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# *Epididymal Sperm Characteristics, Testicular Morphometric Traits and Growth Parameters of Rabbit Bucks Fed Dietary Saccharomyces cerevisiae and/or Zinc Oxide*

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## ABSTRACT

The objectives of this twelve-week feeding trial were to determine the effects of dietary *Saccharomyces cerevisiae* and/or zinc oxide on epididymal sperm characteristics, testicular morphometric traits, and growth parameters of bucks. 16 (New Zealand White) bucks 16-wk-old, weighing 2.8kg were randomly allotted to one of 4 treatment groups. Each treatment was replicated four times, with 1 buck per replicate, in a completely randomized design (CRD). Each treatment group was randomly assigned to one of the four commercial experimental diets designated thus: $T_A$ = control diet with no additives, $T_B$ = 0.12g/kg *Saccharomyces cerevisiae*, $T_C$ = 150mg/kg zinc oxide and $T_D$ = 0.12g/kg *Saccharomyces cerevisiae* +150 mg/kg zinc oxide. Although treatment had no effect ($p>0.05$) on final body weight, average daily weight gain and feed conversion ratio, epididymal sperm characteristics and testicular morphometric traits differed significantly ($p<0.05$). Bucks on $T_B$(*Saccharomyces cerevisiae*-based diet) had improved ($p<0.05$) sperm concentration, motility and live sperm, tubule diameter, epididymal volume, volume fraction of duct, and total duct volume, but decreased testicular volume. Bucks fed $T_A$ (control diet) had improved volume fraction of tubule but recorded the highest incidence of head and tail sperm abnormality. Though, $T_C$(zinc oxide-based diet) enhanced ($p<0.05$) seminal vesicle volume, sperm pH was better among bucks fed $T_D$ (*Saccharomyces cerevisiae* + zinc oxide-based diet). It can be concluded that dietary inclusion of *Saccharomyces cerevisiae* at 0.12g/kg of feed improved epididymal sperm characteristics and testicular morphometric traits of rabbit bucks.

## INTRODUCTION

Feeding is an integral component of livestock and poultry enterprises. As an essential resource in livestock production, feed not only supplies livestock with nutrients which they require for normal metabolic processes, but also, its intake by animals, culminate in the production of valuable products (Fink-Gremmels, 2004). Animal feed consists of major and minor essential nutrients that are fed with intention of bringing about increased productivity. In view of this, feed additives like antibiotic growth promoters became increasingly utilized in livestock production in order to sustain large-scale animal production, while ensuring improved growth, feed efficiency, health and well-being of animals, and also meeting the protein demand of teeming human population (Peterson *et al*., 1991; Dibner & Richards, 2005; Huyghebaert *et al*., 2011; Hamasalim, 2016).

Although, antibiotic growth promoters have remarkable growth promoting and prophylactic effects (Niewold, 2007), their long term-use portends deleterious impact on public health (Nanung, 2013). Even

at low therapeutic doses, dietary inclusion of antibiotic growth promoters in animal nutrition has been linked to cases of gram-negative bacteria resistance in animals and humans (Butaye *et al*., 2003), carcinogenicity in human consumers of animal products (Torres *et al*., 2010), environmental degradation (Bekele & Ahanafi, 2010) etc. These concerns have prompted a widespread need and search for alternative feed additives that can bring about improved productive performance in the same manner as AGPs (Huyghebaert*et al*., 2011). Probiotics is among several extensively researched alternative feed additives that has received increased attention over the years (Yan & Kim, 2014). Probiotics are non-pathogenic live microbial feed additives that have been shown to improve productive capacity and immune status of animals by enhancing the composition and balance of the animal's gut microflora (Hill *et al.,* 2014). According to Scott (2012), trace minerals like zinc oxide have also gained prominence in recent times.

