Original Research Article

Effects of prolonged fasting on cloacal microbiota of *Crotalus durissus* with different corporal scores

ABSTRACT

Ai**ms:** This study provides a detailed analysis of the composition and diversity of the bacterial community within the cloacal cavity of *Crotalus durissus* (Cascavel) under the influence of diet and the corporal score of breeding snakes through metagenomics analysis. For this aim, the study also provides a body condition score protocol for the classification of snakes asthin, normal, and obese.
Study design: The experimental groups were established from snake body conditions such as visibility of ribs, the aspect of the side view, the upper and ventral view, and the aspect of the final portion, of the neck and square bone.
Place and Duration of Study: The research was conducted at S-Inova Laboratory at Don Bosco Catholic University, between December 2017 and February 2019 (Mato Grosso do Sul, Brazil).
Methodology: There are 3 groups of score corporations: thin (n=3), normal (n=3), and obese (n=3). The first sample of each animal was obtained 7 days after feeding and the second sample after 50 days of fasting. The metagenomic DNA was extracted from all 18 *swab* samples through DNeasy® PowerSoil® Kit (Qiagen). Thereafter the samples were submitted to sequencing, performed using the 2 x 250bp paired-end sequencing on a single run of an IlluminaHiSeq, followed by the application of bioinformatics tools.
Results: The most representative phyllo for all the samples was Proteobacteria (69,4%), followed by Bacteroidetes (19,2%) and Synergists (11,2%). In the group of obese snakes, the phylum Proteobacteria still being the group with the higher relative abundance in the two moments, with a decrease of 9,6%, as well as the phylum Bacteroidetes which decreased by 5,5% in the second moment (after fasting). Differently, the groups of the normal and thin have a decrease of 37,2% and 29,4% respectively of the phylum Proteobacteria and an increase of the relative abundance of 35% and 27,9% of the phylum Bacteroidetes. Before prolonged fasting the groups obtained 213 shared OTUs which reduced to 206 after the prolonged fasting, suggesting that, in general, the treatment influences microbial communities of the groups, which was possible to observe when analyzed and compared individually. The index of Shannon diversity showed that before the fasting, the groups of obese snakes and normal presented a bigger richness of species, differently from the thin group that showed more diversity after 50 days of fasting. The variance analysis showed that the obese snakes presented a bigger proximity to the samples collected before the prolonged fasting. The samples showed greaterproximity in PCA analyses and ANOVA when considering the prolonged fastingtime than when

compared by body score.
Conclusion: With this study, it was possible to conclude that prolonged fasting can influence the cloacal microbiota of the snake *Crotalus durissus*, except in the individuals considered obese.

KEYWORDS: 16S rDNA gene. Body Condition Score. *Crotalus durissus*.

1. INTRODUCTION

In Brazil the most important reptile group for public health is the species of Viperidae family (Jararacas, Cascavéis and Surucucus) which stars most of the ophidian accidents recorded, [1]. This group also is the most interesting for the management of captivity both for conservation and for bioprospecting of venom [2].

The maintenance of a vivarium involves wide factors to guarantee the health status and well-being of the snakes, which includes specialized professionals, efficient air conditioning, environmental enrichment, appropriate techniques of breeding, and an adequate feeding regime [3, 4, 5]. In captivity, adequate management is crucial to the good health status of each snake, given the fact that as reptiles the snakes are very sensitive to ambient conditions. However, the captive condition itself is capable of inducing stress conditions that involve microbiome disturbance.

It is remarkable the participation of the gut microbiota in this context, which means the entire community of microorganisms associated with the gut. Facing the difficulties of acclimating to the new environment, it was reported that the gut microbiome, as all microbiota associated with the snake, suffers alterations that can lead to bacterial diseases, viral and protozoa infections. [6]. Being essential to life, not only for reptiles but for inclusive human beings, the microorganisms directly participate in digestion and its deeply enrolled in the immunity system, influencing normal physiology [7].

Captive animals can face the consequences of nutritional imbalances from the changes in appetite, digestion, nutrient assimilation, or even inappropriate feed management [8,9]. These stressing factors added to the limitation of the animals to a microenvironment can lead to a disturbance in the gut microbiome and finally to obesity, which is a common disturbance in captive snakes, even with adequate feed management [10,11]. The different microbial communities can affect energetic management and homeostasis, which reinforces the hypothesis that the predisposition to obesity is given by a gut microbiome capable of extracting or storing nutrients more efficiently [12]. Considering that the host diet is the key to the gut microbiome composition, the feed regime presents a big influence on microbiome modulation, correlating to obesity and disease. And some authors highlight obesity in captive reptiles by the fact of the animal having available food, without foraging and/or energetic waste to obtain that food, as it is in nature [8,9,11].

Many snake species pass long periods of fasting in natural environments, which can be years between one feed and another [13], although there is minimum known about the impact of fasting on the gut microbiome, and even, there is a large gap on our understanding of the non-mamalian vertebrate microbiome, especially among wild reptiles [14,15,16]. In C*hinese alligator*

was reported that the gut microbiome can be directly impacted by periods of fasting [16]. Also, by analyzing the gut microbiome it is possible to evaluate the potential environmental adaptability of a host [17] in this case, in captivity, where the snakes are subject to many situations of stress.

In this way, with the recent technological advances in omics sciences, such as metagenomics, it is possible to characterize the microbiota, directly from the environmental samples [18,19]. Previous research on snake gut microbiomes utilized swabbing the cloacal cavity as a non-invasive method and effective for metagenomic analysis. To analyze the relation of gut microbiome alterations in fasting to the condition of each snake obese, thin snake, or normal, it is extremely important to classify them by corporal score analysis based on their morphology.

