Effect of herbal extracts on piglet performance and small intestinal epithelial villi

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ABSTRACT: The effect of a mixture of herbal extracts on piglet performance was estimated on 178 piglets allocated to 3 groups comprising 6 litters each. Group I (control) was fed with the standard barley-wheat-soybean mixture. Group II received the same mixture supplemented with a blend of formic and propionic acids. Group III received the basal diet supplemented with a mixture of water extracts from sage, lemon balm, nettle and coneflower (20, 30, 30, and 20%, respectively) at 500 mg/kg feed. The experiment lasted for 84 days but on day 56 six piglets from each group were slaughtered and their gastrointestinal tract was removed. Apparent digestibility was estimated using the Cr_2O_3 indicator method. Acidity of digesta was measured in the stomach, ileum, and caecum, and volatile fatty acid content was evaluated in the ileum and caecum. Amounts of bacteria and morphological structure were evaluated in the ileal digesta and epithelium, respectively. In the experimental groups less dead and culled piglets were observed than in the control group. Piglets in the herb-supplemented group grew faster than control animals and showed significantly higher final average body weights. There was no significant difference in feed utilization. Acetic acid content was higher in both groups receiving supplements. The amount of propionic acid in the caecum of animals from the herb-supplemented group was lower than in animals from groups I and II. There were no significant differences in bacteria population in ileum chyme. The herbal extract improved the structure of the ileal epithelium by significantly increasing villus height. Better digestibility of nutrients could be due to higher villi in this group.

Keywords: piglet nutrition; ileum; intestine morphology; plant extracts

Herbs and their extracts have been used in human and veterinary medicine for a long time (Viegi et al., 2003). Plants contain many active substances such as phenolic compounds (mainly tannins and flavonoids), essential oils, and others (Reuter et al., 2007; Schnitzler et al., 2008). Herbs can be used as dried parts of plants (Kong et al., 2007a) or as extracts prepared mainly using water or ethanol as solvents (Kong et al., 2007b). Such extracts are more practical than dried plants because of their higher content of active substances and easier application. Herbs probably may replace antibiotics and synthetic growth promoters banned by the European Union (Namkung, 2004).

The positive effect of herbs in pig feeding was found by Mellor (2000). Paschma (2004) examined the influence of herbal supplements on sows' performance. The beneficial effect of herbs added to feed mixture for fatteners on their weight gains, carcass meat content, and meat quality was noted by Szewczyk et al. (2006) and Hanczakowska et al. (2007). Sage (*Salvia officinalis*) contains essential oils which have strong antimicrobial and antioxidant activities (Bozin et al., 2007). Another plant with similar properties is lemon balm (*Melissa officinalis*), the antimicrobial activity of which was found by Mahady et al. (2005). Also purple coneflower (*Echinacea purpurea*), previously used in botanical medicine, is now the subject of research and its immunological activity is known (Goel et al., 2005). Herbal extracts can be especially useful in feeding of piglets sensitive to harmful environmental effects. Oetting et al. (2004) obtained beneficial results by adding the herb extract to piglet feed but only in a relatively high dose (about 2000 ppm). In a study of Namkung et al. (2004) the antibiotic

limited the growth of harmful and beneficial bacteria while herb extracts inhibited only the growth of pathogenic bacteria.

The aim of this experiment was to evaluate the effects of a mixture of herbal extracts on rearing results, performance, intestinal microbiota, and structure of mucosal epithelium of the ileum in piglets and to compare them with the traditional acidifier.

MATERIAL AND METHODS

Animal management, treatments, and experimental design

The present experiment consisted of two parts: a growing experiment and a digestibility trial. All experimental procedures involving the use of live animals were approved by the Local Ethics Committee for Experiments with Animals. The study was conducted under continual veterinary supervision.

