The effectiveness of enzymatic replacement therapy measured by turbidimetry and the lipaemic index in exocrine pancreatic insufficient young, growing pigs, fed a high-fat diet

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ABSTRACT

Purpose: Conventionally, the management of exocrine pancreatic insufficiency (EPI) involves the consumption of a specific diet as well as the replacement of pancreatic enzymes, the effectiveness of which is usually measured by a classical method of blood analyses of non-esterified fatty acids (NEFA) and triglycerides (TG). Dietary supplementation with a pancreatic enzyme preparation (PEP), in conjunction with a high-fat diet, on growth performance, digestibility and absorption (analysed using turbidimetry) of dietary fat in pigs with EPI was investigated.

Materials/Methods: EPI was developed by surgical ligation of the pancreatic duct of six male pigs, 6 weeks of age. The pigs were fed a high fat diet (twice daily). A PEP containing 1800 mg entercoated pancreatin was included in the high fat meals. Blood, urine and faecal samples were collected. The urine and faeces were analysed for dry matter, crude protein and fat content. The lipaemic index and plasma lipid profiles were assessed.

Results: EPI completely stopped growth of the pigs. Treatment with PEP significantly increased (P<0.05) growth and body mass as well as the digestibility of dry matter and crude protein. PEP significantly improved the coefficient of fat absorption, the lipaemic index (measured by turbidimetry methods) and caused significant changes in plasma nonesterified fatty acids and triglyceride concentrations.

Conclusions: The short term enzymatic replacement therapy together with a high fat meal has immediate beneficial effects on diet digestibility and on the growth retardation observed in EPI pigs. The turbidimetry method used to measure lipaemic index is a reliable, quick and efficient technique in measuring plasma lipid profiles and thus a good tool for assessing fat absorption.

Key words: exocrine pancreatic insufficiency, turbidimetry, pigs, growth, digestibility, high-fat diet

INTRODUCTION

Exocrine pancreatic insufficiency (EPI) refers to a state of impaired pancreatic secretion, usually resulting from the destruction of the pancreatic acinar tissue, either through an inflammatory process or the progressive atrophy of the tissue. Destruction of this pancreatic tissue leads to the inadequate production of pancreatic digestive enzymes resulting in maldigestion and malabsorption of essential nutrients. The diagnosis of EPI is based on the presence of typical clinical signs including weight loss, steatorrhea, voluminous faeces and polyphagia, together with various pancreatic function tests which are carried out when EPI is suspected [1-4].

Cystic fibrosis (CF) is one of the major causes of EPI and is an autosomal recessive disease involving all exocrine secreting organs such as the exocrine pancreas, liver and gut [5,6]. CF is characterised by increased viscosity of all exocrine
secretions, resulting in the blockage of most ducts within the exocrine glands [7-9]. In most CF patients the pancreatic duct is primarily affected due to impaired transmembrane fluid and electrolyte transport, which results in a limited flow of protein-rich pancreatic juice, protein precipitation within the duct and eventually blockage, fibrosis and the development of small cysts [1,8,9].

Malabsorption as a result of EPI is the main gastrointestinal problem in approximately 80-90% of all CF patients, resulting in a poor nutritional status and an increased morbidity and mortality [10-12]. Thus, it is important to find good strategies for the nutritional management of EPI in order to improve the nutritional status of patients. Even though great advances have been made in the treatment of EPI, additional studies are necessary in order to further improve therapy in general [13].

Conventional treatment of EPI involves replacement of the pancreatic enzymes. However, despite high doses of pancreatic enzyme extracts used during therapy, normalisation of digestion does not always occur and only partial correcions of the malnutrition have been reported [10,14]. Restriction of dietary fat intake in the treatment of EPI is commonly suggested, however recent studies have suggested that the consumption of a high-fat diet can be well tolerated by EPI patients and can restore optimal body mass and nutritional status of the patients [3].

The measurement of the effectiveness of enzyme therapy is usually done through the postprandial measurement of blood levels of NEFA or TG. The conventional analytical methods for which are time consuming or expensive.

