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Effect of Gonadotrophin (Pergonal®) on semen characteristics, hormonal profile and biochemical constituents of the seminal plasma of West African Dwarf (WAD) bucks

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ABSTRACT: Sixteen healthy West African Dwarf (WAD) bucks aged 2.0 to 2.6 years, weighing between 8.50 kg and 8.52 kg were used to study the effect of gonadotrophin (Pergonal®) on semen characteristic, hormonal profile and biochemical constituents of the seminal plasma. The 16 WAD bucks were divided into 4 treatment groups. Each treatment group consisted of 4 bucks with one buck per replicate in a Completely Randomized Design (CRD) with 4 levels of Pergonal® as treatment. The levels of Pergonal® were 0.00 i.u (T_1), 9.00 i.u (T_2), 18.00 i.u (T_3), and 27.00 i.u (T_4) Pergonal® injections (Ferring Labs USA). The group which contained no Pergonal® served as the control (T_1), administered with 1.00 ml physiological saline. All the treatments were given by intramuscular injections. The results showed significant differences ($P<0.05$) among the treatment groups in all the parameters for semen quality: semen volume, mass motility, semen pH, individual motility, sperm concentration, proportion of live, normal and dead sperm cells. The results further showed that there were significant differences ($P<0.05$) among the treatment groups in luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone levels in the serum. Similarly, the results showed that there were significant differences ($P<0.05$) among the treatment groups in sodium, potassium, calcium, magnesium, fructose, bicarbonate, urea, Ascobic acid and citric acid in the seminal plasma. The results of this study showed that Pergonal$^{(R)}$ enhanced semen quality and was not detrimental to the hormonal profile and biochemical constituents of the seminal plasma of the WAD bucks.

Keywords: Hormones, Pergonal$^{(R)}$, semen quality, seminal plasma constituents, WAD bucks.

INTRODUCTION

The West African Dwarf (WAD) goat is predominantly found in the rain forest belt of the west coast of Africa (Iheukwumere, 2006). In rural South Eastern Nigeria, WAD goat rearing is usually integrated into the traditional farming system. In this region and elsewhere, WAD goat production fulfils important economic as well as social functions, with over 80% of rural farmers keeping them primarily as an investment and source of manure or for meat at home or during festivals (Iheukwumere, 2006). West African Dwarf goats are hardy and short-legged animals. Their body colour varies from white to black or black with white spots or white with black spots. Mature goats may have bears (Iheukwumere, 2008). They are good scavengers and are resistant to trypanosomiasis, but have slow growth rate (Oni, 2002). The West African Dwarf goat supplies excellent quality meat, milk, skin and other products.

The general reproductive performance of goats has been noted to be low in terms of offspring produced per female per year, though the occasional occurrence of triplets has been recorded. The WAD goat is still largely unimproved. The improvement of the tropical breeds of

goats generally and the WAD goat in particular for higher productivity as has been successfully done with goats in France (Leboeuf et al., 1998) will require information on the semen characteristic of bucks. Thus, knowledge of the semen characteristics of pubertal bucks is very useful in the early selection of sires for planned breeding programmes. In addition, older bucks constitute a problem as their libido and reaction time decline with increasing age (Bitto and Egbunike, 2012). Inspite of recent reports in WAD bucks on sperm production rate and sperm storage capacity (Bitto and Egbunike, 2006a), testicular morphometry (Bitto and Egbunike, 2006b), some biochemical characteristics of spermatozoa and seminal plasma (Bitto and Egbunike 2007) as well as the histometry of the tests (Bitto and Egbunike, 2008), information on the use of Gonadotrophin for induction of spermatogenesis in WAD bucks is still lacking.

The primary aim of induction of spermatogenesis is to improve semen quality (Ameh, 2004; Abu et al., 2006). Spermatogenesis involves the use of follicle stimulating hormone (FSH), and luteinizing hormone (LH) (Abu et al., 2006). Most of these preparations of FSH and LH are very expensive perhaps because of the brand names, some of them requires cold chain strong and often deteriorate because of inadequate storage and handling (Herbert et al., 2000).

There is therefore the need to examine some generic preparations that could induce spermatogenesis in animals but at the same time are cheap, readily available and easily managed under developing countries conditions. Pergonal(R), is a fertility drug of ferring Labs. USA (also known as Humegon or Mentrophin and with similar constituents as plusset(R)). It is a gonadotrophin preparation lyophilized in vials containing a mixture of gonadotrophins consisting of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in a ratio 1:1 (Iheukwumere et al., 2004). Follicle stimulating hormone and LH present in Pergonal(R) play vital roles in the initiation of spermatogenesis (Egu, 2016). The hormone preparation is cheap, readily available and does not require cold chain storage (Iheukwumere, 2005).

