Prebiotics mannan-oligosaccharides accelerate sexual maturity in rats: A randomized preclinical study

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Abstract

Background and Aim: The prebiotics, mannan-oligosaccharides (MOS), demonstrate the ability to increase probiotic microorganisms and fixation and removal of pathogens associated with chronic systemic inflammation in the digestive system. Inflammatory processes play an important role in modulating the brain-intestinal axis, including maintaining male reproductive function and spermatogenesis and regulating stress. The aim of the present study was to evaluate the action of MOS on testosterone and corticosterone concentrations and the reproductive system development of rats in the growth phase as an animal model.

Materials and Methods: In total, 128 male rats were used, randomly divided into four experimental groups (n=32): Control; MOS 1; MOS 2; and MOS 3. From each group, eight animals were sacrificed in four experimental moments (14, 28, 42, and 56 days, respectively, moments 1, 2, 3, and 4) and hormonal measurements and histological evaluations were performed.

Results: The results revealed the effect of diet, MOS, and timing on testicle weight (p<0.05). At moments 3 and 4, the groups supplemented with MOS showed higher concentrations of testosterone and decreased corticosterone levels throughout the experimental period. Groups supplemented with MOS showed an increase in the frequency of relative sperm and sperm scores. The radii of the seminiferous tubules presented a significant statistical effect of the diet, moments, and diet + moment interaction.

Conclusion: It was concluded that the three different MOS prebiotics brought forward sexual maturity.

Keywords: corticosterone, murine, prebiotic, reproductive system, testosterone, yeast.

Introduction

The prebiotics, mannan-oligosaccharides (MOS), are mostly complex molecules that are linked to portions of proteins derived from the cell wall of the yeast *Saccharomyces cerevisiae* [1] and their production originates mainly from the sugar and alcohol, beer, and bakery sectors [2]. These functional foods, undigested by enzymes, salts, and acids produced by the body, are selectively fermented by microorganisms of the gastrointestinal (GI) tract [3]. Supplementation with prebiotics has been studied due to their ability to increase probiotic microorganisms, especially the genera *Bifidobacterium* and *Lactobacillus*, beneficially modulating the native microbiota [4], as well as increasing short-chain fatty acid (SCFAs) production, decreasing intestinal pH, and beneficially modulating immune, neurobehavioral, and metabolic functions in animals and humans [5].

When supplemented in the appropriate dose and frequency, prebiotics confer immediate beneficial effects associated with the fixation and removal of possible pathogens in the digestive system [6]. These pathogenic agents containing endotoxins are found at very high levels in the mammalian intestine, inducing chronic systemic inflammation [7]. Inflammatory processes play an important role in the etiology of various...
diseases, and involve multiple pathophysiological systems in the modulation of the brain-intestinal axis, among which is the endocrine system, notably, and gonadal hormones [8,9]. The androgens, testosterone, and corticosterone are steroid hormones involved in several processes, including maintaining male reproductive function, spermatogenesis, and stress regulation [10,11], while estrogens play an important endocrine role in the physiology and pathology of the GI tract, including the regulation of motor and sensory function [12]. Low testosterone levels are associated with an increase in pro-inflammatory factors and the administration of testosterone reduces the levels of these factors, reinforcing the anti-inflammatory effect of testosterone. This indicates that the uncontrolled inflammatory responses in the host contribute to the testicular atrophy phenotype in older mice [13].

A study using prebiotic and probiotic supplementation in the diet reported improvements in the volume of the ejaculate, the concentration of spermatozoa, the number of ejaculated spermatozao, motility, and the percentage of typical forms, when administered to patients with idiopathic oligoasthenoteratospermia. In addition, supplementation increased follicle-stimulating hormone (FSH), LH, and blood testosterone levels [13].

Therefore, the objective of the present study was to evaluate the action of different MOS on the plasma concentrations of testosterone and corticosterone, as well as the development of the reproductive system, using histomorphometric parameters of reproductive organs of rats in the growth phase, as an animal model.

