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Influence of NSP-degrading enzymes on pH, ammonia and volatile fatty acids concentration in the stomach and small intestine of growing pigs

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ABSTRACT

Thirty-six male castrated pigs (German Landrace; body weight: 26-40 kg) received a diet consisting of (%): barley, 31; rye, 30; wheat bran, 18; soyabean meal, 15.5 and premixes 5.5, during a 3-week period. The diet contained 18.9% non-starch-polysaccharides (NSP) with 4.5% β-glucan, 9.4% arabinoxylan and 2.9% cellulose and was fed to another 18 pigs. This diet was supplemented with a commercially available enzyme premix (600 FXU xylanase and 56 FBG 1.4-β-glucanase per kg feed) and was fed to another18 pigs. At the end of the feeding period the animals were killed at three different times after feeding (1, 3 and 6 h postprandial). pH, ammonia concentration and volatile fatty acids (VFA) were determined in the digesta of the stomach and of the small intestine, which was divided into two sections of equal length.

The pH in the upper digestive tract was not significantly influenced by enzyme supplementation. The concentration of NH_3 in these sections was slightly lower compared with the unsupplemented control ($P>0.05$). Propionate was the most important volatile fatty acid in the stomach (44.7 molar percentage of VFA) and in the small intestine (76.6 and 75.3 molar percentage in the first and second section). It was found that the VFA concentration tended to be lower in the stomach (acetate: $P<0.05$) and higher in the small intestine (particularly propionate, $P>0.05$) if NSP degrading enzymes were added.

KEY WORDS: growing pigs, NSP, NSP-degrading enzymes, digesta, pH, NH_3, VFA

INTRODUCTION

Non-starch polysaccharides (NSP) affect digestion in the intestinal tract in many ways. They influence the viscosity in the small intestine, the absorption of nutri-

ents, and modify transit time and microbial fermentation (Antoniou and Marquardt, 1981; Kamphues, 1987; Smits and Annison, 1996; Simon, 1997; Aulrich and Flachowsky, 1998). Both mammals and birds have a natural lack of NSP-degrading enzymes. In monogastric animals the precaecal microbial population capable of producing such enzymes is small when compared with ruminants. Therefore, feeding diets high in NSP (e.g. rations rich in barley and rye) results in poorer performance in poultry and, to a lesser extent, in pigs. In order to improve feed conversion, NSP-hydrolyzing enzymes are used in poultry and pig nutrition. Many studies have been performed to measure the effects of NSP-degrading enzymes on performance parameters of nonruminants, as reviewed for pigs by Haberer and Schulz (1998).

In contrast to efficiency studies, little is known about the effects of enzymes on physiological processes in the digestive tract of pigs. Recently some studies were done to measure the effects of enzymes on digesta composition and disappearance of nutrients from the digestive tract (e.g. Bedford et al., 1992; Haberer et al., 1998a,b; Inborr et al., 1993; Jensen et al., 1998; Rattay, 1998).

Physiological parameters such as the pH, NH_3-concentration, and concentration of volatile fatty acids of the digesta are not described in these papers. The objective of the present experiment was to investigate the influence of supplementing β-glucanase and xylanase in pig diets on pH, NH_3-concentration and concentration of volatile fatty acids in various segments of the digestive tract depending on postprandial (ppr) time.

MATERIAL AND METHODS

Experimental design

Thirty-six male castrated pigs (German Landrace) with an initial mean bodyweight (BW) of 26.5 kg were divided into two groups of 18 and used in this experiment. The pigs were individually housed in pens (air conditioned: 20°C, relative humidity: 55-60%).

The experimental diets (Table 1) were based on barley, rye, wheat bran and soyabean meal and fed either unsupplemented (group C) or supplemented (group E) with a commercially available enzyme premix (ZY 28, microbial source *Humicola insolens*; Lohmann Animal Health, Cuxhaven, Germany) providing 600 FXU xylanase (EC 3.2.1.8) and 56 FBG 1,4-β-glucanase (EC 3.2.1.4) per kg feed as the main enzyme activities. FXU is defined as Colour-Xylanase-Unit, determined by colour reaction using Remazol Brilliant Blue-xylan (Cowan, 1994).

