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SHORT COMMUNICATIONS

THE EFFECT OF AGE AND THE TYPE OF LIQUID FEED ON THE INSULIN AND INSULIN RECEPTOR ISOFORMS mRNA EXPRESSION IN THE JEJUNUM OF NEONATAL CALVES

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ABSTRACT

The objective of the study was to determine the effect of age and the type of the liquid feed on the mRNA expression of insulin and two isoforms of its receptor (IR-A and IR-B) in the jejunum of neonatal calves. Thirty bull calves were randomly allocated to five experimental groups (6 animals per group). Calves from group 1 were slaughtered immediately after allocation to the trial at the age of 5 days. Calves from the group 2, 3 and 4 were fed milk replacer and slaughtered at week intervals (on 12., 19., 26. day of life, respectively). Calves from group 5 were fed whole milk and slaughtered at the 26. day of life. Groups 2, 3, 4 and 5 were offered starter mixture *ad libitum* during whole study period. Tissues from the jejunum were collected subsequently to slaughter. Expression of mRNA of insulin and insulin receptor isoforms were evaluated using the semi-quantitative RT-PCR method. Ins mRNA expression increased linearly together with calves age whereas IR-B mRNA expression showed the quadratic trend of changes. Type of liquid feed had no effect on the expression of investigated genes in jejunum of neonatal calves.

Keywords: age, feed, insulin, jejunum, calves

INTRODUCTION

The insulin (Ins) is the one of the key regulatory peptides that influences on the metabolism of all groups of nutrients: proteins, lipids and carbohydrates **(Liu *et al*., 2009)**. Ins binds to the specific receptor which was shown to be alternatively spliced in the tissue-specific manner **(McGrattan *et al*., 2000)**. Process of alternative splicing leads to the generation of shorter (IR-A, devoid of exon 11) and longer isoform (IR-B) of insulin receptor. Isoforms differ also in binding affinity for the Ins (IR-A > IR-B) **(McGrattan *et al*., 1998)**. Despite of numerous research, the role of Ins in the gastrointestinal tract development is not clear. We presumed that this role changes with age and so changes the mRNA expression of Ins and both insulin receptor isoforms. Thus, the primary objective of the current study was to determine the effect of age on the mRNA expression of insulin and two isoforms of its receptor (IR-A and IR-B) in the jejunum of neonatal calves.

Milk replacers (MR), popularly used in calf rearing instead of whole milk (WM), due to the hard conditions of the production processes, are lacking in the bioactive peptides such as Ins **(Seegraber and Morrill, 1986; Montagne *et al*., 1999)**. Bioactive components of the colostrums and WM play an important role in whole organism development, especially intestines, during the first month of calf's life. Hence we hypothesized that the lack of bioactive substances in the MR will influence on the mRNA expression of Ins and its receptor isoforms. In order to examine this issue, we analysed the mRNA expression of Ins and both insulin receptor isoforms in the jejunum of calves fed MR or WM.

MATERIAL AND METHODS

Thirty 5 day old bull calves were randomly allocated to five experimental groups (6 calves per group). Up to 5 day of life all animals were fed colostrum and WM. Calves from group 1 were slaughtered immediately after allocation to the trial. Calves from group 2, 3 and 4 were fed with MR and slaughtered at week intervals (on 12., 19., 26. day of life, respectively). Calves from the group 5 were fed WM and slaughtered at the 26. day of life. The liquid feed dose (MR or WM) was stable during the whole trial and amounted 10% of initial body weight of each calf. Starter mixture was offered *ad libitum* to group 2, 3, 4 and 5 during whole study period.

Whole thickness tissue samples from the middle jejunum were taken for analyses promptly after slaughter. Total RNA isolation was carried out according to procedure

described by **Chomczyński and Sacchi (1987)** and subsequently subjected to the reverse transcription reaction. Expression of Ins **(Figliuzzi *et al.*, 2008)** as well as IR-A and IR-B **(Pfaffl *et al.*, 2002)** mRNA was evaluated using a semi-quantitative RT-PCR method whereas the β-actin (ACTB) mRNA expression was taken as a housekeeping gene **(Moore *et al.*, data not published, GenBank no. BC142413.1)**. PCR products were run in a 3.0% agarose gel which were previously stained with ethidium bromide. The density of the gel band was evaluated using the Scion Image for Windows (Scion Corporation, Maryland, USA).

