



β -mannanase-supplemented diets containing xylanase reduced by 85 kcal of metabolizable energy/kg promotes benefits in fecal alpha diversity in lactating sows

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ABSTRACT - Enzyme-supplemented diets can influence the intestinal microbiome in an intricate interplay with the immune system. Therefore, the effects of β -mannanase supplementation in metabolizable energy (ME)-reduced diets containing xylanase were investigated on cytokine concentrations and fecal microbiota profile in lactating sows ($n = 60$, 248.4 ± 2.4 kg) assigned to a randomized block design with three dietary treatments: a diet containing xylanase (Control; reduction of 40 kcal of ME/kg, CD40), CD40 + β -mannanase (0.3 g/kg) and a reduction of 45 kcal ME/kg (CD85), and CD40 + β -mannanase (0.3 g/kg) and reduction of 60 kcal ME/kg diet (CD100). The ME reduction in CD40, CD85, and CD100 diets was based on a requirement of 3,400 kcal/kg, with diets formulated to contain to have 3,360, 3,315, and 3,300 kcal/kg, respectively. The study period lasted 26 days. Serum cytokines and fecal microbiota were assessed on day 18 and 21, respectively. The fecal microbiota profile was characterized by 16S rRNA gene sequencing. Sows fed CD85 had ($P = 0.014$) higher alpha diversity richness than CD100 based on the Simpson index. The Acutalibacteraceae family was more abundant ($P = 0.044$) with CD100 than CD85, but CAG-508 and NSJ-53 families exhibited ($P < 0.05$) a higher abundance in sows fed CD85 than CD100. The *Fimenecus* genus exhibited ($P < 0.05$) a lower abundance on CD85 compared to CD40 or CD100, while the *NSJ-53* genus showed ($P = 0.044$) a higher abundance in sows fed CD85 diet than in those fed the CD100 diet. Similarly, were observed ($P < 0.05$) for the *Fimenecus* sp004556705 and *NSJ-53* sp014384795 species. In conclusion, a diet supplemented with β -mannanase and containing xylanase reduced by 85 kcal/kg fed to lactating sows positively affected fecal alpha diversity.

Keywords: anti-inflammatory cytokines, carbohydrases, energy reduction, exogenous enzymes, fecal microbiota, lactating sows



1. Introduction

Plant-based ingredients commonly used in pig diets contain significant amounts of antinutritional compounds (Liu et al., 2019; Baker et al., 2021; Vangroenweghe et al., 2021). To mitigate their adverse effects, exogenous enzymes are supplemented to diets to improve the digestion and absorption of nutrients in non-ruminant animals (Kiarie et al., 2013; Kipper et al., 2020). Furthermore, this nutritional strategy improves nutrient digestibility and the gain-to-feed ratio, allowing a reduction in ME in the diet formulation (Genova et al., 2023b).

Lactation is a vital phase in sow reproduction that demands increased nutrition for maintenance, growth, and milk production, which may lead to excessive mobilization of body reserves (Kim et al., 2018). Additionally, it is imperative to understand the changes during lactation when implementing nutritional strategies, as this provides specific insights into potential effects on the health of sows and their progeny. Therefore, supplementing β -mannanase can be a viable strategy to mitigate limitations affecting feed digestion in lactating sows.

Previously, it was reported that dietary supplementation of β -mannanase increased nutrient digestibility and mitigated body weight loss in lactating sows (Kim et al., 2018). Studies involving the performance responses of pigs fed diets containing β -mannanase (Kipper et al., 2020), the role of enzymes added to pig diets (Kiarie et al., 2022) and supplementation with dietary β -mannanase in piglets (Vangroenweghe et al., 2021) attributed the decrease in immune response induced by feed intake and the consequent reduction in energy expenditure for immune system activation to the hydrolysis of β -mannans.

The impact of dietary supplementation with exogenous enzymes in ME-reduced diets, alone or in combination, on favorable aspects of the immune response and the fecal microbiota in sows remains uncertain. Notably, ME-reduced diets have previously been documented as inducing changes in the fecal microbiome in pigs, regardless of the use of combined enzymes on microbial population abundance (Genova et al., 2023b).

β -mannanase-xylanase supplementation in ME-reduced diets improves nutrient utilization and minimizes potential intestinal disturbances. By degrading mannans, the enzyme reduces anti-nutritive impacts such as intestinal inflammation and digesta viscosity (Genova et al., 2023b). This reflects the ability of β -mannanase-xylanase supplementation to promote favorable conditions for intestinal microbial ecology. For example, it improves the digestibility of targeted non-starch polysaccharides (e.g., arabinoxylan and mannans) (Tiwari et al., 2018) and promotes the diversity of beneficial bacteria (Genova et al., 2023a), resulting in beneficial modulation of the intestinal microbiota. This modulation manifests itself as a reduction in pathogenic bacteria and attenuated intestinal inflammation, as evidenced by studies conducted by Kiarie et al. (2022), who assessed the effect of β -mannanase supplementation, and by Genova et al. (2023a,b), who tested β -mannanase in diets fed to growing pigs (Genova et al., 2023a) and the combined effect of β -mannanase, xylanase, and phytase in diets fed to finishing pigs (Genova et al., 2023b).

Reducing ME in diets is a viable economic approach, but it can negatively affect intestinal and immune function due to the energy demand required to maintain these processes. Given the critical role of immune function and intestinal health in lactating sow productivity and piglet development, understanding how enzyme supplementation influences cytokine profiles and microbiota composition is essential. Therefore, dietary supplementation with β -mannanase-xylanase may have economic, environmental, nutritional, and health impacts. This study aimed to assess the associated effects of these enzymes in diets with reduced ME on cytokine concentrations and fecal microbiota profile in lactating sows.

2. Material and methods

2.1. Ethics statement

The experiment was conducted on a commercial farm of Western Paraná (Toledo, PR, Brazil, 24° 42'50" S, 53° 44' 34" O), with lactating sows from the weaned piglet production unit. The Animal Use Committee of the Universidade Estadual do Oeste do Paraná approved the protocol for the experiment (number 17-2022). All methods were carried out in accordance with relevant guidelines and regulations. All methods were reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org/arrive-guidelines>).

2.2. Animals, experimental design, housing, and dietary treatments

A total of 60 hybrid sows at 110 days of gestation (248.4 ± 2.4 kg BW) from a commercial lineage (Landrace $\times$ Large White, Agroceres PIC®) were selected for the study. The sows were housed in farrowing crates for a period of 26 days (5 days of acclimatization and 21 days of lactation). The sows were allocated to a randomized block design to 1 of 3 dietary treatments, with 5 sows per treatment in each block ($n = 4$ blocks of rounds in time), resulting in 20 sows/treatment. The sows were classified according to their BW as light-, medium-, or heavy-weight and according to farrowing order into 3 groups: P1, primiparous (13 sows); P2, sows with 2 or 3 farrowings (29 sows); and P3, sows with 4 or 5 farrowings (18 sows). The average farrowing order of sows assigned to each treatment was 2.8, 2.6, and 2.7 farrowings for the groups fed the diet containing xylanase (CD40), CD40 + β -mannanase (CD85), and CD40 + β -mannanase (CD100), respectively.

Five days before farrowing, all sows were transferred to the same house in individual conventional farrowing crates ($2.4 \times 1.6 \times 1.3$ m), with a slatted metal floor and equipped with front gutter feeders and nipple drinkers, with water provided *ad libitum*. A heated creep area ($0.98 \times 0.68 \times 0.66$ m) was accessible to piglets to maintain thermal comfort between 28 °C and 32 °C. The farrowing crates were installed in a commercial facility with a concrete floor and side curtains. The ambient temperature (25.3 ± 0.4 °C) and relative humidity ($60.0 \pm 1.8\%$) were recorded throughout the experimental period using a datalogger (UNI-T®, model UT330B digital USB; Beijing, China) positioned at the center of the facility.

The experimental diets were corn and soybean meal-based, supplemented with synthetic amino acids, and formulated to meet the nutrients requirements of the animals (Rostagno et al., 2017). The diets were provided in mash form, varying only in soybean oil and neutral energy replacement (kaolin, an inert compound) contents (Table 1). The experimental diets were provided according to the reproductive phase of the sows, beginning at 110 days of gestation and ending at 21 days of lactation. In the prepartum period (5 days before parturition), feeding was controlled by providing 2 kg of diet per animal once a day. The daily diet was weighed and stored in labeled plastic bags. Sows were not fed on the day of farrowing. During lactation, the sows were fed 4 times a day (07:30, 11:30, 14:00, and 17:30), and the average daily diet consumption was 7 kg.

