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Original Research

Effects of graded dose of probiotics on packed cell volume, live body weight and gonadal and extragonadal sperm reserves of rabbit buck

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ABSTRACT

Probiotics are live microorganisms, and when administered in sufficient amounts they grant health benefits to the host. This study was designed to determine the effects of a graded dose of probiotics on packed cell volume (PCV) live body weight and semen characteristics of the rabbit buck. Twelve healthy adult rabbit bucks were purchased and kept in the Laboratory Animal Research Unit of the Large Animal Clinic, Faculty of Veterinary Medicine, University of Maiduguri. The animals were randomly grouped into four A, B, C and D. Groups A, B and C rabbits were supplemented in their feeds with probiotics at the dose of 300 g, 200 g, and 100 g respectively. Group D, served as control and fed only a basal diet without probiotics throughout the research period. Drinking water was provided *ad libitum* throughout the study period. Thereafter, blood samples were collected aseptically via the ear vein once every week for six weeks and the live body weight of each rabbit in all the groups was measured with weighing balance once every week for six weeks. At the end of the six weeks, the rabbit bucks were aseptically prepared for orchidectomy and their testes were used for evaluation of gonadal and extragonadal sperm reserves. The results reveal that the (PCV) and the live body weight were significant ($P < 0.05$) among the treated groups when compared to the control (group D). However, the Gonadal morphometric gonadal and extragonadal sperm reserves were not significant ($p > 0.05$) among the treated groups when compared to the control (group D). In conclusion, Probiotics have the potential and influence on the (PCV) and live body weight of the rabbits in a dose-dependent manner, with no significant effects on the semen characteristics of the rabbits bucks.

Keywords: bucks rabbit, gonadal and extragonadal sperm reserves, gonadal morphometric, semen characteristics, probiotics

INTRODUCTION

Currently, most of the developed countries around the globe ban the use of antibiotics in livestock industries. This decision becomes a big challenge to find alternatives to antibiotics used for treatment, prophylaxis and feed additives (Salah *et al*., 2019). One of the most common and effective substances to replace antibiotics in livestock industries and beyond is probiotics. This might potentially benefit the host animal by improving its intestinal physiology and enhancing the absorption of vital nutrients required by the body (Salahuddin *et al*., 2013). Probiotics are known as yeast, live bacteria or bacterial spores which can prevent enteric disease in rabbits (Ewuola *et al*., 2012). It also served as a growth promoter and gastrointestinal flora stabilizer for eubiotics in rabbits (Bhatt *et al*., 2017) Probiotics usually work against harmful micro-organisms thereby reducing the intestinal pH with the production of lactic acid and promoting the digestion process by producing enzyme transformation of the nutrient and assimilation of vitamins, micronutrients and macronutrients (Ezema and Eze, 2012). The availability of quality nutrients in the system facilitates the performance of the reproductive system and the effective process of production in rabbits.

Following the termination of the experiment, the rabbits were aseptically and testes were removed surgically after the administration of local anesthesia around the scrotum. The buck was restrained and the scrotum was infiltrated with 1.5 ml of lidocaine. The testes were examined grossly and assessed for testicular weight, volume, gonadal and extragonadal sperm reserves. The right and left testes and their epididymes were evaluated for individual rabbits of

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The amount of good-quality spermatozoa produced by the testis depends usually on the percentage of packed
cell volume and body weight of the animal (Lucas, 1998). This will determine the reproductive performance and output of quality semen. The ability of the testes to produce quality semen and store it in the gonads or the gonadal sperm reserves is a reflection of the basic index of a selection of rabbit bucks for breeding purposes (Iliyasu *et al*., 2023). Several scholars reported that a high correlation existed between haematological parameters, age, body weight and gonadal sperm reserves in Bunaji bulls and Yankasa rams as reported by Iliyasu *et al*. (2020). Evaluation of the testes, penis, body weight and haematological parameters is an important part of the physical examination to establish the status of rabbit buck breeding fitness. A series of studies have been carried out on the sperm concentration, volume and motility of semen of livestock (Plachot *et al*., 1984; Segerson *et al*., 1981; Hafez, 1987) to establish rabbit buck that is viable for reproduction.

