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, ...
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 routine-ly 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 KCI, 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 Pulawy, Pulawy, 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 cm² 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 eosin for morphometry analysis under light microscope. Morphometry analysis involved measurements of the rumen wall (rumen papilla 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 cm³) of the ruminal cranial dorsal and ventral sac, abomasal, duodenal, and jejunal as well as liver tissue were placed in RNAAlater (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 -80°C. The RNA integrity was electrophoretically verified using ethidium bromide staining, whereas the RNA purity was measured by optical density (OD) absorption ratio OD260nm/OD280nm. 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 μl of Ribonuclease Inhibitor and 5 μg of total RNA.

Complementary DNA was subsequently used for PCR reaction with following primers: IGF-1 forward: 5’-GCCTGCAGCATTTGGAATAGCTCT-3’; IGF-1 reverse: 5’-TGGGATCTATTACCAGCAGT-3’; IGF-2 forward: 5’-TGGACACCTCAGTTGCTCTGTT-3’; IGF-2 reverse: 5’-TCGAAACAGCATCTCTATCCAG AT -3’ (Fleming et al. 2005); β-actin forward: 5’-GTCATCACCATCGGCA GTAG-3’; β-actin reverse: 5’-AATCGGCAGATTCCATG-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 MgCl₂, 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 MgCl₂, 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.

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

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Weight (% BW)</th>
<th>Length (cm x 10^2/kg BW)</th>
<th>Mid duodenum (μm)</th>
<th>Mid jejunum (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day of life</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>Entire small intestine</td>
<td>2.06</td>
<td>2.14</td>
<td>2.38</td>
<td>2.43*</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.08</td>
<td>0.09</td>
<td>0.10</td>
<td>0.12**</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.88</td>
<td>1.95</td>
<td>2.18</td>
<td>2.19*</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>Liver</td>
<td>2.05</td>
<td>1.63**</td>
<td>1.59**</td>
<td>1.63**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire small intestine</td>
<td>2934</td>
<td>2063**</td>
<td>2745</td>
<td>3064</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.66</td>
<td>0.54</td>
<td>0.65</td>
<td>0.72</td>
</tr>
<tr>
<td>Jejunum</td>
<td>2778</td>
<td>2339**</td>
<td>2623</td>
<td>2919</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.99</td>
<td>0.59**</td>
<td>0.56**</td>
<td>0.86</td>
</tr>
</tbody>
</table>

1 L – linear, Q – quadratic, C – cubic; NS – non significant (P>0.10); *P<0.05, **P<0.01 as compared to 5 day of life

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
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 papilla length and tunica muscularis thickness. Shen et al. (2004 and 2005) found that an increase of ruminal papilla number 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 papilla number and 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 stomachs development (Tamate et al. 1998, Blattler et al. 2001), is connected with the gastrointestinal tract growth and 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 muscularis 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 villus 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


