Effect of Gonadotrophin (Pergonal(R)) on Haematology, Immune Leucocyte Status and Serum Metabolites Ofmature Yankasa Rams Treated for Sperm Production

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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® 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® represented as T 1 T 2, T 3 and T 4 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 Ogunsami, 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®), a fertility drug (Ferring Labs., USA) also known as Humagon or Mentrophin with similar constituents as Plusset® 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
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 *Aspilia Africana* (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 T1, T2, T3 and T4 respectively. The group that was placed on T1, 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 T2, T3 and T4 received different doses of Pergonas as indicated in Table 1 while the group on T1 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 of each ram using a one ml syringe with 0.01ml graduation. 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 Neubauerhaemocytometer 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 Crumin. 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 T3 recorded the highest value in PCV which differed significantly (P<0.05)from rams on T2 and T4 which were also significantly different from each other. However, there was no significant difference (P>0.0) between rams on T1 and T2 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

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

<table>
<thead>
<tr>
<th>Day</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.09</td>
<td>0.18</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>0.12</td>
<td>0.21</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>0.00</td>
<td>0.12</td>
<td>0.27</td>
<td>0.33</td>
</tr>
<tr>
<td>Total</td>
<td>0.00</td>
<td>0.33</td>
<td>0.66</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**Table 2: Concentration of Pergonal administered on mature Yankasa Rams**

<table>
<thead>
<tr>
<th>Day</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>15.72</td>
<td>30.00</td>
<td>57.78</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>21.03</td>
<td>37.50</td>
<td>57.78</td>
</tr>
<tr>
<td>3</td>
<td>0.00</td>
<td>21.03</td>
<td>51.00</td>
<td>57.78</td>
</tr>
<tr>
<td>Total</td>
<td>0.00</td>
<td>57.78</td>
<td>118.5</td>
<td>173.34</td>
</tr>
</tbody>
</table>
Table 3: Effect of pergonal administration on haematology of yankasa rams

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1 (0.00)</th>
<th>T2 (57.78)</th>
<th>T3 (11850)</th>
<th>T4 (17334)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC (%)</td>
<td>40.45b</td>
<td>44.65b</td>
<td>49.35a</td>
<td>48.76a</td>
<td>0.62</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.30b</td>
<td>15.40b</td>
<td>16.35a</td>
<td>17.25a</td>
<td>0.63</td>
</tr>
<tr>
<td>WBC (x10^9/l)</td>
<td>9.33a</td>
<td>7.41b</td>
<td>7.56b</td>
<td>7.65b</td>
<td>0.45</td>
</tr>
<tr>
<td>RBC (x10^12/l)</td>
<td>4.85b</td>
<td>5.30b</td>
<td>5.54ab</td>
<td>5.65a</td>
<td>0.07</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>80.00b</td>
<td>85.35a</td>
<td>86.24b</td>
<td>87.15a</td>
<td>1.60</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>33.00b</td>
<td>33.00</td>
<td>33.00</td>
<td>33.00</td>
<td>0.00</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.30</td>
<td>34.34</td>
<td>34.30</td>
<td>34.30</td>
<td>0.00</td>
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</table>

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

The WBC values obtained in this study were within the normal range of 4-12(x10^9/L) reported for sheep (Radostits et al., 1997). This was an indication that Peronal injections were tolerated by the rams. White blood cell values obtained in this study were higher than the value of 7.38 (x10^9/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 suggest 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 Peronal administered increased. However, there was no significant difference (P>0.05) between rams on T4 and T3 in RBC values. The RBC values obtained in this study were lower than the normal range of 9.0 – 15.0 (x10^12/L) reported for sheep (Radostits et al., 1997) but higher than 2.33±0.18 – 3.17±0.09 (x10^12/L) reported in WAD sheep (Sowande et al., 2008).

Rams in all Peronal treated groupsrecorded significantly (P<0.05) higher MCV values than rams on the control treatment (T1). However, there were no significant differences (P>0.05) among rams on T2, T3 and T4 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 (Lazzaro2003).

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 MCHC 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 (Peronal®) are shown in Table 4.

Rams on T1 had the highest neutrophil value which differed significantly (P<0.05) from rams on T1, T2 and T4. There was no significant difference (P>0.05) between rams on T1 and T2 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(x10^9/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 T1 had the highest lymphocytes value which differed significantly (P<0.05) from rams on the Peronal 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 T2 and it differed significantly (P<0.05) from the counts of T1, T3 and T4. These values were within the normal range (1-10%) for sheep (Merck, 2010). This is an indication that the administration of Peronal did not trigger allergic reactions in the rams.

Monocyte counts showed that rams on T4 had the highest value which differed significantly (P<0.05) from rams on T1, T2 and T3. Rams on T1 and T3 were similar to each other but differed significantly from rams on T2. Monocyte values obtained in this study were within the normal range of 0 – 6% or 0 – 0.75(x10^3/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 – Nwiyi et al 2000) rather than Peronal. Basophils were not detected among the treatment groups. The result of serum metabolites of Yankasa rams treated with gonadotrophin (Peronal®) are shown in Table 5.

Rams on T1 had the highest urea value which differed significantly (P<0.05) from rams on T2, T3 and T4. However there was no significant difference (P>0.05) between rams on T3 and T4 and these differed significantly (P<0.05) from T2. The concentration of urea in the blood declined as the dosage of Peronal 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 T4 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; Odugwu et al 1999; Ahamefule et al., 2005).

Serum glucose concentration was highest in rams on T1 which differed significantly (P<0.05) from rams on T1, T2 and T4. 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

availability of nutrient to the animal (Esonu et al., 2001; Iheukwumere and Herbert, 2002).
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 T1, T2 and T3 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 T3 and T4. It is possible that higher doses of Pergonal injection increased metabolism and efficient utilization of nutrients. High cholesterol level in the serum suggests inadequate or insufficient protein and good quality of protein in the diet which supported basic metabolic and physiological activities. There was equally a decline in total protein in the diet which supported basic metabolic and physiological activities. There was equally a decline in total protein in the diet which supported basic metabolic and physiological activities.

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 IU 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.

REFERENCES


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment (Pergonal)(IU)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1 (0.00)</td>
<td>T2 (57.78)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>40.36b</td>
<td>40.34b</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>58.53a</td>
<td>54.46b</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.10b</td>
<td>1.45a</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.00f</td>
<td>3.85b</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

abcMeans within row having different superscript are significantly (P<0.05) different; SEM = Standard error of means

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

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


