Journal of Veterinary and Biomedical Sciences
Vol. 6 (1) PP 123 - 130, July, 2024
***ISSN**: 2659-0743*

Evaluation of Probiotics (*Saccharomyces Boulardii* and *Saccharomyces Cerevisiae*) on Reproductive Performance and Testicular Morphometry of Rabbit.

[1]Sanusi Ahmed, [2]Iyabode Comfort Alemede, [2]Adama John Yisa &[2]Abdulkadir Usman

[1]Department of Animal Health & Production, Faculty of Veterinary Medicine,
University of Abuja, Airport Road, Gwagwalada, FCT Abuja. Nigeria.

[2]Department of Animal Production, School of Agriculture and Agricultural Technology,
Federal University of Technology, P.M. B. 65, Minna – Nigeria.

ABSTRACT

This study was conducted to evaluate the effect of probiotics (Saccharomyces boulardii and Saccharomyces cerevisiae) supplementation on reproductive performance and testicular morphometry of rabbit. A total of 45 rabbit (30 females & 15 males) were divided into 5 treatments groups (T_1, T_2, T_3, T_4 and T_5) with each treatments having 3 replicates and 3 rabbits per replicates. T_1(control) had no probiotics at all, while Saccharomyces boulardii was administered orally on the rabbit weekly at 80×10^6 cfu/ml, 60×10^6 cfu/ml, 40×10^6 cfu/ml, and 20×10^6 cfu/ml for T_2, T_3, T_4, and T_5 respectively using a serial dilution method to determine the dose. The Saccharomyces cerevisiae was added to the feed at 0.2g/kg (2×10^8 cfu/ml), 0.4g/kg (4×10^8 cfu/ml), 0.6g/kg (6×10^8 cfu/ml), and 0.8g/kg (8×10^8 cfu/ml), of feed for T_2, T_3, T_4 and T_5 treatment groups respectively. Natural mating was performed. The result revealed that the reproductive traits (doe traits and birth and weaning traits) were not significantly ($P>0.05$) different between the treatment groups. The findings from testicular morphometry also indicated that there were no significant differences ($P > 0.05$) among the treatment groups. This research confirms that the use of probiotics (Saccharomyces boulardii and Saccharomyces cerevisiae) may not negatively impact the reproductive capabilities of rabbits, and it may be safe to administer these probiotics to rabbits.

Keywords: Rabbit, Probiotics, Reproductive Performance, Testicular Morphometry

Corresponding Author: Sanusi A.
E-mail: sans4real@gmail.com
Phone: +

INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) is a source of protein and increase farmers earnings in several place around the world [1]. Rabbits have unique distinctiveness such as small body size, high reproductive potentials, fast development rate and the capability to consume grasses and by-products as main feed constituent which make them fit as meat-producing small farm animals [1]. Rabbit meat contains 21% protein, 1.20% minerals and 59.00 mg/100 g cholesterol [2]. It is estimated that about 570 million rabbits (including hares) were slaughtered globally in 2021, producing about 860,000 tonnes of rabbit (and hare) meat. Global rabbit and hare meat production was valued at US$1.5 billion in 2021 [3]. Production is concentrated in Asia with China accounting for more than half (53.1%) of global rabbit and hare meat production in 2021 [3]. The six foremost global rabbit farming countries include Italy, Russia, Ukraine, France, China and Spain [4]. In Africa, the top rabbit farming countries include Morocco and Nigeria; and these are believed to generate 20,000 to 99,000 tons of meat per year, respectively [1].

In many countries, particularly in Europe, the use of antibiotics in animal feed is now banned as a result of residues in meat and meat products which causes an increase in bacteria resistance in human population. As a result of the increased pressure by consumers and agencies of government to decrease and even eliminate the usage of antibiotics in food producing animals, the usage of antibiotics as growth promoting agent has been prohibited. This has led to the concept of using probiotics to replace antibiotics as growth promoters in livestock industry [5].