Materials and Methods

Ethical approval

This study was carried out after approval from the Ethics Committee on Animal Use (Protocol no. 1175 and 1177), from the Universidade do Oeste Paulista, Presidente Prudente, SP, Brazil.

Study period and location

The study was carried out from July 2017 to June 2018 at the Biotério Experimental and laboratory analyses were carried out in the Department of Functional Sciences at the Universidade do Oeste Paulista, located in Presidente Prudente, State of São Paulo (Brazil).

Animals, care and experimental design

One hundred and twenty-eight male Wistar rats, 21 days of age, with a mean initial body mass of 46.17±4.99 g, were kept in individual cages, under the same standard lighting conditions (light/dark cycle of 12/12 h) and controlled temperature of 22±1°C. The diets and drinking water were provided at will during the 56 days of the experimental period (Figure-1). Concealment of allocation, treatment, and animal management strategy, blinding during and after the intervention, and concealment of evaluations and analysis of results were performed to reduce bias in the study [14].

Four die were formulated (Table-1), comprising the treatments: Control (C), basal diet (SD); MOS1, SD diet supplemented with 1% of the MOS 1; MOS 2, SD diet supplemented with 10 g.Kg⁻¹ of the MOS 2; and MOS 3, SD diet supplemented with 4 g.Kg⁻¹ of the MOS 2; and MOS 3, SD diet supplemented with 10 g.Kg⁻¹ of the MOS 3. Vitamin mix/Kg: Nicotinic acid, 30 mg; pantothenade, 15 mg; pyridoxine, 6 mg; thiamine, 5 mg; riboflavin, 6 mg; folic acid, 2 mg; biotin, 0.2 mg; Vitamin B₁₂, 25 mg; Vitamin E, 75 IU; Vitamin A, 4000 IU; Vitamin D₃, 1000 IU; Vitamin K, 900 mg; Choline, 1000 mg.; and Mineral mix mix/Kg: Calcium, 5000; Phosphorus, 1.561; Potassium, 3600; Sulfur, 300; Sodium, 1019; Chlorine, 1.574; Magnesium, 507; Ferro, 35; Zinc, 30; Manganese, 10; Copper, 6; Iodine, 0.2; Molybdenum, 0.15; Selenium, 0.15.

MOS: Mannan-oligosaccharides

SD diet supplemented with 1% of the MOS 1 prebiotic (MOS derived from the cell wall of the yeast S. cerevisiae, strain 1026, composed of 17% α-mannan [Bio-Mos, Alltech Inc., Brazil]); MOS 2, SD diet supplemented with 0.4% of the prebiotic MOS 2 (Active fraction, α-1,3 and α-1,6 mannanprotein, derived from a mannan oligosaccharide, presenting 30% α-mannan [S. cerevisiae, Alltech Inc., Brazil]); and MOS 3, SD diet supplemented with 1% of the MOS 3 prebiotic (β-glucans, mannans, mannose polymers, chitins [glycosamine polymers], and galactans [galactose polymers] derived from the yeast strain S. cerevisiae [ImmunoWall®, ICC Brazil]). The dosages of prebiotics supplemented in the diets were as recommended by the manufacturers. The animals were randomly assigned to the four experimental groups.
Euthanasia and collection of samples

Water and diet consumptions were measured every 3 days and body weight was measured weekly. At each moment: 1, 2, 3, and 4 (respectively, 14, 28, 42, and 56 days after the beginning of the experimental period), eight animals from each of the four experimental groups were anesthetized intraperitoneally, with a dosage of 30 mg.Kg\(^{-1}\) of live weight of Tiopental 50 mg.mL\(^{-1}\) (Thiopenxat, Cristália – Produtos Químicos Farmacêuticos Ltda. – São Paulo/SP, Brazil) [16]. Blood samples were collected through cardiac puncture, centrifuged to obtain plasma, and later transferred to microtubes and stored in a freezer at -80°C. After blood collection, the animals were euthanized by exsanguination [17]. After death, the organs (testicles and adrenal glands) were removed and weighed on a digital analytical scale (Shimadzu - model AY220, Japan).

