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Daily Sperm Production, Gonadal and Extra-Gonadal Sperm Reserves of Rabbits Fed Prebiotic and Probiotic Supplemented Diets

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Abstract

In a 12-week feeding trial, an investigation was conducted with 32 weaned crossbred rabbits with average weight of 691±61.00g to assess the testicular and epididymal sperm reserves and sperm production of rabbits fed dietary prebiotics (Biotronic®) and probiotics (BIOVET®-YC) at the recommended rates of 4kg/tonne and 500g/tonne respectively. The control diet 1 had neither probiotic nor prebiotic while prebiotic, probiotic and symbiotic (prebiotic + probiotic) were added, at the recommended rates, to diets 2, 3 and 4 respectively. The 32 rabbits were randomly and equally allotted to the diets and housed individually. At the end of the feeding trial, 5 animals per treatment were sacrificed and their reproductive tracts dissected. The testes and epididymides were carefully sampled, weighed and processed. Results showed that the right, left and paired testes weights of the animals were not significantly different among the dietary treatments. However, the testicular sperm reserves were significantly ($P<0.05$) influenced by the treatments. Sperm reserves in both left and right testicles of animals fed diets 2 and 3 were similar to the control animals but they were significantly ($P<0.05$) lower than those fed diet 4 containing the combination of prebiotic and probiotic. The epididymal sperm reserves were significantly ($P<0.05$) higher in rabbits fed prebiotic and symbiotic diets than those fed diet 3 and the control. The daily sperm production and sperm production efficiency were significantly ($P<0.05$) affected by the treatments with the rabbits on diet 4 recording significantly ($P<0.05$) highest values (21.29 $x10^6$ spermatozoa/ml and 10.80 $x10^6$ spermatozoa/ml respectively).This study suggests that prebiotics (Biotronic®) and the combination of pre- and pro- biotics (Biovet®-YC) possess a potential to improve reproductive efficiency of rabbit bucks.

Keywords: Prebiotics, probiotics, rabbits, sperm reserves, sperm production.

Introduction

The amount of good quality live spermatozoa produced by the testis and the ability to store them effectively are the basic index of selection of male animal for breeding purpose. Osinowo *et al.* (1981) reported that a high correlation existed between age, body weight and sperm reserves in Bunaji bulls. Evaluation of the ejaculate is an important part of the physical examination of male animals. Series of studies have been carried out on the concentration, volume and motility of semen (Plachot *et al.*, 1984; Segerson *et al*., 1981; Hafez, 1987) to ascertain male that is viable for reproduction.

Nutrition is an important factor in growth as it enhances the hereditary make-up, resulting in maximum growth. There is a relationship between nutrition and productivity which has a great effect on the production and reproductive performance of rabbits. Effects of nutrition on reproduction of farm animals have been reported (Oyedipe *et al.*, 1982; Vincent *et al.*, 1985). Onset of puberty is a function of body weight than age. The age at puberty is influenced by many factors, including the physical environment, photoperiod, age, environmental temperature, growth rate and body weight. The activity of growth promoters such as prebiotics and probiotics in enhancing increased body weight may have an improved effect on reproduction in farm animals. There are fewer studies with prebiotics and probiotics in rabbits than in other monogastric species. Several studies so far are limited to the assessment of the effect on growth, feed conversion, caecal activity and digestibility. A probiotic is a live microbial feed supplement that has beneficial effects on the host animal by improving its intestinal balance (Fuller, 1989) and has beneficial effects on the health of the host (Lee *et al.*, 1999). While prebiotics refer to oligosaccharides which are not readily digested by the animal enzymes, but can selectively stimulate certain intestinal bacteria species, which have potential beneficial effects on the host health and growth rate (Mosenthin and Baller, 2000). With the above in mind coupled with the fact that body weight has direct influence on sperm production, this prompted the design of this investigation to assess the testicular and epididymal sperm reserves and sperm production of rabbits fed prebiotic and probiotic-supplemented diets.

Materials and methods

Experimental site, animal management and general procedures

The experiment was carried out at the rabbitry unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The site is located on the latitude $7^0$20'N and 3^0 50'E, 200m above the sea level. Four diets were formulated including the control (diet 1) with crude protein of 16.19%, crude fibre of 10.18% and digestible energy of 2744kcal/kg. Diets 2, 3, and 4 contained the same ingredients like the control diet but supplemented with Prebiotics (Biotronic® at 4 kg/tonne), Probiotics (Biovet®-VT at 500g/tonne), and Symbiotic (the combination of both pre- and pro-biotics at recommended rate indicated above) respectively. Biotronic® contains fructo-oligosaccharides and organic acids while BIOVET®-YC contains *Lactobacillus acidophilus* (45,000 million c.f.u), *Saccharomyces cerevisiae* (125,000 million c.f.u), and *Saccharomyces boulardii* (30,000 million c.f.u).

