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Effect of Intensity of Feeding on the Intestinal Microflora of Pigs

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Abstract

In individual, single-phase feeding animals were fed extensively (group E – 7 animals) or intensively (group I – 7 animals) in *semi ad libitum* system. The mixtures differed in composition as well as energy and nutritional value, with constant ratio of protein to energy of 13.12:1 in intensive feeding and 13.04:1 in extensive feeding. Fibre content per 1 kg mixture was 3.43% in group I and 12.3% in group E. For microbiological studies samples were taken from the duodenum, ileum, jejunum and large intestine and both quantitative and qualitative differences in the microflora of the differently fed groups was found.

Key words: fatteners, feed intensity, intestinal microflora

Introduction

In order to obtain a high quality product both genetically and environmentally based approaches in the breeding of pigs are being undertaken. These include the selection of breeds and lines for crossing with indigenous breeds, the withdrawal of antibiotics used in feed and their replacement with biostimulators and the extensive husbandry and nutrition of animals (Libudzisz and Kowal, 2000; Fabijańska *et al.*, 2001; Gajewska *et al.*, 2001, 2002; Rekiel *et al.*, 2004).

Fibre can affect the length and capacity of select segments of the gastrointestinal tract in pigs (Turski and Batorska, 1991; Drochner and Coenen, 1986; Drochner, 1993; Schleicher, 1997). The type of fibre affects the development of the alimentary tract. A favourable increase in the volume of the large intestine of growing pigs was observed when the feed was supplemented with dry grass, whereas the use of wheat bran resulted in disadvantageous shortening and reduced volume of the small intestine (Leroch, 2003).

The composition of the intestinal microflora is relatively constant in the individual stages of life but can change with the type of feed ingested (Stavric and Kornegay, 1995). It can thus be assumed that feed components, including the type and amount of fibre, can affect the quantitative and qualitative composition of the intestinal microflora.

The aim of the current study was to determine the effect of varied feed intensity of growing pigs on the microflora of the small and large intestine.

Experimental

Materials and Methods

The experiments embraced 14 young mixed-breed pigs, porkers and sows, divided into two groups. The mean body weight of the animals at the beginning of the experiment was 25 kg. The animals were fed intensively (group I) or extensively (group E). Fattening was single-phase and the feed was given individually in *semi ad libitum* system. The mixes used in the nutrition of the

Table I
Composition of the mixes, their energetic and nutritional value

Feed components	Type of feeding					
	intensive			extensive		
	participation of resources %	total protein* g	metabolic energy* MJ	participation of resources %	total protein* g	metabolic energy* MJ
Barley grits	64.0	75.65	7.507	64.0	75.65	7.507
Wheat grits	10.0	11.05	1.188	–	–	–
Soybean meal after extraction	13.5	62.57	1.667	–	–	–
Meat and bone meal	5.0	28.25	0.744	–	–	–
Lard	7.0	–	2.422	–	–	–
Premix	0.5	–	–	0.5	–	–
Dried plants of the Papilionaceae family	–	–	–	35.5	52.36	2.307
Total	100.0	177.52	13.53	100.0	128.00	9.814

* – calculated according to Polish Norm of Pigs Nutrition (1993)

growing pigs differed in composition and energetic and nutritional value, with constant protein to energy ratio (Table I). In intensive feeding this ratio was 13.12 : 1 and in extensive feeding 13.04 : 1. The fibre content per 1 kg mixture was: in group I – 3.43%, in group E – 12.03%.

Following fattening the pigs were slaughtered. For *in vitro* microbiological examinations approximately 1 g samples were taken under sterile conditions from the individual parts of the gastrointenstinal tract, that is the duodenum, jejunum, ileum and large intestine. After dilution, the removed material was used for plating, using poured plates and surface spread. Single colonies from the mixture of various microorganisms were obtained using the streak plate method.

Morphological observations of the microorganisms embraced macroscopic examinations of the colonies, that is size, shape, surface, consistency, border type and colour, and microscopic observations after Gram staining of the cells. Examination and observations of microorganisms were made on King B medium (for growing bacteria belonging to the genus *Pseudomonas*), Sabouraud medium (for fungi), blood agar medium (for total bacterial count), on McConkey medium (for *Enterobacteriaceae*) and Eijkman medium (for acid-producing bacteria), (Kunicki-Goldfinger, 2001; Grabińska-Łoniewska, 1999; Duszkiewicz-Reinhard, 1999).

After collecting samples from the intestines and spreading them on media, quantitative determinations were made. To allow for correct interpretation of the results determinations of dry weight of the intestinal mass were also carried out. For isolation and identification the following media were used: MPA agar (for plate streaking), McConkey, King and APT agar.

Identification of bacteria to species was carried out using the following biochemical tests from bioMerieux: API 50CH for the identification of bacteria belonging to the genus *Lactobacillus*; API 20A for the identification of gram-positive and gram-negative anaerobic bacteria; API Staph for the identification of staphylococci and micrococci.

