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Original article

Insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) mRNA levels in relation to the gastrointestinal tract (GIT) development in newborn calves

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Abstract

The study was aimed at determining the age-depended changes in insulin-like growth factors 1 and 2 (**IGF-1** and **IGF-2**) mRNA levels in relation to the gastrointestinal tract (**GIT**) development in newborn calves. Twenty four male calves (5-day-old) were randomly allocated to 4 groups which were slaughtered at day 5, 12, 19 and 26 of their life, respectively. Tissue samples from the rumen, abomasum, duodenum, jejunum and liver were taken for analysis, and the level of IGF-1 and IGF-2 mRNA was determined using RT semi-quantitative PCR method. Both IGF-1 and IGF-2 mRNA level was the biggest on 5 day of life, significantly decreased up to day 12-19 in most of analyzed GIT parts, and started to increase thereafter. In the rumen the reduction in the amount of IGFs transcripts was associated with the reduction in papillae length and tunica muscularis thickness. The abomasum weight and tunica mucosa thickness increased from the 5 day up to 19 day of life, whereas abomasal IGF-1 mRNA level decreased together with calf's age up to 19 day of life, and the level of IGF-2 mRNA did not change. The reduction in IGFs mRNA level after 5 day of life in the duodenum (IGF-1 and IGF-2) and in the jejunum (IGF-1) was associated with reduction in villi length (duodenum and jejunum), and the increase of crypt depth (duodenum).

Key words: postnatal period, small intestine development, rumen development, gene expression

Introduction

Development of GIT is modulated by various endogenous and exogenous factors among which the insulin-like growth factor ligand system is considered to be especially important (Ontsouka et al. 2004). This system consists of three structurally related ligands which are insulin-like growth factor 1 (**IGF-1**), insulin-like growth factor 2 (**IGF-2**) and insulin (Polk and Barnard 1999). IGF-2 exerts its actions mostly during prenatal period and is responsible mainly for fetal growth and development (Georgieva et al. 2003,

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Ontsouka et al. 2004). Moreover, IGF-2, besides insulin, has been demonstrated to be involved in the mechanisms governing the intestinal epithelium differentiation in calves (Georgiev et al. 2003). IGF-1 in turn is an important mediator of tissues growth and differentiation mainly during the postnatal period (Hammon and Blum 2002). It influences enterocyte proliferation and maturation as well as morphology and functions of calf GIT (Georgiev et al. 2003, Roffler et al. 2003).

It was proven that the levels of IGFs in calf GIT changes within first week of life and may be modulated by the type of liquid feed, i.e. colostrum, milk or milk replacers (Hammon and Blum 1997, Cordano et al. 2000). High levels of IGFs in colostrum and whole milk indicate that they are important for growth and maturation of GIT (Hammon and Blum 2002). Knowledge about ontogenetical changes in IGFs levels with calves age may help to understand better the mechanisms of GIT development. Therefore, this study was designed to determine the age-dependent changes in IGF-1 and IGF-2 mRNA levels in relation to the weight and morphology of different parts of gastrointestinal tract in neonatal calves.

Materials and Methods

Animals and diets

The animal study protocol was approved by the Local Ethics Committee prior to the study. Twenty four clinically healthy male calves (Holstein or Holstein × Limousine) at the mean age of 5 days (±1) were randomly divided into four experimental groups (6 calves per group) which were slaughtered, respectively, on their day 5 (±1), 12 (±1), 19 (±1), and 26 (±1) of life. In order to simplify in subsequent text only mean age of calves in each experimental group will be used. The calves were kept in ventilated barn, in individual pens (1.5 × 1.2 m) with rubber floor covered with sawdust to avoid straw intake.

Before the onset of the trial all calves were routinely fed with colostrum (day 1-2 postnatal) and than with 2.5 L of whole milk (**WM**, day 3-4) given twice a day, without access to the starter diet or hay. After starting the experiment calves were fed individually with restricted volume of milk replacer (**MR**) and *ad libitum* starter diet (only animals slaughtered on day 12, 19 and 26). The MR (Primolac, Polmass S.A., Bydgoszcz, Poland; 220 g crude protein (**CP**)/kg of dry matter (**DM**) and 4.4 Mcal metabolizable energy (**ME**)/kg of DM) was mixed with warm water (about 40°C) in the ratio of powder to water of 1:9 (wt/wt) and fed twice a day from bucket with a teat. The daily dose of MR was stable during the whole experimental period and equalled to 10% of initial BW of the calf. Refusals of MR were recorded daily.

Commercial pelleted starter mixture (Concentrate KCJ, Polmass S.A., Bydgoszcz, Poland; 380 g CP/kg of DM and 3.0 Mcal ME/kg of DM) was mixed with whole corn grain (50/50; wt/wt) and offered as a starter diet (240 g CP/kg of DM and 3.2 Mcal ME/kg of DM) once daily, beginning on the first day of the experiment. Samples of feeds were collected weekly. They were then pooled and analyzed for DM, ash, CP, crude fat and crude fibre using standard procedures (AOAC, 2000). ME content was calculated based on NRC (2001) equations. The starter diet refusals were collected daily and samples from each calf were divided by sieving into starter mixture and corn grain for exact calculation of DM, CP and ME intake.