Most studies in this field are focused on mammals, highlighting that our knowledge about reptile microbiota is still very limited [16]. Still, it is essential to expand the knowledge about the health and better management of such important animals as snakes. For this reason, this present study providesan analysis of the composition and diversity of the bacterial community associated with the cloacal cavity of *Crotalus durissus* (Cascavel), under the influence of food management and the corporal score of breeding snakes through metagenomics analysis. In this way, the large-scale sequencing of metagenomic DNA of bacterial communities associated with the cloacal cavity of *Crotalus durissus* allows the increase of the acknowledgment of abundance, diversity, and changes in these communities in face of aprolonged period of fasting, as for treating captive animals with mensal feeding management, as described by Melgarejo (2002) [10]. In addition within three days was possible to see alterations in the intestinal microbiota related to the availability of nutrients [20].

2. METHODOLOGY

2.1. Body Score classification

The snake used in this study was *Crotalus durissus* (Cascavel), which originated from squads of intense captivity of Vivarium (Biotério) of Dom Bosco Catholic University in Campo Grande, Mato Grosso do Sul.

For them to be distributed in certain groups, they had a minimum of three years in captivity, with a monthly routine of food, was created criteria that classify them as obese, thin, and normal.

The score of corporal condition developed in this present study was grounded on concepts used for mammals, which includes the visual aspects already observed for corn snakes [21] through visual and palpable inspections, allowing verifying the fat reservation and muscle mass, and classifying the animals on a scale of one to six. Therefore, the chart developed has the categories, S1, S2, S3, S4, S5, and S6, considering S1 very thin, S4 animal wanted, and S6 obese.

The middle categories, S2, thin, S3, thin with muscularity, and S5, overweight, were created aiming group animals that, according to the parameters, could not be included in external categories.

For them to be grouped as standard the evaluation of the ribs, side vision, higher vision, ventral vision, final portion, and neck/square bone. The definition for each criterion in each category was based in first place on the acknowledgment of the biology of the animals, and on the

perceptionsabout the anatomy of *Crotalus durissus*. Later, by analyzing each squad animal, it's possible to make some settings in each criterion.

In the area of the ribs, the thinner is the animal, it's easier to visualize the ribs, and it's possible to see the muscle layer during palpation. In obese ones, it isn't possible to make this type of visualization and it's possible to palpate the fat layers.

When evaluating the side and the higher parts, it was considered if the transverse and thorny spin were visible. Therefore, it was necessary to consider that *Crotalus durissus* has a body in a keel shape with a flat belly and that even in obese snakes the thorny process is still minimally visible. By palpation, it's possible to feel the quantity of local musculature and the presence of fat, as well as the rounded shape that some obese animals could have. For those considered desirable or healthy, the transverse is not visible, and in the thin ones, there is a muscle layer loss.

Chart 1: Table developed on this study for the body score evaluation, enabling the classification of snakes of *Crotalus durissus*within six groups: (S1) Very thin, (S2) Thin, (S3) Thin with muscles, (S4) Wanted animal, (S5) Overweight, (S6) Obese.

	S1	S2	S3	S4	S5	S6
Ribs	Very evident ribs to the touch, with very reduced musculature	Very evident ribs (visible), eassily touched with a thin layer of muscles	Evident ribs (visible), easily touched with a layer of musculature	Low evident ribs (not visible), easily touched and with developed musculature	Not visible ribs, with a layer of developed musculature and observations of fat	Not visible ribs, with thick layer of fat
Side view	Evident spinous process, with reduced musculature through the body	Very visible spinous process with low presence of musculature though the body	Visible spinous process with presence of musculature through the body, except on terminal portion	Visible and touchable spinous process and uniform musculature distribution through the body, except on terminal portion	Touchable spinous process, with presence of musculature with points of fat through the body, except on terminal portion	Rounded body with evident fat
Upper view	Very evident transverse process, easily touched and low musculature	Evident transverse process with soft musculature layer, easily touched	Evident transverse process, with barely visible musculature but easily touched	Low evident transverse process or not visible and hardly touched	Transverse process not visible and hardly touched	Rounded body, with evident fat, and with visible prickly process until the middle region of the body

Ventral View	Presence of ventral groove and internal structures easily touched.	It's possible to visualize the ventral groove on final portion, with easy identification of the internal structures through the ventral touch	With no ventral groove, and with possibility of identification of internal structures through ventral touch	With no ventral groove and difficult to identify the internal structures through the touch	Internal structures hardly touched, with points of fat through ventral touch	Difficult palpation, with impossibility to identify the abdominal contents due to the huge presence of fat
Final portion	Tapered with low musculature	Body with reduced musculature and skin touching the skeleton	Uniform body with low musculature	Uniform body with no strangulation on final portion of the body, with a uniform tapering of the body	Bulky body, with reduced and tapered final portion	Strangulation on final portion of the body, abrupt tapering
Neck/Square bone	Low muscular structure with no fat observation, and with possible identification of the extremities of the square bone	Uniform muscular structure, with no fat observed	Uniform muscular structure	Muscular structure well distributed, uniform, with no fat observed	Muscular structure well distributed, uniform	Muscular structure well distributed, uniform, with possibility of fat presence

Anatomically, snakes don’thave a sternum bone, therefore, when they are weak it's a generation of a groove that makes possible the identification of some internal structures through touch, making this a feature of the condition. This identification is not very easy to apply in healthy individuals and impossible in obese since they present a fat mass in the respective region.

Inthe final portion it was possible to observe that in obese animals, independently of the sex, there is a huge tuning between the cloaca and the tail start, which doesn’t occur in animals classified by normals, that way the tuning is proportional with the animal body. Thus, in the weak animals, it's possible to see a tuning that can be considered proportional, however with palpation there is less musculature.

