Ann. Anim. Sci., Vol. 25, No. 1 (2025) 271–279 *DOI: 10.2478/aoas-2024-0068*

sciendo

STUDY OF THE POSSIBILITY OF MODULATING THE COMPOSITION OF THE GASTROINTESTINAL MICROBIOME OF RABBITS FED FERMENTED RAPESEED MEAL*

Łukasz Wlazło[1], Dorota Kowalska[2], Paweł Bielański[2], Mateusz Ossowski[1♦], Anna Czech[3], Marcin Łukaszewicz[4], Bożena Nowakowicz-Dębek[1]

[1]Department of Animal Hygiene and Environmental Hazards, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
[2]Department of Small Livestock Breeding, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
[3]Department of Biochemistry and Toxicology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
[4]Department of Biotransformation, University of Wrocław, Plac Uniwersytecki 1, 50-137 Wrocław, Poland
♦Corresponding author: mateusz.ossowski@up.lublin.pl

Abstract

Understanding digestive functions and the role of microorganisms in the prevention of gastrointestinal and systemic diseases may be a strategy for preventing intestinal dysbiosis during critical periods of animal rearing, strengthening the immune system and reducing herd mortality. The aim of the study was to determine the effect of the addition of fermented rapeseed meal (FRSM) to the diet of rabbits on the composition of the bacterial microbiota of the caecal contents. The experiment was conducted using 40 35-day-old rabbits (*Oryctolagus cuniculus*) assigned to four groups of 10 animals each. Animals in the control group (group C) were fed a standard diet, while the experimental groups received 4% (group E1), 8% (group E2) or 12% (group E3) dried FRSM in place of the previously used soybean meal (SBM). After 120 days, six rabbits (three males and three females), of average size and intended for slaughter, were selected from each group. The contents of the caecum were collected from these animals for metagenomic analysis. The research demonstrated that the microbiome of the caecum of rabbits shows low diversity at higher phylogenetic levels, but is highly diverse at lower levels. The study showed no directly proportional relationship between the various groups of microorganisms inhabiting the digestive tract and the share of fermented rapeseed meal used in the diet. To the best of our knowledge, this is the first study to characterize the microbiome of rabbits fed diets with the inclusion of fermented feed components.

Key words: fermented rapeseed meal, rabbits, metagenomic analyses

The community of intestinal bacteria in rabbits consists of 100–1000 billion microorganisms per gram of caecum, belonging to 1000 different species (Combes et al., 2011; Montes-Caretto et al., 2021). In both humans and animals the intestinal microbiota is considered to function as a 'microbial organ', which is essential to preserving health (Cotozzolo et al., 2020; Binek, 2012). The population colonizing the intestines of mammals is the largest of all microbial communities. It is involved in the digestion of nutrients, protects against external pathogens, supports metabolism and immunity, and also takes part in neurobiological processes (Collins and Bercik, 2009; Xu et al., 2007; Guarner and Malagelada, 2003).

Fermented rapeseed meal (FRSM) is currently one of the most commonly analysed alternative feed components. It is regarded as equivalent to imported soybean meal (SBM), which is usually obtained from genetically modified organisms (GMOs) (Grela et al., 2019; Sobotka et al., 2012). The use of feedstuffs derived from GMOs raises concerns among the public, which is increasingly focused on the composition and origin of food products (Niwińska et al., 2019). Various replacements for SBM are used in the diet of rabbits, including FRSM, which has higher nutritional value than non-fermented rapeseed meal (Tian et al., 2017; Wlazło et al., 2021). Moreover, by providing bioactive substances, FRSM has proven to be a valuable ingredient in the diet of rabbits, supporting the immune system and helping to maintain a healthy intestinal microbiome (Wlazło et al., 2021). An argument in favour of the use of rapeseed products is the fact that rapeseed is the most commonly grown oilseed plant in Poland, and fermented products are enriched with bioactive substances (Sońta and Sońta, 2021). The fermentation process increases the population of lactic

*Work financed as part of the core research of the Department of Animal Hygiene and Environmental Risks of the University of Life Sciences in Lublin and the research project 'Production of a multifunctional feed component (material) (FSB) with unique synbiotic properties, which is a response to market needs reported by feed producers', financed by the European Regional Development Fund under the Smart Growth Operational Program – 2014–2020.

acid bacteria (LAB), mainly of the genus *Lactobacillus*, leading to an increase in the amount of lactic acid (Nowakowicz-Dębek et al., 2021; Niba et al., 2009). Intake of fermented feedstuffs has also been observed to cause the death of pathogenic bacteria of the family *Enterobacteriaceae* (Wang et al., 2018). In herbivores, such as rabbits, intestinal organisms take part in the breakdown of feed components which cannot be digested by the host. The dominant group is the phylum Firmicutes (Cotozzolo et al., 2020; Velasco-Galilea et al., 2018), believed to play a major role in degradation of cellulose. In studies on the composition of the microbiome, traditional microbiological culture methods are inadequate, due to the limited growth of these microbes *in vitro* on artificial media. Studies on the rabbit genome are thus an essential element in designing biomedical research and learning the role of the intestinal microbiota and mechanisms associated with diseases in rabbits. Whole genome analyses constituting reference data, deposited in the National Center for Biotechnology Information (NCBI) genome database, have been an important source of new knowledge about the rabbit genome. Recent research in this area added two high-quality rabbit reference genomes (Liu et al., 2021). The few studies conducted on the composition of the intestinal microbiota of rabbits have been based on amplicon analysis of the intestinal microbial community. The development of metagenomic analyses using modern sequencing technology seems to be particularly important in view of anticipated changes in preventive and therapeutic procedures in animals. This type of analysis makes it possible to thoroughly assess the effect of specific factors on populations of intestinal microbes and to modulate the microbiome composition, in order to improve rearing quality and preserve animal health. Therefore, both biomedical research and breeding practice require knowledge of the genome of the rabbit intestinal microbiota.

