EFFECT OF MENOGON® ON HAEMATOLOGICAL PARAMERERS AND BODY WEIGHT OF RABBIT BUCKS

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ABSTRACT

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Haematological traits and body weight of rabbit bucks treated with Menogon were studied. Twenty-four Crossbreed rabbit bucks weighing 1.3 – 1.6 kg at 15 – 17 weeks were randomized into four treatment Menogon doses of 0 I.U. (control), 7.5 I.U., 15.0 I.U., and 22.5 I.U in a completely randomized design for 56 days. Each treatment was replicated three times with two bucks per replicate. Samples of blood were collected weekly from the ear vein for haematology. Results of haematology showed that the packed cell volume (%) of 15.0 I.U (33.45 %) was significantly (P<0.05) higher than those of 22.5 I.U (29.33), 7.5 I.U (29.04) and control (28.67) groups. Significant (P<0.05) increase observed in lymphocyte count (%) was higher in the control (51.92) than in 7.5 I.U (49.34), 15.0 I.U (45.83), and 22.5 I.U (43.25) groups. Results of body weight showed higher significant (P<0.05) difference in the average weght gain (0.79 kg) and final weight gain (2.20 kg) of bucks at 22.5 I.U. dose of Menogon. These results revealed that Menogon significantly increased the weight of the rabbit bucks without any adverse effects since the haematological values were within normal range.

Keywords: Menogon, haematology, body weight, rabbit bucks.

INTRODUCTION

Gonadotrophin therapy remains an essential component in the routine management for enhancing reproductive capability of farm animals. This therapy was originally designed for use in female animals where it stimulates the ovaries to produce multiple follicles, thus making them more fertile (Borth et al., 1954). Lately, gonadotropin has been reported to be effective in improving semen quality of local cocks (Abu et al., 2006). Human Menopausal Gonadotropins (Menotropins) is a purified preparation of gonadotropins that are extracted from the urine of post-menopausal women (Van De Weijer et al., 2003), usually LH and FSH (Lunenfeld, 2004). Menotropin is an active substance for the treatment of fertility disturbances achieved by triggering FSH and LH production in the body (Liu et al., 2008). However, there is a concern that hormones used repeatedly in animal production endanger the health of animals and man.

Haematological values are widely used to determine systemic relationship and physiological / pathological adaptations including the evaluation of general health condition, diagnosis and prognosis of various types of animals’ physiological disorder (Muhammed et al., 2000; Shah et al., 2007, Njidda and Isidahomen, 2011). Thus, Glawisching et al. (1977) postulated that normal values of blood must be used in the diagnostic laboratory as “working values”. Although studies have been conducted on the haematological parameters of rabbits, like many other livestock (Fudge, 2000; Hein and Hartmann, 2003; Chineke et al., 2006; Amata, 2010; Njidda and Isidahomen, 2011), to the best of the authors knowledge, there is no information on the effect of human menopausal gonadotropin on such values in rabbits. Therefore, this study was carried out to evaluate the effect of Menotropin (Menogen) on the haematological and body weight parameters of rabbit bucks.

MATERIALS AND METHODS

Location of study: The experiment was carried out at the Rabbity Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike. Umudike is situated in Ikwunor, Abia State in South Eastern Nigeria in the west of Africa. Its geographical coordinates are 5° 28’ North, 7° 33’ East and at an altitude of 112m above sea level in the tropical rainforest zone. Umudike has an average rainfall of about 2177mm per annum with relative humidity of about 72% and a monthly temperature range of 22 °C to 36 °C (N.R.C.R.I, 2010).

Experimental materials and management

A total of 24 Crossbred rabbit bucks aged 15-17 weeks weighing 1.3 - 1.6 kg were used in this study. The rabbits were managed intensively. They were quarantined for 3 weeks during which they were treated with Ivomec® injection for the control of haemoparasite, internal and external parasites. The bucks were individually kept in cages (50x55x40 cm) of a three tier hutch and each cage was provided with a feeder and a drinker. The experimental animals were given ad libitum access to water. Commercial diet (15 % crude protein and 2500 kcal
kg\(^{-1}\) metabolizable energy) was supplied in the morning and supplemented with Tridax procumbens, Centrosema pubescens, Calopogonium mucunoides and Panicum maximum in the evening. Ambient temperature (°C) and relative humidity (%) inside the rabbit building were measured daily throughout the experimental period between 0900 and 1100h using mercury thermometer (to the nearest 0.1 °C) and wet and dry bulb hygrometer (to the nearest 1%). The ambient temperature and relative humidity were averagely recorded as 24 °C and 89 %, respectively. Menogon\(^\text{®}\) bearing batch No: CE0310B was purchased from a Pharmacy. A pack of Menogon\(^\text{®}\) contained 5 ampoules of dry substance and an accompanying 5 ampoules of diluents. One ampoule of Menogon\(^\text{®}\) contains menotrophin (human menopausal gonadotrophim [HMG]) corresponding to 75 IU. FSH and 75 IU. LH. One ampoule of diluents contained isotonic sodium chloride solution. Packs of Menogon\(^\text{®}\) used in the study were stored in the refrigerator (below 25 °C) and protected from light.

