INFLUENCE OF *Saccharomyces cerevisiae* ON TESTICULAR AND ACCESSORY ORGAN INDICES IN MALE RATS

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ABSTRACT

Saccharomyces cerevisiae (*S. cerevisiae*) is commonly added to animal diets for its probiotic and nutritional benefits, particularly in promoting digestion and growth. However, its effects on male reproductive health are not well understood. This study evaluated how different dietary levels of *S. cerevisiae* affect testicular and accessory organ development in male Wistar rats. Twenty male rats were divided into four groups and supplemented with 0 (control), 0.5, 1.0, or 1.5 g/kg body weight of *S. cerevisiae* for 35 days. Final assessments included body weight, organ weights, and testicular morphometry. Rats receiving higher yeast doses gained significantly less weight. Absolute testicular and epididymal weights declined with increasing supplementation, while relative weights increased—likely due to reduced overall body mass. Testicular circumference, length, diameter, and volume also decreased in a dose-dependent manner. Non-reproductive organs such as the liver, kidney, and spleen showed reduced absolute weights without changes in relative values. These results indicate that excessive *S. cerevisiae* may impair reproductive organ development, highlighting the need for regulated supplementation. Further research is needed to understand the underlying mechanisms and potential long-term reproductive effects.

Keywords: Dietary supplementation, male fertility, organ weight, *Saccharomyces cerevisiae*, testicular morphology

INTRODUCTION

Male reproductive health plays a vital role in the continuity of animal species, relying heavily on the proper structure and function of the testes and accessory reproductive organs. In recent years, dietary strategies aimed at improving fertility have gained considerable interest, particularly those involving probiotics and yeast-based supplements (Blancafort and Llácer, 2023; Khani *et al*., 2025). Among these, *Saccharomyces cerevisiae*—a well-known yeast—has received significant attention due to its beneficial effects on gut health, nutrient utilization, and immune function in both livestock and laboratory animals (Perricone *et al*., 2022; Xu *et al*., 2025). Its inclusion in animal feed has been associated with enhanced feed efficiency and improved overall growth performance (Pang,*et al*., 2022; Lin *et al*., 2023).

Despite the widespread use of *S. cerevisiae* as a dietary additive, most of the existing studies

have focused on its impact on general health and growth, especially in monogastric species. In contrast, relatively few studies have looked closely at how it affects the male reproductive system. When reproductive effects are reported, the emphasis is often on hormone levels or sperm quality, leaving a significant knowledge gap regarding the structural and anatomical responses of reproductive organs—particularly the testes and epididymis (Emmanuel *et al*., 2019; Besseboua and Ayad, 2021; Chao *et al*., 2023). Some emerging evidence even suggests that high doses of yeast supplementation might disrupt endocrine function or cause subtle tissue damage in reproductive organs (Omar *et al*., 2018; Ahn and Jeung, 2023), though such findings have not been consistently validated, especially in rodent models.

This gap is particularly important given the growing interest in functional feed additives and the need to balance their benefits against potential reproductive risks. Specifically, how different doses of *S. cerevisiae* affect organ development, both in absolute size and relative to body weight remains unclear. Understanding whether such changes indicate healthy adaptation, toxicity, or hormonal imbalance is essential, especially for developing safe and effective nutritional interventions.

Based on this background, the current study set out to examine the effects of graded dietary supplementation of *S. cerevisiae* on the reproductive organs of male rats. We evaluated both absolute and relative organ weights, along with testicular morphometry and body weight changes, to provide a more complete picture of how *S. cerevisiae* supplementation might influence male reproductive health. Our findings aim to inform future research and guide safe usage levels of *S. cerevisiae* in animal nutrition programs.

MATERIALS AND METHODS

Experimental Animals and Housing Conditions

Twenty healthy male albino Wistar rats, aged between 7 and 8 weeks and weighing approximately 100–110 grams, were used for this study. The animals were sourced from the Animal House of the Department of Veterinary Physiology and Biochemistry, University Nigeria, Nsukka. Upon arrival, the rats were housed in clean, well-ventilated polypropylene cages (Jiangsu Jining Xinda Medical Equipment Co., Ltd., China), with five animals per cage. They were maintained under standard laboratory conditions: a 12-hour light/dark cycle, ambient temperature of 22 ± 2 °C, and relative humidity of 55–65%.

