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EFFECTS OF PROBIOTICS THERAPY ON SEMEN QUALITY TRAITS OF WEST AFRICAN DWARF BUCKS

***Udoh, U.H. and Inyang, U.A.**

Department of Animal Science, University of Uyo, Akwa Ibom State, Nigeria.

* Corresponding author's E-mail: utibudy4good@yahoo.com

ABSTRACT

Effects of diets fortified with combinations of baker's yeast (Saccharomyces cerevisiae) and lactic acid bacteria (Lactobacillus acidophilus) were examined on semen quality traits of West African Dwarf (WAD) bucks. Forty-two (42) WAD bucks were randomly allocated to six diets or treatments (14.28% CP and 1788.3kcal/kg ME) viz: T_1 (Panicum maximum + concentrate feed mixture), T_2 (Panicum maximum + concentrate feed + 1 g of Lactobacillus acidophilus /head/day), T_3 (Pniicum maximum + concentrate feed + 2.50 g of Saccharomyces c. /head/day), T_4 (Panicum maximum + concentrate feed + 2.50 g of Saccharomyces c. /head/day), T_5 (Panicum maximum + concentrate feed + 2.50 g of Lactobacillus a. and Saccharomyces c. /head/day) and T_6 (Panicum maximum + concentrate feed + 5.0 g of Lactobacillus a. and Saccharomyces c./head/day). The experimental design was completely randomized design. Data obtained was subjected to ANOVA of SAS (version 6.03). Dietary supplementation of probiotics improved semen quality traits in the following pattern: T_2, T_3 for testis weight (62.33g); T_6 for sperm motility (95.00%); T_6 for semen volume and concentration (0.86 mL and 293.00 x 10^6), total motile sperm and morphology (2.41 x 10^9 and 93.33%); T_2, T_3, T_4, T_5, T_6 for live: dead sperm; but similar for final live weight. Dietary supplementation of probiotics (Saccharomyces c. + Lactobacillus a. combinations) should be used for the improvement of semen quality traits of West African Dwarf (WAD) bucks.

Keywords: West African Dwarf bucks, *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, Semen quality traits.

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INTRODUCTION

Goats *(Capra hircus)* are believed to be the second animal domesticated after the dog. Goat semen is a whitish secretion of the buck's reproductive organs, obtained at ejaculation. It contains spermatozoa. Probiotics are viable micro-organisms that improve the gut microflora by enzymes, organic acids, vitamins and non-toxic antibacterial substances that the microbes secrete once ingested (Jun *et al*., 2002). Probiotics may also be a functional food, but more specifically it is a live microbial feed supplement that beneficially affects the host beyond correcting the traditional nutrient deficiencies by improving intestinal balance (Fuller, 1999). The effect of probiotics is achieved through stimulation of beneficial bacteria. Probiotics only change the profile of microbial intestine contents in animals by increasing the numbers of micro-organisms belonging to the group of favorable on account of unfavourable pathogens or toxicogenic microflora (Agazzi *et al.*, 2014).

Probiotics have a lot of benefits on male reproductive system. Probiotics can improve appetite, egg size in chickens, body weight of animals and semen quality. In order to exert their beneficial effects, probiotics must

survive the gastrointestinal tract, persist in the host and prove safe for consumers (Devries *et al.,* 2006). *Saccharomyces cerevisiae (baker's yeast) and Lactobacillus acidophilus (lactic acid bacteria)* are probiotics. *Lactobacillus acidophilus* is a strain of probiotics which confers health benefits on the host. It improves libido in treated animals, stimulates the immune system, makes the reproductive organs fertile and has been proved to enhance the functions of the lower gut (Ross *et al.,* 2010). They are generally recognized as safe (GRAS) organisms and can be safely used as probiotics for medical and veterinary applications (Fuller, 1989). *Saccharomyces cerevisiae* is a commercial yeast product used specifically for animal feeding. It has a broad range of biomodulatory properties and is a growth promoter for ruminants. It protects against genotoxicity and mitigates its spermatoxic effects. This might in part be due to its ability to absorb these mycotoxins, where several studies have reported the absorption mechanisms in vitro (Baptista *et al.,* 2004).

This study was therefore conducted to determine the effects of mixed probiotics (*Lactobacillus acidophilus,* and *Saccharomyces cerevisiae)* on the semen quality traits of West African Dwarf bucks.