The aim of the study was to determine the effect of the addition of FRSM to the diet of rabbits on the composition of the bacterial microbiota of the caecum contents.

Material and methods

Animals and diet

The experiment was conducted using 40 35-day-old rabbits (*Oryctolagus cuniculus*), which were crosses of two typical meat breeds: New Zealand White and Popielno White. The experimental period was from 35 days after birth until slaughter at the age of 120 days. All tests on animals were conducted with the approval of the Local Ethics Committee for experiments on animals (approval no. 65/2020). The animals were divided into four groups of 10 individuals each, with similar body weight and equal shares of the sexes (5 females and 5 males). The rabbits were kept in cages at two per cage to create five replicates of the sample in each group. The experimental design was as follows: the animals in the control group (group C) were fed a standard diet, and the experimental groups received 4% (group E1), 8% (group E2) or 12% (group E3) dried FRSM, in place of SBM. The chemical composition of the diet in all groups was appropriately balanced, and the content of nutrients was in accordance with recommendations for nutrition of fur-bearing animals (2011), as presented by Wlazło et al. (2021). The animals were fed *ad libitum* and had free access to drinking water. After 120 days, six rabbits (3 male and 3 female) with average body weight for the group were selected from each group for slaughter. The rabbits were slaughtered in accordance with Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. An incision was made in the abdominal wall, after which the entire gastrointestinal tract (GIT) was immediately removed from each carcass. The GIT was transported under refrigeration (4°C) to a laboratory, and the caecum contents of each slaughtered animal were collected. GIT samples were collected in sterile conditions using an MSC Advantage class II biosafety cabinet with HEPA filters (Thermo Scientific, Waltham, MA, USA). From each part of the caecum three samples of digesta (about 10 g) were collected and pooled and then transported to a laboratory for metagenomic analysis.

Metagenomic analysis

The bacterial populations were analysed using 16S ribosomal RNA (rRNA), which is part of the small 30S ribosomal subunit in prokaryotic cells, containing nine hypervariable regions (V1–V9) of about 30–100 bp, involved in the secondary structure of the small ribosomal subunit. Quality control (QC) tests were performed throughout the analytical process, from DNA sampling to obtaining the final results. The DNA concentration of the samples was measured prior to library preparation by the fluorimetric method using PicoGreen reagent (Life Technologies). The measurement was performed in a Tecan Infinite plate reader. To amplify the region and prepare the library, specific sequences of primers 341F and 785R were used. PCR was carried out using Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs), with reaction conditions as recommended by the manufacturer. Sequencing was performed in the MiSeq sequencer, in paired-end (PE) technology, 2×300 nt, using the MiSeq Reagent Kit v3 (600-cycle) (Illumina) in accordance with the protocol. Bioinformatic analysis enabling classification of reads to species level was carried out using QIIME software and the SILVA v138 database of reference sequences. Classifications with numbers amounting to less than 5% were assigned to the category 'OTU less than 5%'. Bioinformatic analysis consisted of the following stages: removal of adaptor sequences (Cutadapt software), analysis of read quality and removal of low-quality sequences (quality < 20, minimum length 30; Cutadapt software), joining of paired sequences (fastq-join algorithm), removal of chimeras (usearch 61 algorithm), clustering based on the database of reference

sequences (uclust algorithm), assignment of taxonomy to the selected reference sequence database (uclust algorithm), and generation of a taxonomic tree (fasttree algorithm). Alpha diversity was assessed based on the observed species, Shannon, Simpson, InvSimpson, and Chao1 indices. Beta-diversity was determined based on the Bray-Curtis or Morisita-Horn method with clustering by the UPGMA method. The analysis was performed in the Genomed Inc. laboratory (Warsaw, Poland).

Results

The analyses of the taxonomic classification of microorganisms in the material are presented in Figure 1. Bacteria made up the largest share of the microbiota in all groups of animals, from 72.48% to 77.08% of the total numbers. The percentage of unclassified microorganisms ranged from 22.66% to 27.27% of the total intestinal microbiota.

Taxonomic identification at the phylum level indicated that the dominant phylum was Firmicutes. Their share in the control group (C) and the experimental group receiving a diet with 12% FRSM (E3) was more than 67% of all identified microorganisms. In groups E1 and E2, the value for this phylum was 62% (Figure 2). Actinobacteriota was the second most common bacterial phylum in the samples of caecum contents. The numbers of Actinobacteriota were similar in all groups; only in group E2, receiving a diet with 8% FRSM, did their percentage increase to 11.91% of the total. In groups E2 and E3, a decrease was noted in the number of microbes of the phylum Verrucomicrobiota. Microorganisms of the phylum Bacteroidota, consisting of three large classes of gram-negative bacteria, were present in similar proportions in the control group (C) and experimental groups E1 and E2. In experimental group E3 there was a decrease in the population of Bacteroidota to 0.19%. Microorganisms forming the superphylum Patescibacteria, which included three phyla belonging to the candidate phyla radiation (CPR) group, were isolated in similar proportions in all experimental groups. Their share was smaller in the control group. Archaeans belonging to the phylum Euryarchaeota were present in varying concentrations in the experimental groups. Their share was lower in the control group (C) than in groups E1 and E2, but their presence was not noted in experimental group E3 (Figure 2).