Growing experiment. The experiment was performed on 178 piglets originating from Polish Landrace × Polish Large White sows mated to a Pietrain × Hampshire boar. After farrowing, piglets were allocated to three experimental groups comprising 6 litters each. Piglets received experimental mixtures *ad libitum* from the 7th day of age to weaning (35th day of age), whereas restricted feeding according to piglet body weight was used from weaning to the end of the experiment (84th day of age). The amount of feed was increased every 7th day by 200 g. On the 35th day of life piglets were weaned and each litter was kept in a separate pen. Animals had free access to water. The experiment lasted until the 84th day of life, but on the 56th day of the experiment 6 piglets

Table 1. Composition of diets for piglets

	Group I	Group II	Group III
Components (g/kg)			
Wheat, ground	419	414	418.5
Barley, ground	200	200	200
Soybean meal	250	250	250
Rape oil	10	10	10
Dried whey	50	50	50
Skim milk powder	40	40	40
Salt	3.5	3.5	3.5
Calcium carbonate	8	8	8
Dicalcium phosphate	12	12	12
L-Lysine	1	1	1
DL-Methionine	1.5	1.5	1.5
Vitamin-mineral premix[1]	5	5	5
Propionic and formic acid	–	5	–
Herbal extracts mixture	–	–	0.5
Nutritional value of 1kg mixture			
Metabolizable energy[2](MJ)	12.56	12.65	12.65
Dry matter (g)	886.5	910.8	881.6
Crude protein (g)	195.0	200.3	207.5
Digestible protein (g)	141.6	146.8	158.7
Crude fat (g)	27.0	26.9	27.0
Crude fibre (g)	28.1	28.5	24.0

[1]composition of vitamin-mineral premix: vit. A 2 700 000 IU, vit. D_3 400 000 IU, vit. E 8.0 g, vit. K_3 0.5 g, vit. B_1 0.5 g, vit. B_2 0.8 g, vit. B_6 0.8 g, vit. B_{12} 0.008 g, pantothenic acid 2.8 g, choline chloride 70 g, folic acid 0.2 g, nicotinic acid 5.0 g, magnesium 10 g, manganese 12 g, iodine 0.1 g, zinc 30 g, iron 20 g, copper 32 g, cobalt 0.06 g, selenium 0.04 g, complete limestone to 1000 g

[2]calculated using the equation of Hoffmann and Schiemann (1980)

from each group (i.e. one piglet from each litter) were slaughtered and their gastrointestinal tract was removed. The individual body weight of all piglets was recorded at 1, 35, 56, and 84 days. Feed intake was measured and feed utilization was calculated for each pen. Piglets were fed with a standard barley-wheat-soybean mixture (Table 1) with no supplement (Group I – control), supplemented with 1 : 1 (w/w) mixture of propionic and formic acids at 5 g/kg of feed (Group II) or with herbal extracts mixture at 500 mg/kg of feed (Group III). Water extracts were produced from sage (*Salvia officinalis*), lemon balm (*Melissa officinalis*), nettle (*Urtica dioica*), and purple coneflower (*Echinacea purpurea*) in amounts of 20, 30, 30, and 20%, respectively. Extracts were commercial preparations produced by Phytopharm Kleka S.A. (Nowe Miasto, Poland).

Digestibility experiment. Between the 56th and 70th day of life, selected piglets from the growing experiment were used for the digestibility trial. Apparent digestibility of nutrients was estimated in 4 litters from each group (10–12 piglets on average), using the indicator method with Cr_2O_3 (3.0 g/kg). The adaptation period lasted 10 days and the balance period 5 days. Feces from one litter were collected once a day and frozen at –20°C. At the end of collection period all daily feces were mixed together and mean sample was prepared for each litter.

Microbiological analyses

Microbiological analyses were performed in ileum digesta. The tests were made with the plate method on agar medium (bioMérieux, Craponne, France), according to European Standards (ISO 16654:2001; ISO 15213:2003; ISO 7937:2004).

Histological analysis

Samples of the ileum were spread on polystyrene plates and fixed in 10% buffered formalin. The intestinal wall was precisely cut and four slides were prepared from each sample. They were stained with hematoxylin and eosin and embedded in paraffin. Villus height and crypt depth were evaluated under a light microscope. Data acquisition was performed with a Carl Zeiss Axioskop microscope (Carl Zeiss GmbH, Jena, Germany) and CDD Camera ZVS-47DE (Optronics Inc., Goleta, USA) connected by RGB line with a graphic card GraBIT PCI (Soft Imaging System GmbH, Munster, Germany) installed in a standard PC computer.

