The objective of this study was to investigate the secretion of pancreatic enzymes and antibacterial activity in weaned pigs of three pure breeds, Pietrain, Duroc and Polish synthetic line 990, to look for eventual differences related to the genotype. Six male pigs of each breed, about 24 kg mean body weight, were equipped with chronic pancreatic duct catheters and duodenal cannulas to assess pure pancreatic juice, and jugular vein catheters for blood withdrawal. Pancreatic juice was collected before and after the morning feeding. Protein output and enzyme activities revealed two distinct profiles: strong manifestation of the prandial phase in Pietrain and line 990 pigs, and weak manifestation in Duroc. The antibacterial activity did not follow the enzyme kinetics, and it was the strongest in pancreatic juice from Pietrain pigs. Postprandial insulinaemia was reduced in the order of: line 990>Pietrain>Duroc. A slight (not significant) tendency towards a reduction of leptin after feeding in synthetic line 990 corresponded with elevated secretion of pancreatic enzymes and plasma insulin. The presented results suggest that the prandial secretion of pancreatic juice differs according to genotype, and the differences may be in part related to release of insulin.

**Key words:** protein output, antibacterial activity, insulin, Pietrain, Duroc

**INTRODUCTION**

In pigs, like in other mammalian species, the secretion of pancreatic juice (PJ) depends on multiple factors involving the composition of food, feeding regime, phase of ultradian and circadian rhythm, as well as methods of pancreatic duct
catheterization and collection of pancreatic juice (1-3). The exocrine pancreas is controlled by a complex neurohormonal mechanism involving gut regulatory peptides (mainly cholecystokinin and secretin) and vagal nerve, pancreas islet hormones acting via the insulo-acinar axis and several other mechanisms (4). The regulatory mechanisms act in concert to establish optimal secretion of pancreatic enzymes for hydrolysis of ingested food. Recently, leptin, a hormone controlling feed intake and energy expenditure, was shown to control the PJ secretion in rats via several distinct pathways (5, 6). In pigs the role of endogenous leptin in controlling the exocrine pancreas remains unknown, though this hormone seems to be involved in regulation of energy metabolism (7).

Botermans and Pierzynowski (8) demonstrated that pancreatic trypsinogen secretion is positively correlated with the daily body weight gain in growing Swedish Landrace pigs, thus the pigs secreting more trypsinogen demonstrated better growth and feed conversion ratios. It is generally accepted that animal genotype affects digestive processes and, consequently, the growth of animals, though to our knowledge, no studies have been conducted to understand to what degree the genotype may affect the magnitude of pancreatic enzyme secretion.

The aim of the present study was to examine the secretion of pancreatic juice, plasma insulin, glucagon and leptin in three pig breeds differing in appetite, daily body weight gain and protein-energy deposition, i.e., in Pietrain, Duroc and Polish synthetic line 990, to look for eventual genotype-related differences. Pietrain pigs have been selected for a low-fat carcass, thus their amino acid turnover is faster and energy deposition lower as compared with the other pig breeds. Pietrain pigs also manifest reduced appetite and low daily body weight gain (9). Among the three examined breeds, line 990 shows the largest potential for depositing protein and high daily body weight gain with relatively low daily food intake. In contrast, Duroc pigs are known for good appetite, large volume of the gastrointestinal tract, and high body weight gain (9). In the present study, an antibacterial activity protein (ABA) was assayed in addition to PJ volume and activity of pancreatic enzymes. ABA was first described in canine pancreatic juice (10) and then in several other mammalian species (11) though details of its synthesis and secretion remain largely unknown. Laubitz and co-workers (12) found ABA in porcine pancreatic juice; the isolated antibacterial protein was identical to a 106 amino acid porcine pancreatic spasmolytic peptide (13), a member of the trefoil peptides family (14). ABA seems to control bacterial growth in the pancreatic acini and duct system as well as the microbial ecology in the upper gut lumen.