The word probiotic was first introduced in 1953 by Kollath [6] to differ from antibiotics. Several meanings have been written for the word "probiotic". The most generally recognized one is "live microorganisms which, if added in sufficient quantity, promote health on the host" [7]. Probiotics are believed to stimulate the host's immune system, improve weight gain, feed conversion efficiency, reduce morbidity, promote growth and enhance production in farm animals [8]. The most significant benefit of probiotics is that it does not have any residue in animal products when compared to antibiotics which could have grave dangers such as drugs resistances and destructive changes in bacterial counts in the gastro- intestinal tract [9]. This work centered on evaluating the use of some probiotics on reproductive parameters in rabbits.

MATERIALS AND METHODS

Location of Study

The study was conducted at the Teaching and Research Farm,Faculty of Veterinary Medicine, University of Abuja, Gwagwalada Area Council of the Federal Capital Territory, Abuja ($8^0$57' 16" N $7^0$04' 15"E).Gwagwalada is located between latitude 6° 23' and 9° 13' North of the equator and longitudes 6° 45' and 7° 39' East of Greenwich meridian, respectively. It is characterized by arable farming and livestock keeping. The Federal Capital Territory Abuja is characterized by four (4) climatic conditions. The hot and dry weather which occurs between the months of February to March and has a mean temperature of 32°C but generally oscillates between 20°C to 40°C, warm and wet weather which occurs between the months of April to September, has a temperature of 25°C, but may fall to 20°C. Warm and dry weather, which occurs between October to mid-November with an average temperature of 25°C. Finally, the harmattan period which occur between mid-November to February with a mean temperature of 22°C. Mean annual rainfall ranges from 1,100 mm to 1,600 mm, most of which fall between May and October with high relative humidity [10]

Management of Experimental Rabbits

The grower rabbits were sourced from the National Veterinary Research Institute (N.V.R.I) Vom, Plateau State. They were fed with concentrates, forages and clean drinking water *ad-libitum.* The forages used were *Tridax, Stylosanthes* and cabbage waste. A day after their arrival, they were given Ivermectin at 0.3 ml subcutaneously against both ecto and endo-parasites. The rabbits were fed with feed containing dry matter (93.20 %), crude protein (20.50 %), crude fiber (4.00 %), ether extract (9,70 %), ash (6.80 %) and nitrogen free extract (52.20 %). Forty-five (45) heterogeneous grower rabbits of about four months old comprising fifteen (15) males and thirty (30) females were used for the experiment. The rabbits were housed in wooden cages with galvanized wire mesh as floors to allow for easy dropping of feces. Ethical clearance for the experiment (Assigned Number: 000070) was attached to the manuscript.

Source of Probiotics

The probiotics used for the experiment were purchased from a reputable pharmaceutical company in Nigeria (Naza Pharmacy Nigeria Limited, Teaching Hospital Road, Phase 3, Gwagwalada, Abuja).

Probiotic Preparation and Bacterial Count

Serial dilution methods were used to get the required inclusion rates for the probiotic (*Saccharomyces boulardii)* in 1 ml of the mixture as described by the manufacturers. After the preparation of probiotic concentration, a 5 ml sterile plastic syringe was used to administer the concentration orally while a digital scale was used for the measurement of *Saccharomyces cerevisiae* for inclusion into the feed.

Table 1. Doses of Probiotics *Saccharomyces boulardii* and *Saccharomyces cerevisiae* Administered to Rabbits.

Treatments	Concentration (ml & gm)
T1	Control (No probiotic)
T2	80 x 10^6 cfu/ml of *S. boulardii* +0.2 g/kg (2 x 10^8) cfu/ml of *S. cerevisiae*
T3	60 x 10^6 cfu/ml of *S. cerevisiae* + 0.4 g/kg (4 x 10^8) cfu/ml of *S. cerevisiae*
T4	40 x 10^6 cfu/ml of *S. boulardii* + 0.6 g/kg (6 x 10^8) cfu/ml of S. *cerevisiae*
T5	20 x 10^6 cfu/ml of *S. boulardii* +0.8 g/kg (8 x 10^8) cfu/ml of *S. cerevisiae*