Throughout the 28-day experimental period, all animals had free access to clean drinking water and were fed a standard commercial rat diet (Vital Feeds®, Grand Cereals Ltd., Jos, Nigeria). The animals were allowed a 14-day acclimatization period prior to the commencement of the experiment. All procedures involving the rats were carried out in line with institutional ethical guidelines.

Experimental Design and Supplementation

The rats were randomly assigned into four groups of five animals each (groups A–D). Group A served as the control and received the basal diet and distilled water only. Group B, C and D received the basal diet supplemented with *S. cerevisiae* at 0.5, 1.0 and 1.5 g/kg body weight respectively.

The yeast supplement used was a commercially available active dry form of *S. cerevisiae* (Levucell® SC, CNCM I-1077 strain, Lallemand Animal Nutrition, Canada). It was freshly measured, dissolved in clean drinking water and administered daily through oral gavage according the dosage per each group.

Body Weight Monitoring and Tissue Collection

Body weights of the rats were recorded at the beginning (Day 0) and end (Day 28) of the experiment using a digital weighing balance (Ohaus Scout SPX2201, Ohaus Corp., USA). The difference between the two readings was used to determine total weight gain.

At the end of the study, the animals were lightly anesthetized using ether inhalation and humanely sacrificed by cervical dislocation.

Organ Weights and Morphometric Evaluation

***In situ* testicular circumference** was measured using a non-elastic tailor's measuring tape (Butterfly™, India) before organ removal.

***Ex situ* testicular length and diameter** were measured after removal using a Mitutoyo 530-122 Vernier caliper (Mitutoyo Corp., Japan).

A midline abdominal incision was made to expose and carefully excise the internal organs, including the testes, epididymis, liver, kidneys, and spleen.

Each excised organ was cleaned of surrounding fat and connective tissue, gently blotted with filter paper, and weighed using a precision digital scale (Kern PFB 200-3, Kern & Sohn GmbH, Germany). Relative organ weight was calculated by expressing the organ weight as a percentage of the animal's final body weight according to the formula:

$$OSI = \frac{Organ\ weight}{Weight\ of\ Animal} \times 100$$

Testicular volume was estimated by water displacement in a **20 mL graduated measuring cylinder (Pyrex®, Corning Inc., USA).**

Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). Group differences were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. Analyses were conducted using **IBM SPSS Statistics version 25.0** (IBM Corp., Armonk, NY, USA).

RESULTS

The findings on how *S. cerevisiae* supplementation affected body weight, testes, epididymides, liver, kidney and spleen weights of the rats are presented in Tables 1,2 and 3.

Effect of *Saccharomyces cerevisiae* on Body Weight Changes

At the beginning of the study, all groups started off with similar body weights (Table 1), showing no significant differences ($p > 0.05$). However, as the experiment progressed, a clear trend emerged. Rats that received *S. cerevisiae* supplementation especially at higher doses gained significantly less ($p < 0.05$) weight compared to the control group. Group A (control) had the highest final body weight (233.14 ± 7.57 g) and weight gain (124.42 ± 3.81 g), while group D (1.5 g/kg BW) recorded the lowest values (185.10 ± 1.74 g and 80.66 ± 2.15 g, respectively).

Table 1: Effects of *Saccharomyces cerevisiae* on Body Weight Gain Changes in Male Rats

Parameter	Group A (control)	Group B (0.5 g/kg bw)	Group B (1.0 g/kg bw)	Group D (1.5 g/kg bw)
Body Weight (g)				
Initial weight (g)	108.72 ± 6.16	103.62 ± 2.15	104.20 ± 2.04	104.44 ± 0.71
Final weight (g)	233.14 ± 7.57^{a}	207.16 ± 5.08^{b}	195.00 ± 3.61^{bc}	185.10 ± 1.74^{c}
Weight gained (g)	124.42 ± 3.81^{a}	103.54 ± 3.48^{b}	90.80 ± 2.64^{c}	80.66 ± 2.15^{d}