Measurements and observations

The calves were individually weighted on day 5 (start), 12, 19, and 26 (end) of the trial. The animal condition was scored at the start of the trial and then daily using five point health status scores: very good-5, very bad-1. Fecal score including fluidity (4 point scale: 1-normal, 4-diarrhea), consistency (5 point scale: 1-normal, 2-frothy, 3-mucous, 4-sticky, 5-hard-constipation) and smell (3 point scale: 1-normal, 3-disgusting) were controlled daily according to Larson et al. (1977). Every abnormal health condition such as fever, bronchitis as well as every veterinary treatment (antibiotic, electrolyte therapy) was documented. Calves with diarrhea were treated with a commercial electrolyte solution (Rehydrat®, Biowet Puławy, Puławy, Poland). Electrolyte therapy was initiated when fecal fluidity equaled to 3 or 4 and antibiotic therapy was initiated when rectal temperature was > 39.5°C.

Tissue sampling and analysis

Within 20 min after slaughter the abdominal cavity was opened, GIT removed immediately, measured for organ weight and size (rumen, abomasum, duodenum, jejunum and liver), and sampled for IGF-1 and IGF-2 mRNA measurements (rumen, abomasum, duodenum, jejunum and liver) and histology analysis (rumen, abomasum, duodenum, jejunum).

Histology and histometry analysis

One cm^2 whole thickness samples from the right side of the cranio-dorsal sack of the rumen, abomasum (near pylorus), mid duodenum, and mid jejunum were

immediately fixed in 4% buffered formaldehyde for 5 days and further stored in ethanol for preparation. The tissues were then embedded in paraffin. Serial histological sections of 5 µm thickness were stained with haematoxylin and eosine for morphometry analysis under light microscope. Morphometry analysis involved measurements of the rumen wall (rumen papillae length and width, muscle layer thickness) in 5 to 8 slides for each tissue sample. In each tissue sample, 30 rumen papillae and rumen muscle layer measurements were performed using optical binocular microscope (OLYMPUS BX 61, Warszawa, Poland) coupled via a digital camera to a PC computer equipped with a Cell^P (OLYMPUS) software. The same procedure was applied for measurements in the abomasum (tunica mucosa thickness), and duodenum and jejunum (villi length, crypt depth, tunica mucosa and tunica muscularis thickness).

IGF-1 and IGF-2 mRNA levels

The RT semi-quantitative PCR method was used to determine IGF-1, IGF-2 and ACTB mRNA levels. Whole thickness wall samples (0.5-1 cm^3) of the ruminal cranial dorsal and ventral sac, abomasal, duodenal, and jejunal as well as liver tissue were placed in RNA*later* (Sigma-Aldrich Corporation, St. Louis, Missouri, USA) right away after collecting to prevent RNA degradation. Unless otherwise stated, all reagents were delivered from Fermentas (Vilnius, Lithuania). Total RNA was extracted from samples (100 mg of examined tissues) according to the method described by Chomczynski and Sacchi (1987). Total RNA was dissolved in pure RNase free water (Promega, Madison, Wisconsin, USA) and kept at -80ºC. The RNA integrity was electrophoretically verified using ethidium bromide staining, whereas the RNA purity was measured by optical density (OD) absorption ratio OD_{260nm}/OD_{280nm}. Only samples with the ratio between 1.8 and 2.0 were taken to further analyses. Total RNA concentration was measured spectrophotometrically using Biophotometer (Eppendorf, Hamburg, Germany). Then, the mRNA was subjected to reverse transcription (**RT**) reaction (Thermocycler Eppendorf AG, Eppendorf, Hamburg, Germany) in order to obtain the first strand of complementary DNA (**cDNA**). Each of 20 µl RT reaction mixture contained 200 U of M-MuLV reverse transcriptase, 0.5 µg of oligo dT primer (IBB PAN, Warszawa, Poland), 1 mM of each dNTP, 20 U of Ribonuclease Inhibitor and 5 µg of total RNA.

Complementary DNA was subsequently used for PCR reaction with following primers: IGF-1 forward: 5'-GCCTGCGCAATGGAATAAAGTCCT-3'; IGF-1 reverse: 5'-TGGGCATCTTCAC-CTGCTTCAAGA-3' (Fleming et al. 2005); IGF-2 forward: 5'- TGGACACCCTCCAGTTTGTCTGT -3'; IGF-2 reverse: 5'-TCGGAAGCAACACTCT TCCACG AT -3' (Pfaffl et al. 2002); β-actin forward: 5'-GTCATCACCATCGGCAA TGAG-3'; β-actin reverse: 5'-AATGCCGCAGGATTCCATG-3' (Moore et al.; GenBank no. BC142413.1). The β-actin (**ACTB**) was taken as a reference gene.

