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Effects of a Probiotic Supplement on the Quantity of Some Bacterial Communities in Fecal Samples of Lactating Sows

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Complex adaptation strategies concerning nutrition, housing technology, and veterinary treatment are required to maintain current production levels under increasingly stringent regulations on the preventive application of antibiotics. The reduced application of antibiotics is recommended for the sustainability of industrial pig production. Probiotic supplementation may contribute to improved sow and piglet health, mitigating the need for antibiotics. The effects of probiotic supplementation on sow performance and the quantity of fecal bacterial communities in lactating sows were investigated. Experimental sows received probiotic supplementation (n=10) and were compared to control sows (n=10). Fecal samples were collected from 20 sows in the second week of lactation. The quantitative measurement of total bacteria, *Prevotella* genus, *Lactobacillus* spp., and *Bifidobacterium* spp. was done by qPCR. Differences in backfat thickness (BFT), BFT loss, and feed intake of control and experimental sows were not significant ($p>0.05$). The amount of total bacteria, *Prevotella*, and *Lactobacillus* spp. was lower ($p<0.05$) in the fecal samples of experimental sows. The *Prevotella* percentage in total bacteria decreased, whereas *Bifidobacterium* spp. the ratio increased in experimental supplemented sows. Overall, probiotic supplementation resulted in notable alterations regarding some of the analyzed bacterial communities.

1. Introduction

The European Union banned the use of antibiotics as growth promoters in 2006 (Regulation (EC) No 1831/2003), and since January 28, 2022 (Regulation (EU) 2019/6), the preventive use of antibiotics has also been restricted in farm animal production due to the emergence of antimicrobial resistance. To mitigate the impact of the stringent regulations, novel adaptation strategies were developed, such as the widespread application of prebiotic and probiotic feed supplements. Prebiotics and probiotics can contribute to improved intestinal health and enhanced immune functions while also improving production traits (e.g., feed conversion efficiency, and growth rate).

The most widely applied probiotics in animal nutrition primarily include species from the genera *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and *Enterococcus*. Among yeasts, *S. cerevisiae* is the most widely used feed supplement in farm animal nutrition. It is rich in digestible proteins, vitamins (B6, thiamine, biotin, riboflavin, nicotinic acid, pantothenic acid), magnesium, and zinc, although it has a low calcium content. The cell wall of *S. cerevisiae* essentially consists of the polysaccharides α-D-mannan, chitin, and β-D-glucan. Notably, *S. cerevisiae* is not an abundant natural component of the gut microbiota in monogastric animals; however, it can function as a bioregulator in various processes, such as immunomodulation through mannoproteins and β-D-mannan (Kiarie et al., 2013). It stimulates the IgA response against pathogenic microorganisms and inhibits pathogen adhesion to the mucosa via the mannan content of its outer cell wall. Additionally, it reduces the harmful effects of toxins and lowers intestinal pH by secreting organic acids (lactic and acetic acid), creating a more favorable environment for the native gut microbiota and decreasing the likelihood of pathogenic

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colonization (Chandrasekaran et al., 2024). Removing oxygen also promotes the proliferation of viable anaerobic bacteria, influencing the composition of the gut microbiome. Supplementing pig diets with live yeast may improve feed efficiency, digestibility, and animal performance and mitigate the impact of negative environmental factors on animal production.

Derived primarily from the yeast *S. cerevisiae*, MOS are recognized for their prebiotic properties and contribute to modulating the gut microbiota. By promoting the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* species (Sohail et al. 2013), MOS improve gut microbial balance, which is crucial for nutrient absorption and immune function in pigs. MOS supplementation can enhance intestinal morphology, including increased villus height, and facilitate nutrient absorption and growth performance. MOS have been shown to bind pathogenic bacteria, such as *Escherichia coli* and *Salmonella typhimurium*, and prevent their adhesion to the gut epithelium reducing the incidence of intestinal infections (Halas and Nochta, 2012). MOS supplementation has been associated with increased levels of IgG immunoglobulins that play a vital role in immune defense in response to pathogenic challenges (Li et al., 2021).

In the present study, the effects of pre- and probiotic feed supplements were analyzed on several production traits of lactating sows (daily feed intake, litter size, piglet weight, sow condition) and on the quantity of selected bacterial communities (total bacteria, *Prevotella* genus, *Lactobacillus* spp., *Bifidobacterium* spp.) in fecal samples of control and experimental sows. The applied feed additives included ActiSaf HR+ (Phileo by Lesaffre, France) yeast probiotics containing a proprietary strain of *Saccharomyces cerevisiae* and Safmannan (Phileo by Lesaffre, France) prebiotics made of *S. cerevisiae* wall fractions rich in mannan-oligosaccharides (MOS) and beta-glucans. Potential changes associated with the experimental supplementation may highlight the benefits of dietary treatments in the efforts to reduce antibiotic pressure in sustainable pig production.

