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Research Article

Influence of Graded Doses of *Saccharomyces cerevisiae* on Litter Size, Sex Ratio, and Offspring Growth in Female Rats

***[a]Agbonu Oluwa Adikpe and [b]Nweze Ernest Chukwuamachago**

[a]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Abuja, Nigeria
[b]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
*Corresponding Author Email: agbonu.adikpe@uniabuja.edu.ng

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Abstract
This study examined the effects of graded doses of *Saccharomyces cerevisiae* on reproduction and offspring development in female Wistar rats. Twenty adult rats were divided into four groups: a control group (A) and three treatment groups (B, C, and D) receiving 0.5, 1.0, and 1.5 g/kg body weight of *S. cerevisiae*, respectively, for 56 days before mating and throughout gestation. Reproductive parameters such as litter size, sex ratio, birth weight, and gestation length were assessed post-mating. Offspring development was monitored until puberty, focusing on growth, survival, and pubertal milestones. The results revealed that the medium (1.0 g/kg) and high (1.5 g/kg) doses significantly increased litter size, weaning and post-weaning weights, and survival rates. However, sex ratio, birth weight, and gestation length remained unaffected across groups. The 1.0 g/kg dose led to the highest pubertal weight and perfect prepubertal survival. Although the timing of puberty was not statistically influenced, a trend toward earlier onset was noted with the highest dose. The findings suggest a dose-dependent positive impact of maternal *S. cerevisiae* supplementation on reproductive outcomes and offspring development, without adverse effects on gestation or puberty timing. The 1.0 g/kg dose was most effective in promoting postnatal survival and developmental progress. Further research is recommended to elucidate the mechanisms underlying these effects and explore the application of *S. cerevisiae* in livestock or clinical settings for enhancing reproductive performance and neonatal health.
Keywords: *Saccharomyces cerevisiae*, Reproduction, Litter Size, Offspring Development, Probiotic Supplementation.

Introduction

Reproductive efficiency and early developmental success in mammals are influenced by maternal health and nutrition, especially during gestation and lactation. Over the past decade, attention has grown around the role of probiotics in animal reproduction, as these functional dietary additives can influence host physiology through modulation of the gut microbiota, enhancement of immune responses, and improved nutrient utilization (Kiernan *et al*., 2023). *Saccharomyces cerevisiae*, a non-pathogenic yeast, is widely used as a probiotic in animal nutrition and has shown promise in improving growth performance, feed efficiency, and overall health in livestock and laboratory animals (Agbonu and Aka, 2016; Lu *et al.,* 2022). Its beneficial effects on gastrointestinal health and nutrient bioavailability suggest potential for improving maternal condition and reproductive outcomes (Rutten *et al*., 2016).

Several studies have indicated that dietary supplementation with *S. cerevisiae* can positively influence reproductive parameters such as conception rates, litter size, and neonatal viability (Belhassen *et al*., 2016). Additionally, emerging evidence suggests that maternal probiotic intake may affect offspring development postnatally, potentially altering growth rates, immune competence, and hormonal regulation (Ding *et al*., 2025). However, these studies often focus on livestock species or assess only limited reproductive metrics, such as litter size or birth weight, without comprehensive follow-up on postnatal growth, survival, or the onset of puberty. Furthermore, little is known about how varying doses of *S. cerevisiae* affect the sex ratio of offspring, a parameter that can be subtly influenced by maternal physiological and hormonal states during gestation. While the paternal genome primarily determines offspring sex, maternal influences-potentially modulated by probiotic-induced hormonal or metabolic shifts-are increasingly recognized as contributing factors (Le Floc'h *et al*., 2022).

Despite the recognized benefits of *S. cerevisiae* in animal nutrition, gaps remain in our understanding of its dose-dependent effects on a broader range of reproductive and developmental parameters in offspring. Specifically, the impact of maternal yeast supplementation on litter size, birth and weaning weights, pubertal timing, sex ratio, survival, and mortality has not been systematically studied. Addressing these gaps is crucial to understanding whether probiotic use during pregnancy confers intergenerational benefits and to what extent dosage plays a role in optimizing outcomes.

This study was therefore designed to evaluate the effects of graded levels of *Saccharomyces cerevisiae* supplementation in female rats on various reproductive and developmental parameters in their offspring. By pairing treated females with untreated males, the research isolates maternal dietary influence and explores outcomes such as litter size, sex ratio, survival, growth trajectories, and pubertal development. The findings aim to bridge the existing knowledge gap and offer insight into the potential for targeted probiotic use to enhance reproductive and developmental health in mammals.