Determination of plasma levels of corticosterone and testosterone and histological analysis of the testicles and adrenals

Circulating plasma corticosterone levels were measured in duplicate using the DPC-MedLab commercial solid-phase Coat-A-Count® Rat-Corticosterone Kit (Ref. TKRC1-100 tubes; Los Angeles-California - Appendix A) according to the manufacturer’s instructions. Total plasma testosterone was assayed using a direct plasma radioimmunoassay technique (Coat-a-Count, DPC, Los Angeles, USA). The lower limit of sensitivity was 0.15 nmol/L, and the assay coefficient of variation was 10.4%. The assay coefficient of variation was 10.4%. The mean plasma dosages as dependent variables. Analysis of variance was performed to assess whether the resulting lines met the conditions of linearity, that is, the slope is different from zero and there is no lack of adjustment. The differences among the slopes of the straight lines of the four treatments/diets were analyzed by the Student’s t-test. The combinations of diet and moments for testicular and adrenal glands weights and radii of the seminiferous tubules were analyzed by two-way analysis of variance with a Tukey post hoc test for main effects (diet and moments) and interaction (diet x moments). The data of the slide readings were calculated, individually, through medians of the scores. To determine whether the frequencies of the median scores differed among experimental groups and the four moments, the Kruskal–Wallis non-parametric test was used. All analyzes were conducted using Bioestat 5.3 software, adopting a 5% significance level.

Results

The results of CFI (g) and percentage WG in the groups supplemented with MOS were found to be lower than in the control group (p>0.05). The mean WC (mL) in the groups supplemented with MOS was higher than in the control group (p<0.05) (Table-2).

There were significant differences in the effect of the diet (p<0.05) on the weight of the adrenal glands only at moments 1 and 2. However, there were significant differences for diet effect and moment effect (p<0.05), no statically differences for moment interaction on the weight of the testicles were seen (p<0.05) (Table-3).

However, the groups supplemented with MOS presented higher concentrations of testosterone at moments 3 and 4 of the experimental period (p<0.05) (Figure-2).
The simple linear regression of the evolution of the corticosterone concentrations as a function of time showed a significant difference (p<0.05) between the angular coefficients of the analyzed lines (Beta), between the groups supplemented with MOS and control. The evolution of corticosterone concentrations in the treatment/control diet increased compared to those treated with different MOS (Figure-3).

The results of the frequencies of the spermatids and spermatozoa relative scores of the histological sections of the testicles are shown in Tables-4 and 5. In the parameter frequency of spermatids relative score, the statistical analysis revealed significant differences between the groups supplemented with MOS and the control group (p<0.01) at moments 1, 2, and 3 (p<0.05), while at the moment 4 no statistical differences were found between the groups supplemented with MOS and the control group (p>0.05). The results of the frequencies of spermatozoa relative score at moments 2, 3, and 4 of the supplemented groups differed significantly (p<0.05) from the control group.

The radius of the seminiferous tubules presented a significant statistical effect of the diet, moments, and diet + moment interaction (Table-6). When comparing the diets, it was found that the MOS showed significant effects on radii of the seminiferous tubules, with higher averages for MOS 3, followed by MOS 2 and MOS1. The interaction among diets and moments is shown in Figure-4.

