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Original Article - Veterinary Medicine

Fecal microbiota characterization in a swine model undergoing experimental bariatric surgery sand clock with gastro-jejunal bypass

[*Caracterização da microbiota fecal em modelo suíno submetido à cirurgia bariátrica experimental relógio de areia com bypass gastrojejunal*]

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ABSTRACT

Bariatric surgery induces a variety of changes, including changes in the composition of the gut microbiota. The investigation evaluated the effect of the experimental gastrojejunal bypass bariatric surgery hourglass (EBSSC G-YB) on the fecal bacterial microbiota in obese Landrace pigs. Twelve six-month-old animals were used, which were supplemented with energy from 2.5 months for a period of 120 days, enough time to reach the condition. Subsequently, they were intervened with the EBSSC G-YB and the changes in the microbiota were characterized, comparing an experimental group with two control groups in a period of 30 days. The result was that the microbiota of the three groups showed *Proteobacteria*, Firmicutes and Bacteroidetes as the ones with the highest relative abundance, however, at the end of the experiment a difference is highlighted, where the Phylum *Spirochaetes* was constant and more frequently in the experimental group, while for control groups 1 and 2 it was the Phylum *Tenericutes*. Although the differences were not significant, qualitatively there was greater diversity in the experimental group, with greater abundance of *Escherichia-Shigella* and *Pseudohongiella*, which may be useful as quantifiable biomarkers of weight loss in future research.

Keywords: bariatric surgery, gastrointestinal microbiome, metabolic surgery, microbiota, obesity

RESUMO

A cirurgia bariátrica induz uma variedade de alterações, incluindo alterações na composição da microbiota intestinal. A investigação avaliou o efeito da cirurgia bariátrica experimental relógio de areia com bypass gastrojejunal (EBSSC G-YB) na microbiota bacteriana fecal em porcos Landrace obesos. Foram utilizados 12 animais, com seis meses de idade, os quais foram suplementados com energia a partir de 2,5 meses, por um período de 120 dias, tempo suficiente para atingir a condição. Posteriormente, foi realizada intervenção com o EBSSC G-YB e avaliadas as alterações na microbiota, comparando-se um grupo experimental com dois grupos controle, em um período de 30 dias. O resultado foi que a microbiota dos três grupos apresentou Proteobacteria, Firmicutes e Bacteroidetes como as de maior abundância relativa, porém, ao final do experimento, destaca-se uma diferença, em que o filo Spirochaetes foi constante e com maior frequência no grupo experimental, enquanto para os grupos controle 1 e 2, foi o filo Tenericutes. Embora as diferenças não tenham sido significativas, qualitativamente houve maior diversidade no grupo experimental com maior abundância de Escherichia-Shigella *e* Pseudohongiella, *o que pode ser útil como biomarcadores quantificáveis de perda de peso em pesquisas futuras.*

Palavras-chave: cirurgia bariátrica, cirurgia metabólica, microbiota, microbioma gastrointestinal, obesidade

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INTRODUCTION

Obesity is the abnormal or excessive accumulation of fat that can have serious consequences for human and animal health and currently represents a growing problem (Rinonapoli *et al*., 2021). By 2016, WHO indicated that 1.9 billion adults over 18 years of age were overweight and by 2020 about 39 million children were overweight or obese (Rinonapoli *et al*., 2021); (Purdy and Shatzel, 2021).

Obesity is considered a multifactorial disease, with serious consequences such as increased bone mineral density, leading to an increased risk of fractures (derived from metabolic factors or a greater predisposition to falls) (Rinonapoli *et al*., 2021), systemic inflammation mediated by interleukins (such as IL-6 and IL-8) and tumor necrosis factor (TNF)-α, which contributes to leukocytosis, increased C-reactive protein and increased platelet count (Purdy and Shatzel, 2021). Additionally, it constitutes the main risk factor for several diseases such as type 2 diabetes mellitus, coronary heart disease and some types of cancer (Davoodi *et al*., 2013);(Powell-Wiley *et al*., 2021); (Fink *et al*., 2022).

Among the main causes of obesity is the energy imbalance between calories consumed and calories expended, derived from the excessive consumption of foods rich in fats and sugars, and an increase in physical inactivity. Since this disease is considered preventable, important efforts have been made worldwide in the generation of policies and programs aimed at healthy eating, physical activity and psychosocial support (Lum *et al*., 2021). Despite their implementation, these have not had sufficient impact and obesity has continued to increase in recent years, making the implementation of more effective treatments and improved prevention mechanisms a priority (Fink *et al*., 2022; Griffith, 2022).

Bariatric surgery helps in the reduction of body weight, so it is indicated for people with obesity and in some countries it is restricted to those for whom other weight loss measures have failed (Douglas *et al*., 2015). Different surgical procedures have been implemented, being sleeve gastrectomy and Roux-en-Y gastric bypass the most frequently applied because of the best results in terms of weight loss; however, dietary modifications are required in patients so that these surgeries have fewer long-term complications (Welbourn *et al*., 2019).

In addition to the significant reduction in body weight and fat mass, bariatric surgery produces changes in metabolic markers, such as plasma levels of adipose-derived mediators, low insulin levels, increased lipolysis, and decreased protein oxidation (Adami *et al*., 2019; Jabbour and Salman, 2021). Most studies have focused on evaluating the effects of bariatric surgery on body composition and metabolic changes; however, recent studies indicate the essential role of the gut microbiota in body weight loss or gain.

The intestinal bacterial microbiota plays important roles at the immunological and metabolic level of the host, including the maintenance of intestinal homeostasis, endocrine regulation, nutrient absorption, satiety regulation, among others (Ejtahed; Hasani-Ranjbar; Larijani, 2017). Studies suggest that, after bariatric surgery, significant changes in the composition and diversity of microbial communities at the intestinal level occur in the host, varying according to the type of procedure performed (Ciobârcă *et al*., 2020). However, the impact of surgical interventions on the modulation of nutrients and intestinal microbiota is a field still little explored.

This study aims to describe the bacterial communities in feces of an obese swine model, operated with a novel experimental bariatric surgery (EBSSC G-YB), which combines the compartmentalization of the stomach with the rapid arrival of nutrients to the last quarter of the small intestine.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and was approved and supervised by the Ethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry of the University of Córdoba - Colombia (Act No.009 of November 30, 2021) for animal studies.

The experimental phase included 12 castrated Landrace pigs, 6.5 months old, average weight 115 kg with signs of overweight or obesity, from two different litters of the same pig program. The animals were housed in individual pens of 2 m^2 each, with bars to allow contact between animals from adjacent pens. They were kept under controlled conditions with a temperature around 23°C (± 2°C), relative humidity between 45-65% and a 12 h/12 h light/dark cycle.

The animals entered the study with an average weight of 20 kg and 2.5 months of age, fed with a commercial finishing diet (PigLevante 1 orange Italcol®); which was enriched with 18% commercial vegetable oil from the company Megaceites S.A.S. and 15% molasses (Tab. 1), to achieve the condition of obesity, based on the information reported (Hernández Hurtado et al., 2016). They were fed twice a day (07:00 and 15:00) and the amount depended on body weight following the manufacturer's instructions. At the beginning of the study, they were clinically evaluated, and blood tests were performed with confirmation of a good state of health.