MATERIAL AND METHODS

Animals and treatment. The animal studies were approved by the Local Ethical Committee. Six healthy male pigs of each pure breed (Pietrain, Duroc and Polish synthetic line 990, Pig Research Station, Pawłowice, Poland), initially of about 24 kg body weight (BW), were used. Animals were
housed individually in metabolic cages with free access to water. The pigs were surgically fitted with chronic accessory pancreatic duct catheters (Silastic®), and T-shaped duodenal silicone cannulas (Silastic®) for collection and subsequent return of pancreatic juice into the duodenum (15). To access blood, a Silastic® catheter was inserted into the right external jugular vein. Surgery was performed under aseptic conditions. After the surgery and between PJ collections the pancreatic catheter and duodenal cannula were externally connected to allow free flow of pancreatic juice into the duodenum. A commercial pelleted diet containing 17% crude protein and 12.6 MJ metabolic energy (EM) was fed at a level of 3.5% BW before the surgery and 2.5% BW during the experimental period. The animals were fed twice a day at 8.00 am and 4.00 pm. The pigs were allowed to recover from the surgery for 8 days.

Pancreatic juice was collected for a total of 8.5 h, starting from 7.30 am to 4.00 pm. The volume of juice was measured every 30 min, and after taking a 1 mL sample the remainder was returned into the duodenum in 2-6 aliquots in the next 30 min period. The PJ samples were stored frozen (-20°C). Blood samples were taken on EDTA and aprotinin, 30 min before feeding and 15, 30, 60, 120 and 240 min after the meal, centrifuged and plasma kept frozen (-80° C) for further analysis of leptin, insulin and glucagon concentrations. Pancreatic juice was collected for antibacterial activity analysis on separate days using an identical sampling schedule. The juice was measured for volume, and after taking a 10 mL sample the remaining juice was infused into the duodenum in 2-6 aliquots in the next 30 min period.

Pancreatic juice and hormone analysis. The pancreatic juice was analyzed for total protein content by the Lowry method (16) modified to be performed in 96-microwell plates. Trypsin activity, following activation of the juice with enterokinase, was measured according to Erlanger et al. (17) using BAPNA (N-α-benzoyl-DL-arginine-p-nitroanilide, Sigma, USA) as the substrate. Amylase activity was determined according to the Walker and Harmon method (18). Lipase activity following prolipase activation was measured using a Lipase-PS kit (Trinity-Biotech, Ireland).

E.coli K12 strain AB1157 (19) was used to analyze PJ antibacterial activity. LB (Luria-Bertani) medium contained 1% Bacto-tryptone, 0.5% yeast extract and 0.5% NaCl. Plates were solidified with 1.5% Difco agar (Becton Dickinson, Sparks, MD). TC medium was Tris-HCl in C-salts (20) pH 8.3 supplemented with 0.5% glucose and 0.2% casamino acids. Bacterial overnight cultures (2x10⁹ cells/mL) were diluted 1:100 in TC medium. Pancreatic juice samples were mixed with a bacterial suspension at a ratio of 1 to 2 or 1 to 4. Colony forming units (CFU) were estimated by counting colonies growing on LB solid medium after 18 hours of incubation at 37°C. Samples for plating were taken immediately after preparing the bacteria-PJ mixture and after 3 and 24 hours of incubation at 37°C with shaking. Controls consisted of bacteria and TC. A decrease in bacterial growth compared with the control devoid of PJ (i.e., the bacterial growth / control bacterial growth (BG/CBG) ratio) to below 1.0 indicated antibacterial activity of PJ. Additionally, bacterial cell damage was observed under light/fluorescence microscope (Zeiss, Germany) following staining with propidium iodide (Molecular Probes, USA) and crystal violet (Sigma, USA).

Plasma leptin, insulin and glucagon concentrations were determined using Multi-species Leptin and Glucagon RIA kits (Linco Research, USA) and Insulin RIA kits (POLATOM, Poland), respectively.