Finally, in the region of the square bone and cervical, it’s possible to observe thatthere is musculature in healthy, that starts on the square bone and keeps by thecervical part and spine. It’s also possible to observe that on thin snakes there is a little musculature, letting clear the ends of square bone.

2.2. Samples collection

After the selection and grouping, nine animals (n=3/group) were fed and after seven days was performed the first collection of cloacal microbiota, was considered as samples of zero time (T0) (Fig. 1). The collection occurs after seven days of alimentation, the second collection of cloacal microbiota occurred 50 days after the first, considered fasting extended period for captive snakes, being an unusual procedure. This last samplewas called fifty time (T50).

The samples were collected from the cloacal cavity of snakes according to Colston (2015) [14]. After the sterilization of the external part of the cloaca with alcohol 70%, in order to make sure that the collected material is not part of the environment microbiota, a sterile swab was gently introduced tothe cloaca (~5-8 mm), was rotated several times by about 1 minute, inside the cloaca. The swabs were stored in microtubes sterile with 2ml at -20°C until the moment of the realization of direct extraction of DNA.

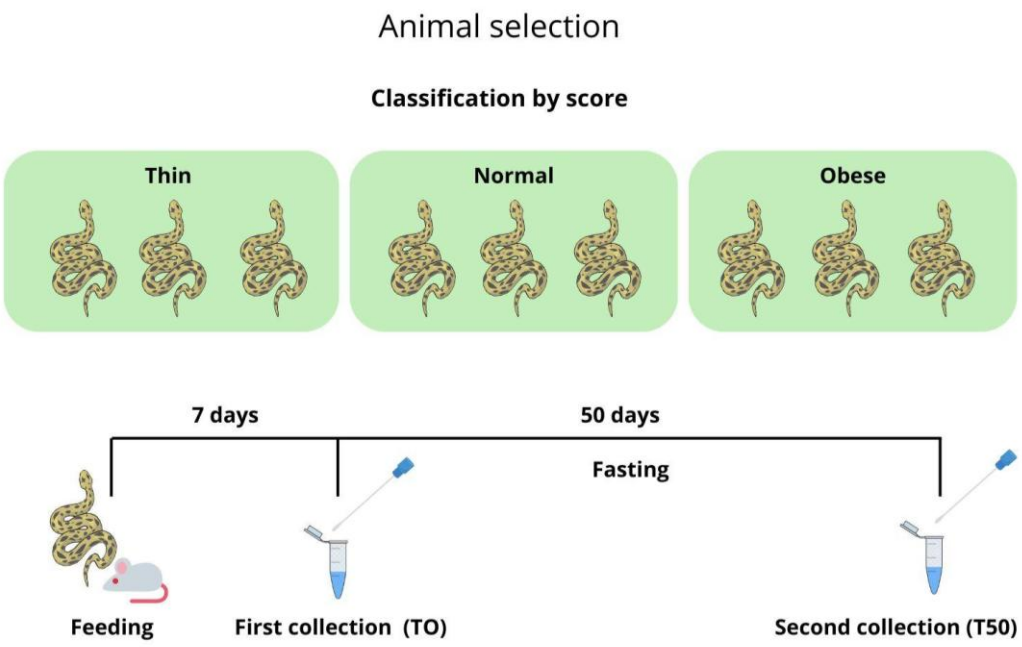

Fig. 1: Selection of the snakes of *Crotalus durissus*using the table of body score condition developed in this study. Three groups with three subjects in each, were maintained in fasting for 50 days; after seven days from the feed occurred the zero collect, followed by the time 50 collection, after the fasting.

2.3. Direct DNA extraction

The entire extraction of DNA from samples was performed using the kit DNeasy® PowerSoil® Kit (Qiagen) with alterations on instructions from manufacturers, including: (1) After adding the C1 solution the samples were incubated at 70°C for 10 minutes. (2) Vortex at maximum speed for 20 minutes in the horizontal position. (3) Was added 30ul of solution C6 to the center of the filter. (4) After adding the solution C6 to the column, it was kept at room temperature for 5 minutes, before the final centrifugation.

The product of the extraction of DNA was quantified using Qubitt® 3.0 Fluorometer and posteriorly rated by agarose gel electrophoresis 0.8% and colored with ethidium bromide. The size can be estimated by comparison with the molecular marker 1kb plus ladder. Confirmed the amplification of 16 rDNA, the samples were prepared to be sequenced.

2.4. Sequencing of DNA followed by Bioinformatics analysis

For complementarity of the results, 18 samples were sent forlarge-scale sequencing to the company GenOne Solutions in Biotechnology, situated in Rio de Janeiro, Brazil. The amplification of region V4 of 16S gene was made using the specific primers: 515F ((5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3'), as well as the sequencing, was made by Illumina HiSeq (http://www.genone.com.br/genomica/), obtained a metagenomics library.

The package QIIME (Quantitative Insights Into Microbial Ecology) V1.7.0 [22] was used to filter raw tags to obtain clean tags with high quality. Then, sequences with a similarity of ≥ 97% were assigned to the same operational taxonomic units (OTUs) using the software Uparse (Uparse v7.0.1001) [23]. A representative sequence for each OTU was noted with the Threshold 0,8 using the software Mothur [24] against the data bank SILVA [25] for a note in each taxonomic classification. The phylogenetic relationship of all the representative sequences of the OTUs can be compared using the software MUSCLE [26].Forvisualizing the OTUs shared between the experimental groups Obese, Normal, and Thin (before and after fasting) the informationon the analysis of the sharing of microbial communities for these groups was detailed on a Venn diagram.

