Reproductive characteristics of rabbit bucks administered *Panax ginseng* extracts

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Abstract

*This study was conducted for 4 months in the quest to discover natural materials, especially of plant origin, capable of substituting the roles of synthetic materials, especially drugs, in reproductive enhancement of livestock species. Such natural materials will help eliminate the menace of residual effects of synthetic materials on livestock products and probably attract more revenue from them. A total of 24 New Zealand White (NZW) weaned rabbit bucks were used and the study was carried out in a Complete Randomized Design (CRD), comprising three treatments replicated four times; MT₁ (control), MT₂ and MT₃, containing 0, 200 and 400 mg/kg body weight of *Panax ginseng* extracts (PGEs), respectively. Assessment for attainment of puberty started from the age of 11 - 14 weeks on a weekly basis and at 72 h interval thereafter by examining their ejaculate and preputial fluid smeared on a glass slide under a microscope for sperm cells. Semen was collected twice a week for 3 weeks after maturity (6 months old) with the aid of an artificial vagina (AV), using rabbit does as teasers. Semen analysis was done within 20 minutes of collection, following standard procedures. Libido was determined weekly for 3 weeks by recording the time it took the matured rabbit bucks to sniff, groom, and mount the rabbit doe in the initial instance (reaction time); and by counting the number of times the matured buck attempts to mount the doe per minute (libido score). Data generated from this study was analyzed using analysis of variance (ANOVA). Age at puberty (MT₁ = 116.00 days; MT₂ = 112.50 days; MT₃ = 100.50 days) was significantly ($P < 0.05$) lower in MT₃ (400 mg/kg b.w), and weight at puberty (MT₁ = 1483.33 g; MT₂ = 1475.00 g; MT₃ = 1378.50 g) recorded a dose dependent significant ($P < 0.05$) decrease in the male rabbits. Semen volume (MT₁ = 0.95 mL; MT₂ = 1.25 mL; MT₃ = 1.55 mL), total sperm count (MT₁ = 115.00×10^6 ; MT₂ = 125.50×10^6 ; MT₃ = 146.20×10^6), progressive sperm motility (MT₁ = 78.50 %; MT₂ = 83.00 %; MT₃ = 82.30 %), live sperm proportion (MT₁ = 80.63 %; MT₂ = 85.40 %; MT₃ = 89.75 %) and libido score (MT₁ = 7.00 mounts/min; MT₂ = 9.00 mounts/min; MT₃ = 11.50 mounts/min) were significantly ($P < 0.05$) higher in adult rabbit bucks administered PGEs, while reaction time (MT₁ = 9.45 s; MT₂ = 3.15 s; MT₃ = 2.60 s) significantly ($P < 0.05$) decreased in PGEs treated rabbits. This study shows that PGEs can orally be administered up to 400 mg/kg body weight in NZW rabbit bucks to enhance their reproductive potential, through early attainment of puberty, improved semen quality and enhanced libido.*

Keywords: Ginseng, libido, puberty, rabbits, semen.

Introduction

Due to the residual effects of synthetic drugs used in livestock production (Iwuji *et al.*, 2019), researchers are delving into studies on non-synthetic materials, especially of plant origin, for use in livestock production. Several plants have

been widely studied for possible benefits in different aspects of animal production like nutrition, reproduction and health. Incidentally, most plants possess pharmacological properties which have been reported to be the reason for their therapeutic potentials (D'Cruz *et al.*, 2010).

Ginseng, which is a plant having different species has been well studied, especially the '*panax*' specie, and has been reported to possess antioxidant properties, enhance immune function and synthesis of nitric oxide (de Jong *et al.*, 2005; Kim *et al.*, 2005). Traditionally, ginseng is being used as an aphrodisiac and it has been reported that *Panax ginseng* enhances nitric oxide synthesis in corpora cavernosa endothelium; and ginsenosides, a major component of ginseng, enhances acetylcholine-induced and transmural nerve stimulation-activated relaxation associated with increased tissue cyclic guanosine monophosphate (Sandroni, 2001). A study in rats (Oremosu *et al.*, 2013) reported that *Panax ginseng* increased serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) which increased spermatogenesis in the rats. Rats that received 5% ginseng experienced increase in blood testosterone level with reduced prostate weight (Fahim *et al.*, 1982). However, while plant species are applied in livestock production for purposes other than reproduction, the reproductive effects of these plants on the animals is of paramount consideration, especially when breeding animals are involved and considering that many plants and plant products have been reported to impede spermatogenesis in various animal species (Saba *et al.*, 2009). Although plants are generally adjudged to aid fertility (Olatunji-Bello *et al.*, 2009), they may also relatively hinder fertility (D'Cruz *et al.*, 2010).