**Experimental design**

The rabbit bucks were randomly assigned to four treatment groups. Each treatment was replicated three times with two bucks constituting a replicate in a Completely Randomized Design (CRD). The treatment groups were as follows:

- **Group A:** This served as the control (No Menogon\(^\text{®}\) treatment).
- **Group B:** 0.1 ml of Menogon\(^\text{®}\) (equivalent to 7.5 IU of FSH and LH) was administered to each rabbit buck.
- **Group C:** 0.2 ml of Menogon\(^\text{®}\) (equivalent to 15.0 IU of FSH and LH) was administered to each rabbit buck.
- **Group D:** 0.3 ml of Menogon\(^\text{®}\) (equivalent to 22.5 IU of FSH and LH) was administered to each rabbit buck.

A vial containing 75 IU FSH and 75 IU LH was reconstituted in 1ml of physiological saline solution and injected intramuscularly. Thus, different doses of Menogon\(^\text{®}\) were administered after every 72 hours for 56 days. Any unused reconstituted material was discarded.

**Blood sample collection**

Samples of blood were collected weekly from the bucks throughout the 56 days following Menogon\(^\text{®}\) administration. Blood samples were collected from the ear vein between 8:00 to 10:00 hours using a 2ml sterile syringe to aspirate 2 ml of blood from each rabbit buck. Aspirated samples were emptied into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles for haematological evaluation. The blood samples were transferred to the laboratory where they were analysed for red blood cells, white blood cells, pack cell volume, haemoglobin, mean cell volume and mean cell haemoglobin concentration (as described by Jain (1986), within 12 hours of collection.

**Estimation of blood parameters**

Packed cell volume (PCV) was determined by the micro-haematocrit method as described by Igene and Oboh (2004). Haemoglobin (Hb) was determined using cyanomethaemoglobin method according to Coles (1986). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using appropriate formulae (Jain, 1986) as follows:

\[
\begin{align*}
\text{MCV} & = \frac{\text{Haematocrit} \times 10 \times 10^3}{\text{RBCs in Millions}} \text{ fl (Femtoliter)} \\
\text{MCH} & = \frac{\text{Haemoglobin (g/dl)} \times 10 \times 10^3}{\text{RBCs in Millions}} \text{ pg} \\
\text{MCHC} & = \frac{\text{Haemoglobin (g/dl)} \times 100}{\text{Haematocrit} \times 100}\%
\end{align*}
\]

**Evaluation of body weight**

The weight of rabbit bucks were taken and recorded on a weekly basis using a weighing scale.

**Statistical analysis**

The data generated were analysed using Analysis of Variance (ANOVA). Significant means were separated using Duncan Multiple Range Test (DMRT). The statistical model for this experiment is:

\[
Y_{ij} = \mu + T_i + e_{ij}
\]

Where:

- \(Y_{ij}\) = Individual observation
- \(\mu\) = Overall mean
- \(T_i\) = Effect of treatment
- \(e_{ij}\) = Error term

All statistical analyses were in accordance with Steel and Torrie (1980).

**RESULTS AND DISCUSSION**

Haematology
The results on the effect of Menogon administration on the haematology of rabbit bucks are shown in Table 1. The RBC counts observed in this study did not show any significant (P>0.05) differences among the treatments. This result agrees with the report of the hekuwuemere et al. (2006). These authors recorded no significant (P>0.05) differences when varying doses of gonadotropin were administered to birds. However, the numerical increase in RBC values among the Menogon treated groups (15.0 I.U. and 22.5 I.U.) tended to confirm the assertion that when normal quantities of testosterone are injected into a castrated adult, the number of RBC per cubic millimeter of blood increases production (Guyton and Hall, 1996). This may be due partly to the increased metabolic rate after Menogon (gonadotrophin) administration which triggered the production of testosterone rather than to a direct effect of Menogon on the red blood cells. The RBC values in this study were in agreement with the mean RBC value of 3.48 ± 0.15×10⁶/mm³ reported by Chineke et al. (2006) but lower than the mean value of 6.0 ± 0.12×10⁶/mm³ and 6.40 ± 0.07×10⁶/mm³ reported by Amata (2010) and Njidda and Isidahomen (2011), respectively. PCV is a measure of the proportion of blood volume that is occupied by RBC. The PCV values of 28.67 to 33.45 % observed in this study fall within the mean value of 28.43 ± 1.08 % reported by Chineke et al. (2006) and the range of 30-50% and 33-50 % reported by Fudge (2000) and Hillyer (1994), respectively. However, the mean PCV value of rabbit bucks on 15.0 I.U dose of Menogon was significantly (P<0.05) higher among the treatment groups.