Statistical analysis. The data are expressed as means and standard errors of mean (SEM). One way-ANOVA followed by post-hoc tests was used to indicate the statistical differences within and between the pig breeds (GraphPad Prism® v. 3.03, GraphPad Software, San Diego, CA, USA). Analysis of the area under the curve (AUC, GraphPad Prism®) was performed to analyse the concentrations of hormones in blood plasma. In all statistical analyses P<0.05 was taken as the level of significance.
RESULTS

Secretion of pancreatic juice.

The secretion of pancreatic juice, output of protein, and activity of trypsin, amylase and lipase are shown in Figures 1 and 2. In the preprandial period, there were no statistically significant differences in PJ volume between the examined breeds. Although there was a tendency towards lower pancreatic enzyme activity in Duroc pigs as compared with Pietrain and line 990, it did not achieve statistical significance. High standard deviations in the examined parameters were the result of collection of juice samples without attention to the periodic pancreatic secretion associated with duodenal myoelectric migrating complexes, MMC (21).

Feeding did not significantly increase the volume of pancreatic juice, though a tendency towards increases was observed during the first prandial 30 min period, particularly in line 990 pigs. One and a half hour after feeding an insignificant tendency towards a transient reduction in PJ volume was observed. Feeding significantly increased the protein output in synthetic line 990 pigs (P<0.01) and to a lesser, but still significant, degree in Pietrain pigs (P<0.05). No significant rise in protein output was found in Duroc pigs. Prandial trypsin and amylase activities showed tendencies toward increases in line 990 pigs, whereas trypsin and lipase, in Pietrain pigs. Again, these preprandial vs. prandial comparisons did not reach statistical significance due to high data scatter.

Analysis of the area under the curve of PJ secreted during the postprandial period showed a number of differences between the examined breeds. The volume of secreted juice during the 8 hours after morning feeding did not show statistical differences and equaled 7.7±0.1 mL/kg BW in Pietrain, 7.2±0.1 mL/kg BW in Duroc, and 7.8±0.1 mL/kg BW in line 990, respectively. However, the

Fig. 1. Pancreatic juice volume (A) and protein output (B) during 4.5 hours collection in Pietrain, Duroc and Polish synthetic line 990 pigs. Bars marked "ctrl" reflect 30 min of preprandial secretion and the following (0.5 - 4) bars represent the 30 min postprandial samplings. Values are means and SEM (n=6).
area under the curve (AUC) calculated for protein output during either the first 4 or 8 prandial hours was the highest for line 990 pigs (P=0.0007 and P=0.001, respectively). In Pietrain and Duroc pigs it was lower by 17% and 42%, respectively, as compared with line 990 pigs. The AUC for trypsin activity in Pietrain was not different from 990 line pigs, but was higher by 58% as compared with Duroc (P=0.01). The AUC for amylase activity showed a tendency similar to that observed in trypsin activity, however, without reaching statistical significance. The comparison of AUC for lipase activity did not show significant differences due to high animal-to-animal variations, though a tendency towards higher lipase activity could be observed in Pietrain pigs.

The antibacterial activity of pancreatic juice showed a pattern distinct from pancreatic protein and enzyme activities as demonstrated in Figure 3. Namely, during the preprandial period ABA was relatively high and showed a biphasic pattern postprandially, no bacteria growth inhibition for the first 2 hours, followed by marked inhibition thereafter. The strongest inhibition of bacterial growth was observed between the postprandial hours 2.5 and 4.5 in Polish synthetic line 990.
pigs (Fig. 3). Inhibition of bacteria growth following incubation with samples of pancreatic juice was the strongest for the samples from Pietrain pigs and was observed after 3 and 24 hours of incubation (Figs. 4, 5). The juice samples from

Table 1. Plasma insulin, glucagon and leptin in Pietrain, Duroc and Polish synthetic line 990 pigs during the preprandial (0 min) and postprandial period (15-240 min)