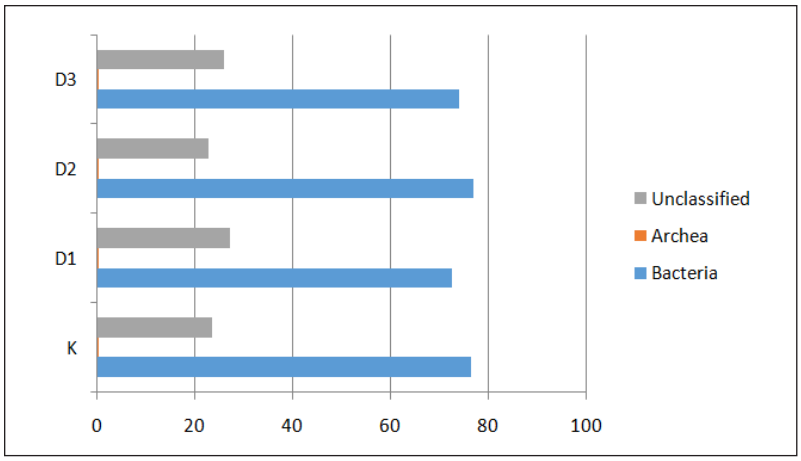


Figure 1. Taxonomic classification of microorganisms according to kingdom (%)

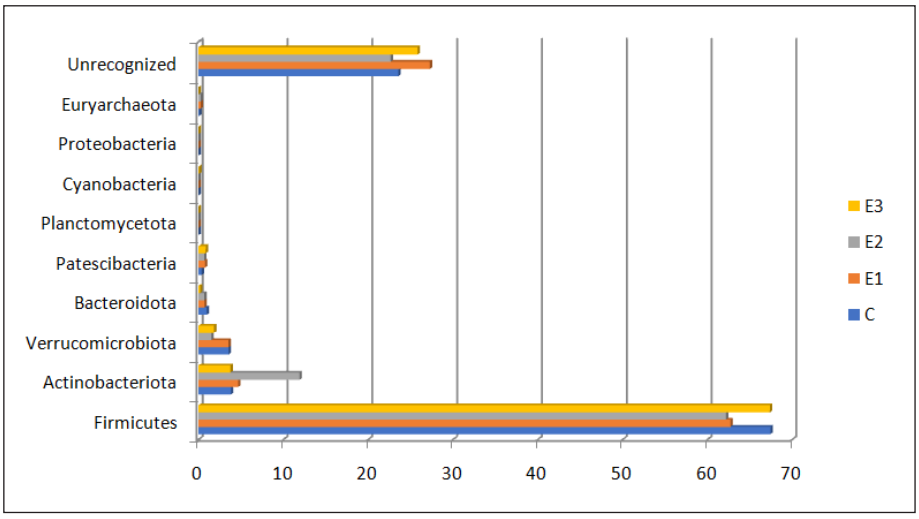


Figure 2. Taxonomic classification of microorganisms according to phylum (%)

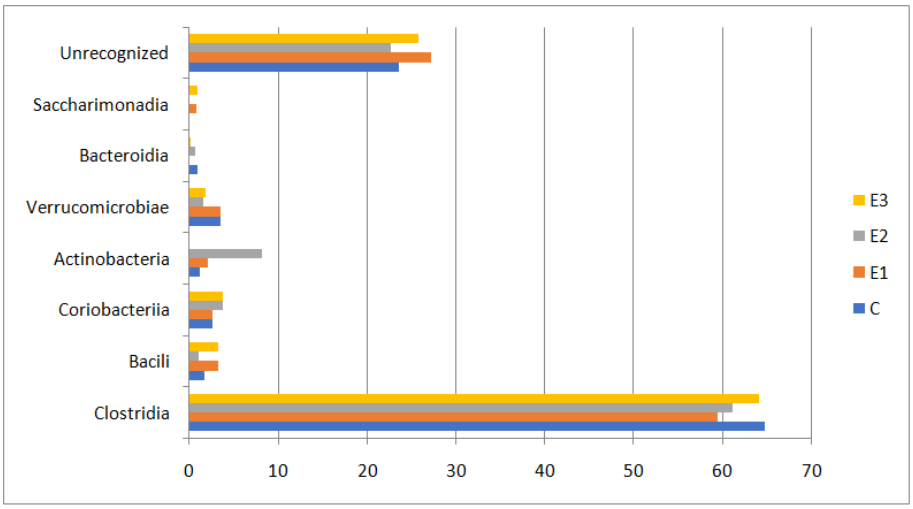


Figure 3. Taxonomic classification of microorganisms according to class (%)

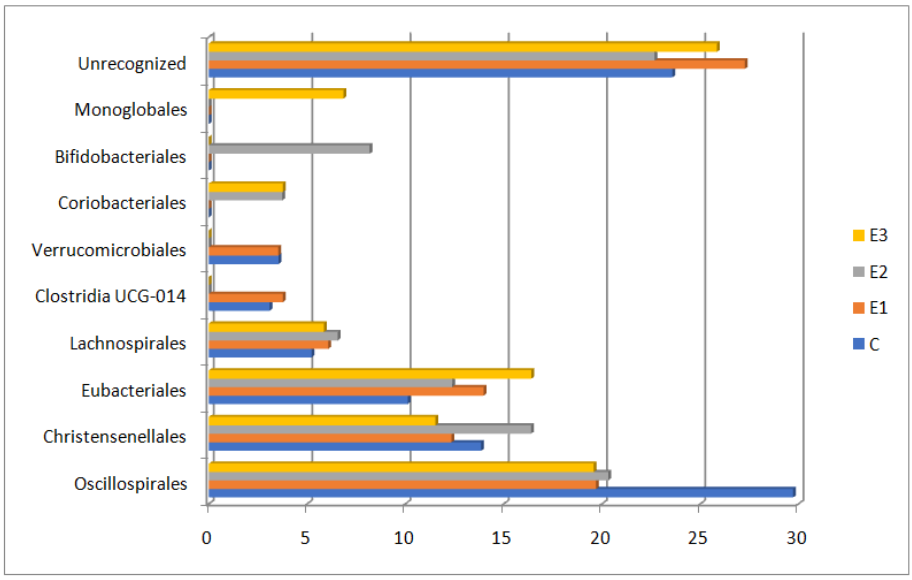


Figure 4. Taxonomic classification of microorganisms according to order (%)

The dominant class of microorganisms in all experimental groups was *Clostridia*, whose proportion ranged from 59.47% in group E1 to 64.71% in the control group C. Bacteria belonging to the classes *Bacilli* and *Coriobacteriia* were the second largest group of microorganisms identified in the material. The classes *Verrucomicrobiae* and *Bacteroidia* were identified as well; their proportions in the total numbers of microorganisms did not exceed 5% and were similar among groups. In group E2 there was an increased proportion of *Actinobacteria* amounting to 8.18% (Figure 3).