Table 1: Mean values of the haematological characteristics of rabbit bucks treated with Menogon

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>0.0 I.U</th>
<th>7.5 I.U</th>
<th>15.0 I.U</th>
<th>22.5 I.U</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10⁶/mm³)</td>
<td>3.14</td>
<td>3.03</td>
<td>3.76</td>
<td>3.24</td>
<td>0.29</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>28.67b</td>
<td>29.04b</td>
<td>33.45a</td>
<td>29.33b</td>
<td>1.00</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>10.82</td>
<td>10.80</td>
<td>11.53</td>
<td>11.53</td>
<td>0.68</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>91.80</td>
<td>96.03</td>
<td>94.10</td>
<td>92.40</td>
<td>5.13</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>34.49</td>
<td>36.47</td>
<td>32.59</td>
<td>36.06</td>
<td>2.48</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>37.46</td>
<td>37.50</td>
<td>34.69</td>
<td>39.20</td>
<td>1.86</td>
</tr>
<tr>
<td>WBC (×10³/mm³)</td>
<td>9.67</td>
<td>9.43</td>
<td>7.79</td>
<td>8.29</td>
<td>0.81</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>51.92a</td>
<td>49.34ab</td>
<td>45.83ab</td>
<td>43.25b</td>
<td>1.81</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>46.00</td>
<td>41.67</td>
<td>39.00</td>
<td>45.50</td>
<td>2.84</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>4.17</td>
<td>3.42</td>
<td>3.33</td>
<td>3.67</td>
<td>0.40</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>1.83</td>
<td>1.83</td>
<td>1.33</td>
<td>1.42</td>
<td>0.46</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>7.83</td>
<td>7.33</td>
<td>5.75</td>
<td>6.15</td>
<td>0.94</td>
</tr>
</tbody>
</table>

ab Means bearing different letters of superscript within the same row differ significantly (P<0.05)

RBC - Red Blood Cells; MCH - Mean Corpuscular Haemoglobin; PCV - Packed Cell Volume
MCHC - Mean Corpuscular Haemoglobin Concentration; HB - Haemoglobin; WBC - White Blood Cells
MCV - Mean Corpuscular Volume

Kopp and Hetesa (2000) reported that higher PCV reading is indicated by a corresponding increase in the number of circulating RBC value. Joel-Gibson (1993) also observed that a decrease in haematocrit is always seen with a decrease in the haemoglobin, thus the two values are linked to one another. Therefore, since haemoglobin is recognized as a carrier of oxygen in the blood and this is released into the tissues through the blood capillaries, the enhanced significant (P<0.05) differences in PCV across the Menogon treated groups indicates sufficient supply of oxygen to the body tissues of these animals resulting in increased metabolism which must have enhanced spermatogenesis and the overall physiological processes in the rabbit bucks.