Different superscripts ^{a,b,bc,c} in a row indicate significant difference between the means ($p < 0.05$)

Effect of *Saccharomyces cerevisiae* on Testicular and Epididymal Parameters

The results of testicular and epididymal data are shown in table 2. Absolute testicular weight was significantly higher ($p < 0.05$) in the control group (3.18 ± 0.06 g) and declined across the treatment groups. Notably, although the actual weight of the testes decreased with supplementation, the relative testicular weight (as a percentage of body weight) increased steadily, from 1.37 ± 0.03% in the control to 1.54 ± 0.01% in group D.

Looking deeper into testicular structure, morphometric assessments revealed consistent reductions in all size-related parameters—including testicular circumference, length, diameter, and volume—as yeast dosage increased. These reductions were statistically significant ($p < 0.05$) with group D showing the most pronounced decline, indicating impaired testicular development at higher supplementation levels.

Similar trends were observed in the epididymis. The absolute weight of the epididymis significantly ($p < 0.05$) declined across the groups, from 0.31 ± 0.00 g in the control to 0.28 ± 0.00 g in group D. Meanwhile, the relative epididymal weight showed a slight but statistically significant ($p < 0.05$) increase with higher yeast doses, from 0.13 ± 0.00% to 0.15 ± 0.00%.

Table 2: Effects of *Saccharomyces cerevisiae* on Organo-Somatic Indices (Relative Organ Weights) and Morphometry of Male Rats' Testes and Epididymides

Parameter	Group A (control)	Group B (0.5 g/kg bw)	Group B (1.0 g/kg bw)	Group D (1.5 g/kg bw)
Final weight (g)	233.14 ± 7.57^{a}	207.16 ± 5.08^{b}	195.00 ± 3.61^{bc}	185.10 ± 1.74^{c}
Testes				
Absolute weight (g)	3.18 ± 0.06^{a}	2.91 ± 0.06^{b}	2.88 ± 0.04^{b}	2.85 ± 0.03^{b}
Relative weight (%)	1.37 ± 0.03^{a}	1.41 ± 0.02^{a}	1.48 ± 0.01^{b}	1.54 ± 0.01^{c}
Epididymis				
Absolute weight (g)	0.31 ± 0.00^{a}	0.30 ± 0.00^{a}	0.29 ± 0.00^{ab}	0.28 ± 0.00^{bc}
Relative weight (%)	0.13± 0.00^{a}	0.14 ± 0.00^{b}	0.15 ± 0.00^{bc}	0.15 ± 0.00^{c}
***In-situ* testicular circumference (cm)**	7.68 ± 0.06^{a}	6.58 ± 0.34^{b}	5.96 ± 0.19^{c}	5.26 ± 0.05^{d}
***Ex situ* testicular length (cm)**	2.27 ± 0.03^{a}	2.07 ± 0.04^{b}	1.93 ± 0.03^{c}	1.72 ± 0.02^{d}
***Ex situ* testicular diameter (cm)**	1.28 ± 0.02^{a}	1.16 ± 0.01^{b}	1.11 ± 0.01^{c}	1.04 ± 0.01^{d}
***Ex situ* testicular volume (cm^3)**	5.94 ± 0.05^{a}	5.62 ± 0.07^{b}	5.14 ± 0.09^{c}	4.40 ± 0.07^{d}

Different superscripts ^{a,ab,b,bc,c} in a row indicate significant difference between the means ($p < 0.05$)

Effect of *Saccharomyces cerevisiae* on Liver, Kidney, and Spleen Indices

Outside the reproductive system, *S. cerevisiae* supplementation also affected other internal organs (Table3). The liver showed a progressive significant ($p < 0.05$) decline in absolute weight across all treatment groups, from 9.76 ± 0.59 g in group A to 7.02 ± 0.15 g in group D. However, relative liver weights remained relatively stable, with no significant differences ($p > 0.05$) observed, suggesting that the liver scaled proportionally with the reduced body size.