Discussion

The supplementation with different MOS did not alter the feeding behavior, food intake, or WG of the rats. Studies in rodents have demonstrated that supplementation with fermentable dietary fiber in the diet can form a link between the result of microbial fermentation in the lower part of the intestine and the metabolic consequences, such as decreased food intake and WG, due to the beneficial modulation of secretion of intestinal peptide similar to glucagon1, glucose-dependent tropic insulin polypeptide, YY peptide, and/or ghrelin [19]. Other studies also did not observe a decrease in food intake or WG in rats with a

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**Table-2:** CFI (g), percentage WG-%, and average WC (mL) of treatments/diets: Control (C), MOS1, MOS 2, and MOS 3, in rats in growth phase. Values presented as mean±standard deviation (n=8 group).

| Parameters | Control | MOS 1 | MOS 2 | MOS 3 |
|------------|---------|-------|-------|-------|
| CFI        | 1053.71 | 994.76 | 977.97± | 976.92± |
|            | ±38.85  | ±40.51 | 58.51 | 60.66 |
| WG %       | 550.5±  | 543.6± | 540.8± | 536.9± |
|           | 12.1    | 11.7   | 12.3   | 9.0   |
| WC         | 26.43±  | 27.42± | 27.87± | 27.61± |
|           | 5.21    | 4.98   | 5.43   | 4.75   |

†There was no statistically significant difference between groups by analysis of variance at 5% probability. ††Control and experimental diets/groups: Control (C): Basal diet (SD); MOS 1, SD diet supplemented with 1% of the MOS; MOS 2, SD diet supplemented with 4 g.Kg-1 of the MOS; and MOS 3, SD diet supplemented with 10 g.Kg-1 of the MOS 3. MOS=Mannan-oligosaccharides, CFI=Cumulative food intake, WG=Weight gain, WC=Water consumption

**Figure-2:** Histograms showing the plasma testosterone concentrations (µg/dL) at the four sample moments (1 [14 days]; 2 [28 days]; 3 [42 days]; and 4 [56 days]), of rats in the growth phase submitted to treatment/control diets and with three different mannan-oligosaccharides. One-way analysis of variance with Tukey’s test during each moment (p<0.05).

**Table-3:** Effects of treatments/diets with MOS (prebiotics), at four sampling moments, on the weight of the adrenal glands and testicles of rats in the growth phase (means±SD).

| Parameters | Diets/groups†† | Moments†† |
|------------|----------------|-----------|
| Adrenal glands (g) | Control | MOS1 | MOS2 | MOS3 | 1 | 2 | 3 | 4 |
|              | 0.0357±0.0039 | 0.0343±0.0067 | 0.0350±0.0051 | 0.0361±0.0023 |
| MOS1        | 0.0450±0.0127 | 0.0445±0.0065 | 0.0434±0.0094 | 0.0432±0.0087 |
| MOS2        | 0.0461±0.0079 | 0.0449±0.0054 | 0.0448±0.0081 | 0.0438±0.0091 |
| MOS3        | 0.0525±0.0127 | 0.0454±0.0059 | 0.0452±0.0056 | 0.0443±0.0075 |
| Testicles (g) | Control | MOS1 | MOS2 | MOS3 | 1 | 2 | 3 | 4 |
|              | 0.4752±0.0643 | 1.2584±0.1054 | 1.6894±0.1540 | 2.1884±0.1619 |
| MOS1        | 0.5563±0.0727 | 1.3272±0.1784 | 1.7663±0.1221 | 2.2572±0.1254 |
| MOS2        | 0.5627±0.0709 | 1.3292±0.1759 | 1.7820±0.1239 | 2.3692±0.1736 |
| MOS3        | 0.5695±0.0505 | 1.3910±0.1773 | 1.8463±0.1660 | 2.4090±0.1949 |

†Sample moments: 1 (14 days); 2 (28 days); 3 (42 days); and 4 (56 days) after the beginning of the experimental period. There was no significant difference between the groups in each moment, using the Tukey test at 5% probability.