At the level of order, *Oscillospirales* bacteria belonging to the class *Clostridia* were dominant in all groups. Their level was highest (29.7%) in the control group (C). In the experimental groups, the share of these microorganisms did not exceed 21%. Microorganisms of the family *Christensenellaceae* and the order *Eubacteriales* had the second and third highest numbers in the material. Their share in the pool of microbes was slightly varied among the groups of animals. The share of microorganisms of the family *Christensenellaceae* ranged from 10.12% (control group C) to 16.41% (group E2) of the total pool of isolates. The family *Lachnospiraceae* belonging to *Eubacteriales* was present in each group of animals, accounting for 5.22% to 6.56% of bacteria. The order *Verrucomicrobiales* was detected only in the control group and experimental group E1, while *Coriobacteriales* bacteria, belonging to the phylum *Actinomycetota*, were noted only in the experimental groups with the highest proportions of FRSM in the diet (groups E2 and E3). The family of bacteria of the order *Bifidobacteriales* was identified only in the material from experimental group E2, amounting to 8.18% of isolates, while the order *Monoglobales* was identified only in the material from group E3, with a share of 6.85%. Microorganisms not identified to order accounted for 22.6% (group E2) to 27.2% (group E1) of all isolates (Figure 4).

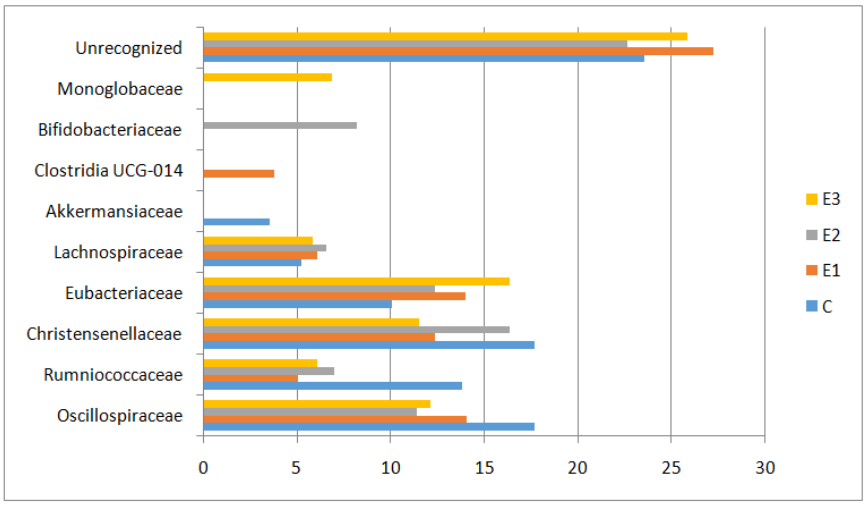


Figure 5. Taxonomic classification of microorganisms according to family (%)

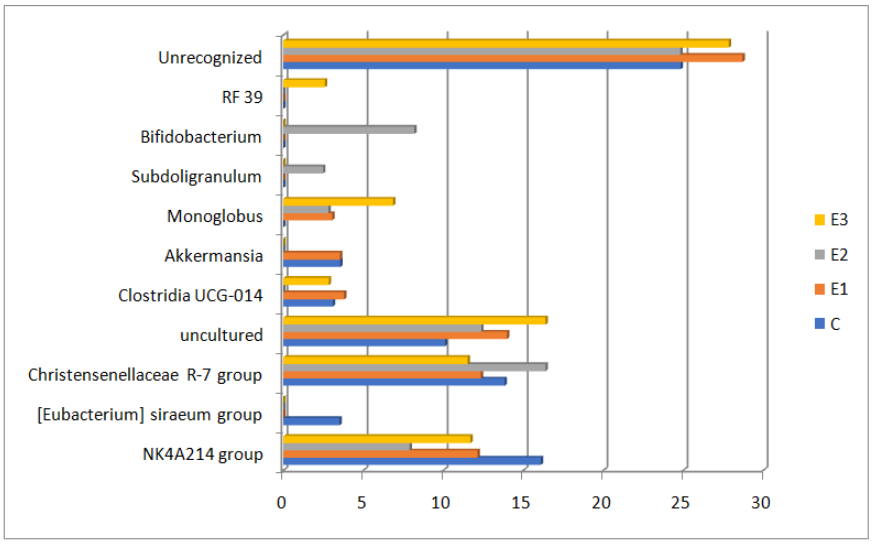


Figure 6. Classification of microorganisms according to taxonomic genus (%)

Microorganisms of the families *Oscillospiraceae*, belonging to *Clostridia*, and *Christensenellaceae* made up the largest share of isolates identified to the level of family. Bacteria belonging to the families *Ruminococcaceae* and *Christensenellaceae* were dominant in the microbiota of the control group and were the two most numerous groups of microorganisms in all groups of animals after *Oscillospiraceae*. As the share of FRSM in the diet increased, there was an increase in the numbers of gram-positive bacteria of the family *Eubacteriaceae* in the order *Clostridiales*. Bacteria of the family *Lachnospiraceae* were present in all groups of animals in similar proportions. The occurrence of microorganisms of the family *Akkermansiaceae* was noted only in the material collected from the control group, and *Clostridia UCG-014* only in the material from group E1. The family *Bifidobacteriaceae* was identified only in samples from animals in group E2, while *Monoglobaceae* was represented only in group E3. From 22.6% (E2) to 27.2% (E1) of the microorganisms in the material were unidentified (Figure 5).