Table 1. Composition of commercial diet and energy supplement (commercial vegetable oil and molasses)

Type of diet	Composition	Quantity (%)
Commercial	Minimal protein	17
	Minimal grease	5
	Maximum humidity	13
	Maximum fiber	7
	Maximum ash	8
Energy supplement	Commercial vegetable oil[1]	18
	Molasses[2]	15

[1] The commercial oil provided 80 calories of fat with a per serving amount of total fat of 9g (14%).

[2] Molasses provided 2100kcal/kg, which for the amount added to the commercial fattening ration corresponds to 787.5kcal.

Pigs with four months of animal management were taken from 2.5 months of age and an experimental phase from 6.5 months of age with a duration of 30 days where weighing, measurements and blood sampling were made, at the beginning of the experimental phase (day 0), on day 15 and on day 30 post-surgical intervention.

Initially, 15 animals were selected, but the sample size was reduced to 12 pigs as this was the number of pigs approved by the ethics committee for the research. They were divided into three subgroups by purposive sampling, where the first control group (CG1) consisted of three animals without surgery and that continued with energy supplementation; a second control group (CG2) consisted of four animals with celiotomy and energy supplementation was withdrawn, and the experimental group (EG) consisted of the five animals with the highest weight that underwent experimental surgery without energy supplementation (Fig. 1).

Yao *et al*. (2019) recommend 10g of fecal matter as a representative amount of the sample, however; approximately 20 to 30g of feces/take were collected per animal at three different times during the experiment, according to the technical guidelines of the company INNOVASEG of Bogotá-Colombia, which oversaw processing the samples. The samples were kept at -90ºC in a refrigerator at the Instituto de Investigaciones Biológicas del Trópico (IIBT) until the samples were filled. The 24 samples collected were then sent refrigerated at 4°C to the laboratory for processing, where DNA was extracted for the description of bacterial communities through complete sequencing of the rRNA-16S gene (Long reads amplicon-based sequencing) using Oxford Nanopore technology.

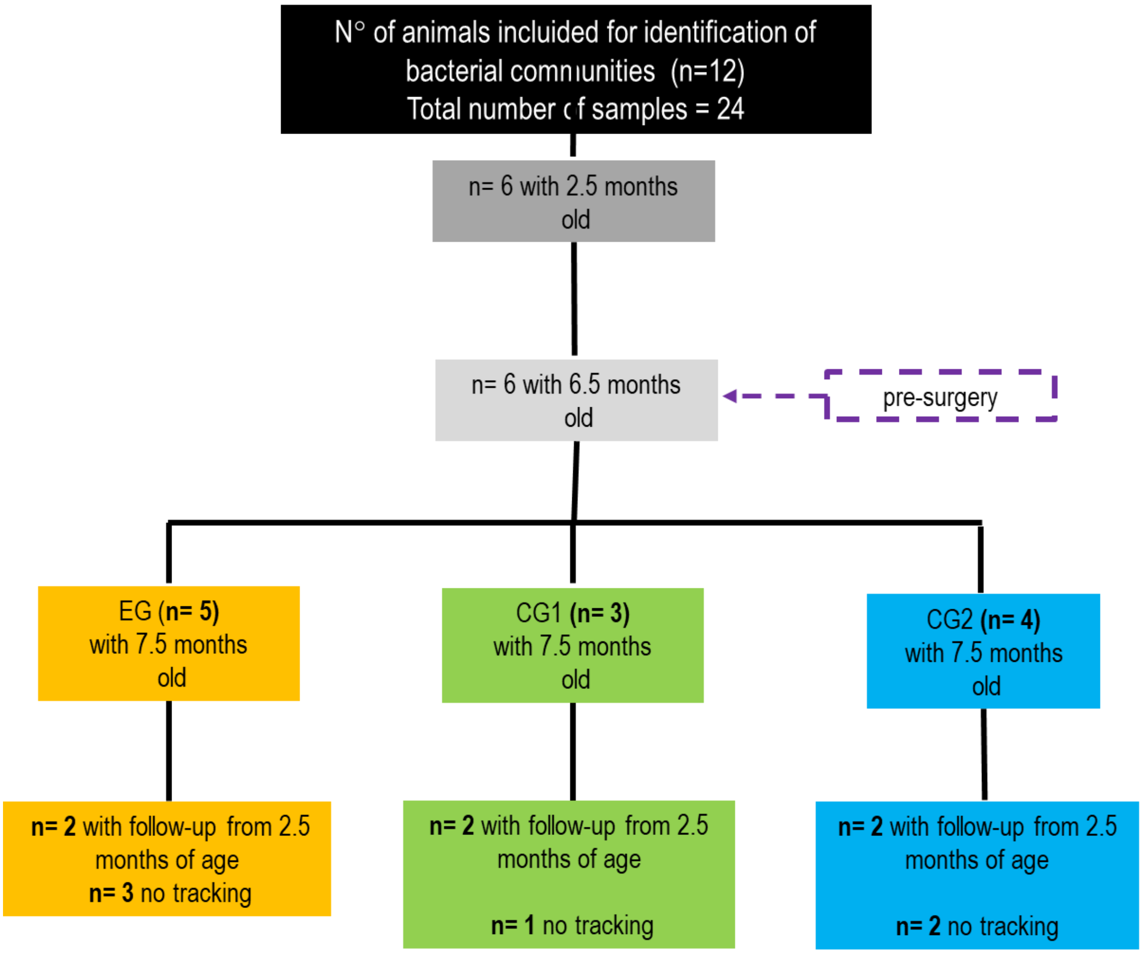

Figure. 1. Experimental design and distribution of Landrace pigs included for identification of bacterial communities according to age stage and feeding management. Where: CG1= control group one without surgery, CG2= control group two with celiotomy and EG= experimental group.

Prior to surgery, the animals were subjected to a 6-hour and 12-hour water and solid fasting periods, respectively. All surgeries were performed under general anesthesia, however, to reduce the dose used, an initial analgesia step was performed, applying intramuscularly flunixin meglumine (1.1mg/kg b.w.) and metoclopramide (0.5mg/kg b.w.). For tranquilization azaperone (4mg/kg bw) was applied and anesthesia was induced with intramuscular injection of xylazine (0.5mg/kg) and ketamine (2 mg/kg) mixture and maintained with isoflurane. Oro-tracheal intubation was performed, and oxygen was supplied throughout the procedure, and heart rate, respiratory rate, temperature, mucosal color, capillary filling time, eyeball position and pupil size were also monitored.

The experimental technique proposed is among those classified as restrictive-malabsorptive. It seeks to reduce the gastric capacity so that the pig has an early and prolonged satiety sensation with a much smaller amount of food than that usually ingested, and that the chyme reaches the last quarter of the small intestine (distal jejunum and ileum) faster. The experimental bariatric technique is not described in the text because it is part of another publication proposal. All surgeries were performed by the author of the experimental technique, who is a veterinary surgeon and junior researcher according to Colciencias categorization. The choice was made to reduce the variability of the surgical technique and maximize its success.