The tested dietary treatments were: 1) a diet containing xylanase (Control; reduction of 40 kcal of ME/kg of diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, reduction of 45 kcal of ME/kg of diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, reduction of 60 kcal of ME/kg of diet, CD100). The ME reduction in each dietary treatment was based on the sows' requirement of 3,400 kcal/kg of diet (Rostagno et al., 2017). The ME of the diets was reduced by reducing the inclusion of soybean oil.

2.3. Characteristics of the enzymes

Xylanase (Sunhy Biology Co., Ltd, Wuhan, HB, China; registration no. PR-08978 03462) was obtained from *Trichoderma longibrachiatum* with an enzyme activity of 10,000 U/g. One unit of xylanase

Table 1 - Composition of diets fed to lactating sows (as-fed basis, %)

Item	Experimental diets ¹		
	CD40	CD85	CD100
Ingredient (%)			
Corn grain (7.88% CP)	55.43	55.43	55.43
Soybean meal (45.4% CP)	27.24	27.24	27.24
Whole soybeans (36% CP)	6.00	6.00	6.00
Sugar	3.00	3.00	3.00
Soybean oil	2.97	2.42	2.24
Fish meal (53% CP)	2.00	2.00	2.00
Dicalcium phosphate	1.61	1.61	1.61
Inert (kaolin)	-	0.51	0.69
Calcitic limestone	0.45	0.45	0.45
Common salt, NaCl	0.44	0.44	0.44
Premix, mineral and vitamin supplement ²	0.30	0.30	0.30
Adsorbent, Mycofix®	0.20	0.20	0.20
L-lysine (54.6%)	0.177	0.177	0.177
DL-methionine (99.5%)	0.056	0.056	0.056
L-threonine (96.8%)	0.061	0.061	0.061
L-tryptophan (99.0%)	0.017	0.017	0.017
L-valine (95.5%)	0.049	0.049	0.049
β -mannanase	-	0.030	0.030
Calculated nutritional requirements			
Metabolizable energy (kcal/kg)	3,360	3,315	3,300
Crude protein (%)	20.50	20.50	20.50
SID lysine (%)	1.080	1.080	1.080
SID methionine + cysteine (%)	0.602	0.602	0.602
SID threonine (%)	0.700	0.700	0.700
SID tryptophan (%)	0.240	0.240	0.240
SID valine (%)	0.892	0.892	0.892
Total calcium (%)	0.80	0.80	0.80
STTD phosphorus (%)	0.43	0.43	0.43
Total sodium (%)	0.200	0.200	0.200
Starch (%)	34.21	34.21	34.21
Crude fat (%)	6.89	6.34	6.16
Crude fiber (%)	2.99	2.99	2.99

SID - standardized ileal digestible; STTD - standardized total tract digestible.

¹ Experimental diets: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).

² Content per kg of premix: vitamin A, 2,080,000 IU/kg; vitamin D3, 312,000 IU/kg; vitamin E, 11,700 IU/kg; vitamin K3, 520 mg/kg; vitamin B1, 260 mg/kg; vitamin B2, 1040 mg/kg; vitamin B6, 390 mg/kg; vitamin B12, 5200 mg/kg; niacin, 6500 mg/kg; pantothenic acid, 4160 mg/kg; folic acid, 260 mg/kg; biotin, 65 mg/kg; choline chloride, 156 g/kg; Zn oxide, 23.94 g/kg; Mn sulfate, 8820 mg/kg; Fe sulfate, 17.66 g/kg; Cu sulfate, 2640 mg/kg; iodine, 217.80 mg/kg; sodium selenite, 79.40 mg/kg; xylanase, 333.33 U/g.

refers to the amount of enzyme capable of releasing 1 micromole of reducing sugar from a xylan solution (5 mg/mL) at 37 °C and pH 5.5 (Genova et al., 2023a,b). β -mannanase (Elanco Animal Health, Inc., São Paulo, SP, Brazil; registration no. SP-59122 30011, Hemicell™ HT) was obtained from *Paenibacillus lentus* and has an enzyme activity of 160,000 U/g. One unit of β -mannanase is defined as the amount of enzyme required to release 0.72 mcg of reducing sugars (equivalent to D-mannose) per minute from goma locust (mannans concentration of 88%) at 40 °C and pH 7.5 (Genova et al., 2023a,b).

2.4. Blood sampling and cytokine analyses

The procedures adopted are in accordance with those described by Genova et al. (2023a,b). Blood collection (without fasting) was performed on day 18 of lactation in 8 sows per treatment, using the

cranial vena cava collection technique, with a 10 mL syringe and a 1.2 × 40 mm needle. The samples were transferred to tubes (glass vacuum blood collection tube, Labingá; Maringá, PR, Brazil) without the addition of anticoagulants. Immediately after collection, the samples were stored in thermal boxes (4 °C) until the end of the blood sampling. Subsequently, blood samples were centrifuged at 1,000 × *g* for 10 min at room temperature, and the serum was carefully collected and stored at -80 °C being sent to the IMUNOVA laboratory (Curitiba, PR, Brazil).

Serum cytokines concentrations were analyzed in technical duplicates, as follows: interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8/CXCL8), interleukin-12/interleukin-23 (IL-12/IL-23p40), interferon- α (IFN- α), interferon- γ (IFN- γ), tumor necrosis factor- α (TFN- α), interleukin-4 (IL-4), and interleukin-10 (IL-10), using specific ELISA kits (ThermoFisher Scientific, Vienna, Austria) according to the manufacturer's instructions.

2.5. Fecal sampling and microbiota

On day 21 of lactation, 10 g of fecal samples were manually collected from 8 sows per treatment. The samples were collected by rectal stimulation, using swabs, and placed in sterile 4 mL Eppendorf-type tubes. Immediately, the sampled material was stored in thermal boxes (4 °C) until the end of the collection period. Subsequently, the samples were stored at -80 °C until the analyses.

A commercial kit (ZR Fecal DNA MiniPrep® from Zymo Research) was used to extract DNA from fecal samples following the manufacturer's instructions. DNA quality and quantification were evaluated using NanoDrop spectrophotometry (Thermo Scientific™). A segment of approximately 460 base pairs of the V3-V4 hypervariable region of the 16S ribosomal RNA gene was amplified using the 375F/805R primer pair for bacterial analysis, under the following PCR conditions: 95 °C for 3 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a step at 72 °C for 5 min. The amplicons were ligated to Illumina® Nextera dual index barcodes under the following conditions: 95 °C for 3 min; 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a step at 72 °C for 5 min. The products were then purified, pooled together, and subsequently sequenced on the Illumina® NextSeq sequencer (Degnan and Ochman, 2012) as paired-end reads of 300 base pairs.

The reads obtained by the sequencer were analyzed on the Quantitative Insights Into Microbial Ecology (QIIME 2) platform, following a workflow that uses forward and reverse sequencing (R1 and R2) in bacterial analysis, including the removal of low-quality sequences, filtration, chimera removal, and taxonomic classification. The sequences were classified into bacterial genera using amplicon sequence variants (ASVs), which involved comparing the homology of the sequences against a database. To compare the amplicons of 16S rRNA gene regions, the 2022 update (Genome Taxonomy Database, GTDB 207 version) of the GTDB bacterial taxonomy database (Degnan and Ochman, 2012) was used, with in silico reads extracted from the same amplified regions.

To generate the classification of the bacterial communities through the identification of ASVs, 75,732 reads per sample were used for the bacterial analysis, with all samples being analyzed. The use of a minimum number of reads per sample aimed at normalizing the data and avoiding statistical comparisons among samples with different sequencing depths.

Families and higher classifications that had uppercase letters as suffixes represented non-monophyletic groupings in the construction of the GTDB reference phylogeny, although there is evidence that these groups are monophyletic. Genera with the same suffix pattern were polyphyletic or subdivided according to the database's reference phylogeny. Species names can also have the same suffix pattern if they categorize a group that may contain ambiguity in the correct nomenclature (Parks et al., 2022). Other codes present in the classifications (e.g.: *NSJ-53* or *sp001543345*) represent the first material used as a reference for clustering into the respective operational taxonomic units (OTUs).