*Saccharomyces cerevisiae* are non-bacteria probiotic supplements (Bai *et al*., 2013) which when used as dietary additives in the nutrition of animals have potential to alter gut microflora by controlling pH (Leeson & Summers, 2008; Ng *et al*., 2009), suppress pathogenic bacteria growth (Stanley *et al*., 2004), modulate immunity (Shashidhara *et al*., 2003; Magalhaes *et al*., 2008), promote intestinal microflora growth (Spring, 2000), improve growth performance (Parks, 2001; Galvao *et al*., 2005), enhance nutrient digestibility and retention (Bradley *et al*., 1995; Ciorba, 2012), affect intestinal mucosal development (Zhang *et al*., 2005), and serve as alternatives in lieu of antibiotic-based drugs in the feed of broiler chicks, thus ensuring food safety for human consumers (Hooge *et al*., 2003; Song *et al*., 2012; Broadway *et al*., 2014) amongst other immense benefits. Zinc is a heavy metal increasingly used alongside other trace minerals in livestock nutrition. These minerals are needed for normal digestive, physiological and biosynthetic functioning of biological systems. The utilization of these trace mineral elements in the nutrition of food-producing animals has revealed their capacity to improve/maintain growth, immunity, and overall metabolic and physiological processes (Attia *et al*., 2012; Scott *et al*., 2012).

According to Richards *et al*. (2010), and Bhowmilk *et al*. (2012), zinc not only serves as a cofactor in a number of cellular enzymatic and transcription processes, it is also involved in maintaining immunity, controlling reproduction, regulating gene expression and cell proliferation, and providing protection against deleterious effect of reactive oxygen species (ROS). Zinc affects semen quality and quantity, particularly in terms of exerting a direct effect on sperm maturity and reproductive epithelium of male sexual glands (Peter *et al*., 2004). Zinc deficiency has been linked to delay in testicular development and spermatogenesis (Peter *et al*., 2004), decreased litter size in pigs (Hoekstra *et al*., 1967), low production levels of luteinizing hormone and follicle-stimulating hormones (Dillin *et al*., 2002), change in dietary preferences in rats (Kennedy *et al*., 1998) low birth weight and number of offspring in ewes (Masters & Fels, 1980). Even though several feeding trials have been done using *Saccharomyces cerevisiae* and zinc oxide in livestock and poultry, there is currently very limited literature on the effect of dietary *Saccharomyces cerevisiea* and zinc oxide inclusion in improving semen and testicular morphometric characteristics in rabbits. Therefore, this study was designed to determine the effect on gross epididymal semen characteristics and testicular morphometrism of rabbit bucks fed dietary inclusions of *Saccharomyces cerevisiae* and/or zinc oxide.

## MATERIALS AND METHODS

### Experimental location and management of experimental animals

The study was carried out at the Rabbit Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. The rabbits were procured from reputable rabbit farms around Nsukka local government area, Nsukka, Nigeria. Before the arrival of the animals, the experimental rabbit hutches, feeding and drinking troughs were thoroughly disinfected using a disinfectant known as Izal®. Upon arrival, the bucks were allowed an acclimatization period of 3 weeks, and subsequently dewormed with ivermectin by subcutaneous means.

### Experimental design and diets

16 New Zealand White rabbit bucks that were 16-wk-old were randomly allotted to 4 dietary treatments with 4 bucks per treatment. Each treatment was replicated four times with 1buck per replicate in a completely randomized design (CRD) with treatment as main effect. Commercial rabbit grower's mash was used for the feeding process. Zinc oxide and/or *Saccharomyces cerevisiae* were procured from an Agro-Chemical Company in Nsukka. Both feed additives were included in the diets at different

levels. The four experimental diets formulated were as follows: $T_A$ = control diet with no additives, $T_B$ = 0.12g/kg *Saccharomyces cerevisiae*, $T_C$ = 150mg/kg zinc oxide and $T_D$ = 0.12g/kg *Saccharomyces cerevisiae* +150mg/kg zinc oxide.