After surgery, pain control was managed with intramuscular injection of flunixin meglumine (1.1mg/kg b.w.), every 12 hours for 3 days. Antibiotic prophylaxis was performed by intramuscular injection of oxytetracycline (5mg/kg b.w.) every 24 hours for three days, complemented with a single intravenous dose of metronidazole (10 mg/kg b.w.) during the operation. It should be noted that the pigs in the two control groups also received this treatment. As for the daily feed ration, it was divided into three portions administered every four 4 hours.

For the extraction of DNA from the stool samples collected throughout the follow-up, an initial step was carried out with mechanical disruption by beating with beads, subsequently for DNA recovery the DNA Stool DNA Isolation kit® (Norgene, Biotek, Corp) was used, following the manufacturer's instructions. The DNA quality of the stool samples was determined with a NanoDrop® 1000 (Thermo Fischer Scientific), the samples included complied with a concentration of >20 ng/µl and an absorbance ratio of ≥1.8/2.0 at 260/280nm.

Amplicons of the 16S rRNA gene were generated using primers 27-F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492-R (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification of the 16S rRNA genes was performed with the KAPA2G™ Robust HotStart ReadyMix PCR kit (Kapa Biosystems). Amplification was performed with the following PCR conditions: initial denaturation at 94 °C for 3 minutes, 30 cycles of 94 °C for 30 seconds, 48 °C for 1 minute and 72 °C for 1 minute, followed by a final extension at 72 °C for 10 minutes. For Barcoding the SQK-NBD114.96 Kit was used and for ligation the SQK-LSK110 kit (Oxford Nanopore Technologies), the amplified DNA was purified with AMPure® XP (Beckman Coulter), the library was loaded onto the R9.4 flow cell (FLO-MIN106; Oxford Nanopore Technologies) and sequenced on the MinION™ Mk1C. Long-read sequencing was performed using the MINKNOW app. 1.11.5 (Oxford Nanopore Technologies).

For basecalling of raw Fast5 files and demultiplexing, Guppy was used to generate approved reads (FASTQ format), then the reads were filtered by removing possible chimeric reads. The average quality score of Phred was evaluated using the NanoPack tool. Finally, taxonomic assignment was performed using the Silva v132.16s database (Fujisaka *et al.*, 2023) and for taxonomic clustering the Kraken tool (Nagaya *et al*., 2021) was used. Alpha diversity analyses were performed for the ASVs of each of the groups. Thus, the Abundance-based Coverage Estimator (ACE) was calculated to obtain an estimate of richness, and the Shannon and Simpson index to obtain an estimate of community diversity in the groups of interest (Bhaskaran *et al*., 2014; Pacheco *et al.*, 2019). Statistical differences in richness and diversity estimates between groups were evaluated using a Mann-Whitney or Kruskall-Wallis test ($p<0.05$). For beta-diversity analyses, a principal coordinate analysis (PCoA) was performed with distances calculated by Bray-Curtis. Analysis of variance permutations using distance matrices (adonis) was used to evaluate significant differences in sample clustering depending on each study group. Alpha and Beta diversity analyses were performed using the R package phyloseq (Manson *et al.*, 1995).

Taking into account the non-normal distribution of the data (Shapiro-Wilks normality test), these were presented in terms of medians together with their corresponding measures of dispersion (interquartile ranges - IQR). For all tests the statistical significance (P value) was set at ≤0.05. STATA17 software® was employed for statistical analyses.

RESULTS

According to Akagbosu *et al.* (2022), bariatric surgery induces changes in digestive tract anatomy, gastric emptying, hormonal status, quantity and choice of ingested nutrients, and bile acid metabolism, which may modify the composition of the intestinal microbiota.

Sequencing of the *rRNA-16S* gene by means of the Oxford Nanopore platform (Leggett and Clark, 2017), generated more than 600,000 reads in the length range of 1.2 to 1.4 kb with a quality score ≥8, which were then taxonomically identified. A total of 36,626 ASVs (Amplicon Sequence Variant) belonging to the Bacteria domain were identified and assigned, being classified as 55 Phyla and 1088 bacterial genera.

The most abundant phyla throughout the follow-up were *Proteobacteria*, *Firmicutes* and *Bacteroidetes*; however, by the third follow-up an increase in the phylum *Tenericutes* was observed (Fig. 2A). At the genus level, differences in relative abundance were observed according to age, for 2.5 months (baseline) the genera *Escherichia-Shigella, Succinivibrio* and *Christensenellaceae R-7 group* were the most abundant, for the age of 6.5 months the genera *Escherichia-Shigella*, *Pseudohongiella* and *Alteromonas*, and finally for 7.5 months *Christensenellaceae R-7 group*, *Escherichia-Shigella* and *Streptococcus* (Fig. 2C).

Regarding the bacterial taxa according to the group analyzed, the results showed *Proteobacteria, Firmicutes and Bacteroidetes as* those with the highest relative abundance at the phylum level (Fig. 2B). However, at the end of the experiment, a higher frequency of the phylum *Proteobacteria* and *Tenericutes* was found in CG1 and CG2 and the phylum *Spirochaetes* in the SG (Fig. 2B).

On the other hand, the results showed differences in the abundance of some genera, according to the group analyzed, being for CG1 *Streptococcus, Escherichia-Shigella*, *Christensenellaceae* R-7 group and *Ruminococcaceae* UCG-014, for CG2 the genera *Succinivibrio*, *Escherichia-Shigella*, *Alteromonas* and *Pseudohongiella*, and for EG the genera *Escherichia-Shigella*, *Christensenellaceae* R-7 group, *Pseudohongiella*, *Lachnospiraceae* NK4A136 group and *Treponema* (Fig. 2D).

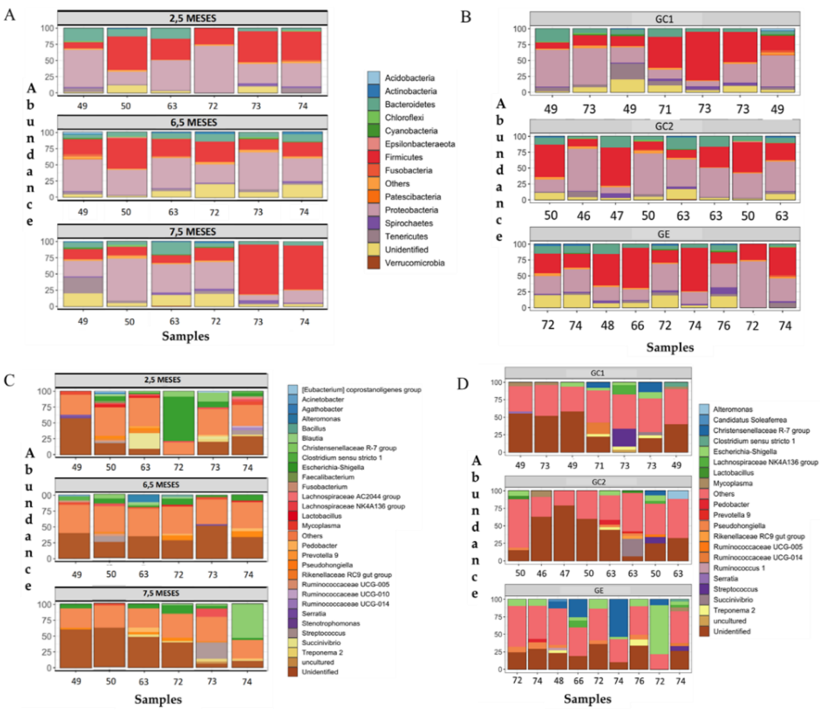

Figure 2. Bacterial composition in stool samples Landrace pigs. (A) Bar chart showing the main bacterial phyla according to follow-up (n=18). (B) Bar chart showing the main bacterial phyla according to the group analyzed (n=24). (C) Bar chart showing the main bacterial genera according to follow-up (n=18). (D) Bar chart showing the main bacterial genera according to the group analyzed (n=24).

