06	−0.05	−0.24	−0.10		−0.22	−0.03	−0.08	−0.30	−0.31	−0.26
Ins_4	0.05	0.40	−0.09	0.16	−0.08		−0.16	0.48	0.00	0.24	0.25
IGF_0	−0.05	0.59	−0.32	−0.25	0.02	0.27		−0.17	−0.22	0.26	−0.13
IGF_4	−0.02	0.69	−0.10	−0.02	−0.14	0.36	0.56		−0.02	0.69	0.62
Bwt_0	−0.13	0.13	−0.22	0.09	0.24	0.03	0.28	0.11		0.12	−0.02
Bwt_4	−0.19	0.42	−0.51	−0.49	0.22	0.39	0.62	0.53	0.01		0.89
Gain	−0.19	0.46	−0.47	−0.44	0.24	0.43	0.63	0.52	−0.06	0.98*	

*Underlined values are statistically significant (r = 0.43; P = 0.05; r = 0.55; P = 0.01)
+$T3_0$, $T3_4$, Cor_0, Cor_4, etc, refer to the parameters measured at 0 and 4.5 months old respectively

Table 3. Multiple regression analysis of the independent variables determined on daily gain (a) and final body weight (b) of Holstein-Friesian calves (n = 43).

Category	Variable	a) Stand. Part. Regres. Coef.	s.e.	Probability	b) Stand. Part. Regres. Coef.	s.e.	Probability
Male	Bwt_0^+	−0.287	0.126	0.035			NS
	$T3_0$	−0.289	0.117	0.022			NS
	Cor_4	−0.375	0.126	0.007	−0.511	0.146	0.002
	Ins_0	0.343	0.121	0.010	0.261	0.145	0.087
	Ins_4	0.352	0.126	0.011	0.338	0.156	0.042
	IGF_0	0.351	0.154	0.033			NS
	IGF_4	0.265	0.146	0.084	0.437	0.155	0.011
	R^2	0.831		<0.001	0.673		0.001
Female	$T3_0$	−0.337	0.165	0.054			NS
	IGF_4	0.670	0.165	0.001	0.691	0.162	<0.001
	R^2	0.494		0.002	0.477		<0.001
Overall	$T3_0$	−0.236	0.124	0.064			NS
	Cor_0			NS	−0.241	0.116	0.044
	Cor_4	−0.254	0.124	0.047	−0.247	0,116	0.039
	IGF_4	0.561	0.123	<0.001	0.596	0.115	<0.001
	R^2	0.412		<0.001	0.484		<0.001

NS - Not statistically significant (P > 0.1)
* Abbreviations as in Table 2.

In contrast, very few of the measured parameters were associated with the rate of gain of the female calves (Table 3). Namely, only the standard partial regression coefficients of final IGF-I concentrations and initial T3 concentrations showed statistically significant Student t-values. These two variables were associated with 49% of the variance in daily gain (R^2 = 0.494).

When all 43 calves were treated as a single group, three variables contributed signifiantly to a multiple regression on gain (final IGF-I, final cortisol and initial T3 concentrations) but only 41% of the variance was associated with this regression (R^2 = 0.412). Similarly, Body weight at 4.5 months was related to a combination of three variables (final IGF-I, initial and final cortisol concentrations), which accounted for about half the variance (R^2 = 0.486).

DISCUSSION

The results obtained here for serum levels of IGF-I in neonatal calves correspond, in general, to those obtained by other authors, which ranged from around 3 nmol/1 (Groenewegen, McBride, Burton and Elsasser, 1990) to 14 nmol/1 (Baumrucker and Blum, 1994; Kerr, Laarveld, Fehr and Manns, 1991). Although it has been shown that IGF binding proteins are not completely removed from rat serum by any of the commonly employed extraction procedures, particularly binding protein-2 using acid-ethanol (Crawford, Martin and Howe, 1992; Rivero, Goya annd Pascual-Lerne, 1994), it seems that the procedure used in this investigation gave adequate results. Serum levels of binding protein-2 appear to be positively associated with growth inhibitory situations in swine (Dauncey, Rudd, White and Shakespear, 1993; Kampman, Ramsay and White, 1993), heifers (Armstrong, Cohick, Harvey, Heimer annd Campbell, 1993) and lacting cows (Vicini, Buonomo, Veenhuizen, Miller, Clemmons and Collier, 1991), so incomplete determination of total IGF-I in sera with relatively large concentrations of this binding protein would tend to ehance any relationship between apparent total IGF-I concentrations and body weight gain. On the other hand, the predominant binding protein (IGFBP-3) in serum is largely removed from the reaction mixture by acid-ethanol. The recoveries obtained here when a reference preparation of IGF-I was added to several calf sera support this proposition.