It has not been determined if the administration of the hormone preparation for spermatogenesis and semen production would induce any side effects on the hormonal profile and seminal plasma constituents of the WAD bucks. This study was therefore conducted to determine the effect of Pergonal(R) administration on the semen quality, hormonal profile and seminal plasma constituents of WAD bucks.

MATERIALS AND METHODS

Experimental animals and their management

Sixteen healthy sexually matured West African Dwarf bucks aged 2.0 to 2.6 years were used for this study. The animals were purchased from the local markets and housed in clean pens constructed in such a way that the bucks could come outside during the day for access to sunlight. The study was conducted at the Sheep and Goat Unit of the Teaching and Research Farm of the Faculty of Agricultural Abia State University, Umudike located near Umuahia Nigeria. The animals were dewormed and routine inspection for cleanliness was carried out. Freshly cut forage consisting of *Panicum maximum, Aspilia Africana, Pennisetum purpureum* (Elephant grass) was fed as basal diet and a commercial concentrate ration of Grower's Mash (containing 2450 Kcal/kg) (Table 1) was used as supplement, the animals were fed twice daily (in the morning and evening). Salt lick was provided as mineral supplement. Water was given *ad libitum* to the animals.

Table 1. Ingredient composition of the commercial concentrate (Top Grower's Mash) fed to WAD bucks.

Ingredient **composition**	**Percent (%)**
Crude Proteins	16.00
Fats and Oil	5.00
Crude Fibre	7.00
Calcium	1.00
Available Phosphorus	0.45
Lysine	0.75
Methionine	0.36
Salt(g)	0.30
Kcal/Kg Metabolisable Energy (ME)	2450

Experimental design and drug administration

The sixteen WAD bucks were divided into 4 treatment groups consisting of 4 bucks per group with one buck per replicate in a completely Randomized Design (CRD). The groups were assigned to 4 levels of Pergonal® as treatment. The levels of Pergonal® were 0.00 i.u, 9.00 i.u, 18.00 i.u and 27.00 i.u Pergonal® represented as T_1, T_2, T_3, and T_4, respectively. T_1, which contained no Pergonal® served as the control. The animals were retreated by intramuscular injections. The injections were as follows:
Pergonal(R) was supplied in 2 vials, each vial containing FSH 75 i.u and LH 75 i.u. The content of the first vial was dissolved in 1.00 ml of physiological saline solution immediately prior to use, resulting in a solution of PFSH 75 i.u plus PLH 75 i.u per ml.

Group T_1 received 1.00 ml physiological saline for 3 days.

Group T_2 received on:
Day 1: 1.50 i.u of PFSH and 1.50 i.u of PLH (0.04 ml)
Day 2: 1.50 i.u of PFSH and 1.50 i.u of PLH (0.04 ml)
Day 3: 1.50 i.u of PFSH and 1.50 i.u of PLH (0.04 ml)
Total: 9.00 i.u of PFSH and PLH (0.12 ml)

Group T_3 received on:
Day 1: 3.00 i.u of PFSH and 3.00 i.u of PLH (0.08 ml)
Day 2: 3.00 i.u of PFSH and 3.00 i.u of PLH (0.08 ml)
Day 3: 3.00 i.u of PFSH and 3.00 i.u of PLH (0.08 ml)
Total: 18.00 i.u of PFSH and PLH (0.24 ml)

Group T_4 received on:
Day 1: 4.50 i.u of PFSH and 4.50 i.u of PLH (0.12 ml)
Day 2: 4.50 i.u of PFSH and 4.50 i.u of PLH (0.12 ml)
Day 3: 4.50 i.u of PFSH and 4.50 i.u of PLH (0.12 ml)
Total: 27.00 i.u of PFSH and PLH (0.36 ml)

Pergonal$^{(R)}$ treatment were given by intramuscular injection on the hind leg (thigh) of each buck using a one ml syringe with 0.01 ml graduation.