This study aimed to investigate the effects of dietary supplementation of PEP, together with a high-fat diet on short term growth performance, apparent digestibilities of dry matter, fat and crude protein using ligation of the pancreatic duct in the pig model of EPI. The study also aimed to use turbidimetry and the lipaemic index as an approach to measuring and analysing the profile of fat absorption in the pig.

MATERIAL AND METHODS

The study was approved by the Lund University Ethics Review Committee on Animal Experiments, Sweden (Ethics clearance no: M 142-06) and the Animal Ethics Screening Committee of the University of the Witwatersrand, South Africa (Ethics clearance no: 2006/70/05).

Animals

Six castrated, male pigs (Swedish Landrace X Yorkshire X Hampshire) weighing approximately 15 kg each, were randomly selected from the University herd at Odarslöv research farm (Swedish Agricultural University), for the study. The pigs had been weaned at four weeks of age and then housed at Odarslöv in individual pens (1.0 x 1.5m) with perforated plastic flooring and wood chips as bedding. All pens were equipped with a dry feeding trough, a drinking nipple and a constant heating lamp (150 W). During the experimental period pigs were individually housed in metabolic cages at the animal unit of the Department of Cell and Organism Biology, Lund University, Sweden. Metabolic cages were also equipped with a drinking nipple and a heating lamp (150 W).

Surgery

All surgery was performed under aseptic conditions.

a) Pancreatic duct ligation and jugular vein catheterization

To artificially induce a state of pancreatic insufficiency, pancreatic duct ligation was performed on pigs at 6 weeks of age, as follows: The pigs were fasted for 12 hours prior to surgery. The pigs were premedicated with azaperone (Stresnil, LEO, Helsingborg, Sweden) 4 mg.kg⁻¹ i.v. and then bathed using surgical soap after which they were anaesthetised using a 0.5-1.5% air mixture of Fluothane (Zeneca, Gothenburg, Sweden) and carrier O₂ at approximately 0.5 l.min⁻¹.

An incision was made posterior to the sternum, along the linea alba. The pancreatic duct was located, isolated and ligated using two silk sutures (Silk 0-3 Ethicon, Johnson and Johnson) and then cut between the sutures. During surgery, all pigs were examined to ensure that there were no accessory pancreatic ducts present. Ampicillin (Doktacillin; Astra Läkemedel, Södertälje, Sweden) was administered as a post-surgical antibiotic at 15 mg.kg⁻¹ i.v. and 50 mg into the incision site. The abdomen was then stitched up, using absorbable sutures for the muscle layers and non-absorbable sutures for the skin.

Six weeks after pancreatic duct ligation surgery (pigs: 12 weeks old), external jugular vein catheters were surgically implanted and ampicillin (Doktacillin; Astra Läkemedel, Södertälje, Sweden) was administered as described above.

b) Post-surgical management

Following both surgeries, the pigs were closely monitored and treated with ampicillin (Doktacillin; AstraZeneca, Södertälje, Sweden), prophylactically at 15 mg.kg⁻¹ i.v. for 3 days.

Feeding

Prior to pancreatic duct ligation surgery, pigs were fed a standard pig diet (“53908 Växtill 320 P BK”, Lantmännen, Sweden) twice daily (5% body mass per meal) at 08:00-09:00 hr and 16:00-17:00 hr. Following surgery and during the experimental period, the pigs were fed the standard diet which was enriched with 15% extra fat composed of 40% rape seed oil (“Rapsolja”, Karlshamn) and 60% cream from cow’s milk (“Vispgrädde”, 40% fat content), twice daily (5% body mass per meal) at 08:00-09:00 hr and 16:00-17:00 hr.

Tab. 1 shows the constituents of the standard pig feed, before the addition of the extra fat. Addition of the extra fat to the standard pig feed, increased the fat content to 18%.