MATERIALS AND METHOD

Experimental Site: This study was carried out in the Goat Unit of the Teaching and Research Farm, Department of Animal Science, Faculty of Agriculture, University of Uyo, Uyo Akwa Ibom State, Nigeria. The area falls within the tropical rainforest zone in Nigeria. The area has two distinct seasons: wet (March-mid November) and dry season (November – March). It has an average annual rainfall range of 2200mm to 3500 mm. The temperature of the area ranges from 26^{o}C to 28^{o}C. Uyo is located between Latitude $4^{0}31$ and $5^{0}30$ north and Longitude $7^{o}30$ and $8^{0}20$ east of the Equator. The mean altitude is 38.1 m above sea level. Sunshine is between 1400 to 1500 hours per year, relative humility is from 71% to 88% annually (University of Uyo Meteorological station, 2014).

Experimental Animals and Management: Forty-two (42) diseases-free yearlings West African Dwarf (WAD) bucks, about 6 – 9 months old were procured from goat farms within Uyo metropolis. Bucks were quarantined for 2 weeks, treated with broad spectrum antibiotics, dewormed and treated against ectoparasites. At the end of the quarantine, bucks were identified individually with neck tags. They were managed under close veterinary watch throughout the study which lasted 109 days (15 weeks, 4days). Prophylactic medications were provided against prevalent goat infections.

Experimental Diets: *Panicum maximum* was harvested fresh daily from the bush and chopped to about 10 cm length. The animals were offered experimental diets at 5% body weight daily. The grass to concentrate ratio was 60:40 (3:2). The proximate composition of *Panicum maximum*, on dry matter basis is as presented in Table 1 below.

Table 1: Proximate composition (%DM) of *Panicum maximum*

Nutrient (%)	***Panicum maximum***
Dry matter	42.75
Crude protein	9.03
Crude fibre	30.00
Ether extract	7.93
Ash	11.07
Nitrogen free extract	41.05

Bucks were randomly allotted to six (6) treatments with seven (7) animals per treatment. During the study, feed was provided at 5% body weight. Formulated concentrate diet and *Panicum maximum* (chopped to about 10cm in length) were served at grass to concentrate ratio of 3:2. Probiotics were top dressed on the concentrate feed per animal, each feeding time. Water was provided *ad libitum.* The experimental diets were:

Treatment 1 (Control or T_1) = *Panicum maximum* + Concentrate feed mixture
Treatment 2 (T_2) = *Panicum maximum* + Concentrate feed mixture+ 1g of antibiotic/head/day.
Treatment 3 (T_3) = *Panicum maximum* + Concentrate feed mixture +2.50 g of baker's yeast (*Saccharomyces cerevisiae)* /head/day.
Treatment 4 (T_4) = *Panicum maximum* + Concentrate feed mixture +5.0 g of baker's yeast (*Saccharomyces cerevisiae)* /head/day.
Treatment 5 (T_5) = *Panicum maximum* + Concentrate feed mixture +2.50 g of Lactic acid bacteria (*Lactobacillus a.*) and baker's yeast combination/head/day.
Treatment 6 (T_6) = *Panicum maximum* + Concentrate feed mixture +5.0 g of Lactic acid bacteria and baker's yeast combination/head/day.

The calculated Crude protein (%) in each of the six (6) treatments was 14.28% and the Metabolizable Energy in the feed mixture was 1788.30 kcal/kg dry matter. The gross composition of the experimental diets mixture (%) is presented in Table 2 below.

Table 2: Gross Composition of the experimental diets' mixture (%)

Treatments/Diets	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
Ingredients:						
Dried cassava peel (DCP)	45.00	45.00	45.00	45.00	45.00	45.00
Brewers dried grain (BDG)	40.70	40.70	40.70	40.70	40.70	40.70
Palm kernel cake	10.00	10.00	10.00	10.00	10.00	10.00
Limestone	2.50	2.50	2.50	2.50	2.50	2.50
Salt	1.50	1.50	1.50	1.50	1.50	1.50
*Vitamin/Mineral premix	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100
Yeast (g)	-	-	2.50	5.00	-	-
Yeast + LAB mixture (g)	-	-	-	-	2.50	5.00
Antibiotic (g)	-	1.00	-	-	-	-
#Crude protein	14.28	14.27	14.47	14.47	14.44	14.50
ME (Kcal/kgDM)	1788.3	1788.3	1788.3	1788.3	1788.3	1788.3