<table>
<thead>
<tr>
<th></th>
<th>After feeding [min]</th>
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<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>120</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>Insulin [U/mL]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pietrain</td>
<td>4.50 ± 0.91</td>
<td>8.91 ± 3.40</td>
<td>10.5 ± 4.03***</td>
<td>21.4 ± 5.49***</td>
<td>14.0 ± 5.81</td>
<td>11.2 ± 6.24</td>
</tr>
<tr>
<td>Duroc 990</td>
<td>3.80 ± 1.21</td>
<td>3.98 ± 1.16</td>
<td>6.19 ± 2.64a</td>
<td>16.9 ± 5.77***</td>
<td>14.9 ± 4.11***</td>
<td>8.34 ± 2.43</td>
</tr>
<tr>
<td></td>
<td>4.75 ±1.31</td>
<td>10.6 ± 4.25</td>
<td>29.7 ± 10.6̄</td>
<td>30.8 ± 16.2̈</td>
<td>21.3 ± 10.1̄</td>
<td>14.0 ± 8.33</td>
</tr>
<tr>
<td></td>
<td>Glucagon [ng/mL]</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Pietrain</td>
<td>0.51 ± 0.04</td>
<td>0.43 ± 0.05</td>
<td>0.44 ± 0.05</td>
<td>0.21 ± 0.06</td>
<td>0.37 ± 0.18</td>
<td>0.46 ± 0.14</td>
</tr>
<tr>
<td>Duroc 990</td>
<td>0.44 ± 0.13</td>
<td>0.33 ± 0.20</td>
<td>0.30 ± 0.17</td>
<td>0.35 ± 0.13</td>
<td>0.45 ± 0.19</td>
<td>0.54 ± 0.28</td>
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<tr>
<td></td>
<td>0.33 ± 0.28</td>
<td>0.32 ± 0.23</td>
<td>0.33 ± 0.18</td>
<td>0.38 ± 0.16</td>
<td>0.58 ± 0.23</td>
<td>0.57 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Leptin [µg/mL]</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pietrain</td>
<td>3.28 ± 0.47</td>
<td>3.38 ± 0.35</td>
<td>3.47 ± 0.27</td>
<td>3.29 ± 0.28</td>
<td>3.52 ± 0.42</td>
<td>3.48 ± 0.44</td>
</tr>
<tr>
<td>Duroc 990</td>
<td>3.38 ± 0.57</td>
<td>3.53 ± 0.47</td>
<td>3.00 ± 0.27</td>
<td>3.01 ± 0.25</td>
<td>3.22 ± 0.59</td>
<td>3.18 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>3.13 ± 0.44</td>
<td>3.45 ± 0.69</td>
<td>2.94 ± 0.67</td>
<td>3.03 ± 0.61</td>
<td>3.09 ± 0.64</td>
<td>3.22 ± 0.60</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=6. ANOVA followed by a post-hoc Tukey-Kramer test. Different letters in upper script indicate a statistical difference between the breeds at a given time point (P<0.05). Asterisks indicate a statistical difference from the preprandial value in the row: * P<0.05, ** P<0.01, and *** P<0.001.
Duroc and 990 line pigs did not inhibit bacterial growth during incubation for 3 hours, but did inhibit the growth of *E. coli* during incubation for 24 hours. The inhibition by PJ from line 990 was, however, stronger than that from Duroc pigs.

**Concentrations of hormones in blood plasma.**

*Table 1* shows plasma concentrations of insulin, glucagon and leptin in the examined Pietrain, Duroc and line 990 pigs. Plasma glucagon and leptin did not show breed-to-breed-related or food-related statistical differences. Interestingly, in Pietrain pigs plasma leptin was particularly stable, whereas in Duroc and line 990 pigs there was a tendency towards a slight decrease after feeding. Plasma insulin showed a prandial peak in all three examined pig breeds. However, the peak in line 990 was the largest, and that in Duroc was the smallest. In Pietrain

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Fig. 4. Inhibition of *E. coli* K12 strain AB1157 growth following incubation for 3 and 24 hours with pancreatic juice collected from Pietrain, Duroc and Polish synthetic line 990 pigs during the prandial phase (i.e. 2-4 hours after start of feeding). The pancreatic juice was mixed with the bacterial suspension at a ratio of 1:2 (A) and 1:4 (B). The results are expressed as the BG/CBG ratio (BG - bacterial growth, CBG - control bacterial growth, >1 - stimulation, <1 - inhibition of growth). Analyses were in triplicate. Arrows indicate time of feeding. Values are means and SEM (n=6).
and line 990, plasma insulin started to increase 30 min postprandially, whereas in Duroc, 30 min later.