**Table 1 –** Composition of commercial feed used for the experiment.

| Nutrient composition | Quantity | Unit |
|---|---|---|
| Crude protein | 20.09 | % |
| Crude fibre | 7.39 | % |
| Fat | 3.04 | % |
| Metabolizable energy | 1970 | Kcal/kg |
| Arginine | 1.42 | % |
| Glycine | 1.16 | % |
| Serine | 1.02 | % |
| Histidine | 0.49 | % |
| Isoleucine | 0.89 | % |
| Leucine | 1.54 | % |
| Methionine | 0.35 | % |
| Cystine | 0.28 | % |
| Phenylalanine | 0.83 | % |
| Tyrosine | 0.72 | % |
| Threonine | 0.72 | % |
| Tryptophan | 0.24 | % |
| Valine | 1.03 | % |
| Linoleic acid | 1.07 | % |
| Calcium | 3.71 | % |
| Potassium | 1.05 | % |
| Available phosphorous | 0.41 | % |
| Magnesium | 0.42 | % |
| Sodium | 0.28 | % |
| Sulphur | 0.22 | % |
| Chlorine | 0.36 | % |

## Performance parameters

Average initial weight of bucks was taken when the experiment started and subsequently weekly. At the end of the experiment, average final buck weight was recorded per buck per replicate. Data collected was used to calculate average weight gain (ADWG) per buck. A weighed amount of feed was given to the bucks daily. Thereafter, daily feed intake per replicate (DFI) was recorded as the difference between feed offered the previous day and that left over in the feeding trough the next day. A summation of DFI per replicate gave the value for the weekly feed intake. Average daily feed intake (ADFI) was then calculated from these values. Feed conversion ratio (FCR) was calculated as feed intake divided by the weight gain.

## Semen collection and evaluation of semen characteristics

At the 8th week of the trial, semen collection commenced using well lubricated artificial vagina whose temperature was maintained at 37°C. The collection was made on a weekly basis from all the bucks for the remaining four weeks of the feeding trial. Semen collected was taken to the laboratory and kept in a hot water bath to maintain the same temperature. The semen samples were thereafter used to determine sperm quality. Semen volume was measured directly from the calibrated tube used for semen collection. Semen pH was determined by dipping a litmus paper into the ejaculate and monitoring the corresponding colour changes. Semen motility was examined as quickly as possible after semen collection. A drop of semen sample was placed on a pre-warmed glass slide, covered with warm cover slip and examined using a microscope at x10 magnification. A scale (0-100) was used to rate the motility. The highest scale (100) reflected the number of sperm cells that showed vigorous and progressive forward motility, whereas, the lowest (0) reflected very minor or immobile cells. Sperm concentration was determined following the procedures described by Azawi & Ismaeel (2012) using Neubaeur haematocytometer. A cover slip was placed on the haematocytometer and two drops of dilute semen was placed under the cover slip. The haematocytometer was carefully placed in a pre-wetted chamber, the lid closed and left for 5 minutes. Thereafter, it was examined using a microscope at ×40 magnification. The sperm cells were counted in five Thoma square of the chamber (i. e. four corners and the center squares). Semen concentration was calculated as follows:

$$\text{Sperm concentration} = \frac{\text{number of sperm cells counted x dilution factor x } 10^6}{\text{area of the haemacytometer x depth of the haemacytometer}}$$

Where: area and depth of haematocytometer = 0.10mm. Percentage live sperm cells were determined using the method described by Esteso *et al*. (2006). A thin smear of semen was made on a clean grease-free slide and stained with two drops of eosin-nigrosin. This technique was based on the principle that eosin-nigrosin penetrates and stains dead sperm cells, while live sperm repel the stain. Dead spermatozoa stained pinkish or reddish, whereas live spermatozoa remained colourless. 100 stained and unstained sperm cells were counted, when the slides were dried using light microscope at ×40 magnification. Percentage of each live sperm cell was then estimated (Esteso *et al*., 2006). Sperm abnormality was determined by making a thin smear of semen sample mounted on clean grease-free glass slide and stained with eosin-nigrosin. 100 sperm cells were counted per slide using hand counter under light microscope (× 40 magnification).

### Evaluation of testicular morphometric traits

At the end of the 12-week study, 8 bucks (2 per treatment) were selected randomly around the mean weight of bucks in each treatment group and used for determination of morphometric traits. The bucks were euthanized, after which, the pairs of testes and epididymis were carefully separated and freed of all adhering connective tissues. Using conventional point counting, fraction of the seminiferous tubules in the testis (epipidymal duct) was estimated. Volume per organ was calculated by multiplying the fraction by the volume of the organ. All testicular sections were observed again at low power magnification (×10) using compound microscope. Each testis round or elliptical profiles of the seminiferous tubules with an apparent lumen was sampled with the frame. Thereafter, the diameter of round profiles or the short axis of elliptical profiles was measured as the tubule diameter. The tubule length per testis was then calculated by dividing the total tubule volume per testis by the cross-sectional area of the tubules.