Semen collection and evaluation

Semen collection was carried out by Electro-ejaculation method (Noakes et al., 2001; Egu and Ukpabi, 2015) seven days after Pergonal$^{(R)}$ injections and continued at 2 weeks interval for 9 weeks. The semen was collected using a standard precision electronic model Eletro-ejaculation, U.S.A, between 8.00 am and 10.00 am. The electro-ejaculator is designed to operate only from deceleration (D.C.) battery power. It consists of a rectal probe and a power control system which gives stepwise control over the applied voltage. The probe delivers an acceleration (A.C.) voltage, usually 12 to 24 volts. The intensity voltage is delivered as pattern 1: 0.0 - 7.0 volts. Electrical probe was inserted into the rectum. The depth of insertion started 1 cm from the anus with 1 cm increment in each depth. Electrical stimulation was applied at the voltage of 1.0 volt, increased by 1.0 volt every 5 seconds to the maximum of 12.0 volts. A transparent graduated tube immersed in a protective jacket containing water at 37^0c with a funnel was used to collect the semen. The animal was restrained and held in an upright position by an assistant. The Vaseline lubricated probe was inserted gently into the rectum. The rhythmic stimulation of the ampullae, lumbar and sacral nerve plexuses caused erection and subsequently ejaculation within few minutes.

The principle of Electro-ejaculation is based on stimulation of the sympathetic-parasympathetic nervous systems. The posterior mesenteric and pelvic plexuses control, to a large degree, the following physiological functions: (i) smooth muscles of the head of the epididymis, vas deferens, ampulla of the vas deferens, ejaculatory duct system, accessory glands and that part of the urethra from the ejaculatory duct area to the external opening; (ii) smooth muscles of the vascular system of the accessory gland area and the shaft of the penis; (iii) diversion of the blood supply to the cavernous areas of the erectile tissue within the penis; (iv) a concurrent relaxation of the retractor penis muscles; (v) erection of the muscle structures of the preputial sheath to accommodate movement to the shaft of the penis within and; (vi) the instantaneous mixture of accessory gland fluids with spermatozoa and forcible ejection of this mixture. In conjunction with these segments of the autonomic nervous system, the spinal nerves and their ganglia control the skeletal muscles in the case of electro-stimulation designed to produce ejaculation. The lumbar and sacral spinal nerves are involved since they are contained in close proximity to the area within the pelvic cavity which receives the probe (Brackett, 2005).

Semen evaluation was carried out as promptly as possible after collection as described by Rodriguez – Martinez and Barth (2007) and Egu and Ukpabi (2016) for qualitative and quantitative parameters such as semen volume and PH, individual motility, sperm concentration, live sperm percentage and normal sperm percentage.

Semen volume

The volume of semen collected was measured in ml using the graduated collection tube.

Mass motility

Using a sterile dropping pipette, a drop of semen was placed on a warm slide while the slide was observed under x10 magnification of a light microscope. The warm slide was obtained by placing the slide on a warm chamber for 2 minutes. The motility estimate was done by taking estimate of sperm waves from three different apexes of the angle and finding the average score (Egu and Ukpabi, 2016; Rodriquez-Martinez and Barth, 2007).

Individual motility

Using a dropping pipette, a drop of semen was placed on the warm slide, two drops of sodium chloride were added, and a cover slide was placed while the slide was examined under x40 magnification of the light microscope. The motility estimate was done by taking estimate from four different apexes of the angle and finding the average according to Egu and Ukpabi (2016).

Sperm concentration

A haemocytometer was used to determine the sperm concentration. A red cell pipette was used to suck up semen to 0.5 ml mark. The semen was diluted by sucking normal saline up to 1.01 ml mark. The pipette was gently rocked to ensure uniform mixture. The first few drops were blown out. The diluted semen was placed on a haemocytometer slide and covered with cover slip. The slide was then placed under the light microscope and

viewed under x40 magnification. Five squares were counted and the average taken to get the semen concentration. This is in line with the method of Iheukwumere and Okere (1990).

Sperm morphology

The morphology of the spermatozoa was evaluated using Eosin-Nigrosin stain. Thin smears of each collected semen sample were made on slides with frosted ends on which the animal's details and date of collection were inscribed in pencil. Smears were done by diluting a drop of the individual's semen sample with 2 to 3 drops of warm Eosin-Nigrosin stain (pH 8.4) and drying the slide by waving it in the air. All smears were left on a warm stage to dry out completely. Slides were thereafter viewed under the light microscope using x200 magnification to find suitable area of good quality on the smear to evaluate. Two hundred sperm cells were counted with a counter and evaluated as they came into view. Data were recorded on a data capture sheet using the classification of Rodriguez-Martinez and Barth (2007) as adopted by the standard operating procedure of the section of reproduction, Faculty of Veterinary Science, Onderstepoort, University of Pretoria. Normal sperm cells are those whose acrosome are intact along with the neck, middle piece and end piece. Whereas abnormal spermatozoa are those whose acrosome, neck, middle piece and end piece have been altered due to injury or sperm ageing. These were observed under the microscope during semen evaluation.