The animals were adapted to the diets over a period of 3 weeks. They received rations twice daily at 07.00 and 19.00 h in amounts of 110 g/kg $BW^{0.75}$ per day, but

in the last 3 days of the adaptation period they were each fed 1700 g/day. The diets were thoroughly mixed with 1 l water at each feed and additional water (1.5 l per animal) was given after feeding.

The final body weight of the pigs was 38.6±3.4 kg in the control and 39.3±2.9 kg in the enzyme-treated group. Only healthy animals were included in the trial. During the adaptation period the animals of both groups gained 585 and 595 g per day, respectively.

TABLE 1

Ingredients (%) and composition (% of DM) of the diets of the control (C) and experimental group (E)

Ingredients/Composition	C	E
Barley „*Gaulois*"	31.00	31.00
Rye „*Rapid*"	30.00	30.00
Wheat bran	18.00	18.00
Soyabean meal	15.35	15.275
Soya oil	2.50	2.50
Vitamin-mineral premix[1]	2.50	2.50
Calciumcarbonate	0.30	0.30
Lysine-HCl	0.25	0.25
DL-Methionine	0.05	0.05
L-Threonine	0.05	0.05
Enzyme complex ZY 28[2]	–	0.075
Organic matter	93.7±0.10	93.7±0.18
Crude protein	17.0±0.29	17.3±0.34
Ether extract	4.1±0.72	4.2±0.22
Crude fibre	6.2±0.07	6.2±0.33
N-free extracts	66.4±0.98	66.0±0.02
Ash	6.3±0.10	6.3±0.18
Starch	33.2±0.03	33.2±0.06
Sugars	5.3±0.05	5.2±0.07
NSP	18.8±0.13	18.9±0.11
$NSP_{insoluble}$	14.3±0.08	14.4±0.36
β-Glucan	4.5±0.01	4.6±0.13
$\beta\text{-Glucan}_{insoluble}$	2.6±0.01	2.7±0.02
Cellulose	2.9±0.03	2.8±0.09
Arabinoxylane	9.4±0.06	9.5±0.02
$Arabinoxylane_{insoluble}$	7.4±0.01	7.6±0.24

[1] provided the following per kg premix: Ca 240 g, P 60 g, Na 55 g, Mg 10 g, Fe 5500 mg, Zn 4000 mg, Mn 2500 mg, Cu 950 mg, I 40 mg, Se 13 mg; Vitamins: A 400000 IE, D_3 40000 IE, E 1200 mg, K_3 40 mg, B_1 40 mg, B_2 125 mg, B_6 80 mg, B_{12} 600 µg, pantothenic acid 245 mg, nicotinic acid 500 mg, choline 2400 mg

[2] main enzyme activities per g ZY 28 (Lohmann Animal Health, Cuxhaven/Germany): endo-1,4-β-xylanase (EC 3.2.1.8) 800 FXU (substrate Remazol Brilliant Blue Xylan), endo-1,4-β-glucanase (EC 3.2.1.4) 75 FBG (substrate Carboxymethylcellulose)

Sampling procedure

After the 3-week adaptation period, six animals per treatment were killed at three times after feeding (1, 3 and 6 h postprandial (ppr)) by electric shock and bleeding. Immediately after slaughter the abdominal cavity was opened and the gastrointestinal tract was ligated at the cardia, the pylorus, about the middle of the small intestine, the ileocaecal junction and the rectum. Then the stomach and small intestine were divided into two segments of exactly equal length (SI 1, SI 2) and removed from the mesentery. Digesta from the three segments were squeezed out carefully and quantitatively collected.

Analyses and statistical procedure

Nutrients of diets were determined as described by Naumann and Bassler (1976), starch determination followed the description by Salomonsson et al. (1984). Non-starch polysaccharides were determined as neutral sugars using the method of Theander et al. (1995).