### Statistical analysis

Data generated from the study were subjected to analysis of variance (ANOVA) procedures for a CRD experimental model. Using the Duncan's multiple-range test as outlined by Obi (2002), significantly different means were separated, and differences were considered to be significant at $p<0.05$. The model for the experimental design was given as follows: $X_{ij} = \mu + T_i + \epsilon_{ij}$. Where: $X_{ij}$ = individual observation; $\mu$ = population mean or overall mean; $T_i$ = treatment mean or effect due to treatment; $\epsilon_{ij}$ = experimental error.

## RESULTS

### Growth performance

Table 1 shows that average final body weight (AFBW), average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were not significantly (($p>0.05$) affected by dietary treatments. It was observed that bucks fed varying dietary levels of *Saccharomyces cerevisae* had numerically higher values for AFBW and ADWG than those that received other dietary treatments.

**Table 2 –** Performance of bucks fed dietary *Saccharomyces cerevisiae* and/or zinc oxide.

| Treatments | A | B | C | D | SEM |
|---|---|---|---|---|---|
| Parameters | | | | | |
| Initial Body Weight (g) | 2850.00 | 2800.00 | 2850.00 | 2800.00 | 31.33 |
| Final Body Weight (g) | 4075.00 | 4425.00 | 4175.00 | 4290.00 | 84.96 |
| Average Daily Weight Gain (g) | 11.00 | 16.00 | 11.00 | 13.00 | 1.16 |
| Average Daily Feed Intake (g) | 30.50 | 30.50 | 31.00 | 31.50 | 0.29 |
| Feed Conversion Ratio | 2.75 | 2.00 | 2.82 | 2.31 | 0.19 |

[a,b,c,d]; Row means with different superscripts differ significantly at $p<0.05$. SEM= Standard error of the mean. Treatment A: control diet with no additives. Treatment B: 0.12 g *Saccharomyces cerevisia*/kg feed. Treatment C: 150 mg Zinc oxide/kg feed. Treatment D: 0.12 g *Saccharomyces cerevisiae* + 150 mg Zinc oxide/kg feed.

### Epididymal sperm characteristics

As shown in Table 2, the effect of dietary treatments on gross epididymal sperm characteristics was significant ($p<0.05$). Bucks fed supplemental *Saccharomyces cerevisiae* (SC) had significantly ($p<0.05$) improved sperm concentration, progressive motility and percent live sperm compared with other treatments. Incidence of head and tail sperm abnormality was also significantly ($p<0.05$) reduced in bucks fed SC-based diets, and highest for control (Treatment A) fed bucks. Sperm concentration in SC supplemented groups was 7.28 compared with 7.10, 6.60 and 5.89 recorded in treatments C, D and A. Progressive motility in SC dietary groups was 80.05, compared with 40.05, 60.05 and 70.05 respectively in treatments C, D and A. Live sperm percent was also higher in SC-supplemented groups, being 94.21 compared with 92.41, 93.60 and 89.40 obtained in treatments C, D, and A. Sperm motility was significantly ($p<0.05$) reduced in bucks fed zinc oxide (treatment C) supplemented diets. There was an observed significant ($p<0.05$) reduction in sperm concentration and percent live sperm in bucks that received the control diet (Treatment A). Bucks fed diets supplemented with a combination of *Saccharomyces cerevisiae* and zinc oxide (Treatment D) had significantly ($p<0.05$) higher sperm pH compared with other treatments. Nevertheless, the least sperm pH value was recorded amongst bucks fed *Saccharomyces cerevisiae*-based diets (Treatment B).