A similar pattern was seen in the kidneys. Absolute kidney weight dropped significantly from 1.65 ± 0.05 g in the control group to

Table 3: Effects of *Saccharomyces cerevisiae* on Organo-Somatic Indices (Relative Organ Weights) of Liver, Kidney and Spleen of Male Rats

Parameters	Group A (control)	Group B (0.5 g/kg bw)	Group C (1.0 g/kg bw)	Group D (1.5 g/kg bw)
Final weight (g)	233.14 ± 7.57^{a}	207.16 ± 5.08^{b}	195.00 ± 3.61^{bc}	185.10 ± 1.74^{c}
Liver				
Absolute weight (g)	9.76 ± 0.59^{a}	8.35 ± 0.24^{b}	7.55 ± 0.05^{bc}	7.02 ± 0.15^{c}
Relative weight (%)	4.21 ± 0.28	4.03 ± 0.05	3.87 ± 0.05	3.79 ± 0.06
Kidney				
Absolute weight (g)	1.65 ± 0.05^{a}	1.47 ± 0.02^{b}	1.40 ± 0.02^{bc}	1.32 ± 0.01^{c}
Relative weight (%)	0.71 ± 0.00	0.71 ± 0.01	0.72 ± 0.01	0.71 ± 0.01
Spleen				
Absolute weight (g)	1.23 ± 0.01^{a}	1.13 ± 0.02^{b}	1.04 ± 0.02^{bc}	1.01 ± 0.00^{c}
Relative weight (%)	0.53 ± 0.02	0.55 ± 0.01	0.53 ± 0.00	0.55 ± 0.00

Different superscripts ^{a,b,bc,c} in a row indicate significant difference between the means ($p < 0.05$)

DISCUSSION

This study revealed clear effects of *Saccharomyces cerevisiae* supplementation on weight and morphometry of reproductive and other organs of male rats, particularly in the testes and epididymides. While the absolute weight of the testes was highest in the control group (3.18 ± 0.06 g), it gradually declined with increasing yeast doses. Interestingly, the relative weight of the testes expressed as a percentage of total body weight increased significantly with higher supplementation levels, reaching its peak in group D (1.54 ± 0.01%). This suggests that even though overall body weight decreased, the testes made up a proportionally larger share of body mass. Such a pattern could indicate a compensatory mechanism or preservation of testicular tissue relative to the rest of the body, as previously observed under nutritional stress conditions (Trapphoff and Dieterle 2023).

The morphometric data tell a different story. Parameters such as *in situ* circumference, length, diameter, and volume of the testes all declined significantly with higher yeast doses. The smallest testicular volume was recorded in group D (4.40 ± 0.07 cm^3), compared to 5.94 ± 0.05 cm^3 in the control group A. These findings suggest that higher doses of *S. cerevisiae* may negatively affect testicular development and structure, potentially interfering with normal spermatogenesis and reproductive function (Barbosa *et al.,* 2019). This could be linked to the bioactive compounds in yeast or to endocrine disruptions, as previously noted by Lobsiger *et al.* (2019).

A similar trend was observed in the epididymis. Absolute weight decreased with increasing yeast supplementation, although relative weights showed a slight upward trend. Since the epididymis is crucial for sperm maturation and storage, its reduced size may reflect compromised sperm quality or reduced androgenic stimulation (Bomfim *et al*., 2025). These changes may stem from impaired testicular output or altered feedback mechanisms within the hypothalamic-pituitary-gonadal axis.

These reproductive changes occurred alongside a noticeable decline in overall growth. While all

groups started with similar body weights, rats receiving yeast supplementation showed significantly lower final weights and weight gains. Group D rats gained the least weight (80.66 ± 2.15 g), compared to 124.42 ± 3.81 g in the control group. This pattern aligns with findings from Lawrence-Azua *et al.* (2018), who reported that high doses of yeast reduced growth in broilers, likely due to reduced feed efficiency or digestive disturbances caused by excessive fermentation byproducts.

Beyond the reproductive system, yeast supplementation also affected other internal organs. Absolute liver weight declined steadily with increasing doses, though the relative liver weight remained statistically unchanged. Similar results were noted in the kidneys and spleen—declines in absolute weights without significant shifts in relative proportions. These findings suggest that yeast may reduce overall body growth without selectively targeting specific organs, supporting a general rather than organ-specific growth inhibition effect (Vanthienen *et al*., 2024).