The PCR reaction for the IGF-1 and IGF-2 contained PCR buffer (Invitrogen, Sao Paulo, Brazil), 0.65 U of Taq DNA polymerase (Invitrogen, Sao Paulo, Brazil), 0.2 mM of each dNTP, 0.12 µM of each primer (IBB PAN, Warszawa, Poland), 2.5 mM $MgCl_2$, 1 µl of cDNA and distilled water (Merial, Saint Priest, France) up to the total volume of 20 µl. For ACTB reaction mix contained: PCR buffer, 0.5 U of Taq DNA polymerase, 0.19 mM of each dNTP, 0.1 µM of each primer (IBB PAN, Warszawa, Poland), 2.4 mM $MgCl_2$, 1 µl of cDNA and distilled water (Merial, Saint Priest, France) up to the total volume of 20 µl. The sample without cDNA was used as a negative control. Amplification conditions for IGF-1 and IGF-2 were as previously described by Fleming et al. (2005).

The PCR products were run in a 2.0% agarose gel (Agarose Type I-A, Low EEO, Sigma-Aldrich Corporation, St. Louis, Missouri, USA), stained with ethidium bromide for visualization, and afterwards were semi-quantitatively determined from the density of the gel band using the Scion Image for Windows (Scion Corporation, Maryland, USA).

Statistical Analysis

All data were subjected to one-way analysis of variance using the GLM procedure of the SAS (SAS, 2002). The assumption of variance homogeneity among experimental groups was assessed using the Levene's test. The statistical model included calves age and breed as main variables. Additionally, the effect of calves age within groups (±1 day of life) was tested. It turned out to be not significant and was excluded from the model. Linear (**L**), quadratic (**Q**), and cubic (**C**) polynomial contrasts were used to test changes in gene abundance with calves age. To aid interpretation of these results, means from day 5 were compared to each subsequent mean using CONTRAST statement of the SAS. Data of mean BW and DM, CP and ME intake in the whole trial were separated using a Tukey t-test. The significance was declared at $P<0.05$ and tendencies at $P<0.10$. Data are presented as mean and SE.

Table 1. Anatomical and histological development of stomach – age-depended changes.

Item	Day of life				SE	Polynomial contrasts[1]		
	5	12	19	26		L	Q	C
Weight (%BW)								
Entire stomach	1.03	1.14	1.50**	1.51**	0.07	<0.01	NS	NS
Reticulorumen	0.39	0.37	0.58*	0.64**	0.04	<0.01	NS	NS
Omasum	0.14	0.15	0.19*	0.18	0.01	0.06	NS	NS
Abomasum	0.50	0.62	0.72**	0.65*	0.03	0.04	NS	NS
% of whole stomach weight								
Reticulorumen	38.6	32.9**	39.5	42.6	1.1	NS	0.02	0.08
Omasum	13.5	12.9	13.0	11.9*	0.4	0.09	NS	NS
Abomasum	47.9	54.2**	47.5	45.5	1.2	NS	0.02	0.07
Rumen – dorsal sac (μm)								
Papillae length	680	515	343**	314**	40	<0.01	NS	NS
Papillae width	98	161**	154**	150**	7	<0.01	0.05	NS
Tunica muscularis thickness	1958	1627	1551	1345**	84	0.04	NS	NS
Abomasum (μm)								
Tunica mucosa thickness	445	541**	534**	500*	13	NS	<0.01	NS

[1] L – linear, Q – quadratic, C – cubic; NS – non significant (P>0.10); *P<0.10, **P<0.05 as compared to 5 day of life

Results

Feed intake and body weight

Daily intake of DM, CP and ME/kg BW from MR fully covered calves needs and did not differ between groups (animals slaughtered on 12, 19 and 26 day of life, data not shown). Total intake of DM, CP and ME/kg BW with liquid and solid feed during the whole trial period also did not differ between experimental groups (data not shown).

Body weight at the beginning of the trial did not differ between groups (data not shown). Up to day 19 of age most of calves lost their BW and increase of BW was observed only between day 19 and 26. Starter diet intake increased with calf age in a group of calves slaughtered on the 19 and 26 day of life (data not shown).

Calves were generally healthy throughout the trial and required only few medical interventions. No statistical differences between groups in body condition score and fecal score were observed (data not shown).

Stomach development

Stomach, reticulorumen and omasum weight increased with calves age, especially beginning on 19 day of their life (L, P≤0.06; Table 1). Similarly, abomasum weight changed together with calves age. It increased up to day 19, and then slightly decreased (L, P=0.04).

The quadratic trend of developmental changes of reticulorumen and abomasum weights expressed as a percent of the whole stomach weight was shown (Q, P=0.02; Table 1.). The reticulorumen weight expressed as a % of the whole stomach weight decreased up to 12 day of life and increased afterwards, whereas abomasum weight expressed as a % of the whole stomach weight increased up to 12 day of life and then decreased.

Rumen papillae length decreased with animals age with pronounced reduction of their length beginning on 19 day of life (L, P<0.01; Table 1). On the other hand, rumen papillae widened up to 12 day of life and slightly narrowed afterwards (L, P<0.01; Q, P=0.05), but they were still wider as compared to their width on 5 day of life. Rumen tunica muscularis thickness decreased linearly with animals age (L, P=0.04).

Quadratic trend of developmental changes for tunica mucosa thickness was found in the abomasum. It increased up to 19 day of life and then slightly decreased (Q, P<0.01).