2.6. Statistical procedures

The serum cytokine concentration was analyzed using GraphPad Prism software version 8. Outliers were identified using the ROUT test ($Q = 1\%$). The data were tested for normality using the Shapiro-Wilk test. Based on the results, if the data met the normality assumption, a linear mixed model was applied, with treatment as a fixed effect and block as a random effect, expressed as:

$$Y_{ijk} = \mu + T_i + b_j + e_{ijk} \quad (1)$$

in which Y_{ijk} = observed value of the dependent variable in the i -th treatment, in the j -th block and in the k -th replicate; μ = overall average; T_i = fixed effect of treatment (group, $i = 1, 2$, and 3); b_j = random effect of block ($j = 1, 2, 3$ and 4); e_{ijk} = residual error term, assumed to be independently and normally distributed with mean zero and constant variance. If the data did not follow normality, an equivalent non-parametric model was used, based on rank analysis, whose null hypothesis is that the distributions between the groups are equal. For both tests, a difference was considered significant when $P < 0.05$.

The statistical analyses and graphs of the fecal microbiome were performed using R software (R Core Team, 2020). The statistical comparison between groups in alpha diversity analyses was conducted using the non-parametric Kruskal-Wallis test followed by Dunn's post hoc. Beta diversity was analyzed using permutational multivariate analysis of variance (PERMANOVA) in the QIIME2 pipeline, with 10,000 permutations, which was expressed as:

$$Y = X\beta + e, \quad (2)$$

in which Y = matrix of pairwise dissimilarities (e.g. Bray-Curtis); X = design matrix encoding group membership; β = estimated coefficients (vector) for group effects; e = error term (residual matrix). Alpha diversity analyses were calculated using the "phyloseq" (McMurdie and Holmes, 2013), "vegan" (Oksanen et al., 2007), and "Microbiome" (Lahti and Shetty, 2018) libraries. To identify specific taxa that may have undergone modulation between the groups, differences in relative abundances were analyzed using the Kruskal-Wallis test with Dunn's post hoc at a 95% confidence level for lower taxonomic classifications: family, genus, and species.

3. Results

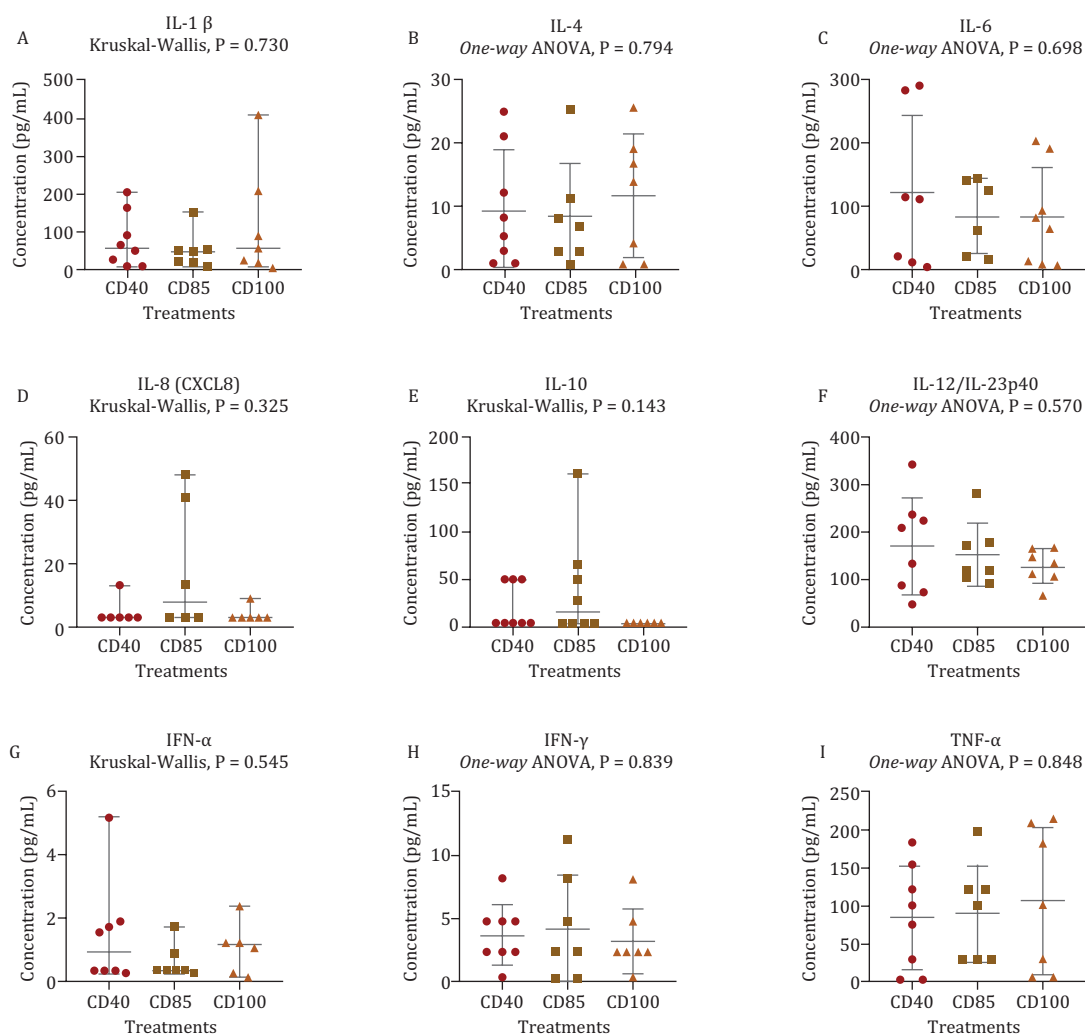
3.1. Serum cytokine concentrations

There was no effect ($P > 0.05$) of dietary treatments on serum cytokines concentrations in lactating sows (Figure 1), but the cytokines IL-12/IL-23p40 exhibited the highest concentrations among all those evaluated (Figure 2).

3.2. Fecal microbiota

Sows fed the CD85 diet showed ($P = 0.014$) higher alpha diversity richness than those fed the CD100 diet based on the Simpson index (0.98 vs. 0.97, Figure 3D). The remaining alpha diversity indices were not altered by the diets (Chao1, observed OTUs, Fisher, Shannon, and Pielou) (Figure 3). No significant effect was observed on beta diversity assessed using Bray-Curtis ($P = 0.527$), Jaccard ($P = 0.526$), UniFrac ($P = 0.687$), and Weighted UniFrac ($P = 0.547$) parameters (Figure 4).

The phyla, classes, orders, families, genera, and species with an average relative abundance above 2% in at least one of the groups tested were plotted in the graphs (Figure 5). In the fecal samples analyzed, the most abundant phylum was Firmicutes, followed by Bacteroidota, Spirochaetota, Actinobacteriota, and Cyanobacteria. Furthermore, the classes Clostridia, Bacilli, Bacteroidia, Negativicutes, Spirochaetia, Coriobacteriia, and Vampirovibrionia showed the highest abundances. The most abundant orders were Christensenellales, Oscillospirales, Peptostreptococcales, Clostridiales, Lachnospirales, Bacteroidales, TANB77, Lactobacillales, Treponematales, Coriobacteriales, Haloplasmales_A, Gastranaerophilales,



Data were presented as means $\pm$ SEM. Differences in serum concentrations of (A) interleukin-1 β (IL-1 β), (B) interleukin-4 (IL-4), (C) interleukin-6 (IL-6), (D) interleukin-8 (IL-8/CXCL8), (E) interleukin-10 (IL-10), (F) interleukin-12/interleukin-23p40 (IL-12/IL23p40), (G) interferon- α (IFN- α), (H) interferon- γ (IFN- γ), and (I) tumoral necrosis factor- α (TNF- α) in sows fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).

Figure 1 - Serum cytokines concentrations on day 18 of lactation in 8 sows per treatment.

and Erysipelotrichales. The families with the highest relative abundance were Peptostreptococcaceae, Clostridiaceae, Oscillospiraceae, Lachnospiraceae, CAG-74, CAG-508, Treponemataceae, Lactobacillaceae, Muribaculaceae, Ruminococcaceae, Acutalibacteraceae, Turicibacteraceae, NSJ-53, Christensenellaceae, Gastranaerophilaceae, Erysipelotrichaceae, and Bacteroidaceae. The most abundant genera were *Clostridium*, *Terrisporobacter*, *CAG-83*, *Romboutsia*, *Sodaliphilus*, *GCA-900199385*, *Limivivinus*, *Onthenecus*, *Limosilactobacillus*, *Turicibacter*, *Merdicola*, *Fimivivens*, and *Fimenecus*. Species that showed relative abundance were *Sodaliphilus* sp004557565, *GCA-900199385* sp900322155, *Clostridium* baratii, *Limivivinus* sp002320035, *Onthenecus* sp900199405, *Turicibacter* sp001543345, *CAG-83* sp900549395, *NSJ-53* sp014384795, *NSJ-63* sp014384805, *Merdicola* sp001915925, *Fimivivens* sp900113995, *Clostridium* butyricum, and *Fimenecus* sp004556705.

