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Effect of dietary protein supply originating from soybean meal or casein on the intestinal microbiota of piglets

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ABSTRACT

Dietary composition is a major factor influencing the intestinal microbial ecosystem of pigs. To alleviate weaning-associated disorders, variations in dietary protein supply may beneficially affect microbial composition in the gastrointestinal tract of piglets. A total of 48 piglets, fitted with simple ileal T-cannulas, was used to examine the effect of protein supply of either highly digestible casein or less digestible, fiber-rich soybean meal (SBM) on the composition of the intestinal microbiota. Gene copies of 7 bacteria groups were determined by real-time PCR in ileal digesta and feces. Ileal counts of total eubacteria, the Bacteroides-Prevotella-Porphyromonas group, *Enterobacteriaceae* and *Clostridium* Cluster XIVa were higher ($P < 0.001$) in the casein-based diets. Fecal counts of all analyzed bacterial groups were higher for the SBM-based diets ($P < 0.001$), apart from *Enterobacteriaceae* ($P < 0.05$) which were higher in the casein-based diets. Ileal counts of lactobacilli linearly increased as the crude protein level was increased up to 335 g/kg ($P < 0.01$). The *Bacteroides–Prevotella–Porphyromonas* group linearly decreased in ileal samples ($P < 0.01$) and increased in fecal samples ($P < 0.05$) as the crude protein level in the SBM-based diet was increased. Both, protein level and protein source may affect intestinal microbial balance. Higher dietary protein levels in combination with diets low in fiber contents might stimulate proliferation of protein fermenting bacteria in piglet's large intestine. Further studies are warranted to clarify, whether this would be associated with intestinal disturbances.

1. Introduction

Weaning of piglets is often associated with a growth check due to the incidence of intestinal disorders such as post-weaning diarrhea (PWD) [1]. Commonly, PWD is associated with an increased microbial fermentation of undigested protein in the gastrointestinal tract (GIT), resulting in the formation of watery feces in combination with reduced growth performance, morbidity and even mortality of piglets [2]. Nowadays, it is increasingly being realized that gastrointestinal health is influenced by the composition of the microbial community and the end-products of bacterial metabolism of diet components [3,4]. Furthermore, differences in composition and function of the microbiota in the GIT of piglets may occur due to changes in diet composition [5,6]. Thus, nutritional strategies aiming to modulate the intestinal microbial ecosystem of weaned piglets appear to be a useful tool to improve GIT health. Within this regard, enhancing the beneficial members of the GIT ecosystem, for instance lactobacilli and bifidobacteria, by supplying specific dietary ingredients such as probiotics and prebiotics, has proven to be an option [7]. Furthermore, growth and activity of potential pathogens may be suppressed by selectively limiting nutrient supply. Although most bacterial species in the GIT are capable of using fermentable protein as energy source some, mainly pathogenic bacteria prefer protein as energy source following the elimination of fermentable carbohydrates from the diet [8]. Bacterial genera in the porcine colon involved in protein fermentation primarily include potentially pathogenic *Bacteroides*, coliform bacteria, and *Clostridium* [9]. Growth of such potentially pathogenic bacteria is often associated with disturbances of the intestinal microbial balance [10] and release of enterotoxins [11]. It was hypothesized that variations in dietary protein intake could modify composition of bacterial groups involved in protein fermentation. For example, lowering protein contents in diets for growing pigs has been shown to limit the amount of protein available for protein fermenting bacteria

Abbreviations: SBM, soybean meal; PWD, post-weaning diarrhea; GIT, gastrointestinal tract; *Bacteroides* group, *Bacteroides–Prevotella–Porphyromonas* group; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; NDO, nondigestible oligosaccharides.

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[12]. Wellock et al. [13] could demonstrate in piglets that a decrease in dietary protein intake results in a higher fecal and colonic lactobacilli to coliform ratio, mainly due to lower coliform population. This, in turn, indicates an improved microbial equilibrium [14]. Additionally, it has been suggested that the amount of dietary protein reaching the large intestine of piglets could be diminished by selecting feed ingredients containing protein of rather high digestibility [15]. However, in studies feeding highly digestible animal protein sources such as fishmeal [16] or casein [13] to weaned piglets, no effect on the composition of the intestinal microbiota could be found, in comparison to plant proteins with a lower protein digestibility such as soybean meal (SBM). However, it has to be considered that SBM is one of the most digestible protein sources among commonly used plant protein supplements in livestock feeding [17]. At present, no comparative studies have been conducted so far, in which potential effects of differences in both protein level and protein source in diets of newly weaned piglets on the composition of bacteria harboring the GIT have been assessed.

In the present study, casein was selected as being representative of a highly digestible protein source, whereas SBM was chosen because of its relatively low protein digestibility and inherent high fiber content compared with casein. These 2 different protein supplements were included at 6 inclusion levels in piglets' diets and fed at 2 feeding levels to obtain a broad range in protein supply between the experimental diets. Piglets fitted with simple T-cannulas at the distal ileum were used to assess the effect of differences in protein source, protein level and feeding level in the diet on microbial composition both at the end of the small intestine and over the entire GIT. To scrutinize any shifts of the intestinal microbial community in response to variations in protein supply, the *Bacteroides–Prevotella–Porphyromonas* group (*Bacteroides* group), a predominant group in pig's intestine [18] was analyzed in addition to *Clostridium* Cluster IV and *Clostridium* Cluster XIVa, known to be responsive to diet [19]. Additionally, *Enterobacteriaceae* were evaluated, containing *Escherichia coli* which is associated with the occurrence of PWD. Moreover, saccharolytic lactobacilli and bifidobacteria were determined as they represent beneficial members of piglets' GIT due to their health-promoting properties [20].

