



## Research Article

# Effect of Gonadotrophin (Pergonal<sup>(R)</sup>) on Haematology, Immune Leucocyte Status and Serum Metabolites of Mature Yankasa Rams Treated for Sperm Production

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**Article History:** Received: September 24, 2015 Revised: October 04, 2015 Accepted: October 10, 2015

### ABSTRACT

Twenty-four Yankasa rams aged 2 – 3 years and weighed between 30.50 and 30.60kg were randomly distributed into 4 groups of 6 animals per group which were further divided into three replicates of two rams each and used to determine the effect of Pergonal<sup>®</sup> on haematology, immune/leucocyte status and serum metabolites. These groups were assigned to 4 levels of Pergonal injection as treatments. The doses were 0.00 U/L, 57.78 U/L, 118.50 U/L and 173.34 U/L Pergonal<sup>®</sup> represented as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. All the treatments were administered by intramuscular injections. The results of this study showed that apart from mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), the haematological, and serum biochemical parameters, and immune status of Yankasa rams may be affected when 57.78 U/L or more of Pergonal are used for induction of spermatogenesis. These parameters should be constantly monitored during Pergonal administration in Yankasa rams.

**Key words:** Pergonal, blood profile, Yankasa rams

### INTRODUCTION

Yankasa sheep is the predominant breed of sheep indigenous to the Guinea and Sudan Savannah belt of West Africa (Iheukwumere *et al.*, 2008). The Nigeria Yankasa rams are typically tall, exceeding a height of 50-70cm at the withers and weigh 30-50kg with an outstanding sexual agility, hence they have been used for artificial insemination programs (Osinowo, 1990).

The significance of determining haematological and serum biochemical indices of domestic animals have been well documented (Obi and Anosa, 1980; Coles, 1986; Taiwo and Anosa, 1988). Similarly, various reports: (Kalamu *et al.*, 1988; Aba-Adulugba and Joshua 1990; Esiewo and Moor 1990; Taiwo and Ogunjami, 2003; Nottidge *et al.*, 1999; Egbe-Nwiiyi *et al.*, 2000; Iheukwumere *et al.*, 2001; Iheukwumere *et al.*, 2004; Iheukwumere *et al.*, 2006; Iheukwumere *et al.*, 2008; Oguike and Ude, 2008) have documented haematological and serum biochemical parameters of domestic species in Nigeria. However, only a few were on sheep particularly the Yankasa sheep raised in the Northern part of Nigeria. Hematological responsiveness of the animal to its external and internal environments may serve as a veritable tool for use in monitoring animal health and nutritional status (Esonu *et al.*, 2001; Churias, 2002).

Human Gonadotrophin (Pergonal<sup>®</sup>), a fertility drug (Ferring Labs., USA) also known as Humagon or Mentrophin with similar constituents as Plusset<sup>®</sup> is a gonadotrophin lyophilized in vials containing a mixture of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in a ratio 1:1 (Dixon and Hopkins 1996). Follicle stimulating hormone and LH present in Pergonal play a vital role in the initiation of spermatogenesis (Abu *et al.* 2006).

The hormone preparation is cheap, readily available and does not require cold chain storage (Iheukwumere *et al.*, 2005). It has not been determined if the administration of the hormone preparation for spermatogenesis would induce any side effects on the blood parameters of treated rams. This study was therefore carried out to determine the effect of Pergonal administration on haematology, immune/leucocyte status and serum metabolites of Yankasa rams.

### MATERIALS AND METHODS

#### Experimental animals and their management

Twenty-four healthy sexually matured Nigerian Yankasa rams aged 2-3 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

**Cite This Article as:** Egu UN and UH Ukpabi, 2015. Effect of gonadotrophin (pergonal<sup>(R)</sup>) on haematology, immune leucocyte status and serum metabolites of mature yankasa rams treated for sperm production. *Inter J Vet Sci*, 4(4): 216-220. www.ijvets.com (©2015 IJVS. All rights reserved)

**Table 1:** Doses of Pergonal Administered on Mature Yankasa Rams

Day	Treatment Dosage (ml)			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
1	0.00	0.09	0.18	0.33
2	0.00	0.12	0.21	0.33
3	0.00	0.12	0.27	0.33
Total	0.00	0.33	0.66	0.99