2. Materials and Methods

The experiment and the sampling were conducted in commercial settings in a pig production facility in Somogytarnóca, Hungary.

2.1 Experimental Animals

In this experiment, first-parity sows produced by crossbreeding Large White and Landrace breeds were involved. The studied population was divided into a control group (n=10) and an experimental group (n=10). Until day 85 of gestation, all sows were fed a gestation diet. From days 104 to 114 of gestation, the sows received a transition diet, and following farrowing (day 115) until weaning (26 days post-farrowing), they were provided with a lactation diet. For the experimental group, feed supplementation included 1.0 kg/t of probiotic Actisaf HR+ and 0.5 kg/t of prebiotic Safmannan alongside the gestation diet, and 0.7 kg/t of Actisaf HR+ and 0.3 kg/t of Safmannan alongside the lactation diet. The experimental animals began consuming the pre- and probiotic yeast products (Phileo by Lesaffre, France) from day 85 of gestation.

Throughout the study, the following main production data were collected: sow live body weight (kg) and backfat thickness (mm) measured by ultrasound upon transfer to the farrowing house (day 110 of gestation), birth litter weight (kg) on day 115, and body weight and backfat thickness at the end of the lactation period. Differences in body weight and backfat thickness were calculated between the time of entry into the farrowing house (day 110) and near the end of the lactation period (21 days post-farrowing). Additionally, average daily feed intake (kg) was determined from the start of the experimental feeding until the end of the lactation period. Randomly selected 26-day-old piglets from both the control (n=100) and the experimental (n=100) groups were individually weighed.

2.2 Sample Collection and Laboratory Analysis

For the analysis of the bacterial composition in fecal samples, fresh individual samples were collected from lactating sows (n=10 control, n=10 experimental). During DNA isolation, fecal samples were centrifuged in phosphate-buffered saline solution, and the resulting pellet was homogenized using TissueLyser LT (Qiagen, Germany). DNA was then isolated using the Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). The integrity of the DNA samples was verified via agarose gel electrophoresis, and their concentration was measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The DNA concentration was standardized to 50 ng/µL.

Specific regions of the bacterial genera (16S rRNA coding regions) were amplified using polymerase chain reaction (PCR) with a Hybrid Px2 thermal cycler (Thermo Fisher Scientific, USA). The 20 µl reaction mixture consisted of 10 µL of 2xPCR Mastermix (containing 1.5 mM MgCl2, 0.6 u Taq DNA polymerase, and 200 µM dNTPs), 1 µl each of forward and reverse primers (0.4 µM each), 1 µL (50 ng) DNA template, and 7 µL nuclease-free water. PCR conditions included an initial denaturation at 95 °C for 3 min, followed by 35 cycles

of denaturation at 95 °C for 1 min, annealing at 52-63 °C for 1 min, elongation at 72 °C for 1 min, and a final elongation at 72 °C for 5 min, with optional storage at 4°C. The presence of specific PCR products was verified by agarose gel electrophoresis, followed by purification using the Promega Wizard SV Gel and PCR Clean-Up System kit. Dilution series (8 standards) were prepared from the purified PCR products to determine the efficiency of the qPCR reactions.

Quantification of the various bacterial genera or families was conducted using qPCR (CFX96 Real-time Detection System, Bio-Rad, USA). The applied primers are listed in Table 1. The 25 µL qPCR reaction mixture comprised 12.5 µL Maxima SYBR Green qPCR Master Mix, 1 µL each of forward and reverse primers (0.4 µM), 2 µL DNA template (100 ng), and 8.5 µL nuclease-free water. The qPCR protocol followed the manufacturer's instructions for the Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific, USA): initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 or 59 °C for 30 s (Table 1), and elongation at 72 °C for 30 s. To determine reaction efficiency and copy number, a tenfold dilution series (standards) of PCR products was prepared. The copy number was calculated according to the instructions by Whelan et al. (2003).