### Testicular morphometric traits

Dietary treatments had significant ($p<0.05$) effect on testicular morphometric traits of rabbit bucks (Table 3). Inclusion of *Saccharomyces cerevisiae* alone in the diet (Treatment B) resulted in significant

**Table 3 –** Epididymal sperm traits of bucks fed dietary *Saccharomyces cerevisiae* and/or zinc oxide.

| Treatments | A | B | C | D | SEM |
|---|---|---|---|---|---|
| Parameters | | | | | |
| Sperm concentration (× $10^9$/ml) | 5.89$^d$ | 7.28$^a$ | 7.10$^b$ | 6.60$^c$ | 2.03 |
| Progressive motility (%) | 70.05$^b$ | 80.05$^a$ | 40.05$^d$ | 60.05$^c$ | 5.59 |
| Live sperm (%) | 89.40$^d$ | 94.21$^a$ | 92.41$^c$ | 93.60$^b$ | 6.99 |
| Head abnormality | 50.50$^a$ | 39.05$^d$ | 46.95$^b$ | 39.65$^c$ | 1.83 |
| Tail abnormality | 47.55$^a$ | 39.85$^d$ | 41.05$^b$ | 44.65$^b$ | 1.14 |
| pH | 7.60$^b$ | 7.50$^c$ | 7.60$^b$ | 7.81$^a$ | 0.42 |

$^{a,b,c,d}$; Row means with different superscripts differ significantly at $p<0.05$. SEM= Standard error of the mean. Treatment A: control diet with no additives. Treatment B: 0.12 g *Saccharomyces cerevisia*/kg feed. Treatment C: 150 mg Zinc oxide/kg feed. Treatment D: 0.12 g *Saccharomyces cerevisiae* + 150 mg Zinc oxide/kg feed.

improvement in tubule diameter, epididymal volume, volume fraction of duct, and total duct volume. Although, dietary supplementation of zinc oxide alone (Treatment C) significantly ($p<0.05$) improved seminal vesicle volume in bucks, volume fractions of tubules were better in bucks fed the control diets. Testicular tubule length and testicular volume was highest ($p<0.05$) for bucks fed treatments A and D while the least ($p<0.05$) testicular tubule length was recorded for treatments B and C. Meanwhile, testicular volume was lowest ($p<0.05$) for bucks fed dietary *Saccharomyces cerevisiae* (Treatment B).

**Table 4 –** Testicular morphometric traits of bucks fed dietary *Saccharomyces cerevisiae* and/or zinc oxide.

| Treatments | A | B | C | D | SEM |
|---|---|---|---|---|---|
| Parameters | | | | | |
| Testicular volume ($cm^3$) | 1.41$^a$ | 1.28$^c$ | 1.38$^b$ | 1.41$^a$ | 0.02 |
| Volume fraction of tubules (%) | 85.05$^a$ | 82.65$^c$ | 84.65$^b$ | 84.75$^b$ | 3.59 |
| Testicular diameter (µm) | 29.85$^c$ | 30.45$^a$ | 30.05$^b$ | 28.65$^d$ | 25.42 |
| Total tubule length (m) | 14.75$^a$ | 14.35$^b$ | 14.25$^b$ | 14.55$^a$ | 0.54 |
| Epididymal volume ($cm^3$) | 0.26$^c$ | 0.33$^a$ | 0.29$^b$ | 0.26$^c$ | 0.11 |
| Volume fraction of duct (%) | 50.65$^b$ | 68.90$^a$ | 49.05$^c$ | 43.60$^d$ | 35.97 |
| Total duct volume ($cm^3$) | 0.20$^b$ | 0.21$^a$ | 0.17$^c$ | 0.11$^d$ | 0.14 |
| Seminal vesicle volume ($cm^3$) | 0.08$^c$ | 0.12$^b$ | 0.17$^a$ | 0.06$^d$ | 0.01 |

$^{a,b,c,d}$; Row means with different superscripts differ significantly at $p<0.05$. SEM= Standard error of the mean. Treatment A: control diet with no additives. Treatment B: 0.12 g *Saccharomyces cerevisia*/kg feed. Treatment C: 150 mg Zinc oxide/kg feed. Treatment D: 0.12 g *Saccharomyces cerevisiae* + 150 mg Zinc oxide/kg feed.