**Table 2:** Concentration of pergonal administered on mature yankasa rams

Day	Concentrations of Pergonal (I.U)			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
1	0.00	15.72	30.00	57.78
2	0.00	21.03	37.50	57.78
3	0.00	21.03	51.00	57.78
Total	0.00	57.78	118.5	173.34

Blood collection and haematological analysis

rams could come outside during the day for access to sunlight. The animals were dewormed and routine inspection for cleanliness was carried out. Four kilograms of freshly cut forage consisting of *Panicum maximum* (2.0kg) and *AspiliaAfricana* (2.0kg) was fed as basal diet and one kilogram of concentrate ration (Growers Mash) per day for each ram 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.

#### Experimental design and drug administration

The Completely Randomized Design (CRD) was used to distribute 24 mature Yankasa rams into 4 treatment groups consisting 6 rams per group. These groups were assigned to 4 levels of Pergonal as treatments. The levels of Pergonal were 0, 57.78, 118.50 and 173.34 I.U represented as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. The group that was placed on T<sub>1</sub>, which contained no Pergonal served as the control. Pergonal was supplied in 24 vials, each vial containing FSH 75 I.U and LH 75 I.U. The content of the first vial was dissolved in 1ml of physiological saline solution immediately prior to use. Groups T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> received different doses of Pergonal as indicated in Table 1 while the group on T<sub>1</sub> received 1.0 ml of physiological solution for three days. Equivalent concentrations of PFSH and PLH are as presented in Table 2. Pergonal treatments were administered intramuscularly on the hind leg (thigh) of each ram using a one ml syringe with 0.01ml graduation. The rams were bled one week after Pergonal injection between 9am and 10.30am from a punctured jugular vein and about 12ml of blood were aspirated from each ram. Two milliliters of each blood sample were poured into Bijou bottles containing ethylene diamine tetra-acetic acid (EDTA) for haematological evaluation. The remaining 10ml of each blood sample were allowed to coagulate to produce sera for blood chemistry analysis.

Blood samples were analyzed within 2 hours of their collection for packed cell volume (PCV) and haemoglobin (Hb). Erythrocyte or red blood cells (RBC) and leucocyte counts were determined as described by Jain (1986). Erythrocyte count was done in a haemocytometer chamber placed under a light microscope. Packed cell volume was determined by the microhaematocrit method

(Jain, 1986) with 75 x 16mm capillary tubes filled with blood and centrifuged at 3000 rpm for 5 minutes. Haemoglobin concentration was also determined by the cyanmethemoglobin method (Jain, 1986). The various red cell indices like mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated from RBC, Hb and PCV (Lazzaro, 2003). Total leucocyte count was carried out using a Neubaerhaemocytometer placed under a light microscope under x10 magnification, after using Natt and Henricks dilution to obtain a 1:200 blood dilution. Differential leucocyte count was achieved using blood smears stained with Wright's dye and each type of cell (neutrophil, lymphocyte, eosinophil, monocyte and basophil) was determined with a counter.

#### Evaluation of blood Chemistry

The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for biochemical evaluation. Total bilirubin, conjugated bilirubin, cholesterol, glucose and calcium concentrations were determined using the analytical kits of Randox Laboratories Limited Crumlin. Co. Anthrax, UK at HASTO Medical Laboratory Enugu, Nigeria.

#### Data analysis

Data collected on haematological and serum biochemical parameters of Yankasa rams were subjected to analysis of variance (ANOVA) using the technique of Steel and Torrie (1980). Significant treatment means were separated using Duncan's New Multiple Range Test as described in Obi (1990).

## RESULTS AND DISCUSSION

The results of the haematological parameters of Yankasa rams treated with gonadotrophin (Pergonal) are shown in Table 3. There were significant differences ( $P < 0.05$ ) among the treatment groups in packed cell volume (PCV), haemoglobin (HB), White blood cell (WBC) red blood cell (RBC) and mean corpuscular volume (MCV) values. However, there were no significant differences among the treatment groups in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values.

