EFFECT OF INTRARUMINALLY AND INTRADUODENALLY INFUSED SHORT-CHAIN FATTY ACIDS (SCFA) ON PANCREATIC JUICE OUTFLOW IN SHEEP

Vaida Šileikienė[1], Rainer Mosenthin[1], Danuta Kruszewska[2], Barbara Krasicka[3] and Stefan G. Pierzynowski[4, 5]
[1] *Institute of Animal Nutrition, University of Hohenheim, D-70593 Stuttgart, Germany, Phone: +497114593938, Fax: +497114592421, E-mail:* sileikie@uni-hohenheim.de; rhmosent@uni-hohenheim.de
[2] *Department of Medical Microbiology, Dermatology and Infection, Lund University, SE-22362 Lund, Sweden*
[3] *Department of Animal Physiology, Warsawa Agricultural University*
Nowoursynowska 166, 02-787 Warsaw, Poland
[4] *Department of Cell and Organism Biology, Lund University, SE-2232 62 Lund, Sweden*
[5] *Sea Fisheries Institute, 82-332 Gdynia, Poland*

Abstract. The effect of intraduodenal and intraruminal infusion of short-chain fatty acids (SCFA) on exocrine pancreatic secretions was studied in 27 Polish Merino sheep that were surgically fitted with a common bile duct catheter, a gall-bladder catheter and two duodenal T-cannulas for collection and subsequent return of pancreatic juice, and with a ruminal cannula. Animals were fed *ad libitum* with silage and grass hay. Saline solution as a control or SCFA adjusted to pH 7.0 or 1.5 were either infused into the duodenum or rumen to overnight fasted sheep. Pancreatic juice was collected 1 h before and 7 h after the infusion had started. The pH value of pancreatic juice was measured immediately after the collection. Intraduodenal or intraruminal infusion of saline had no affect on the pancreatic juice outflow. No differences in the pancreatic juice outflow were obtained after intraruminal infusion of SCFA in comparison to saline infusion. However, a tendency to a decreased juice outflow was observed after intraruminal infusion of saline and neutralized SCFA (pH 7.0). The intraduodenal infusion of SCFA at pH 7.0 and pH 1.5 in comparison to saline resulted in a decreased pancreatic juice outflow. This effect was more pronounced when neutralized SCFA were infused. The results suggest that SCFA may affect pancreatic juice outflow both *via* pH dependent and pH independent mechanisms located in the mucosa of the proximal small intestine.
Keywords: short-chain fatty acids (SCFA), sheep, pancreatic secretion, rumen, duodenum.

Į DIDĮJĮ PRIESKRANDĮ IR DVYLIKAPIRŠTĘ ŽARNĄ ĮLIETŲ LAKIŲJŲ RIEBALŲ RŪGŠČIŲ (LRR) ĮTAKA AVIŲ KASOS SEKRECIJAI

Santrauka. Tiriant lakiųjų riebalų rūgščių (LRR) įtaką kasos sekrecijai, bandymas atliktas su 27 Lenkijos merinosų veislės avimis (kūno masė bandymo pradžioje 45–55 kg). Avims į didįjį prieskrandį, dvylikapirštę bei klubinę žarnas buvo įsodinti kasos latako kateteriai ir paprastosios T-kaniulės. Bandymo dieną avys buvo alkinamos. LRR tirpalai (pH 7,0 ar 1,5) buvo įliejami į didįjį prieskrandį ar dvylikapirštę žarną. Kasos sulčių mėginiai buvo imami 1 h prieš ir 7 h po infuzijos. Kas valandą matuojamas ištekėjusių kasos sulčių kiekis ir pH vertė. LRR (pH 1,5 ar 7,0) bei izotoninio tirpalų injekcijos į didįjį prieskrandį kasos sulčių sekrecijai įtakos neturėjo ($p>0,05$), tačiau įliejus LRR tirpalo (pH 1,5), kasos sulčių sekrecija buvo didesnė palyginti su izotoninio bei LRR (pH 7,0) tirpalų injekcijomis.