Live sperm percentage

A drop of semen was put on a clean slide; a drop of eosin nigrosine stain was added. The two drops were mixed using another clean slide and observed under the microscope. The percentage of live spermatozoa was assessed by identifying those with intact cell membrane, from dye exclusion or by hypotonic swelling (Rodriguez-Marthinez and Barth, 2007)

Hormonal assay

Blood samples (5 ml each) were obtained with needle and syringe by jugular vein puncture of the twelve bucks one week after Pergonal$^{(R)}$ injections, for testosterone, FSH and LH evaluation. The blood samples were cooled immediately after collection in iced water and transferred to the laboratory, refrigerated at 4^0C for 1 hour and the serum separated by centrifugation at 5,000 rpm for 10 minutes. The sera were stored immediately at -20^0C until enzyme immune assayed (EIA) with Immunometrics limited Kit (UK) for testosterone, FSH and LH as described by Micallef et al. (1995).

Biochemical constituents of seminal plasma

Semen samples used for estimation of biochemical constituents of seminal plasma were centrifuged at 15,000 rpm for 15 minutes (Iheukwumere et al., 2001; Egu and Ukpabi, 2015). The seminal plasma samples were immediately subjected to laboratory analysis for the following biochemical parameters: Sodium, Potassium, bicarbonate, urea, fructose, calcium, magnesium, citric acid and ascorbic acid. Sodium and potassium concentrations were estimated with a flame photometer on samples suitably diluted with deionized water (Iheukwumere et al., 2001). While urea and bicarbonate concentrations were determined according to the method of Baker and Silverton (1986). Fructose concentration in the seminal plasma was determined according to the procedure of Singgh (2004). Calcium and Magnetic concentrations were estimated by means of atomic absorption spectrophotometer on seminal plasma using the methods of Iheukwumere (2008). Magnetic resonance spectroscopy was used to determine the concentrations of Ascobic acid and Citric acid (Robert et al., 2000).

Data analysis

Data obtained on semen characteristics, hormonal profile and biochemical constituents of the seminal plasma of West African Dwarf bucks were subjected to one – way analysis of variance (ANOVA) using the technique of Steel and Torrie (2006). Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi (2002).

RESULTS AND DISCUSSION

The results of gonadotrophin (Pergonal$^{(R)}$) administration on semen characteristics of West African Dwarf bucks are shown in Table 2. There were significant differences ($P<0.05$) among the treatment groups in semen volume. Bucks on T_3 recorded the highest value of 1.10 ml in semen volume and this differed significantly ($P<0.05$) from bucks on T_1 (0.50 ml) which were similar ($P<0.05$) to bucks on T_2 (0.70 ml) in semen volume. There were no significant differences ($P<0.05$) among bucks on T_2, T_3, T_4 in semen volume. The lowest value in semen volume was observed in bucks on T_1. The values for volume of semen obtained in this study were higher than the mean value of 0.47 ± 0.02 ml reported for adult WAD bucks by Bitto and Egbunike (2012), and 0.30 ± 0.02 ml reported by Oyeyemi et al. (2011) in WAD bucks. Iheukwumere et al. (2008) noted that method of semen collection, season of the year, bred, age, body weight of animal, scrotal circumference and frequency of semen harvest can affect ejaculate volume in animals.

There were significant differences ($P<0.05$) among the

Table 2. Effect of Pergonal(R) on semen characteristics of WAD bucks.

Parameters	Treatment (Pergonal® i.u)				SEM
	T_1 (0.00)	T_2(9.00)	T_3 (18.00)	T_4 (27.00)	
Semen Volume (ml)	0.50[b]	0.70[ab]	1.10[a]	1.00[a]	0.14
Semen pH	8.00[b]	9.00[a]	8.00[b]	9.00[a]	0.29
Mass motility	1.50[ab]	1.00[b]	3.00[a]	0.50[b]	0.54
Individual motility (%)	75.00a[b]	65.00[b]	80.00[a]	65.00[b]	3.75
Sperm concentration (X10^9/ml	0.29[b]	0.65[a]	0.80[a]	0.47[ab]	0.11
Proportion of live sperm cells (%)	70.32[b]	73.00[ab]	85.00[a]	64.00[b]	4.40
Proportion of normal sperm cells (%)	68.00[a]	65.15[ab]	78.10[a]	45.23[b]	6.88
Proportion of dead sperm cells (%)	29.68[a]	27.00[ab]	15.00[b]	36.00[a]	4.40