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Experimental procedure

a) General experimental procedure
Following implantation of jugular vein catheters, pigs were housed in metabolic cages and allowed an adaptation period of 7 days prior to commencing the feeding trial, with the high fat diet. The feeding trial experimental period lasted 15 days, the first 7 days served as a control period after which the pigs were supplemented with PEP (Creon 10 000, Solvay Pharmaceuticals GmbH, Hannover, Germany) for 7 days. 24 hour faecal and urine samples were collected on day 5, 6, 7, 12, 13 and 14. Blood was collected on day 6, 8 and 14. On day 15 the pigs were weighed and killed by an intravenous overdose of Pentobarbital (Mebumal, Nordvacc, Stockholm, Sweden).

b) Administration of PEP
For each pig, 12 PEP capsules were administered with the morning meal and another 12 capsules with the evening meal. Each PEP capsule contained 150 mg pancreatin equivalent to 10 000 active lipase units, 8000 active amylase units and 600 active protease units. The PEP was administered with 20 g of Vanilla yoghurt (3% fat; Skäne Mejerier AB, Malmö, Sweden) and 20 g of the pig’s meal. Once the PEP preparation mixture was consumed, the pigs were weighed and killed by an intravenous overdose of Pentobarbital (Mebumal, Nordvacc, Stockholm, Sweden).

c) Feed sample processing
Samples (100 g) of the feed mixture administered to the pigs were taken daily during the experimental procedure in order to determine selected nutrient intake.

d) Collection and processing of faecal and urine samples
The total sample weight for faecal samples collected on each day was recorded and the faecal samples were homogenised and stored at -20ºC until further analysis.

The total sample weight for urine samples collected on each day was recorded and the urine samples were stored at -20ºC until further analysis. 2 M sulphuric acid was added to samples during collection to keep pH below 3.

e) Blood sampling and plasma processing
Five millilitre blood samples were taken via the jugular vein catheters an hour prior to administration of the morning meal. Subsequently, samples were obtained 30 minutes after administration of food, and then at 1, 2, 3, 4, 6, 8, 12, and 24 hours after the morning food administration. The blood samples were collected into 10 ml glass vials containing 0.5 ml of a mixture of both Trasylol (aprotinin 10000 KIE ml⁻¹; Bayer, Germany) and EDTA (dihydrate, sodium salt of ethylenediaminetetraacetic acid 0.04 g ml⁻¹; Merck, Switzerland) and chilled until they were centrifuged at 3000 G, at 4ºC for 15 minutes, approximately 30-60 minutes after collection. The plasma was stored in 1 ml aliquots at -20ºC until further analysis.

Sample Analysis
a) Feed, faecal and urine samples
All feed, faecal and urine samples were analysed by a certified specialist laboratory (Lantmännen Analyacen AB, Lidköping, Sweden) for dry matter, nitrogen and fat content, using standard ‘Association of Official Analytical Chemists’ procedures:

Dry matter content: Both feed and faecal samples were analysed for dry matter content by desiccation at 103ºC for 5 hours. The dry matter content was used to calculate dry matter digestibility (%) using the following formula:

\[
\text{Digestibility (}\%\text{)} = \frac{(\text{DM intake}) - (\text{DM faeces})}{(\text{DM intake})} \times 100
\]

Nitrogen content: Feed, faecal and urine samples were analysed for raw nitrogen content using the Kjeldahl method [15] with an N factor of 6.25 to convert to crude protein.

Fat content: Both feed (with pre-extraction) and faecal (without pre-extraction) samples were analysed for fat content using the standard gravimetric method for fat analysis on a Tecator instrument (Tecator AN 301, 2001). The co-efficient of fat absorption (CFA) was calculated using the following formula:

\[
\text{CFA} = \frac{(\text{fat intake}) - (\text{fat in faeces})}{(\text{fat intake})} \times 100
\]

b) Blood samples
The blood samples were used to determine lipaemic index and plasma lipid profiles, using standard turbidimetry and clinical chemistry methods, respectively. Turbidimetry procedures were undertaken in the Department of Cell and Organism Biology, Lund University, Sweden. The plasma lipid profiles were carried out in a specialist laboratory, Medilab, Tarnaby, Sweden.

The lipaemic index was calculated using the method formula below [16]:

\[
\text{Lipaemic index} = \frac{[\text{plasma absorbance at 660nm}] - [\text{plasma absorbance at 700nm}]}{100}
\]

Turbidimetry Methods:
200 µl of each plasma sample was added to a 96-well plate (96F MicroWell™ Plates, Product no: 269620, Nune, Denmark) which was then loaded into a Spectra Max M, plate reader (Molecular Devices, USA) and the absorbance of the plasma samples was measured at wave lengths of 660 and 700 nm. Absorbance results were processed using a SoftMax Pro 4.6 processor (Molecular Devices).