The alpha diversity was estimated using the index of Shannon, which attributes bigger importance to the rare species, using the QIIME and showed by using the software R (Version 2.15.3). The indexes were compared between the samples through the Tukey method, considering (P=0,05).

The beta diversity, to demonstrate the similarity between the samples, was calculated through the analysis of mean components (PCA) on the QIIME platformusing the headquarters of distance Weighted UniFrac, the weighted distance that considers both the participation of a community and the relative abundance, that is why it is a quantitative metric. To evaluate the similarity between the experimental groups it was calculated by the variance analysis (ANOVA), using the Tukey-Kramer method of multiple comparisons, with correction of Bonferroni (p>0,05) (with nominal coverage of 99%). This analysis allows us to verify if there exists a significant difference between the averages of the groups with a variance inside the groups, creating dice that enable an estimateofwhether the groups are part of a population or distinctive populations with different features.

To identify the bacterial group's presence or absence in front of fasting or corporal score was used the method of the size of the effect of linear discriminatory analysis (LDA) using the software LEfSe [25], making it possible to compare, through a histogram, the differential abundances of bacteria between the groups on familial and genetic levels. The cladogram identifies the biomarkers of relative abundance between the groups before and after fasting.

The statistical test of taxonomic differences was calculated by the software Statistical Analysis of Metagenomic Profiles (STAMP) version 2.0.0 [27]with multiple corrections of Bonferroni (P<0,05) (nominal roof of 95%).

3.RESULTS

3.1. Analysis of the composition of bacterial community

On the general relative abundance analysis, the most representative phyllo for all the samples was Proteobacteria (69,4%), followed by Bacteroidetes (19,2%), the Synergists (11,2%) (Fig. 2). Therefore, it's necessary to highlight that there are differences in representativity of the phylum Proteobacteria and Bacteroidetes when compared with the treatment, before and after prolonged fasting. In the group of obese snakes, the phylum Proteobacteria still was the group with the higher relative abundance in the two moments, with a decrease of 9,6%, as well as the phylum Bacteroidetes which decreased by 5,5%. Differently, the groups of the normal and thin have a decrease of 37,2% and 29,4% respectively of the phylum Proteobacteria and an increase of the relative abundance of 35% and 27,9% of the phylum Bacteroidetes.

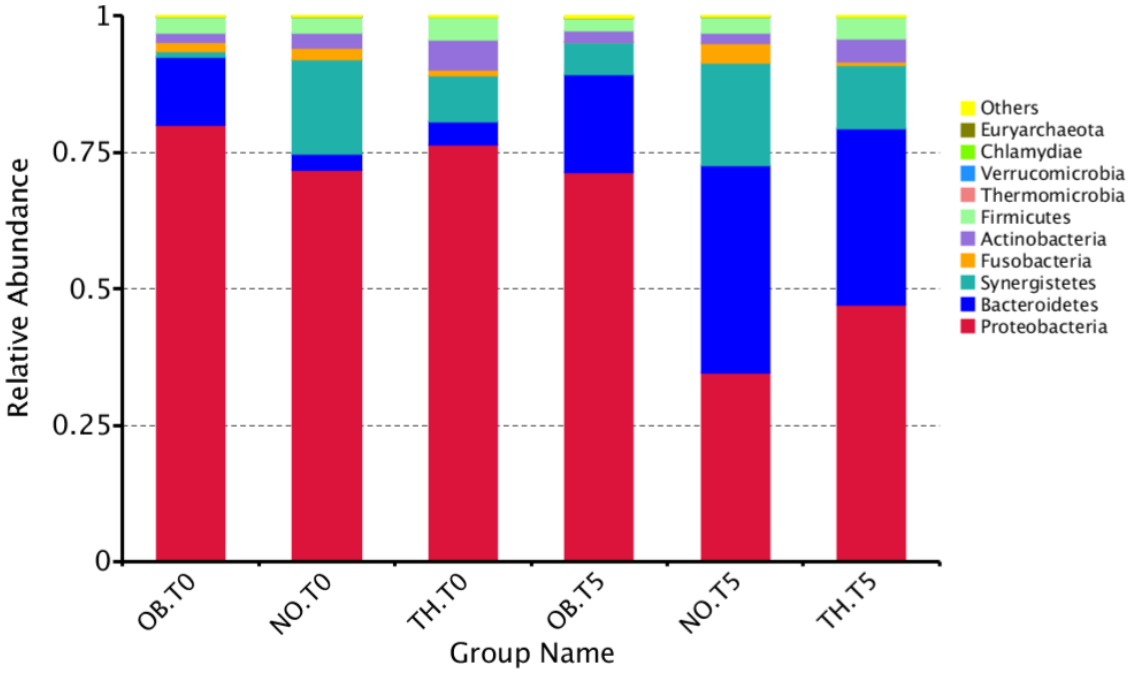

Fig. 2: Graphic of the Relative abundance analysis of the bacterial phylum from the cloacal microbiota of *Crotalus durissus* with different body scores.

The main classes identified are composed by Betaproteobacteria, Gammaproteobacteria, Bacteroidia, Synergistia, followed by Sphingobacteriia and Clostridia. At the genus level, the most abundant identified groups include *Stenoxybacter*, non-identifiable groups, *Bacteroides,Jonquetela*, *Salmonella,* and *Actinomyces*.