[ab]: means within row having different superscript are significantly (P<0.05) different. **SEM** = Standard error of means.

treatment groups in semen pH. Bucks on T_2 and T_4 recorded the highest value of 9.00 in semen pH each and these differed significantly (P<0.05) from bucks on T_1 (8.00) and T_3 (8.00) which were similar (P<0.05) to each other in semen pH. Semen pH values obtain in T_1 and T_3 were within the normal range of 7 to 8 reported by Meacham (2000), while the semen pH values in T_1 and T_4 were higher than the normal range. The measured pH depends on the length of time since ejaculated and it tends to increase shortly after ejaculation as a result of loss of CO_2 (Meacham, 2002; Egu and Ukapi, 2015; Egu and Ukpabi, 2016). Semen maintains its pH near neutral in the acidic vaginal environment providing the sperm with the opportunity to enter the neutral pH of the cervical mucus (Meacham, 2002; Egu and Ukpabi, 2016).

There were significant differences (P<0.05) among the treatment groups in mass motility. Bucks on T_3 recorded the highest value of 3.00 in mass motility and this differed significantly (P<0.05) from bucks on T_2 (1.00) and T_4 (0.50) which were similar (P<0.05) to each other and similar (P<0.05) to bucks on T_1 in mass motility. There was no significant difference (P<0.05) between bucks on T_1 and T_3 in mass motility. The lowest value in mass motility was observed in bucks on T_4. The values for mass motility obtained in this study were within the normal range of 1 to 5 (Brackett, 2005). However, the values for mass motility obtained in this study were lower than the mean value of 4.25±0.5 reported for WAD bucks by Bitto and Egbunike (2012).

There were significant difference (P<0.05) among the treatment groups in individual motility. Bucks on T_3 recorded the highest score of 80% in individual motility and this differed significantly (P<0.05) from bucks on T_2 (65.00%) and T_4 (65.00%) which were similar (P<0.05) to each other and similar (P<0.05) to bucks on T_1 in individual motility. There was no significant difference (P<0.05) between bucks on T_1 and T_3 in individual motility.

The highest score in individual motility obtained in this study (80.00%) was higher than the mean value of 74.66% reported for adult WAD bucks by Bitto and Egbunike (2012). This could be attributed to high capacity for induction of spermatogenesis and improvement of fertility by Pergonal(R) injection. Sperm motility is a critical indicator of semen quality and fertility potential because it is required for penetration of cerical mucus, transport through the female genital tract, and penetration through the corona rediata and zona pellucida before oocyte fertilization (Iheukwumere, 2006; Egu and Ukpabi, 2016). Semen motility is also affected by frequency of semen collection (Iheukwumere et al., 2008; Egu and Ukpabi, 2016).

There were significant differences (P<0.05) among the treatment groups in sperm concentration. Bucks on T_3 recorded the highest value of 0.80x10^9/ml in sperm concentration and this differed significantly (P<0.05) from bucks on T_1 (0.29x10^9/ml) which were similar (P<0.05) to bucks on T_4 (0.47x10^9/ml) in sperm concentration. There were no significant differences (P<0.05) among bucks on T_2, T_3, and T_4 in sperm concentration. The lowest value in sperm concentration was observed in bucks on T_1. The highest sperm concentration obtained in this study (0.80x10^9/ml) was within the range of 0.53±0.04 to 1.65±0.07 reported by Bitto and Ebgunike (2012) in pubertal and adult WAD bucks. This was within the normal range of 200 to more than 1,000 million spermatozoa/ml reported by Rodriguez-Martinez and Barth (2007). Normally, an increase in the semen collection frequency is associated with a decrease in spermatozoa concentration (Iheukwumere and Okere, 1990; Arroita et al., 2000; Egu and Ukpabi, 2016).

There were significant differences (P<0.05) among the treatment groups in percentage of live sperm cells. Bucks on T_3 recorded the highest percentage of live sperm cells (85.00%) and this differed significantly (P<0.05) from bucks on T_1 (70.32%) and T_4 (64.00%) which were similar (P<0.05) to each and similar (P<0.05) to bucks on T_2 in percentage of live sperm cells. The lowest percentage of live sperm cells was observed in bucks on T_4. The percentages of live sperm cells obtained in this study were lower than the range of 88.91±0.61 to 91.88±0.60 reported by Bitto and Egbunike (2012) in pubertal and adult WAD

Table 3. Effect of Gonadotrophin on hormonal profile of WAD bucks.