Improving the rabbit production industry in Nigeria, which has been regarded as emergent or rudimentary (Iwuji *et al.*, 2019), will include enhancing or optimizing their reproductive ability. Although rabbits are regarded as highly prolific animals (Iwuji, 2016), they are also prone to reproductive impediments or difficulties emanating from different causes, which

may be environmental, nutritional, physiological, and managerial or pathogenic (Mailafia *et al.*, 2010). It is possible that *Panax ginseng* may enhance reproductive abilities of rabbit bucks as reported in mice and rodents treated with *Panax ginseng* (Leung and Wong, 2013). Earlier studies on *Panax ginseng* with rabbits recorded significant increases in serum levels of follicle stimulating hormone (FSH), testosterone (T), triiodothyronine (T3) and tetraiodothyronine (T4) of treated rabbit bucks (Iwuji *et al.*, 2017), while milk yield was significantly decreased in rabbit does administered *Panax ginseng* extracts (Iwuji *et al.*, 2018). Sequel to the reported roles of *Panax ginseng* in different animal models, it became imperative for this experiment to evaluate the effects of *Panax ginseng* extracts (PGEs) on age and weight, at puberty, of growing rabbit bucks and their semen characteristics and libido, at maturity.

Materials and methods

Location and site of the experiment

The experiment lasted for 4 months and was carried out at the Department of Animal Science and Technology Teaching and Research Farm, Federal University of Technology, Owerri, Imo State. Imo State is situated in South Eastern agro-ecological zone of Nigeria, and lies between latitude 4°4' and 6°3' N, and longitude 6°15' and 8°15' E. Owerri is about 100 m above sea level with mean annual rainfall of 2500 mm, temperature range of 26.5 – 27.5 °C, and humidity range of 70 – 80 %. Dry season duration (months with less than 65 mm rainfall) is three months, which takes place during the months of December, January and February. (Ogbuwu *et al.*, 2014).

Experimental material

The experimental material for this study is *Panax ginseng* extracts (PGEs). A commercially available capsulated pure

Panax ginseng extracts (Mason natural® Korean Ginseng 518 mg) produced by MASON VITAMINS, INC. USA, was purchased from a reputable pharmacy. Each day, the contents of the *Panax ginseng* extracts capsule which weighed 500 mg without the capsule were dissolved in distilled water at the rate of 200 mg/mL and administered orally to the rabbits used in this experiment, according to their treatment group. 20 capsules or 10,000 mg of the *Panax ginseng* extracts (solute) were emptied into a graduated test tube. Distilled water (solvent) was then added and gradually stirred until all the solute were dissolved in the solvent and the volume of the solution made up to 50 mL.

Experimental animals and their management

Twenty four New Zealand White (NZW) growing rabbit bucks (average age of 2 months) were used for this study. The rabbits were purchased from a reputable farm in Umuahia, Abia State. On arrival, the rabbits were housed separately in two hutches of twelve cages each and allowed two weeks to acclimatize before administering the treatments. During the acclimatization period, the rabbits were injected with 0.1 mL vitoxy (an antibiotic) as a prophylactic measure. The rabbits were then divided into three experimental groups and randomly allocated to three treatments; MT₁, MT₂ and MT₃; containing 0, 200 and 400 mg PGEs per kg body weight, respectively. The treatments were orally given to the rabbits, using a syringe, between the hours of 7am and 9am, daily. Feed and water were given freely to the animals, while other standard management practices described by Mailafia *et al.* (2010) were carried out throughout the experiment.