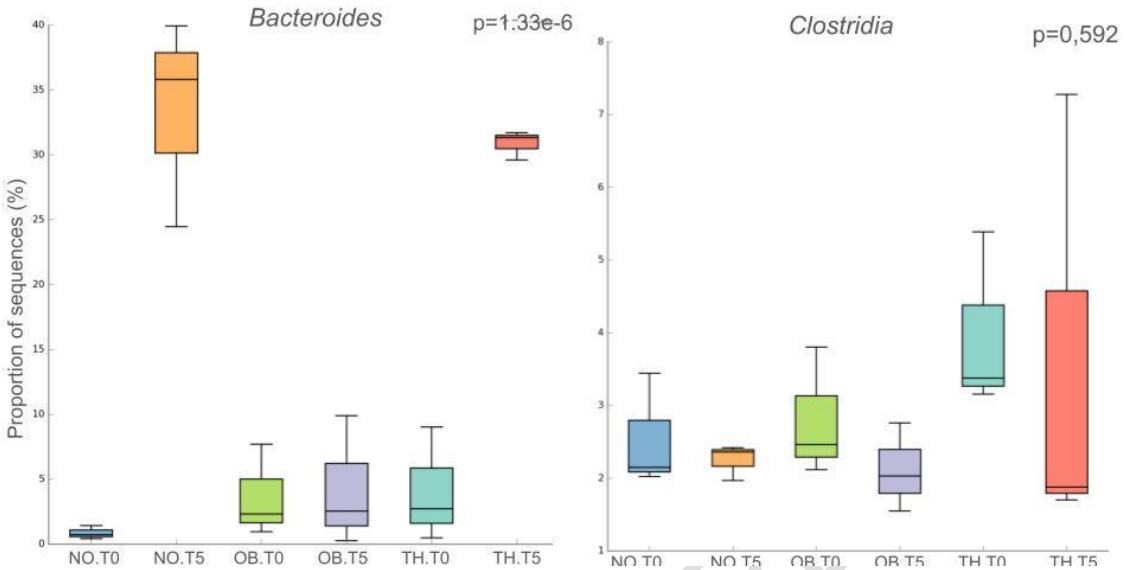

Fig. 3: Box plot of the proportion of sequences identified of genus *Bacteroides* and *Clostridia* in each experimental group before and after fasting.

It is possible to observe that the *Bacteroides* gender present in obese groups do not presented expressive variations after fasting, (Fig. 3) which can indicate a resistance to modulation by feed approach in the established microbiome. The *Clostridia* gender, which was identified in the three groups, presented more expressive changes after fasting in the thin group.

Analyzing the OTUs, it was possible to observe that there is a sharing of OTUs between the groups, as well as there, were OTUs that were present exclusively in one experimental group. Before prolonged fasting the groups obtained 213 shared OTUs that suffered reduced to 206 after the prolonged fasting, suggesting that, in general, the treatment influences the microbial communities of the groups, which was possible to observe when analyzed and compared individually. It's important to highlight that the increase or the decrease in the number of OTUs does not necessarily reflect the decrease or increase of the richness of species in each group but corroborates with the results obtained in relative abundance.

The index of Shannon diversity (Fig. 4) showed that before the fasting, the groups of obese snakes and normal presented a bigger richness of species, differently from the group of thin that showed more diversity after 50 days of fasting.

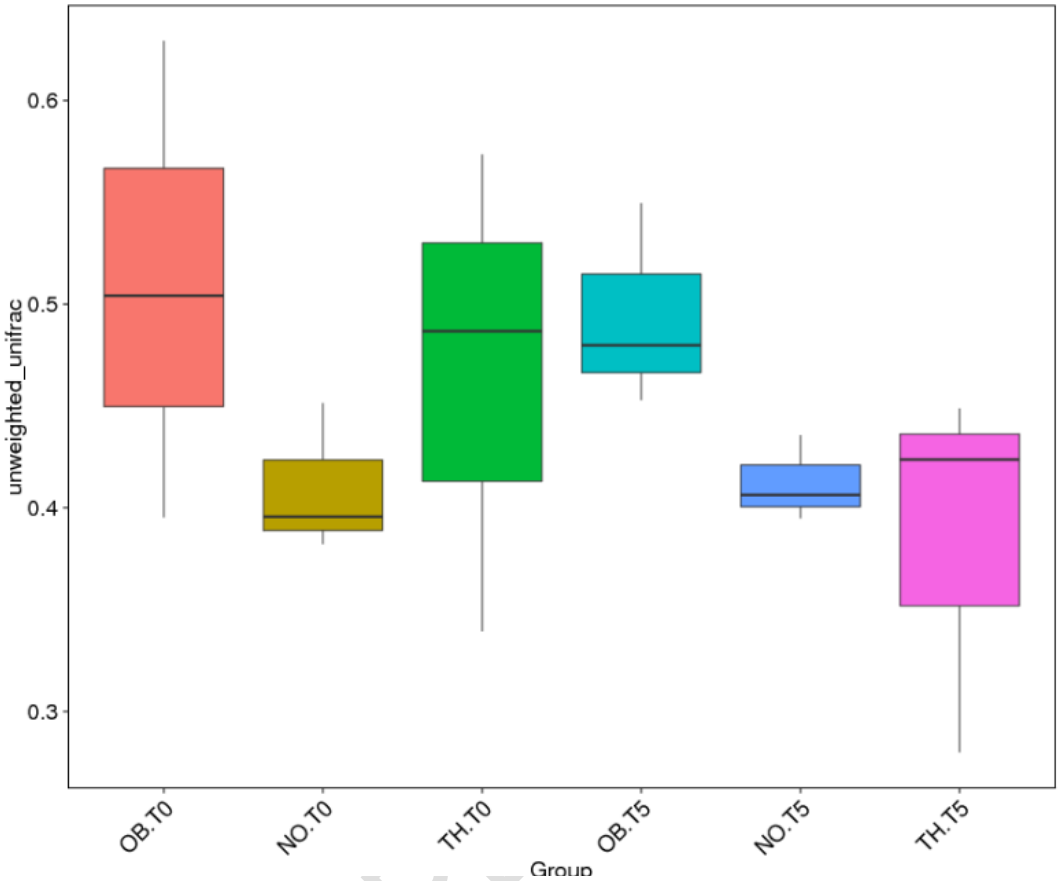

Fig. 4: Alfa diversity of the cloacal microbiota of *Crotalus durissus* analyzing the experimental groups concerning the treatment of prolonged fasting.