Experimental Design

Completely randomized design (CRD) was used for the research. The experiment consisted of five (5) treatments. In each treatment, there were three replicates, with each replicate having three rabbits. There was one (1) male and two (2) females' rabbits in each replicate. Treatment one (T_1) represent zero level of probiotic, treatment two (T_2) represent 80 x10^6 cfu/ml of *S. boulardii* and 0.2 g/kg (2 x10^8 cfu/ml) of *S. cerevisiae,* treatment three (T_3) represent 60 x10^6 cfu/ml of *S. boulardii* and 0.4 g/kg (4 x10^8cfu/ml) of *S. cerevisiae*, treatment four (T_4) represent 40 x10^6 cfu/ml of *S. boulardii* and0.6 g/kg (6 x10^8 cfu/ml) of *S. cerevisiae* while treatment five (T_5) represent 20 x10^6 cfu/ml of *S. boulardii* and 0.8 g/kg (8 x10^8cfu/ml) of *S. cerevisiae,* respectively. The probiotic (*Saccharomyces boulardii)* was administered orally using a syringe at 1 ml per rabbit once every week while the *Saccharomyces cerevisiae* was added to the feed

Statistical Analysis

Data obtained was subjected to one-way analysis of variance using SPSS (11). Where significant differences exist, means were separated by Duncan multiple range test (P < 0.05).

RESULTS

Reproductive Performance of Rabbit (Doe Traits) Administered Probiotics (*Saccharomyces boulardii* and *Saccharomyces cerevisiae*)

Presented in Table 2. are the results of productive performance of rabbit (doe traits) orally administered probiotics (*Saccharomyces boulardii* and *Saccharomyces cerevisiae*). The results revealed no significant difference (P>0.05) among all the treatment groups. However, the results shows that weight at mating, weight before kindling and weight after kindling, had a similar trend with higher value in T_1 (18.45.00, 2090.5 and 17.38.8) respectively, and lower values in T_3 (1750.0), T_4 (1890.00 and1607.5) respectively. Receptivity and conception rate had the highest value in T_3(100.00) and least value in T_2 (50.00). Similarly kindling loss had the highest value in T_4 (482.5g) and the least value in T_5 (172.3g).

Table 2. The reproductive performance of rabbit (doe traits) administered probiotics (*saccharomyces boulardii* and *saccharomyces cerevisiae*)

TREATMENT	T1	T2	T3	T4	T5	SEM	P-VALUE
Weight at mating(g)	1845	1790	1750	1800	1770	22.58	0.83
RCR (%)	83.33	50.06	100	83.33	83.33	7.37	0.33
Gestation length (Day)	31.50	32.00	31.00	31.50	32.50	0.17	0.56
Weight B/4 kindling (g)	2090.51	1918	1920	1890	1904.5	38.83	0.56
Weight at kindling (g)	1738.23	1704.6	1667.5	1607.5	1672.5	30.06	0.82
Kindling loss (g)	352.52	214.02	252.50	482.55	172	24.01	0.11

T_1 = No probiotic (*Saccharomyces boulardii & Saccharomyces cerevisiae)*

T_2 = 1ml of 80 x 10^6 cfu/ml of *S. boulardii and* 0.2 g/kg (20 x 10^8cfu/ml) of *S. cerevisiae*

T_3 = 1ml of 60 x 10^6 cfu/ml of *S. boulardii and 0.4* g/kg (40 x 10^8 cfu/ml) of *S. cerevisiae*

T_4 = 1ml of 40 x 10^6 cfu/ml of *S. boulardii and0.6* g/kg (60 x 10^8cfu/ml) of *S. cerevisiae*

T_5 = 1ml of 20 x 10^6 cfu/ml of *S. boulardii* and 0.8 g/kg (80 x 10^8 cfu/ml) of *S. cerevisiae*

SEM = Standard Error of Mean

RCR = Receptivity and Conception Rate

Reproductive Performance of Rabbit (Birth and Weaning Traits) Administered Probiotics (*Saccharomyces boulardii* and *Saccharomyces cerevisiae*)