Parameters	Treatment (Pergonal® i.u)				SEM
	T_1 (0.00)	T_2 (9.00)	T_3 (18.00)	T_4 (27.00)	
Follicle stimulating Hormone (iu/L)	3.08^{ab}	2.30^{bc}	3.16^{ab}	2.15^{c}	0.26
Luteirnizing hormone (iu/L)	3.21	3.21	3.22	3.21	0.10
Testosterone (ng/mL)	18.35^{b}	19.34^{a}	19.50^{a}	19.45^{a}	0.27

^{ab}: Means within row having different superscript are significantly (P0<05) different, **SEM =** Standard error of means.

bucks. This disparity in the percentage of live sperm cells may not be unconnected to the differences in environment, season of the year and nutritional status of WAD the bucks.

There were significant differences (P<0.05) among the treatment groups in percentage of normal sperm cells. Bucks on T_3 recorded the highest percentage of normal sperm cell (78.10%) and this differed significantly (P<0.05) from bucks on T_4 (45.25%) which were similar (P<0.05) to bucks on T_2 (65.15%) in percentage of normal sperm cells. The percentage of normal sperm cells obtained in this study were lower than the range of 85.90± 0.72 to 94.15±0.60 reported by Bitto and Egbunike (2012) in pubertal and adult WAD bucks. This disparity in the percentage of normal sperm cells could be attributed to the differences in environment, season of the year and nutritional status of the WAD bucks.

There were significant differences (P<0.05) among the treatment group in percentage of dead sperm cells. Bucks on T_4 recorded the highest percentage of dead sperm cells (36.00%) and this differed significantly (P<0.05) from bucks on T_3 (15.00%) which were similar (P<0.05) to bucks on T_2 (27.00%) in percentage of dead sperm cells. There were no significant differences (P<0.05) among bucks on T_1, T_2, and T_4 in percentages of dead sperm cells. The lowest percentage of dead sperm cells was observed in bucks on T_3.

The observation in this study that the group that received the highest dose of Pergonal(R) recorded the lowest percentage of live sperm cells, normal sperm cells and highest percentage of dead sperm cells suggests that 27.00 ml gonadotrophin/buck administered within 3 days could have deleterious effect on sperm cells.

The result of gonadotrophin administration on hormonal profile of West African Dwarf bucks are shown in Table 3. There were significant differences (P<0.05) among the treatment groups in follicle stimulating hormone (FSH). Bucks on T_3 recorded the highest value of 3.16 iu/L in FSH and this differed significantly (P<0.05) from bucks on T_2 (2.30 iu/L) and T_4 (2.15 iu/L) which were similar (P>0.05) to each other in FSH value. There was no significant difference (P<0.05) between bucks on T_1 and T_3 in FSH values. Bucks on T_1 were also similar (P>0.05) to bucks on T_2 in FSH value. The lowest value in FSH was observed in bucks on T_4. The observation in this study that the FSH value in bucks treated with 18.00 iu gonadotrophin (T_3) was higher (P<0.05) than in the group that received higher dose of the drug suggest that a high dose of the drug such as 27.00 iu gonadotrophin/buck within 3 days given in this study could excite suppressive effects on the hypothalamus. This observation is an agreement with the report of Iheukwumere (2005) in goats and Egu and Ukpabi (2006) in sheep.

There were no significant differences (P<0.05) among the treatment groups in luteinizing hormone (LH) levels in the serum. Bucks on T_3 recorded the highest numerical value of 3.22 iu/L in LH. Similarity (P>0.05) in LH values among the treatment groups was an indication that the administration of the test drug had no deleterious effects on LH secretion. Luteinizing hormone as an interstitial cell stimulating hormone (ICSH) stimulates the interstitial of leydig to produce testosterone which facilitates the process of spermatogenesis (Herbert et al., 2000; Egu and Ukpabi, 2016).

Bucks on T_3 recorded the highest value of 19.50 ng/ml in serum testosterone and this differed significantly (P<0.05) from bucks on T_1 (18.35 ng/ml). There were no significant differences (P<0.05) among bucks on T_2, T_3 and T_4 in testosterone concentrations. The lowest testosterone value was observed in bucks on T_1. Testosterone values obtained in this study were within the range of 0 to 20 ng/ml reported by king et al. (1993). However, the testosterone values obtained in this study were higher than the range of 3.60±0.09 to 7.31±0.59 reported by El-speily and El-Hanoun (2013) in male rabbits and the range of 0.60 to 0.95 ng/ml reported by Egu and Ukpabi (2016) for WAD rams. This disparity in serum testosterone could be attributed to differences in genotype and species.

