HAEMATOLOGICAL PROFILE AND BODY WEIGHT OF RABBIT BUCKS ADMINISTERED HUMAN MENOPAUSAL GONADOTROPIN (MENOGON)

ANSA, Anietie Archibong

Department of Animal Science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
Email: aa.ansa@unizik.edu.ng, **Phone:** +234 813 068 7781

ABSTRACT

The study investigated the effect of Menogon treatment on haematological parameters and body weight of male rabbits. Twenty-four bucks of Chinchilla × Dutch breed, weighing between 1.3 – 1.6 kg and aged 15 – 17 weeks, were divided into four groups receiving different doses of Menogon (0.0 IU as control, 7.5, 15.0, and 22.5 IU) for 56 days in a completely randomised design. Each dose group was replicated three times with two bucks per replicate. Blood samples were collected weekly from the ear vein for haematological analysis. The results showed that the packed cell volume was significantly (p<0.05) higher in the group receiving 15.0 IU (33.45 ± 1.00%) compared to the 22.5 IU (29.33 ± 1.00%), 7.5 IU (29.04 ± 1.00%), and control (28.67 ± 1.00%) groups. Additionally, there was a significant (p<0.05) increase in lymphocyte count in the control group (51.92 ± 1.81%) compared to the 7.5 IU (49.34 ± 1.81%), 15.0 IU (45.83 ± 1.81%), and 22.5 IU (43.25 ± 1.81%) groups. Regarding body weight, there was a significantly (p<0.05) higher average weight gain (0.79 ± 0.03 kg) and final weight gain (2.20 ± 0.03 kg) observed in bucks treated with 22.5 IU of Menogon. These findings indicated that Menogon administration led to a significant increase in rabbit bucks' weight without adverse effects, as haematological parameters remained within normal ranges.

Keywords: Menogon, Blood, Growth performance, Male rabbits

INTRODUCTION

Gonadotrophin therapy remains crucial for optimizing the reproductive capacity of farm animals. Although, originally intended for use in female animals to stimulate ovarian follicle production and increase fertility (Schneider *et al.*, 2006; Leão and Esteves, 2014), gonadotropin has been reported for its effectiveness in improving the semen quality of Nigerian indigenous chickens (Abu *et al.*, 2006).

Human menopausal gonadotropins (hMG), derived from the urine of post-menopausal women (Van De Weijer *et al.*, 2003), primarily consisting of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Lunenfeld, 2004; Liu *et al.*, 2008), are employed to treat fertility issues by stimulating the production of these hormones in the body. However, there are concerns about the repeated use of such hormones in animal husbandry and its potential impact on animal and human health.

The assessment of haematological values serves as a fundamental indicator of health status, reflecting alterations in blood cell counts, haemoglobin levels, and other essential parameters (Shah *et al.*, 2007; Njidda and Isidahomen, 2011). Changes in these parameters can signify physiological adaptations or potential adverse effects induced by exogenous hormone administration. Similarly, alterations in body weight serve as a tangible measure of metabolic changes and overall well-being. Despite the extensive use of hMG in animal physiology, limited research has

specifically investigated its impact on the haematological parameters and body weight of animals, leaving a significant gap in our understanding of the physiological responses of male animals to hMG treatment. Therefore, this study aims to address this gap by comprehensively examining the haematological profile and body weight of rabbit bucks following hMG administration.

By elucidating the effects of hMG treatment on haematological parameters and body weight of male rabbits, this research contributes to our understanding of the potential physiological consequences of exogenous hormone exposure to rabbits. Furthermore, the findings of this study may have implications for the use of hMG in reproductive management programs, and contribute to the welfare, and health assessment protocols of male rabbits undergoing hormone therapy.

MATERIALS AND METHODS

Location of Study: The experiment was conducted in the Rabbitry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, between October 27, 2023 – January 12, 2024.

Ethics: This experiment was approved and conducted according to the provisions of the Animal Research Ethics Committee on the use of animals for biomedical research at the Nnamdi Azikiwe University, Awka, Nigeria.

Experimental Materials and Management: This investigation used 24 matured male rabbits, a hybrid of Chinchilla and Dutch breeds, aged between 15 and 17 weeks, weighing between 1.3 and 1.6 kg. They were managed intensively in a three-tier hutch and quarantined for 21 days. During this period, the rabbits were closely monitored for signs of physiological aberration. They had free access to feed and water, receiving a standard diet (Top Feed grower) containing 15.1% crude protein and 2663.3 kcal/kg metabolizable energy in the morning and free access to a combination of forage (*Tridax procumbens, Centrosema pubescens, Calopogonium mucunoides,* and *Panicum maximum*) in the evening.

Environmental conditions within the rabbit enclosure were consistently measured using a digital thermometer for temperature and a hygrometer for humidity. The average temperature was maintained at 24.0 ± 6.0°C and the humidity at 89.0 ± 11.0%. The Menogon (batch number CE0310B) used in the study was sourced from Ferring Pharmaceuticals, Saint-Prex, Switzerland. Each Menogon package contained five ampoules of menotrophin (75 IU FSH and 75 IU LH) and five ampoules of isotonic sodium chloride solution as a diluent. All Menogon packages for the study were stored in the refrigerator and away from light.