Erythrocyte indices (MCV, MCH, and MCHC) are helpful constants in the initial classification of anaemia (Garby, 2002; Awodi et al., 2005) and also serve as a useful index of the capacity of bone marrow to produce RBCs (Awodi et al., 2005). The results of erythrocyte indices obtained in this study did not reveal any significant (P>0.05) differences among the treatment groups. Apart from the values of MCHC which were consistent with the reference value within the range of 34-37 % reported by Burke (1994), Gillet (1996) and Fudge (2000), the values of MCH and MCV as shown in Table 1 were inconsistent with the range of 18-24 pg and 50-75 fl respectively reported by Burke (1994), Gillet (1996) and Fudge (2000). Barger (2003) demonstrated that any change in MCV, MCH, and MCHC of rabbits above or below the normal range indicates macrocytic and hypochronic anaemia, probably due to the increased activity of bone marrow and deficiency of some haemopoietic factors influencing the capacity of bone marrow to produce red blood cell (Awodi et al., 2005). However, since MCHC is recognized as the most significant in the diagnosis of anaemia (Njidda and Habangda, 2006) and the values of PCV, RBC, WBC, Hb and MCH in all the treatments were within the normal ranges for healthy rabbits, the higher values of MCV and lower values of MCH observed in this study may not pose a serious problem. The result of the haematological study further demonstrated no significant (P>0.05) differences in the WBC counts. The mean values for leucocytes in all treatments were within the values of 3-12×10³/mm³ reported by Hein and Hartmann (2003). It was observed that the values of WBC counts numerically decreased with increased level of Menogon administration up to the rate of 15.0 I.U. which has the lowest mean value of 7.79×10³/mm³.
In the consideration of WBCs differentials, the mean value of lymphocytes for bucks on 22.5 I.U. dose was significantly (P<0.05) lower than those in the control group but not those in 7.5 and 15.0 I.U. dose which were not significant (P>0.05) from the control group. Also, the mean values obtained for basophil, monocyte, eosinophil and neutrophil were not significantly (P>0.05) different but followed a trend which numerically decreased with increased dose of Menogon up to the dose of 15.0 I.U.

Body weight
The result on body weight of rabbit bucks treated with different levels of Menogon is presented in Table 2. It could be observed from Table 2 that though the initial body weight (IBW) of the bucks were closely equalized among the treatments at the beginning of this study, the final body weight (FBW) and weight gain of the bucks were significantly (P < 0.05) different. There are possible explanations for the significant (P < 0.05) differences in body weight observed in these findings.

First, it could be assessed from the point of increased physiological activity after the administration of Menogon which had a direct effect on the stimulation of the Leydig cells of the testis to produce testosterone. Perhaps the most contributory function of testosterone in weight increment is its anabolic effect and it is known to increase the rate of metabolism. This increased rate of metabolism is possibly an indirect result of the effect of testosterone on protein metabolism (Hansel and McEntee, 1977; Guyton and Hall, 1996). Kochakian and Tillotson (1957) carried out an experiment where they observed that the muscles of the head, neck, shoulder, back and abdominal wall of castrated guinea pigs are stimulated by testosterone administration out of proportion to the increase in body weight. Testosterone increases the synthesis of protein and decreases the rate at which amino acids are broken down, thus resulting in the general increase in muscle mass. Additionally, Guyton and Hall (1996) and Brackett (2004) reported that after prolonged injection of testosterone the bones grow considerably in thickness and deposit considerable additional calcium salts. Thus, testosterone increases the total quantity of bone matrix and causes calcium retention. The increase in bone matrix is believed to result from the general protein anabolic function of testosterone and the deposition of calcium salts to result secondarily to the increased bone matrix. Hansel and McEntee (1977) reported that gonadotrophin can stimulate glycogen deposition in skeletal muscles and consequently increasing the body mass of an animal.

Table 2: Body weight of rabbit bucks treated with different levels of Menogon®

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0.0 I.U</th>
<th>7.5 I.U</th>
<th>15.0 I.U</th>
<th>22.5 I.U</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight (kg)</td>
<td>1.43</td>
<td>1.44</td>
<td>1.44</td>
<td>1.42</td>
<td>0.04</td>
</tr>
<tr>
<td>Final Weight (kg)</td>
<td>1.77bc</td>
<td>1.82bc</td>
<td>2.00bc</td>
<td>2.20a</td>
<td>0.06</td>
</tr>
<tr>
<td>Weight Gain (kg)</td>
<td>0.33c</td>
<td>0.38c</td>
<td>0.56c</td>
<td>0.79c</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Means bearing different letters of superscript within the same row differ significantly (P < 0.05)

Another possible explanation could be as a result of increased plasma ghrelin which may have been stimulated by Menogon injection. This was reflected in the increased quantity of feed consumed by the bucks that received higher doses of Menogon (15.0 I.U. and 22.5 I.U). Greenman et al. (2009) showed that there was a positive correlation between ghrelin and total testosterone (r = 0.5, P < 0.039) and bioavailable testosterone (r = 0.719, P < 0.001) in male subjects. Ghrelin is however involved in the regulation of growth hormone secretion and appetite control (Casanueva and Dieguez, 2004)

CONCLUSION
The results of this study revealed that human menopausal gonadotropin (Menogon®) had no adverse effect on haematological parameters and significantly increased weight gain of rabbit bucks. This suggested that Menogon® may be promising in enhancing productivity in rabbit bucks without any physiological deleterious effect. However, the determination of the effect of gonadotropin administration above the dose used in this study and some toxicological and safety studies can be conducted.

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