Table 1: Primer sequences for respective groups of bacteria, based on (Heinritz et al., 2016)

Target	Primer sequence (5'-3')	Annealing (°C)	Length (bp)	Efficiency (%)
Total bacteria	GTGSTGCAYGGYYGTCGTCA, ACGTCRTCCMCNCCTTCCTC	55	147	96.8±5.2
Prevotella genus	CACRGTAAACGATGGATGCC, GGTCGGGTTGCAGACC	59	121	94.6±3.5
Lactobacillus spp.	AGAGGTAGTAACTGGCCTTTA, GCGGAAACCTCCCAACA	59	391	92.2±5.2
Bifidobacterium spp.	CGCGTCCGGTGTGAAAG, CTTCCCGATATCTACACATTCCA	59	126	95.9±4.2

2.3 Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows v.20.0 (IBM Corp., USA). The normality of data distribution was assessed by the Shapiro–Wilk test. Group comparisons were conducted using the Mann–Whitney U test or independent samples t-test.

3. Results and Discussion

The effects of probiotic supplementation were determined on sow productivity based on main production traits that were monitored during the experimental period and on the composition of selected fecal bacterial communities.

3.1 Sow Production Traits

Main production traits were collected and analyzed in the control and experimental groups of lactating sows (Table 2). Based on the results, the inclusion of *S. cerevisiae* and MOS in the diet did not affect significantly ($P>0.05$) the overall body condition of the sows. According to Tao et al. (2023), dietary supplementation with yeast and yeast-derived products can help maintain body condition by supporting gut health and nutrient absorption, although significant effects are often observed more prominently under conditions characterized by nutritional or environmental stress. The slightly mitigated decrease in backfat thickness (BFT) of the experimental group might indicate a moderate improvement in energy utilization during lactation, although the difference was not statistically significant ($P>0.05$). The trend towards lower average daily feed intake and slightly improved nursing skills in the experimental group could indicate improved feed efficiency. This observation is supported by research showing that yeast supplements can improve feed efficiency by enhancing gut health, which may allow animals to derive more nutrients from less feed (Zhao et al., 2022).

Méndez-Palacios et al. (2018) found no differences in average daily gain, daily feed intake, or feed efficiency between the control group and the group supplemented with 4 kg/t *S. cerevisiae* during the 21-150 days fattening period of pigs. In contrast, Fu et al. (2019) reported that dietary yeast hydrolysate increased daily gain and feed intake during the finishing phase in fattening pigs, as well as overall daily gain throughout the experiment. Similar results were observed in weaned piglets with yeast extract supplementation (Carlson et al., 2005). However, Waititu et al. (2016) did not find a positive association between growth traits and the consumption of yeast or hydrolysates in pigs.

Table 2: Production traits of the control and the experimental sow groups

Traits	Control sows	Experimental sows	P-value
Starting weight (kg)	256.7±28.1	252.52±31.7	0.622
Sow weight at weaning (kg)	195.8±20.9	188.64±30.9	0.341
Weight loss during lactation (kg)	60,8±19.6	63.88±15.5	0.552
Starting back fat thickness (mm)	19.2±3.7	18.16±3.1	0.286
Back fat thickness at weaning (mm)	14.6±3.1	13.92±3.4	0.463
Decrease in back fat thickness (mm)	4.6±3.2	4.2±3.9	0.727
Daily feed intake (kg)	6.3±1.5	5.9±1.3	0.412
Weaning to estrus interval (day)	$8,0^{a}$±4,8	$5,4^{b}$±3,2	0.043
Piglets born alive (pcs)	14.2±4.1	14.3±2.6	0.902
Litter weight at birth (kg)	19.5±5.7	19.6±3.4	0.979
Average piglet weight at weaning (kg)	7.3±1.7	7.5±1.9	0.582

^{a,b} Means with different superscripts differ significantly (P<0.05)

In line with the findings of Jang et al. (2013), no significant differences (P>0.05) were observed in average litter weight at birth and the number of live-born piglets (Table 2). However, other studies (Mariella et al., 2009) reported more live-born piglets in groups treated with yeast supplements. Piglets from experimental sows had slightly higher average weaning weights, although the difference was not statistically significant (P>0.05). In contrast, Shen et al. (2011) reported that supplementation of live yeast in the diet of pregnant and lactating sows improved the average daily weight gain of their piglets.

Most authors have demonstrated the health benefits of probiotic yeast in piglets; supplementation with yeast or its derivatives increased daily feed intake and growth (Kiros et al., 2019). It stimulated mucosal immunity by increasing the activity of IgA and IgM against pathogens, improved intestinal development and function, bound mycotoxins, influenced gut microbiota, and reduced post-weaning diarrhea in piglets (Jiang et al., 2015). However, Kornegay (1995) did not observe a positive effect of yeast supplementation on daily weight gain in their studies with weaned piglets.