Įliejus LRR tirpalo (pH 7,0) į dvylikapirštę žarną, kasos sulčių sekrecija sumažėjo ($p<0,01$) pirmą ir antrą, lyginant su izotoninio tirpalo (0,9 % NaCl) injekcija, bei antrą ir trečią valandą nuo įliejimo pradžios, lyginat su LRR tirpalo (pH 1,5) injekcija. Įliejus LRR tirpalo (pH 1,5), kasos sulčių sekrecija buvo mažesnė ($p>0,01$) pirmą, antrą ir trečią valandą nuo įliejimo pradžios, lyginant su izotoninio tirpalo injekcija. Galima daryti išvadą, kad LRR gali turėti įtakos kasos sekrecijai.
Raktažodžiai: lakiosios riebalų rūgštys, avys, kasos sekrecija, didysis prieskrandis, dvylikapirštė žarna.

Introduction. Ruminants obtain their energy from short-chain fatty acids (SCFA), the major end-products of microbial fermentation in the reticulo-rumen. However, SCFA are not only an important energy source, but also a potential humoral factors that regulate exocrine pancreatic secretions in ruminants. In early studies, Taylor (1963) and Magee (1961) had shown that the application of SCFA directly into the ruminal lumen or duodenum via fistula increased both pancreatic juice outflow and amylase output in sheep. The authors attributed this effect to the duodenal acidification due to the application of SCFA. However, a decrease in the pH of intestinal digesta after infusion of hydrochloric acid into the small intestine of sheep resulted in an enhanced secretion of pancreatic juice only, whereas amylase output was not affected (Kato et al., 1987; Krzeminski et al., 1990). On the other hand, intraduodenal infusion of propionate elicited a transient increase in amylase output and a decrease in pancreatic juice outflow as well (Johnson et al., 1986). In agreement with these results, Katoh and Yajima (1989) and Katoh and Tsuda (1984) demonstrated under *in vitro* conditions that SCFA have the potential to induce the release of amylase in pancreatic tissue from sheep. However, in a recent study by Swanson (2003), using an *in vitro* model with calf pancreatic tissue, it was shown that SCFA did not increase pancreatic enzyme release and in some cases releases were inhibited. However, a better understanding of the mechanisms regulating pancreatic secretions in ruminants could lead to the development of optimized feeding strategies.

The objective of the present study was to investigate the effect of short-term intraruminal or intraduodenal infusions of SCFA adjusted to pH 7.0 or 1.5 on the pancreatic juice outflow in sheep.

Materials and methods. The experiments were performed on 27 Polish Merino sheep (initial body weight 45-55 kg). They were fed *ad libitum* good quality silage of sugar beets and grass hay. The animals had free access to water and mineral supply. Sheep were surgically fitted with ruminal fistulas according to Jarrett (1948) as described by Taylor (1962). After two weeks, the animals were fitted with catheters into the duodenum, common bile duct and gall bladder. Anesthesia was induced after 36 h of starvation with Nembutal (Pentobarbital, Abbot, USA), and an incision of 20 cm was made parallelly to and directly behind the last rib on the right side of the animal. The bile duct was ligated. A silicone catheter was inserted into the common bile duct for collection of pure pancreatic juice. Another catheter was inserted into the gall bladder for collection of bile. Two simple T-cannulas were inserted into the duodenum. One of them was inserted distal to the pylorus and the second cannula was inserted about 50 cm proximately of the common duct. The abdominal wall was closed with interrupted sutures. In the periods between sample collections pancreatic juice was diverted to the duodenum *via* the duodenal T-cannula.

The study was approved by the Warsaw Agricultural University Ethical Review Committee for Animal Experiments and conducted according to the European Community regulations concerning the protection of experimental animals. Experiments started one week after surgery. During the days of sample collection, the animals had free access to water, whereas the feed was withheld. To minimize the influence of diurnal variations, all measurements were performed between 06.00 h and 14.00 h. The infusion of SCFA or saline as a control was performed between 07.00 – 07.45 h. Pancreatic juice was collected over a period of 8 h. The volume of secretion was recorded at 60 min intervals. The pH value of the samples was determined immediately after collection. After measuring the volume of pancreatic juice and its pH, the pancreatic juice was returned to the duodenum *via* the duodenal T-cannula.

The sheep were assigned to 3 treatments. Control animals (n=7) received the intraruminal or intraduodenal infusion of saline; 10 animals obtained intraduodenal infusions of SCFA at pH 7.0 or 1.5, respectively, whereas another 10 animals were loaded intraruminally with a solution of SCFA at pH 7.0 and 1.5, respectively.