**DISCUSSION**

*Secretion of pancreatic juice in Pietrain, Duroc and synthetic line 990 pigs.*

After overnight fasting, before the meal was offered, PJ volume and enzyme output in the three breeds did not statistically differ due to high data scatter. High
variability in pancreatic secretion results from the periodic character of pancreatic secretion in pigs. The cycles form an ultradian rhythm of a period of 60 to 70 min and are closely associated with duodenal MMC (22). Using precise flow-meters, Kiela and co-workers (2) demonstrated that the kinetic profile of pig PJ secretion shows remarkable fluctuations within the cycles, even 20-fold differences between the PJ secretion in peak and nadir. In the present study we did not record the pancreatic and MMC cycles due to the methodological complications introduced by implantation of electrodes on the small intestine, frequent collection of pancreatic juice, i.e. every 5 min, and simultaneous recording of myoelectric activity and juice flow. We arranged instead for a long-term collection of PJ in the postprandial period that should cover the postprandial pattern and several secretory cycles, and analyzed integrated data using AUC (21).

Analysis of the cumulative flow and enzyme output revealed the existence of two general secretory profiles: synthetic line 990 and Pietrain responded strongly to feeding with significantly elevated protein output, while Duroc hardly responded to feeding. Thus, Pietrain pigs selected for lean carcass, and consequently characterized by low daily body weight gain, and line 990 pigs with the largest potential to deposit proteins and increase body weight, secreted more pancreatic enzymes (in particular trypsin and amylase) than Duroc. This difference may be explained by the large size of the gastrointestinal tract in Duroc, which may help in more efficient enzymatic digestion with less pancreatic enzyme supply. In this strategy, the costs of pancreatic enzyme synthesis in Duroc pigs are reduced and thereby more energy can be spent on muscle protein synthesis than in the other two breeds. In older animals this extra energy can presumably also be spent on fat deposition. On the other hand, Pietrain pigs have the smallest gastrointestinal tract and show a worse feed conversion ratio than Duroc and line 990, during both ad libitum and restricted feeding (Fandrejewski, Skiba, Żebrowska, et al - unpublished data). Thus, high synthesis and secretion of pancreatic enzymes and ABA proteins is in agreement with low protein deposition in Pietrain pigs and may substantially contribute to the energy balance. In synthetic line 990 pigs, high pancreatic protein output corresponds with high daily body weight gain and energy deposition (9). Moreover, it is mirrored in high plasma insulin suggesting that insulin may play a role in this mechanism (see below).

The presented breed-to-breed comparison of pancreatic exocrine secretion does not agree with the previous observations made within one breed, which suggested that higher pancreatic enzyme output is associated with a higher body weight gain and better feed conversion ratio (8). Also in Yorkshire pigs with exocrine pancreatic insufficiency induced by ligation of the pancreatic duct, supplementation of pancreatic enzymes normalized growth rates, although supplementation in non-operated pigs was ineffective (23). Similar effects were observed in miniature pigs with experimental pancreatic insufficiency (24). These results contrast with a common belief coming from canine studies and human surgery practice that the exocrine pancreas overproduces digestive enzymes, and
a remarkable portion of the pancreas (up to 80%) can be removed without serious consequences in food digestion. This might be true in adults but not in fast growing infants in whom the amount of digestive juices seems to contribute to the kinetics of body growth.