Chemical analyses

Gross composition of feeds and feces was analyzed according to AOAC (2005). Chromium contents in feed and feces were determined after nitric acid × perchloric acid wet ash preparation (AOAC, 2005). Apparent digestibility coefficients (ADC) were calculated using the following equation:

$$ADC\ (\%) = 100 - [100 \times ((a/b) \times (c/d))]$$

where:

a = chromium content in feed (%)

b = chromium content in feces (%)

c = nutrient content in feces (%)

d = nutrient content in feed (%)

Acidity of stomach, ileum, and caecum contents was measured with a CP-411 pH meter (Elmetron, Zabrze, Poland) equipped with a Metron 12-01 electrode (Metron, Troun, Poland). Short-chain fatty acids (SCFA) in the ileum and caecum were separated on column CP-Wax 58 (Varian BV, Middelburg, the Netherlands) (25 m, 0.53 mm, 1 m, carrier gas – helium, 6 ml/min), with a column oven temperature program from 90 to 200°C, using Varian 3400 gas chromatograph (Varian Associates Inc., Walnut Creek, USA) equipped with a Varian 8200 CX autosampler (2000 C), FID detector (2600 C), and Star Chromatography Workstation Software.

Antioxidant activity of the extracts mixture was estimated with the spectrophotometric method *in vitro* using synthetic radicals ABTS (Re et al., 1999). Results are expressed as Trolox Equivalent Antioxidant Capacity (TEAC). Content of phenolic compounds was analyzed spectrophotometrically according to Singleton and Rossi (1965) using the Folin-Ciocolteu reagent. Results are expressed as Gallic Acid Equivalent (GAE).

The contents of tannins, phenolic acids, and flavonoids were determined spectrophotometrically according to Polish Pharmacopoeia (2002).

Statistical analysis

Statistical analysis of treatment effects was conducted using analysis of variance with compari-

son of means by Duncan's multiple range test at $P \leq 0.05$ and $P \leq 0.01$ levels of significance using STATISTICA Version 5.1 package (StatSoft, 1996).

RESULTS

While the content of tannins and phenolic acids was similar in all extracts, the content of flavonoids in the extract from sage was almost three times higher than that in the extract from lemon balm (Table 2).

Sage extract had the highest content of polyphenols (59 GAE) and the highest antioxidant activity (2528 TEAC). Both these values were the lowest (16.8 GAE and 601 TEAC) in the case of coneflower extract (Table 3).

There were less dead and culled piglets (Table 4) in group III (8.5%) than in the control group (10.2%), but slightly more than in group II (6.5%). At the beginning of the experiment average body weight of experimental piglets was lower than that of controls (Table 4). At weaning (day 35) body weight of all piglets was similar (7.61–8.25 kg), but the difference between group III and the control group was about 8%. At the end of the experiment the body weight of piglets fed herbs (25.08 kg) was significantly higher ($P \leq 0.05$) than that of control and acid-supplemented pigs (22.75 and 22.94 kg). In the first period of the experiment (1^{st}–35^{th} day of age) piglets from herb-supplemented group III grew faster by 15.7% ($P \leq 0.01$) than control animals and by 9.3% when compared with acid-supplemented group II. At later periods piglets fed with herbal extract still grew faster than those from groups I and II, but these differences were not significant. However, during the whole experiment (1^{st}–84^{th} day of age) differences in piglets' body weight gain between group III (284 g) and the other two groups (253 and 256 g) were significant ($P \leq 0.05$) and amounted to 12.2 and 10.9%, respectively.

During the whole experiment (1^{st}–84^{th} day) the average feed utilization in group III receiving the diet with herbal extracts was significantly better, by about 10%, than in groups I and II (Table 3). Statistically significant differences were also observed between feed conversion of piglets fed with herb mixture and the control group; they were about 4% during nursing (7^{th}–35^{th} day of age) and 12% after weaning (35^{th}–84^{th} day of age).

Table 5 shows the results of digestibility study. The herbal extracts significantly improved digestibility of all nutrients when compared to the control group, while in the case of dry matter and crude fat also compared to the acid-supplemented group ($P \leq 0.05$). In contrast to the control group, piglets receiving the herbal supplement were characterized by better protein digestion (by 2.6 percentage points), and better crude fat and crude fibre digestion (by about 12.6 and 9.3 percentage points, respectively).