†Control and experimental diets/groups: Control (C): basal diet (SD); MOS 1, SD diet supplemented with 1% of the MOS 1; MOS 2, SD diet supplemented with 4 g.Kg-1 of the MOS 2; and MOS 3, SD diet supplemented with 10 g.Kg-1 of the MOS 3. MOS=Mannan-oligosaccharides
weight considered healthy after prebiotic, supplementation in the diet [20,21], as well as in humans [22,23]. However, in obese or overweight rodents, prebiotics have been shown to assist in weight control by decreasing food intake [24].

WC did not alter with the inclusion of different MOS in the diet, as demonstrated in other studies with the use of the probiotics galursan HF 7K, B-GOS®, Larch gum (from Larix occidentalis), inulin, and oligofructose [23].

The weight of the adrenal glands had no effect on time, moments, or diet. However, the results revealed the effect of diet and time on testicle weight. Although only the weight of the testicles analyzed did change with the supplementation of MOS in the diet, the evaluations for the plasmatic levels of corticosterone and testosterone hormones were beneficially modified in plasma from the groups supplemented with the three different MOS. This may be the result of the indirect effect of these indigestible compounds, through the beneficial modulation of the amounts of beneficial bacteria classified as probiotics (mainly of the genera Bifidobacterium and Lactobacillus), in the complex community of microorganisms that resides in the intestinal tract of mammals [3,25]. These probiotic bacteria process insoluble dietary fibers, prebiotics, into SCFAs that can be directly or indirectly involved in communication along the microbiota-gut-brain axis due to their neuroactive properties and their effects on other intestinal-brain signaling pathways, including the immune and endocrine systems [25,26]. In addition, many studies demonstrated that testosterone in male rats was significantly predictive by testicular weight [27] and similar associations between testis
The results revealed anticipation of an increase in the number of spermatic cells, spermatozoa, in the groups supplemented with MOS. This finding is consistent with previous studies [27-29]. The results also revealed an increase in the number of spermatogonia in the groups supplemented with MOS, which suggests a positive effect on spermatogenesis. Additionally, the study confirmed that MOS has a beneficial effect on the hypothalamic-pituitary-gonadal axis, inhibiting the secretion of gonadotropin-releasing hormone from the hypothalamus and decreasing the release of gonadotropin from the pituitary gland. These findings are consistent with previous studies [34,35], mainly in stress situations [37].

The results also demonstrated that MOS supplementation had a positive effect on the frequency of spermatids in groups supplemented with MOS. This beneficial action in the MOS-treated groups was also observed in the groups treated with MOS. This finding is consistent with previous studies [38], which reported an increase in the frequency of spermatids in groups supplemented with MOS.

A reduction in plasma testosterone concentration may be due to the indirect action of the monocytes and macrophages present in the plasma. MOS supplementation may also have a beneficial effect on the immune system, reducing the inflammatory response and improving the ability of the body to deal with infections. This may contribute to an increase in plasma testosterone concentration in the MOS-treated groups.

On the other hand, when there is an imbalance in the intestinal microbial community due to diet or stress, bacterial species such as Gram-negative bacteria can induce behaviors associated with increased anxiety and stress responses. These behaviors may be linked to changes in the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the release of glucocorticoids (cortisol in humans and rodents) and is involved in the regulation of the stress response. MOS supplementation may have a beneficial effect on the HPA axis, improving the ability of the body to deal with stress and reducing the release of glucocorticoids.

In summary, the results of this study suggest that MOS supplementation has a beneficial effect on spermatogenesis and plasma testosterone levels in male rats. The results also highlight the importance of maintaining a healthy intestinal microbial community and reducing stress to maintain optimal reproductive health.
with previous studies in which glucocorticoids affect spermatogenesis and can cause meiotic arrest in germ cells, since glucocorticoids have receptors located in Leydig cells and in spermatocytes in meiosis prophase I [40,41]. The regulation of spermatogenesis depends on FSH and testosterone. While FSH plays a major role in the development of the immature testicle, causing the proliferation of Sertoli cells and the progression of spermatogonia A to spermatogonia B, testosterone alone can maintain complete spermatogenesis [42]. FSH and testosterone have receptors on Sertoli cells and modulate spermatogenesis by regulating the function of Sertoli cells [43].