Small intestine development and liver weight

The weight of entire small intestine tended to increase (L, P=0.05; Table 2), and the weight of duodenum and jejunum increased together with calves age (L, P≤0.04), whereas ileum weight did not change in the examined period of animals life. On the other hand, the quadratic trends in developmental changes of the entire small intestine, jejunum and ileum lengths together with calves age were observed (Q, P≤0.02). The jejunum length decreased on 12 day of

Table 2. Anatomical and histological development of small intestine and liver – age-depended changes.

Item	Day of life				SE	Polynomial contrasts[1]		
	5	12	19	26		L	Q	C
Weight (% BW)								
Entire small intestine	2.06	2.14	2.38	2.43*	0.06	0.05	NS	NS
Duodenum	0.08	0.09	0.10	0.12**	0.01	<0.01	NS	NS
Jejunum	1.88	1.95	2.18	2.19*	0.05	0.04	NS	NS
Ileum	0.11	0.11	0.10	0.15	0.01	NS	NS	NS
Liver	2.05	1.63**	1.59**	1.63**	0.06	0.03	<0.01	NS
Length (cm x 10^2/kg BW)								
Entire small intestine	2934	2063**	2745	3064	142	NS	0.01	0.09
Duodenum	0.66	0.54	0.65	0.72	0.03	NS	NS	NS
Jejunum	2778	2339**	2623	2919	87	NS	0.02	NS
Ileum	0.99	0.59**	0.56**	0.86	0.08	NS	<0.01	NS
Mid duodenum (μm)								
Villi length	653	519**	583	541**	21	0.04	NS	0.03
Crypt depth	249	257	293**	257	6	NS	0.07	0.02
Tunica mucosa thickness	876	915	886	850	25	NS	NS	NS
Tunica muscularis thickness	319	352	449*	393	17	NS	NS	NS
Mid jejunum (μm)								
Villi length	504	406*	420*	392**	19	0.05	NS	0.04
Crypt depth	196	171	171	179	7	NS	NS	NS
Tunica mucosa thickness	686	597	636*	584*	31	0.08	NS	NS
Tunica muscularis thickness	233	315	325*	340**	16	<0.01	NS	NS

[1] L – linear, Q – quadratic, C – cubic; NS – non significant (P>0.10); *P<0.10, **P<0.05 as compared to 5 day of life

life and then increased to the length larger than observed on 5 day of life, and the ileum length decreased on 12 and 19 day of life and increased afterwards. No significant changes in the length of duodenum together with calves age were noticed.

Duodenal villi length decreased up to 12 day of life, then increased up to day 19, and again decreased, but their length on day 26 was higher than observed on day 12 (L, P=0.04; C, P=0.03; Table 2). Crypt depth increased up to 19 day of calves life and decreased afterwards (Q, P=0.07; C, P=0.02). Similar tendency of developmental changes in the duodenum was observed for tunica muscularis thickness, however it was not statistically proved. Tunica mucosa thickness did not change during the whole examined period of calves life.

The villi length in mid jejunum changed together with calves age showing the linear and cubic trend (L, P=0.05; C, P=0.04; Table 2). It decreased up to 12 day of life, then slightly increased on day 19, and again decreased to the lowest value on 26 day of life. On the other hand, tunica mucosa thickness tended to increase (L, P=0.08) whereas tunica muscularis thickness increased with calves age (L, P<0.01). The crypt depth in the mid jejunum did not change with calves age.

The liver weight decreased up to 19 day of calves life and then slightly increased (L, P=0.03; Q, P<0.01; Table 2).

IGF-1 and IGF-2 mRNA levels

The IGF-1 and IGF-2 mRNA level was found in all analyzed parts of GIT. Both IGF-1 and IGF-2 mRNA level was the highest on 5 day of life, significantly decreased up to day 12-19 in most of analyzed GIT parts, and started to increased thereafter.

The level of IGF-1 mRNA in the cranial dorsal sac of the rumen was the lowest on 12 day of life and did not change within next examined periods of life (L, P=0.04; Fig. 1). In the cranial ventral sac of the rumen, the level of IGF-1 mRNA decreased up to day 19, and then increased (L, P=0.02; Q, P=0.08). In the abomasum and duodenum IGF-1 mRNA level significantly decreased up to 19 day of life and then increased (Q, P≤0.01). Tendency towards quadratic trend in IGF-1 mRNA level with calves age was found in the jejunum (Q, P=0.09). The level in this part of GIT was the lowest on 19 day of life, and then slightly increased. In the liver, in turn, it decreased with calves age and was the lowest on 19 and 26 day of life (L, P=0.03). The level of IGF-1 mRNA on day 5 was the biggest in the dorsal sac of the rumen and the lowest in the liver, while on day 26 the level was the highest in the abomasum and the lowest in the liver. On the 12 and 19 day of life the IGF-1 transcript level was comparable between examined tissues.

