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## Effects of CCK and gastrin on pancreatic secretion in vivo in the calf

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**Influence of fish oil supply on diet digestibility and ruminal metabolism in cows.** M Doreau, Y Chilliard (*Laboratoire sous-nutrition des ruminants, Inra, Theix, 63122 Saint-Genès Champanelle, France*).

Lipid supplementation often decreases diet digestibility in ruminants and leads to a shift of ruminal fermentations towards a propionate production. The digestive effects of fish oil intake are not well known and have been studied in two trials on lactating Holstein cows receiving diets made of maize silage and concentrates (65 and 35% on dry matter basis, respectively).

In trial 1 (six cows, 3 × 3 Latin square), a control diet (C1) was compared to diets supplemented with 200 (FO1) or 400 (FO2) mL/day fish oil of Menhaden type, given per os. Organic matter digestibility (OMD) was higher ( $P < 0.01$ ) for diet FO2 (73.5%) than for diet C1 (69.2%), diet FO1 being intermediate (72.2%). Neutral detergent fibre digestibility (NDFD) was higher ( $P < 0.05$ ) for diet FO2 (47.3%) than for diet C1 (42.7%), diet FO1 being intermediate (46.2%).

In trial 2 (six cows fitted with ruminal and duodenal cannulas, 3 × 3 Latin square), a control diet (C2) was compared to diets supplemented with 300 mL/day fish oil continuously infused in the rumen (FOR) or in the duodenum (FOD). Fish oil ruminal supply increased ( $P < 0.05$ ) OMD and NDFD (OMD: 72.6, 75.5 and 74.2% for treatments C2, FOR and FOD; NDFD: 59.4, 64.5 and 60.2 for treatments C2, FOR and FOD). Ruminal fish oil infusion increased ( $P < 0.01$ ) the proportion of propionate (C2, 18.8%; FOR, 23.9%) at the expense of acetate (C2, 65.0%; FOR, 60.0%) in ruminal volatile fatty acids. With treatment FOR, in which fatty acids (FA) from fish oil represented more than 60% of FA supply, FA reaching the duodenum comprised 0.33% of C20:5n-3 and 0.51% of C22:6n-3, whereas fish oil contained 21.7 and 7.3% of these acids; however the saturation pro-

cess was uncomplete (0.45% of C20:0 and 0.60% of C22:0) and many intermediate compounds were found. Moreover a high proportion (36.0%) of octadecenoic FA, of trans configuration for a major part, was found, suggesting a change in the hydrogenation process of dietary 18-carbon polyunsaturated FA.

These trials showed an unexpected positive effect of fish oil on diet digestibility and an extensive hydrogenation of 20- and 22-carbon FA.

**Effects of CCK and gastrin on pancreatic secretion in vivo in the calf.** G Le Drean, I Le Huerou-Luron, M Gestin, D Fourmy, P Guilloteau (*Laboratoire du jeune ruminant, Inra, 65, rue de Saint Brieuc, 35042 Rennes cedex, France*).

In the calf, beyond the first month of age, pancreatic CCK-B/gastrin receptors [with similar affinities for cholecystokinin (CCK) and gastrin] are predominantly expressed compared to pancreatic CCK-A receptors [Le Meuth et al (1993), *Endocrinology* 133, 1182-1191]. The only other species in which this particularity is observed is man. The effects of CCK and gastrin perfusions were examined on pancreatic secretion in vivo in the calf. The experiment was carried out on seven milk-fed Holstein calves aged between 2 and 4 months and fitted with two catheters (pancreatic duct and duodenum). The pancreatic juice was continuously collected and simultaneously reintroduced after sampling. The study, which was carried out during the interdigestive period (4.5 to 7.5 h after meal), was divided into six successive periods: basal (15 min), perfusion into the jugular vein of saline (1 mL/min; 30 min) and of saline containing CCK-9 or gastrin 13S (30 pmol/min/kgPV; 45 min) and post-perfusion (3 × 30 min). Since the pancreatic juice and protein flow as well as the protein concentration were similar for the two first periods (*t* test), they were put