For the preparation of infusion solutions, the acetic and propionic acid at concentrations of 71 and 40 mmol, respectively, was dissolved in 150 ml of duodenal fluid (for duodenal infusion) or in 150 ml of ruminal content (for ruminal infusion). The pH of the solutions was adjusted to pH 7.0 and 1.5 using 5 N NaOH or 1 N HCl, respectively.

Statistical analyses of data was performed using Student's test and ANOVA**.**

Results. During the experimental period animals were clinically healthy and exhibited normal behaviour.

As shown in Figure 1, a marginal but not significant decrease in the pancreatic juice outflow was obtained for the intraruminal infusion of SCFA at pH 7.0 and saline compared with the infusion of SCFA at pH 1.5.

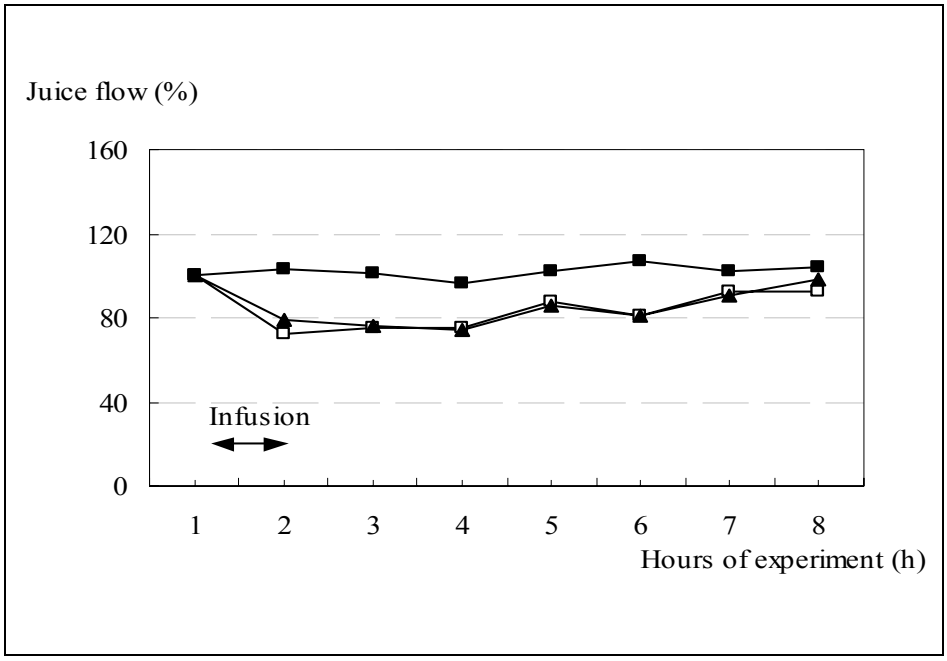

Figure 1. **Effect of intraruminal infusion of saline and SCFA at pH 1.5 and 7.0 on pancreatic juice outflow in sheep**

The intraduodenal infusion of saline (Figure 2) had no effect on the pancreatic juice outflow in comparison to pancreatic juice outflow before the infusion. The pancreatic juice outflow after intraduodenal infusion of SCFA at pH 7.0 was significantly ($p \leq 0.01$) lower over a period of 3 h after the start of infusion compared to the outflow before infusion. Compared to the intraduodenal infusion of SCFA infused at pH 7.0 there was a significantly higher production of pancreatic juice at the first and second ($p \leq 0.01$) as well as at the third and fifth hour ($p \leq 0.05$) post infusion. The pancreatic juice outflow

for the intraduodenal infusion of SCFA at pH 1.5 was significantly inhibited ($p \leq 0.01$) over a period of 3 h following infusion. After the intraduodenal infusion of the SCFA infused at pH 1.5, pancreatic juice outflow was significantly ($p \leq 0.05$) lower at the second hour after the start of infusion compared to the saline infusion. The pancreatic juice outflow for the intraduodenal infusion of SCFA at pH 7.0 compared to the intraduodenal infusion of SCFA at pH 1.5 differed significantly at the second and third ($p \leq 0.01$) as well as at the fifth hour ($p \leq 0.05$) after the start of infusion. The effect of intraduodenal infusion of saline and SCFA at pH 7.0 and 1.5 on the production of pancreatic juice is shown in Figure 2.

Intraduodenal as well as intraruminal infusions of saline and SCFA did not affect pH of secreted pancreatic juice (data not shown).