To estimate the alpha diversity for the ASVs encountered the richness estimator (ACE) was calculated, and the Shannon Weaver and Simpson indices were calculated to estimate the diversity of the bacterial communities (Fig. 3). The diversity analysis revealed higher richness in samples from 7.5 months old animals compared to 2.5 and 6.5 month old animals (ACE, p=0.0832; Shannon, p=0.635; Simpson, p=0.3722) (Mann-Whitney, Kruskall-Wallis) (Fig. 3A). For the analyzed group, the results showed higher indices (diversity and richness) in

EG animals (ACE, p=: 0.8756; Shannon, p=0.6743; Simpson, p=0.7205 (Kruskall-Wallis), compared to the control groups (Fig. 3B). However, despite the differences these indices were not statistically significant, probably due to the number of samples analyzed. To estimate the variability of the bacterial communities, beta diversity was evaluated using principal coordinates analysis (PCoA) based on Bray-Curtis differences, where the results showed a separation in the groups analyzed (Fig. 3). PERMANOVA analysis using distance matrices (adonis) showed no statistically significant differences in the distribution of centroids according to age (r2= 0.10, p= 0.24) (Fig. 3A) and group analyzed (r2=0.18, p=0.21) (Fig. 3B). Although statistical significance was not found, it is possible to observe a grouping of the controls compared to the experimental group.

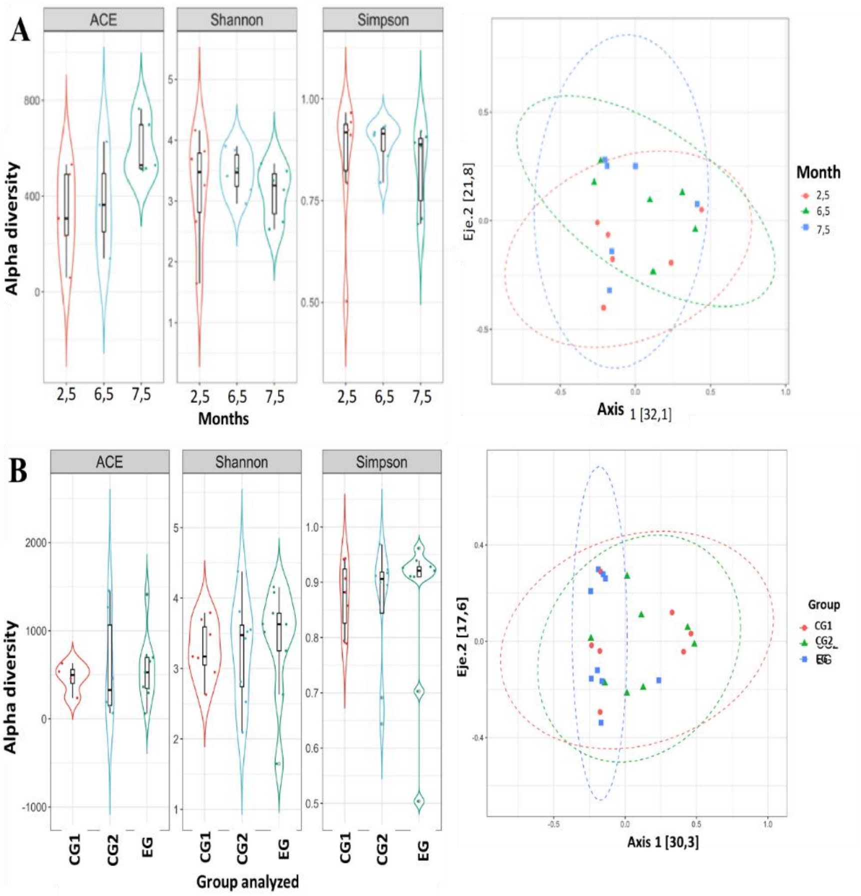

Figure 3. Analysis of alpha and beta diversity of bacterial communities in fecal matter of Landrace pigs according to age and study group. (A) diversity indices and NMDS plot with respect to follow-up (n=18). (B) diversity indices and NMDS plot with respect to the analyzed group (n=24).

On the other hand, when comparing the relative abundance of the phyla according to the group evaluated, the results showed that for the SG the phyla *Firmicutes* and *Spirochaetes* were the most abundant, for CG1 it was the phylum *Ternericutes*, while for CG2 it was the phylum *Bacteriodetes* and *Proteobacteria* (Fig. 4)*;* however, these differences were not significant.

