PRELIMINARY TRIAL OF AN EX-VIVO MODEL OF PORCINE INTESTINAL SACS TO STUDY THE EFFECT OF MEAT PEPTIDES ON IRON ABSORPTION.

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INTRODUCTON

Iron deficiency and iron deficiency anemia are some of the most common micronutrient deficiencies in the world. The supplements classically prescribed for iron deficiency (ferrous sulfate, gluconate or fumarate) are only poorly absorbed in healthy patients and in patients with inflammatory bowel disease the absorption is even lower. [1]. Therefore, large doses are given, which cause unpleasant side effects. It has been shown previously that meat proteins increase the absorption of iron from a phytate-rich meal in humans [2]. Experiments with intestinal cells have shown that peptides of salt-soluble myofibrillar proteins are most efficient in promoting iron uptake [3]. However, monocultures of intestinal enterocytes are overly simple models of absorption the gut. The objective of this work is to develop a more realistic model of iron absorption using sacs of pig duodenum *ex vivo*, and to examine the effect of meat protein peptides on the uptake of iron from ferrous gluconate in this model.

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MATERIALS AND METHODS

Preparation of meat peptides: Commercially available bovine *M. Longissimus thoracis* was obtained at 48 h post-mortem and stored until 7 days post-slaughter at 4 ° C. The muscle was minced and homogenized with distilled water (4 ° C) to removed sarcoplasmic proteins. The myofibrillar proteins in the insoluble fraction were extracted with 0.3 M NaCl solution (0.3 M), separated from insoluble proteins by filtration and re-precipitated by salting out in distilled water (20: 1; v: m) for 24h at 4°C. The salt-soluble proteins were redissolved in 0.3 M NaCl with 40 µM Ferrous Gluconate and hydrolysed with pepsin for 1 h, at 37 ° C, pH 2.0. The efficiency of hydrolysis was evaluated by SDS-PAGE. The protein content of the hydrolysate was quantified by the Bradford method [4] and the concentration of Fe^{+2} quantified by a colorimetric method [5]

Preparation of intestinal sacs: The initial 1.5 m portion of pig duodenum was obtained from commercially available animals at slaughter. The sample was washed, immersed in Eurocolins buffer and immediately transferred to the laboratory at 4 C. In the laboratory, 15 cm sections of the duodenum from known positions were made into closed sacs by tying their ends with cotton thread and 20 mL of Tyrode's buffer placed inside, either with 40 µM free ferrous gluconate (GluFe), 40 µM ferrous gluconate with peptides (Pep Fe) or without ferrous gluconate or peptides (control). Each intestinal sac was then incubated in 200 mL of Tyrode's buffer at 37 ° C with 95%O_2 / 5%CO_2 for 90 minutes with constant agitation. Samples (1 mL) of the fluid within each sac were taken at 0, 30 and 90 min, with replacement of the sample with fresh Tyrode's solution containing glucose. The viability of each preparation was determined by monitoring the decrease in glucose concentration in the internal medium. A commercial glucose kit (Wiener labs) was used to measure the amount of glucose. After 90 minutes, the sacs were opened, and the intestinal mucosa obtained by scraping, was homogenized in Tyrode's buffer (1:2 p/v) and centrifuged at 800 x g for 10 min. The supernatant was used for the determination of iron in the mucosa by means of the colorimetric method of Riemer et al [5]

RESULTS AND DISCUSSION

Viability of intestinal sacs: Glucose absorption. A decrease in glucose concentration with time was seen in the internal environment of all duodenal sacs (fig.1.) In the control sacs, this indicated that preparations were still metabolically functional during the experiment. In comparison with the control sacs, glucose usage tends to be lower in sacs containing ferrous gluconate (GluFe) or ferrous gluconate plus meat peptides (PepFe), indicating that the presence of iron altered the metabolism of the tissue.

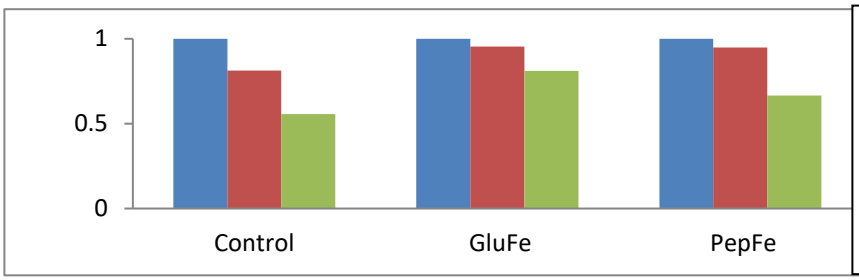

Fig.1. Average glucose concentration within the duodenal sacs expressed, as a proportion of the starting concentration, at 0 min (blue), 30 min (red) and 90 min (green). Control= no added FE^{2+} or peptides; GluFe = added FE^{2+} only; PepFe = added FE2+ plus meat peptides

Iron absorption: Fig.2 shows the iron content in the mucosal layer of the gut after 90 mins. The intestines incubated with ferrous gluconate (GluFe) alone and those incubated with ferrous gluconate with peptides (PepFe) showed an increased absorption of iron compared to the control. In the first 90 cm of the intestine the absorption of iron is greater when meat peptides are present. In the subsequent 45 cm (the proximal jejunum) absorption from ferrous gluconate alone was increased and was similar to absorption from ferrous gluconate plus peptides. This preliminary result may indicate that iron absorption in the proximal small intestine (duodenum) is different than in more distal (jejunal) regions.

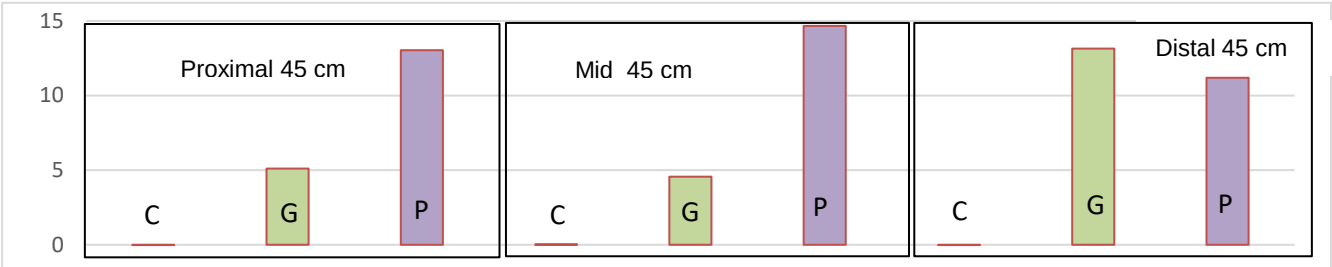

Fig.2. Iron concentration (left axis, μM) after 90 min in mucosa of intestinal sacs containing buffer only (C), buffer plus ferrous gluconate (G) or buffer plus ferrous gluconate plus meat peptides (P). Results from preparations in the first 45 cm of the intestine (proximal), second 45 cm (mid) and third 45 cm (distal) of the intestine shown separately.

CONCLUSION

This intestinal bag technique allows the monitoring of absorption processes in the presence of all tissue components, while maintaining the integrity of the tissues. Preliminary results from this assay demonstrate its potential to observe the absorption of iron by pig intestine and how this is affected by the presence of peptides from meat proteins. In the first 90 cm (duodenum), meat peptides appear to potentiate iron uptake. Improvement and further testing of this technique will allow the development of new supplements containing bioactive peptides from meat which may be valuable solutions to health problems due to deficiency of this mineral.

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