



# Abundance of mRNA of the somatotrophic axis and insulin receptor in different layers of the jejunal and ileal walls of neonatal calves

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## Background

- The intestinal wall contains mucosal and submucosal layers that consist of structurally and functionally different enterocytes and of follicles of Peyer's patches (PP) and other gut-associated lymphoid tissues (GALT)
- The mRNA of IGF-1 and -2, of IGF type-1 and -type2 receptors (IGF-1R and IGF-2R), of insulin receptors (InsR), growth hormone receptors (GHR) and of IGF-binding proteins (IGFBP-1, -2 and -3) in calves are present in the entire gastrointestinal tract (GIT) and are influenced by GIT sites and nutrition

## Goals

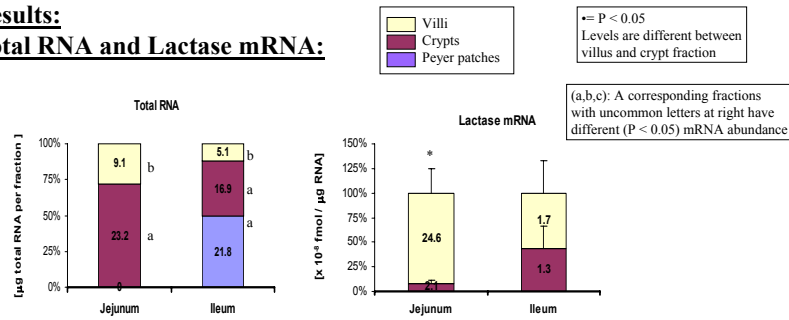
- Measure by RT-PCR the mRNA levels of IGF-1 and -2, IGFBP-2 and -3, IGF-1R, IGF-2R, IR and GHR in villus and crypt fractions (mucosa) and in PP containing fractions (submucosa) in jejunum and ileum
- Test the validity of cryostat sectioning for the isolation of villus, crypt and PP fractions in gene expression analysis in calves intestine

## Material and Methods

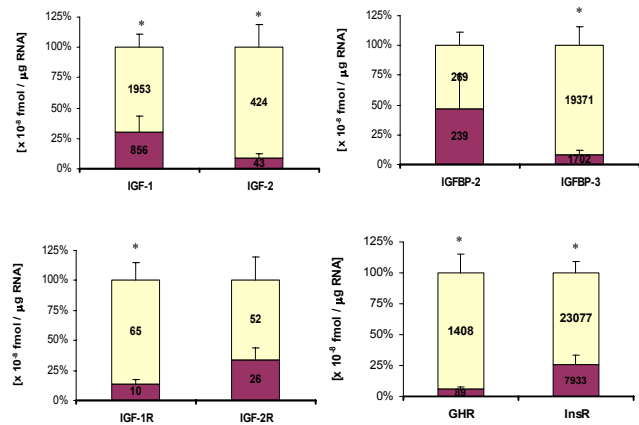
- Animals:** calves 5d old, fed colostrum, were euthanized by i.v. injection of 80 mg pentobarbital per kg body weight 60 min before they were injected with 500 mg of 5-bromo-2-deoxyuridine (BrdU) dissolved in 20 mL of saline.
- Tissue preparation:** Segments of jejunum and ileum were opened longitudinally and flattened onto the glass slides, serosa side down and frozen in liquid nitrogen. Pieces of 5x5mm<sup>2</sup> were cut and covered with supporting medium (O.C.T.). Morphologically identical slices were collected and combined as a fraction.
- Characterization and Evaluation of analysed fractions:**
  - The first and the last slices of a fraction were stained with haematoxylin eosin to confirm histological similarity
  - The slice before the last was used for cell proliferation assay based on the incorporation of BrdU into the DNA.
  - Measure of lactase mRNA levels
- Total RNA and cDNA synthesis:**
  - Total RNA was extracted from combined 12 slices (2 to 13<sup>th</sup>) using Trizol®, then one µg RNA was reverse transcribed into cDNA with 100 U of RNase-free H- reverse transcriptase (Gibco Life Tech.) with 100 µM random primers.

## Results:

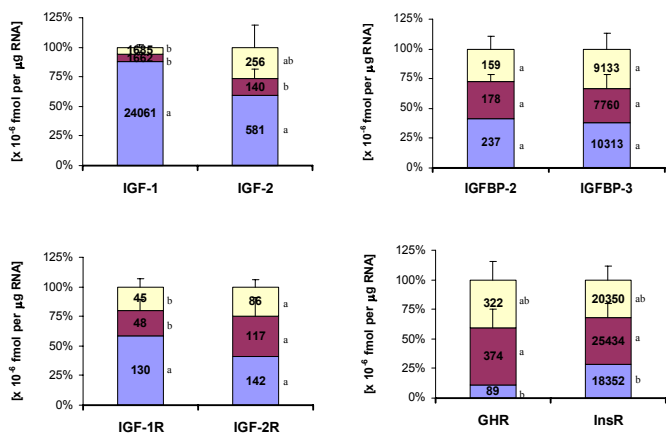
### Total RNA and Lactase mRNA:



### mRNAs of IGFs, IGFBPs, GHR, IGF-Rs and IR in jejunal fractions:



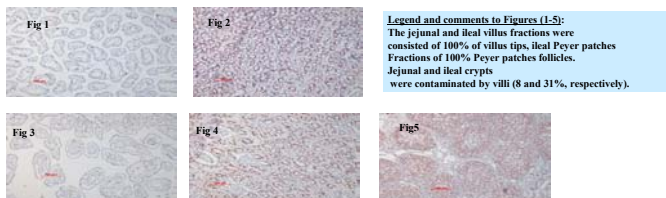
### mRNAs of IGFs, IGFBPs, GHR, IGF-Rs and IR in ileal fractions:



## PCR quantification: by means of external standard curves

- Standards were generated by PCR products after cloning into plasmids and transformation in *Escherichia Coli* (Pfaff MW, Georgieva TM, Georgiev IP, Ontsouka E, Hageleit M, Blum JW, DAE 2002; 22(2): 91-102)
- Lactase mRNA was quantified based on an standard curve generated by purifying the corresponding PCR products
- PCR efficiency calculated as  $E=1/10^{-\text{slope}}$  and was between 1.78 and 1.89

## Result: histomorphology of villus tips, crypts and Peyer's patches



## Comments to results:

- Amounts of total RNA in crypts were 2.5 times higher (P < 0.05) than in villus fractions (jejunum), and in PP were numerically (1.3 times) higher than in crypt fractions, but 4.3 times higher (P < 0.05) than in villus fractions
- In jejunum mRNA levels of IGF-1, IGF-2, IGF-1R, InsR, GHR and IGFBP-3 were higher (P < 0.05) in villi than in crypts
- In ileum, mRNA levels of IGF-1 were 20 times higher (P < 0.05) in PP than in villus and crypt fractions.

## Conclusions:

- Cryostat sectioning permits to isolate pure fractions of villus tips and Peyer's patches, but due to intrinsic morphology the crypt fraction was less pure
- mRNA of IGFs, IGFBPs and GHR, IGF-Rs and IR are variably expressed in different small intestinal layers
- Levels of measured traits in villus relative to crypt fractions in jejunum differed in part markedly from those in ileum
- The high levels of IGF-1 mRNA in Peyer's patches indicates an important role of IGF-1 for lymphocytes