Table 1. Constituents (%) of standard pig feed as supplied by manufacturer.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>%</th>
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<tbody>
<tr>
<td>Fat</td>
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<tr>
<td>Protein</td>
<td>17.6</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>61.38</td>
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<tr>
<td>Ash</td>
<td>5.12</td>
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<tr>
<td>Water</td>
<td>12.4</td>
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<tr>
<td>Total Energy</td>
<td>12.6 MJ kg⁻¹</td>
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Clinical chemistry methods:
Plasma lipid profiles, including total cholesterol, low density lipoproteins, high density lipoproteins, triglycerides and non-estrified fatty acids (NEFA) contents were determined by Medilab, Tarnaby, Sweden; using standard calorimetric kits (RocheDiag nostic, Switzerland and Wako Chemicals, Germany) on a Hitachi 912 Multianalyzer (RocheDiag nostic, Switzerland).

Data Analysis
All data are expressed as mean (±SD). Faecal and urine profiles, nitrogen digestibility, coefficient of fat absorption (CFA), blood profiles as well as body mass data were analysed using a repeated measures ANOVA. A Tukey post hoc test was used when significant differences or effects were detected by the repeated measures ANOVA. All statistics were performed using GraphPad Instat version 3.00 for Windows 95 (GraphPad Software, San Diego, California, USA). P<0.05 was considered significant.

RESULTS

Body mass and feed intake
Administration of the PEP, together with the high fat diet, significantly increased body mass (P=0.016) (Fig. 1). Feed intake (g) was not significantly different during both control and treatment periods (P=0.3329).

Dry matter and Apparent Digestibility
a) Dry matter (Faecal and Urine)
Faecal dry matter during the PEP treatment period was significantly lower than that measured during the control period (P<0.001) (Fig. 2). The dry matter digestibility on the last two collection days during the PEP treatment (day 13 and 14) were significantly higher than that on the first day of control collections (day 5) (P=0.0166) (Fig. 3). Urine dry matter content (g) was not significantly affected by treatment with PEP (P=0.1183).

b) Nitrogen balance, Crude protein (Faecal and Urine)
Faecal crude protein content was significantly reduced by the PEP (P=0.005), (Fig. 4). This was reflected by a significant increase in the digestibility of crude protein (P=0.013) (Fig. 5). Urinary nitrogen content (g) was not significantly affected by treatment with PEP (P=0.326).

c) Fat
Inclusion of PEP in the diet significantly lowered faecal fat content compared to the control period (P<0.0001) (Fig. 6). CFA values calculated from all three collections during the
PEP treatment period, were significantly higher than the CFA value calculated from the first day of control collections (P=0.003) (Fig. 7).

**Plasma lipids**

*a* Turbidimetry

The lipaemic index during the control period (day 6) did not differ significantly between the various sampling points (Fig. 8). On the first day of treatment with PEP preparation (day 8) significant differences between the various sampling points were observed, as well as in the trends observed across the 24 hour sampling period compared to that of day 6. Following the first day of PEP treatment (day 8) the lipaemic index increased slowly with time reaching its peak on the 12th hour after the morning food administration. On the last day of treatment with the PEP preparation (day 14), the lipaemic index also increased with time and started to increase sooner than observed on day 8, reaching its peak on the 6th hour after the morning food administration.

*b* Plasma lipid profile

Although treatment with PEP did not significantly affect the plasma concentration of cholesterol, high density lipoproteins and low density lipoproteins (data not shown), significant changes were observed in both the NEFA and triglyceride concentrations between the various blood sampling time points as well as between the different collection days. The time at which the peak NEFA concentration was reached, after morning food administration varied significantly between the various sampling days (Fig. 9). The peak NEFA concentration was reached at the 24th hour after morning food administration during the control period (day 6) and at the 6th hour after morning food administration on the first (day 8) and last days (day 14) of treatment with the PEP preparation.
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The effectiveness of enzymatic replacement therapy measured by turbidimetry and the lipaemic index in exocrine pancreatic insufficient young, growing pigs, fed a high-fat diet observed in the triglyceride concentration were not as well defined as those observed in the NEFA concentration; however definite trends were demonstrated on the first and last days of treatment with the pancreatic enzyme preparation (day 8 and 14) (Fig. 10).