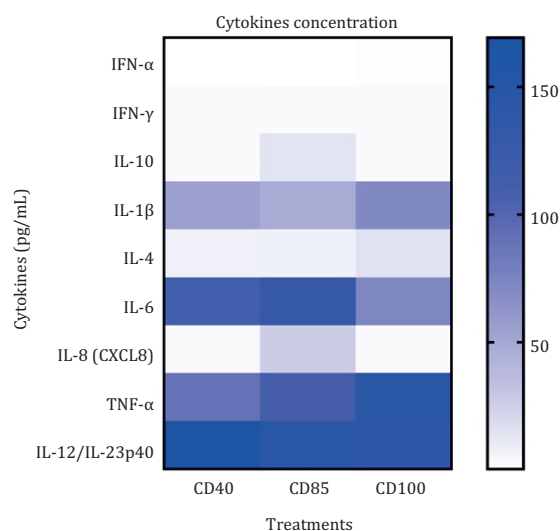


Figure 2 - Heatmap of cytokines concentrations on day 18 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).

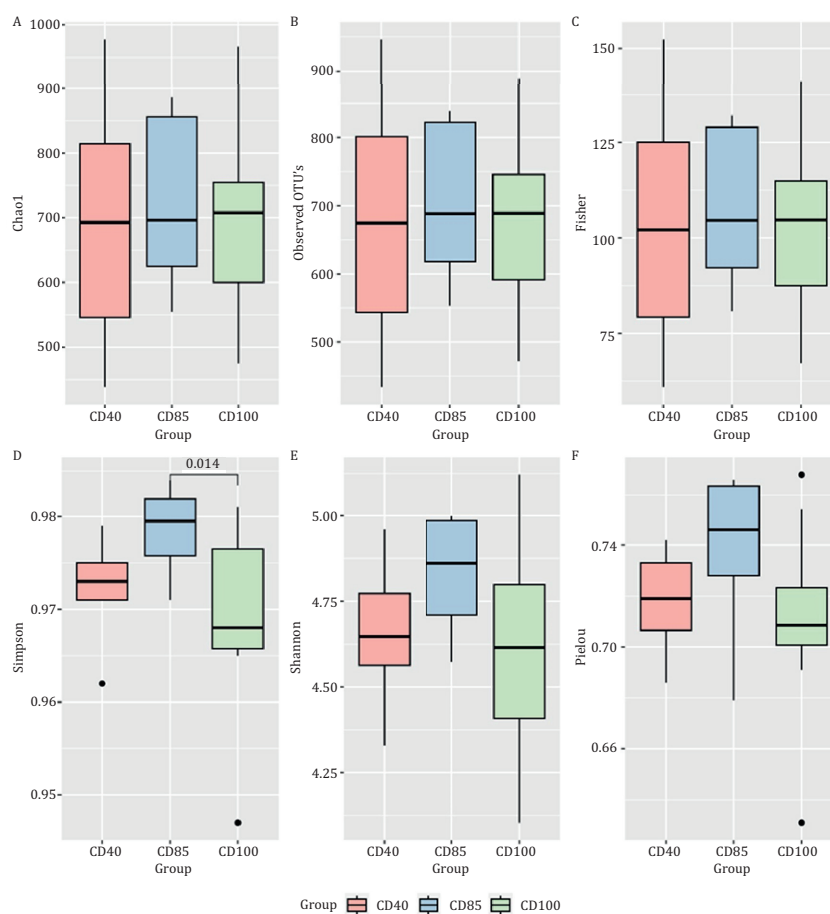


Figure 3 - Estimated alpha-diversity by Chao1 (A), Observed OTUs (B), Fisher index (C), Simpson index (D), Shannon index (E), and Pielou's Evenness (F) on day 21 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).

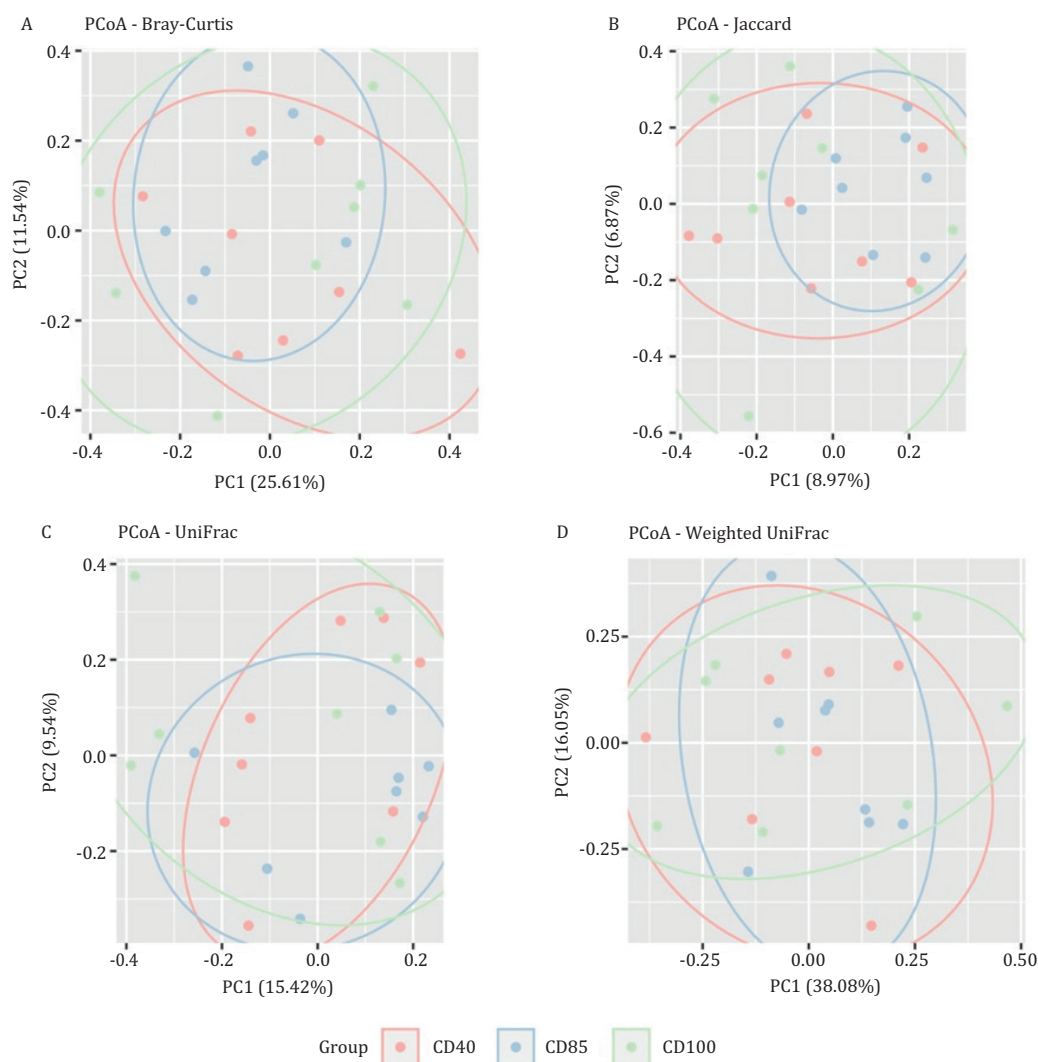


Figure 4 - Beta diversity estimated by Bray-Curtis (A), Jaccard (B), UniFrac (C), and Weighted UniFrac (D) parameters on day 21 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).

No differences were observed between the groups for the Firmicutes:Bacteroidetes ratio (Figure 6). The Acutalibacteraceae family was more abundant in the sows fed CD100 diet than in those fed CD85 diet ($P = 0.044$, Figure 7A). Furthermore, sows fed CD85 diet had a higher abundance of the CAG_508 ($P = 0.012$, Figure 7B) and NSJ_53 ($P = 0.044$, Figure 7C) families than those fed CD100 diet.

The *Fimenecus* genus exhibited a lower abundance in sows fed CD85 diet compared to sows fed CD40 ($P = 0.007$, Figure 8A) or CD100 diets ($P = 0.005$, Figure 8A), while the NSJ-53 genus showed a higher abundance in sows fed CD85 diet than in those fed CD100 diet ($P = 0.044$, Figure 8B). Similarly, these results followed ($P < 0.05$) for the *Fimenecus* sp004556705 (Figure 9A) and NSJ-53 sp014384795 (Figure 9B).