There were no significant age-depended changes in the IGF-2 mRNA level in the cranial ventral sac of

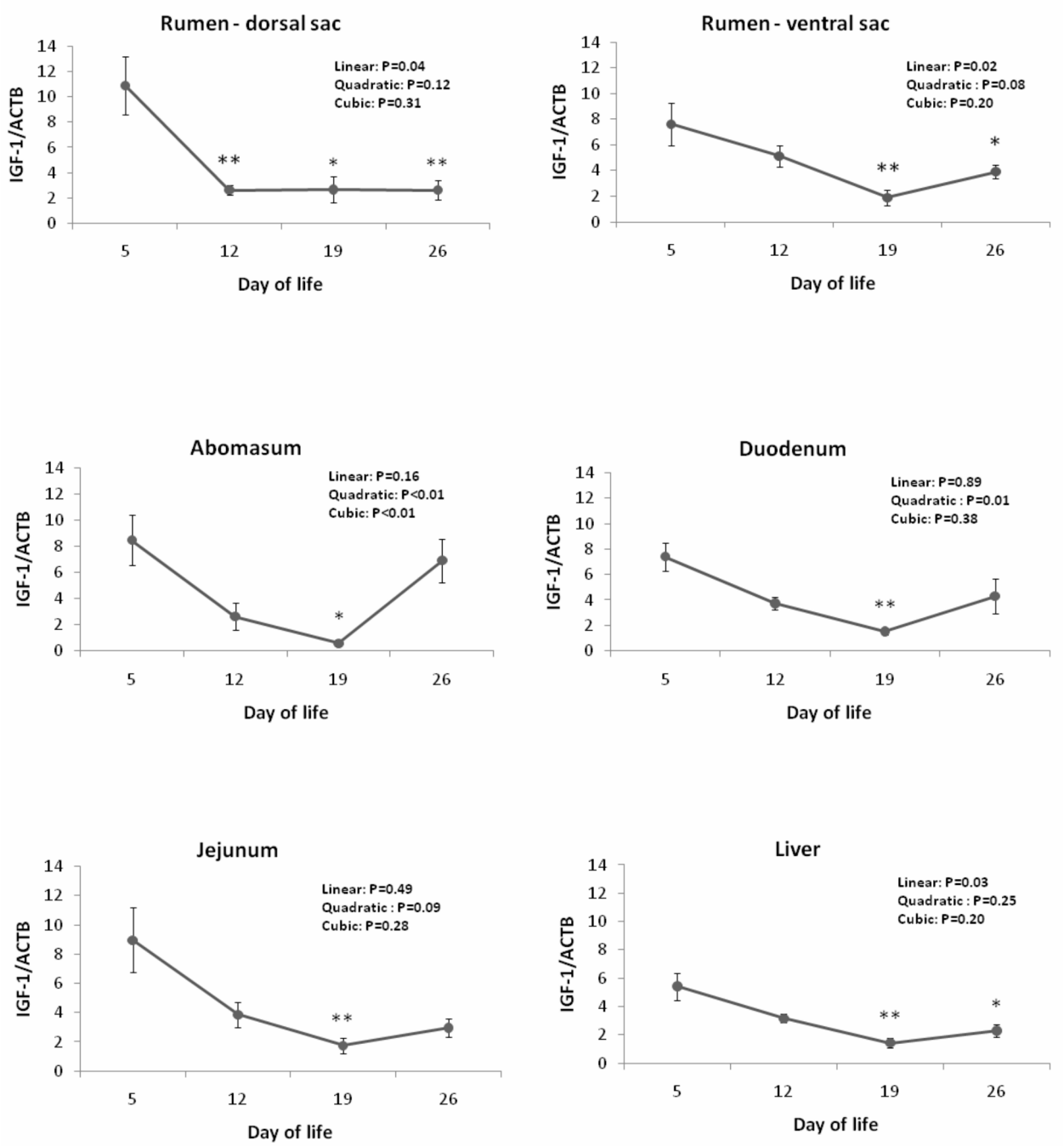

Fig. 1. Postnatal profile of IGF-1 mRNA level in calf gastrointestinal tissues from 5 to 26 day of life. *P<0.10, **P<0.05 compared to 5 day of life.

rumen and jejunum (Fig. 2). Tendency towards quadratic trend in the level was found in the cranial dorsal sac of the rumen (Q, P=0.10) where the amount of IGF-2 transcript tended to decrease up to day 12, and slightly increased afterwards. In the abomasum and duodenum the IGF-2 mRNA level changes also showed a quadratic trend (Q, P=0.04, P<0.01, respectively). In the liver, the IGF-2 transcript value decreased with calves age with the lowest value on day 12 and 19, and then increased on day 26 (Q, P=0.04 and C, P=0.08).

Discussion

Colostrum and to a lesser extent WM, contains a wide range of non-nutritional factors like hormones, growth factors, enzymes and others that support and regulate process of calf GIT development (Buhler et al. 1998, Blattler et al. 2001). Beside direct effect, the intake of bioactive components affects GIT development by regulation of local production of some growth factors, including IGF-1 and IGF-2 (Ontsouka et al. 2004). Thus, the highest IGF-1 and IGF-2

