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ORIGINAL ARTICLE

Prandial and postprandial exocrine pancreatic secretion after duodenal infusion of alpha-ketoglutarate, formic acid and potassium di-formate in pigs

Rafał Filip[1,2], Danuta Kruszewska[1], Jose L. Valverde Piedra[3], Sylwia E. Szymańczyk[3], Tadeusz Studziński[3], Radosław P. Radzki[3], Marek Bieńko[3], Stefan G. Pierzynowski[1]

[1] *Department of Cell and Organism Biology, Lund University, Lund, Sweden*
[2] *Institute of Agricultural Medicine, Lublin, Poland*
[3] *Department of Animal Biochemistry and Physiology, Faculty of Veterinary Medicine, Agricultural University , Lublin, Poland*

Abstract: The positive effect of acidifiers on performance (growth) may be associated with the stimulation of the pancreatic secretion since some studies have shown a direct relationship between pancreatic enzyme outflow and body weight gain and feed conversion. The aim of the study was to investigate the pancreatic juice (PJ) composition after treatment with alpha-ketoglutarate (AKG), formic acid (FA) and its potassium salt (KDF). Experiments were conducted on 9 pigs (15 ± 5 kg. bw.) surgically fitted with a pancreatic duct catheter, a T-cannula in the duodenum and a jugular vein catheter. The piglets were fed a standard diet twice daily and had free access to tap water. During the prandial period, duodenal infusions of KDF, FA, AKG or saline 0.9% NaCl (2.5ml/h/kg bw.) were carried out in experimental and control pigs, respectively. PJ was analyzed for volume, protein content and trypsin activity, and blood assayed for cholecystokinin (CCK). Both AKG and KDF had stimulatory effects on the PJ outflow. The PJ volume increased significantly in the prandial phase ($p<0.05$) in the AKG group, and increased significantly in both the prandial and postprandial phases ($p<0.05$) in the KDF group. Formic acid did not stimulate exocrine pancreatic secretion. There were no effects on protein and trypsin output and plasma CCK levels after the treatment. In conclusion, the exocrine pancreatic secretion can be stimulated by oral supplementation with AKG and KDF, and this effect is mediated mainly by secretin since only volume outflow was altered in the pigs.

Key words: Pancreas, Exocrine, Formic acid, Potassium di-formate, Alpha-ketoglutarate.

Abbreviations: FA – Formic acid; KDF – Potassium di-formate; FA 2 – Formic acid pH 2; FA 5 – Formic acid pH 5; KDF 3.6 – Potassium di-formate pH 3.6; KDF 5 – Potassium di-formate pH 5; AKG – alpha ketoglutarate; CCK – Cholecystokinin; GIT – Gastrointestinal tract

INTRODUCTION

During suckling, exocrine pancreatic secretions remain low and are not stimulated by the ingestion of milk [1]. On the other hand, Pierzynowski et al. (1995) [2] have demonstrated that there is an increase in the pancreatic juice volume and levels of protein and trypsin solely dependent on the shift from milk to solid food after weaning. This also coincides with increased hydrochloric acid production in the stomach [3].

Organic acids are widely distributed in nature as normal constituents of plants and animals tissues. They are also formed through microbial fermentation of carbohydrates, predominantly in the large intestine. Inclusion of 1.2% formic, 1.6% lactic and 2.4% sorbic acid in pig feeds have been shown to improve the daily weight gain and feed conversion rates by 22.1 and 7.5, 8.1 and 1.8, and 26.7 and 6.5%, respectively, in pigs weaned at 3-4 weeks [4]. However, the underlying mechanism remains unclear. It is generally believed that dietary organic acids or their salts may decrease the gastric pH which results in an increase in the activity of the gastric proteolytic enzymes together with the gastric retention time. On the other hand, acidification of the diet reduces its buffering capacity which may cause more efficient proteolysis of the digesta in the stomach and therefore result in a higher protein digestibility, and/or organic acids are able to change from the undissociated to the dissociated form depending on the enviromental pH. In the undissociated form, the acid is able to freely diffuse through the semipermeable membranes of the microorganisms into the cell cytoplasm. Once inside the cell, where the pH is maintained near 7, the acids dissociate into protons (H^+ ions) and anions R($HCOO^-$ ions) which can have a disruptive effect on bacterial protein synthesis. This in effect reduces bacterial multiplication in the GIT, which can spare the ingested nutrients [5, 6].