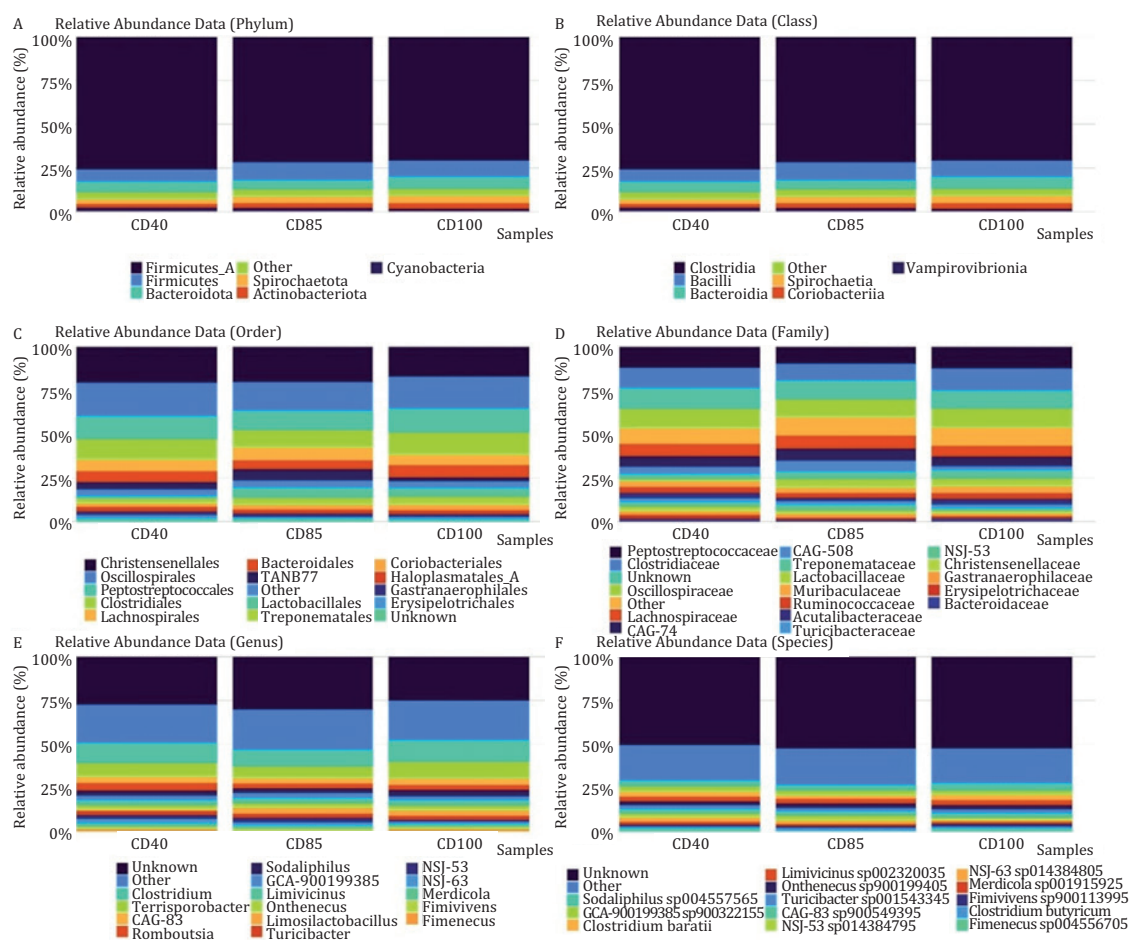


Figure 5 - Relative abundance of phyla (A), classes (B), orders (C), families (D), genera (E), and species (F) presents on day 21 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).

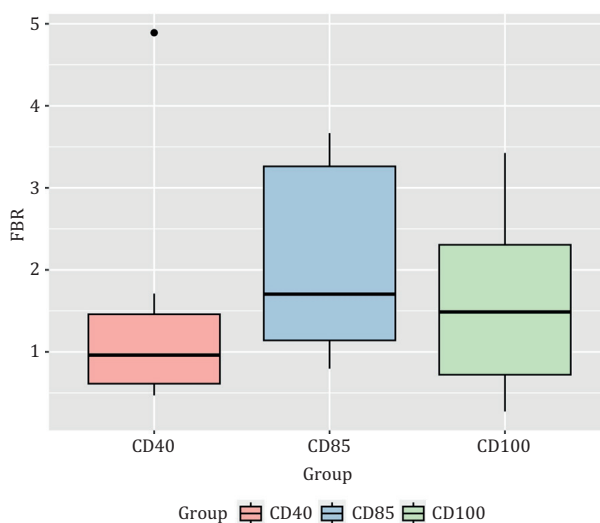
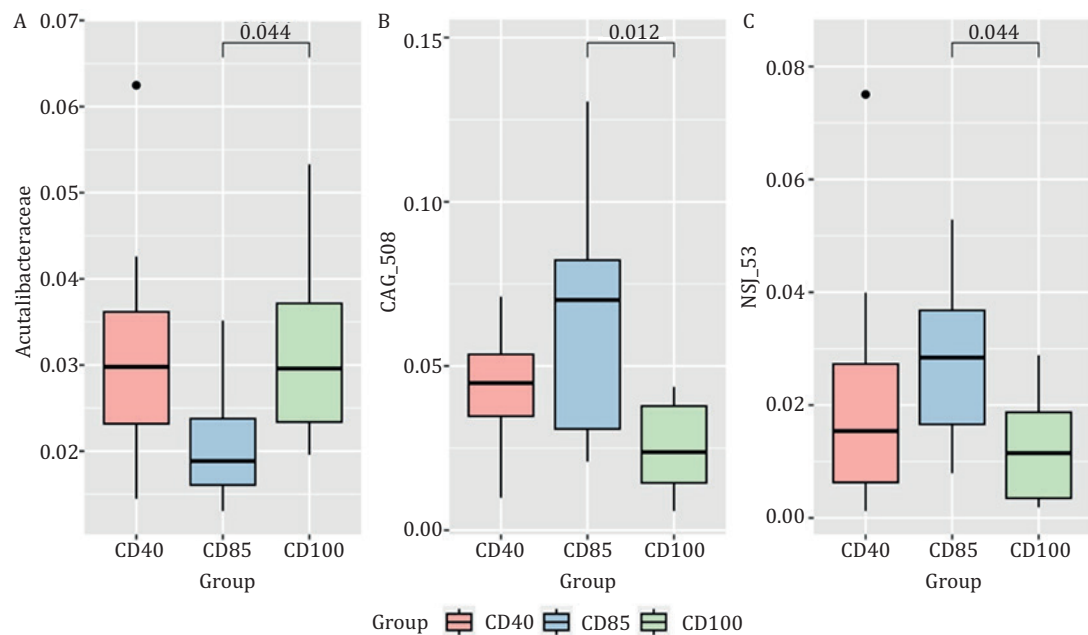
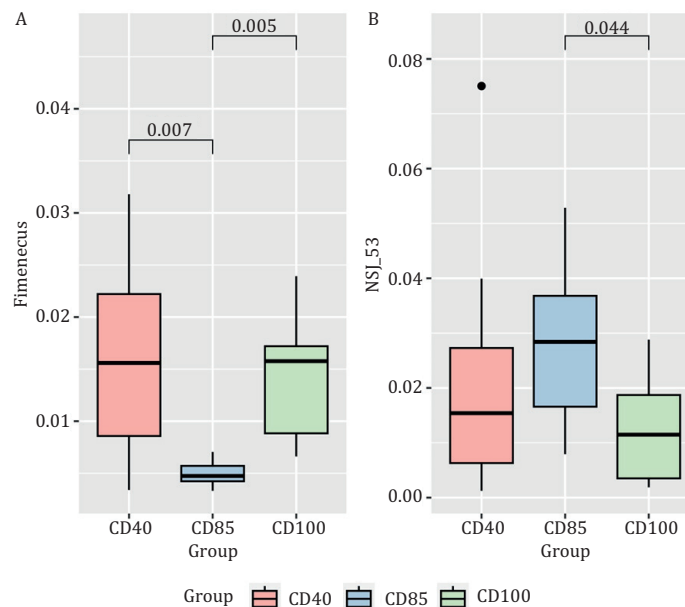


Figure 6 - Firmicutes:Bacteroidetes ratio (FBR) on day 21 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).