A total of thirty-two weaned rabbits with average weight of 691.00g were randomly assigned to the four dietary treatments such that each treatment had 8 animals housed individually, in a completely randomized designed experiment that lasted for twelve (12) weeks. The animals were fed *ad libitum* twice daily at 08.00h and 16.00h. At the end of the feeding trial, the animals were anesthetized and sacrificed and their reproductive tracts were dissected. The testes and epididymides were carefully removed and weighed. The right testis, the left testis and the different part of epididymides (caput, corpus, cauda) were homogenized separately in 0.154M NaCl (physiological saline) at the rate of 5ml/g testis. The suspensions were mixed and filtered through a double layer of sterile gauze into clean glass test tubes and the sperm concentrations therein determined by direct haemocytometric count after proper dilution (1:20 v/v) in 0.154M NaCl (Igboeli and Rakha, 1971, Egbunike *et al*., 1975).

Determination of daily sperm production (DSP)

The daily sperm production was estimated from the testicular sperm reserves. The estimation of DSP from testicular homogenates was based on the fact that the nuclei of elongated spermatids are resistant to physical destruction at some point during spermatogenesis. The DSP of the rabbits was therefore calculated with the formula proposed by Amann (1970) as follows:

$$DSP = \frac{\text{Testicular sperm count}}{\text{Time divisor (3.43)}}$$

Data analysis

All data obtained from this investigation were subjected to analysis of variance of completely randomized design using the SAS (1999) package and the means were separated using Duncan multiple range test of the same software.

Results and Discussion

The mean weights of the rabbits at the end of the feeding trial were 1710.00g, 1810.71g, 1708.00g and 1824.27g for rabbits fed diets 1, 2, 3 and 4 respectively. The testicular weights and sperm reserves of rabbits fed prebiotic and probiotic diets are shown in Table 1. The right, left and paired testes weights of the animals were not significantly different among the dietary treatments. However, the testicular sperm reserves were significantly ($P<0.05$) influenced by the treatments. Sperm reserves in both left and right testicles of rabbits fed diets 2, 3 and control were similar but they were significantly ($P<0.05$) lower than those fed diet 4 that contained the combination of prebiotics and probiotics. The epididymal weight and sperm reserves of the experimental animals are shown in Table 2. The weight distribution was not significantly different among the treatments. The sperm reserves in caput, corpus and caudal epididymis and both left and right of the organ followed the same trend. The sperm reserves were significantly ($P<0.05$) higher in rabbits fed prebiotic and symbiotic diets than those fed diet 3 and the control diet. Conversely, the daily sperm production and sperm production efficiency (SPE) (Table 3) were significantly ($P<0.05$) affected by the treatments with the rabbits on symbiotic diet, recording significantly ($P<0.05$) highest values (21.29×10^6 spermatozoa/ml and 10.80×10^6 spermatozoa/ml respectively). The DSP of rabbits fed diets 2 and 3 were statistically identical to those on the control diet, while rabbits on probiotic diet recorded the least SPE value. The effects observed for T4 in this study could probably be attributed to the positive synergistic effect of the prebiotics and probiotics that encouraged symbiotic relationship. The prebiotics provided a substrate for the probiotics to thrive and consequently improved the growth performance of the same animals (Ewuola *et al*., 2011). It has been established that high correlation exists between body weight, age and sperm reserves of animals (Osinowo *et al.*, 1981). However, onset of puberty is a function of body weight than age. Improved body weight by the use of growth promoting agent may induce onset of puberty which will directly influence sperm production in the testis and sperm reserves in the epididymides. Another possibility is the fact that prebiotics and probiotics in a diet improve intestinal mucosal development which enhances nutrient digestibility (Fuller, 1989; Mosenthin and Baller, 2000, Ewuola *et al*., 2011) that nourish the sertoli cells and seminal fluid that nurse the germ cells. The sperm reserves in the epididymis was similar to that reported for matured rabbits by Ogunlade *et al*. (2006) and Ewuola and Egbunike (2010) with caudal epididymides accounting for about half of the total epididymal sperm reserves followed by the caput and corpus epididymides as also observed by Egbunike and Elemo (1978) for boars. The disparity observed between percentage of the total spermatozoa in each of the three-epididymal compartments in rabbits fed prebiotic and symbiotic diets as compared to probiotic diet and the control diet may be attributed to the potential of the Biotronic® and its combination with Biovet® in improving growth (Ewuola *et al*., 2011) and reproductive efficiency in rabbits. Probiotics (Biovet®) used in this study was unable to elicit this response probably because it was without substrate supplement.