Nutrition is an important factor in reproduction and growth as it enhances semen production in male animals. 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 by Oyedipe *et al*., (1982). The 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, body weight and the status of the haematology. The activity of growth promoters such as prebiotics and probiotics in enhancing increased body weight and optimum utilization of nutrients may have improved effects on reproduction in livestock. There are fewer research documents in the study area with probiotics in rabbits or other monogastric animal species. Several studies so far are limited to the appraisal of the effect on growth, feed conversion, caecal activity and digestibility.

Based on The fact that body weight has a direct influence on sperm production, prompted the design of this investigation to assess the packed cell volume, and gonadal and extragonadal sperm reserves of rabbits fed graded doses of probiotics-supplemented diets.

MATERIALS AND METHODS

STUDY AREA

This study was carried out at the Faculty of Veterinary Medicine University of Maiduguri, Borno State, North Eastern, Nigeria. Maiduguri is Cosmopolitan in nature, located at an elevation of 354 meters above sea level between latitudes 110 and 140N and longitudes 100 and 140E, within the Sahel region and has a total land mass of 50,778 square kilometres (BMLS, 2007). It has a population density of 1,738 people per square kilometer, and a total population of 521,492 (NPC, 2006). The temperature ranges from 35-40^{0}C for most parts of the year with two distinct seasons, a rainy season with mean annual rainfall of 647mm from July to October and a prolonged dry season for the rest of the year (LCRI, 2007). The state geographical features are favourable to livestock and agricultural produce (NPC, 2006).

EXPERIMENTAL ANIMALS AND MANAGEMENT

Twelve, healthy rabbits aged 1-8 months with an average body weight of 5.0 ± 1.3 kg were purchased from the market. The rabbits were examined to ensure that they were healthy. The Rabbit-bucks were kept in the laboratory research cages of the Large Animal Clinic Unit, Veterinary Teaching Hospital University of Maiduguri. The rabbits were kept for 6 weeks before the commencement of the Experiment. Thereafter, the rabbits were acclimatized for two weeks before the commencement of the research. The rabbits were kept under intensive management and fed on grasses, groundnut and salad leaves water was provided ad libitum throughout the research period.

SOURCE OF PROBIOTICS

Five kilograms (Kg) of Probiotics were purchased from Afro-Vet Veterinary Diagnostic Centre, Ibrahim Taiwo Estate, Maiduguri Metropolis, Borno State.

EXPERIMENTAL DESIGN

The rabbits were randomly selected into four groups (A, B, C and D) of three rabbits per group. Groups A, B and C were supplemented with probiotics at different doses of 300, 200 and 100 g respectively. Group D served as an untreated control and was fed normal ratio. The Probiotics were supplemented in the treated group diet for 6 weeks. Thereafter, Body weight and PCV were recorded once every week throughout the investigational period. At the end of the trial, 2 rabbits were selected randomly from each group for surgical removal of their testes.

SURGICAL REMOVAL OF THE TESTES OF THE RABBIT

Following the termination of the experiment, the rabbits were aseptically and testes were removed surgically after the administration of local anesthesia around the scrotum. The buck was restrained and the scrotum was infiltrated with 1.5 ml of lidocaine. A surgical blade was used to incise the scrotum and expose the testicular tissue the testicle was separated from the blood vessels and spermatic cord, and the major blood vessels and spermatic cord were ligated with chromic catgut. The fascia was incised to reach the tunica albuginea, then, the epididymis was also separated from the testes using sharp scissors, and the tunica albuginea was incised and separated from the testicular parenchyma

using a scalpel blade as described by Iliyasu *et al*. (2023).