The genus represented in the highest numbers was *Ruminococcaceae* NK4A214 group, whose share was highest in the control group – 16.09%. The percentage shares of genera belonging to the *Christensenellaceae* (*Christensenellaceae* group R7) and of unculturable bacteria showed slight variation and were similar in all groups. In the material from the control group, *Eubacterium siraeum* group accounted for 3.51% of identified microorganisms. Probiotic bacteria *Clostridia-UCG-14* associated with tryptophan metabolism were identified only in the control group and in experimental group E1, at similar levels. The same was noted for the probiotic bacterium of the genus *Akkermansia.* Microorganisms of the genus *Monoglobus* were noted in all groups receiving FRSM (E1, E2 and E3), but not in the control group. *Sub-*

doligranulum and *Bifidobacterium* were noted only in the caecum contents of animals in group E2, and *Enterococcaceae* bacterium RF39 only in the material from group E3. Unidentified microorganisms made up from 24.8% of isolates from the control group to 28.7% from group E1 (Figure 6).

In the vast majority of cases, metagenomic analysis did not allow for species identification of microorganisms. The proportion of unidentified species in the pool of DNA sequences was lowest in group C (98.39%) and highest in group E3 (99.8%). *Ruminococcus albus*, *Clostridium* sp., *Bifidobacterium magnum* and *Methanobrevibacter smithii* were also identified in the material from all groups. *Desulfovibrio* species were found only in the caecum contents of animals in the control group. For the other species identified, no relationship with the use of FRSM was shown (Figure 7).

The number of bacterial species observed was highest in the control group C – 1190 species. A slightly smaller number of species was noted in experimental group E3, amounting to 1131. Experimental groups E1 and E2 had similar numbers of identified bacterial species (1098 and 1070, respectively), but fewer than in group E3.

Indicators of biodiversity, i.e. the Shannon, Simpson, Chao1 and InvSimpson indices, were also determined in the study. Beta-diversity was presented as the results of PCoA. The higher the Shannon index, the higher the biological diversity in the sample. The values obtained for this parameter indicate similar bacterial species composition in all groups and moderate genetic diversity. This means that although the diet contained diverse species of bacteria, this diversity was not very high. The value close to 1 for the Simpson index also means that nearly the entire diversity in the sample consisted of a small number of dominant species, while the remaining species were poorly represented. This was confirmed in all experimental groups and the control group. The values for the Simpson index indicate that the species were evenly distributed and that one or a few species were dominant over the rest of the population. Estimation of the total species diversity in the samples from each group using the Chao1 index indicates that the diversity of species composition was highest in the control group C. The experimental groups had similar, slightly lower values for this index. The inverse Simpson index (InvSimpson), which measures the dominance of species in a sample, was higher in the experimental groups (E1, E2 and E3) than in the control group C. An increase in the percentage share of FRSM in the feed of the experimental groups was shown to be associated with an increase in this parameter (Table 1).

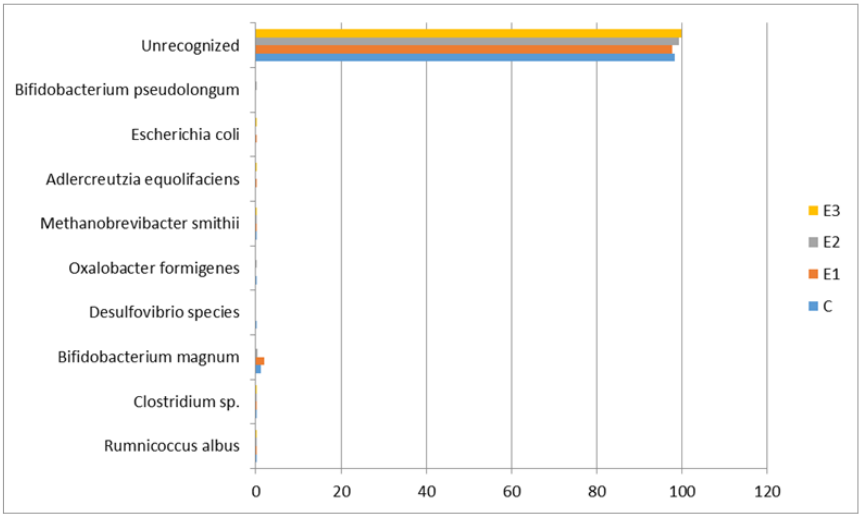


Figure 7. Taxonomic classification of microorganisms according to species (%)

Table 1. Alpha diversity in groups

Sample	Observed species	Shannon	Simpson	Chao1	InvSimpson
E1	1098	5.13	0.98	1137.46	52.98
E2	1070	5.12	0.98	1129.21	60.20
E3	1131	5.28	0.99	1183.73	67.86
C	1190	5.22	0.98	1249.02	50.88