The pH of digesta was measured potentiometrically. The ammonia concentration was determined by a modified Conway-method (Voigt and Steger, 1967).

The concentration of volatile fatty acids (VFA) in digesta was determined by gas chromatography (Hewlett Packard 5580 with FID), with 15% dioctylsebacinate and sebacinic acid with Kieselgur (60-100 mesh) as described by Honig and Rohr (1973). The samples were centrifuged at 5000 rpm for 5 min, then 1.5 ml of 25% metaphosphoric acid and 0.5 ml of formic acid were added to 10 ml of the supernatant and again centrifuged at 5000 rpm for 20 min. Finally one drop of saturated $HgCl_2$solution was added before analysis. Further details of materials and methods are described by Haberer (1997).

Data of measurements are given in the tables as average ($\bar{x}$) and standard deviation ($\pm S$). Data were analyzed with SAS® for Windows™ version 6.10 with the GLM procedure MANOVA. Differences between control and enzyme-group were tested with Fisher's least-significance-difference test.

RESULTS

Significant differences in the pH of digesta from various segments of the digestive tract were found (Table 2). The stomach pH was much lower (4.06) than in the small intestine. In SI 1 the pH was lower (6.06) than in SI 2 (6.77). Apart from one exception (SI 2, group E, 6 h ppr) the pH decreased in all segments of the digestive tract depending on the time ppr (from 6.05 to 5.58 and 5.26 on the average, Table 2). Enzyme supplementation did not significantly influence the pH of the digesta.

The ammonia concentration was relatively low in the digesta of all segments of the digestive tract (Table 3). The highest values were measured in SI 1 h ppr. Except for these values there were no significant differences between the digesta of various segments, time after feeding, or enzyme supplementation.

The highest concentrations of total volatile fatty acids were measured in the stomach (Table 4). There were some differences in VFA concentration between the intestine segments. One hour ppr the digesta of SI 1 showed higher values; 3 and 6 h ppr higher concentrations were measured in SI 2 (Table 4). Animals of the control group showed higher VFA concentrations in the stomach than enzyme-supplemented animals at any time, but because of the small number of animals (n = 6) and high standard deviation, the differences between groups were not significant ($P>0.05$). Except 6 h ppr in SI 1, enzyme-supplemented pigs showed higher concentrations of VFA in the digesta of the small intestine in all assays (Table 4). Molar concentrations of acetate, propionate and butyrate depended on the segment of the digestive tract, time ppr and enzyme supplementation (Table 5). Propionate

TABLE 2

pH in digesta depending on segment of digestive tract and time ppr (n = 6,$\bar{x}$, SD)

Time	Segment of digestive tract					
	stomach		small intestine 1		small intestine 2	
h ppr	C	E	C	E	C	E
1	4.89±0.16	4.74±0.40	6.17±0.15	6.27±0.17	7.08±0.10	7.16±0.12
3	4.00±0.25	4.21±0.12	6.02±0.14	6.03±0.28	6.62±0.30	6.59±0.24
6	3.18±0.31	3.36±0.39	5.95±0.15	5.91±0.19	6.43±0.25	6.73±0.35
$\bar{x}$	4.02	4.10	6.05	6.07	6.71	6.83

TABLE 3

Concentration of ammonia (mmol l^{-1}) depending on segment of digestive tract and time ppr (n = 6,$\bar{x}$, SD)

Time	Segment of digestive tract					
	stomach		small intestine 1		small intestine 2	
h ppr	C	E	C	E	C	E
1	2.6±0.7	2.2±0.5	4.0±0.6	3.9±1.0	2.5±0.9	3.1±1.2
3	3.5±1.8	2.8±1.3	3.2±0.3	3.1±0.7	3.0±0.7	3.1±1.0
6	3.7±1.1	3.5±1.0	3.6±1.0	3.1±0.5	3.6±1.5	3.2±1.0
$\bar{x}$	3.3	2.8	3.6	3.4	3.0	3.1