Together these data indicate an acceleration of puberty in male rats treated with MOS prebiotics due to the change in the steroidogenic axes and the increased spermatogenesis at 51 and 65 days of age. This may also be due to the decrease in corticosterone levels, which have an inhibitory effect on reproduction by intervening in the HPA axis [35], since corticosterone acts on the intracellular receptors that interact with transcription factors and is capable of regulating gene expression of enzymes and causing a variety of responses [39].

We also observed greater development of seminiferous tubules in the groups treated with MOS, which reinforces the hypothesis that treatment with the prebiotic accelerated the sexual maturity of the rats. A study demonstrated that glucocorticoids negatively influence puberty of male rats, since the application of dexamethasone led to a disorder in the morphology of seminiferous tubules, presenting an irregular, and disorganized basal membrane in some areas, with stratified germinal epithelium cells showing vacuoles in their cytoplasm. The period of puberty is critical and corticosterone has a great influence in this phase on the secretion of testosterone and in the proliferation of Sertoli and germ cells [42].

These results have important clinical relevance since the MOS prebiotic in pubertal rats led to a change in steroidogenesis, representing a decrease in cortisol and an increase in plasma testosterone, improving the reproductive profile, verified by the higher proportion of spermatids and spermatozoa in treated animals and greater development of seminiferous tubules. In view of the results observed, we suggest that further studies should be carried out in adult and older rats, seeking improvement in the endocrine and reproductive profile in humans to bring benefits to the health of individuals.

Other studies report that supplementation with prebiotics demonstrated direct benefits, for example, carbohydrate structures similar to host glycans can block adhesion to host cells [44] or indirect benefits by encouraging the proliferation of intestinal microbiota bacteria whose metabolites directly affect the intestinal environment or host gene expression, for example, the effect of SCFAs on intestinal pH and on the modulation of immune functions and regulation of axis development (HPA) [45,46]. The role of the intestinal microbiota was evidenced in germ-free mice, where mild restrictive stress induced an exaggerated release of corticosterone and adrenocorticotrophic hormone compared to specific pathogen-free controls. This has since been replicated independently, again after a mild stressor, both in mice [47] and in rats [48].

Although the rats used in the current experiment were not purposely exposed to stressful procedures, all animals were regularly subjected to routine maintenance or monitoring procedures, such as personnel entering the animal room, moving about, and cleaning cages, feeding and fasting cycles, body weight collection, and physical examination, and although these procedures can be considered incidental so that their effects on the welfare of laboratory animals can be neglected by the ethical review committees, these routine procedures can cause significant stress to animals, although apparently benign [49]. Studies report that handling murines leads to a significant increase in blood corticosterone concentration [50,51], as well as changing the cage [50] and the environment [52]. Such hypothesis seems to be strengthening by the work of Mucignat-Caretta et al. that demonstrated how young mice are more responsive to mild stressors, such as environmental ones, with higher corticosterone plasmatic peaks [53]. Corticosterone, a result of the rapid activation of the HPA axis, is considered a gold standard biomarker of acute stress [54,55].

Finally, environmental factors such as diet, drugs (e.g., antibiotics), pathogens, and toxic and environmental pollutants can directly and indirectly stress the

### Table 6: Measurements of the radii of the seminiferous tubules of testicles of rats in the growth phase supplemented with different MOS. Values expressed as mean±standard deviation.

| Groups   | Moments†/Seminiferous tubules (µm) |
|----------|-----------------------------------|
|          | 1       | 2       | 3       | 4       |
| Control  | 33.02±0.99^A | 43.12±1.32^a | 48.73±1.01^c | 54.83±1.86^f |
| MOS 1    | 30.90±0.76^A | 50.21±1.85^CD | 56.44±1.00^e | 63.65±2.76^G |
| MOS 2    | 31.07±0.71^A | 53.11±1.30^DE | 60.96±2.05^f | 65.57±3.32^G |
| MOS 3    | 31.37±0.71^A | 54.29±1.23^f | 61.95±1.91^f | 65.72±3.15^G |