Experimental Design: The rabbit bucks were divided into four treatment groups in a completely randomized design (CRD). The groups received the following treatments: Group A, Control group without Menogon treatment; Group B, received 0.1 ml of Menogon, equivalent to 7.5 IU of FSH and LH, per rabbit buck; Group C, received 0.2 ml of Menogon, equivalent to 15.0 IU of FSH and LH, per rabbit buck; and Group D, received 0.3 ml of Menogon, equivalent to 22.5 IU of FSH and LH, per rabbit buck. These treatments were administered intramuscularly every 72 hours for 56 days.

Blood Sample Collection and Evaluation: Blood samples were obtained weekly from the male rabbits for 56 days after they were given Menogon. The samples were taken from the ear vein of each rabbit between 0800 and 1300 hours using a 2 ml sterile syringe. These samples were then placed into Ethylenediaminetetraacetic acid (EDTA) bottles for haematological assessment. Subsequently, the blood samples were analyzed for various parameters including red blood cell (RBC) count, white blood cell (WBC) count, haematocrit or packed cell volume (PVC), haemoglobin concentration (Hb), erythrocyte indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] and WBC differential counts (basophils,

monocytes, eosinophils, and neutrophils) according to the procedure outlined by Jain (1986). Analysis was conducted within 12 hours of sample collection.

Evaluation of Body Weight: The weights (g) of rabbit bucks were taken and recorded every week using a weighing scale.

Statistical Analysis: The data generated were analyzed using Analysis of Variance (ANOVA). Significant means were separated using the Duncan Multiple Range Test (DMRT). Significantly different means were accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Haematology: The results of the effect of Menogon administration on the haematology of rabbit bucks indicated that the RBC counts did not show any significant differences ($p > 0.05$) among the treatments (Table 1). This result was in agreement with the report of Iheukwuemere *et al.* (2006) who reported no significant differences ($p > 0.05$) when varying doses of gonadotropin were administered to birds. However, the numerical increase in RBC values among the Menogon-treated groups (15.0 IU and 22.5 IU) tended to confirm the assertion that when normal quantities of testosterone are injected into a castrated adult, the number of RBC per cubic millimetre of blood increases (Bachman *et al.,* 2014). This may be due partly to the increased metabolic rate after Menogon administration which triggered the production of testosterone rather than to a direct effect of Menogon on the RBCs. The RBC values in this study were in agreement with the mean RBC value of $3.48 \pm 0.15 \times 10^6/mm^3$ reported by Chineke *et al.* (2006), but lower than the mean value of $6.0 \pm 0.12 \times 10^6/mm^3$ and $6.40 \pm 0.07 \times 10^6/mm^3$ 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 $28.43 \pm 1.08\%$ reported by Chineke *et al.* (2006) and the range of 30 – 50% reported by Fudge (2000).

However, the mean PCV value of rabbit bucks on a 15.0 IU dose of Menogon was significantly higher ($p < 0.05$) than for the other treatment groups. Kopp and Heteša (2000) and Mondal and Lotfollahzadeh (2023) reported that when the PCV is high, there tends to be a corresponding rise in the number of circulating RBCs in the blood. Similarly, Kishimoto (2020) observed that a reduction in haematocrit typically accompanies a decrease in haemoglobin levels, indicating a close relationship between these two measures. Since haemoglobin is responsible for transporting oxygen in the bloodstream, which is then delivered to tissues through capillaries, the higher significant differences ($p < 0.05$) in PCV among the groups treated with Menogon suggest an ample supply of oxygen to the tissues of these animals. This likely led to increased metabolism, which could enhance spermatogenesis and other physiological processes in the male rabbits.

Furthermore, parameters such as MCV, MCH, and MCHC are valuable indicators of anaemia (Awodi *et al.*, 2005). They also provide insight into the bone marrow's ability to produce red blood cells (Awodi *et al.*, 2005). The results of erythrocyte indices (MCV, MCH and MCHC) obtained in this study did not reveal any significant differences ($p > 0.05$) 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 Fudge (2000) and Varga (2014), the values of MCV and MCH (Table 1) were inconsistent with the range of 50 – 75 fl and 18 – 24 pg respectively reported by Fudge (2000) and Varga (2014). Barger (2003) demonstrated that any deviation from the normal levels of MCV, MCH, and MCHC in rabbits suggests the presence of macrocytic and hypochromic anaemia, likely due to increased bone marrow activity and a deficiency in certain haematopoietic factors affecting red blood cell production (Awodi *et al.*, 2005). However, since MCHC is considered the most crucial parameter in anaemia diagnosis (Njidda and Hambagda, 2006), and since the levels of PCV, RBC, WBC, Hb, and MCH in all treatments fell within the healthy range for rabbits, the slightly elevated MCV and decreased MCH values observed in this study may not pose any significant treat.