# DISCUSSION

## Growth performance

According to Gibson & Roberfroid (2008), supplementation of *Saccharomyces cerevisiae* (SC) in the diet of animals can improve the quality of their feed and also their performance and productive traits. However, as observed in the present study (Table 2), mean live weights of bucks at the end of the feeding trial were 4075 g, 4425 g, 4175 g, 4290 g for A (control), B (SC), C (zinc oxide-ZNO) and D (SC + ZNO) respectively. This indicates that dietary inclusion SC or ZNO alone or a combination of both feed additives did not exert any significant ($p>0.05$) effect on performance of bucks. The result of the present study on growth performance is in line with the work of Collins & Moran (1999), whose study revealed that supplementing zinc oxide in the diet of broiler birds did not significantly ($p>0.05$) enhance body weight and feed efficiency of the birds. The result however contradicts the reports of Ezema & Eze (2015), who reported that rabbits fed diets supplemented with SC had improved average weight gain and daily feed intake compared to those in the control group. The result also disagrees with the work of Alcicek *et al*. (2004), who found that dietary inclusion of 10ppm zinc oxide resulted in significant improvement in rabbit performance. Although, Yirga (2015) noted that results obtained on the growth promoting effects of probiotics in livestock nutrition are somewhat inconsistent, probiotic feed additives are largely capable of inducing improved growth responses in animals. Thus, the exact reason for the non-significant effect on growth performance in both the *Saccharomyces cerevisiae* and zinc oxide supplemented groups as observed in this study is not known. However, it may be due to factors associated with diet preparation and composition, quantity of feed additives used, feeding strategy, or other physiologic or environmental issues.

## Epididymal spermcharacteristics

Cheng *et al*. (2002) pointed out that semen quality assessment is an important marker in selection of

breeding males, and also for effective monitoring of the male's reproductive capacity. Usually, sperm traits such as sperm motility, percentage of live or dead sperm, morphological features among others often determines the inseminating capacity of particular semen (Okoro *et al*., 2016). As shown in Table 3, supplemental SC had more pronounced ($p$<0.05) effect on gross epipidymal semen characteristics of rabbit bucks compared to their counterparts that received other dietary treatments. According to Wysokinska & Kondracki (2013), sperm motility is a vital ejaculate attribute that has an enormous impact on egg cell fertilization. The rate of motility of a sperm determines the extent it can gain entry into the vestments (the cumulus oophorus, zona pellucida, and other coatings) around the oocyte, in order to reach the site of fertilization as well as achieve fertilization (Hidiroglou *et al*., 1978). It is evident from the result on semen characteristics obtained in the present study that semen from bucks fed SC had superior motility, higher sperm concentration, higher percentage of sperm livability and lower number of abnormal spermatozoa. This improvement in semen characteristics may be attributed to the antioxidant property of SC as a probiotic-based feed additive (Uskova & Kravchenko, 2009; Spyropoulos *et al*., 2011). The result of the present study on epididymal sperm characteristics agrees with the findings of Mangiagali *et al*. (2012) whose study showed that inclusion of lycopene in the water of rabbits, particularly at 0.5g/L, significantly affected sperm motility, forward progressive motility, sperm viability and other sperm quality parameters. Nevertheless, Castellini *et al*. (2003) reported that supplemental antioxidants such as vitamin C and E had no significant effect on sperm parameters of rabbits.

As reported by Subeve *t al*. (2005) and Mourvaki *et al*. (2010) semen contains lipids as important constituents. Structurally, seminal lipids help to maintain membranous integrity, absorption, utilization and inseminating capacity of spermatic cells. Nonetheless, these lipids are prone to attack by ROS. Physiologically, ROS are needed during sperm capacitation and fusion of sperm to ova (Aggarwal *et a*l., 2003). Again, when the body's ability to neutralize free radicals is below the rate at which the radicals are produced, oxidative stress becomes the resultant effect (Khan, 2011). Hence, according to Castellini *et al*. (2003) the fertilizing ability of sperm cell depends largely on the ideal balance of polyunsaturated fatty acids and antioxidant effects. Unlike other mammals, sperm from rabbits have been shown to contain high concentrations of n-6 than n-3 polyunsaturated fatty acids (Gliozzi *et al*., 2009). This predisposes the spermatozoa to faster oxidative damage under storage conditions, with consequent damaging effect on quality of the sperm (Bansal & Bilaspuri, 2011). Nevertheless, there are scientific evidence (Castellini, 2008; Mourvaki *et al*., 2010) to corroborate the claim that compounds with antioxidant properties such as *Saccharomyces cerevisiae* have potential to inhibit oxidative damage in cell membrane of sperm cells and fragmentation of sperm DNA brought about by the harmful activities of free radicals. According to Aggarwal *et al*. (2004) these beneficial effects of antioxidants in turn helps improve sperm motility and other seminal traits.