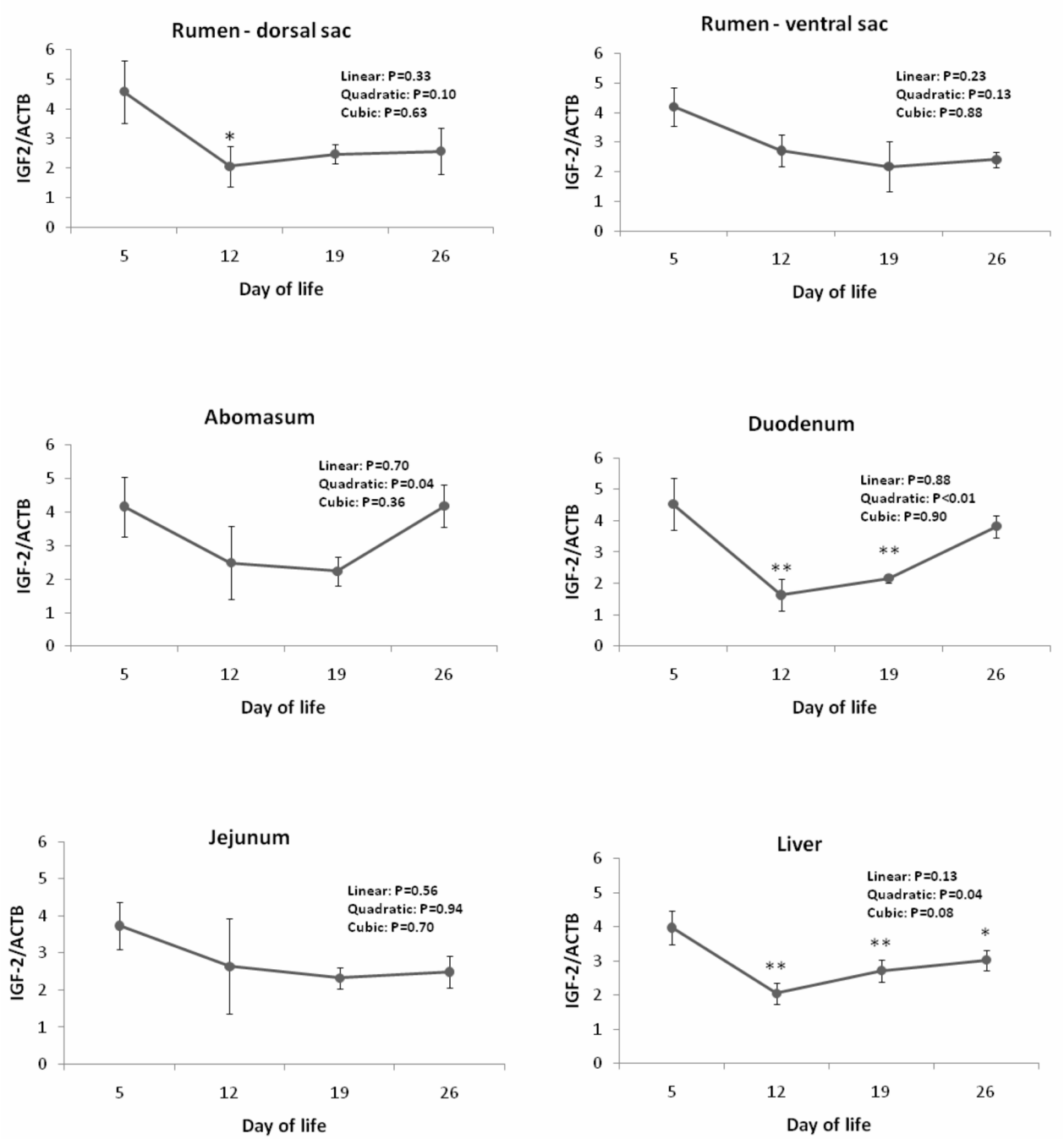

Fig. 2. Postnatal profile of IGF-2 mRNA level in calf gastrointestinal tissues from 5 to 26 day of life. *P<0.10, **P<0.05 compared to 5 day of life.

mRNA level on 5 day of life observed in our calves in all examined GIT fragments was not a surprise. It was a period of colostrum and milk consumption and thus intensive GIT development. MR intake that took place after day 5 of age, due to a lack of bioactive components and perhaps a presence of antinutritional factors, did not sustain optimal stimulation for GIT development, or even negatively affected this process in newborn calves what corroborates with earlier findings by Blättler et al. (2001). It may explain a decrease of IGFs mRNA level with calves age following switch from WM to MR. Furthermore, a negative effects of MR found in this study on calf GIT development may partially explain BW loss of calves. BW loss is often observed in newborn calves, especially when calves are shifted from colostrum or WM to MR (Le Huerou-Luron et al. 1992), what may be associated with stress, decrease of feed intake and thus negative energy balance. In our study, calves willingly consumed offered MR, and protein and energy require-

ments were fully covered. Thus BW loss was probably mainly associated with a change in nutrients source (WM to MR) and negative effect of offered MR on GIT development. It is also worth to notice that BW loss that was observed in most of calves from 5 to 19 day of life corresponded with changes in the IGFs mRNA level in the majority of investigated GIT tissues.