AKG is a key metabolite in the intracellular Krebs cycle [7]. It was shown, that the addition of organic acidifier to the diet of the weaned pig, or intraduodenal infusion, stimulates the exocrine pancreas secretion which results in the increase of the pancreatic juice volume, protein content and trypsin

Corresponding author: Prof. Stefan G. Pierzynowski, Department of Cell and Organism Biology, Lund University, Helgonavagen 3b, 223 62 Lund, Sweden. E-mail: stefan.pierzynowski@telia.com

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activity [8]. Bearing in mind that one the most important factors in the stimulation of pancreatic secretion is the pH in the upper duodenum, it remains unclear what determines the quantity and the composition of the pancreatic juice secreted after organic acidifiers treatment. However, stimulation of pancreatic secretion by the organic acids is dependent on the pH and the length (specificity) of the carbon chain. It was shown that there is a direct relation between pancreatic enzyme outflow, feed conversion and increased body gain. Therefore, it may be concluded that the positive effect of organic acidifiers on animal performance (growth) may be associated with the stimulation of the pancreatic secretion [9].

The aim of the study was to investigate the quantitative and qualitative composition of prandial and postprandial exocrine pancreatic secretions after treatment with AKG, FA and KDF.

MATERIALS AND METHODS

Animals

Animal studies were approved by the Local Ethical Committee. Experiments were carried out on 9 pigs aged 8 weeks (15 ± 5 kg. b.w) obtained from the Swedish Agricultural University, Department of Agricultural Biosystems and Technology, Alnarp. Pigs were housed individually in 1.0 × 1.5 m pens and equipped with a dry feeding trough, a drinking nipple and a heating lamp (150 W, 24 hours/day). All the pigs had visible contact with each other and could always move freely. The light was automatically controlled with a light period between 06.00-18.00 hours and a dark period between 18.00-06.00 hours. Under general Halothane anesthesia (Fluothane – Astra Zeneca, Sweden) a silicone catheter was inserted into the external jugular vein and the accessory pancreatic duct, and 2 silastic cannulae (Dow Corning, Midland USA) were placed in the duodenum for chronic collection of pancreatic juice (PJ) according to the method described by Pierzynowski et al. (1988). For 3 days after surgery, the pigs were treated with strepcillin (Strepcillin, Novo Industries Co., Bagsvaerd, Denmark). Standard feeding times between 10.00-11.00 and 16.30-17.30 were maintained. All the pigs received a standard diet for growing – finishing pigs (Slaktfoder, Sweden) at a dose of 2.0% of their body weight per meal while water was allowed *ad libitum*. The feed contained 12.6 MJ metabolizable energy, 140 g crude protein and 8.5g lysine/kg. Actual food consumption was monitored daily while the weight gain was checked once a week. After a post surgical recovery period of 1 week, and after training the animals, the prandial pancreatic secretions were studied.

Experimental Protocol

Experiments were conducted with 3 separate sets of pigs (FA, KDF and AKG) randomized to all the treatments in each set according to the Latin square principle protocol.

Formic acid (FA)

In the control pigs, pancreatic juice was re-introduced into the duodenum and physiological saline was infused (2.5ml/h/kg b. wt., id.). In the experimental pigs, a formic acid solution with pH 2 (FAS-2 group – 0.75mmol in 2.5ml saline/h/kg b. wt.) and a pH 5 (FAS-5 group – 0.75mmol in 2.5ml saline/h/kg b. wt.) was administered and PJ reintroduced.

Potassium diformate (KDF)

In the control pigs, pancreatic juice was re-introduced into the duodenum and physiological saline was infused (2.5ml/h/kg b. wt., id.). In the experimental pigs, a KDF solution with pH 2 (KDF-2 group – 0.06mmol in 2.5ml saline/h/kg b.wt., id.) and a pH 5 (KDF-5 group – 0.06mmol in 2.5ml saline/h/kg b. wt., id.) was administered and PJ reintroduced.