TABLE 4

Total concentration of volatile fatty acids (mmol l^{-1}) in digesta depending on segment of digestive tract and time ppr (n = 6,$\bar{x}$, SD)

Time	Segment of digestive tract					
	stomach		small intestine 1		small intestine 2	
h ppr	C	E	C	E	C	E
1	22.5±10.9	20.8± 7.7	14.8±5.3	17.9±9.3	13.6±3.2	14.3± 7.3
3	25.3±11.2	21.0±12.0	11.0±7.4	16.5±7.2	15.3±5.8	21.3± 7.8
6	25.7± 8.0	21.4± 8.4	8.7±0.3	8.0±3.5	19.0±6.1	23.0±10.9
$\bar{x}$	24.5	21.1	11.5	14.5	16.0	19.5

TABLE 5

Molar percentages of acetate, propionate and butyrate on total volatile fatty acids in digesta depending on segment of digestive tract and time ppr (n = 6,$\bar{x}$, SD)

Time	Segment of digestive tract					
	stomach		small intestine 1		small intestine 2	
h ppr	C	E	C	E	C	E
Acetate						
1	29.3±19.5	27.9±15.9	9.5± 2.0	6.1± 2.2	12.5± 4.4	14.0± 4.2
3	51.0±31.2	41.9±29.5	10.0± 1.8	8.5± 1.2	17.0± 4.6	14.6± 4.2
6	54.1±22.2	48.6±16.8	13.8± 3.4	15.0± 3.8	19.5± 6.3	18.3± 3.9
Propionate						
1	57.3±10.6	58.6±11.0	75.0±10.8	78.8± 8.5	72.8± 6.8	74.8± 7.3
3	39.1± 7.6	45.7± 8.6	74.5± 6.8	79.4± 6.7	72.5± 5.4	77.0± 8.1
6	33.5± 4.6	37.4± 5.8	71.3± 5.6	71.3± 6.2	74.2± 5.2	77.4±10.2
Butyrate						
1	9.3± 4.9	11.0± 2.9	14.9 ± 3.4	14.0± 4.5	14.0± 2.2	10.5± 2.8
3	8.3± 2.4	10.0± 7.6	14.5 ± 6.3	11.5± 4.2	10.4± 4.6	8.0± 1.4
6	10.5± 5.4	10.3± 5.6	12.6 ± 4.6	12.5± 5.0	5.8± 2.6	3.9± 1.7

was the dominating volatile fatty acid, especially in the small intestine. Regardless of the time ppr, the molar proportion of propionate in SI amounted to more than 70 molar percentage. It decreased from 58 to 35 in the stomach from 1 to 6 h ppr.

Enzyme supplementation increased the propionate concentration in all segments of the digestive tract and at each time. The molar acetate concentration was usually decreased and the butyrate concentration was lower in the small intestine after enzyme supplementation (Table 5).

DISCUSSION

Enzyme supplementation did not significantly influence the pH of the digesta (Table 2). In some cases we observed a higher pH after enzyme addition, in other situations we did not find any influence or a small reduction. Similar data are reported by Inborr et al. (1991), Bedford et al. (1992), and Rattay (1998). Van der Meulen and Bakker (1991) postulated that enzyme supplementation may decrease the buffer capacity of cell wall fractions resulting in a lower pH of digesta. Increased microbial fermentation may also decrease the pH of digesta.

Only a few studies examined the influence of enzyme supplementation on NH_3-concentration of digesta. In agreement with our data (Table 3) Hackl et al. (1995) and Rattay et al. (1998) found no significant influence of added NSP-degrading enzymes on NH_3-concentration of digesta in the stomach and small intestine. Some authors measured a lower pH of enzyme-supplemented groups in the stomach (e.g. Hackl et al., 1995) or in the small intestine (e.g. Sudendey and Kamphues, 1995).