**Leptin and insular hormones in controlling the exocrine pancreas in pigs.**

Lack of significant differences in serum leptin concentrations confirm the results obtained by Berg et al. (7), and may result from similar fat contents in the adipose tissue in examined breeds. McNeel (25) reported, however, that in 20 kg pigs, the plasma leptin concentration was the same in obese and lean pigs. Also, Maćkowiak et al. (26) reported no differences in plasma leptin of various breeds, though in their study they examined 100 kg pigs. No information in the literature is available concerning leptin levels in synthetic line 990. The tendency towards a lowered leptin ratio (0.5 - 2 h postprandial) in these pigs may be one of the reasons for the highest postprandial pancreatic response, since this hormone, when given intravenously, inhibits exocrine pancreas secretion via multiple mechanisms (5, 6). Leptin is also known to inhibit the synthesis and release of insulin from islet cells (27). In line 990 pigs the trend towards lowered plasma leptin corresponds to a high plasma insulin ratio. It may therefore be concluded that the high rate of postprandial pancreatic juice secretion in line 990 is due to enhanced insulo-acinar axis activity. On the other hand, in growth-retarded pigs the interdigestive synthesis and release of insulin was low and its secretory response to nutrients and 2-deoxy-D-glucose, reduced (28). These findings coincided with low secretion of pancreatic juice, though the exocrine pancreas was capable of responding to vagal and gut regulatory peptide stimulation. Amylase activity in the pancreatic juice and in the pancreas of growth retarded piglets was, however, significantly reduced even though trypsin and chymotrypsin activities were not different. This means that the status of the endocrine and exocrine pancreas has an important impact on pig growth after weaning. The endocrine-exocrine pancreas relationships are complex andregulated in part at the duodenal level. According to Rerat and co-workers (29), total pancreatic juice drainage in pigs depressed the absorption of glucose and amino acids, reduced insulin production by 64%, and did not change glucagon production. However, in our study the postprandial plasma glucagon showed a tendency toward reduction in Pietrain and Duroc pigs. In this aspect, our results are closer to the observation in adult Goettingen minipigs by Meier et al. (30). In their study high statistical significance (P < 0.01) was, however, obtained thanks to the recognition of glucagon pulses of approximately 4 min duration.

**Antibacterial activity in porcine pancreatic juice of different breeds.**

We have shown for the first time a food-related kinetic pattern of the antibacterial activity of pancreatic juice. In pigs, this pattern is distinctly different...
from that for pancreatic enzyme outputs. In the early prandial phase, the BG/CBG ratio increased instead of the expected increase in antibacterial activity, and following the first two prandial hours, the BG/CBG ratio dropped to below one and was maintained so until the end of the collection period. Previously, Pierzynowski et al. (11) suggested that the antibacterial activity of pancreatic juice may escape from known hormonal (cholecystokinin, secretin) mechanisms that regulate exocrine pancreas function. It is difficult to explain this biphasic pattern because a direct method for measuring ABA protein is not known, and we used a bioassay instead. Cephalic stimulation leads to secretion of pancreatic juice rich in proteins. During the ABA assay, juice proteins are heat denatured, and can presumably be utilized by bacteria for their growth. ABA is heat-resistant (12). In the sequential postprandial juice samples, the total protein contents could have been much lower relative to ABA, thereby unmasking the antibacterial activity in pancreatic juice. Consequently, in the preprandial pancreatic juice, ABA was also high due to a low protein content. This is in agreement with the microscopic observation showing destroyed bacterial cells following incubation with pancreatic juice. The antibacterial activity also tended to be breed-specific, however, again it was not related to any of prandial patterns in pancreatic juice. In Pietrain pigs, inhibition was obvious during the first 3 hours of incubation with the bacterial culture; after 24 hours of incubation, inhibition was evident in all samples.

In conclusion, the presented study shows that prandial secretion of pancreatic enzymes and antibacterial activity protein depend on pig genotype. In general, Pietrain and line 990 pigs secreted more pancreatic enzymes than Duroc. Also the antibacterial activity of pancreatic juice was the strongest in Pietrain, and the weakest in Duroc, though the ABA kinetic profile was distinctly different from that of pancreatic digestive enzymes. Plasma insulin also showed a non-uniform pattern in which the prandial responses in line 990 and Pietrain pigs were stronger than in Duroc.

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REFERENCES


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