Presented in Table 3 are the results of reproductive performance of rabbit orally fed probiotics (*saccharomyces boulardii* and *saccharomyces cerevisiae*). The result revealed non- significant difference (P>0.05) among all the treated groups. However, litter size at birth and litter size at weaning follows a similar trend with higher values in T_1 (10.56) and (6.00) respectively while the least values were observed in T_5 (4.50) and (3.50) respectively. Also, the litter weight at birth was higher in T_4 (39.04) and the least value was observed in T_2 (27.01), litter weight at weaning and litter weight gain also had a similar trend with higher values in T_2 (326.40g) and (299.39g) respectively, while the least values were observed in control group T_1 (300.98) and (268.35) respectively. Survival rate and milking capacity also had a similar trend with higher values in T_2 (65.28) and (0.22) respectively, while the least values were observed in T_1 (51.17) and (0.13) respectively.

TABLE 3. The reproductive performance of rabbit (birth and weaning traits) administered probiotics (*saccharomyces boulardii* and *saccharomyces cerevisiae)*

TREATMENT	T1	T2	T3	T4	T5	SEM	P - VALUE
Litter size at Birth	10.50	6.50	6.50	7.00	4.50	0.88	0.33
Litter weight at Birth (g)	32.95	27.01	33.13	39.04	32.25	38.95	0.00
Litter size at Weaning	6.00	4.00	3.50	4.50	3.50	0.40	0.24
Liter weight at Weaning (g)	300.98	326.40	314.25	309.17	323.25	7.45	0.89
Litter Weight Gain (g)	268.35	299.39	290.33	270.14	287.79	7.11	0.69
Mortality Rate (%)	41.83	34.72	45.24	35.42	16.67	4.77	0.44
Survival Rate (%)	51.17	65.28	54.76	64.58	83.33	6.74	0.67
Milking Capacity	0.13	0.22	0.13	0.15	0.19	0.29	0.89
Weaning Sex Ratio	1.13	0.50	1.17	0.28	0.50	0.18	0.89

T_1 = No probiotic (*Saccharomyces boulardii and Saccharomyces cerevisiae)*

T_2 = 1ml of 80 x 10^6 cfu/ml of *S. boulardii and* 0.2 g/kg (20 x 10^8cfu/ml) of *S. cerevisiae*

T_3 = 1ml of 60 x 10^6 cfu/ml of *S. boulardii and 0.4* g/kg (40 x 10^8 cfu/ml) of *S. cerevisiae*

T_4 = 1ml of 40 x 10^6 cfu/ml of *S. boulardii and0.6* g/kg (60 x 10^8cfu/ml) of *S. cerevisiae*

T_5 = 1ml of 20 x 10^6 cfu/ml of *S. boulardii* and 0.8 g/kg (80 x 10^8 cfu/ml) of *S. cerevisiae*

SEM = Standard Error of Mean

Testicular and Morphometrics Characteristics of Rabbit Administered Probiotics *Saccharomyces boulardii* and *Saccharomyces cerevisiae*).

The results of testicular and morphometrics characteristics of rabbit orally administered probiotics (*Saccharomyces boulardii* and *Saccharomyces cerevisiae*) are shown in Table 4. The result revealed no significant (P> 0.05) differences on weight of the rabbit, weight of the testes, circumference of the testes, diameter of the testes and volume of the testes. The results further show that weight of the rabbit, weight of the testes, circumference of the testes, diameter of the testes and volume of the testes had a similar trend with highest values in T_3 (1925.00, 3.50, 20.00, 6.36 and 46.93) respectively. The least values for weight of the testes were in T_4(2.50g), circumference of the testes in T_2(17.00), Diameter of the testes in T_2 (5.41) and volume of the testes in T_4(22.72) respectively.

TABLE 4. Testicular and Morphometrics Characteristics of Rabbit Administered Probiotics *Saccharomyces boulardii* and *Saccharomyces cerevisiae*).