α-Ketoglutarate (AKG)

In the control pigs, pancreatic juice was re-introduced into the duodenum and physiological saline was infused (2.5ml/h/kg b. wt., id.). In the experimental pigs, an AKG solution with pH 2 (AKG-2 group – 0.75mmol in 2.5ml saline/h/kg b. wt., id.) and a pH 5 (AKG-5 group – 0.75mmol in 2.5ml saline/h /kg b. wt., id.) was administered and PJ reintroduced.

Experimental procedure

Before each experiment, the pancreatic catheter was disconnected from the duodenal cannula and the PJ allowed to flow freely for about 30 min. to allow its stabilization. Experiments were performed on overnight fasted pigs for a period of 3 hours (1 h preprandial, prandial and postprandial period) daily starting 09.00. PJ was collected in 30 min. periods, measured, and two 0.5 ml samples were taken for analysis and stored at (-20°C), while the rest was reintroduced into the duodenum in small portions in between and before the next collection period. FA, KDA and AKG infusions started at the beginning of the prandial period and lasted for 1 hour. Basal and postprandial blood sampling for CCK assay was carried out before the start of the infusions, and just before the end of the experiment.

Analyses

PJ was analysed for total protein content using the method of Lowry et al. (1951) [10], modified to be performed on a microwell plate, with bovine serum albumin (A - 7638, Sigma Chemical Co., St. Louis, MO, USA) as a standard. The trypsin activity was determined by the hydrolysis of N-α-benzyl-Arg-*p*-nitroanilide (L-BAPNA), (modified Erlanger et al. 1966) [11]. The enzyme activity is expressed as units (U). 1U being defined as the amount of enzyme that hydrolyses 1μmol of substrate/min. One-way ANOVA followed by unpaired Student t - test was used for the comparison of 2 sets of data. The differences were recognised as significant when $p<0.05$. Statistical analysis was carried put with InStat v2.03 software (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Compared to the control, both AKG and KDF intraduodenal infusions had stimulatory effects on the exocrine pancreas activity with resultant volume output ($p < 0.05$) (Figure 1a, 1c). The increases were achieved in the prandial phase for AKG at pH 2, and in the prandial and postprandial phases for KDF at pH 2 and 5. There was a striking pattern in the preprandial – prandial – postprandial decrease in PJ output for AKG at both pH 2 and 5 ($p < 0.05$), and an ascending preprandial – prandial – postprandial volume output pattern for KDF at both pH 2 and 5 ($p < 0.05$), while FA had no stimulatory effect on the pancreas compared with the control (Figure 1a, 1b, 1c).

There were no effects on protein output in either the prandial or postprandial periods after treatments with AKG,

FA and KDF (Figure 2a, 2b, 2c). However, postprandial trypsin output was lowered by AKG at pH 5 when compared with the postprandial period in the control ($p < 0.05$) (Figure 3a). There was no effect of treatments on plasma CCK levels.