**DISCUSSION**

We found that the short term dietary supplementation of the PEP, together with the high fat diet, had immediate beneficial effects on the growth performance as well as the digestion and absorption of fat in a pig model of EPI. The dose of PEP used in this study has previously been shown to improve digestibility in pancreatic-duct ligated pigs [14].

EPI was successfully developed in all the pigs via pancreatic duct ligation. The significantly increased body mass after PEP treatment was in agreement with most studies in pigs with EPI [17,18], demonstrating that sufficient exocrine pancreatic secretion is essential not only for digestion, but also the subsequent assimilation of nutrients to ensure growth and thus development of the animal as recently suggested by Rengman *et al.* [19]. The increased assimilation was reflected in the significantly lower faecal dry matter and crude protein content, increased faecal dry matter and crude protein digestibilities during the PEP treatment.

The urinary nitrogen content was unaffected by PEP treatment, however following PEP treatment, nitrogen digestibility was improved, thus the pigs were able to assimilate more nitrogen which was used to build protein, resulting in improved growth performance.

PEP treatment also improved steatorrhea. The co-efficient of fat absorption (CFA) calculated for all three collections during the treatment period were significantly higher than those without treatment. Previous studies have reported similar results as well as a reduction in faecal wet weight in CF patients receiving pancreatic enzyme therapy compared to those receiving a placebo [20,21].

The present study shows that the inclusion of a high fat diet in the treatment of EPI is beneficial, at least in the short term. The PEP together with the high-fat diet was well tolerated by the pigs and alleviated some of the detrimental effects of EPI. Before extrapolating these positive findings to humans, one must take into account the differences in growth rates between young and older pigs and between fast growing animals such as pigs compared to humans. In conclusion, we have demonstrated an immediate improvement in growth performance in young, growing EPI pigs while receiving the high fat diet together with the PEP.

Using turbidimetry, we were able to rapidly determine the effect of PEP on the lipaemic index. The lipaemic index remained relatively constant on the day of control collections (day 6) and significant changes in the lipaemic index were observed following PEP treatment (day 8 and 14). The peak lipaemic index value increased and reached a peak at a much faster rate following treatment compared to the control collection period (day 6). Apart from a previous study which successfully made use of the lipaemic index as a tool in the assessment of triglyceride concentrations [16], there is a dearth of literature evaluating the plasma lipid profile of EPI pigs using turbidimetry and lipaemic indices. The temporal, postprandial changes observed in the lipaemic index were mirrored in both the plasma fatty acid and triglyceride concentrations before and after PEP treatment. The changes observed in the lipaemic index values following PEP treatment were mainly due to improved absorption of NEFA and triglycerides, as none of the other blood parameters measured (cholesterol, low density lipoproteins, high density lipoproteins) displayed any significant changes following PEP treatment. These observations led us to conclude that the turbidimetry methods used as an approach to analyzing the overall plasma lipid profile in EPI pigs was effective and capable of detecting changes in plasma lipid content.

**CONCLUSIONS**

Results from the present study have shown that the short term treatment with a PEP in EPI pigs, fed a high fat diet immediately and essentially improved the digestibility of the macronutrients assessed, as well as the growth performance. These results could provide useful insight and could be considered in the recommendations of dietary approach by clinicians for young, growing human patients suffering from EPI. The use of turbidimetry as an approach to measuring and analysing the plasma lipid profile in EPI pigs, before and after treatment proved to be effective and is recommended as a quick tool for assessing digestion and absorption of fat in similar future studies.

*Figure 10. Triglyceride (TG) concentration (mmol.l⁻¹) measured during the control period (day 6) and on the first (day 8) and last days (day 14) of treatment.*
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REFERENCES