Bacterial suspensions were prepared from single colonies. After confirming the purity of the cultures by streaking out, the strains were transferred to agar medium. After 24 hour incubation at 37°C new suspensions were made. These were used to inoculate the API media API 20, API Staph, API 50CHL, API 50CHB (depending on the test) using the McFarland scale as recommended by the producer, which are then used to inoculate the test strips. The results were read using the APILAB computer program, which identifies the species or gives the percent probability of identification. The identity of the species was corroborated using Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons,1974).

Results and Discussion

Table II presents a quantitative compilation of the microorganisms from the individual sections of the digestive tract. In group E the number of bacteria belonging to the family *Enterobacteriaceae* was lower than in group I and moreover, the total number of microorganisms in the individual parts of the digestive tract was lower in this group. In the four studies segments of the intestines in pigs of the extensive groups the number of acid-producing bacteria, with potential probiotic activities, was more numerous than in the intensive group. These bacteria represented the genera *Lactobacillus, Enterococcus* and *Leuconostoc* (Rolfe 2000; Banach 2001).

Several microorganisms representing auto- and allochtonous microflora were isolated. These were bacteria of the genera *Lactobacillus*, *Escherichia*, *Enterococcus*, *Bacteroides*, *Sarcina*, *Pseudomonas*, *Bacillus* and others (Table III). Some of them were found in both studied groups, some only in either group I or E.

Bacteria belonging to the genera *Escherichia* or *Pseudomonas* grew on nutrient agar after 18–24 hours, as opposed to bacteria of the genus *Lactobacillus* that grew much slower. Moreoever, the latter required specific growth media, such as APT agar.

Table II
Number of microbial cells in individual segments of the digestive tract in the intensively (I) and extensively (E) fed groups, per 1 g dry weight of intestine content

Segment of digestive tract	Type of medium used									
	King B		McConkey		Sabouraud		Eijkman		Nutrient agar + blood	
	group-feeding type:									
	I	E	I	E	I	E	I	E	I	E
Duodenum	8.7×10^7	5.7×10^7	8.5×10^7	2.1×10^6	5.6×10^3	2.3×10^3	7.2×10^6	3.0×10^8	7.1×10^9	3.3×10^8
Jejunum	9.2×10^7	1.3×10^8	1.2×10^8	3.5×10^6	9.0×10^3	5.3×10^3	7.4×10^6	3.0×10^8	1.7×10^{10}	8.4×10^8
Ileum	1.7×10^8	5.9×10^8	5.0×10^7	2.1×10^7	5.2×10^3	5.6×10^3	8.0×10^6	6.1×10^8	1.8×10^{10}	1.7×10^9
Large intestine	1.6×10^8	6.2×10^8	5.5×10^7	3.8×10^6	9.9×10^3	7.2×10^3	9.0×10^6	1.7×10^8	1.3×10^{10}	7.9×10^8

Table III
Bacteria isolated from animals fed intensively (I) or extensively (E)

Group/feeding	Identified bacteria
Intensive (I)	*Escherichia coli*; *Pseudomonas* sp.; *Sarcina; Micrococcus sedentarius* (*Kytococcus sedentarius*); *Staphylococcus cohnii* subsp. *cohnii*; *Bacillus subtilis*;
Extensive (E)	*Lactobacillus acidophilus*1; *Leuconostoc mesenteroides* subsp. *mesenteroides Bacillus subtilis*; *Bacillus licheniformis*; *Bacteroides melaninogenicus* subsp. *intermedius* (*Prevotella intermedia*); *Escherichia coli; Pseudomonas* sp.; *Sarcina* sp.;

From among the isolated bacteria several randomly selected strains, namely: *Lactobacillus acidophilus* 1, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus cohnii* subsp. *cohnii*, *Micrococcus sedentarius* (*Kytococcus sedentarius*), *Bacteroides melaninogenicus* subsp. *intermedius* (*Prevotella intermedia*) were identified using Api tests and APILAB computer program. Moreoever, the bacteria*: Escherichia coli*, *Pseudomonas* sp. and *Sarcina* sp. were identified. The physiological, morphological and biochemical characteristics of the isolated strains (*e.g. Lactobacillus acidophilus* 1, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Bacillus subtilis*, *Bacillus licheniformis*) points to their probiotic properties. Such organisms have a beneficial action on the organisms of animals (Lonvaud-Funel 1999; Reid 1999; Kailasapathy and Chin, 2000; Casula and Cutting, 2002; Heyman and Ménard, 2002; Fernández *et al.*, 2003). These potentially probiotic strains were much more numerous in the case of the group E animals, which received feed containing greater fibre content. Moreover, the increase in number of probiotic species was accompanied by a decrease in the number of potentially pathogenic bacteria, such as *E. coli* and *S. cohnii* subsp. *cohnii.*

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