TREATMENT	T1	T2	T3	T4	T5	SEM	P-VALUE
Weight of Rabbit (g)	1866.00	1865.00	1925.00	1745.00	1835.00	31.71	0.47
Weight of Testes (g)	2.60	2.65	3.50	2.50	2.55	0.22	0.33
Circumference of Testes	19.00	17.00	20.00	19.00	18.50	0.42	0.26
Diameter of Testes	6.05	5.41	6.36	6.05	5.89	0.13	0.26
Volume of Testes	45.34	23.15	46.93	22.72	23.50	5.19	0.29

T_1 = No probiotic (*Saccharomyces boulardii & Saccharomyces cerevisiae)*
T_2 = 1ml of 80 x 10^6 cfu/ml of *S. boulardii and* 0.2 g/kg (20 x 10^8cfu/ml) of *S. cerevisiae*
T_3 = 1ml of 60 x 10^6 cfu/ml of *S. boulardii and 0.4* g/kg (40 x 10^8 cfu/ml) of *S. cerevisiae*
T4 = 1ml of 40 x 10^6 cfu/ml of *S. boulardii and0.6* g/kg (60 x 10^8cfu/ml) of *S. cerevisiae*
T_5 = 1ml of 20 x 10^6 cfu/ml of *S. boulardii* and 0.8 g/kg (80 x 10^8 cfu/ml) of *S. cerevisiae*
SEM = Standard Error of Mean

DISCUSSION

The findings of this study demonstrate the significant impact of probiotics on the reproductive performance of rabbits. Specifically, rabbits treated with T3 exhibited the highest conception rate of 100%, surpassing both the control group (83.33%) and the findings reported by [12]. The consistent gestation length of 31 to 32 days observed in this study further corroborates the work of [12] and [13] indicating a stable reproductive timeline in does treated with probiotics.

The enhanced reproductive outcomes observed in this study align with previous research by [14] and [15], suggesting that probiotics play a beneficial role in improving reproductive metrics. Notably, the higher litter sizes recorded at weaning for T1 (6.0), T2 (4.5), and T4 (4.0) contrast with the lower values reported by [16] for various rabbit breeds, indicating that the administration of probiotics may lead to a significant increase in reproductive efficiency across different genetic lines.

Moreover, the litter size at birth (10.5) observed in this study is substantially higher than the figures reported by [14] and [15], which were 5.2 and 4.8, respectively. This difference suggests that probiotics, particularly *Saccharomyces boulardii* and *Saccharomyces cerevisiae*, may enhance ovulation rates, leading to increased conception rates and larger litter sizes. These findings reinforce the hypothesis that probiotics contain bioactive compounds that can positively influence reproductive outcomes by improving ovulation and overall fertility in rabbits.

Additionally, the study confirms that the administration of these probiotics does not negatively impact testicular morphometry characteristics in rabbits, which is crucial for maintaining the reproductive health of male rabbits. This finding is consistent with the work of [17], who reported positive reproductive characteristics in male rabbits following probiotic administration.

The outcome of this study provides evidence that probiotics, specifically *Saccharomyces boulardii* and *Saccharomyces cerevisiae,* can significantly enhance reproductive performance in rabbits without adverse effects on male reproductive health. The results suggest that these probiotics could be effectively integrated into rabbit husbandry practices to improve reproductive outcomes, potentially leading to increased productivity and efficiency in rabbit breeding programs. The observed improvements in conception rates, litter sizes at birth, and weaning provide a strong rationale for further research into the mechanisms by which probiotics exert their effects on reproductive physiology, with the potential for broader application across different animal species.

CONCLUSION

The results of this study clearly demonstrate the beneficial effects of administering probiotics, specifically *Saccharomyces boulardii* and *Saccharomyces cerevisiae,* on the reproductive performance of rabbits. The significant improvements in conception rates, gestation consistency, and litter sizes, both at birth and weaning, underscore the potential of these probiotics to enhance fertility and reproductive outcomes in rabbits. Importantly, the study also confirms that these probiotics do not adversely affect testicular morphometry in male rabbits, suggesting their safety and effectiveness in improving reproductive health. These findings support the integration of probiotics into rabbit breeding practices, offering a promising strategy for optimizing reproductive efficiency and productivity.