A significant reduction was found in the weaning to estrus interval in the group of the supplemented experimental sows. This suggests that the yeast probiotics and prebiotics (MOS and beta-glucans) may have supported quicker recovery and readiness for the next reproductive cycle. This aligns with research indicating that *S. cerevisiae* and MOS can modulate immune function and improve gut integrity, leading to better overall reproductive performance (Jang et al., 2013). The quicker return to estrus may be attributed to the enhanced metabolic and immune status of the sows, which are critical during the post-weaning period.

3.2 Composition of Fecal Bacterial Communities

The copy numbers of selected bacterial communities were determined in the control and the experimental groups (Table 3).

Table 3: Copy numbers of the analyzed bacterial communities (log_{10} values±SD)

Bacterial groups	Control sows	Experimental sows
Total bacteria	9.78^{a}±0.27	9.21^{b}±0.20
Prevotella genus	9.45^{a}±0.22	8.64^{b}±0.21
Lactobacillus spp.	7.17^{a}±0.14	6.71^{b}±0.13
Bifidobacterium spp.	6.08±0.10	6.36±0.11

^{a,b} Means with different superscripts differ significantly (P<0.05)

The total bacterial count in the experimental sows was significantly (P<0.05) lower compared to the control sows. The considerable decrease in total bacterial load suggests that the supplementation with *S. cerevisiae* and prebiotics (MOS and betaglucans) have had a modulating effect on the gut microbiota, potentially reducing the overall bacterial load by selectively promoting beneficial bacteria while suppressing pathogenic or non-beneficial genera. This finding is consistent with Law et al. (2024) that have observed a balancing effect of yeast and MOS on the gut microbiota, leading to a more stable and beneficial microbial community.

The *Prevotella* genus – widely associated with the degradation of complex carbohydrates and fiber – also showed a significant (P<0.05) reduction in the experimental sows. The reduction in *Prevotella* could be due to the competition with other bacterial groups that were promoted by the supplementation, or it could indicate a shift in the substrate utilization patterns within the gut of the supplemented sows. Previous research has indicated that dietary interventions, including yeast-derived prebiotics such as MOS, can influence the relative

abundance of the *Prevotella* genus, and may reduce it in favor of other beneficial bacteria (Fouhse et al., 2019).

The *Lactobacillus* spp. copy numbers also decreased in the experimental group compared to the control group. *Lactobacillus* spp. are generally considered beneficial, contributing to gut health through the production of lactic acid and other antimicrobial compounds.

In contrast to the reductions observed in other bacterial groups, the absolute quantity of *Bifidobacterium* spp. showed an increase in the experimental sows. However, the difference was not significant ($P>0.05$). *Bifidobacterium* is another genus of beneficial bacteria associated with the fermentation of oligosaccharides, leading to the production of short-chain fatty acids that contribute to gut health. The increase in *Bifidobacterium* may be attributed to the prebiotic effects of MOS (Kango et al., 2022).

Due to remarkable differences in absolute quantities presented in $\log_{10}$ copy numbers in Table 3, the quantity of selected bacterial communities was also calculated relative to total bacteria (Table 4). The relative values indicate a notable shift in the significant ($P<0.05$) reduction of the *Prevotella* genus and an impressive relative increment in *Bifidobacterium* spp., while *Lactobacillus* spp. relative quantities did not differ ($P>0.05$); however, the increasing trend (0.24 vs 0.31) indicated the beneficial effects of the supplements on *Lactobacilli*, as well.

Table 4: Percentage (%) of the analyzed bacterial communities relative to total bacteria

Bacterial groups	Control sows	Experimental sows
Prevotella genus	45.72^{a}±16.39	26.37^{b}±9.50
Lactobacillus spp.	0.24±0.07	0.31±0.12
Bifidobacterium spp.	0.0194^{b}±0.0038	0.1378^{a}±0.0169

^{a,b} Means with different superscripts differ significantly ($P<0.05$)

4. Conclusions

The supplementation of *S. cerevisiae* and yeast-derived prebiotics (MOS and beta-glucan) in the diet of late-gestation and lactating sows demonstrated remarkable effects on the gut microbiota composition and moderate effects on production traits. The experimental group exhibited a significant ($P<0.05$) reduction in total bacteria and other bacterial communities (*Prevotella* and *Lactobacillus* spp.), while an increase was observed in *Bifidobacterium* spp. Based on the relative quantities, the supplementation essentially promoted *Lactobacilli* and *Bifidobacteria* and notably decreased the ratio of the *Prevotella* genus. This shift suggests potential benefits in enhancing nutrient absorption and immune functions and may lead to a shorter weaning-to-estrus interval and improved readiness for the next reproductive cycle. Further studies are needed to explore the mechanisms behind these effects and refine dietary strategies that support sustainable pig production under the increasingly stringent regulations on antibiotic use.

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