

## Effects of hesperidin on the oestrous cycle of Yankasa ewes

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### ABSTRACT

The study was conducted to investigate the effect of hesperidin on the oestrous cycle of Yankasa ewes. Twenty-six Yankasa ewes (26 ewes) aged 8 – 18 months were randomly assigned into treatment (n = 16) and control (n = 10) groups. The treatment group was given hesperidin at a dose rate of 200 mg/kg BW, once weekly for 4 weeks, while the control group received - 10 mL of a mix of DMSO and normal saline. The signs of oestrus were observed twice daily between 6.00 – 11.00 a.m. and 2.00 – 6.00 p.m. The time of onset of oestrus (TOO), oestrus response rate (ORR), and oestrus duration (OD) were determined for 3 oestrous cycles. The result showed that the TOO of the hesperidin-fed group was significantly ( $p \leq 0.05$ ) lower than those of the control group in the 2<sup>nd</sup> and 3<sup>rd</sup> oestrous cycles. ORR and OD were significantly ( $p \leq 0.05$ ) higher in the hesperidin-fed group compared to the control in the 2<sup>nd</sup> and 3<sup>rd</sup> oestrous cycles. The study indicates that hesperidin administration improved the TOO, ORR and OD in Yankasa ewes.

Keywords: Hesperidin, oestrus response rate, oestrus duration, onset of oestrus, Yankasa ewes.

### INTRODUCTION

Sheep contributes enormously to the protein requirement of most developing countries (Mandal *et al.*, 2007). In Sub-Saharan Africa, sheep provide almost 30% of the meat and 16% of milk consumed by most Nigerians (Awobajo, 2018). Sheep play a significant role in food security and the socio-cultural and economic development of Nigerians, especially among rural populations (Ajala *et al.*, 2008). They are used as cash savings by small farmers and are slaughtered for meat during ceremonies and festivals (Umaru *et al.*, 2009).

The oestrous cycle is an important phase in the reproductive cycle of female animals. It is a recurring physiological phenomenon induced by reproductive hormones, with progesterone and estrogen as principal players. The cycle is divided into two phases; the follicular phase (proestrus and oestrus stages) and the luteal phase (metoestrus and diestrus). However, the oestrous cycle in Yankasa ewes is constrained by several factors such as extreme environmental conditions (thermal stress, solar irradiation, wind speed, and relative humidity), nutritional deficiency, water scarcity, disease burdens, poor management practices and others (da Silva *et al.*, 2021).

Thermal stress is known to cause oxidative stress through the generation of free radical species or reactive oxygen species (ROS) resulting in oxidant/antioxidant imbalance, thereby resulting in poor reproductive performance in ewes (Celi & Gabai, 2015; Sa'ayinzat *et al.*, 2021). It impacts negatively on male and female reproductive capacities, resulting to overall poor reproductive performance (Boudjellaba *et al.*, 2018). Studies on the influence of climatic seasons on the oestrous cycle revealed a significant reduction in the reproductive performance of ewes (Igono *et al.*, 1982) and gilts (Dimitrov, 2014).

Hesperidin is an exogenous phytochemical antioxidant obtained from citrus and lemon fruit peels (Pyrzynska, 2022). It is a flavanone glucoside that belongs to a group of bioflavonoids containing phenolic moieties in their structures (Jadeja and Devkar, 2014). Hesperidin has been reported to have several biological and therapeutic properties such as ROS scavenging (Arafa *et al.*, 2009; Suarez *et al.*, 2011), antimicrobial (Iranshahi *et al.*, 2015), antiviral (Saha *et al.*, 2009), and anti-inflammatory properties (Kuntic *et al.*, 2014; Rotimi *et al.*, 2016). In addition, hesperidin has been reported to improve sperm production and function in males

(Khaki *et al.*, 2011) and also improve maternal and foetal outcomes in females (Agarwal *et al.*, 2005; Agarwal *et al.*, 2006a; Agarwal *et al.*, 2006b). Its ameliorative effects through scavenging of ROS and protection against microbes may result in better conception rates, reduced embryonic mortality and foetal deaths in ewes (Zhong and Zhou, 2013; Abuelsaad *et al.*, 2014; Miller *et al.*, 2014; Iranshahi *et al.*, 2015). This study aimed to determine the effects of hesperidin on the oestrous cycle parameters in Yankasa ewes.