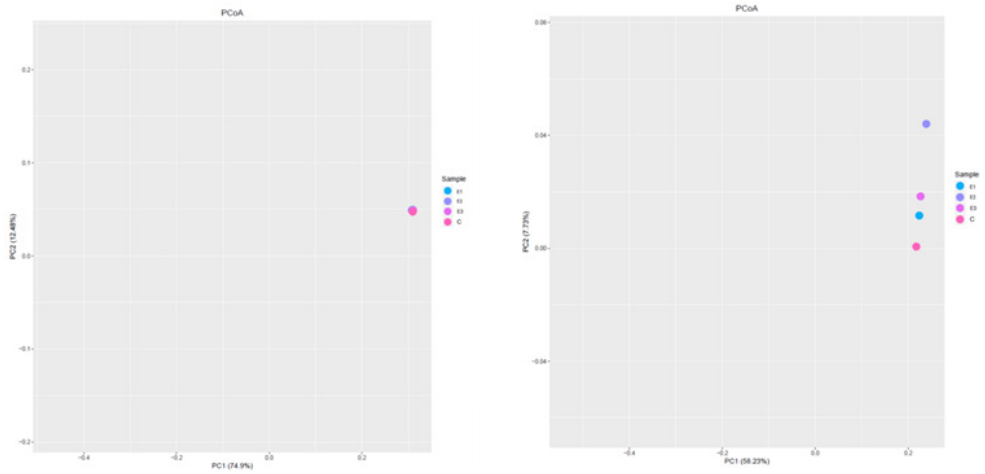


Figure 8. PcoA for the analysed research groups. β-diversity – based on the Bray-Curtis and Morisita-Horn methods

PCoA, carried out on the basis of indicators of genetic similarity between bacteria and taking into account the relationships between samples by presenting the distances in a set of coordinates, showed very high similarity of all groups. The distances observed for the groups indicate that they all had a similar species or functional composition, as confirmed by the short distances between data sets, seen in Figure 8. Analysis of the Morisita-Horn index indicates that the set of microorganism in group E2 was more genetically diverse and differed in species composition from the other groups (Figure 8).

Discussion

The study showed low diversity of the intestinal microbiome at higher phylogenetic levels, but high diversity at lower levels. A similar pattern was reported by Xu et al. (2021), according to whom there are relatively few phyla and classes of microbes at higher phylogenetic levels. The analyses conducted in the present study indicate high stability of the microbial composition of rabbits. There was no directly proportional relationship between groups of microorganisms colonizing the GIT and the increasing share of FRSM in the feed. The phylum Firmicutes was predominant in all experimental groups, as confirmed by Hu et al. (2021), but the results differ significantly from those presented by Monteils et al. (2008), who identified more than 90% of isolates in rabbit intestinal samples as Firmicutes. In the present study, the next largest phylum after Firmicutes (62.2–67.5%) was Actinobacteriota, whose proportion ranged from 3.77% in the control group to 11.91% of isolated DNA sequences in group E2. This is a much larger share than that reported by Combes et al. (2017) and Velasco-Galilea et al. (2018). Earlier studies often focused on determining the composition of the microbial community in faeces, and this may affect the size of groups of microorganisms. *Bacillus subtilis* strain Y87, which was used to produce FRSM, did not significantly affect the numbers of microorganisms. Within the phylum Firmicutes, *Clostridia* was the largest class, followed by *Bacilli*, accounting for from 0.9% to 3.3% of DNA isolates. No relationship was observed between the share of FRSM in the rabbits' diet and the number of *Bacilli* identified. Velasco-Galilea et al. (2018) and Zeng et al. (2015) reported a significant percentage of Bacteroidota in the microbiota of the caecum and faeces of rabbits. In the present study, the percentage of this phylum did not exceed 1% of isolates, while Actinobacteriota and Verrucomicrobiota were present in higher numbers. The share of Verrucomicrobiota bacteria decreased as the inclusion of FRSM in the diet increased. This difference may be due to feeding conditions, the breed of rabbits, the composition of the diet, or feed components. The discrepancies may also result from the research methods used, such as sequencing of the 16S rRNA gene region and taxonomic annotation. At the level of order, *Oscillospirales* was highly represented, with a share of ~19% in the experimental groups (E1, E2 and E3) and the highest share in group C (29.58% of isolates). These microorganisms, as typical intestinal microbiota, are the main producers of butyric acid. Butyrate production is an important marker that can be used to identify potential next-generation probiotic microbes (Wu et al., 2022). We found no information in the literature about the share of this group in the intestinal microbiota of rabbits, so it is difficult to compare values. The values obtained in the present study differed from those presented by Agradi et al. (2023) for *Christensenellaceae*. The proportion of this family in the population of microbes ranged from 11.53% in group E3 to 16.39% in group E2, while Agradi et al. (2023) reported that the share of this group in the intestinal contents did not exceed 3.5%. The authors included bovine colostrum as a nutraceutical in the diet of rabbits to prevent intestinal dysbiosis, strengthen the immune system, and reduce mortality in the wean-