Two independent statistical models were used for statistical analysis (SAS 8.01). In the first one a one-way analysis of variance and preplaned polynomial contrasts were used to investigate linear (L), quadratic (Q) and cubic (C) changes in the mRNA expression with calves age. Moreover, the comparison between the 12., 19. and 26. day with reference to the 5. day of life was conducted using orthogonal contrasts. In the second statistical model, to evaluate the effect of the type of the liquid feed (MR vs WM), data obtained for groups 4 and 5 were subjected to a one-way analysis of variance. The significance was declared at $P<0.05$ and tendencies at $P<0.10$.

RESULTS AND DISCUSSION

Ins mRNA expression increased together with calves age, showing the linear trend of changes ($P=0.04$) and reaching the maximal value in the 26 day of life ($P\leq0.05$; Figure 1). These changes were accompanied by the alterations in the IR-B mRNA expression (Q: $P=0.06$; Figure 3) whereas IR-A mRNA expression did not change (Figure 2). Expression of IR-B transcript decreased during the first two weeks of the trial (from 5 to 19 day of life) and then increased to the value similar to observed in the 5 day of life.

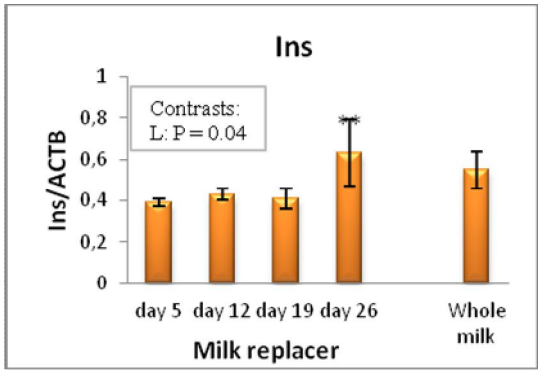

Figure 1 Insulin mRNA expression in relation to β-actin mRNA expression in the jejunum of examined groups of calves; $^{**}P\leq0.05$ in comparison to 5 day of life

There was no significant difference between the levels of all three kinds of transcripts in the groups of calves fed different types of liquid feed (MR vs WM; Figure 1-3).

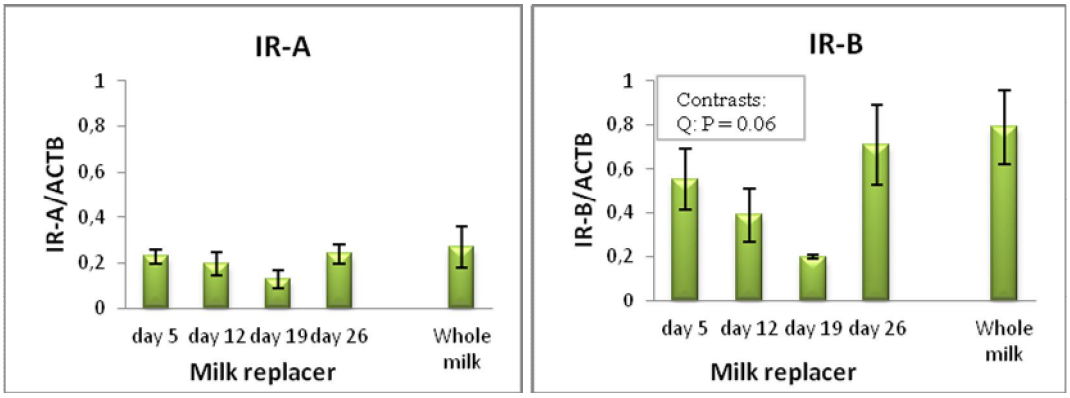

Figure 2 and 3 Insulin receptor isoforms (IR-A and IR-B, respectively) mRNA expression in relation to β-actin mRNA expression in the jejunum of examined groups of calves

It was shown that the liver of neonatal calves is not mature enough to deal with the biological functions and still needs maturations **(Cordano *et al.*, 2000)**. Hence increasing local expression of Ins in the jejunum may support the regulation of metabolic processes in the enterocytes.

Results obtained for Ins receptor isoforms are difficult to discuss because most of the studies concerning insulin receptor treat it like a one transcript or peptide. Existence of two different isoforms was discovered relatively recently. However, considering the fact that IR-A isoform has greater binding affinity to Ins, our results suggest that the role of Ins in the development of this part of calf gastrointestinal tract may be insignificant and Ins influence on the intestinal cells may be modulated only by the number of IR-B receptors. Though, in order to confirm this hypothesis, further studies are required, including the investigation of the IR-A and IR-B protein level in the jejunum, since it is a well known fact that the level of protein not always reflects the level of transcript.

CONCLUSION

The Ins and IR-B mRNA expression in the calf jejunum changed together with calves age. A type of liquid feed had no effect on the expression of Ins, IR-A and IR-B in this part of calf gastrointestinal tract.

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