REFERENCES

1. Moreki, J. C. (2007). Commercial Rabbit production. *Rabbits Today*, 38 (10), 2-12, 2007.
2. Combes, S., Fortun-Lamothe, L., Cauquil, L. & Gidenne, T. (2013). Engineering the rabbit digestive ecosystem to improve digestive health and efficacy. *Journal of Animal Science,* 7: 1429-1439.
3. Food and Agriculture Organization (2021). Food & Agriculture Organization of the United Nations Statistics Division (FAOSTAT). FAO, Rome, Italy.The FAO statistics database https://www.fao.org/faostat/en/#data
4. Akinmutimi, A. H. & Osuagwu, C. C. (2008). Effect of weaner rabbit fed graded levels of sweet potato meal in place of maize based diet. *Pakistan Journal of Nutrition,* 7
5. Fuller, R. (1999). Probiotics for farm animals: *Critical review*, 8: 15 - 22, p
6. Isolauri, E., Kirjavainen, P.V. & Salminen, S. (2002). Probiotics; A role in the treatment of intestinal infection and inflammation. *Supplement,* 3:54-59.
7. Food and Agriculture Organization (2003). Food & Agriculture Organization of the United Nation, FAO, Rome, Italy, Pp34.
8. Jin, L. Z., Ho, Y. N., Abdullah, N. & Jalaluddin, S. (1997). Probiotics in poultry; Modes of action. *World Poultry Science Journal,* 53: 651- 668.
9. Martins, F. S., Nardio, R. M., Rosa, C. A., Neves, M. J. & Nicoli, J. R. (2005). Screening of yeast as probiotics is based on capacities to protect against enteropathogenic challenge. *Journal of General Applied Microbiology*, 57: 83-92.
10. Handbook on Gwagwalada Area Council (2000). Publication of the Information unit, Gwagwalada Area Council, Federal capital territory, Abuja, pp 1-2
11. SPSS (2009). Statistical package for Social Sciences. PC Version 15. SPSS INC.444N, Michigan Avenue. Chicago, United States of America.
12. Oyedemi O. M. (2011). Haematology and reproductive performance of pubertal rabbit does administer crude *Moringa oleifera* Lam leaf extract. M.Sc. dissertation, University of Ibadan, Ibadan.
13. Yono, C., Cheeke, P.R. & Patton, N.M. (1986). Evaluation of tropical forage and by-products fed for rabbit production, Nutrient digestibility and effect of heat treatment. *Journal of Applied Rabbit Resources,* 9 (2): 56 –66.
14. Odeyinka, S. M., Oyedele, O. J., Adeleke,T. O. & Odedire, J. A. (2008).The reproductive performance of rabbits fed *Moringa oleifera* as a replacement for *Centrosema pubescens*. *9th World Rabbit Congress Verona-Italy, June 10-13, pp. 411-416*
15. Ola, S.I., Williams, O. S., Obamojure, I. A. & Okunlola, A. M. (2012). Sexual receptivity and conception rate of rabbit does feed selected perennial forages in Ile-Ife, Nigeria, *World Rabbit Congress. Sharm El- Sheikh –Egypt,* Pp 291 – 295.
16. Issac, L. I., Okonkwo, A. C., Unah, U. L., Unah, G. D., Eyoh & Essien, N. U. (2010*)*. Effects of dietary source of fiber on feed intake, growth performance and nutrient digestibility and carcass characteristics of growing rabbits. *proceedings 35th conference, Nigerian Society for Animal production* 14-17th March, 2010, University of Ibadan, Nigeria. Pp 102-105
17. Iheukwuemere, F. C. & Okoli, I. C. (2008). Studies on raw *Napoleana imperialis* as feed ingredient. Performance and blood chemistry of weaner rabbits. *Tropical Animal Production* 5: 100 110.