ing period. The role of this family in the microbiological composition of the gastrointestinal contents of animals is little known, and reports of the occurrence of *Christensenellaceae* are limited to its numbers in the total pool of microorganisms. However, studies on the role of the human microbiome and its effect on health indicate that the abundance of *Christensenellaceae* in the human intestines is inversely proportional to body mass index (BMI). These results suggest a strong and repeatable association between BMI and the ecology of microbes in the human intestines and metabolic diseases. The population size of this family in the human intestines is also inversely correlated with BMI and the occurrence of inflammatory bowel disease. Waters and Ley (2019) showed that the proportion of *Christensenellaceae* in the pool of intestinal microorganisms is highly heritable, although the specific human genes underlying this heritability are as yet unknown. The discrepancies observed may therefore result from both environmental factors, including diet, and the breed of rabbits used in the experiment. The families *Oscillospiraceae* and *Ruminococcaceae*, responsible for the degradation of cellulose from plant fibres, which can be considered analogous to digestion of dietary fibre in ruminants, were highly represented in the pool of microorganisms in all groups of animals. According to Zeng et al. (2015), these microorganisms are represented in large numbers in the microbiota of the soft faeces of rabbits, which undergo caecotrophy. These groups of microbes are highly specialized in degradation of insoluble fibre and utilization of polysaccharides. Their share in the pool of isolates was highest in the control group C, but did not differ from the values presented by Hu et al. (2021). At the genus level, there was high variation and low repeatability in the detection of DNA sequences in individual groups. The most highly represented was the NK4A214 group (Firmicutes). Their share ranged from 7.89% in group E2 to 16.09% in the control group, which was much higher than the value obtained by Hu et al. (2021), who reported that the NK4A214 group was present in the gastrointestinal contents of rabbits with an average share of 4.1%. Similar values to those obtained in the present study for this genus were reported by Jin et al. (2018), who found a 7.27% share of the NK4A214 group in the small intestine. These bacteria are capable of degrading plant polysaccharides in order to produce volatile fatty acids (VFA), which play an important role in maintaining intestinal health by supplying energy for the colonic epithelium, while at the same time reducing the occurrence of inflammation and functioning as a modulator of immune functions (Jin et al. 2018). Thus the increase in the share of this group of microorganisms observed in the present study should be considered to be beneficial and to promote microbial transformations in the digesta. Identification of microbes to species level was low, most likely due to the relatively sparse information on rabbit metagenomes in the taxonomic reference database. The share of unidentified taxa ranged from 97.7% in group E2 to 99.8% in group E3. The results indicate significant diversity of microbes and the need for further study. The most highly represented species was *Bifidobacterium magnum*, although it was not present in group E3. Due to the lack of information on the species composition of bacteria from the contents of the GIT of rabbits, the results are difficult to interpret. Representatives of this species isolated from rabbit faeces were previously included among LAB, but are currently a separate taxonomic unit. The first information is found in a work by Scardovi and Zani (1974) and concerned large acidophilic bifidobacteria isolated from rabbit faeces. The taxonomy of the species *Bifidobacterium magnum* and its occurrence in the GIT of rabbits remains little known and poorly indexed. The development of metagenomics has demonstrated the important role of the intestinal microbiome in the competitive exclusion of pathogens and in the development and maturation of the immunity of the intestinal mucosa (Arrazuria et al., 2016).

The number of species of the microbiota observed in the intestinal contents was highest in the control group (C). This indicates that the complexity of the species identified in the intestinal environment of the experimental groups decreased with the increasing inclusion of the feed ingredient tested in the study. This is also confirmed by the Chao1 estimator. Therefore it can be concluded that the addition of FRSM led to the dominance of individual species of the intestinal microbiota in the experimental groups. The share of fermented feed components leading to a restructuring of the intestinal microbiota, narrowing the pool of microbes, has been well described in studies on many animal species (Hui et al., 2021; Umu et al., 2020). The results obtained in the experimental groups and the control group indicate high similarity of species composition and functional composition. The phylogenetic distances between microbes also confirm the similarity of the species present. The differences are minor and demonstrate the high stability of the intestinal microbiota of the rabbits, as confirmed in previous research conducted for cultured groups of bacteria (Wlazło et al., 2021).

The composition and size of microbial communities is not only a valuable marker of pathological conditions, but also contributes to knowledge and understanding of the pathogenesis of chronic inflammatory diseases of the intestines (Walker et al., 2011). Diet is known to be one of the factors modelling the microbial community in all animals, including hind-gut fermenters such as rabbits. To our knowledge this is the first study to characterize the microbiome of rabbits fed a diet containing fermented ingredients. Learning the digestive functions of microorganisms and their role in the prevention of gastrointestinal and systemic diseases may be a strategy for preventing intestinal dysbiosis during sensitive periods in rearing, strengthening the immune system, and reducing mortality in the herd. Therefore there is a need for research on the ecology of intestinal microbes, aimed at finding links between the intestinal microbiota and host health. This will make it possible to

develop strategies for using diet components as therapeutic agents.

References

Agradi S., Sulce M., Menchetti L., Vigo D., Castrica M., Barbato O., Andoni E., Quattrone A., Munga A., Marongiu L.M., Curone G., Brecchia G. (2023). Dietary supplementation with n-3 polyunsaturated fatty acids: Effects on reproductive and productive performance and meat quality in rabbit breeding. Anim. Nutr., 14: 70–78.

Arrazuria R., Elguezabal N., Ramon A., Hooman J.D., Khafipour E. (2016). *Mycobacterium avium* subspecies *paratuberculosis* infection modifies gut microbiota under different dietary conditions in a rabbit model. Front. Microbiol., 7: 00446.

Binek M. (2012). Human microbiome – health and disease (in Polish). Post. Mikrobiol., 51: 27–36.

Collins S.M., Bercik P. (2009). The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology, 136: 2003–2014.

Combes S., Massip K., Martin O., Furbeyre H., Cauquil L., Pascal G., Bouchez O., Le Floc'h N., Zemb O., Oswald I.P., Gidenne T. (2017). Impact of feed restriction and housing hygiene conditions on specific and inflammatory immune response, the cecal bacterial community and the survival of young rabbits. Animal, 11: 854–863.

Cotozzolo E., Cremonesi P., Curone G., Menchetti L., Riva F., Biscarini F., Marongiu M.L., Castrica M., Castiglioni B., Miraglia D., Luridiana S., Brecchia G. (2020). Characterization of bacterial microbiota composition along the gastrointestinal tract in rabbits. Animals, 11: 31.

Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing (Text with EEA Relevance). Available online: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009R1099

Grela E.R., Czech A., Kiesz M., Wlazło Ł., Nowakowicz-Dębek B. (2019). A fermented rapeseed meal additive: Effects on production performance, nutrient digestibility, colostrum immunoglobulin content and microbial flora in sows. Anim. Nutr., 5: 373–379.

Guarner F., Malagelada J. (2003). Gut flora in health and disease. Lancet, 361: 512–519.