Age and weight at puberty

The age and weight of the growing rabbit bucks at puberty were determined and recorded. The assessment for attainment of

puberty started from the age of 11 - 14 weeks on a weekly basis and at 72 h interval thereafter by examining their ejaculate and preputial fluid smeared on a glass slide under a microscope for sperm cells (Ewuola and Egbunike, 2010).

Semen collection and evaluation

Semen was collected from the rabbit bucks at an average age of 6 months with the aid of teaser does, using artificial vagina (AV) as described by Herbert and Adejumo (1995). The bucks were trained to ejaculate into the AV for 2 weeks, after which semen for analysis was collected twice within the 4th week of the experiment at 3 days interval between the hours of 8.00 am and 9.00 am. Collected semen were analyzed immediately after liquefaction, following procedures outlined by Adeyemi *et al.* (2014). Semen volume was read off the graduated collection tube in the AV. Concentration of spermatozoa per mL was measured with an improved Neubauer haemocytometer. Motility was estimated from a small representative drop of semen that was held at temperature of 37 °C. Smears were made from each ejaculate for evaluation of sperm morphology and for determining the proportion of abnormal spermatozoa. The differential staining (one drop of semen to two drops of eosin) on a slide and observation under the microscope aided the determination of the total live sperm. Total spermatozoa per ejaculate were calculated by multiplying semen volume by sperm concentration.

Reaction time and libido

Reaction time and libido of the rabbit bucks were assessed and recorded weekly through the introduction of a teaser doe to the bucks and monitoring their sex drive. Reaction time was measured as the time it took the rabbit buck to sniff, groom, and mount the rabbit doe, while libido score was measured as the number of times the buck attempts to mount the doe per minute. Both parameters were measured with the aid of an electronic

stop watch.

Experimental design and data analysis

The experiment was carried out in a Complete Randomized Design (CRD). It was made up of three treatments; MT₁, MT₂ and MT₃ containing 0, 200, and 400 mg of *Panax ginseng* extracts (PGEs), respectively. Each treatment contained 8 rabbits with 4 replicates of 2 rabbits per replicate. The statistical model for this experiment is;

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where;

Y_{ij} = Individual observation

μ = Overall mean

T_i = Treatment effect

e_{ij} = Random error, which is assumed to be independently, identically and normally distributed with zero mean and constant variance.

Data collected were subjected to analysis of variance (ANOVA) using SAS (2004). Significantly ($P < 0.05$) different means were separated using the same software.

Results and discussion

Age and weight at puberty

Table 1 presents the average ages and weights of the growing rabbit bucks at puberty. Weight of rabbit bucks on MT₃ (1378.50 g) were significantly ($P < 0.05$) lower than those of bucks on MT₁ (1483.33 g) and MT₂ (1475.00 g), while bucks on MT₁ significantly ($P < 0.05$) recorded the highest weight at puberty.

Age and weight are among the factors affecting attainment of puberty in farm animals and since puberty is a gradual quantitative phenomenon rather than an acute and qualitative endocrinological event, growth rate then becomes a factor intertwined with age and live weight of the animal when puberty is considered (Lawrence and Fowler, 2002). The significant ($P < 0.05$) decrease in age and weight at which the male growing rabbits administered PGEs attained puberty is an indication that PGEs can be used to hasten onset of puberty in male rabbits, thereby enhancing reproductive efficiency and corroborating the spermatogenic/androgenic (endocrine effects) and fertility enhancing properties of ginseng reported in many literatures (Salvati *et al.*, 1996; Schardt, 1999; Chen *et al.*, 1999). The reduced age and weight at puberty of the male rabbits treated with PGEs is probably due to increased growth rate recorded in these animals. This is sequel to the fact that the relationship between growth rate, age and live weight at puberty are very complex and it is virtually impossible to separate the effects of growth rate *per se* from those of live weight and/or age. Hence selection for high growth rates in animals under domestication has led to puberty being attained at younger ages and sometimes at lighter live weights and therefore at lower proportions of mature live weight, compared with the situation in unselected populations from which such animals have been derived (Lawrence and Fowler, 2002).