Table 2. Content of phenolic compounds in the extracts (g/kg)

Extract	Tannins	Phenolic acids	Flavonoids
Sage (*Salvia officinalis*)	38.7	39.7	14.2
Lemon balm (*Melisa officinalis*)	33.7	28.5	5.3
Coneflower (*Echinacea purpurea*)	36.4	26.3	13.2
Nettle (*Urtica dioica)*	26.3	25.3	9.4

Table 3. Antioxidant activity and polyphenols content in preparations

Extract	Antioxidant activity TEAC[1]	Content of polyphenols GAE[2]
Herbal extracts mixture	1917 ± 86.6	43.4 ± 0.88
Sage *(Salvia officinalis)*	2528 ± 96.8	59.0 ± 0.86
Lemon balm *(Melissa officinalis)*	1300 ± 31.9	36.5 ± 0.86
Nettle (*Urtica dioica)*	743 ± 18.8	23.3 ± 0.88
Coneflower (*Echinacea purpurea)*	601 ± 16.8	16.8 ± 0.86

[1]μmol of trolux per g of preparation
[2]mg of gallic acid per g of preparation

Table 4. Indices of piglet performance

Indices	Group I	Group II	Group III	SEM
No. of litters in treatment	6	6	6	–
No. of born piglets per treatment	59	60	59	–
Average No. of piglets born per litter	9.83	10.0	9.83	–
Average No. of piglets weaned per litter	8.83	9.33	9.00	–
Average No. of piglets at 84th day per litter	8.83	9.00	9.00	–
Dead and culled piglets (%)	10.2	6.5	8.5	–
Body weight (kg) **on days of age**				
1st	1.75^{B}	1.68^{B}	1.48^{A}	0.027
7th	2.68^{b}	2.62^{b}	2.36^{a}	0.054
35th	7.61	7.87	8.25	0.147
56th	11.13^{a}	11.36^{ab}	12.30^{b}	0.207
84th	22.75^{a}	22.94^{a}	25.08^{b}	0.438
Average daily gain (g) **in periods of life** (days)				
1–35	172^{A}	182^{AB}	199^{B}	4.018
35–56	167	166	193	5.527
56–84	415	413	456	10.982
35–84	309	308	343	7.333
1–84	253^{a}	256^{a}	284^{b}	5.195
Feed conversion ratio (kg/kg) **in periods of life** (days)				
7–35	0.114^{b}	0.113^{b}	0.109^{a}	0.002
35–56	1.50^{B}	1.50^{B}	1.32^{A}	0.029
56–84	2.32^{B}	2.33^{B}	2.03^{A}	0.066
35–84	2.06^{B}	2.10^{B}	1.81^{A}	0.035
1–84	1.47^{B}	1.46^{B}	1.32^{A}	0.019

^{a,b}mean values in the same row with different letters differ significantly at $P \leq 0.05$

^{A,B}mean values in the same row with different letters differ significantly at $P \leq 0.0$

Acetic acid was the most abundant acid both in the ileum and caeca (Table 6). Its content was very similar (about 5 µmol/g wet weight) in the ileum of animals from all groups. The only significant difference in the ileal chyme of piglets was in the contents of valeric and isovaleric acids, but the amount of both these acids was very low. The contents of short-chain fatty acids in caecum

Table 5. Apparent digestibility coefficients of nutrients (%)

	Group I	Group II	Group III	SEM
No. of litters	4	4	4	–
Dry matter	81.6^{a}	81.8^{a}	83.2^{b}	0.306
Crude protein	72.9^{a}	73.3^{ab}	75.5^{b}	0.532
Crude fat	32.3^{aA}	34.6^{aAB}	44.9^{bB}	2.131
Crude fibre	18.5^{a}	21.1^{ab}	27.8^{b}	1.570
N-free extract	93.1^{A}	93.1^{AB}	93.4^{B}	0.119

^{a,b}mean values in the same row with different letters differ significantly at $P \leq 0.05$

^{A,B}mean values in the same row with different letters differ significantly at $P \leq 0.01$

Table 6. Short-chain fatty acids (SCFA) content of piglets' ileum and caecum chyme (μmol/g wet weight)