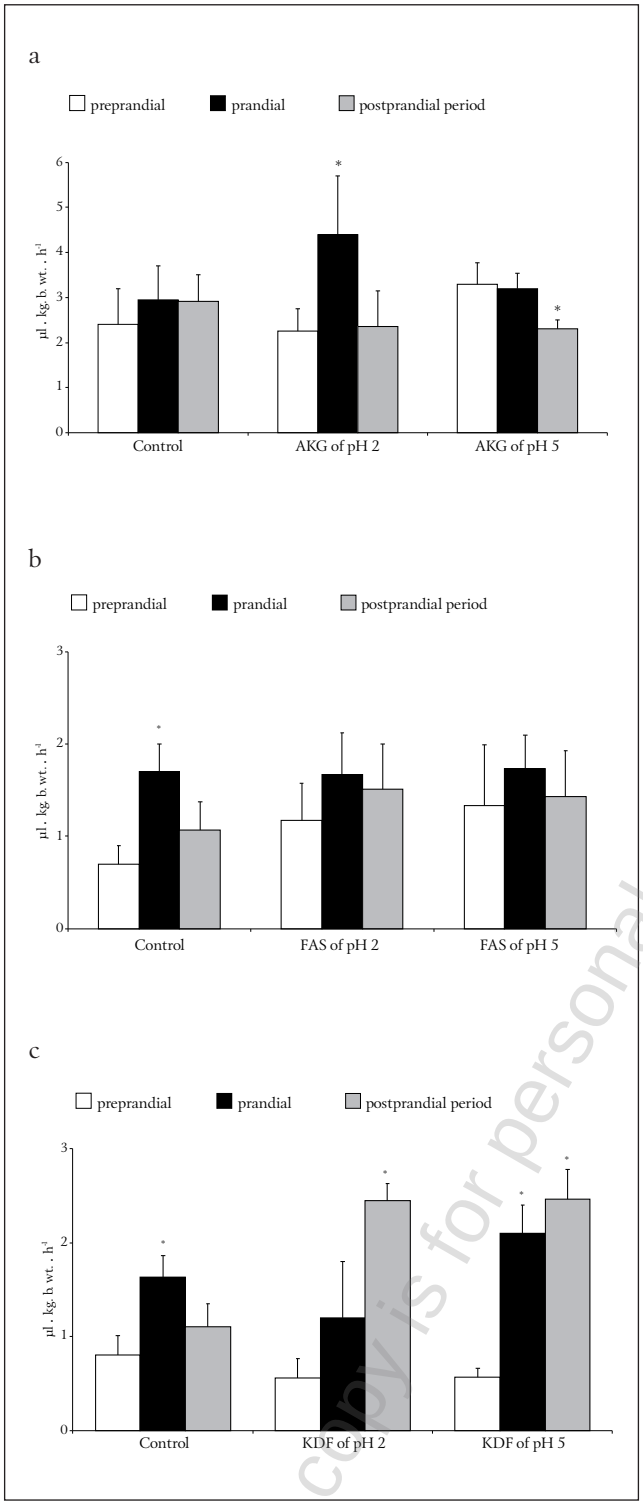

Figure 1 Pancreatic juice (PJ) output in conscious pigs. a) α-Ketoglutarate (AKG, n=3), b) Formic acid (FA, n=3) c) Potassium diformate (KDF, n=3). In control pigs pancreatic juice was re-introduced into the duodenum and physiological saline was infused. In the experimental pigs AKG solution of pH 2 and pH 5, formic acid solution of pH 2 and pH 5 and KDF solution of pH 2 and pH 5. In experimental pigs PJ was also reintroduced into the duodenal lumen.
*Mean value of n=3±SE was significantly different from the respective control (unpaired Student t-test, $p<0.05$).