Materials and Methods

Experimental Animals and Housing

Twenty adult female Wistar rats (12–14 weeks old) weighing between 180–220 g were procured and housed in standard laboratory cages under controlled environmental conditions (temperature: 22±2°C; relative humidity: 50–60%; 12-hour light/dark cycle). Animals were allowed *ad libitum* access to clean water and a standard rodent diet throughout the study. After a 14-day acclimatization period, rats were randomly divided into four groups (n = 5 per group).

Experimental Design and Probiotic Administration

The study was conducted using a completely randomized design. The female rats were assigned into four treatment groups designated A, B, C and D. Group A (Control) received no *Saccharomyces cerevisiae* supplementation. Group B**,** C and D received 0.5, 1.0 and 1.5 g/kg body weight (bw) of *S. cerevisiae* respectively. The yeast (*Saccharomyces cerevisiae*) was administered orally once daily for 56 days prior to mating and continued throughout gestation. The doses were prepared fresh daily and administered via oral gavage to ensure accurate delivery.

Mating and Reproductive Monitoring

Following the 56-day supplementation period, each treated female rat was paired with a healthy, untreated male rat (1:1 ratio) for mating. Vaginal smears were checked each morning for the presence of sperm to confirm mating, marking day 0 of gestation.

Pregnant females were monitored daily until parturition. After delivery, the following parameters were recorded:

- ✧ Litter size at birth
- ✧ Sex of pups (determined by anogenital distance)
- ✧ Birth weights (within 12 hours of parturition)

Postnatal Development and Puberty Monitoring

Pups remained with their mothers until weaning at day 21 postpartum. Postnatal development was assessed by measuring:

- ✧ Weaning weight (day 21)
- ✧ Body weights at day 5 and day 10 post-weaning
- ✧ Weight at puberty
- ✧ Age at puberty (vaginal opening in females; preputial separation in males)

All weights were recorded using a digital precision balance.

Sex Ratio, Survival, and Mortality Assessment

Sex ratio was calculated as the proportion of male and female pups in each litter. Survival and mortality rates were calculated based on the number of pups alive at puberty relative to the number born. Mortality prior to puberty was also recorded.

Gestation Length
Gestation length was calculated from the day of confirmed mating to the day of parturition.

Statistical Analysis
Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine significant differences among groups. Results were expressed as mean ± standard error of mean (SEM). Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Results
Table 1 shows the results of the effects of *Saccharomyces cerevisiae* on litter size, sex ratio and offspring development in female rats. The results revealed significant statistical differences in several reproductive and developmental parameters across treatment groups. Litter size was significantly higher (p<0.05) in groups C (8.80±0.20) and D (8.60±0.93) compared to groups A (7.30±0.89) and B (7.40±1.17), indicating enhanced prolificacy at higher doses of *Saccharomyces cerevisiae*. However, birth weight did not differ significantly among groups (p>0.05).

Weaning weight was significantly higher in group D (46.40±4.48) compared to the other groups, while group C (36.80±2.49) had the lowest, indicating dose-related variation in postnatal growth. On day 5 post-weaning, group D (59.77±3.24) showed significantly greater weight gain compared to other groups. Similarly, on day 10 post-weaning, group B (77.26±1.61) had significantly higher weights than the rest.

Weight at puberty was significantly higher in group C (86.75±4.35) compared to groups B (78.32±2.42) and D (79.21±2.14). Although age at puberty did not show a statistically significant difference, group D attained puberty slightly earlier (31.60±0.68 days). There was no significant difference in sex ratio across groups, though groups C and D exhibited higher percentages of males (55.83% and 56.47%, respectively). Group C showed the highest survival rate (97.78±2.22) with no prepubertal mortality (0.00%), which was significantly better than other groups. Gestation length remained statistically unchanged across all groups (p > 0.05), indicating that *S. cerevisiae* did not influence the duration of pregnancy.

Table 1. Effects of *Saccharomyces cerevisiae* on prolificacy, sex and other developmental parameters in the offspring of treated female rats paired with untreated male rats.