It is notable that bucks fed zinc oxide (Treatment C) supplemented diets recorded the least sperm progressive motility compared to the control (treatment A), and other dietary treatments. Notwithstanding, the motility value of 40.05% recorded for zinc oxide fed bucks was still within the range reported by Pascual *et al*. (2004) as shown in Table 3. This result on decreased sperm motility associated with zinc oxide supplementation contradicts the findings of Kumar *et al*. (2006) whose work showed that at different levels of dietary zinc inclusion significantly improved mass and individual sperm motility of crossbred cattle bulls. Rahman *et al*. (2014) also reported significant increase in sperm motility in rabbit bucks fed zinc sulphate supplemented diets. It has been shown that zinc is actively involved in energy movement along the adenosine triphosphate energy pathway, basically by controlling phospholipid energy reserves, improving sperm oxygen uptake, and thus sperm motility (El-Masry *et al*., 1994). Zinc is also a potent antioxidant compound that scavenges superoxide radicals present in seminal plasma, and thus, improves sperm motility (Kumar *et al*., 2006). As reported by Negamine *et al*. (1990) and Kurmal *et al*. (2006) enzymes such as sorbitol dehydrogenase and lactose dehydrogenase are major biochemical constituents of zinc and increase in these enzymes led to increase in sperm motility. It is surprising though, that this was not the case in the present study. Zinc supplementation led to significant ($p$<0.05) decrease in motility of spermatic cells. This result is however similar to that obtained from the reports of Khallare & Shrivastav (2003) and Alvarez-Gomez *et al*. (2007) who attributed reduced sperm motility in animals fed neem leaf meal based diets to blockage in energy metabolic route.

Although, dietary treatments had significant ($p$<0.05) effects on sperm characteristics of rabbit

bucks in the present study (Table 3), the percentage head and tail abnormal sperm cells obtained in all the supplemented dietary groups exceeded 20 %. Research showed that 20 % head and tail abnormality is the upper limit recommended as the lowest value of abnormal spermatozoa indicative of good reproductive and prolific functions in male animals used for either natural mating or artificial insemination purposes (Zemjanis, 1977; Oyeyemi & Okediran, 2007). More so, there are considerable evidence that supports the existence of positive genetic correlation between body weight gain and sperm concentration (Rodriguez-De Lara *et al*., 2015), and ejaculate volume (Lavara *et al*., 2011) in rabbits, and also between daily body weight gain and volume of ejaculate, abnormal sperm, and sperm pH in broilers (Ramamurthy *et al*., 1989). This implies that heavier animals tend to possess more sperm reserves. This was however not the case in the present study, as final body weight and average body weight gain did not differ among different treatment groups, and as a result, had no determining effect on reproductive performance of bucks. The results of the present study also showed a significant ($p$<0.05) reduction in pH in bucks on *Saccharomyces crevisia*e diet, whereas the pH of those on zinc supplemented and control diets were comparable ($p$>0.05). However, a marked significant improvement in pH was recorded among bucks fed a combined dietary mixture of *Saccharomyces cerevisiae* and zinc oxide. This showed that the combined effects of the probiotic (*Saccharomyces cerevisiae*) and zinc supplementation resulted in enhanced semen pH. The result obtained on pH does not agree with the findings of Attia *et al*. (2013) who reported that including either vitamin C, or vitamin E, combined with selenium or probiotic in the diets of rabbit bucks did not affect semen pH. The findings of Kumar *et al*. (2006) indicated that there was significant ($p$<0.01) reduction in semen pH values in bucks fed zinc sulfate supplemented diet.