Ram on T<sub>3</sub> recorded the highest value in PCV which differed significantly ( $P < 0.05$ ) from rams on T<sub>2</sub> and T<sub>1</sub> which were also significantly different from each other. However, there was no significant difference ( $P > 0.05$ ) between rams on T<sub>3</sub> and T<sub>4</sub> in PCV values. The PCV values obtained in rams treated with higher doses of Pergonal were higher than the normal range of 27.0-45.0% reported in sheep (Radostits *et al.*, 1997) and higher than 44.33% reported in West African dwarf sheep (Oguike and Ude, 2008). This suggests that the test drug might have affected the physiological state of the animals.

The haemoglobin values obtained in the Pergonal treated groups were higher than the normal range of 9.0-15.0g/dl reported for sheep (Radostits *et al.*, 1997). It is possible that the gonadotrophin injections increased metabolism and efficient utilization of nutrients. Haemoglobin concentration in the blood has been associated with

**Table 3:** Effect of pergonal administration on haematology of yankasa rams

Parameters	Treatment (Pergonal)(IU)				SEM
	T <sub>1</sub> (0.00)	T <sub>2</sub> (57.78)	T <sub>3</sub> (11850)	T <sub>4</sub> (173.34)	
PVC (%)	40.45 <sup>c</sup>	44.65 <sup>b</sup>	49.35 <sup>a</sup>	48.76 <sup>a</sup>	0.62
Hb (g/dl)	14.30 <sup>b</sup>	15.40 <sup>ab</sup>	16.35 <sup>a</sup>	17.25 <sup>a</sup>	0.63
WBC (x10 <sup>9</sup> /l)	9.33 <sup>a</sup>	7.41 <sup>b</sup>	7.56 <sup>b</sup>	7.65 <sup>b</sup>	0.45
RBC (x10 <sup>12</sup> /l)	4.85 <sup>c</sup>	5.30 <sup>b</sup>	5.54 <sup>ab</sup>	5.65 <sup>a</sup>	0.07
MCV (fl)	80.00 <sup>b</sup>	85.35 <sup>a</sup>	86.24 <sup>a</sup>	87.15 <sup>a</sup>	1.60
MCH (pg)	33.00	33.00	33.00	33.00	0.00
MCHC (g/dl)	34.30	34.34	34.30	34.30	0.00

<sup>abc</sup>Means within row having different superscript are significantly (P<0.05) different; SEM = Standard error of means

availability of nutrient to the animal (Esonu *et al.*, 2001; Iheukwumere and Herbert, 2002).

The WBC values obtained in this study were within the normal range of 4-12(x10<sup>9</sup>/L) reported for sheep (Radostits *et al.*, 1997). This was an indication that Pergonal injections were tolerated by the rams. White blood cell values obtained in this study were higher than the value of 7.38 (x10<sup>9</sup>/L) reported in West Africa dwarf sheep (Oguike and Ude, 2008). This may be attributed to breed difference. The normal values of WBC obtained in this study also depicts absence of infection since elevations of WBC suggests infection by microorganisms especially bacteria (Aka *et al.*, 2008; Sowande *et al.*, 2008).

The values for RBC increased significantly (P<0.05) as the dosage of Pergonal administered increased. However, there was no significant difference (P>0.05) between rams on T<sub>4</sub> and T<sub>3</sub> in RBC values. The RBC values obtained in this study were lower than the normal range of 9.0 – 15.0 (x10<sup>12</sup>/L) reported for sheep (Radostits *et al.*, 1997) but higher than 2.33±0.18 – 3.17±0.09 (x10<sup>12</sup>/L) reported in WAD sheep (Sowande *et al.*, 2008).

Rams in all Pergonal treated groups recorded significantly (P<0.05) higher MCV values than rams on the control treatment (T<sub>1</sub>). However, there were no significant differences (P>0.05) among rams on T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> in MCV values. The MCV values obtained in this study were much higher than the normal range of 28 – 40fl reported for sheep (Reece and Swenson 2004). Mean corpuscular volume is an indication of the average volume of blood cells (Lazzaro 2003).

There were no significant differences (P>0.05) among the treatment groups in MCH and MCHC values. The MCH value obtained in this study (30.00pg) was higher than the normal range (8–12 pg) in sheep (Reece and Swenson 2004). The MCHC values obtained in this study were within the normal range of 31.0 – 34.0 (g/dl) reported for sheep (Radostits *et al.*, 1997). The observed variations in MCV and MCH values may be attributed to differences in season, nutritional and physiological status of the rams (Esonu *et al.*, 2001). The results of differential leucocytes count of Yankasa rams treated with gonadotrophin (Pergonal<sup>®</sup>) are shown in Table 4.