DETERMINATION OF GONADAL AND EPIDIDYMAL SPERM RESERVE

Gonadal and epididymal sperm reserves were determined as described by Iliyasu *et al*. (2023). The testicular parenchyma was sliced and homogenized with a high-speed blender for two minutes with 50 ml of 0.9 % NaCl solution containing antibiotics (sodium penicillin G, 100 IU/ml and streptomycin sulphate 1 mg/ml) to prevent bacterial growth. For determining the epididymal sperm reserves, the caput, corpus and cauda epididymides were isolated, and minced with a pair of scissors separately in 20 ml of 0.9 % NaCl solution. All tissues were homogenized for 2-6 hours after minced. Testicular homogenates and epididymal samples were refrigerated overnight. After 24 hours, the samples were filtered through gauze and the filtrate volumes were measured. The gonads were homogenized with 50 ml of normal saline and filtered. The filtrate was diluted at a ratio of 1: 2 one millilitre of filtrate was diluted separately with 2 ml of normal saline solution before examination as described by Iliyasu *et al*. (2020). Sperm concentration of the testes and epididymal sample were determined using a haemocytometer and light microscope, as described by (Iliyasu *et al.,* 2014).

MEASUREMENT OF GONADAL ORGAN

A digital weighing scale (Essae® Colorado USA) was used to determine the weight of testicular parenchyma while a cylinder and water were used to measure testicular volume according to the method described by Iliyasu *et al*. (2014). Based on gross anatomy, the left and right epididymides were divided into the corpus, caput, and cauda sections and each component was weighed. Thereafter, each component of the extragonadal sperm reserves was placed in normal saline for onward estimation of epididymal sperm reserves using a haemocytometer.

STATISTICAL ANALYSES

Data were analyzed using GraphPad Prism (Instat®). Data were subjected to one-way ANOVA and Dunnett Posthoc test. Values were summarized and expressed as mean ± SEM and the results were considered significant at $p < 0.05$.

RESULTS

The mean body weight and Parked cell volume PCV of rabbit bucks of the treated group increased significantly ($p < 0.05$) in a dose-dependent manner throughout the research period when compared to the control untreated group as presented in (Table 1) and (Table II) respectively.

The gonadal dimension, gonadal and extra gonadal sperm reserves of the rabbit buck treated with graded dose of Probiotics were significantly ($P < 0.05$) increase in a dose dependant manner compared to the control (group D).

However, the testicular sperm reserves were significantly ($P<0.05$) influenced by the treatment regimen among the groups as presented in (Table III). Sperm reserves in both left and right testicles and epididymis were significantly increase compared to the control group D. Similar findings were observed on the paired corpus, carput and caudal of the extragonadal sperm reserves but was not statistically ($p>0.05$) significant as shown in (Table IV)

DISCUSSION

The goals of the current study were to clarify the effects of dietary supplementation of a graded dose of probiotics on live body weight, percentage of Parked cell volume (PCV), and gonadal and extragonadal sperm reserves of rabbit bucks.

PCV is one of the conventional haematological parameters used to appraise the health status of livestock (Adhikari *et al*., 2018). It is an index which explains a direct reflection of the effects of dietary management on the animals in terms of the potential and quality of the ingested supplement by the animal to enhance its haematological and biochemical process (Abdel-Hamid *et al*., 2019). The PCV from this study was an indication that the rabbit's health status was normal. This agreed with the findings reported by Chinwe *et al*. (2021) who stated that high PCV can be recorded in animals that are on a symbiotic diet with a clear presentation of normal red blood cell counts and haemoglobin concentration as an indication that the animals are not anaemic. Similar evidence has been reported in sheep, rats (Salahuddin *et al*., 2013), pigs (Czech *et al*., 2010) and chickens (Al-Saad *et al*., 2014). It was also reported by several scholars that increase in packed cell volume of rats dosed with Lactobacillus plantarum (Aboderin and Oyetayo, 2006) is an indication of normal physiological activities and sound health of the animals.

The increased Packed Cell Volume (PCV) among the treated groups compared to the non-treated control group is an indication of the influence of the Probiotics on the rabbit bucks. This agrees with Dimcho *et al*. (2005) who reported high hematopoietic function of rabbits fed supplemented doses of prebiotics and probiotics, particularly the PCV but the supplement did not affect other components of the blood constituents which include haemoglobin concentrations and Red blood cell (RBC) concentration (Nwachukwu, et al., 2021). This concurs with the increased PVC reported by Doaa e*t al*. (2015) and similar findings on level of PCV were reported during maternal intake to determine the health status of an offspring as reported by Céline *et al*. (2022). The current study agrees with the findings reported by Shoeib *et al*. (1997) who recorded a high percentage of RBC count and PCV of the rabbits supplemented with probiotics.