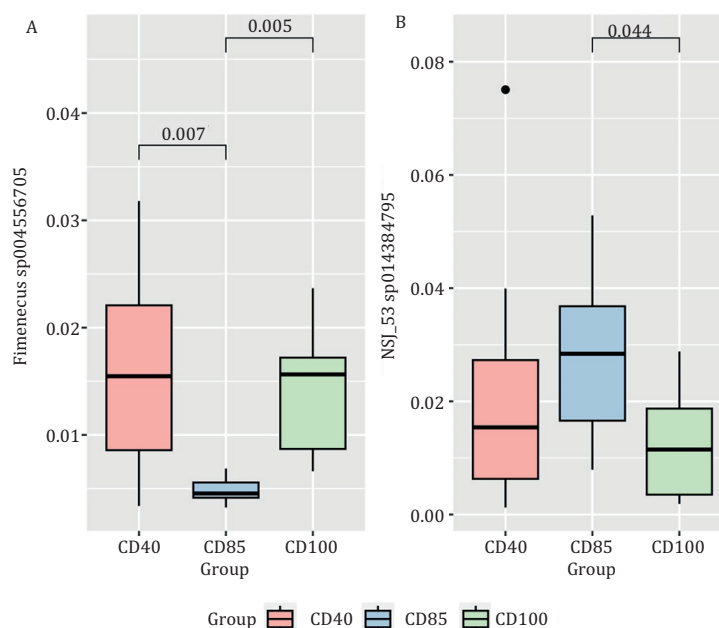
Statistical tests were performed using the Kruskal-Wallis test and Dunn's post hoc test, with $P < 0.05$.

Figure 7 - Differential abundance of taxa between groups for *Acutalibacteraceae* (A), *CAG_508* (B) and *NSJ_53* (family code) (C) families on day 21 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).



Statistical tests were performed using the Kruskal-Wallis test and Dunn's post hoc test, with $P < 0.05$.

Figure 8 - Differential abundance of taxa between groups for *Fimenes* (A) and *NSJ_53* (genus code) (B) genera on day 21 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).



Statistical tests were performed using the Kruskal-Wallis test and Dunn's post hoc test, with $P < 0.05$.

Figure 9 - Differential abundance of taxa between treatments for *Fimenecus sp004556705* (A) and *NSJ-53 sp014384795* (B) species on day 21 of lactation in 8 sows per treatment fed to one of three dietary treatments: 1) a diet containing xylanase (Control; valorization of 40 kcal of ME/kg diet, CD40), 2) CD40 + β -mannanase (0.3 g/kg, valorization of 45 kcal ME/kg diet, CD85), and 3) CD40 + β -mannanase (0.3 g/kg, valorization of 60 kcal ME/kg diet, CD100).

4. Discussion

4.1. Serum cytokine concentrations

In the present study, dietary treatments, including β -mannanase supplementation in ME-reduced diets containing xylanase, did not affect serum cytokine concentrations in lactating sows. Cytokines such as IL-1 β , IL-6, IL-10, TNF- α , and IL-12/IL-23p40 serve as indicators of immune status and inflammation (Meizlish et al., 2021). The absence of treatment effects on these markers indicates that, under the present experimental conditions, the enzymatic strategy adopted did not alter systemic inflammatory or regulatory immune pathways. Monitoring cytokine profiles provides information about the immune response, but may not fully capture localized or subtle immune modulations driven by the microbiota, which could occur at the intestinal level without systemic reflection.

In the present study, IL-12/IL-23p40 showed the highest concentrations among the cytokines evaluated in all treatment groups, which is consistent with its central role in innate immune activation and modulation of inflammatory responses (Cavaillon et al., 2020; Splichalova et al., 2023). However, because IL-12/IL-23p40 and the other cytokines did not differ between dietary treatments, these patterns likely reflect the physiological immune status of lactating sows rather than a diet-induced response. Thus, the combination of xylanase and β -mannanase in ME-reduced diets did not induce overt systemic inflammation or cytokine "storm".

The relationship between dietary enzymes and immune responses is complex and may depend on the specific substrates available in the gastrointestinal tract (Kiarie et al., 2022). Our data suggest that even though β -mannanase and xylanase altered the intestinal environment, these effects were not enough to

translate into measurable changes in systemic cytokines. Therefore, any potential immune modulation is likely to be local or of low magnitude and cannot be demonstrated by the current serum data.

The reduction of ME at up to 100 kcal/kg in the diets did not appear to compromise the sows' immune status, as serum cytokine levels remained unaffected. Immune activation, while essential for pathogen defense, involves energetic costs that can impact nutrient partitioning and overall productivity (Wang et al., 2023). For example, changes in the dietary starch-to-fat ratio altered inflammatory cytokine concentrations during critical periods such as parturition (Wang et al., 2023). This suggests that within the parameters evaluated, moderate reductions in energy do not cause systemic immune activation (Rodrigues et al., 2022). The moderate reduction in ME applied here did not cause detectable systemic immune activation, suggesting that the nutritional adjustment was well tolerated by lactating sows based on the serum cytokine profile. Nonetheless, localized or subclinical effects cannot be excluded and would require tissue-level assessments in future studies.

While a systematic review indicates that β -mannanase supplementation reduced inflammatory responses caused by β -mannan and released prebiotic-like products into the intestinal tract (Kiarie et al., 2022), our findings do not support systemic immunomodulation by β -mannanase under the current conditions, and fecal microbiota data point to limited microbial ecological adjustments rather than functional changes.

4.2. Fecal microbiota

In the present study, the hypothesis that β -mannanase supplementation could influence the fecal microbiome composition and diversity in lactating sows was supported by the observed increase in microbial alpha diversity, specifically based on the Simpson index. The fecal microbiota is influenced by several factors, including host genetics (Spor et al., 2011), diet (Wu et al., 2011), and the immune system (Slack et al., 2009). The microbiota plays an important role in various aspects of animal physiology, such as the inflammation process, metabolic syndromes (Chassaing et al., 2014), energy metabolism (Donohoe et al., 2011), and immune responses (Peng et al., 2021). In this study, β -mannanase supplementation in ME-reduced diets containing xylanase resulted in limited changes within the fecal microbial community.

Lactating sows fed the CD85 diet exhibited a slight but statistically significant increase in alpha diversity based on the Simpson index compared to those fed the CD100. This difference was slight in magnitude and was not accompanied by changes in other alpha diversity indices (Chao1, observed OTUs, Shannon, Pielou) or in beta diversity metrics (Bray-Curtis, Jaccard, UniFrac, weighted UniFrac), indicating that the overall profile of the microbial community remained stable across all treatments.

Consistent with previous research (Vojinovic et al., 2019), the observed microbial modifications highlight the ability of the microbiota to adapt subtly in response to dietary interventions. These modest modifications are compatible with the concept that the intestinal microbiota of lactating sows is relatively resilient and may adjust to energy-reduced, enzyme-supplemented diets with only limited changes in dominant taxa.

The balance between Firmicutes and Bacteroidetes in fecal microbiota is often studied for its potential influence on intestinal health and metabolism, given that their proportions can be associated with several physiological and pathological conditions. In this study, the Firmicutes-to-Bacteroidetes ratio was not significantly affected by the dietary treatments, suggesting that the energy reduction and supplementation strategies employed did not markedly alter these predominant microbial groups. This lack of response may suggest the resilience of these microbial profiles to dietary interventions or indicate that the modifications primarily impacted other microbial populations or functions without significantly changing the relative abundances of these major phyla.

Across all treatment groups, Firmicutes remained the dominant phylum, accounting for over 80% of the microbial community. These findings are consistent with previous research documenting the predominance of Firmicutes in the intestinal microbiota of lactating sows (Liu et al., 2019). The high relative abundance of Firmicutes is often associated with an enhanced capacity for energy extraction

from the diet (Turnbaugh et al., 2006), which may support the increased energetic demands during lactation. However, the observed microbial composition remained stable across diets, indicating that the dietary modifications did not substantially alter the profile at the phylum level.

At the class level, Clostridia was the predominant bacterial class in all groups of lactating sows, aligning with prior studies that analyzed the dynamic changes in intestinal microbiota during the perinatal period (Sun et al., 2020) and lactation (Xiong et al., 2019). Additionally, the Clostridia class of bacteria has a multitude of enzymes (e.g.: butyryl-CoA:acetate-CoA transferase) that facilitate the breakdown of polysaccharides into short-chain fatty acids (SCFA). Consistently, research on the *Clostridium* genera showed that they can produce butyrate (Holman et al., 2017; Vital et al., 2014; Parada Venegas et al., 2019) and have associated butyrate with the prevention and treatment of intestinal disorders, by reducing inflammation in the host's intestine, allowing intestinal epithelial cells to use it as a source of energy (Hamer et al., 2008). Nevertheless, the present data do not allow us to infer functional consequences at the host level, particularly because systemic cytokines remained unchanged among treatments.