Volatile fatty acids in the digesta arise mainly from microbial fermentation of carbohydrates. The total concentration of volatile fatty acids in the stomach and small intestine (Table 4) agrees with measurements by Friend et al. (1963), Bach Knudsen et al. (1991) and Rattay et al. (1998). Van der Meulen and Bakker (1991) found a lower concentration of VFA in the stomach and small intestine. The total concentration of volatile fatty acids of the control group was higher in the stomach, but enzyme supplementation insignificantly increased ($p>0.05$) the concentration of VFA in the small intestine. A higher concentration of VFA may result from the hydrolyzing activities of enzymes and higher microbial activity.

Few measurements providing the molar proportions of volatile fatty acids in the digesta of the stomach and small intestine are available. Most authors found that *in vitro* (Bardon and Fioramonti, 1983; Barry et al., 1995) and in vivo acetate was the dominating volatile fatty acid in the small intestine of pigs (Bach Knudsen et al., 1991; Ehle et al., 1982; Stanogias and Pearce, 1985; Gaedeken et al., 1989; Vervacke et al., 1989). Some newer data by Gollnisch (1998) and Rattay et al. (1998) confirm the values presented in this report (Table 5) and show 55 to 75% molar percentages of propionate in digesta in the small intestine of pigs. Jamroz et al. (1996) measured a high proportion of propionate in the digesta of poultry.

Some of the reasons for the variance in the data among different authors could be:

- various sources and amounts of NSP and other carbohydrates (i.e. sugars and starch),
- different experimental methods (e.g. time ppr.),
- short time of adaptation of animals.

Our present data demonstrate that microbial fermentation differs from processes in the rumen and the large intestine. Normally 50 to 60 molar percentage of

acetate and 20 to 30% of propionate are found in both compartments (e.g. Rohr and Kaufmann, 1975; Demeyer et al. 1995; Rattay et al., 1998).

Differences in microbial colonization and the available nutrients between the small intestine on the one hand and the large intestine or rumen on the other resulting from different physiological situations could be responsible for differences in the fatty acid pattern. Beginning in the stomach, enzyme supplementation increased the molar propionate concentration in all segments of the digestive tract (Table 5). These data seem to confirm an effect of NSP-hydrolyzing enzymes on fibre degradation and fermentation of smaller fractions. On the other hand, concentration levels do not allow any conclusions to be drawn about fermentation rate or fermented amount because of absorption of fermentation products. Therefore, the data should not be overestimated.

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STRESZCZENIE

Wpływ dodatku enzymów rozkładających polisacharydy nieskrobiowe (NSP) na pH, stężenie amoniaku i lotnych kwasów tłuszczowych (LKT) w żołądku i jelicie cienkim rosnących świń

Trzydzieści sześć wieprzków rasy niemieckiej zwisłouchej, o masie ciała 26-40 kg, żywiono przez 3 tygodnie dawką złożoną z (%): jęczmienia, 31; żyta, 30; otrąb pszennych, 18; śruty poekstrakcyjnej sojowej, 15,5 oraz premiksu, 5,5. Dawka zawierała 18,9% NSP, w tym 4,5% β-glukanu; 9,4% arabinoksylanu oraz 2,9% celulozy.

Dietę dla połowy zwierząt uzupełniano dodatkiem handlowego preparatu enzymatycznego (600 FXU ksylanazy i 56 FBG 1,4- β-glukanzay w kg paszy). Na zakończenie doświadczenia zwierzęta ubijano po 1, 3 lub 4 godzinach po odpasie. W treści żołądka i jelita cienkiego, które podzielono na 2 części równej długości, oznaczono pH, stężenie amoniaku oraz LKT.

Dodatek enzymów nie wpłynął istotnie na pH górnej części przewodu pokarmowego ani na stężenie NH_3. Propioniany stanowiły istotną część kwasów w żołądku (44,7% sumy LKT) i w jelicie cienkim (76,6 i 75,3% mol, w pierwszej i drugiej części, odpowiednio). Przy dodatku enzymów do diety stwierdzono tendencję do zmniejszenia stężenia LKT w żołądku (kwas octowy, $P<0,05$), a zwiększenie w jelicie cienkim (szczególnie propionianów, $P>0,05$) .