Testosterone is a steroid hormone produced by the interstitial cells of the testes (leydig cells) and it causes the development of secondary sex characteristics of the male and the development and maintenance of male reproductive tract as well as stimulates and maintains spermatogenesis (Brackett, 2005).

The results of gonadotrophin administration on biochemical constituents of seminal plasma of WAD bucks are shown in Table 4. There were significant differences (P<0.05) among the treatment groups in sodium level in the seminal plasma. Bucks on T_3 recorded the highest value of 175.20 mg/100ml in sodium level in the seminal plasma and this differed significantly (P<0.05) from bucks on T_2 (101.00 mg/100ml) and T_4 (66.30 mg/100ml) which

Table 4. Effect of Gonadotrophin on biochemical constituents of the seminal plasma of WAD bucks.

Parameter	Treatment (Pergonal® i.u)				SEM
	T_1 (0.00)	T_2 (9.00)	T_3 (18.00)	T_4 (27.00)	
Sodium (mg/100ml)	145.20^{ab}	101.00^{b}	145.20	66.30^{b}	23.98
Potassium (mg/100ml)	13.50^{ab}	34.00^{ab}	44.00^{a}	10.50^{b}	8.08
Calcium (mg/100ml)	6.15^{ab}	9.10^{a}	10.10^{a}	4.30^{b}	1.36
magnesium(mg/100ml)	4.20^{b}	11.40^{a}	12.00^{a}	4.10^{b}	2.18
Fructose (mg/100ml)	170.00^{a}	130.50^{ab}	184.20^{a}	110.25^{b}	19.08
Bicarbonate (mg/100ml)	20.15^{b}	20.23^{ab}	23.20^{a}	20.20^{ab}	0.03
Urea (mg/100ml)	41.00^{ab}	40.20^{b}	42.00^{a}	40.30^{b}	0.42
Ascobic Acid (mg/100ml)	6.00^{b}	6.30^{b}	6.80^{ab}	7.70^{a}	0.37
Lactic acid (mg/100ml)	43.00	43.00	43.00	43.00	0.00

^{ab}**:** means within row having different superscript are significantly (P<0.05) different, **SEM =** Standard error of means.

were similar (P>0.05) to each other and similar (P>0.05) to bucks on T_1 in seminal plasma sodium. There was no significant difference (P>0.05) between bucks on T_1 and T_3 in seminal plasma sodium. The lowest value for sodium was observed in bucks on T_4. A positive and significant correlation has been established between sodium concentration and sperm concentration in bucks (Akpa et al., 2013).

There were significant differences (P<0.05) among the treatment groups in seminal plasma potassium. Bucks on T_3 recorded the highest value of 44.00 mg/100ml in potassium and this differed significantly (P<0.05) from bucks on T_4 (10.50 mg/100ml) which were similar (P>0.05) to bucks on T_1 and T_2 in potassium value. There were no significant differences (P>.0.05) among bucks on T_1, T_2, and T_3 in potassium values. The lowest value in potassium was observed on bucks on T_4 indicating that the administration of 27.00 i.u Pergonal$^{(R)}$/buck within 3 days given in this study decreased this element. Sperm concentration is also positively correlated with potassium concentration in the seminal plasma. This trend was equally observed in this study. Increasing potassium concentration in the seminal plasma is negatively corrected with progressive motility of sperm in rams and bucks while sodium has the opposite effect (Abdel-Rahman et al., 2000; Akpa et al., 2013).

Bucks on T_3 recorded the highest value of 10.10 mg/100.l in seminal plasma calcium and this differed significantly (P<0.05) from bucks on T_4 (4.30 mg/100ml) which were similar (P>0.05) to bucks T_1 (6.15mg/100ml). There were no significant differences (P<0.05) among bucks on T_1, T_2, and T_3 in calcium values. The lowest value in calcium was observed in bucks on T_4 indicating that the administration of 27.00 i.u Pergonal$^{(R)}$/buck within 3 days given in this study decreased this cation.

Bucks on T_3 recorded the highest value of 12.00 mg/100ml in seminal plasma magnesium and this differed significantly (P<0.05) from bucks on T_1 (4.20 mg/100ml) and T_4 (4.10 mg/100ml) which were similar (P>0.05) to each other in magnesium value. There was no significant difference (P>0.05) between bucks on T_2 and T_3 in magnesium values. The lowest value in seminal plasma magnesium was observed in bucks on T_4 indicating that the administration of 27.00 i.u Pergonal$^{(R)}$/buck within 3 days given in this study could have decreased metabolism and efficient utilization of nutrients that caused a decreased in this cation. Males on high plane of nutrition produce increased levels of fructose and cations in their seminal plasma (Iheukwumere et al., 2001).