1Sample moments: 1 (14 days); 2 (28 days); 3 (42 days); and 4 (56 days) after the beginning of the experimental period. Control and experimental diets/groups: Control (C): basal diet (SD); MOS 1, SD diet supplemented with 1% of the MOS 1; MOS 2, SD diet supplemented with 4 g.Kg^-1 of the MOS 2; and MOS 3, SD diet supplemented with 10 g.Kg^-1 of the MOS 3. Data were analyzed by two-way analysis of variance with a Tukey post hoc test at 5% probability. Different letters indicate significant statistical differences. MOS=Mannan-oligosaccharides.
intestinal microbiota, through alterations in inflammation, oxidative stress, immune function, and the GI environment. The composition of the diet, in particular, is an important factor that influences the intestinal microbiota, due to the intake of nutrients that directly affect the types and nutrients available to the intestinal microbes and the countless effects of different nutrients on the physiology of the host [56,57]. All animals were exposed to the same possible stressors, which should result in the same blood concentrations of corticosterone; however, the groups supplemented with the three different MOS demonstrated a significant reduction in corticosterone, indicating a beneficial effect in response to these possible stressor agents.

In addition, the radii of the seminiferous tubules showed greater development in the groups treated with MOS. These results confirm the previous data and reinforce the hypothesis that treatment with the prebiotic accelerated the sexual maturity of the rats. A study demonstrated that hormones of the glucocorticoid class, such as corticosterone, negatively influence the puberty of male rats and lead to a disorder in the morphology of the seminiferous tubules, presenting an irregular and disorganized basal membrane in some areas, with stratified germina epithelium cells presenting vacuoles in their cytoplasm. The period of puberty is critical and corticosterone has great influence in this phase in the secretion of testosterone and in the proliferation of Sertoli and germ cells [42]. Therefore, the decrease in blood corticosterone concentration observed in the current study in groups supplemented with the three different MOS may be responsible for the increase in testosterone and, indirectly, in the maturation of seminiferous tubules.

These results have important clinical relevance since the prebiotic MOS led to a change in steroidogenesis in pubertal rats, representing a decrease in cortisol and an increase in plasma testosterone, improving the reproductive profile, verified by the higher proportion of spermatids and spermatozoa in treated animals and greater development of seminiferous tubules. In view of the observed results, we suggest that other studies should be carried out in adult and older rats, seeking improvement in the endocrine and reproductive profile in humans to bring benefits to the health of individuals.

**Conclusion**

It was concluded that the three different MOS prebiotics tested in this experiment anticipated sexual maturity in growing Wistar rats through higher plasma concentrations of testosterone and reduced cortisol, as well as increased spermatid production and, consequently, higher spermatozoa production.

**Authors’ Contributions**

HB: Made substantial contributions to the conception or design of the work, preparation of diets, the acquisition, analysis, and interpretation of data; drafted the work, and approved the submitted version. LER: Made substantial contributions to the analysis and interpretation of data; drafted the work and approved the submitted version. MMK: Made substantial contributions to the conception or design of the work and animal management. RK: Made substantial contributions to the design, analysis and interpretation of data, and approved the submitted version. HRSC: Made substantial contributions to the acquisition, analysis and interpretation of data and approved the submitted version. MNR: Made substantial contributions to the acquisition, analysis and interpretation of data measurements, and approved the submitted version. OCS: Made substantial contributions to the histological analysis and interpretation of data, and approved the submitted version. RG: Made substantial contributions to the analysis and interpretation of data and approved the submitted version. ICG: Made substantial contributions to the analysis or design of the work and interpretation of data; drafted the work, and approved the submitted version. All authors have read and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.

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