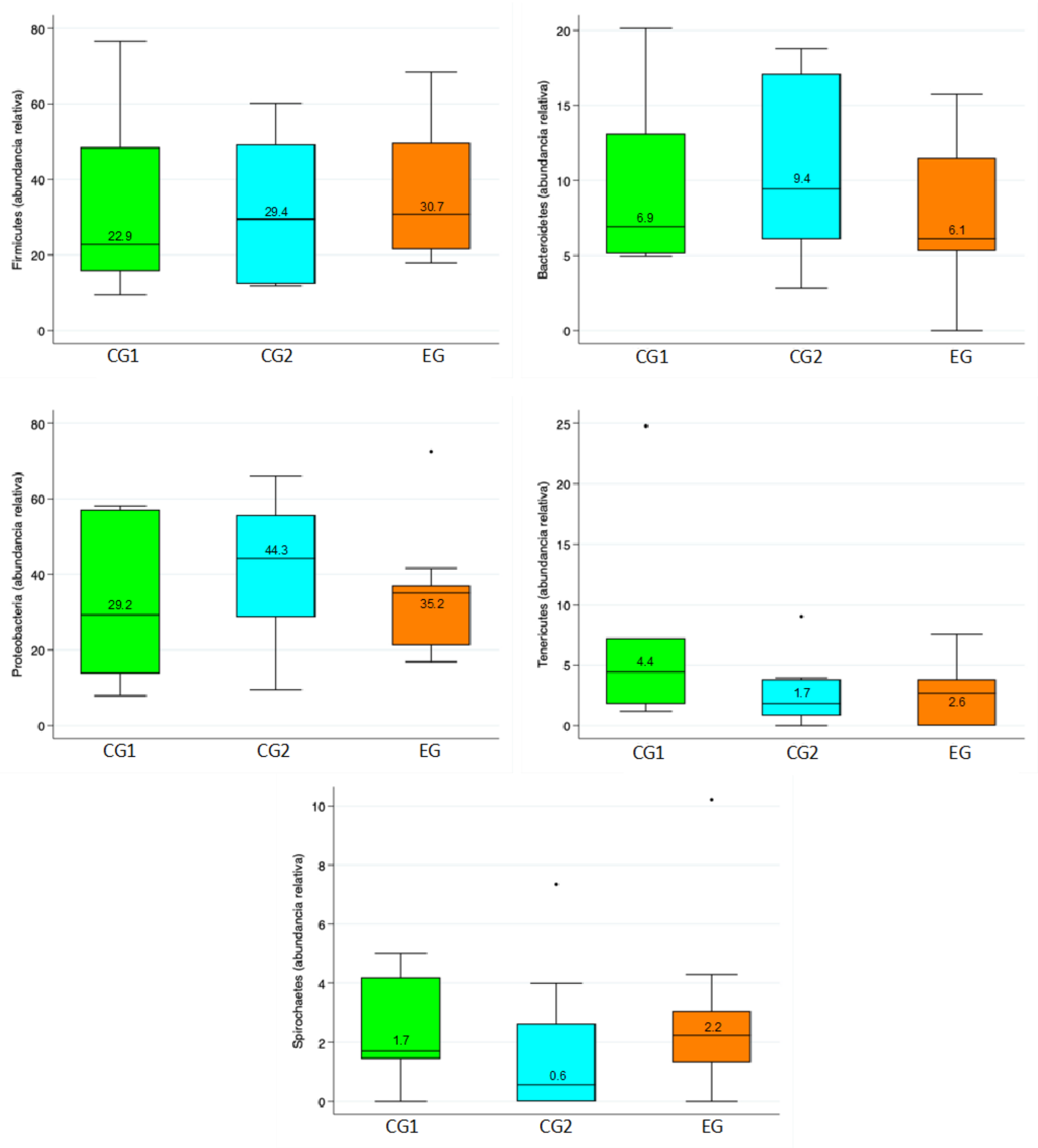

Figure 4. Differences between the relative abundance of the most abundant phyla according to the group Landrace pigs evaluated at the end of the experiment.

Finally, the differences in relative abundances between the most frequent bacterial genera in the analyzed population were compared, and it was found that *Escherichia-Shigella* and *Pseudohongiella* were significantly more abundant in the SG compared to the control groups (Fig. 5).

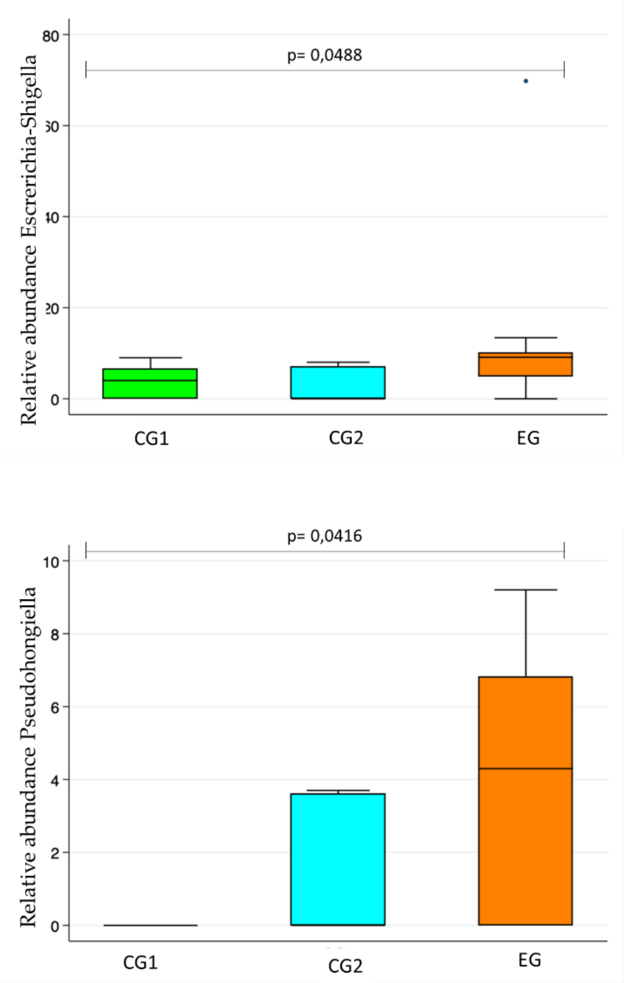

Figure 5. Differences between the relative abundance of *Escherichia-Shigella* and *Pseudohongiella* according to the group Landrace pigs evaluated.

DISCUSSION

The porcine model and its easy handling make it an excellent biological model that facilitates the understanding of organic functioning, which can be extrapolated to other animal species, including humans. In this sense, this model is widely used in the implementation of surgical techniques including bariatric techniques (Silva Ribeiro *et al*., 2020) and research aimed at the description of bacterial communities (microbiota) at the intestinal level and their impact on organ physiology and phenotype. At the microbiota level, studies suggest that particular bacterial taxa contribute to metabolic processes, which could affect energy regulation in obesity (Akagbosu *et al*., 2022). However, considering that this condition is of multifactorial etiology, factors additional to the microbiota such as environmental or geographical, behavioral, genetic, dietary, microbial and biological factors also contribute to its etiopathogenesis, and differences in results and conclusions apart from the above may also be influenced by study designs and sample size (Akagbosu *et al*., 2022).

According to Akagbosu *et al*. (2022), reduced bacterial biodiversity at the gut level in individuals consuming a Western diet (high in fats, sugars and animal proteins) was associated with an increased incidence of obesity, coronary vascular disease and metabolic syndrome, with the obese condition being a common parameter found in pigs from the two CGs.

With respect to the microbiota, when eubiosis is present, the pig's intestine will be in optimal condition for good functioning and will be considered healthy. The characteristics of microbial dysbiosis are not completely clear; however, it has been defined as an intestinal imbalance identified by a marked decrease in the amount of obligate anaerobic (beneficial) bacteria, such as members of the Phylum *Bacteroidetes* and *Proteobacteria*, and a relative increase in the abundance of facultative anaerobic bacteria (pathobionts), where some members of the Phylum *Firmicutes* are found (Fenske *et al*., 2020). According to Fujisaka *et al*. (2023), dysbiosis causes the so-called "leaky gut" syndrome in which the intestinal barrier function is impaired, with increased permeability, through decreased intestinal mucus and tight junction protein content in the intestinal epithelium. With increased intestinal permeability, the bacterial cell wall component endotoxin enters circulation and causes hyperendotoxemia. The endotoxin activates Toll-like receptor (TLR) 4, which in turn promotes chronic inflammation in adipose tissue and liver and exacerbates obesity-induced insulin resistance. The inclination of the balance towards one or another bacterial phylum influences the lean or obese body condition (Fenske *et al*., 2020), so it is presumed that the animals in the study and in particular those of the EG, had a healthy intestine with higher indices of bacterial diversity and richness, which are important for the eubiosis status.