LAB = Lactic acid bacteria; ME = Metabolizable energy; DM = Dry matter.
# = Determined crude protein
**Supplied per kg diet: Vit. A, 10 x 10^6 ; Vit. D_3, 2 x 10^6 I.U; Vit. E, 2 x 10^4mg; Vit. K_3, 2 x 10^3mg; Vit. B, 3000mg; Vit. B_2,5,000mg; Niacin, 45,000mg; Calcium pantothenate, 10g; Vit. B_6, 4g; Vit. B_{12}, 20mg; Choline chloride, 300g; Folic acid, 1g; Biotin, 50mg; Manganese, 300g; Iron, 120g; zinc, 80g; Copper, 8.5g; Iodine, 1.5g; Cobalt, 300mg; Selenium, 120mg; Anti-oxidant 120g.*

Data Collection: Semen samples were collected from each buck in the goat pen once a week between 6.30 am and 8.00 am by means of an electro-ejaculator. The experiment lasted for 109 days (15 weeks, 4 days). The volume of escalate collected was read directly from graduated collection tubes. Progressive motility was examined in Animal Science Laboratory immediately after collection, by placing a drop of semen on a glass slide and examining under a microscope at x40 objective lens magnification. Physical evaluation was done on

each ejaculate (i.e. sperm volume, motility, morphology, sperm concentrate, live: dead, total motile sperm/ejaculate, testis weight and final weight.

Data Analysis: All data collected were subjected to one way analysis of variance with Completely Randomized Design (CRD). Means were separated using Duncan's multiple range test. All statistical analyses were done using procedure of ANOVA of SAS (1998), version 6.03.

RESULTS AND DISCUSSION

The effects of probiotics on semen quality traits (Testis weight, sperm motility and morphology, semen volume, semen concentration, live: dead sperm ratio, total motile sperm per ejaculation) and final live weight of West African Dwarf bucks are as presented in Table 3 below.

Effects of Probiotics on Testis Weight: Testis weight was influenced by all the experimental diets. Adetoro and Ewuola (2014) observed that this attribute can be influenced by nutrition and environment. The testis weight in this study was generally poor. Bucks on T1, T_2 (1 g of antibiotic/head//day) and T_3 (2.50 g of baker's yeast/head/day) improved testis weight to the standard range of between 55.00 and 77.28 g as proposed by Gofur *et al.,* (2007). The reason for the variation may be attributed to age and physiology in terms of reproduction. Sole inclusion of either antibiotics at 1 g/head/day or baker's yeast at 2.50 g/head/day can therefore be recommended for the improvement of testis weight in WAD bucks. The effects of T_2 and T_3 on testis weight (62.33 g respectively) were however not significantly ($p > 0.05$) different from those on T1 but different from those on T_4, T_5 and T_6 (51.00, 52.67 and 54.00 g respectively). Result obtained in this study revealed that inclusion of baker's yeast up to 5.0 g or its incorporation with lactic acid either at 2.50 g or 5.0 g reduces testis weight and should be discouraged.

Effects of Probiotics on Sperm Motility: Sperm motility was not influenced by T_2 (1 g of antibiotic/head/day), T_3 (2.5 g of baker's yeast/head/day) and T_5 (2.50 g of Lactic Acid Bacteria and baker's yeast combination/head/day). Bucks on treatment 4 (5.0 g of baker's yeast (*Saccharomyces cerevisiae)* /head/day) had a negative influence (85.00 %) by reducing sperm motility but was similar ($p > 0.05$) with response from T1, T2, T3 and T5 (were all 88.33 %). Therefore, when the improvement of sperm motility is considered, treatments 2, 3, 4 and 5 should not be administered. Bucks on Treatment 6 (5.0 g of lactic acid bacteria and yeast combination/head/day) recorded an improved sperm motility significantly ($p < 0.05$) at 95.00 %. The increase in sperm motility might be caused by an increase in ATPase activity which might be linked to improvement in the ultra structure of the sperm (Thangaraj *et al.,* 2003) thereby boosting the motility of the bucks. The results from this study revealed that 5.0g of lactic acid bacteria and baker's yeast combination can be administered to WAD bucks to improve their sperm motility.

Effects of Probiotics on Semen Volume: Semen volume was most improved in bucks fed Treatment 6 (5.0 g of Lactic Acid Bacteria and baker's yeast combination/head/day) with 0.86mL though similar ($p > 0.05$) with those on T_5, T_2, T_1 (0.80, 0.77 and 0.73mL respectively) but different from bucks on T_4 and T_3 (0.70 and 0.67mL respectively). Treatments 3 (2.5 g of baker's yeast/head/day) and T_4 (5.0 g of baker's yeast/head/day) had negative influences by reducing the semen volume. Treatment 2 (1 g of antibiotic per head/day) was not significantly different ($p > 0.05$) from T_5 (2.50 g of lactic Acid Bacteria and baker's yeast combination per head/day). The reason for the increase in semen volume of bucks on T6 can be attributed to some lactic acid bacteria which have been shown to increase colonic NADPH-cytochrome P-450 reductase activity (Pool-Zobel, 2005) and glutathione S-transferase levels (Challa *et al*., 1997). Obviously, these enzymes which are involved in several

metabolic processes including spermatogenesis and steroidogenesis might enhance such physiological functions in the treated bucks.