## MATERIALS AND METHODS

### STUDY AREA

The experiment was carried out at the Livestock Investigation Department, National Veterinary Research Institute, Vom, Plateau State, Nigeria. It is situated between latitude 9°44' N and longitude 8°47' E at 1,285 m above sea level with a mean relative humidity of 55 % (Ntem, 2015). Ethical approval was obtained from the Animal Use and Care Committee of the National Veterinary Research Institute, Vom, Plateau State, Nigeria (Approval Number AEC/02/36/17).

### EXPERIMENTAL ANIMALS

A total of 29 healthy Yankasa sheep comprising 26 ewes and 3 rams were used for the study. The animals were purchased from *Tilde Fulani* village, about 31 miles from the study area. The ewes were 8-18 months and weighed between 18 – 22 kg, while the rams were males with proven reproductive history aged between 18 – 24 months old with an average weight of 37.7 kg. The sheep were stocked at one sheep/m<sup>2</sup> and housed in well-ventilated pens under proper hygienic conditions. They were given a daily ration of 24 kg (1.00 kg/animal) containing 2500 mKcal and 15% crude protein concentrates and a mix of silage and hay, while water was provided *ad libitum*. Premixes containing vitamins and minerals were excluded from the ration to ensure they do not influence the outcomes of the study. The animals were pre-conditioned for four weeks before the commencement of the experiment. During this period, they were screened for parasites and those infected were treated accordingly.

### SOURCE AND CONSTITUTION OF HESPERIDIN

Three kilograms of high-grade commercial hesperidin powder (98.5% purity) was purchased from ANIMED, Connecticut, USA, and was used for the experiment. The powder was dissolved in 30 mL dimethyl sulphoxide (DMSO) (99.5%). The solution obtained was diluted five times with normal saline to reduce the concentration of DMSO and obtained a final concentration of 200 mg/ml. The prepared solution was administered at a dose rate of 200 mg/kg body weight *per os* to 16 ewes in the treatment group every 7 days, while the 10 ewes in the control group received

a mix of 10 mL of DMSO and normal saline without hesperidin throughout the studies. The weekly dosage of hesperidin used in the current study was based on literature reports and studies conductive on the therapeutic use of hesperidin and studies on the lethal dose of hesperidin in various animals (Australian Government CMEC 44, 2004; Bhalekar *et al.*, 2016; Jadeja & Davekar, 2014; Zanwar *et al.*, 2014).

### EXPERIMENTAL DESIGN

The ewes were synchronized using an intravaginally controlled internal drug release (CIDR) insert (CIDR, EAZI-BREED<sup>TM</sup>, New Zealand) for 11 days placed intravaginally. After the withdrawal of CIDR on the 11th day, the ewes were given intramuscularly 10 mg dinoprost tromethamine, IM (Prostaglandin F2 $\alpha$ ) to facilitate luteolysis. At the end of the first oestrous cycle, the animals were randomly divided into two groups comprising the control group (n=10) and the treatment group (n=16). One ram was placed in the controlled group while the remaining 2 were placed in the hesperidin-treated group. Each ewe in the hesperidin-treated group received 200 mg/kg body weight of hesperidin by drenching every 7 days for 4 weeks. The overt signs of oestrus were monitored twice daily between 6.00 – 11.00 a.m. and 2.00 – 6.00 p.m. during the study using aproned rams. Standing to be mounted by other ewes (homosexual mounting) and standing to be mounted by the rams (heterosexual mounting) were the primary and sole criteria for judging the evidence of oestrus in the Yankasa ewes. At the end of three oestrous cycles, the time of onset of oestrus (TOO), oestrus response rate (ORR), and oestrus duration (OD) were determined. The time of onset of oestrus (TOO) was defined as the time interval (in days) when the ewe first stood to be mounted by the ram after withdrawal of the CIDR and expressed in percentages. Oestrus response rate (ORR) was considered as the number of ewes showing standing oestrus (heat) in a group divided by the number of ewes in that group and expressed as mean  $\pm$  SEM. The end of estrus was considered as the time when the ewe refused to be mounted by the aproned ram. Oestrus duration (OD) was defined as the interval between the onset and end of oestrus (heat) and expressed as mean  $\pm$  SEM.