Certainly the results confirm that serum IGF-I levels appear to reflect growth rate in young cattle (Lund-Larsen et al., 1977; Groenewegen et al., 1990), even though birth weight of our calves was not correlated with IGF-I concentrations. The statistically significant relation found by Breier, Gluckman and Bass (1988) may be a reflection of the greater range in birth weight in their calves. Birth weight was not directly related with any of the parameters determined here, although it emerged as one of the variables with a statistically significant negative association with subsequennt gain in male calves. Although differences in the examined parameters described 83% of the variance associated with daily gain of these calves, the contribution of variables obtained at birth alone was not sufficiently large to allow prediction of subsequent growth, because they only accounted for 0.60 of the variance associated with daily gain.

In the female calves parameters other than those examined here appeared to have a dominant influence on body weight gain, possibly oestrogens. Thus, Elsasser and coworkers (1993) showed that perturbations in IGF-I regulation imparted by the administration of exogenous T3 could be partly overridden by use of the oestradiol-progesterone anabolic adjunct Synovex-S.

Kahl and Bitman (1983) reported a positive correlation between T3 concentration and body weight in dairy calves from 6 to 22 weeks of age, the males exhibiting higher T3 levels than the females. Similar mean hormone values but the reverse sex related difference were found here. It is possible that this may be related to differences in the timing of circadian and ultradian rhythms which have been studied in cows (Bitman, Kahl, Wood and Lefcourt, 1994) but not, it seems, in calves.

The detection of a significant negative association of cortisol levels in relation to body weight gain confirms the conclusion of other authors (Hafs, Purchas and Pearson, 1971; Trenkle and Topel, 1978; Trenkle, 1981) about the important influence of glucocorticoids on growth and carcass characteristics in cattle. Baumrucker and Blum (1994) mentioned cortisol concentrations of approximately 30 nmol/L in neonatal calves which are in the range of the values obtained here. Lower levels were reported for 4 month old Charolais and Angus steers (Trenkle and Topel, 1978), but this may be a breed difference because we recently found higher serum cortisol levels in Holstein-Friesian calves than in Charolais crosses at 15 days of age on the same farm (Nikolić, Šamanc, Damjanović, Ratković, Radojičić and Begović, 1995).

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KONCENTRACIJA NEKIH HORMONA I INSULINU-SLIČNOG FAKTORA RAST-I U SERUMU MUŠKIH I ŽENSKIH TELADI I NJIHOVA MOGUĆA POVEZANOST SA BRZINOM RASTA ORGANIZMA

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SADRŽAJ

Koncentracije insulina, kortizola, trijodotironina (T3) i insulina-sličnog faktora rasta (IGF-I) su određene u krvnom serumu muških i ženskih teladi holjštajnske rase, pre uzimanja kolostruma (0 meseci starosti) i ujutro pre hranjenja starosti od oko 4.5 meseci.

Dobijene vrednosti su bile slične kod oba pola osim što su nivoi kortizola (0 meseci) i IGF-I (4.5 meseci) bili viši a nivo T3 (4.5 meseci) niži kod mužjaka ($n = 21$) nego kod ženski ($n = 22$). Koncentracije insulina, kortizola i T3 su se snižavale, a koncentracije IGF-I povećavale sa starošću životinja. Pored toga, nađene su statistički značajne korelacije između neonantalnih nivoa T3 i kortizola, kao i između nivoa IGF-I i brzine prirasta kod oba pola. Statističke analize više faktornom regresijom su pokazale da su samo dva od devet ispitivanih parametara (neonatalni T3 i IGF-I pri starošću od 4.5 meseci) imala značajnu povezanost sa brzinom prirasta ženskih teladi ($R^2 = 0.495$). Međutim, na sedam od ispitivanih parametara zajedno otpala 83% varijanse za brzinu prirasta muških teladi ($R^2 = 0.831$). Međutim, udeo rezultata dobijenih odmah posle rođenja u celini nije bio dovoljno veliki da dozvoljava predviđanje budućeg prirasta pojedinih životinja, jer je na te parametre otpalo samo 60% varijanse vezane za brzinu prirasta u toku ispitivanog intervala.