2. Materials and methods

The research protocol was approved by the German Ethical Commission for Animal Welfare. Care of the animals used in this experiment was in accordance with the guidelines issued by the German regulation for care and treatments of animals [21].

2.1. Animals and housing

Four consecutive experiments, consisting of 2 periods each, were conducted, every experiment with 12 German Landrace × Piétrain piglets (48 piglets in total). The piglets were weaned at 16–17 days of age with an initial bodyweight (BW) of 5.9 ± 0.94 kg. They were obtained from the Research Station of the University of Hohenheim, and were kept in pairs until surgery to adapt them to their metabolic crates (0.75 m × 1 m). After surgery, piglets were housed individually in their metabolic crates. The temperature in the research unit was regulated by an automated temperature control system, and was 27 °C at the beginning of each experiment and gradually decreased to 21 °C until the end of the experiments. Each crate was equipped with an infrared heating lamp and a low pressure drinking nipple. From weaning until surgery, the piglets were fed a commercial milk replacer.

2.2. Surgical procedure

On days 2 and 3 post weaning, 6 pigs each were fitted with simple T-cannulas at the distal ileum according to the procedure described by Li et al. [22]. The cannulas were prepared from high molecular weight polyethylene. The internal diameter of the barrel of the cannulas was 12 mm, the total diameter was 15 mm, the length of the barrel was 45 mm and each of the 2 wings was 43 mm in length. The washer was 40 mm in diameter and had a short barrel of 15 mm in length. Natural rubber plugs were used as stoppers.

2.3. Experimental procedure

The piglets were fed semi-synthetic, cornstarch-based assay diets either supplemented with graded levels of SBM or casein at the expense of cornstarch. These 2 assay diets were formulated to meet or to exceed the National Research Council (NRC) [23] nutrient recommendations for piglets from 5 to 20 kg of BW. Ingredient composition and nutrient contents of the diets are shown in Tables 1 and 2. In addition, these diets were fed at 2 different feeding levels, referred to as low (30 g/kg BW) and high (60 g/kg BW) to assess the effect of variations in feeding level on intestinal microbial composition. Piglets were weighed at the beginning of each experimental period to adjust their daily feed allowances according to the assigned feeding level, and they were fed 2 equal meals in mash form twice daily (at 0700 and 1900 h). In each Experiment 1, the 12 experimental diets (2 protein sources × 6 protein levels) were randomly allocated to the 12 experimental animals within period 1. Pigs, which received SBM or CAS in period 1, received CAS or SBM in the opposite order in period 2. Feeding levels were equally distributed over protein sources in period 1 (3

Table 1
Composition of the soybean meal-based diets (g/kg, as fed) and analyzed nutrient content.

Ingredient	Crude protein, g/kg (as fed)					
	85	135	185	235	285	335
Soybean meal[a]	212.5	338.0	463.5	589.0	714.5	840.0
Cornstarch[b]	632.1	506.6	381.1	255.6	130.1	4.6
Dextrose[b]	45.0	45.0	45.0	45.0	45.0	45.0
Lactose[c]	10.0	10.0	10.0	10.0	10.0	10.0
Oil[d]	29.0	29.0	29.0	29.0	29.0	29.0
Cellulose[e]	30.0	30.0	30.0	30.0	30.0	30.0
Monocalciumphosphate	5.0	5.0	5.0	5.0	5.0	5.0
Sodium chloride	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin E[f]	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin and mineral premix[g]	30.0	30.0	30.0	30.0	30.0	30.0
TIO_2	5.0	5.0	5.0	5.0	5.0	5.0
Analyzed nutrient content						
Dry matter (DM), g/kg	909.8	907.4	906.4	899.1	898.0	898.7
Crude protein, g/kg DM	106.9	165.7	224.3	277.8	331.6	385.5
Lysine, g/kg DM	6.1	9.2	13.2	16.5	19.2	21.6
Neutral detergent fiber, g/kg DM	93.4	106.3	128.4	152.2	176.9	202.6
Acid detergent fiber, g/kg DM	54.1	68.0	81.7	107.9	120.2	133.8

[a] 94% soybean meal: WLZ Niederlassung, BayWa AG, Nürtingen, Germany + 6% soy hulls: ADM, Mainz, Germany.
[b] Roquette, Frankfurt, Germany.
[c] Meggle, Wasserburg, Germany.
[d] 25% soybean oil + 75% rapeseed oil.
[e] Rettenmaier & Soehne, Rosenberg, Germany; from wood.
[f] BASF, Ludwigshafen, Germany; dl-α-tocopheryl acetate, >50% purity.
[g] AGRAVIS Futtermittel GmbH, Münster, Germany; provided the following quantities of minerals and vitamins per kg diet: 6.7 g calcium, 2.4 g phosphor, 1.5 g sodium, 0.4 g magnesium, 17,999 IU vitamin A, 1980 IU vitamin D3, 60.0 mg vitamin E, 4.8 mg vitamin K, 4.8 mg vitamin B1, 7.8 mg vitamin B2, 6.0 mg vitamin B6, 60.0 μg vitamin B12, 54.0 mg niacin, 16.2 mg pantothenic acid, 72.0 μg biotin, 300.0 mg choline chloride, 88.5 mg zinc oxide, 15.0 mg copper, 124.5 mg iron, 82.5 mg manganese, 1.8 mg potassium iodate, 0.3 mg selenium.