Hu X., Wang F., Yang S., Yuan X., Yang T., Zhou Y., Li Y. (2021). Rabbit microbiota across the whole body revealed by 16S rRNA gene amplicon sequencing. BMC Microbiol., 21: 312.

Hui Y., Tamez-Hidalgo P., Cieplak T., Satessa G.D., Kot W., Kjærulff S., Nielsen M.O., Nielsen D.S., Krych L. (2021). Supplementation of a lacto-fermented rapeseed-seaweed blend promotes gut microbial- and gut immune-modulation in weaner piglets. J. Anim. Sci. Biotechnol., 12: 1–14.

Jin D.X., Zou H.W., Liu S.Q., Wang L.Z., Xue B., Wu D., Tian G., Cai J., Yan T.H., Wang Z.S., Peng Q.H. (2018). The underlying microbial mechanism of epizootic rabbit enteropathy triggered by a low fiber diet. Sci. Rep., 8: 1–15.

Liu C., Wang S., Dong X., Zhao J., Ye X., Gong R., Ren Z. (2021). Exploring the genomic resources and analysing the genetic diversity and population structure of Chinese indigenous rabbit breeds by RAD-seq. BMC Genom., 22: 1–14.

Monteils V., Cauquil L., Combes S., Godon J.J., Gidenne T. (2008). Potential core species and satellite species in the bacterial community within the rabbit caecum. FEMS Microbiol. Ecol., 66: 620–629.

Montes-Carreto L.M., Aguirre-Noyola J.L., Solís-García I.A., Ortega J., Martinez-Romero E., Guerrero J.A. (2021). Diverse methanogens, bacteria and tannase genes in the feces of the endangered volcano rabbit (*Romerolagus diazi*). Peer J., 17: PMC8378336.

Niba A.T., Beal J.D., Kudi A.C., Brooks P.H. (2009). Potential of bacterial fermentation as a biosafe method of improving feeds for pigs and poultry. Afr. J. Biotechnol., 8: 1758–1767.

Niwińska B., Szymczyk B., Szczurek W. (2019). Prospects for domestic production of animal feeds and non-gm animal foods (in Polish). Wiad. Zoot., 4: 107–120.

Nowakowicz-Dębek B., Wlazło Ł., Czech A., Kowalska D., Bielański P., Ryszkowska-Siwko M., Łukaszewicz M., Florek M. (2021). Effects of fermented rapeseed meal on gastrointestinal morphometry and meat quality of rabbits (*Oryctolagus cuniculus*). Livest. Sci., 251: 104663.

Scardovi V., Zani G. (1974). *Bifidobacterium magnum* sp. nov., a large, acidophilic bifidobacterium isolated from rabbit feces. Int. J. Syst. Bacteriol., 24: 29–34.

Sobotka W., Pomianowski J., Wójcik A. (2012). Effect of genetically modified soybean and "00" rapeseed meals on pig fattening performance and technological and sensory properties of pig meat (in Polish). Żywn. Nauka Technol. Jakość, 1: 106–115.

Sońta M., Sońta M. (2021). Żywienie tuczników bez soi (in Polish). Hod. Trz. Chlew., 11–12.

Tian G., Wang L., Yu B., Chen H., Luo Y., Chen D., Liu S. (2017). Comparison of nutritional value between rapeseed meal and solid-state fermented rapeseed meal for growing rabbits. Chin. J. Anim. Nutr., 29: 798–805.

Umu Ö.C.O., Mydland L.T., Øverland M., Press C.M., Sørum H. (2020). Rapeseed-based diet modulates the imputed functions of gut microbiome in growing-finishing pigs. Sci. Rep., 10: 9372.

Velasco-Galilea M., Piles M., Vinas M., Rafel O., Gonzalez-Rodriguez O., Guivernau M., Sánchez J.P. (2018). Rabbit microbiota changes throughout the intestinal tract. Front. Microbiol., 9: 21–44.

Walker A.D., Sanderson J.D., Churcher C., Parkes G.C., Hudspith B.N., Rayment N., Brostoff J., Parkhill J., Dougan G., Petrovska L. (2011). High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol., 11: 3032643.

Wang C., Shi C., Zhang, Y., Song D., Lu Z., Wang Y. (2018). Microbiota in fermented feed and swine gut. Appl. Microbiol. Biotechnol., 102: 2941–2948.

Waters J.L., Ley R.E. (2019). The human gut bacteria *Christensenellaceae* are widespread, heritable, and associated with health. BMC Biol., 28: 6819567.

Wlazło Ł., Kowalska D., Bielanski P., Chmielowiec Korzeniowska A., Ossowski M., Łukaszewicz M., Czech A., Nowakowicz-Debek B. (2021). Effect of fermented rapeseed meal on the gastrointestinal microbiota and immune status of rabbit (*Oryctolagus cuniculus*). Animals, 11: 11030716.

Wu L., Zhou K., Yang Z., Li J., Chen G., Wu Q., Lv X., Hu W., Rao P., Ai L., Ni L. (2022). Monascuspiloin from *Monascus*-fermented red mold rice alleviates alcoholic liver injury and modulates intestinal microbiota. Foods, 11: 3048.

Xu J., Mahowald M.A., Ley R.E., Lozupone C.A., Hamady M., Martens E., Henrissat B., Coutinho P., Minx P., Latreille P., Cordum H., Van Brunt A., Kim K., Fulton R.S., Fulton L.A., Clifton S.W., Wilson R.K., Knight R.D., Gordon J.I. (2007). Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol., 5: e156.

Zeng B., Han S., Wang P., Wen B., Jian W., Guo W., Yu Z., Du D., Fu X., Kong F., Yang M., Si X., Zhao J., Li J. (2015). The bacterial communities associated with fecal types and body weight of rex rabbits. Sci. Rep., 5: 9342.

Received: 21 X 2023
Accepted: 29 V 2024