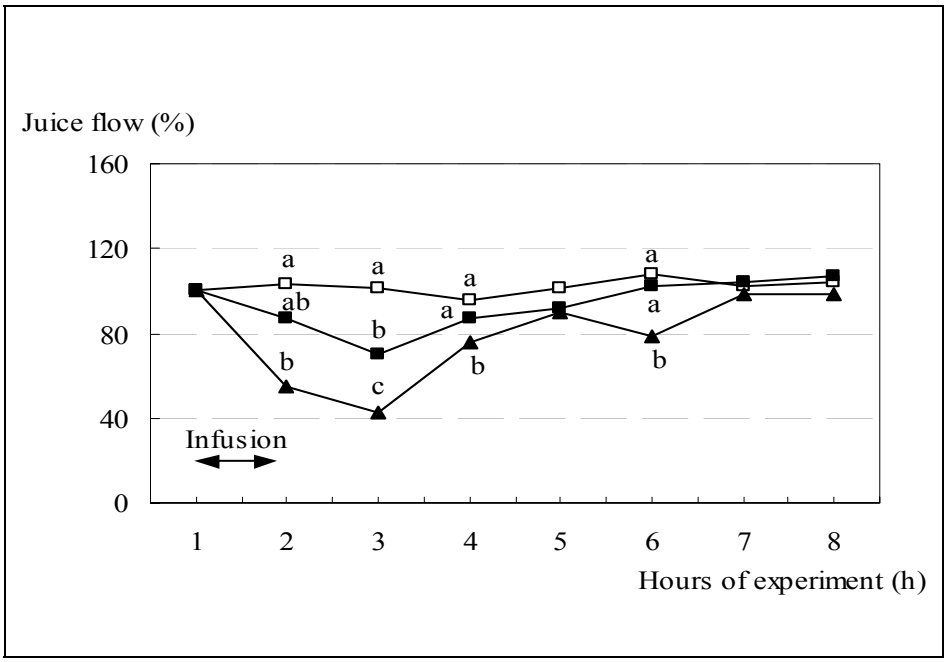

Figure 2. **Effect of intraduodenal infusion of saline (□) and SCFA at pH 1.5 (■) and pH 7.0 (▲) on pancreatic juice outflow in sheep. The different superscripts indicate significant ($p<0.05$) differences at any time of measurement**

Discussion. A short-term ruminal infusion of saline as control and SCFA at pH 7.0 resulted in a numerical decrease of pancreatic juice outflow (Figure 1) and an unchanged pH of the ruminal content (data not shown) in experimental animals. A transient decrease of the pH in ruminal content pH (data not shown) and a numerical increase in the flow of pancreatic juice, compared to the saline infusion, were obtained following ruminal infusion of SCFA at pH 1.5. These results suggest that the flow of acidic ruminal digesta to the duodenum may stimulate the pancreatic juice outflow to a certain level. Accordingly, Zabielski and Pierzynowski (2001) reported that in ruminating calves the continuous flow of acidic digesta through the abomasum and small intestine during the preprandial period might enhance exocrine pancreatic secretions. Taylor (1962) also showed in a study with adult sheep that the pancreatic secretory response increases after loading of acetic acid into the rumen, while the response did not occur when the flow of digesta into the duodenum was interrupted. The authors concluded that the increase in HCl secretion is responsible, mediated through secretin, for the pancreatic response.

An increase in pancreatic juice outflow as well as in amylase and protein output were obtained in ruminants following intravenous injections (Harada and Kato, 1983; Kato et al., 1989; Mineo et al., 1990) as well as intraduodenal infusions (Magee, 1961) of SCFA. In contrast, in the present study the short-term duodenal infusion of SCFA resulted in a transient decrease in pancreatic juice outflow. Moreover, the pancreatic juice flow after SCFA infusion at pH 7.0 was significantly lower than for SCFA infusion at pH 1.5 (Figure 2). Obviously, SCFA may affect pancreatic secretion independent of changes in pH. Johnson et al. (1986) found in sheep that intraduodenal propionate infusions resulted in a transient decrease in pancreatic juice outflow and an increase in amylase output on the first day of infusion. Swanson et al. (2003), using an *in vitro* model with calf pancreatic tissue, showed that SCFA did not increase amylase and trypsin release and in some cases releases of the enzymes were inhibited. Moreover, neurohormonal mimics (caerulein, cholecystokinin mimic; carbachol, acetylcholine analogue) did not influence enzyme release when tissues were incubated with SCFA. It has been suggested that in contrast to the supraphysiological concentrations used in the study of Katoh and Yajima (1989), more physiological concentrations of SCFA may have the potential to influence exocrine pancreatic secretions. Secondly, SCFA might reduce the response of tissue to neurohormonal stimulation. Cholecystokinin (CCK) had a direct receptoral effect on the pancreatic acinar cells and an indirect duodenal–mucosa-located mechanism that controls the pancreatic juice secretion *via* a long vago-