In the rumen, the reduction in the amount of IGFs transcript was associated with the reduction in papillae length and tunica muscularis thickness. Shen et al. (2004 and 2005) found that an increase of ruminal papillae length and width was associated with higher concentration of IGF-1 in plasma and higher IGF-1 receptor mRNA level in the rumen tissues in young goats but not in 4 years old bulls. Žitňan et al. (2005) found a positive correlation between plasma IGF-1 concentration and rumen epithelium development in newborn calves. In this study, IGF-1 mRNA level was the highest in the rumen. These observations indicate that IGFs, especially IGF-1, may have an important trophic effect on ruminal mucosa in bovine species at early postnatal stage. However, it is of interest that papillae width in the current study increased independently of IGFs mRNA level. Moreover, significant increase (from 19 day of calves life) of the solid feed intake, a factor considered as the main stimulator of forestomachs development (Tamate et al. 1962), did not stimulated papillae length and tunica muscularis thickness growth, although reticulorumen weight increased with calves age. It is known that solid feed intake may reduce papillae length as a result of its abrasive effect on rumen epithelium and desquamation of rumen epithelial cells (Greenwood et al. 1997, Beharka et al. 1998). However, in this study it seems that rather low solid feed intake was inadequate for stimulation of local or systemic IGFs concentration and thus stimulation of rumen epithelial cell proliferation and papillae length growth, as it was shown in young goats (Shen et al. 2004), and tunica muscularis development.

It is well known that during early postnatal period the stomach grows faster than the rest of the body (Xu 1996). According to the above, in our study, the abomasum weight and tunica mucosa thickness increased from 5 up to 19 day of life. Stimulation of cell growth and proliferation is usually associated with an increase of the IGF-1 gene expression. Surprisingly, in the current study abomasal IGF-1 mRNA level decreased together with calves age up to 19 day of life, whereas the level of IGF-2 mRNA did not change, what altogether may indicate a minor role of IGFs in the postnatal development of abomasum in calves.

The reduction in IGFs mRNA level after 5 day of life in the duodenum (IGF-1 and IGF-2) and in the jejunum (IGF-1) was associated with reduction in villi length (duodenum and jejunum) and the increase of crypt depth (duodenum). Slight changes in duodenal (IGF-1 and IGF-2) and jejunal (IGF-1) IGFs mRNA level after 19 day of life have not caused the change of intestinal villi length. However, crypt depth decreased. Ontsouka et al. (2004) found that IGF-1 mRNA level was negatively correlated with villus circumferences and heights (on 5 day of life) and positively correlated with crypt depth (on 1 and 5 day of life) in calves. On the contrary, in the studies on 64 day old calves, Velayudhan et al. (2008) did not found any correlation between histological parameters and the level of IGF-1 and its receptor mRNA level, although the higher level of IGF-1 in ileum was accompanied by the shortest villi. Based on this, we can assume that the role of IGFs is changing during the postnatal period but the current study does not allow to fully confirm this hypothesis. Observed reduction of villus size and an increase of crypt depth may also be a result of the offered diet. Milk-based formula fed calves had shorter jejunal villi and showed a tendency towards greater jejunal crypt depths than calves fed with colostrum, even when the milk replacer had a similar nutrient content as the colostrum (Buhler et al. 1998, Blattler et al. 2001). In this study, the IGF-2 mRNA abundance was being changed in the duodenum but not in the jejunum what may suggest IGF-2 has rather minor influence on the growth processes in this part of GIT.

In the current study, the level of IGF-1 transcript in the liver, the main place of its production, was surprisingly the lowest on 5 and 26 day of life in comparison to all examined tissues. Cordano et al. (2000) found that IGF mRNA level is the lowest in the liver of 8 day old calves in comparison to 4 month old veal calves, 1.5-years-old castrated bulls and 2-3 years old intact bulls. This observations indicate that in so young animals the organs, including liver, are not mature enough yet to deal with their biological functions and colostral and milk support and local tissue gene expression play a major role in governing GIT growth and maturation.

Levels of bioactive peptides like IGFs changes with the age during early postnatal stages of life what is connected with the gastrointestinal tract growth and development. This abundance may partly reflect the stadium of maturity of particular organs and tissues. However, in the first weeks of life, GIT development is in the delicate balance that can be easily disturbed. Access to the nutrients is of a special importance. In this study the local IGFs mRNA level decreased in all GIT compartments after diet switch from WM to MR. The alterations in IGFs mRNA abundance were accompanied by the morphological changes in several parts of GIT, showing that the role of the particular IGFs depends on the GIT segment.

Acknowledgments

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References

AOAC (**2000**), AOAC, Official Methods of Analysis. 17th ed., Association of Official Analytical Chemists, Washington, DC.

Beharka AA, Nagaraja TG, Morrill JL, Kennedy GA, Klemm RD (**1998**) Effects of form of the diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. J Dairy Sci 81: 1946-1955.

Blättler U, Hammon HM, Morel C, Philipona C, Rauprich A, Romé V, Le Huèrou-Luron I, Guilloteau P, Blum JW (**2001**) Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. J Nutr 131: 1256-1263.

Bühler C, Hammon H, Rossi GL, Blum JW (**1998**) Small intestinal morphology in eight-day-old calves fed colostrum for different durations or only milk replacer and treated with long-R3-insulin-like growth factor I and growth hormone. J Anim Sci 76: 758-765.

Chomczyński P, Sacchi N (**1987**) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159.

Cordano P, Hammon HM, Morel C, Zurbriggen A, Blum JW (**2000**) mRNA of insulin-like growth factor (IGF) quantification and presence of IGF binding proteins, and receptors for growth hormone, IGF-I and insulin, determined by reverse transcribed polymerase chain reaction, in the liver of growing and mature male cattle. Domest Anim Endocrinol 19: 191-208.