The modest increases in relative weights of reproductive organs, especially at higher yeast doses, might reflect the immune and metabolic influence of components like β-glucans and mannans found in *S. cerevisiae*. These compounds are known for their roles in enhancing immunity and gut health (Baek *et al*., 2024). However, the results from this study show that higher concentrations may do more harm than good, particularly when it comes to reproductive tissue integrity.

CONCLUSION

In summary, this study highlights that while *Saccharomyces cerevisiae* is often valued for its nutritional and probiotic benefits, its supplementation—especially at higher doses—may negatively affect male reproductive structures in rats. The observed reductions in testicular and epididymal size, along with diminished body weight, suggest that excessive yeast intake could impair reproductive development rather than support it. Although relative organ weights increased, this was likely a reflection of overall body mass reduction rather than enhanced reproductive growth. By focusing on both absolute and relative organo-somatic indices, this study fills an important gap in the current understanding of yeast supplementation and reproductive health. These findings underscore the need for cautious dosing and open new avenues for future research into the hormonal and functional consequences of dietary yeast on male fertility.

RECOMMENDATION

Given the findings of this study, it is advisable to use *S. cerevisiae* supplements in male diets with caution, particularly at higher doses, as they may negatively impact reproductive organ development. To ensure safe and beneficial use, further research should explore the underlying hormonal and cellular effects, including detailed sperm analysis and tissue evaluations, to better understand how yeast supplementation influences male fertility over time.

REFERENCES

Khani, N., Ashkezary, M. R., Hosseinzadeh, N., Aghapour, B., Hosseini, A., Abedi-Soleimani, R., Houshmandi, S., Shokouhian, S. M. J. and Homayouni-Rad, A. (2025). The role of functional foods based on probiotics in improving fertility: A review. *Journal of Functional Foods, 129*, 106871. https://doi.org/10.1016/j.jff.2025.106871.

Blancafort, C. and Llácer, J. (2023). Can probiotics enhance fertility outcome? Capacity of probiotics as a single intervention to improve the feminine genital tract microbiota in non-symptomatic reproductive-aged women. *Frontiers in Endocrinology, 13*, 1081830. https://doi.org/10.3389/fendo.2022.1081830.

Xu, J., Wen, C., Song, G., Lesaux, A. A., Zhang, H. and Luo, Y. (2025). Effect of yeast probiotic *Saccharomyces cerevisiae* on the gut health of dogs undergoing rapid dietary transition. *Frontiers in Microbiology, 16*, 1561660. https://doi.org/10.3389/fmicb.2025.1561660.

Perricone, V., Sandrini, S., Irshad, N., Comi, M., Lecchi, C., Savoini, G. and Agazzi, A. (2022). The role of yeast *Saccharomyces cerevisiae* in supporting gut health in horses: An updated review on its effects on digestibility and intestinal and fecal microbiota. *Animals, 12*(24), 3475. https://doi.org/10.3390/ani12243475

Pang, Y., Zhang, H., Wen, H., Wan, H., Wu, H., Chen, Y., Li, S., Zhang, L., Sun, X., Li, B. and Liu, X. (2022). Yeast probiotic and yeast products in enhancing livestock feeds utilization and performance: An overview. *Journal of Fungi, 8*(11), 1191. https://doi.org/10.3390/jof8111191.

Lin, J., Comi, M., Perricone, V., Agazzi, A., Qiu, K., Wang, J., Wu, S., Qi, G. and Zhang, H. (2023). Effects of *Saccharomyces cerevisiae* hydrolysate on growth performance, immunity function, and intestinal health in broilers. *Poultry Science, 102*(1), 102237. https://doi.org/10.1016/j.psj.2022.102237.

Emmanuel, D. C., Amaka, A. E., Okezie, E. S., Sunday, U. P. and Ethelbert, O. C. (2019). Epididymal sperm characteristics, testicular morphometric traits and growth parameters of rabbit bucks fed dietary *Saccharomyces cerevisiae* and/or zinc oxide. *Revista Brasileira de Ciência Avícola*, *21*(1), 1–10. https://doi.org/10.1590/1806-9061-2018-0803.