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

Parameters	0.0 IU	7.5 IU	15.0 IU	22.5 IU
RBC ($\times 10^6$/mm^3)	3.14 ± 0.29	3.03 ± 0.29	3.76 ± 0.29	3.24 ± 0.29
PCV (%)	28.67 ± 1.00[a]	29.04 ± 1.00[a]	33.45 ± 1.0[b]	29.33 ± 1.00[a]
Hb (g/dl)	10.82 ± 0.68	10.80 ± 0.68	11.53 ± 0.68	11.53 ± 0.68
MCV (fl)	91.80 ± 5.13	96.03 ± 5.13	94.10 ± 5.13	92.40 ± 5.13
MCH (pg)	34.49 ± 2.48	36.47 ± 2.48	32.59 ± 2.48	36.06 ± 2.48
MCHC (%)	37.46 ± 1.86	37.50 ± 1.86	34.69 ± 1.86	39.20 ± 1.86
WBC ($\times 10^3$/mm^3)	9.67 ± 0.81	9.43 ± 0.81	7.79 ± 0.81	8.29 ± 0.81
Lymphocytes (%)	51.92 ± 1.81[b]	49.34 ± 1.81[ab]	45.83 ± 1.81[ab]	43.25 ± 1.81[a]
Neutrophil (%)	46.00 ± 2.84	41.67 ± 2.84	39.00 ± 2.84	45.50 ± 2.84
Eosinophil (%	4.17 ± 0.40	3.42 ± 0.40	3.33 ± 0.40	3.67 ± 0.40
Basophil (%)	1.83 ± 0.46	1.83 ± 0.46	1.33 ± 0.46	1.42 ± 0.46
Monocyte (%)	7.83 ± 0.94	7.33 ± 0.94	5.75 ± 0.94	6.15 ± 0.94

[a,b]*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, and MCV = Mean Corpuscular Volume*

The result of the haematological study further demonstrated no significant differences (p>0.05) in the WBC count. The mean values for leucocytes in all treatments were within the values of 3 – 12 ×10^3/mm^3 reported by EBMCONSULT (2024). It was observed that the values of WBC count numerically decreased with increased levels of Menogon administration up to the rate of 15.0 IU which has the lowest mean value of 7.79 ×10^3/mm^3.

In the context of WBC differentials, the average lymphocyte counts in bucks receiving a 22.5 IU dose was significantly lower (p<0.05) when compared to those in the control group. However, there was no significant difference (p>0.05) observed between the control group and those receiving 7.5 and 15.0 IU doses. Similarly, the mean values for basophils, monocytes, eosinophils, and neutrophils did not show significant differences (p>0.05), but they demonstrated a trend of decreasing values with higher doses of Menogon, up to 15.0 IU. In all these, the mean values of WBC differentials across all treatment groups fell within the reference range for healthy male rabbits, as reported by Moore *et al.* (2015).

Body Weight: The result of the body weight of rabbit bucks treated with different levels of Menogon presented in Table 2 revealed that although the initial body weight (IBW) of the bucks was similar across all treatments at the start of the study, there were significant differences (p<0.05) in the final body weight (FBW) and weight gain.

One explanation for the observed weight differences may be attributed to the increased physiological activity following Menogon administration, directly stimulating the Leydig cells of the testis to produce testosterone (O'Donnell *et al.*, 2017). Testosterone's anabolic effect likely contributes to weight gain by enhancing metabolism, as it promotes protein synthesis and reduces the breakdown of amino acids (Wang *et al.*, 2012). Tang *et al.* (2009) carried out an experiment where they observed that the muscles of the head, neck, shoulder, back and abdominal wall of castrated guinea pigs were stimulated by testosterone administration out of proportion to the increase in body weight. Additionally, Brackett (2004) and Bachman *et al.* (2014) 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 in the increased bone matrix.

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

Parameters	0.0 IU	7.5 IU	15.0 IU	22.5 IU
Initial Weight (kg)	1.43 ± 0.04	1.44 ± 0.04	1.44 ± 0.04	1.42 ± 0.04
Final Weight (kg)	1.77 ± 0.06[a]	1.82 ± 0.06[b]	2.00 ± 0.06[bc]	2.20 ± 0.06[c]
Weight Gain (kg)	0.33 ± 0.03[a]	0.38 ± 0.03[a]	0.56 ± 0.03[b]	0.79 ± 0.03[c]

[a,b,c] *Means bearing different letters of superscript within the same row differ significantly (p<0.05)*

Jensen *et al.* (2011) reported that gonadotrophin can stimulate glycogen deposition in skeletal muscles and consequently increase the body mass of an animal.

Another potential explanation may be a result of a possible increase in plasma ghrelin which may have been stimulated by Menogon, resulting in increased feed intake by the bucks receiving higher doses (15.0 and 22.5 IU) of Menogon. Research by Greenman *et al.* (2009) reported a positive correlation between ghrelin levels and testosterone, indicating a possible link between ghrelin, testosterone, and increased feed consumption. Ghrelin, known for its role in regulating growth hormone secretion and appetite, may influence weight gain through its effects on appetite control, as highlighted by Pradhan *et al.* (2013) and Khatib *et al.* (2014).

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 causing any harmful physiological effects. However, the effect of gonadotropin above the dose used in this study, as well as conducting toxicological and safety assessments needs to be investigated.

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