Table 1: Average age and weight at puberty of growing rabbit bucks administered *Panax ginseng* extracts

Parameters	MT ₁	MT ₂	MT ₃	SEM
Age (days)	116.00 ^a	112.50 ^a	100.50 ^b	2.23
Weight (g)	1483.33 ^a	1475.00 ^b	1378.50 ^c	18.19

^{abc}: Means within a row with different superscripts are significantly ($P < 0.05$) different. MT = Male treatment; SEM = Standard error of means.

Semen characteristics and libido

Table 2 presents the semen characteristics and libido evaluation results of the experimental rabbits. Semen volume was significantly ($P<0.05$) higher in rabbits on MT_3 (1.55 mL) than in rabbits on MT_1 (0.95 mL), but similar ($P>0.05$) to the semen volume of the rabbits in MT_2 (1.25 mL) which was similar ($P>0.05$) to MT_1 . Total sperm count was significantly ($P<0.05$) higher in rabbits on MT_3 (146.20×10^6) than in rabbits on MT_2 (125.50×10^6) which was significantly ($P<0.05$) higher than total sperm count of rabbits on MT_1 (115.00×10^6). Sperm concentration of the experimental rabbits was significantly ($P<0.05$) higher in rabbits on MT_1 ($123.68 \times 10^6/\text{mL}$) than in rabbits on MT_2 ($98.40 \times 10^6/\text{mL}$) and MT_3 ($96.16 \times 10^6/\text{mL}$) which were similar ($P>0.05$). Rabbit bucks on MT_2 (83.00 %) and MT_3 (82.30 %) have similar ($P>0.05$) percentage of progressive motile spermatozoa, which were significantly ($P<0.05$) higher than percentage of progressive motile spermatozoa recorded for rabbits on MT_1 (78.50 %). Percent live sperm proportion was significantly ($P<0.05$) higher in rabbits on MT_3 (89.75 %) than in rabbits on MT_2 (85.40 %) which was significantly ($P<0.05$) higher than the percent live sperm proportion observed in rabbits on MT_1 (80.63 %). The percent abnormal sperm of the rabbits was similar between rabbits on MT_1 (14.01 %) and MT_2 (12.60 %), but significantly ($P<0.05$) lower in rabbits on MT_3 (9.45 %). Reaction time was significantly ($P<0.05$) higher in rabbits on MT_1 (9.45 secs) than in rabbits on MT_2 (3.15 secs) and MT_3 (2.60 secs) which were similar ($P>0.05$). Libido score was significantly ($P<0.05$) higher in rabbits on MT_3 (11.50 mounts/min.) than in rabbits on MT_2 (9.00 mounts/min.) which was significantly ($P<0.05$) higher than libido score for rabbits on MT_1 (7.00

mounts/min.).

Semen analysis of the adult rabbit bucks recorded a dose dependent increase in the volume, which became significant at 400 mg administration of *Panax ginseng* extracts. Previous studies have recorded increased and decreased semen volumes in different animals treated with *Panax ginseng* (Akram *et al.*, 2010; Abdel-Hamed *et al.*, 2014). However, since *Panax ginseng* has been reported to enhance endocrine and tissue functions of organs and increase blood flow through smooth muscle relaxation (Park *et al.*, 2005), the significant increase in the semen volume of MT_3 rabbits can be appropriately concluded to be the effect of PGEs on the accessory sex glands that produce fluids which forms greater portion of the semen volume (Frandsen *et al.*, 2009). Furthermore, variation in animal species and ginsenoside content, coupled with difference in age and method of extract preparation of the ginseng used, could also be contributory factors in the conflicting semen volume obtained in experiments with ginseng (Akram *et al.*, 2010; Abdel-Hamed *et al.*, 2014). Total sperm count of the experimental rabbits recorded a dose dependent increase, which is similar to total sperm count results in most studies with *Panax ginseng*, recorded in both animal and human models (Hosseini *et al.*, 2010; Leung and Wong, 2013). The significantly lower sperm concentrations recorded in animals receiving PGEs (MT_2 and MT_3) were due to the increased volume recorded in these groups; this will not affect fertility of the animals since total sperm count was significantly higher in the PGEs treated animals. The significantly higher percentages of progressive motile spermatozoa, live sperm proportion and low abnormal sperm percent recorded in the rabbit bucks receiving PGEs could be due to L-arginine content (Polan *et al.*, 2004), antioxidant property, nitric oxide