When tracing a comparison of estimated bacterial communities, based on observed OTUs, considering the time of fasting, the rarefaction curve obtained presented changes in the amount of OTUs after reaching 46.335 sequences. This result allows us to affirm that there was a reduction in the number of OTUs after prolonged fasting.

The beta diversity was calculated with the analysis of principal components (PCA) (Fig. 5), using the weighted distance metric (Weighted UniFrac) and showed similarity of the bacterial community between the analyzed samples, based on the groupings of OTUs, this way, each graphic point represents a sample and the color of the group in which it is inserted. Therefore, in this first graphic it was possible to observe that, even after the prolonged time of fasting, the samples of Time 0 presented similarities with the corresponding samples on Time 50.

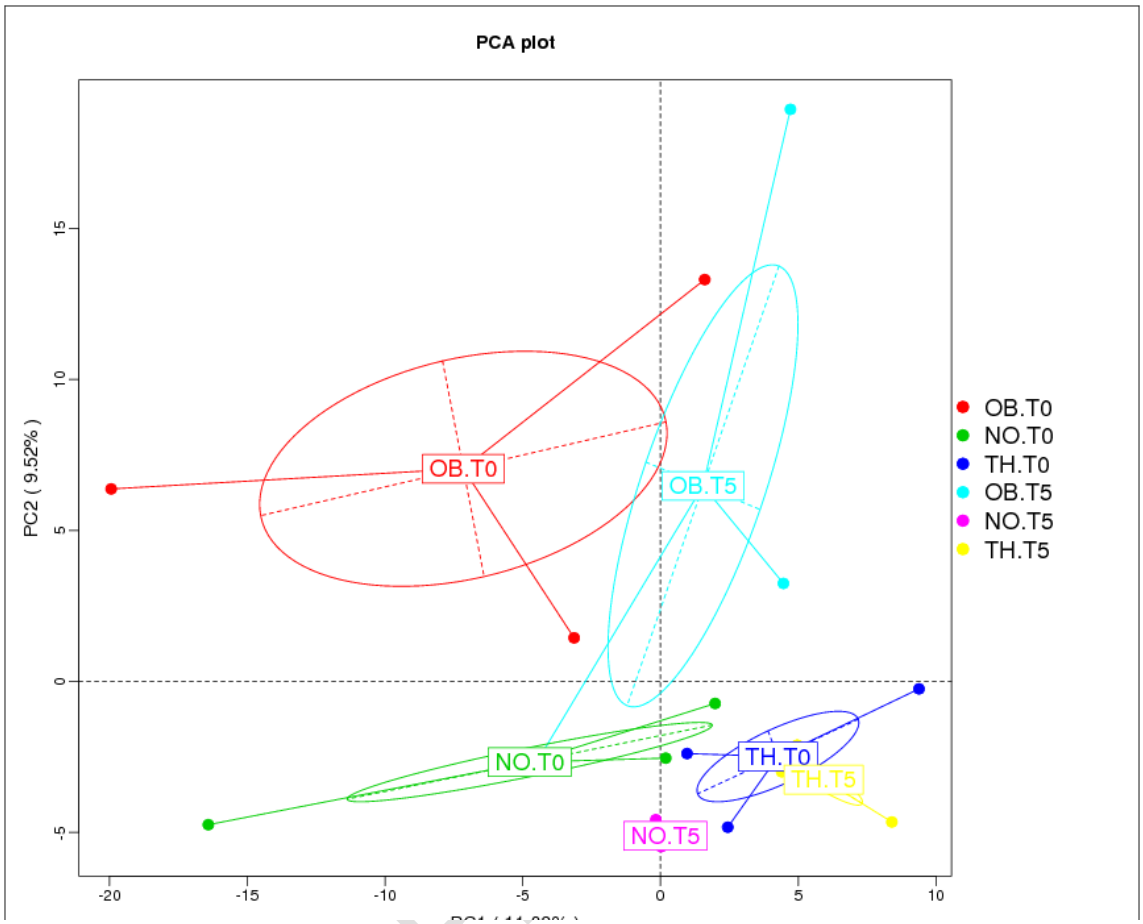

Fig. 5: Spatial graphic of Principal Components Analysis (PCA) using the matrix of distance Weighted UniFrac, measuring the distance of each sample into the groups and between the experimental groups.

The variance analysis (Fig. 6) showed that the experimental groups have significant differences between their communities, as can be observed in the clear separation of the groups in different quadrants, suggesting that prolonged fasting can influence the characteristics of the same. But still possible to observe that the samples that represent the obese snakes (points linked by a red circle represent the same individual), present bigger proximity with the samples of the groups before the prolonged fasting. That corroborates with the results of relative abundance, in which the proportion of the present phylum on the samples doesn't present significant variations in front of the prolonged fasting.