VFA	Group I	Group II	Group III	SEM
No. of piglets	6	6	6	–
Ileum				
Acetic	4.59	5.93	5.28	0.678
Propionic	0.805	0.617	0.562	0.117
Isobutyric	0.032	0.297	0.078	0.068
Butyric	0.320	0.017	0.103	0.046
Isovaleric	0.037^{A}	0.351^{B}	0.209^{AB}	0.043
Valeric	0.016^{a}	0.199^{b}	0.059^{a}	0.029
Total acids	5.804	7.567	6.291	0.840
Caecum				
Acetic	53.234	68.747	61.490	5.308
Propionic	38.432	37.310	32.978	2.115
Isobutyric	0.472	1.160	0.757	0.149
Butyric	20.332	14.754	17.668	1.576
Isovaleric	0.222	0.576	0.308	0.080
Valeric	3.217	3.554	3.597	0.362
Total acids	115.909	126.104	116.800	8.125

^{a,b}mean values in the same row with different letters differ significantly at $P \leq 0.05$

^{A,B}mean values in the same row with different letters differ significantly at $P \leq 0.01$

digesta did not differ significantly between the groups.

The only significant difference in acidity of the contents of digestive tracts was higher pH in the ileum and jejunum of piglets receiving herb extracts (Table 7).

Supplementation of the herbal extracts lowered the amount of *Escherichia coli* when compared to the remaining groups but this difference was not significant (Table 7). There was also no significant difference in total amount of anaerobic bacteria, though acids in group II significantly lowered the number of

Table 7. Acidity of digesta in different parts of digestive tract and microbial population in small intestine (ileum) digesta

Item	Group I	Group II	Group III	SEM
No. of piglets	6	6	6	–
Digesta pH				
Stomach	1.65	1.26	1.58	0.095
Small intestine				
Duodenum	5.76	5.29	5.72	0.188
Jejunum	5.87^{a}	5.86^{a}	6.30^{b}	0.080
Ileum	5.58^{A}	5.96^{AB}	6.52^{B}	0.147
Caecum	5.50	5.68	5.72	0.064
Number of microorganisms in digesta (Log_{10}CFU/1g chyme)				
Escherichia coli	9.01	8.98	7.67	0.633
Anaerobic bacteria	7.01	7.64	9.27	0.474
Clostiridium	2.14^{b}	0.41^{a}	2.36^{b}	0.336

^{a,b}mean values in the same row with different letters differ significantly at $P \leq 0.05$

^{A,B}mean values in the same row with different letters differ significantly at $P \leq 0.01$

Table 8. Ileum mucosa epithelium structure

Item	Group I	Group II	Group III	SEM
No. of measurements	206	142	206	–
Villus height (μm)	257^{A}	285^{B}	316^{C}	3.56
Villus width (μm)	111^{A}	121^{B}	133^{C}	1.19
No. of measurements	107	156	111	–
Crypt depth (μm)	280^{A}	381^{C}	335^{B}	4.66
Villus height/crypt depth	0.917	0.748	0.943	–

$^{a-c}$mean values in the same row with different letters differ significantly at $P \leq 0.05$

$^{A-C}$mean values in the same row with different letters differ significantly at $P \leq 0.01$

clostridia (0.41 Log_{10}CFU/1 g) when compared to the control group and the group receiving herbal extracts (2.14 and 2.36 Log_{10}CFU/1 g, respectively).

Both additives used in the experiment significantly ($P \leq 0.01$) improved the ileum mucosa structure by increasing all villi measurements as well as the crypt depth (Table 8). The villi height observed in the intestine of piglets receiving herbal extracts was increased by about 23 or 11% when compared with the control or acid-supplemented groups, respectively.

DISCUSSION

A slightly higher number of dead and culled piglets receiving herb extracts than those receiving acids suggests that herbs as an antimicrobial agent are not so good as organic acids that have been used for many years as preservatives of animal feeds (Partanen and Mroz, 1999). On the other hand, however, they had a positive effect on piglets when compared to control which is in accordance with the results of Kuhn et al. (2005), who found immunostimulatory effects of *Echinacea purpurea* in piglets. Also Strompfová and Lauková (2009) reported probiotic properties of sage extract, which was also one of the extracts used in this experiment.