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release and hormonal effects of *Panax ginseng* (Kim *et al.*, 2005; Jang *et al.*, 2012; Al-Ebady *et al.*, 2012). Other properties of *Panax ginseng* like anti-aging, protein synthesis and steroid-like effects, among others, may have also played a role in the semen characteristics of these rabbits receiving PGEs (Metori *et al.*, 1997).

Reaction time in seconds, which is the time it takes the rabbit buck to sniff, groom and try to mount the doe for intromission was significantly lower in the rabbits receiving PGEs. This means that it took lesser time, from the time of introduction of the rabbit does into the cages of the PGEs treated rabbit bucks, for the rabbits to mount the rabbit does. Libido score (mounts/minute) was significantly higher in a dose dependent manner among the experimental rabbits, showing that PGEs has a strong

positive effect on libido of the rabbit bucks, which is in agreement with earlier studies (Yoshimura *et al.*, 1998; Sandroni, 2001; Jang *et al.*, 2008). These effects were attributed to the ability of PGEs to cause enhanced erection in the animals through the release of nitric oxide (NO) which relaxes the smooth muscles of the erectile tissues of the penis (corpus cavernosum) for increased blood flow. The ginsenoside, Rg1 (a component of ginseng) has been implicated for this role (Wang *et al.*, 2010). Furthermore, studies on rodents reveals that *Panax ginseng* or ginsenoside Rg1 can facilitate copulatory behaviour and a dose dependent increase in mounting, intromission, and penis licking in mice which are exposed to estrous females; but such effects were not observed in mice treated with ginsenoside Rb1, Rb2, and Ro (Yoshimura *et al.*, 1998).

Table 2: Semen characteristics and libido of adult rabbit bucks administered *Panax ginseng* extracts

Parameters	MT ₁	MT ₂	MT ₃	SEM
Semen volume (mL)	0.95 ^b	1.25 ^{ab}	1.55 ^a	0.18
Total sperm (x 10 ⁶)	115.00 ^c	125.50 ^b	146.20 ^a	6.63
Sperm concentration (x 10 ⁶ /mL)	123.68 ^a	98.40 ^b	96.16 ^b	4.75
Progressive sperm motility (%)	78.50 ^b	83.00 ^a	82.30 ^a	1.22
Live sperm proportion (%)	80.63 ^c	85.40 ^b	89.75 ^a	1.38
Abnormal sperm (%)	14.01 ^a	12.60 ^a	9.45 ^b	1.02
Reaction time (secs)	9.45 ^a	3.15 ^b	2.60 ^b	1.18
Libido score (mounts/minute)	7.00 ^c	9.00 ^b	11.50 ^a	0.62

^{abc}: Means within a row with different superscripts are significantly (P<0.05) different. MT = Male treatment; SEM = Standard error of means.

The male sex steroid, testosterone, is synthesized in the Leydig cells under the control of luteinizing hormone (LH), which is produced by the anterior pituitary (Leung and Wong, 2013). Testosterone levels are strongly correlated with libido and studies with *Panax ginseng* in rats have been reported to significantly increase blood testosterone levels. Ginsenoside Rb1 found in ginseng has been reported to increase the secretion of luteinizing hormone (LH) by acting directly on the anterior pituitary gland (Tsai *et al.*, 2003). It has also been suggested that ginseng may regulate the pituitary-testis axis at both hormonal and

neuronal levels (Shi *et al.*, 2013).

Conclusion

The relevance of *Panax ginseng* extracts (PGEs) in rabbit buck production is being demonstrated in this study, especially in respect of their reproduction. Rabbit farmers can use up to 400 mg/mL of PGEs to induce early puberty with enhanced semen quality and libido. This will serve as a veritable option to synthetic drugs used for same purpose.

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