Fleming JM, Leibowitz BJ, Kerr DE, Cohick WS (**2005**) IGF-I differentially regulates IGF-binding protein expression in primary mammary fibroblasts and epithelial cells. J Endocrinol 186: 165-178.

Georgiev IP, Georgieva TM, Pfaffl M, Hammon HM, Blum JW (**2003**) Insulin-like growth factor and insulin receptors in intestinal mucosa of neonatal calves. J Endocrinol 176: 121-132.

Georgieva TM, Georgiev IP, Ontsouka E, Hammon HM, Pfaffl MW, Blum JW (**2003**) Abundance of message for insulin-like growth factors-I and -II and for receptors for growth hormone, insulin-like growth factors-I and -II, and insulin in the intestine and liver of pre- and full-term calves. J Anim Sci 81: 2294-2300.

Greenwood RH, Morrill JL, Titgemeyer EC (**1997**) Using dry feed intake as a percentage of initial body weight as a weaning criterion. J Dairy Sci 80: 2542-2546.

Hammon H, Blum JW (**1997**) The somatotropic axis in neonatal calves can be modulated by nutrition, growth hormone, and Long-R3-IGF-I. Am J Physiol 273: E 130--138.

Hammon HM, Blum JW (**2002**) Feeding different amounts of colostrum or only milk replacer modify receptors of intestinal insulin-like growth factors and insulin in neonatal calves. Domest Anim Endocrinol 22: 155-168.

Le Huerou-Luron I, Guilloteau P, Wicker-Palaquart C, Chayvialle JA, Burton J, Mouats A, Toullec R, Puigserver A (**1992**) Gastric and pancreatic enzyme activities and their relationship with some gut regulatory peptides during postnatal development and weaning in calves. J Nutr 122: 1434-1445.

Larson LL, Owen FG, Albright JL, Appleman RD, Lamb RC, Muller LD (**1977**) Guidelines toward more uniformity in measuring and reporting calf experimental data. J Dairy Sci 60: 989-991.

NRC (**2001**), NRC, Nutrient requirements of dairy cattle. National Academy Press. Washington D. C.

Ontsouka CE, Sauter SN, Blum JW, Hammon HM (**2004**) Effects of colostrum feeding and dexamethasone treatment on mRNA levels of insulin-like growth factors (IGF)-I and -II, IGF binding proteins-2 and -3, and on receptors for growth hormone, IGF-I, IGF-II, and insulin in the gastrointestinal tract of neonatal calves. Domest Anim Endocrinol 26: 155-175.

Pfaffl MW, Georgieva TM, Georgiev IP, Ontsouka E, Hageleit M, Blum JW (**2002**) Real-time RT-PCR quantification of insulin-like growth factor (IGF)-1, IGF-1 receptor, IGF-2, IGF-2 receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species. Domest Anim Endocrinol 22: 91-102.

Polk DB, Barnard JA (**1999**) Hormones and growth factors in intestinal development. In: Sanderson IR, Walker WA (eds) Development of the Gastrointestinal Tract, BC Decker Inc.: Hamilton, Ontario, pp. 37-55.

Roffler B, Fah A, Sauter SN, Hammon HM, Gallmann P, Brem G, Blum JW (**2003**) Intestinal morphology, epithelial cell proliferation, and absorptive capacity in neonatal calves fed milk-born insulin-like growth factor-I or a colostrum extract. J Dairy Sci 86: 1797-1806.

SAS Institute (**2002**), SAS Institute. 2002. The SAS System, Version 9.1, SAS Institute Inc., Cary, NC, USA.

Shen Z, Seyfert HM, Lohrke B, Schneider F, Zitnan R, Chudy A, Kuhla S, Hammon HM, Blum JW, Martens H, Hagemeister H, Voigt J (**2004**) An energy-rich diet causes rumen papillae proliferation associated with more IGF type 1 receptors and increased plasma IGF-1 concentrations in young goats. J Nutr 134: 11-17.

Shen Z, Kuhla S, Zitnan R, Seyfert HM, Schneider F, Hagemeister H, Chudy A, L°hrke B, Blum JW, Hammon HM, Voigt J (**2005**) Intraruminal infusion of n-butyric acid induces an increase of ruminal papillae size independent of IGF-1 system in castrated bulls. Arch Anim Nutr 59: 213-225.

Tamate H, McGilliard AD, Jacobson NL, Getty R (**1962**) Effect of various dietaries on the anatomical development of the stomach in the calf. J Dairy Sci 45: 408-420.

Velayudhan BT, Daniels KM, Horrell DP, Hill SR, McGilliard ML, Corl BA, Jiang H, Akers RM (**2008**) Developmental histology, segmental expression, and nutritional regulation of somatotropic axis genes in small intestine of preweaned dairy heifers. J Dairy Sci 91: 3343-3352.

Xu RJ (**1996**) Development of the newborn GI tract and its relation to colostrum/milk intake: a review. Reprod Fertil Dev 8: 35-48.

Žitňan R, Kuhla S, Sanftleben P, Bliska A, Schneider F, Zupcanova M, Voigt J (**2005**) Diet induced ruminal papillae development in neonatal calves not correlating with rumen butyrate. Vet Med – Czech 50: 472-479.