According to geography, the research results resemble the results of two studies cited by (Scheithauer *et al*., 2022) in work conducted in France who found *Proteobacteria, Bacteroides* and *Escherichia as the* most abundant Phyla and genus post Roux-en-Y gastric bypass or sleeve gastrectomy and also resemble the results advanced in Paise Netherlands by Scheithauer *et al*. **(**2022), who found *Proteobacteria* and *Akkermansia* as the most abundant post-gastric bypass Roux-en-Y phylotype and genus, but differ from other studies such as Liu *et al*. (2017) who found Bacteroidetes and Bifidobacterium as the most abundant in work performed in China post-gastric bypass in Roux-en-Y, and with that of **(**Sánchez-Alcoholado *et al*., 2019) who found *Bacteroidetes* and *Akkermansia* as the most abundant in work performed in Spain post Roux-en-Y gastric bypass or sleeve gastrectomy, as also differs from the results of Ciobârcă *et al*. (2020) who found *Bacteroidetes* and *Fusobacteria* as the most abundant phylum and genus post adjustable banding.

According to age, (Yang *et al*., 2022) reported that Phylum *Firmicutes* and *Bacteroidetes* were the most abundant in rectal fecal samples of piglets from Jinfen White and Mashen breeds. According to (Zhao *et al*., 2015); (Vigors *et al*., 2016); Zhang *et al*. (2022) the intestinal microbial richness of pigs varies significantly with age and with intestinal segment. According to age according to Zhao *et al*. (2015), *Proteobacteria* in adult pigs represents 2% of the abundance while that of *Firmicutes* is greater than 90%. According to the segment, the ileum of the pig is colonized mainly by *Firmicutes* and *Proteobacteria*, and the abundance of *Bacteroidetes* in the cecum and colon increases significantly (Liu *et al*., 2017; Zhang *et al*., 2022), however these results do not coincide with those observed in the study pigs, where it was found that the most abundant Phyla throughout the follow-up (2.5, 6.5 and 7.5 months of age) and independent of the study group were *Proteobacteria, Firmicutes* and *Bacteroidetes*; with an increase in the Phylum *Tenericutes* by the third follow-up. These variations in order of abundance when compared with the works cited in the paragraph, may be related to the constituents of the diet that has among its components vegetables such as corn and the supplementation used to achieve the obese condition, which could acidify the gastro-intestinal pH to levels that favored or stimulated the formation of bacteria of the phylum *Proteobacteria* as reported by Akagbosu *et al*. 2022. The non-difference between phyla could be influenced by the sample size and the follow-up time, which did not allow identifying significant changes, being advisable for future research a larger number of animals and a longer follow-up time.

When talking about diversity between groups, the experimental group showed the highest diversity, which opens the door to raise the experimental surgery as influential in the results, towards the training of *Proteobacteria* which, as a Phylum; is much less abundant than *Firmicutes* in fecal matter of adult pigs according to Zhao *et al*. (2015). The results also seem to agree with those of Scheithauer *et al*. (2022) who report post-analysis of 40 pre-RYBG and post-RYBG cases in humans, increase in proinflammatory

Proteobacteria with improvement of chronic inflammation post-surgery. They substantiate the improvement based on findings of increased lipopolysaccharide (LPS) and flagellin-specific immunoglobulin A (IgA), but total fecal IgA content remained unchanged. Thus, an increase in intestinal IgA after bariatric surgery neutralized immunogenic bacteria and their components, leading to an improvement of systemic inflammation. The results of the present investigation could possibly be influenced by the consequent lower pH of chyme emptying in distal jejunum where *Proteobacteria* are resistant and functionally associated with a beneficial profile characterized by decreased systemic inflammation, improved glucose homeostasis and with body weight loss (Debédat *et al.*, 2019) which were results obtained in the EG.

The results related to the increase in microbiota diversity at different taxonomic levels were similar to the result obtained by(Juárez-Fernández *et al*., 2021) in obese patients after bariatric surgery, in addition; they highlight a reduction of the Phylum *Firmicutes* compared to the same patients before the intervention; therefore, the results suggest that bariatric experimental surgery had a greater impact on the results, than the energy restriction derived from sugar and fat.

Studies such as Guo *et al*., 2018 have reported that bariatric surgery can induce increase in some classes of *Proteobacteria* that correlate with lower serum insulin concentration Guo *et al.*, 2018 lower BMI in patients after Roux-en-Y gastric bypass according to the report of (Sánchez-Alcoholado *et al*., 2019). In addition, higher *Proteobacteria* abundance has been associated with less food addiction after bariatric surgery according to **(**Oduro-Donkor *et al*., 2020) Whereas, higher concentrations of *Firmicutes* which was the predominant Phylum in the two CGs, have been related to increased metabolic degradation of energy sources, resulting in increased caloric absorption and consequently higher weight gain. In this regard, increased *Firmicutes* abundance has been consistently associated with obesity (Crovesy *et al.*, 2020). It should be noted that CG1 and CG2 pigs at the end of the study were still obese (detailed information in another article) with similar BMI (=0.99), while GE pigs involuted their condition from obesity to leanness with BMI going from 0.99 to 0.93, so promoting a shift towards a healthy profile (less *Firmicutes* more *Proteobacteria*) may be a determinant of the beneficial effects of experimental bariatric surgery.

At the end of the experiment, it is worth noting a difference between the groups, where the phylum *Spirochaetes* was constant and with higher frequency in all the animals of the SG, while for the animals of groups CG1 and CG2 it was the phylum *Tenericutes*. The EG results agree in part with a previous study by LI et al., 2023 and Wang *et al*., 2023 who reported Phylum *Spirochaetes* as the third most abundant of the four phyla found in healthy growing pigs fed rice bran and in the work with finishing Creole sows, respectively.

In terms of genus, in the EG there was greater bacterial diversity; led by *Escherichia-Shigella (Proteobacteria*), followed by *Christensenellaceae R-7 group (Firmicutes), Pseudohongiella (Proteobacteria), Lachnospiraceae NK4A136 group (Firmicutes)*, and *Treponema (Spirochaetes*); for CG1 the genera *Streptococcus (Firmicutes), Escherichia-Shigella (Proteobacteria), Christensenellaceae R-7 group and Ruminocococcaceae UCG-014 (Firmicutes)* and for CG2 the genera *Succinivibrio (Proteobacteria), Escherichia-Shigella (Proteobacteria), Alteromonas* and *Pseudohongiella (Proteobacteria*) (Figure 2D). At the genus level, the results of this study are discordant with respect to a previous report by Wang *et al*., 2023 , who found *Prevotella, Treponema* and *Lactobacillus* as the most abundant genera in finishing sows, with some differences between animals in the study, explained by other characteristics such as the animal's own gene pool, breed, sex, age stage and environmental conditions such as management and feeding. According to Wang *et al.*, 2023 say that the genus *Treponema* is related to low feed conversion, so it may be of particular interest and should be taken into account as a possible biomarker in future works of evaluation of the standardized bariatric technique in the research, since it is the genus not common among the three groups of the study and is present in the EG.