Rams on T<sub>3</sub> had the highest neutrophil value which differed significantly (P<0.05) from rams on T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub>. There was no significant difference (P>0.05) between rams on T<sub>1</sub> and T<sub>2</sub> in neutrophil values. The neutrophil values obtained in this study were within the normal range of 10 – 50% (Radostits *et al.*, 1997) or 0.70 – 6.00(x10<sup>3</sup>/μL) reported for sheep (Merck, 2010). Neutrophils have phagocytic and bactericidal capabilities which mean that they play an important role in inflammatory conditions. They are very important for

defense whenever acute infection is present (Banerjee 2005).

Results showed that rams on T<sub>1</sub> had the highest lymphocytes value which differed significantly (P<0.05) from rams on the Pergonal treatment which were similar (P>0.05) to each other. The lymphocyte values obtained in this study were within the normal range (40 – 75%) for sheep (Merck, 2010). This suggests that the administration of the test drug was not detrimental to the functioning of the immune system (Iheukwumere *et al.*, 2008). White blood cell and lymphocyte counts are known to increase during infection.

The highest eosinophil count was recorded in rams on T<sub>2</sub> and it differed significantly (P<0.05) from the counts of T<sub>1</sub>, T<sub>3</sub> and T<sub>4</sub>. These values were within the normal range (1-10%) for sheep (Merck, 2010). This is an indication that the administration of Pergonal did not trigger allergic reactions in the rams.

Monocyte counts showed that rams on T<sub>4</sub> had the highest value which differed significantly (P<0.05) from rams on T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. Rams on T<sub>1</sub> and T<sub>3</sub> were similar to each other but differed significantly from rams on T<sub>2</sub>. Monocyte values obtained in this study were within the normal range of 0 – 6% or 0 – 0.75(x10<sup>3</sup>/μL) reported for sheep (Radostits *et al.*, 1997; Merck 2010). The observed variations in monocyte counts may be ascribed to other physiological factors (Mahmood *et al.*, 1994; Egbe – Nwiiyi *et al* 2000) rather than Pergonal. Basophils were not detected among the treatment groups. The result of serum metabolites of Yankasa rams treated with gonadotrophin (Pergonal<sup>®</sup>) are shown in Table 5.

Rams on T<sub>1</sub> had the highest urea value which differed significantly (P<0.05) from rams on T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. However there was no significant difference (P>0.05) between rams on T<sub>3</sub> and T<sub>4</sub> and these differed significantly (P<0.05) from T<sub>2</sub>. The concentration of urea in the blood declined as the dosage of Pergonal administered increased. Serum urea values obtained in this study were slightly higher than the normal range of 2.85-7.14(mmol/L) reported in sheep (Kaneko *et al.*, 1997) except in rams on T<sub>4</sub> which was within the normal range. High levels of urea in the blood have been reported to indicate a lowered utilization of protein, poor protein quality or excess protein catabolism associated with protein deficiency (Oduye and Adadevoh 1976; Oduguwa *et al* 1999; Ahamefule *et al.*, 2005).

Serum glucose concentration was highest in rams on T<sub>3</sub> which differed significantly (P<0.05) from rams on T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub>. The values did not follow a definite trend but were within the normal range of 2.78 - 4.44 (mmol/L) reported for sheep (Kaneko *et al.*, 1997). Glucose is one of the metabolites measured as an indicator of the energy

**Table 4:** Effect of Pergonal on differential leucocyte count of Yankasa rams

	Treatment (Pergonal)(IU)				
	T <sub>1</sub> (0.00)	T <sub>2</sub> (57.78)	T <sub>3</sub> (118.50)	T <sub>4</sub> (173.34)	
Neutrophils (%)	40.36 <sup>b</sup>	40.34 <sup>b</sup>	43.18 <sup>a</sup>	40.06 <sup>c</sup>	0.03
Lymphocytes (%)	58.53 <sup>a</sup>	54.46 <sup>b</sup>	55.70 <sup>b</sup>	54.09 <sup>b</sup>	0.67
Eosinophils (%)	1.10 <sup>b</sup>	1.45 <sup>a</sup>	1.11 <sup>b</sup>	1.10 <sup>b</sup>	0.09
Monocytes (%)	0.00 <sup>c</sup>	3.85 <sup>b</sup>	0.00 <sup>c</sup>	4.75 <sup>a</sup>	0.20
Basophils (%)	0.00	0.00	0.00	0.00	0.00