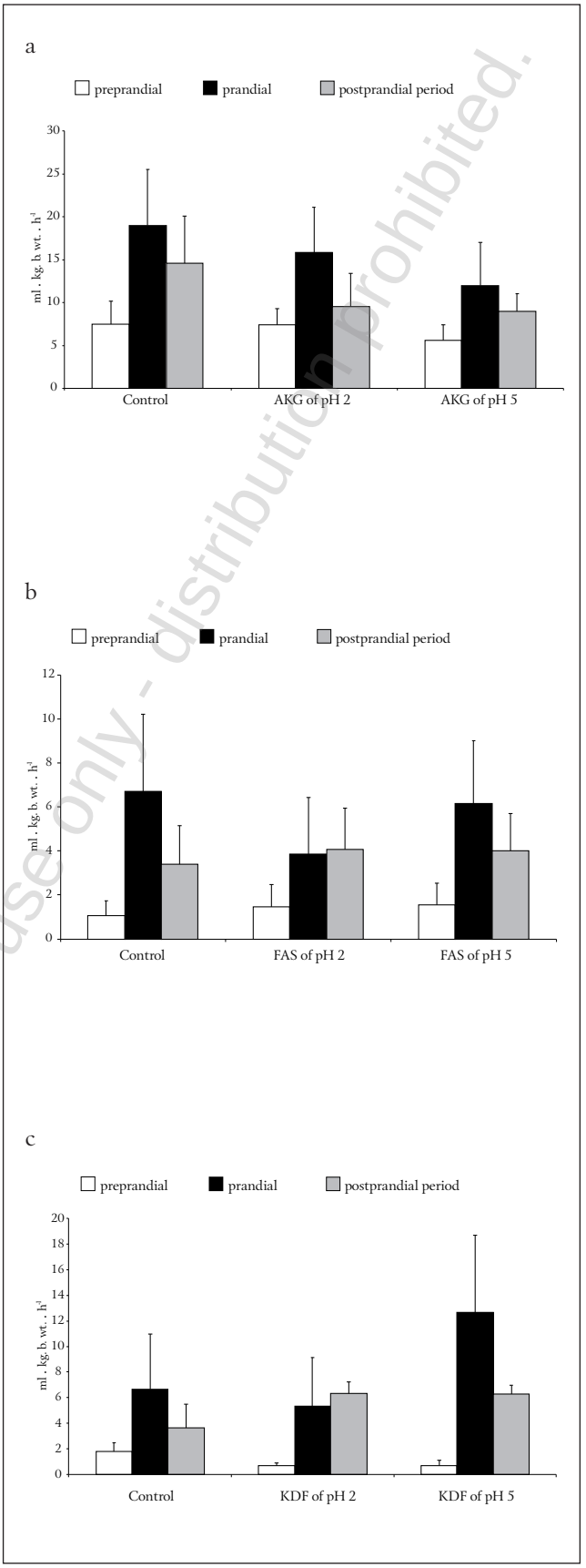

Figure 2 Pancreatic juice (PJ) protein output in conscious pigs. a) α-Ketoglutarate (AKG, n=3±SE), b) Formic acid (FA, n=3±SE) c) Potassium diformate (KDF, n=3±SE). In control pigs pancreatic juice was re-introduced into the duodenum and physiological saline was infused. In the experimental pigs AKG solution of pH 2 and pH 5, formic acid solution of pH 2 and pH 5 KDF solution with pH 2 and pH 5. In experimental pigs PJ was also reintroduced into the duodenal lumen.