On the other hand, the modifying role in the intestinal microbiota achieved in the EG after surgery, mostly represented by the genus *Escherichia-Shigella;* was also observed in another study, where the values were higher in people after laparoscopic gastric sleeve surgery when compared to the values of people with normo-weight (Albaugh *et al*., 2017), further suggesting; that this entero-bacterial genus could be used as a bio-marker or possible quantifiable indicator of a biological condition or change; such as that achieved after experimental surgery.

The increase in *Escherichia/Shigella* genus in EG may in part agree with the results of (Akagbosu *et al*., 2022)), who demonstrated alterations with a tendency to increase in *Proteobacteria* such as *E. coli* in patients with gastric sleeve (GS) and Roux-en-Y bariatric surgery (RYGB). The explanation for this alteration could be associated with an increase in luminal acidity and dissolved oxygen after these procedures, as well as the ability of *E. coli* to efficiently harvest energy after bariatric surgery. Regarding *Escherichia coli, it* can synthesize the best studied enteric bacterial protein enteric protein (ClpB), and which can act as a mimetic of alpha-melanocyte stimulating hormone (αMSH) with similar anorexigenic effects. Furthermore; *E. coli-derived* ClpB is able to enhance PYY and GLP 1 secretion, and directly activate anorexigenic neurons and subsequently induce satiety with effect on weight loss (Arnoriaga-Rodríguez *et al*., 2020; Han *et al*., 2021) coinciding part of the above statements , with results obtained in EG pigs.

Regarding the EG, it is presumed that the distal enteric medium where the post-surgery chyme arrives may have a very low pH due to the rapid delivery of gastric contents, less buffering and less dilution due to the short enteric pathway and possibly due to the higher concentration of gastric juices in proportion to the ration consumed, which corresponded to 1/3 of that of the CG, variables that could influence the development of bacteria resistant to acidity and with the power to digest energy sources derived from poorly digested vegetables, properties that characterize *Proteobacteria,* among which the *Escherichia/Shigella* genus can be found (Akagbosu *et al*., 2022).

Taking into account the results of this study and previous report (Aron-Wisnewsky *et al*., 2019), the application of bariatric surgeries, could affect the composition of the intestinal microbiota by inducing alterations in entero-environmental and systemic factors, as well as by anatomical changes in the digestive tract (Aron-Wisnewsky *et al*., 2019). Furthermore, the metabolic improvement that occurs after surgery cannot be explained only by caloric control and weight loss, but also by changes in microbial communities. Although changes in the composition of the intestinal microbiota may be caused by adaptation of microorganisms to caloric control, derived from bariatric surgeries leading to a decrease in energy harvesting, by alterations in fermentation activity and subsequent sustainable weight loss (Alqahtani *et al*., 2023; Wang *et al*., 2023).

The results of this study suggest that experimental hourglass bariatric surgery with gastro-jejunal bypass had modulatory effects on gut microbiota. The statement agrees with the findings of Scheithauer *et al*. (2022) who state that**,** metabolic improvements mediated by bariatric surgery are mainly associated with immune activities that are mediated by microbial alterations and by changes in incretin secretion.

Understanding gut microbial changes will provide key information on how weight loss is modulated in the face of bariatric surgery. In addition, characterization of the gut microbiota before and after bariatric surgery may generate preliminary data for the development of therapies based on bacterial taxa that contribute to the treatment of animal and human obesity. These therapies could improve outcomes after surgery, or even decrease the need for surgery in the future by becoming a weight loss option.

Considering that bariatric experimental surgery remodeled the intestinal bacterial microbiota in a 30-day post-surgery period, it is necessary to propose future research with a larger sample size, several cut-off points in time and a longer study time. The approach is based on the concepts of recurrence/weight maintenance and transitional/permanent microbiota, which need to be identified. The statistical model would consider a prospective investigation comparing the same animals with obese condition before and after experimental surgery and another possible comparison with a control group of non-obese animals.

CONCLUSIONS

In this first study a novel bariatric surgery titled EBSSC G-YB was standardized, which according to the statistical analysis did not show significant differences between the experimental groups. However, the results suggest that the intervention could influence the bacterial communities such as the increase achieved in the Phylum *Spirochaetes* with higher indexes of bacterial richness and diversity and high abundance of the genus *Escherichia/Shiguella* and *Pseudohongiella*, which could be considered as intestinal biomarkers in future research.

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REFERENCES

ADAMI, G, F.; CARBONE, F.; MONTECUCCO, F. *et al*. Adipose tissue composition in obesity and after bariatric surgery. *Obes. Surg.*, v.29, p.3030-3038, 2019.

AKAGBOSU, C.O.; NADLER, E.P.; LEVY, S.; HOURIGAN, S.K. The role of the gut microbiome in pediatric obesity and bariatric surgery. *Int. J. Mol. Sci*., v.23, p.15421, 2022.

ALBAUGH, V.L.; BANAN, B.; AJOUZ, H. *et al*. Bile acids and bariatric surgery. *Mol. Aspects Med*., v.56, p.75-89, 2017.

ALQAHTANI, S.J.; ALFAWAZ, H.A.; NADINE, M.S. *et al*. Bariatric surgery as treatment strategy of obesity in saudi people: effects of gut microbiota. *Nutrients*, v.15, p.361, 2023.

ARNORIAGA-RODRÍGUEZ, M.; MAYNERIS-PERXACHS, J.; BUROKAS, A. *et al*. Gut bacterial ClpB-like gene function is associated with decreased body weight and a characteristic microbiota profile. *Microbiome*, v.8, p.59, 2020.

ARON-WISNEWSKY, J.; PRIFTI, E.; BELDA, E. *et al*. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. *Gut*, v.68, p.70-82, 2019.

BHASKARAN, K.; DOUGLAS, I.; FORBES, H. *et al*. Body-mass index and risk of 22 specific cancers: a populationbased cohort study of 5·24 million UK adults. *Lancet*, v.384, p.755-765, 2014.

CIOBÂRCĂ, D.; CĂTOI, A.F.; COPĂESCU, C. *et al*. Bariatric surgery in obesity: effects on gut microbiota and micronutrient status. *Nutrients*, v.12, p.235, 2020.

CROVESY, L.; MASTERSON, D.; ROSADO, E.L. Profile of the gut microbiota of adults with obesity: a systematic review. *Eur. J. Clin. Nutr*., v.74, p.1251-1262, 2020.

DAVOODI, S.H.; MALEK-SHAHABI, T.; MALEKSHAHI-MOGHADAM, A. *et al*. Obesity as an important risk factor for certain types of cancer. *Iran. J. Cancer Prev*., v.6, n.4, 2013.

DEBÉDAT, J.; CLÉMENT, K.; ARON-WISNEWSKY, J. Gut microbiota dysbiosis in human obesity: impact of bariatric surgery. *Curr. Obes. Rep*., v.8, p.229-242,-2019.

DOUGLAS, I.J.; BHASKARAN, K.; BATTERHAM, R.L.; SMEETH, L. *et al*. Bariatric surgery in the United Kingdom: a cohort study of weight loss and clinical outcomes in routine clinical care. *PLoS Med.*, v.12, p.e1001925, 2015.