The results from this study revealed that 5.0 g of Lactic acid bacteria and baker's yeast combination can be administered to WAD bucks to significantly ($p < 0.05$) improve their semen volume.

Effect of Probiotics on Sperm Morphology: Sperm morphology was most influenced ($p<0.05$) by treatment 6 (5.0 g of Lactic acid bacteria and yeast combination/head/day) at 93.33 % and similar ($p>0.05$) with T1, T2 (1 g of antibiotic/head/day), T_4 (5.0g of baker's yeast /head/day) and T_5 (2.50 g of lactic acid bacteria and baker's yeast combination/head/day) at 88.33, 90.00, 88.33 and 91.67 % respectively. T_3 (2.5 g of baker's yeast/head/day) had a negative influence by reducing sperm morphology to 86.67%. Therefore when the improvement of sperm morphology is considered T_6 should be adopted while T_2, T_3, T_4 and T_5 should not be administered. Microorganisms (pathogens) possess a deleterious effect on sperm function, both directly by altering the structure of the sperm (morphology), by affecting its motility (Depuydt *et al*., 1998) or by provoking a premature acrosome reaction (Kohn *et al*., 1998). However, probiotics have shown several mechanisms in the live mammalian body. Such mechanisms include but not limited to production of antibacterial peptides from paneth cells and an increase in brush border enzyme activity (Satoh, 1988) thereby excluding the unwanted microbes from altering the form of sperm cells. This study revealed that inclusion of baker's yeast and lactic acid bacteria combination at 5.0 g increases sperm morphology and should be encouraged.

Effects of Probiotics on Semen Concentration: Semen Concentration was most influenced ($p<0.05$) by treatment 6 (5.0 g of lactic acid bacteria and baker's yeast combination/head/day). Bucks semen concentration from T1, T_3 (2.5 g of baker's yeast/head/day), T_4(5.0 g of baker's yeast /head/day) and T_5(216.67, 215.67, 214.33 and 225.00 $x10^6$) were similar ($p>0.05$) and lower ($p < 0.05$) than those on T_2 (250.00 x 10^6). Therefore their (T_1–T_5) use should be discouraged. Pathogenic organisms within the semen are noted to not only affect morphology but also induce the putrefaction of diluents components of biological origin, and the utilization of metabolic substrates (Lamming, 1984), and indirectly stimulating the production of antibodies that can be directed against the sperm glycocalyx complex (Kurpisz and Alexander, 1995) and this in turn affects semen concentration and sperm cells. Al-Sobayil *et al.* (2008) had reported a range of 1.22 – 2.11 $x10^9$ mL for low and high doses of synbiotics on Damascus bucks while a range of 2.68 – 2.71 $x10^9$ mL for Aradhi bucks treated with low and high doses of the same synbiotics. Administering 5.0 g of lactic acid bacteria and baker's yeast combination improved semen concentration and therefore should be encouraged.

Effects of Probiotics on live: dead of Sperm: Live: dead of sperm was most influenced by treatment 6 (5.0 g of lactic acid bacteria and baker's yeast combination/head/day) which recorded 97.00% but was similar ($p>0.05$) with those of T_5 and T_4 (93.33 and 93.33% respectively). The ratio for T2, T3, T_4 (5.0 g of baker's yeast /head/day) and T_5 (2.50 g of lactic acid bacteria and baker's yeast combination/head/day) were not significantly ($p>0.05$) different. Bucks on T1 (Control) recorded the least on live to dead ratio and should not be encouraged. Some reports have indicated that metabolic products such as endotoxins from some bacteria (pathogens) appear to have detrimental effects on the survival of sperm (Almond and Poolperm, 1990). Hence, semen quality and the quantity of viable sperm cells may be reduced with bacterial contamination. However, the action of the probiotics might have led to exclusion of these deleterious organisms thereby favouring the increase in live sperms cells. Moreso, it has

been found that probiotic bacteria exert an antioxidant effect on sperm to protect against the surrounding free radicals (Yang *et al.*, 2012). The results from this combination should be encouraged to improve the live: dead of sperm.