Table I: Effects of graded dose of probiotics on live body weight (kg) of Rabbits.

Parameters/groups and doses of Probiotics	Group A / Dose 300 g	Group B/ Dose 200 g	Group C/ Dose 100 g	Group D/ normal ratio
Week 1	2.23±0.60^{b}	2.00±0.37^{ab}	2.30±0.88^{a}	2.33±0.21^{a}
Week 2	3.25±1.10^{ab}	2.63±0.60^{b}	2.90±0.22^{ab}	2.83±0.60^{a}
Week 3	3.67±0.67^{ab}	2.73±0.50^{b}	3.67±0.67^{ab}	2.85±0.30^{a}
Week 4	4.21±0.01^{ab}	3.50±0.50^{ab}	3.89±0.27^{ab}	2.93±0.70^{ab}
Week 5	5.00±0.00^{ab}	4.50±1.19^{ab}	4.31±0.17^{ab}	3.50±1.21^{ab}
Week 6	6.33±0.42^{ab}	5.50±1.10^{ab}	5.67±0.33^{ab}	4.50±1.19^{ab}

The increase in the erythrogram or the PCV could be attributed to the ability of the probiotics to improve the health status of the intestine and the overall physiological well-being of the rabbits supplemented with probiotics (Alkhalf *et al.,* 2010). Similarly, findings were reported by Abdelhady & El-El-Abasy (2015) who recorded that a diet supplemented by prebiotics inulin 4 % has increased iron bioavailability in iron-deficient pigs. Similarly, Piglets fed with a diet supplemented with prebiotics inulin 4 % revealed 15 % higher haemoglobin (Hb) concentration after five weeks of intervention compared with the piglets fed with a basal diet (Crespo-Piazuelo *et al.*, 2022).

Table II: Effects of graded dose of probiotics on percentage of Parked Cell Volume PCV of Rabbit

Parameters/groups and doses of Probiotics	Group A/Dose 300 g	Group B/Dose 200 g	Group C/Dose 100 g	Group D/ normal ratio
Week 1	38.38±0.00^{a}	36.18±0.00	37.38±0.00^{a}	39.38±0.00^{b}
Week 2	38.38±0.10^{a}	37.38±0.02^{a}	37.42±0.20^{a}	39.38±0.00^{b}
Week 3	39.30±0.01^{ab}	37.88±0.23^{a}	38.28±0.10^{a}	39.38±0.00^{b}
Week 4	39.38±0.00^{ab}	39.48±0.00^{ab}	39.08±0.00^{a}	39.78±0.0^{b}
Week 5	40.58±0.58^{ab}	40.88±0.50^{ab}	39.88±0.50^{ab}	39.90±0.00^{ab}
Week6	41.28±0.58^{ab}	40.98±0.58^{ab}	40.38±0.00^{ab}	40.58±0.58^{ab}

Values were expressed as mean ± SEM values with different superscripts $^{a, b}$ within the columns are significantly different at ($P < 0.05$).

Table III. Effects of graded dose of Probiotics on gonadal dimension, gonadal and extra gonadal sperm reserves of rabbit.

Groups /parameter	Right Gonadal weight (g)	Left Gonadal weight (g)	Right Gonadal Volume (CM^3)	Left Gonadal Volume (CM^3)	Right Gonadal Sperm Reserves	Left Gonadal Sperm Reserves	Right Epididymal Sperm Reserves	Left Epididymal Sperm Reserves
Group A/Dose 300g	7.65±0.0^{ab}	7.10±0.2^{ab}	7.61±1.3^{ab}	7.00±0.0^{ab}	245.51±0.5^{ab}	200.50±0.00^{a}	88.70±0.00^{ab}	75.08±0.3^{ab}
Group B/Dose 200g	7.40±1.1^{ab}	7.00±1.0^{ab}	7.37±0.1^{ab}	6.80±1.0^{a}	231.30±2.9^{ab}	201.50±0.00^{a}	70.90±0.00^{ab}	70.75±0.4^{ab}
Group C/Dose 100g	6.65±0.0^{b}	6.55±0.0^{a}	6.59±1.2^{a}	6.05±0.2^{a}	211.15±0.0^{ab}	202.50±0.00^{a}	68.20±0.00^{ab}	65.25±0.5^{ab}
Group D/ normal ratio	6.30±0.6^{b}	6.00±0.1^{a}	6.38±0.1^{a}	6.00±0.0^{a}	209.25±0.5^{ab}	203.50±0.00^{a}	65.90±0.00^{ab}	61.42±1.6^{a}