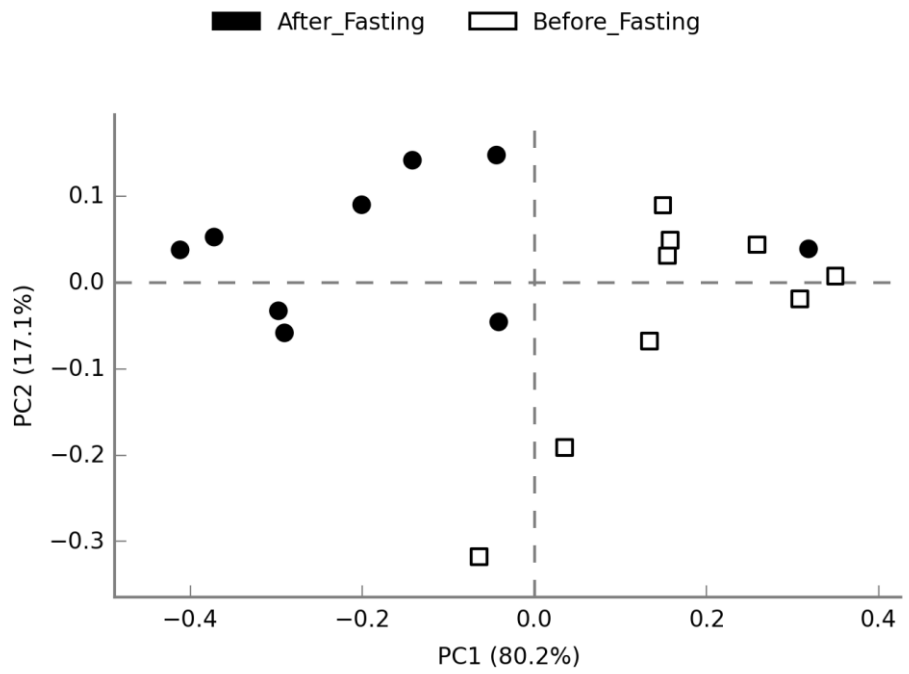

Fig. 6: Variance Analysis (ANOVA), using the Tukey-Kramer method of multiple comparisons, with Bonferroni correction (P=.05) (with nominal coverage of 99%).

For the statistics results of this present study, comparisons were made among peers, where p-value>0,05 for all the samples. Then, it was possible to verify significant statistical differences in the groups of normal snakes and thin. The results allow us to affirm that prolonged fasting has a statistical influence on the relative abundance of the Proteobacteria and Bacteroidetes phylum, having an increase in the abundance of OTUs of the Bacteroidetes phylum and a decrease in Proteobacteria, after fasting. The experimental group of snakes considered obese by corporal score, don't present statistical significance.

4. DISCUSSION

The present study aimed to characterize the bacterial microbiota of snakes in front of a condition faced in nature, in comparison to your feed habit, fasting. And evaluated if the difference in the body score, which suffers variations when imposed in captivity, makes a difference in this condition.

The body score condition evaluation for many aims is commonly realized by two different approaches. The body condition score (referred in the literature as BCS) is a classification method based on visual evaluation and is commonly used for veterinarian analysis. Despite being relatively subjective, de BCS provides satisfactory animal classification based on the pattern characteristics of healthy animals [21] and just by evaluating the corporal condition of the individual in visual and tactual ways, independent of size and/or physiologic state, to estimate the nutritional state, is more viable than measuring it by the weight [28]. Specifically for snakes, the BCS parameters are scarce. Gimmel and collaborators (2021) [21] elaborate for

corn snakes some factors to be analyzed considering the visible body condition by palpation based on BCS described for other species.

In this study were presented results related to the development of a chart of classification by the score of the corporal condition for snakes of the species *Crotalus durissus* that have received the same food management. This procedure for *C. durissus,* until this moment, is the first scientific study to establish BCS (body score condition) parameters including obese snakes in the experimental group, facilitating a better vision of the body condition of the animal without deeper exams.

Despite the appropriate environmental enrichment, the captive becomes for the animal limiting factor, [8,9,11,29] and some authors highlight obesity in captive reptiles by the fact of the animal has available food, without foraging and/or energetic waste for obtain that food, as it's in nature.

The microbiota of *Crotalus durissus* captive in the vivarium of UCDB has a dominance of the Proteobacteria phylum, followed by Bacteroidetes and Synergistes when in the condition of alimentation. A study that aimed to know the associated microbiota to *Crotalus horridus* showed that the dominance in the gastrointestinal tract is of Proteobacteria, as is reported in this study, followed by Firmicutes and Bacteroidetes, in lower portions [30].

The hypothesis that can explain this difference is that the animal used in the research was freshly captured from nature and, therefore, does not have a controlled alimentation, since it was found DNA of trace elements of mollusks and arthropods, beyond belonging to different locations, in this case in North America, differently from this study where the animals were fed with production mice and controlled sterilization. Differences in the bacterial community can be assigned to several factors, as location, type of alimentation, and even specie, how evaluated in lizards [31,32,33] mice [34], turtles [35], and bats [36].

When evaluating the bacterial community of different regions of the gastrointestinal tract of snakes *Agkistrodon piscivorus* (Cottonmouth), Colston and collaborators (2015) [14] reported that in samples from cloaca, the dominant phylum was Proteobacteria, as well as the Bacteroidetes phylum appears between the three dominant phyla, what corroborates with the results of this present study.

In that regard, considering fasting, Costello and collaborators (2010)[37], evaluated the remodeling of the microbiota of the gastrointestinal tract of *Python molurus* born in captive, after a period of 30 days of feed privacy and found that the fasting is associated with a improve of abundance and dominance of the Bacteroidetes phylum and the presence of Firmicutes, Proteobacteria, and Synergistes, having yet a decrease of the diversity of all the groups present in the bacterial microbiota of this animal, what explains the change of the bacterial microbiota configuration in front of the prolonged fasting presented in this research. This result can also be observed on the mice cecum, private of alimentation by 24 hours [38].