Conclusion and Recommendation

This study has demonstrated that prebiotics (Biotronic®) and the combination of pre- and pro- biotics (Biovet®-YC) showed a tendency to improve reproductive potential of rabbit bucks indirectly. Further investigation is suggested to assess the mechanism of action of these growth promoting agents on fertility.

Table 1: Testicular weights and sperm reserves of rabbit bucks fed prebiotic and probiotic supplemented diets

Treatments Parameters	1(Control)	2 (Prebiotics)	3 (Probiotics)	4 (Symbiotic)
Testicular Weights (g)				
Right Testis	1.03±0.07	1.03±0.08	0.90±0.23	0.78±0.004
Left Testis	1.05±0.13	1.04±0.78	0.79±0.20	1.19±0.18
Paired Testes	2.08±0.13	2.07±0.14	1.69±0.10	1.97±0.25
Testicular sperm reserves ($x10^6$)				
Right Testis per g testis	$31.55±6.44^b$	$37.25±4.43^b$	$26.11±2.96^b$	$53.37±5.99^a$
Per testis	$32.5±6.08^b$	$38.37±4.06^{ab}$	$23.50±2.71^b$	$41.63±7.99^a$
Left Testis per g testis	$28.46±2.79^a$	$26.81±2.13^b$	$25.32±2.67^b$	$26.37±2.44^b$
Per testis	$29.88±4.55^b$	$27.88±3.07^b$	$20.00±1.474^b$	$31.38±3.87^a$
Paired Testes per g testis	$29.99±2.59^b$	$32.00±3.24^{ab}$	$25.74±3.53^b$	$39.87±3.04^a$
Per testes	$62.38±5.57^b$	$66.25±5.06^b$	$43.50±2.09^c$	$73.01±5.55^a$

^{abc}Means in the same row with different superscripts are significantly ($P < 0.05$) different.

Table 2: Epididymal weights and sperm reserves of rabbit bucks fed prebiotic and probiotic supplemented diets

Treatments Parameters	1(Control)	2 (Prebiotics)	3 (Probiotics)	4 (Symbiotic)
Epididymal weight (g)				
Right Epididymis	0.46±0.06	0.57±0.03	1.03±0.36	0.64±0.01
Left Epididymis	0.46±0.06^{ab}	0.50±0.04^{a}	0.36±0.03^{b}	0.58±0.03^{a}
Paired Epididymis	0.92±0.11	1.07±0.05	1.39±0.37	1.22±0.05
Epididymal Sperm Reserves ($x10^6$)				
Total Caput	36.28±5.27^{b}	55.50±5.30^{a}	23.38±2.85^{b}	63.63±8.64^{a}
Total Corpus	21.28±3.53^{b}	30.38±5.63^{ab}	24.96±3.96^{b}	31.50±2.59^{a}
Total Cauda	50.63±9.16^{b}	65.63±8.73^{ab}	62.13±11.61^{b}	72.50±8.42^{a}
Right Epididymis	42.53±5.17^{b}	72.63±6.12^{ab}	69.13±12.50^{ab}	81.50±9.25^{a}
Left Epididymis	65.63±6.27^{ab}	78.88±7.38^{a}	41.35±4.13^{b}	86.13±8.15^{a}
ESR*	108.15±9.44^{b}	151.51±11.84^{a}	110.48±14.92^{b}	167.63±10.83^{a}
ESR/g Epididymis	117.21±11.18^{b}	141.60±12.35^{a}	95.79±13.63^{b}	137.40±11.31^{a}

^{abc}Means in the same row with different superscripts are significantly ($P < 0.05$) different.
SEM = Standard Error of Mean *ESR =Epididymal Sperm Reserves

Table 3: Daily sperm production and testicular sperm reserves of rabbit bucks fed prebiotic and probiotic supplemented diets

Treatments Parameters	1(Control)	2 (Prebiotics)	3 (Probiotics)	4 (Symbiotic)
Paired Testes weight (g)	2.08±0.13	2.07±0.14	1.69±0.10	1.97±0.25
Testicular sperm reserves ($x10^6$)	62.38±5.57^{b}	66.25±5.06^{b}	43.50±2.09^{c}	73.01±5.55^{a}
DSP* (per testis $x10^6$)	18.19±2.25^{ab}	19.31±2.46^{ab}	12.68±2.19^{b}	21.29±3.83^{a}
SPE** (DSP per g testis $x10^6$)	8.74±1.22^{b}	9.33±2.40^{ab}	3.70±1.94^{c}	10.80±2.01^{a}

abc: Means in the same row with different superscripts are significantly ($P < 0.05$) different.
SEM: Standard Error of Mean *DSP = Daily Sperm Production *SPE = Sperm Production Efficiency

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