Values were expressed as mean ± SEM values with different superscripts $^{a, b}$ within the columns are significantly different at ($P < 0.05$). Gonadal and extragonadal sperm reserves units is $\times 10^{9}$

Table IV: Effects of graded dose of Probiotics on extragonadal dimension and extra gonadal sperm reserves of rabbit

Groups /Parameters	Paired Corpus weight (g)	Paired Carput weight (g)	Paired Caudal weight (g)	Paired Epididymal Sperm Reserves
Group A/Dose 300g	2.25±0.0	1.10±0.2^{b}	3.31±1.3^{b}	155.70±0.00^{a}
Group B/Dose 200g	2.10±1.1^{b}	1.03±1.0^{b}	3.37±0.1^{b}	141.90±0.00^{b}
Group C/Dose 100g	2.05±0.0	1.02±0.0	2.59±1.2	137.20±0.00
Group D/ normal ratio	2.00±0.6^{a}	1.00±0.1^{a}	2.38±0.1^{a}	166.90±0.00^{a}

Values were expressed as mean ± SEM values with different superscripts a, b within the columns are significantly different at ($P < 0.05$). Gonadal and extragonadal sperm reserves units is×109

The amplifying outcome on the body weight gain of the treated groups of rabbit bucks remains in dose dose-dependent manner this agreed with the findings reported by Niu, *et al*. (2006).This might be attributed to the influence of probiotics in feed conversion through manipulation of the physiological ways within the gastrointestinal tract, thereby improving digestion by balancing the resident normal micro-flora of the gut as they are known to improve the integrity of the intestinal mucosal and digestion process (Angelaski, 2017). It also improves absorption and facilitates the transportation of nutrients within the intestine and this can ensure optimum reproduction and productivity as reported in porcine production Zhang *et al*. (2020).

It has been recognized by several scholars that a high correlation usually exists between body weight and sperm reserves of rabbit bucks (Ewuola and Egbunike, 2010) and this agreed with the findings reported by Iliyasu *et al*. (2014) in Rams and Osinowo *et al*. (1981) in boars respectively. Similar findings were also reported by Iliyasu et al. (2023) in rabbit buck treated with a graded dose of honey. However, some scholars (Ewuola, *et al*., 2011) linked the onset of puberty with an increase in body weight than age, as an investigation by Galbraith, (1982) has revealed that improved body weight by the use of growth-promoting agents and steroids may induce the onset of puberty on livestock which will directly influence sperm production and sperm reserves within the testes and the epididymides, respectively.

This can also be linked to the prospect of probiotics as supplements that have the potential to improve the physiology of the intestinal mucosal which might enhance nutrient digestibility, absorption and assimilation of macro and micronutrients required by the animals (Ewuola *et al*., 2011). This might augment the nourishment of the Sertoli cells and boost seminal fluid productions that are required for nursing the germ cells (Niu *et al*., 2020). The sperm reserves in the epididymis especially the corpus and caudal were similar to that reported for rabbits treated with honey by Iliyasu *et al*. (2023) and rabbit-fed fumonisin-contaminated diets as reported by Ogunlade, (2006). While the disparity observed between the percentage of the total spermatozoa in each of the three anatomical compartments of the epididymides of the rabbit bucks fed probiotics supplement was higher in the caudal part than what was reported by Iliyasu *et al*. (2023) in rabbit and goat by Raji *et al*. (2017). This may be attributed to the potential of the probiotics to improve the physiology of the intestine which translates into the process of increased assimilation of nutrients that are required for optimum growth and overall physiological performance of livestock.

CONFLICT OF INTEREST

We declared no conflict of interest concerning this manuscript.

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