Regarding specific microbial families, sows fed the CD100 diet exhibited a higher relative abundance of the Acetivibacteraceae family compared to the CD85 diet. However, the information available on the relevance of this family to the porcine intestinal microbiota is limited (Barducci et al., 2024). On the other hand, sows fed the CD85 diet exhibited a higher relative abundance of the CAG-508 and NSJ_53 families compared to the CD100 diet. These taxa remain poorly characterized in pigs, and their biological relevance in lactating sows is currently uncertain (Qing-Bo et al., 2023). Therefore, the observed differences in their relative abundance should be interpreted as exploratory signals that warrant further investigation rather than as evidence of specific functional changes.

The Christensenellaceae family, represented by the NSJ_53 genus, has been proposed as a potential probiotic candidate in other species with some evidence linking it to metabolic regulation and intestinal homeostasis (Waters and Ley, 2019; Alemán et al., 2018). In pigs, the specific role of Christensenellaceae remains to be elucidated. While the literature suggests potential beneficial effects (Waters and Ley, 2019; Alemán et al., 2018), the current data do not support a definitive functional role for this taxon in systemic health or immune modulation.

The *Fimenes* genus has limited characterization in the context of porcine microbiota, though studies in humans report high pectin-degrading activities, including arabinases, galacturonases, and galacturonidases (Calvete-Torre et al., 2023). Its potential role in pig intestinal health and immune function remains speculative (Wiese, 2019), warranting further research. Therefore, the microbial shifts observed, such as modest increases in diversity and specific taxa, likely reflect minor ecological adjustments. These subtle microbial adaptations may play a key role in maintaining intestinal stability during dietary interventions.

5. Conclusions

The β -mannanase supplementation in diets containing xylanase and reduced by 85 kcal of metabolizable energy/kg fed to lactating sows showed a positive impact on fecal alpha diversity based on the Simpson index, without affecting other alpha diversity indices, beta diversity, or systemic cytokine concentrations. Under the present experimental conditions, this nutritional strategy was well tolerated by sows based on systemic immune assessment and was associated with only subtle ecological adjustments in the fecal microbiota. Furthermore, more research is needed to investigate the mechanisms underlying the regulation of the intestinal microbiota in lactating sows and to determine whether these modest taxonomic shifts have measurable functional consequences for sow performance.

Data availability

The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization: Carvalho, P. L. O. **Data curation:** Genova, J. L. **Formal analysis:** Genova, J. L.; Vaz, P. A. and Eyng, C. **Funding acquisition:** Kipper, M. and Carvalho, P. L. O. **Investigation:** Engelsing, E. F. R.; Bickel, A. G.; Boelhouwer, M.; Nunes, R. V.; Alves, R. H. R. A. and Rodrigues, C. C. **Methodology:** Engelsing, E. F. R.; Carvalho, S. T.; Eyng, C. and Carvalho, P. L. O. **Project administration:** Carvalho, S. T.; Eyng, C. and Carvalho, P. L. O. **Resources:** Carvalho, S. T.; Kipper, M. and Carvalho, P. L. O. **Supervision:** Carvalho, S. T.; Kipper, M.; Eyng, C. and Carvalho, P. L. O. **Writing – original draft:** Lima, J. P. S. **Writing – review & editing:** Lima, J. P. S.; Genova, J. L.; Poveda-Parra, A. R.; Eyng, C.; Costa, L. B. and Carvalho, P. L. O.

Conflict of interest

Author Marcos Kipper was employed by Elanco Company. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

- Alemán, J. O.; Bokulich, N. A.; Swann, J. R.; Walker, J. M.; De Rosa, J. C.; Battaglia, T.; Costabile, A.; Pechlivanis, A.; Liang, Y.; Breslow, J. L.; Blaser, M. J. and Holt, P. R. 2018. Fecal microbiota and bile acid interactions with systemic and adipose tissue metabolism in diet-induced weight loss of obese postmenopausal women. *Journal of Translational Medicine* 16:244. <https://doi.org/10.1186/s12967-018-1619-z>
- Baker, J. T.; Duarte, M. E.; Holanda, D. M. and Kim, S. W. 2021. Friend or foe? Impacts of dietary xylans, xylooligosaccharides, and xylanases on intestinal health and growth performance of monogastric animals. *Animals* 11:609. <https://doi.org/10.3390/ani11030609>
- Barducci, R. S.; Santos, A. A. D.; Pacheco, L. G.; Putarov, T. C.; Koch, J. F. A.; Callegari, M. A.; Dias, C. P.; Carvalho, R. H. and Silva, C. A. 2024. Enhancing weaned piglet health and performance: The role of autolyzed yeast (*Saccharomyces cerevisiae*) and β -glucans as a blood plasma alternative in diets. *Animals* 14:631. <https://doi.org/10.3390/ani14040631>
- Calvete-Torre, I.; Sabater, C.; Margolles, A. and Ruiz, L. 2023. Fecal microbiota cooperative metabolism of pectins derived from apple pomace: A functional metagenomic study. *LWT* 187:115362. <https://doi.org/10.1016/j.lwt.2023.115362>
- Cavaillon, J. M.; Singer, M. and Skirecki, T. 2020. Sepsis therapies: Learning from 30 years of failure of translational research to propose new leads. *EMBO Molecular Medicine* 12:e10128. <https://doi.org/10.15252/emmm.201810128>
- Chassaing, B.; Ley, R. E. and Gewirtz, A. T. 2014. Intestinal epithelial cell Toll-like receptor 5 regulates the intestinal microbiota to prevent low-grade inflammation and metabolic syndrome in mice. *Gastroenterology* 147:1363-1377. <https://doi.org/10.1053/j.gastro.2014.08.033>
- Degnan, P. H. and Ochman, H. 2012. Illumina-based analysis of microbial community diversity. *ISME Journal* 6:183-194. <https://doi.org/10.1038/ismej.2011.74>
- Donohoe, D. R.; Garge, N.; Zhang, X.; Sun, W.; O'Connell, T. M.; Bunger, M. K. and Bultman, S. J. 2011. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism* 13:517-526. <https://doi.org/10.1016/j.cmet.2011.02.018>
- Genova, J. L.; Azevedo, L. B.; Rupolo, P. E.; Cordeiro, F. B. C.; Vilela, H. L. O.; Careli, P. S.; Toledo, D. C. F.; Carvalho, S. T.; Kipper, M.; Rennó, L. N.; Faveri, J. C. and Carvalho, P. L. O. 2023a. β -mannanase supplemented in diets saved 85 to 100 kcal of metabolizable energy/kg, supporting growth performance and improving nutrient digestibility in grower pigs. *Scientific Reports* 13:12546. <https://doi.org/10.1038/s41598-023-38776-5>
- Genova, J. L.; Rupolo, P. E.; Azevedo, L. B.; Henz, D.; Carvalho, S. T.; Kipper, M.; Gonçalves, G. A. C.; Vilela, H. L. O.; Pasquetti, T. J.; Oliveira, N. T. E.; Dietrich, A. R. M. and Carvalho, P. L. O. 2023b. β -mannanase supplementation in diets reduced in 85 kcal metabolizable energy/kg containing xylanase-phytase improves gain to feed ratio, nutrient usage, and backfat thickness in finisher pigs. *Frontiers in Veterinary Science* 10:289. <https://doi.org/10.3389/fvets.2023.1144692>