## Testicular Morphometric traits

According to Ewuola (2013), two important factors that guide breeders in selecting breeding males include the quantity of good quality spermatogenic cells with good livability produced by the testis, and its ability to effectively store the produced spermatozoa. Oyeyemi *et al*. (2002) also opined that having adequate information on the morphometric traits of the testis and other reproductive organs of a particular breeding male is a vital pointer to its breeding value and fecundity. This is due to the fact that the spermatozoa-storing ability of the testis of a male animal determines its fertility level. Tubular measurement is usually the traditional method employed in assessing spermatogenic activity in any investigation involving testicular functions (Silva *et al*., 2006). As shown in Table 4,dietary *Saccharomyces cerevisiae* (SC) improved ($p$<0.05) testicular parameters of bucks, mainly tubule diameter, epididymal volume, volume fraction of duct, and total duct volume, compared to those on the control diet. This implies that SC promotes the growth and development of testicular glands and gonadal sperm reserve. Colenbrander & Kemp (1990) asserted that testicular weight and quantity of sperm produced are correlated. Morris *et al*. (1999) also stated that within a species of animals, there often exist positive correlation between spermatozoa production, testicular size and testicular length. Some authors (Oyeyemi & Okediran, 2007; Oyeyemi *et al*., 2002; Britto *et al*., 2004) have also shown through their reports that testes that are larger in size tend to possess more sperm producing ability than smaller ones. Larger testes weight of bucks fed SC based diet would be said to contain more seminiferous tubules, leydig cells, Sertoli cells, and thus, produce larger amount of sperm.

Mosenthin & Baller (2000) and Ewuola *et al*. (2011) reported that dietary supplementation of probiotics has been linked with enhanced intestinal mucosal development which has significant effect on improved nutrient digestibility. The improved intestinal mucosal development helps to promote the growth and concentration of Sertoli cells and seminal fluids. According to (Berndtson *et al*., 1987) testes with more Sertoli cells are larger in size and tend to produce more spermatozoa than those with less number of Sertoli cells. However, it was observed that bucks fed mixture of *Saccharomyces cerevisiae* and zinc oxide had reduced testicular diameter compared to the other treatment dietary groups. This may probably indicate that the synergistic effect of both additives had a depressing impact on testicular growth of rabbit bucks. Morton (1988) attributed decrease in testis size to extensive loss of seminiferous epithelial cells, invariably signifying lower levels of spermatozoa production. Ogbuewu*et al*. (2009) reported significant reduction in seminiferous tubule diameter of rabbit bucks fed 15% neem leaf-based diet. Whereas, testicular volume was significantly improved by dietary supplementation of zinc oxide + *Saccharomyces cerevisiae*, it was lowest among bucks on dietary *Saccharomyces cerevisiae* and zinc oxide. The work of Ansa *et al*. (2017) showed that supplementation of cadmium reduced testis volume and

epididymal carrying capacity and length. El-Neweshy *et al*. (2013) attributed reduction in testicular volume to reduced testosterone levels, and this consequently results in low sperm production. However, this study recorded improved testis volume among zinc oxide +*Saccharomyces cerevisiae* supplemented groups. This aligns with the findings of Ansa *et al*. (2017) who reported that methanolic extract of Date palm (*Phoenix dactylifera*) resulted in significant improvement of testis volume and density among rabbit bucks exposed to cadmium inclusion.

Dietary supplementation of zinc oxide alone (treatment C) significantly ($p$<0.05) improved seminal vesicle volume in bucks. This result agrees with the findings of Amara *et al*. (2008) whose study showed that dietary inclusion of zinc not only reduced DNA oxidative damage, but also reversed reduction in sperm and testosterone production induced by cadmium exposure in the testis of Wistar rats. The work of Oteiza *et al*. (1999) had shown that zinc deficiency resulted in concomitant cell damage in the testis of rats. Results of the present study also revealed that seminal vesicle volume was markedly ($p$<0.05) reduced in bucks fed treatments D and A (*S. cerevisiae* + zinc oxide diet, and control diet). This result is in consonance with the reports of Predes *et al*. (2010) and Elgawish & Ghanem (2014) whose works revealed that there was significant reduction in seminal vesicle volume and prostate gland of Wistar rats exposed to 1.2 mg/kg cadmium supplemented diet.

## CONCLUSION

Dietary inclusion of *Saccharomyces cerevisiae* at 0.12g/kg of feed, improved epididymal sperm characteristics and testicular morphometrism of rabbit bucks.

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