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Diet supplemented with pancreatic-like enzymes of microbial origin restores the hippocampal neuronal plasticity and behaviour in young pigs with experimental exocrine pancreatic insufficiency

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ABSTRACT

It is postulated that exocrine pancreatic insufficiency (EPI) can evoke neurological disorders. In the present study pancreatic-like enzymes of microbial origin (PLEM) were examined as a functional food component, with the goal of improving cognitive function and brain structure in a pig model of EPI. EPI in the present study induced alterations in the behaviour of the pigs as well as degenerative changes within the morphological structure of the hippocampus. EPI leads to a reduced number of pyramidal neurons and decreased levels of neural cell adhesion molecules (NCAM) in the CA1 area of the hippocampus. Here, we provide evidence that the use of PLEM as a functional food, in the form of dietary supplementation with PLEM for 10 days, restored pig behaviour and the histo-morphology of the hippocampus in EPI pigs. Thus, we suggest that the use of PLEM as a functional food ingredient should be considered in the prevention and/or postponement of the development of EPI-related encephalopathy.

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1. Introduction

Lifestyle factors such as eating behaviour, the quality of nutrition and physical activity have been shown to affect the health of more than 50% of the world's population. It is commonly recognised that consumption of functional food can ameliorate the effects of maldigestion and thus, malnutrition. Maldigestion, as well as all the consequences related to malnutrition, are mainly an effect of the lack of exocrine pancreatic enzymes. It is commonly recognised that malnutrition is somewhat destructive in terms of brain function, with all cognitive and behavioural functions as well as brain morphology being demolished under conditions of malnutrition.

At infancy in mammals, a physiologically low level of pancreatic enzymes is observed (Pierzynowski, Weström, Svendsen, Svendsen, & Karlsson, 1995; Zoppi, Andreotti, Pajno-Ferrara, Njai, & Gaburro, 1972). In humans, pancreatic lipase is especially low, since the only source of lipase at that stage is from the mother's breast milk (Jensen, Buist, & Wilson, 1986). In elderly humans, production of pancreatic enzymes is also low (Al-Kaade, 2013; Majumdar, Jaszewski, & Dubick, 1997). In addition, there are several diseases that lead to the loss of pancreatic parenchyma (pancreatitis, cystic fibrosis or obstruction of the main pancreatic duct; decreased pancreatic stimulation, celiac disease) and/or the acid-mediated inactivation of pancreatic enzymes (Zollinger–Ellison syndrome). Moreover, some – actually very modern – surgical techniques, which are now commonly used to treat obesity, such as gastric by-pass surgery, also lead to a lack of pancreatic enzymes in the gut lumen (Zingg & Oertli, 2012). In humans, independent of the initial cause of pancreatic insufficiency, the resulting lack of exocrine pancreatic enzymes is commonly treated with dietary pancreatic enzyme replacement therapy.

Pancreatic insufficiency is often associated with marked neurological alterations related to cognitive and sensory motor function (Jongsma et al., 2011). Many patients with chronic pancreatitis report symptoms that are associated with a decrease in cognitive function, such as depressive symptoms (Gallassi, Morreale, & Pagni, 2001; Gomez et al., 2006; Oosterman, Derksen, van Wijck, Veldhuijzen, & Kessels, 2011), sleep disturbances (Goel, Rao, Durmer, & Dinges, 2009) and the use of opioid medication (Sjogren, Thomsen, & Olsen, 2000).

Surprisingly, studies dedicated to the investigation of brain function and morphology under conditions of malnutrition caused by EPI and subsequent effects of dietary supplementation with pancreatic enzymes, are lacking – in both human and animal models. For the current study, we made use of well established EPI pig model (Goncharova et al., 2014).

The main aim of the present study was to evaluate the effects of experimental functional food created by mixing PLEM with regular (3% fat) pig diet, which was additionally supplemented with fat (in total 18% fat), on the behavioural activity and the histo-morphological structure of the hippocampus of a pig model with experimental EPI.

2. Materials and methods

2.1. Ethics statement

The recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health were strictly followed. The protocol was approved (approval № M142-06) by the University of Lund Ethics Review Committee on Animal Experiments (Lund, Sweden). All efforts were made to minimise animal suffering.

2.2. Animals and housing

The experiment was carried out at the research farm of the Department of Agricultural Biosystems and Technology, Swedish University of Agricultural Sciences, Lund, Sweden. Fifteen castrated male piglets (breed: Swedish Landrace × Yorkshire × Hampshire), 6 ± 2 weeks of age, weighing 11.3 ± 0.9 kg at surgery, were used in the study. The pigs were randomised into 3 groups – pigs with intact pancreas (Control group, n = 5), pigs in which EPI was induced by pancreatic duct ligation (EPI group, n = 5) and EPI pigs which received PLEM (EPI+PLEM group, n = 5).

The pigs were housed in identical, individual pens with the dimensions of 1.0 m × 1.5 m. The pens were designed such that the pigs were able to maintain visual contact with one another and were equipped with a dry feeding trough, a drinking nipple and constant heating lamp (150 W, 24 hours a day, a red light). All pens had perforated plastic flooring and wood chips were used as bedding. The size of each individual pen allowed for the pigs to move around freely. Lighting was controlled automatically, with lights on from 06.00 a.m. to 06.00 p.m. Heating lamps operated 24 hours per day.

2.3. Surgery and experimental design

EPI was artificially induced by pancreatic duct ligation (Gewert et al., 2004). Briefly, after a one-week adaptation period to individual pens, pigs were subjected to pancreatic duct ligation surgery. Prior to surgery all pigs were fasted for approximately 12 hours. Pigs were sedated using azaperone (Stresnil, LEO, Helsingborg, Sweden) at 4 mg/kg bw, i.m, then washed using surgical soap and shaved from the ventral cervical. The pigs were then anaesthetised using 0.5–1.5% air mixture of Fluothane (Zeneca, Gothenburg, Sweden) and O_2 as a carrier gas, at approximately 0.5–1 L/min in a close-circuit respiratory system (Komesaroff Medical Developments, Melbourne, Australia). The surgery was performed under aseptic conditions. A 14–18 cm long incision was made posterior to the sternum, along the *linea alba*. The accessory pancreatic duct was isolated and ligated at 2 and 3 cm distance from the duodenal papilla with double silk sutures (Ethicon 0.3; Jonson and Jonson Medical Products, Peterborough, ON, Canada) and then cut between the ligatures. The abdomen was then stitched up using 3 layers of sutures, absorbable sutures for the muscle layers/pleura and non-absorbable sutures for the skin. Postoperative pain was prevented by administration of buprenorphine i.m. 0.01 mg/kg bw (Temgesic®, Schering-Plough AB, Stockholm, Sweden) for 3 days after surgery. Ampicillin (Doktacilline, Astra Lakemedel, Sodertalje, Sweden)