Grela et al. (2006) found slightly better body weights in piglets receiving extracts from lemon balm, nettle, and purple coneflower compared to unsupplemented piglets, but contrary to this study Utiyama et al. (2004) found no improvements in the weight gains of piglets receiving extracts from garlic, cloves, cinnamon, pepper, and thyme. They probably used an insufficient amount of the extract, because in another experiment (Oetting et al., 2004) they achieved better results by increasing the extract dose.

It seems that in the present experiment higher body weight gains of piglets receiving herb extracts resulted from an increase in nutrient digestibility, which in turn was an effect of positive development of the intestinal structure. Intestinal villi are the main site of nutrient absorption (Ray et al., 2002) and their better development could be the reason for higher nutrient absorption (Mekbungwan et al., 2002) as well as for better performance of piglets (Swiatkiewicz and Hanczakowska, 2006). Velazques et al. (2005) reported positive effects of herb extract on the development of the intestinal tract. Also according to Kong et al. (2009) the digestibility of protein and intestinal absorption of amino acids can be improved by adding herbal powder to the feed for young pigs.

It is known that the amount of volatile fatty acids in the digestive tract of pigs increases from its proximal to distant parts. In a study by Nyachoti et al. (2006) with early-weaned pigs, concentrations of acetic and propionic acids changed from 0.907 and 0.343 mmol/l in the duodenum to 70.29 and 56.92 mmol/l in the ileum, respectively. The lack of significant differences between particular groups of piglets in fatty acids content of both parts of the digestive tract suggests that herb extract and organic acids were not sufficiently active antimicrobial factors to lower volatile fatty acids production (Mroz, 2005).

Literature data concerning the effect of organic acids on pH in the digestive tract are not consistent. While Fevrier et al. (2001) found a reduction of pH in the stomach when feed for piglets was supplemented with formate, Canibe et al. (2001) did not find such a relationship. No difference in the pH of intestinal contents of hens after supplementing feed with formic and propionic acids was found by Thompson and Hinton (1997) and a similar result was obtained by Manzanilla et al. (2004) who used formic acid and plant extracts in the experiment on piglets. According to these last authors stomach acidity is mainly affected by the buffering capacity of solid meal and water. Also Namkung et al. (2004)

found no difference in pH of ileum chyme after supplementing feed with organic acids, probably due to buffering capacity of the secretions into intestine.

In the present experiment the differences in the amount of microorganisms in the ileum digesta were generally small, which is consistent with the results of Namkung et al. (2004) who also found no difference in coliform populations in the ileum and colon of piglets receiving organic acids and herb extracts. According to Castillo et al. (2006) who used butyric acid, the effect of this acid and plant extracts was not related to a reduction in the number of total bacteria inhabiting different sections of the gastrointestinal tract but rather to changes in the ecological structure and metabolic activity of the microbial community. On the other hand, the small amount of *clostridia* in the ileum chyme of animals receiving acids in this experiment could be the result of antimicrobial activity of propionic and formic acids (Roth and Kirchgessner, 1998). The decrease in the number of *E. coli* and *clostridia* in the intestinal digesta was also observed when fumaric or medium chain fatty acids were used in feed for piglets (Hanczakowska et al., 2011).

As mentioned above, there is a relationship between villi height and nutrient absorption and digestibility (Mekbungwan et al., 2002). Also Pappenheimer and Michel (2003) proved a decisive role of villi in the intestinal absorption of nutrients. The positive effect of extracts from medicine plants on piglet villi height was reported also by Fang et al. (2009). Thus it is likely that changes in villi structure under the influence of the herb extract improved nutrient absorption and, in turn, piglet performance. Now we do not have a satisfactory explanation of the beneficial effect of herbal extracts and their antioxidative activity on ileal mucosa structure and piglet performance. It is possible that it is due to free radical-scavenging activities of polyphenolic compounds (Rice-Evans et al., 1997; Asfar et al., 2003). Perhaps an increased villus height is paralleled by an increased expression of brush border enzymes and improved nutrient transport system (Viveros et al., 2011).

CONCLUSION

Summing up the results obtained, it can be stated that extracts from the chosen plants can have positive effects on piglet performance mainly due to positive changes in small intestine morphology.

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