There were significant differences (P<0.05) among the treatment groups in seminal plasma fructose. Bucks on T_3 recorded the highest value of 184.20 mg/100ml in fructose and this differed significantly (P<0.05) from bucks on T_4 (110.25 mg/100ml) which were similar (P>0.05) to bucks on T_2 (130.50 mg/100ml). There were no significant differences (P>0.05) among bucks T_1, T_2, and T_3 in fructose values. The lowest value in fructose was observed in bucks on T_4 indicating that 27.00 i.u Pergonal$^{(R)}$/buck within 3 days given in this study could have decreased metabolism and efficient utilization of nutrient that caused a decrease in seminal plasma fructose. Gonzales et al. (1993) reported a wide range of 136 to 628 mg/100ml fructose for semen. Owen and Katz (2005) reported that fructose is a measure of seminal vesicle function, being a source of energy for the sperm. Fructose is the primary source of lactic acid in semen.

There were significant differences (P<0.05) among the treatment groups in seminal plasma bicarbonate. Bucks on T_3 recorded the highest value of 23.20 mg/100ml and this differed significantly (P<0.05) from bucks on T_1 (20.15 mg/100ml) which were similar (P>0.05) to bucks on T_2 and T_3 in bicarbonate value. There were no significant differences (P>0.05) among bucks on T_2, T_3, and T_4 in bicarbonate values. The lowest value in seminal plasma bicarbonate was observed in bucks on T_1 indicating that the administration of gonadotrophin increased this element. The values of bicarbonate obtained in this study ranged from 20.15 to 23.20 mg/100ml and were similar to the value (20.00 mml/L) recorded by Okamura et al. (2006), who also inferred that sodium bicarbonate in

seminal plasma stimulates sperm motility.

There were significant differences ($P<0.05$) among the treatment groups in seminal plasma urea. Bucks on T_3 recorded the highest value of 42.00 mg/100ml in urea and this differed significantly ($P<0.05$) form bucks on T_2 (40.20 mg/100ml) and T_4 (40.30 mg/100ml) which were similar ($P>0.05$) to each other and similar ($P>0.05$) to bucks on T_1 in seminal plasma urea. There was no significant difference ($P>0.05$) between bucks on T_1 and T_3 in urea values. The lowest value in urea was observed in bucks on T_4. Cortada et al. (2000) reported that a sharp increase in plasma urea level could result in gonadal degeneration and infertility with reduced sperm production and loss of libido.

There were significant differences ($P<0.05$) among the treatment groups in Ascobic acid concentration in the seminal plasma. Bucks on T_4 recorded the highest value of 7.70 mg/100ml in ascobic acid and this differed significantly ($P<0.05$) form bucks on T_1 (6.00 mg/100ml) and T_2 (6.30 mg/100ml) which were similar ($P>0.05$) to each other and similar ($P>0.05$) to bucks on T_3 in ascobic acid value. There was no significant difference ($P>0.05$) between bucks on T_3 and T_4 in ascobic acid values. The lowest value in ascorbic acid was observed in bucks on T_1. Ascobic acid concentration increased with increased levels of gonadotrophin administration. Studies have shown that vitamin C plays a vital role in increasing semen volume, sperm concentration and motility in goats and rams (Sonmez and Demirci, 2003; Fazeli et al., 2010; Egu and Ukpabi, 2016) and keeping them strong by protecting them from free radicals (Dawson et al., 1992; Fazeli et al., 2010; Egu and Ukpabi, 2016). Results of this study indicate that the administration of gonadotrophin in bucks enhanced the concentration of ascobic acid in the seminal plasma which is very important in assessing semen quality and fertility in male animals.

There were no significant differences ($P>0.05$) among the treatment groups in lactic acid concentration in the seminal plasma. The value recorded was 43.00 mg/100ml across the treatments. Owen and Katz (2005) inferred that an interaction between lactic acid and CO_2 concentration can lead to pH changes.

Conclusion

The results of this study showed that the administration of up to 18.00 i.u of gonadotrophin improved sperm quality of WAD bucks. The main intension of the administration of gonadotrophin was to stimulate spermatogenesis and enhance semen quality. A higher dosage exerted a suppressive effect on the proportion of live and normal sperm cells and the level of follicle stimulating hormone in the serum of the WAD bucks. Though most of the values for the parameters measured fall within the normal ranges for adult goats, there is need to constantly monitor blood and hormonal profiles of West African Dwarf bucks under gonadotrophin treatment for spermatogenesis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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