vagal reflex in preruminant calves (Zabielski and Pierzynowski, 2001). It may be speculated if SCFA are involved in the neurohormonal regulation of the pancreatic secretion in sheep. The mechanism for the inhibition of exocrine pancreatic secretions due to intraduodenal infusion of SCFA remains unclear. Obviously, these infused SCFA can mimic rumen acidosis in terms of SCFA concentration resulting from impaired absorption or overproduction of SCFA in the rumen. In each of these cases, such signals may inhibit pancreatic juice outflow which, in turn, may correspond with inhibited emptying of the abomasum. Obviously, the pH of the duodenal content is an essential regulator of pancreatic secretion in pre-ruminant calves (Zabielski and Pierzynowski, 2001).

Conclusions and prospective. It seems likely that SCFA in adult sheep may affect the pancreatic juice outflow both *via* pH dependent and pH independent mechanisms located in the mucosa of the proximal small intestine. However, further studies are needed to evaluate the possible effect of SCFA on the output of protein and enzymes in pancreatic juice, as well as possible hormonal pathways in which the SCFA may affect the exocrine pancreatic secretion. The results obtained may contribute to the development of optimized feedings strategies in ruminants.

References

1. Harada E., Kato S. Effect of short-chain fatty acids on the secretory response of the ovine exocrine pancreas. Am. J. Physiol. 1983. Vol. 244 (Gastrointest. Liver Physiol. 7). P. G284-G290.

2. Johnson D.D., Mitchell G.E., Tucker R.E., Muntifering R.B. Pancreatic amylase, plasma glucose and insulin responses to propionate and monensin in sheep. J. Dairy Sci. 1986. Vol. 69. P. 52-57.

3. Kato S., Ando T., Adachi N., Mineo H., Ushijima J. The effect of atropine on pancreatic responses to intravenous secretin and intraduodenal HCl in sheep. Jpn. J. Zootech. Sci. 1987. Vol. 58. P. 65-71.

4. Kato S., Asakawa N., Mineo H., Ushijima J. Effect of short-chain fatty acids on pancreatic secretion in calves aged 2 weeks and 13 weeks. Jpn. J. Vet. Sci. 1989. Vol. 51. P. 1123-1127.

5. Katoh K., Tsuda T. Effects of acetylcholine and short-chain fatty acids on acinar cells of the exocrine pancreas in sheep. J. Physiol. 1984. Vol. 356. P. 479-489.

6. Katoh K., Yajima T. Effects of butyric acid and analogues on amylase release from pancreatic segments of sheep and goats. Pflügers Arch. 1989.Vol. 413. P. 256-260.

7. Krzeminski R., Mikolajczyk M., Kulasek G. The effect of intraduodenal infusion of 0.1 N HCl on the volume and composition of pancreatic juice and bile in wethers. J. Anim. Physiol. Anim. Nutr. 1990. Vol. 64. P. 139-142.

8. Magee D.F. An investigation into the external secretion of the pancreas in sheep. J. Physiol. 1961. Vol. 158. P. 132-143.

9. Mineo H., Kanai M., Kato S., Ushijima J. Effects of intravenous injection of butyrate, valerate and their isomers on endocrine pancreatic responses in conscious sheep (*ovis aries*). Comp. Biochem. Physiol. 1990. Vol. 95 A, No 3. P. 411-416.

10. Swanson K.C., Matthews J.C., Woods C.A., Harmon D.L. Influence of substrate and/or neurohormonal mimic on in vitro pancreatic enzyme release from calves postruminally infused with partially hydrolyzed starch and/or casein. J. Anim. Sci. 2003. Vol. 81. P. 1323-1331.

11. Taylor R.B. Pancreatic secretion in the sheep. Res. Vet. Sci. 1962. Vol.3. P. 63-77.

12. Zabielski R. and Pierzynowski S.G. Development and regulation of pancreatic juice secretion in cattle. State-of-the art. J. Anim. Feed Sci. 2001. Vol. 10. P. 25-45.