<sup>abc</sup>Means within row having different superscript are significantly (P<0.05) different; SEM = Standard error of means

**Table 5:** Effect of pergonal on serum metabolites of yankasa rams

Parameter	Treatment (Pergonal)(IU)				SEM
	T1	T2	T3	T4	
	0.00	57.78	118.50	173.34	
Urea (mmol/L)	7.21 <sup>a</sup>	7.18 <sup>b</sup>	7.15 <sup>c</sup>	7.13 <sup>c</sup>	2.76
Glucose (mmol/L)	3.04 <sup>b</sup>	2.45 <sup>c</sup>	3.35 <sup>a</sup>	2.45 <sup>c</sup>	0.07
Cholesterol (mg/dl)	1.38 <sup>b</sup>	1.35 <sup>b</sup>	2.25 <sup>a</sup>	2.35 <sup>a</sup>	0.27
Calcium (mmol/L)	2.14 <sup>b</sup>	2.35 <sup>a</sup>	2.31 <sup>a</sup>	2.28 <sup>a</sup>	0.05
Total bilirubin (mmol/L)	7.32 <sup>a</sup>	8.50 <sup>a</sup>	8.45 <sup>a</sup>	6.35 <sup>b</sup>	0.51
Conjugated bilirubin (mmol/L)	3.77 <sup>a</sup>	3.77 <sup>a</sup>	3.65 <sup>b</sup>	3.65 <sup>b</sup>	0.03

<sup>abc</sup>Means within row having different superscript are significantly (P<0.05) different; SEM = Standard error of means

status of animals. Normal glucose levels in the rams indicate adequate synthesis in the liver from propionate metabolites as the major glucose precursor (Sowande *et al.*, 2008).

The serum cholesterol levels from rams on T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were within the normal range of 1.10-2.30 (mmol/L) reported for sheep (Merck, 2010). However, the values increased significantly (P<0.05) as the dosage of Pergonal increased in T<sub>3</sub> and T<sub>4</sub>. It is possible that higher doses of Pergonal injection increased metabolism and efficient utilization of nutrients. High cholesterol level in the serum has been implicated in the etiology of arteriosclerosis and other heart diseases in man (McDonald *et al.* 1995; Ramos *et al.*, 2003).

Serum calcium values obtained in this study were slightly lower than the normal range of 2.88 - 3.20 (mmol/L) reported in sheep (Kaneko *et al.*, 1997) and 9.60±1.60 reported for WAD sheep (Oduye and Adadevoh 1976). The similarity observed in rams on T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> indicates probable electrolyte balance in the animals' body caused by gonadotrophin administration. This observation is in agreement with the report of Iheukwumere *et al.* (2004) in goats.

The total bilirubin values obtained in this study were within the normal range of 1.71-8.55 mmol/L (Kaneko *et al.*, 1997) or 0.70-8.60 mmol/L (Merck, 2010). Total and conjugated bilirubin values are indicators of protein adequacy. The normal values of total bilirubin in this study suggest adequate or sufficient protein and good quality of protein in the diet which supported basic metabolic and physiological activities. There was equally a decline in total bilirubin value as the dosage increased beyond T<sub>2</sub>.

Rams on T<sub>1</sub> and T<sub>2</sub> recorded significantly (P<0.05) higher conjugated bilirubin values than those on T<sub>3</sub> and T<sub>4</sub> which were similar to each other. The values obtained in this study were within the normal range of 0.0 - 4.61 (mmol/L) reported for sheep (Kaneko *et al.*, 1997). High levels of conjugated bilirubin indicate that bile is not being properly excreted; therefore an obstruction may be present in the bile duct or gall bladder.

## Conclusion

The result of this study showed that apart from MCH and MCHC, the hematological and serum biochemical parameters and immune status of Yankasa rams may be affected when 57.78 I.U or more of Pergonal are used for induction of spermatogenesis. Though, most of the hematological and serum biochemical values fall within the normal ranges for adult sheep, there is need to constantly monitor blood profile of Yankasa rams under Pergonal treatment for spermatogenesis.

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