- Hamer, H. M.; Jonkers, D.; Venema, K.; Vanhoutvin, S.; Troost, F. J. and Brummer, R. J. 2008. Review article: the role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics* 27:104-119. <https://doi.org/10.1111/j.1365-2036.2007.03562.x>
- Holman, D. B.; Brunelle, B. W.; Trachsel, J. and Allen, H. K. 2017. Meta-analysis to define a core microbiota in the swine gut. *mSystems* 2:e00004-17. <https://doi.org/10.1128/mSystems.00004-17>
- Kiarie, E. G.; Steelman, S. and Martinez, M. 2022. Does supplementing β -mannanase modulate feed-induced immune response and gastrointestinal ecology in poultry and pigs? An appraisal. *Frontiers in Animal Science* 3:875095. <https://doi.org/10.3389/fanim.2022.875095>
- Kiarie, E.; Romero, L. F. and Nyachoti, C. M. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. *Nutrition Research Reviews* 26:71-88. <https://doi.org/10.1017/S0954422413000048>
- Kim, J. S.; Hosseindoust, A.; Ju, I. K.; Yang, X.; Lee, S. H.; Noh, H. S.; Lee, J. H. and Chae, B. J. 2018. Effects of dietary energy levels and β -mannanase supplementation in a high mannan-based diet during lactation on reproductive performance, apparent total tract digestibility and milk composition in multiparous sows. *Italian Journal of Animal Science* 17:128-134. <https://doi.org/10.1080/1828051X.2017.1345663>
- Kipper, M.; Andretta, I.; Quadros, V. R.; Schroeder, B.; Pires, P. G. S.; Franceschina, C. S.; Hickmann, F. M. W. and França, I. 2020. Performance responses of broilers and pigs fed diets with β -mannanase. *Revista Brasileira de Zootecnia* 49:e20180177. <https://doi.org/10.37496/rbz4920180177>
- Lahti, L. and Shetty, S. 2018. Introduction to the microbiome R package. *Bioconductor*. <https://doi.org/10.18129/B9.bioc.microbiome>
- Liu, H.; Hou, C.; Li, N.; Zhang, X.; Zhang, G.; Yang, F.; Zeng, X.; Liu, Z. and Qiao, S. 2019. Microbial and metabolic alterations in gut microbiota of sows during pregnancy and lactation. *FASEB Journal* 33:4490-4501. <https://doi.org/10.1096/fj.201801221RR>
- McMurdie, P. J. and Holmes, S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. <https://doi.org/10.1371/journal.pone.0061217>
- Meizlish, M. L.; Franklin, R. A.; Zhou, X. and Medzhitov, R. 2021. Tissue homeostasis and inflammation. *Annual Review of Immunology* 39:557-581. <https://doi.org/10.1146/annurev-immunol-061020-053734>
- Oksanen, J.; Kindt, R.; Legendre, P.; O'Hara, B. and Stevens, M. H. H. 2007. The vegan package: community ecology package.
- Parada Venegas, D. P.; De la Fuente, M. K.; Landskron, G.; González, M. J.; Quera, R.; Dijkstra, G.; Harmsen, H. J. M.; Faber, K. N. and Hermoso, M. A. 2019. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Frontiers in Immunology* 10:277. <https://doi.org/10.3389/fimmu.2019.00277>
- Parks, D. H.; Chuvochina, M.; Rinke, C.; Mussig, A. J.; Chaumeil, P. A. and Hugenholtz, P. 2022. GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Research* 50:D785-D794. <https://doi.org/10.1093/nar/gkab776>
- Peng, J.; Tang, Y. and Huang, Y. 2021. Gut health: The results of microbial and mucosal immune interactions in pigs. *Animal Nutrition* 7:282-294. <https://doi.org/10.1016/j.aninu.2021.01.001>
- Qing-Bo, L.; Meng, J. X.; Ma, H.; Liu, R.; Qin, Y.; Qin, Y. F.; Geng, H. L.; Ni, H. B. and Zhang, X. X. 2023. Description of gut mycobiota composition and diversity of caprine animals. *Microbiology Spectrum* 11:e02424-22. <https://doi.org/10.1128/spectrum.02424-22>
- R Core Team. 2020. R: A language and environment for statistical computing. Available at: <<http://www.r-project.org/index.html>>. Accessed on: Mar. 31, 2024.
- Rodrigues, L. A.; Koo, B.; Nyachoti, M. and Columbus, D. A. 2022. Formulating diets for improved health status of pigs: Current knowledge and perspectives. *Animals* 12:2877. <https://doi.org/10.3390/ani12202877>
- Rostagno, H. S.; Albino, L. F. T.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saraiva, A.; Teixeira, M. L.; Rodrigues, P. B.; Oliveira, R. F.; Barreto, S. L. T. and Brito, C. O. 2017. Tabelas Brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. UFV, Viçosa, MG.
- Slack, E.; Hapfelmeier, S.; Stecher, B.; Velykoredko, Y.; Stoel, M.; Lawson, M. A.; Geuking, M. B.; Beutler, B.; Tedder, T. F.; Hardt, W. D.; Bercik, P.; Verdu, E. F.; McCoy, K. D. and Macpherson, A. J. 2009. Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* 325:617-620. <https://doi.org/10.1126/science.1172747>
- Splichalova, A.; Kindlova, Z.; Killer, J.; Neuzil Bunesova, V.; Vlkova, E.; Valaskova, B.; Pechar, R.; Polakova, K. and Splichal, I. 2023. Commensal bacteria impact on intestinal Toll-like receptor signaling in *Salmonella*-challenged gnotobiotic piglets. *Pathogens* 12:1293. <https://doi.org/10.3390/pathogens12111293>
- Spor, A.; Koren, O. and Ley, R. 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology* 9:279-290. <https://doi.org/10.1038/nrmicro2540>
- Sun, L.; Zhang, Y.; Chen, W.; Lan, T.; Wang, Y.; Wu, Y.; Liao, X. and Mi, J. 2020. The dynamic changes of gut microbiota during the perinatal period in sows. *Animals* 10:2254. <https://doi.org/10.3390/ani10122254>

- Tiwari, U. P.; Chen, H.; Kim, S. W. and Jha, R. 2018. Supplemental effect of xylanase and mannanase on nutrient digestibility and gut health of nursery pigs studied using both *in vivo* and *in vitro* models. *Animal Feed Science and Technology* 245:77-90. <https://doi.org/10.1016/j.anifeedsci.2018.07.002>
- Turnbaugh, P. J.; Ley, R. E.; Mahowald, M. A.; Magrini, V.; Mardis, E. R. and Gordon, J. I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444:1027-1031. <https://doi.org/10.1038/nature05414>
- Vangroenweghe, F.; Poulsen, K. and Thas, O. 2021. Supplementation of a β -mannanase enzyme reduces post-weaning diarrhea and antibiotic use in piglets on an alternative diet with additional soybean meal. *Porcine Health Management* 7:8. <https://doi.org/10.1186/s40813-021-00191-5>
- Vital, M.; Howe, A. C. and Tiedje, J. M. 2014. Revealing the bacterial butyrate synthesis pathways by analyzing (meta) genomic data. *mBio* 5:e00889-14. <https://doi.org/10.1128/mbio.00889-14>
- Vojinovic, D.; Radjabzadeh, D.; Kurilshikov, A.; Amin, N.; Wijmenga, C.; Franke, L.; Ikram, M.; Uitterlinden, A.; Zhernakova, A.; Fu, J.; Kraaij, R. and van Duijn, C. M. 2019. Relationship between gut microbiota and circulating metabolites in population-based cohorts. *Nature Communications* 10:5813. <https://doi.org/10.1038/s41467-019-13721-1>
- Wang, W.; Yu, Z.; Yin, X.; Wang, Z.; Xu, S.; Shi, C.; Zang, J.; Liu, H. and Wang, F. 2023. Effect of dietary starch-to-fat ratio on lipid metabolism, inflammation, and microbiota of multiparous sows and newborn piglets. *Agriculture* 13:1069. <https://doi.org/10.3390/agriculture13051069>
- Waters, J. L. and Ley, R. E. 2019. The human gut bacteria *Christensenellaceae* are widespread, heritable, and associated with health. *BMC Biology* 17:83. <https://doi.org/10.1186/s12915-019-0699-4>
- Wiese, M. 2019. The potential of pectin to impact pig nutrition and health: feeding the animal and its microbiome. *FEMS Microbiology Letters* 366:fnz029. <https://doi.org/10.1093/femsle/fnz029>
- Wu, G. D.; Chen, J.; Hoffmann, C.; Bittinger, K.; Chen, Y. Y.; Keilbaugh, S. A.; Bewtra, M.; Knights, D.; Walters, W. A.; Knight, R.; Sinha, R.; Gilroy, E.; Gupta, K.; Baldassano, R.; Nessel, L.; Li, H.; Bushman, F. D. and Lewis, J. D. 2011. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334:105-108. <https://doi.org/10.1126/science.1208344>
- Xiong, Y.; Pang, J.; Lv, L.; Wu, Y.; Li, N.; Huang, S.; Feng, Z.; Ren, Y. and Wang, J. 2019. Effects of maternal supplementation with rare earth elements during late gestation and lactation on performances, health, and fecal microbiota of the sows and their offspring. *Animals* 9:738. <https://doi.org/10.3390/ani9100738>