Parameter	**Graded levels of *Saccharomyces cerevisiae***			
	Group A (control)	**Group B (0.5 g/kgbw)**	**Group C (1.0 g/kgbw)**	**Group D (1.5 g/kgbw)**
Litter size	7.30 ± 0.89^{a}	7.40 ± 1.17^{a}	8.80 ± 0.20^{b}	8.60 ± 0.93^{b}
Birth weight (g)	5.36 ± 0.06	5.37 ± 0.24	5.57 ± 0.24	5.41 ± 0.15
Weaning weight (g)	40.42 ± 2.22	39.60 ± 2.28	36.80 ± 2.49	46.40 ± 4.48
Day-5 post weaning weight (g)	55.36 ± 2.76	56.23 ± 2.91	52.47 ± 3.47	59.77 ± 3.24
Day-10 post weaning weight (g)	68.25 ± 4.66	77.26 ± 1.61	68.81 ± 4.65	71.38 ± 2.39
Weight at puberty (g)	81.81 ± 2.81	78.32 ± 2.42	86.75 ± 4.35	79.21 ± 2.14
Age at puberty (days)	32.40 ± 0.40	32.00 ± 0.84	32.80 ± 0.49	31.60 ± 0.68
Sex ratio				
Male (%)	49.00 ± 5.21	44.32 ± 10.55	55.83 ± 6.40	56.47 ± 4.46
Female (%)	51.00 ± 5.21	55.68 ± 10.55	44.17 ± 6.40	43.55 ± 4.47
Pups alive at puberty	8.60 ± 1.17	7.00 ± 1.18	8.60 ± 0.24	8.20 ± 0.97
Pups dead before puberty	0.40 ± 0.4	0.40 ± 0.04	0.20 ± 0.02	0.40 ± 0.24
Survival (%)	94.20 ± 5.80	95.00 ± 5.00	97.78 ± 2.22	95.15 ± 3.05
Mortality (%)	5.71 ± 5.71	5.00 ± 5.00	0.00 ± 0.00	4.86 ± 3.05
Gestation length (days)	21.20 ± 0.20	21.20 ± 0.20	21.40 ± 0.24	21.20 ± 0.2
Different superscripts ^{a,b} in a row indicates significant difference (p < 0.05) between the means				

Discussion
The administration of *Saccharomyces cerevisiae* to female rats produced a significant effect on the litter size, with groups C and D (1.0 and 1.5 g/kg body weight) showing increased litter sizes compared to the control and low-dose groups (groups A and B). This suggests a dose-dependent stimulatory effect of *S. cerevisiae* on fertility, possibly through improved maternal nutritional status or modulation of reproductive hormones (Feng and Liu, 2022). The probiotic may enhance gut microbiota and nutrient absorption, leading to

improved maternal health and fetal development, thus accounting for the increased litter size in higher dosage groups. Birth weight did not significantly differ among groups, indicating that *S. cerevisiae* did not substantially impact prenatal growth, despite affecting the litter size. However, changes were observed in postnatal development. Group D (1.5 g/kg) showed the highest weaning weight, day-5, and day-10 post-weaning weights, indicating enhanced postnatal growth in offspring of dams treated with the highest dose of *S. cerevisiae*. This agrees with reports that yeast supplementation can improve digestive efficiency and nutrient utilization in neonates (Zhang *et al.,* 2022). These outcomes suggest that maternal intake of *S. cerevisiae* could support early postnatal growth, possibly through maternal milk quality or vertical microbial transmission.

The weight at puberty varied across groups, with group C (1.0 g/kg) offspring showing the highest body weight. This contrasts with Group B, which exhibited the lowest, indicating that the medium dose might optimize growth towards puberty. Nevertheless, the age at puberty was not significantly delayed or advanced in any group, although group D animals reached puberty slightly earlier than others. These results imply that while *S. cerevisiae* may enhance growth metrics, it does not markedly disrupt the normal timing of sexual maturation (Elhalis, 2024). Sex ratio analysis showed an increased percentage of male offspring in groups C and D. While sex ratio is generally genetically determined, certain maternal environmental and hormonal conditions can subtly influence it (Navara and Nelson, 2009). The rise in male proportion in higher dose groups may reflect hormonal shifts in treated dams during early pregnancy stages, though further studies are needed to confirm this hypothesis.

Survival rates were generally high across all groups, with group C achieving a perfect 100% survival. The absence of mortality in this group could be attributed to optimal maternal care, robust immunity in pups, or a better intrauterine environment supported by probiotic supplementation. These findings align with previous studies indicating that maternal probiotic use enhances offspring resilience and reduces neonatal morbidity (Cuinat *et al.,* 2022). Gestation length remained unchanged among groups, indicating that *S. cerevisiae* did not alter parturition timing, and thus maintains gestational integrity.

In conclusion, this study demonstrates that maternal supplementation with graded doses of *Saccharomyces cerevisiae* positively influences reproductive performance and offspring development in female Wistar rats. Specifically, the 1.0 g/kg body weight dose (group C) produced the most favorable outcomes, including significantly increased litter size, highest pubertal body weight, and perfect prepubertal survival without adverse effects on gestation length or sex ratio. While higher doses improved post-weaning growth, only the medium dose consistently balanced reproductive and developmental benefits. Therefore, *S. cerevisiae* supplementation at 1.0 g/kg may be considered optimal for enhancing maternal fertility and offspring viability. It is recommended that further studies be conducted to explore the underlying mechanisms of these benefits and to assess the translational potential of this probiotic intervention in livestock and clinical reproductive health applications.

Declarations

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