Besseboua, O. and Ayad, A. (2021). Effect of *Saccharomyces cerevisiae* feed supplementation on haematology and reproductive parameters for Algerian rabbits. *Journal of Applied Life Sciences and Environment*, *LIV*(2[186]), 111–122. https://doi.org/10.46909/journalalse-2021-011.

Chao, H. H., Zhang, Y., Dong, P. Y., Gurunathan, S. and Zhang, X. F. (2023). Comprehensive review on the positive and negative effects of various important regulators on male spermatogenesis and fertility. *Frontiers in Nutrition*, *9*, 1063510. https://doi.org/10.3389/fnut.2022.1063510.

Ahn, C. and Jeung, E.-B. (2023). Endocrine-Disrupting Chemicals and Disease Endpoints. *International Journal of Molecular Sciences*, *24*(6), 5342. https://doi.org/10.3390/ijms24065342.

Omar, A. R., El-Din, E. Y. S., Abdelrahman, H. A. and Mohamed, H. R. (2018). Evaluation of dietary supplement intake (yeast tablets) on pregnant albino rats and their fetuses (implications of yeast tablets on rats & fetuses). *Brazilian Archives of Biology and Technology*, *61*, e181602 69. https://doi.org/10.1590/1678-4324-2018160269

Trapphoff, T. and Dieterle, S. (2023). Cryopreservation of ovarian and testicular tissue and the influence on epigenetic pattern. *International Journal of Molecular Sciences, 24*(13), 11061. https://doi.org/10.3390/ijms241311061.

Barbosa, L. P., Barbosa, L. P., Silva, R. R., Silva, F. F., Araújo, A. A., Pereira, L. G. R. and Detmann, E. (2019). Intake, digestibility, performance, and feeding behavior of crossbred heifers fed diets containing different levels of castor meal treated with calcium oxide. *Revista Brasileira de Zootecnia, 48*, e20180803. https://doi.org/10.1590/1806-9061-2018-0803.

Lobsiger, N., Venetz, J. E., Gregorini, M., Christen, M., Christen, B. and Stark, W. J. (2019). YestroSens, a field-portable *S. cerevisiae* biosensor device for the detection of endocrine-disrupting chemicals: Reliability and stability. *Biosensors and Bioelectronics, 146*, 111710. https://doi.org/10.1016/j.bios.2019.111710.

Bomfim, L. P., Silva, M. S. dos S., da Costa, I. R., *et al.* (2025). Consumption of low-protein or high-fat diets during peripubertal age alter spermatozoa, testis and epididymis of pubertal rats. *Journal of Developmental Origins of Health and Disease, 16*, e15. https://doi.org/10.1017/S204017442500008X.

Lawrence-Azua, O. O., Awe, A. O., Saka, A. A., Okotie, U. J., Awodele, O. A. and Isegbe, E. I. (2018). Effect of yeast (*Saccharomyces cerevisiae*) supplementation on the growth performance, haematological and serum biochemical parameters of broiler chicken. *Nigerian Journal of Animal Science, 20*(1), 191–199.

Vanthienen, W., Fernández-García, J., Baietti, M. F., Claeys, E., Van Leemputte, F., Nguyen, L., Goossens, V., Deparis, Q., Broekaert, D., Vlayen, S., Audenaert, D., Delforge, M., D'Amuri, A., Van Zeebroeck, G., Leucci, E., Fendt, S.-M. and Thevelein, J. M. (2024). *The novel family of Warbicin® compounds inhibits glucose uptake both in yeast and human cells and restrains cancer cell proliferation. Frontiers in Oncology, 14*, Article 1411983. https://doi.org/10.3389/fonc.2024.1411983

Baek, K. R., Ramakrishnan, S. R., Kim, S. J. and Seo, S. O. (2024). Yeast cell wall mannan structural features, biological activities, and production strategies. *Heliyon, 10*(6), e27896. https://doi.org/10.1016/j.heliyon.2024.e27896