The identified gender highlight the significative presence of pathogenic species, and according to Hu et. al. (2024)[39] highlight that the snakes may serve as a natural reservoir of zoonotic diseases.*Clostridia* gender is directly associated with human and animal diseases [40], such as *Salmonella* [41] and *Actinomyces* that is known as a potentially pathogenic group [42]. Meanwhile *Bacteroides* comprehend mainly commensal bacteria [43]

Beyond the difference in the bacterial composition of the microbiota, the samples presented a decrease in the number of OTUs and also the shared reinforced by the alpha diversity that

presents the low Shannon index in the samples after the fasting, corroborating with the result found in *Python morulus* [37]

The results presented in this study for the experimental group of obese snakes demonstrated a pattern with the other groups before fasting, although has no significant alterations on the bacterial microbiota composition after prolonged fasting, practically keeping the same configuration, which results that reflect the lack of statistic results and on proximity of the samples on the charts of PCA (Fig. 5) and ANOVA (Fig. 6).

Most of the studies related to snakes have been aimed to study ecology and venom prospection and a few aimed to study the snake's microbiota. Although the advancement of molecular tools helped Hill and collaborators (2008) [44] to determine, for the first time, the bacterial diversity present in the intestine of *Crotalus horridus* and *Agkistrodon piscivorus*, using the gel electrophoresis technique of denaturing gradient (DGGE) from the 16s rDNA gene, resulting in a dominance of the phylum Bacteroidetes and Firmicutes.

The phylum Firmicutes, which is related to getting energy, is favored by a high-fat diet and present species with genes associated with membrane transport, transcription, and cellular motility. In another way, this type of diet causes a decrease in the Bacteroidetes phylum [45].

In the case of snakes considered obese, after prolonged fasting there has been an increase of 5,5% in the proportion of Bacteroidetes and a decrease of 0,6% in Firmicutes when deprived of nutrients. It is worth empathizing that in all the experimental groups the proportion of Bacteroidetes was bigger than the Firmicutes.

Although what draws attention to these results is the proportion of Proteobacteria, which is a phylum with a big variety of pathogenic species such as *Salmonella, Klebsiella*, *Pseudomonas,* and *Brucella*, this last, acting as a differential on linear discrimination analysis of the experimental group of thin snakes, is that this phylum has a decrease of 8,6% in its proportion on obese snakes after fasting.

At this point, it's important to highlight that the research that evaluated samples directly from the gastrointestinal tract or fecal content obtained as a result of a dominance of Bacteroidetes and Firmicutes phylum [37,32] differentially of research that worked with cloaca samples that have obtained a dominance of Proteobacteria [14,31] This suggests that the predominance of this phylum on cloaca can be a normal condition.

Although most of the studies, in the sense of understanding the role of the microbiota and your relationship with the host health, have been only directed to humans or mice for an application for humans [46,47,48]. On this hand, this research indicates that the abundance of Proteobacteria phylum on the gastrointestinal tract is low in healthy individuals. The increase or the stability of this phylum can be an indicator of metabolic disturbances or intestinal inflammations. It also affirms that an equilibrate microbiota has a good symbiotic interaction with the immunological system of the host, which confers the capability of suppressing the proliferation of the Proteobacteria phylum [49].

So that it is possible to confirm the hypothesis of metabolic disturbances or intestinal inflammation in the experimental group of obese snakes, it would be necessary complementary research in order to verify if the results of the cited research are applicable to snakes.

About the *Brucella* genre that appears as a differential group on LDA of the experimental group of normal snakes, there is just one report of this genre in reptiles. Ali and collaborators (2018)[50] tested the presence of antibodies of *Brucella* in lizards, snakes, and turtles (n=34) in

Pakistan. Of the total number of reptiles in the sampling, ten turtles were positive for the presence of the antibody of *Brucella*. Therefore, complementary exams need to be done to complete the results found in this present study, in that way, the animals can be forwarded for due treatment. It's good to highlight that this is the first report of the *Brucella* genre in snakes.

5. CONCLUSION

With the chart of body score classification of the corporal condition proposed by the present study, it was possible to separate the experimental groups of this research in a way that the results showed as satisfactory in face of the objectives that were proposed. Beyond that, furthermore, becomes an auxiliary tool for snake management of *Crotalus durissus* species in captivity.

The composition of the cloacal microbiota of *Crotalus durissus* snakes with different body scores kept in captivity on UCDB Vivarium presented a dominance of the phylum Proteobacteria and Bacteroidetes before the fasting with a decrease in the proportion of Proteobacteria phylum on the groups of thin and normal snakes after 50 days of fasting and with no significant alterations on the proportion of Proteobacteria on the experimental group of obese snakes. Beyond the decrease in the number of OTUs present in the samples after the prolonged fasting.

When comparing the experimental groups for the number of OTUs, the obese snakes showed a bigger amount of observed OTUs than normal, and the thin before and after prolonged fasting, which do not significate a differencein species richness, once that was the only group that doesn't present statistical significance.

With this study, it was possible to conclude that prolonged fasting can influence the cloacal microbiota of the snake *Crotalus durissus*, except in theindividuals considered obese, which requires complementary exams to suggest a metabolic syndrome and intestinal inflammation.

It's important to highlight that this present study, beyond the characterization of the cloacal bacterial microbiota, is the first to relate the results with the body score of the corporal condition of *Crotalus durissus*.

Ethical Approval

The present study was accomplished according to the Authorization and Information System of Biodiversity (Authorization and Information System in Biodiversity - SISBIO) under registration 47695-1, of (Environment Institute of Mato Grosso do Sul - IMASUL) and the Committee of Ethics and Utilization of Animals (CEUA) of Don Bosco Catholic University, under protocol nº022/2017

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