together to constitute a reference period. CCK perfusion led to an increase of 50% in the protein concentration and of 80% in the protein flow ( $P < 0.05$ ), whereas the juice flow did not change significantly. Gastrin perfusion induced an increase of 90% in the protein flow and concentration and a decrease of 40% in the juice flow ( $P < 0.05$ ). The protein concentration and flow remained high (+90%,  $P < 0.05$ ) over the 30 min following gastrin perfusion but not CCK perfusion.

In conclusion, under our experimental conditions, gastrin was shown to stimulate pancreatic protein secretion in a more substantial and durable way than CCK. Since Le Meuth et al (1993) have described CCK-B/gastrin in calf from 1 month of age, it would be of a great interest to study pancreatic secretion by blocking these receptors with specific antagonists. It would be then possible to characterize the functional role of pancreatic CCK-B/gastrin receptors in the calf. The decrease in the juice flow might be due to the action of gastrin, unmediated by secretin. However, further investigations are required to precise the mechanism implicated. (This work was supported by the Region Bretagne).

**Regulation of bovine pancreatic elastase II expression during ontogeny.** M Gestin, I Le Huerou-Luron, G Le Drean, P Guilloateau (*Laboratoire du jeune ruminant, Inra, 65, rue de St Briec, 35042 Rennes cedex, France*).

In newborn infants, a deficiency in the proteolytic function in the digestive tract could partially account for intolerance to some milk proteins. Pancreatic elastase II may be responsible for this deficiency. In the calf, the specific activity of this hydrolase is high at birth but decreases sharply during post-natal development [Gestin et al (1996), *Reprod Nutr Dev*, n° 6]. This evolution has also been observed for other enzymes which

play an important role in milk digestion. In order to determine the regulation level of elastase II expression during ontogeny, we quantified the mRNAs of this enzyme using RT-PCR in six groups of milk-fed calves aged 0, 2, 7, 21, 28 and 119 days. On the basis of the cDNA sequences of several species, two oligonucleotides corresponding to identical regions were chosen. These two primers bind to the bovine cDNA regions +42 to +62 and +787 to +804, which enabled us to obtain a PCR product with a size of 763 pb. Preliminary studies were carried out to check the existence of a linear relation between the amounts of matrix-cDNA and the products of amplification. The mRNAs were quantified by extrapolating the intensity of the signal of the amplicon against a standard curve. This curve is drawn on the basis of successive dilutions of a determined quantity of specific elastase II cDNA. The expression rate of bovine elastase II specific mRNAs does not significantly change with age (*Kruskal-Wallis's test*) although it tends to increase. Indeed, the minimum and the maximum values are observed respectively in calves aged 0 day ( $85.8 \pm 18$  fg/ $\mu$ g of total RNA) and 119 day ( $197.6 \pm 47$  fg/ $\mu$ g of total RNA). This evolution differs considerably from that of elastase II activity, which decreases by 96% between these two stages. A mainly post-transcriptional regulation of bovine elastase II mRNAs could therefore account for the existence of these non parallel profiles. (This work has been supported by the Region Bretagne and the CNIEL).

**Amylase in *Pecten maximus* (mollusca, bivalve): protein and cDNA characterization.** S Le Moine<sup>1</sup>, D Sellos<sup>2</sup>, J Moal<sup>1</sup>, JY Daniel<sup>1</sup>, F San Juan<sup>3</sup>, JF Samain<sup>1</sup>, A Van Wormhoudt<sup>2</sup> (<sup>1</sup>*Ifremer, centre de Brest, BP 70, 29280 Plouzané*; <sup>2</sup>*Collège de France, BP 225, 29182 Concarneau, France*; <sup>3</sup>*Universidad de Vigo, Apto 874, 36200 Vigo, Spain*).