EJTAHED, H.S.; HASANI-RANJBAR, S.; LARIJANI, B. Human microbiome as an approach to personalized medicine. *Altern. Ther. Health Med*., v.23, p.8-9, 2017.

FENSKE, G.J.; SUDEEP GHIMIRE, S.; ANTONY, L. *et al*. Integration of culture-dependent and independent methods provides a more coherent picture of the pig gut microbiome. *FEMS Microbiol. Ecol*., v.96, p. fiaa 022, 2020.

FINK, J.; SEIFERT, G.; BLÜHER, M. *et al*. Obesity surgery—weight loss, metabolic changes, oncological effects, and follow-up. *Dtsch. Ärztebl. Int*., v.119, p.70-80, 2022.

FUJISAKA, S.; WATANABE, Y.; TOBE, K. The gut microbiome: a core regulator of metabolism. *J. Endocrinol*., v.256, p.e220111, 2023.

GRIFFITH, R. Obesity, poverty and public policy. *Econ. J.*, v.132, p.1235-1258, 2022.

GUO, Y.; HUANG, Z.P.; LIU, C.Q. Modulation of the gut microbiome: a systematic review of the effect of bariatric surgery. *Eur. J. Endocrinol*., v.178, p.43-56, 2018.

HAN, H.; YI, B.; ZHONG, R. *et al*. From gut microbiota to host appetite: gut microbiota-derived metabolites as key regulators. *Microbiome*, v.9, p.162, 2021.

HERNÁNDEZ HURTADO, L. et al. Changes on Adipose Tissue Distribution After Laparoscopic Roux-en-Y Gastric Bypass in Obese Göttingen Minipig. Effects on Glucose Metabolism. Obesity Surgery, v. 26, n. 12, p. 3001–3006, dez. 2016.

JABBOUR, G.; SALMAN, A. Bariatric surgery in adults with obesity: the impact on performance, metabolism, and health indices. *Obes. Surg*., v.31, p.1767-1789, 2021.

JUÁREZ-FERNÁNDEZ, M.; ROMÁN-SAGÜILLO, S.; PORRAS, D. *et al*. Long-term effects of bariatric surgery on gut microbiota composition and faecal metabolome related to obesity remission. *Nutrients*, v.13, p.2519, 2021.

LEGGETT, R.M.; CLARK, M.D. A world of opportunities with nanopore sequencing. *J. Exp. Botany,* v.68, p.5419-5429, 2017.

LI, X., LI, M., HAN, J. Effects of different starch diets on growth performance, intestinal health and faecal microbiota of growing pigs. Journal of Animal Physiology and Animal Nutrition, p. jpn.13810, 18 fev. 2023.

LIU, R.; HONG, J.; XU, X. *et al*. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat. Med*., v.23, p.859-868, 2017.

LUM, M.; GRADY, A.; FALKINER, M. *et al*. Assessing the implementation of healthy eating and physical activity policies and practices in the family day care setting: a cross sectional study. *Health Promot. J. Aust*., v.32, p.116-125, 2021.

MANSON, J.E.; COLDITZ, G.A.; HENNEKENS, C.H. Body weight and mortality among women. *New Engl. J. Med*., v.333, n.11, 1995.

NAGAYA, M.; HASEGAWA, K.; UCHIKURA, A. *et al*. Feasibility of large experimental animal models in testing novel therapeutic strategies for diabetes. *World J. Diabetes*, v.12, p.306-330, 2021.

ODURO-DONKOR, D.; TURNER, M.C.; FARNAUD, S. *et al*. Modification of fecal microbiota as a mediator of effective weight loss and metabolic benefits following bariatric surgery. *Exp. Rev. Endocrinol. Metabol*., v.15, p.363-373, 2020.

PACHECO SÁNCHEZ, D.; PINTO FUENTES, P.; ASENSIO DÍAZ, E. Actualización en cirugía bariátrica/metabólica. *Nutr. Clin. Med*., v. 13, p.113-127, 2019.

POWELL-WILEY, T.M.; POIRIER, P.; LORA E. *et al*. Obesity and cardiovascular disease: a scientific statement from the american heart association. *Circulation*, v.143, p.e984-e1010, 2021.

PURDY, J.C.; SHATZEL, J.J. The hematologic consequences of obesity. *Eur. J. Haematol*., v.106, p.306-319, 2021.

RINONAPOLI, G.; PACE, V.; RUGGIERO, C. *et al*. Obesity and bone: a complex relationship. *Int. J. Mol. Sci*., v.22, p.13662, 2021.

SÁNCHEZ-ALCOHOLADO, L.; GUTIÉRREZ-REPISO, C.; GÓMEZ-PÉREZ, A.M. *et al*. Gut microbiota adaptation after weight loss by Roux-en-Y gastric bypass or sleeve gastrectomy bariatric surgeries. *Surg. Obes. Relat. Dis*., v.15, p.1888-1895, 2019.

SCHEITHAUER, T.P.M.; DAVIDS, M.; WINKELMEIJER, M. *et al*. Compensatory intestinal antibody response against pro-inflammatory microbiota after bariatric surgery. *Gut Microbes*, v.14, p.2031696, 2022.

SILVA RIBEIRO, M.; ZORRON, R.P.A.S.; SILVA, S.J.Q. *et al*. New intragastric sleeve technique reduces adipose tissue in pig experimental model: tomographic study. *Sci. Rep.*, v.10, p.18122, 2020.

VIGORS, S.; O'DOHERTY, O.V.; KELLY, A.K. *et al*. The effect of divergence in feed efficiency on the intestinal microbiota and the intestinal immune response in both unchallenged and lipopolysaccharide challenged ileal and colonic explants. *PLoS One*, v.11, p.e0148145, 2016.

WANG, Y.; HE, Y.; LIANG, Y. *et al*. Fecal microbiota transplantation attenuates Escherichia coli infected outgrowth by modulating the intestinal microbiome. *Microb. Cell Fact.*, v.22, p.30, 2023.

WELBOURN, R.; HOLLYMAN, M.; KINSMAN, R. *et al*. Bariatric surgery worldwide: baseline demographic description and one-year outcomes from the fourth IFSO global registry report 2018. *Obes. Surg*., v.29, p.782-795, 2019.

YANG, B.; LIU, C.; HUANG, Y. *et al*. The responses of lactobacillus reuteri LR1 or antibiotic on intestinal barrier function and microbiota in the cecum of pigs. *Front. Microbiol*., v.13, p.877297, 2022.

YAO, G.; WU, S.; ZENG, X. *et al*. Different gut microbiome composition in obese Guizhou minipigs between female and castrated male. *Folia Microbiol*., v.64, p.889-898, 2019.

ZHANG, L.; WANG, X.; ZHANG, X. Modulation of intestinal flora by dietary polysaccharides: a novel approach for the treatment and prevention of metabolic disorders. *Foods*, v.11, p.2961, 2022.

ZHAO, W.; WANG, Y.; LIU, S. *et al*. The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. *PLoS One*, v.10, p.e0117441, 2015.