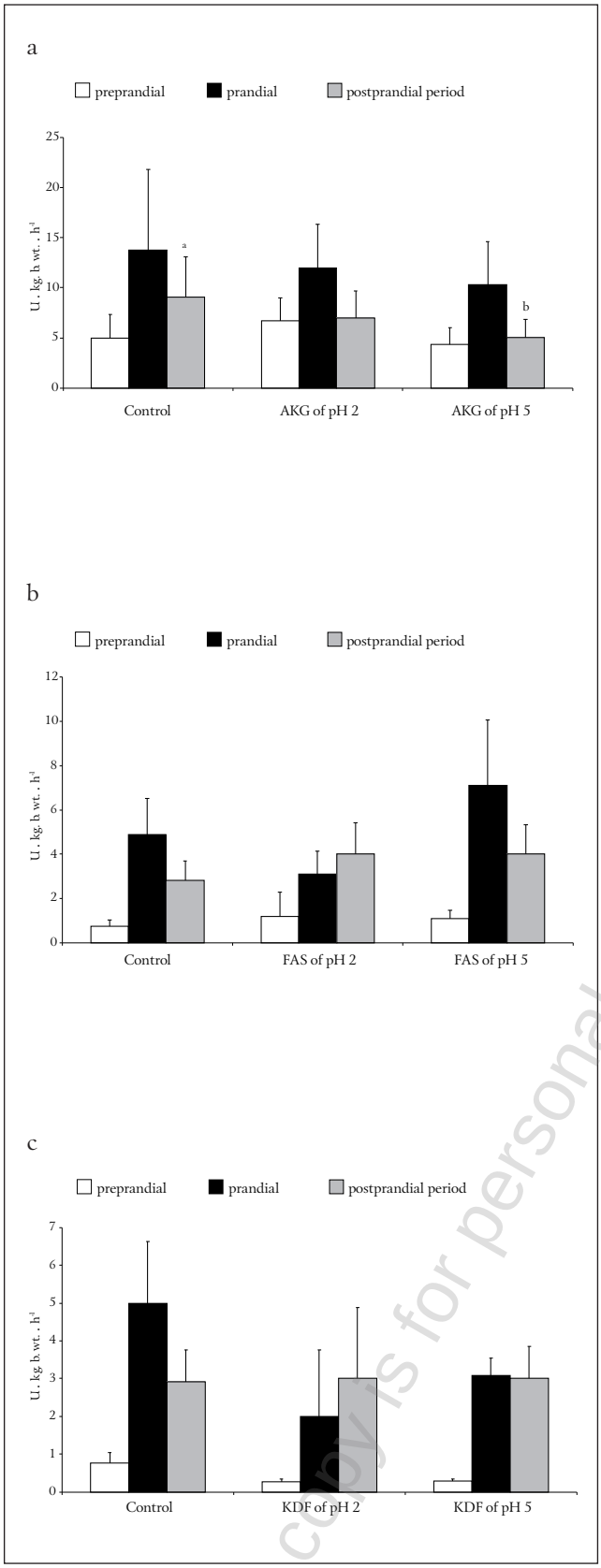

Figure 3 Pancreatic juice (PJ) trypsin output in conscious pigs. a) α-Ketoglutarate (AKG, n=3±SE), b) Formic acid (FA, n=3±SE) c) Potassium diformate (KDF, n=3±SE). In control pigs pancreatic juice was re-introduced into the duodenum and physiological saline was infused. In the experimental pigs AKG solution of pH 2 and pH 5, formic acid solution of pH 2 and pH 5 KDF solution of pH 2 and pH 5. In experimental pigs PJ was also reintroduced into the duodenal lumen. Small letters indicates significantly different from the respective phase in the control experiment ($p<0.05$).

DISCUSSION

The improvements in growth performance attained by dietary acidification with organic acids are attributed to the reduced dietary pH and buffering capacity of feed in the stomach, although no growth promoting effects have been demonstrated in piglets by lowering the pH value and the buffering capacity of the feed with inorganic acids such as phosphoric and hydrochloric acid, according to Roth and Kirchgessner (1998) [4].

The effects of organic acids on the intermediary metabolism have not been well documented and the available literature show very divergent findings. Tschierschwitz et al. (1982) [12] and Grassmann and Klasna (1986) [13] investigated the effects of dietary fumaric acid supplementation on intermediary metabolism in the rat liver. In both studies, fumaric acid had no effect on the activites of the enzymes in the citric acid cycle. Similar studies carried out by Grassmann and Kirchgessner (1979) [14] and Grassmann and Klasna (1986) [13] with citric acid also showed no effects, although succinate dehydrogenase activity was increased in one study and decreased in the other. Grassmann and Kirchgessner (1979) [14] reported that citric acid supplementation did not affect the activities of liver transaminases, while Grassmann and Klasna (1986) [13] have reported increased liver glutamate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities. Kirchgessner and Roth (1988) [15] have proposed that organic acids may stimulate intermediate metabolism, resulting in improved energy or protein/amino acid utilisation.

In the presented study, both AKG and KDF stimulated an increase in pancreatic juice volume compared with the control. The most interesting observation was the contrasting manner in which these acidifiers stimulated the increased volume output. The AKG increase was achieved at pH 2, and for KDF at pH 2 and 5. A comparison of the preprandial, prandial and postprandial periods of volume secretions in AKG treated pigs, shows that at pH 2 there was a marked increase in prandial, and a decrease in postprandial secretion by about 45%. Recent experiments (data not shown) with 10 × bigger dose (0.676 mmols in 2.5ml saline/h/kg b wt) of AKG at both pH values have confirmed the short lasting nature of stimulated exocrine pancreatic secretion. KDF, on the other hand, showed an ascending preprandial – prandial – postprandial PJ output at both pH values.

According to Singer (1987) [16], gastric acid entering the duodenum stimulates increased pancreatic juice volume and bicarbonate through the hormone secretin, while peptides and amino acids stimulate secretion of pancreatic enzymes through the release of CCK. In the presented study, the feed and AKG, FA and KDF infusions were administered simultaneously; however, the results do not show any stimulatory effect on protein output nor trypsin activity. However, Thaela et al. (1998) [8] reported increased protein output with intraduodenal infusions of FA in weaned and overnight fasted pigs.

In our study, no significant changes in plasma CCK were observed after AKG, FA and KDF treatments. Pierzynowski et al. (1993) [17] have proposed that CCK has an indirect action on the porcine exocrine pancreas through reflexes initiated by a hormone acting intraduodenally. Therefore, the effects of the acidifiers on the exocrine pancreas activity should be investigated under the influence of both the humoral (CCK)

and the vagovagal reflexes that activate cholinergic neurons in the pancreas, since qualitative and quantitativee exocrine secretions are closely linked.

CONCLUSION

In conclusion, exocrine pancreatic secretion can be stimulated by oral supplementation with AKG and KDF, and the effect is mediated mainly by secretin since only fluid secretion was altered in the pancreatic juice of pigs. However, pancreas exocrine function after organic acid, used as feed additives or preservatives in human and animal nutrition, needs further investigation to clarify their possible impact.

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