Effect of Probiotics on Total Motile Sperm per Ejaculate: Total motile sperm per ejaculate was most influenced ($p<0.05$) by treatment 6 (5.0g of lactic acid bacteria and baker's yeast combination/head/day) at 2.41 $x10^9$. Total motile sperm/ejaculate ranged from 1.27 – 2.41 x 10^9 ml across all treatments. T_3 (2.5 g of baker's yeast/head/day) and T_4 (5.0 g of baker's yeast /head/day) reduced total motile sperm/ejaculate (1.27 and 1.29 $x10^9$) which were similar ($p>0.05$) with T_1 and T_5 (1.40 and 1.59 $x10^9$ respectively) and should be discouraged. Inclusion of 5.0 g of lactic acid bacteria and baker's yeast combination can therefore be recommended for the improvement of total motile sperm per ejaculate. The reason for the positive effect of the mixed probiotics (T_6) cannot be farfetched as this can be attributed to the synergistic effect on spermatogenesis (Challa *et al*., 1997).

Effect of Probiotics on Final Live Weight of Bucks: Final live weight of bucks was influenced ($p<0.05$) by the treatments. Bucks on T_6 (5.0 g of lactic acid bacteria and baker's yeast combination/head/day) and T_5(2.50 g of lactic acid bacteria and baker's yeast combination/head/day) at 11.75 and 11.63 kg respectively, improved the final live weight numerically above T_1(11.53 kg). Bucks on T_2 (11.23 kg) and T_4(11.40 kg) were similar with each other and similar ($p>0.05$) to T_6, T_5, and T_1 while those on T_3 (2.50 g of baker's yeast/head/day) at 10.60 kg were the least ($p<0.05$) when compared to others. Inclusion of 5.0 g of lactic acid bacteria and baker's yeast combination/head/day is recommended for the improvement of final live weight of West African Dwarf (WAD) bucks. The reason for this can be attributed to the synergistic effect between the probiotic organisms and rumen microorganisms to enhance their functions in rumen degradations and absorptions (Al-Sobayil *et al.*, 2008) and in turn body weight increase or growth as observed for bucks on T6.

Table 3: Semen Quality Traits of Goats Fed Probiotics

Parameters	Treatment						
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**SEM**
Testis weight (g)	62.33^a	62.33^a	62.33^a	51.00^b	52.67^b	54.00^b	7.33
Sperm motility (%)	88.33^b	88.33^b	88.33^b	85.00^b	88.33^b	95.00^a	1.80
Sperm volume (ml)	0.73^{ab}	0.77^{ab}	0.67^b	0.70^b	0.80^{ab}	0.86^a	0.05
Mophology (%)	88.33^{ab}	90.00^{ab}	86.67^b	88.33^{ab}	91.67^{ab}	93.33^a	1.52
Concentration ($x10^6$)	216.67^c	250.00^b	215.67^c	214.33^c	225.00^c	293.00^a	3.49
Live:dead (%)	86.67^c	91.67^{bc}	88.33^{bc}	93.33^{ab}	93.33^{ab}	97.00^a	1.57
TMS/E ($x10^9$)	1.40^c	1.69^b	1.27^c	1.29^c	1.59^{bc}	2.41^a	0.11
Final LW of bucks(kg)	11.53^a	11.23^a	10.60^b	11.40^a	11.63^a	11.75^a	0.85

^{abc} Means along rows bearing different superscripts are significantly different ($p<0.05$).
TMS/E = Total motile sperm/ejaculation; SEM = standard error of mean; LW = Live weights.

CONCLUSIONS

- Dietary supplementation of probiotics influenced and improved semen quality traits of West African Dwarf bucks.
- Treatment with 2.50g/day of yeast improved testis weight; 5.00g/day of yeast and lactic acid bacteria improved sperm motility, semen concentration and total motile sperm; 2.50 and 5.00g/day of yeast and lactic acid bacteria improved semen volume, sperm

morphology and final semen weight; 1g antibiotic, 2.50g yeast, 5.00g/day yeast, 2.50g/day yeast with lactic acid bacteria and 5.00g/day of yeast with lactic acid bacteria improved live:dead sperm ratio.

- Generally, bucks treated with 2.50g/day yeast and lactic acid bacteria and 5.00g/day of yeast and lactic acid bacteria performed optimally on semen quality characteristics.

RECOMMENDATION

- Dietary supplementation of probiotics (*Saccharomyces cerevisiae, Lactobacillus acidophilus* + *Saccharomyces cerevisiae* combination) should be employed in the improvement of semen quality traits of West African Dwarf bucks. Further research need to be conducted to investigate the efficiency of probiotics with more variable ratios to attain the optimum level which may lead to the most economic efficiency in returns.

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