### DATA ANALYSIS

Data generated from the study were subjected to an independent t-test using the statistical analysis System (SAS, Institute Inc., Release 8.2, Cary, NC, USA, 2013), and presented in bar charts. Values of  $p \leq 0.05$  were considered significant.

### RESULTS

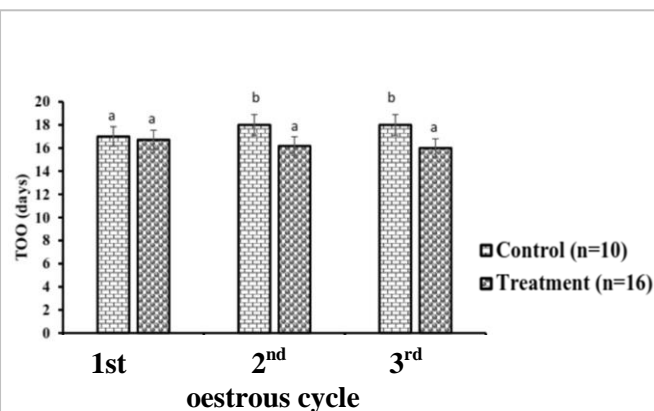
The effects of hesperidin on the time of onset of oestrous (TOO) are presented in Figure I. The TOO was not significantly ( $p > 0.05$  days) different between the control

group ( $17.00 \pm 0.69$  days) and the hesperidin-fed group ( $16.71 \pm 2.98$  days) in the first oestrous cycle. However, the TOO of the hesperidin-fed groups in the second ( $16.17 \pm 3.13$  days) and third ( $16.00 \pm 3.3$  days) oestrous cycles were significantly ( $p \leq 0.05$ ) lower than the control groups ( $2^{\text{nd}}$  -  $18.00 \pm 2.83$  days;  $3^{\text{rd}}$  -  $18.00 \pm 1.41$  days). The result of the effect of hesperidin on the oestrous response rate (ORR) is shown in Figure II. There was no significant ( $p > 0.05$ ) difference in ORR during the first oestrous cycle between the hesperidin-fed group ( $70.00 \pm 19.00$ ) and the control group ( $66.50 \pm 17.00$ ). In the second and third oestrous cycles, there was a significant ( $p \leq 0.05$ ) increase in ORR between the hesperidin-fed ( $2^{\text{nd}}$  -  $87.50 \pm 10.00$ ;  $3^{\text{rd}}$  -  $75.00 \pm 6.00$ ) and the control ( $2^{\text{nd}}$  -  $80.00 \pm 8.00$ ;  $3^{\text{rd}}$  -  $67.00 \pm 6.00$ ) groups. The effect of hesperidin on oestrus duration (OD) is

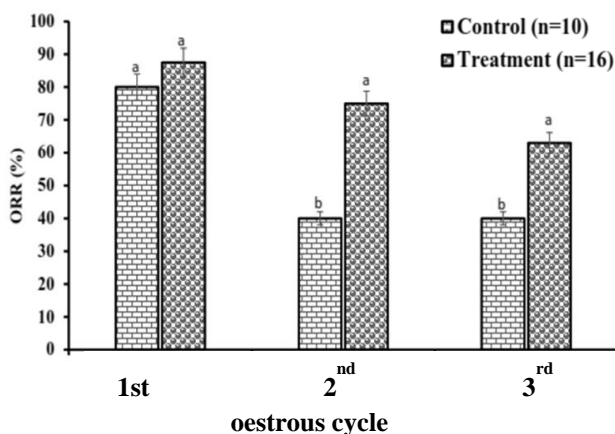
presented in Figure III. In the first oestrous cycle, there was no significant ( $p > 0.05$ ) difference in OD between the hesperidin-fed group ( $28.3 \pm 7.0$  hours) and the control group ( $26.60 \pm 5.30$  hours). In the second and third oestrous cycle, OD was significantly ( $p \leq 0.05$ ) higher in the hesperidin-fed group ( $2^{\text{nd}}$  -  $33.40 \pm 10.16$ ;  $3^{\text{rd}}$  -  $30.00 \pm 5.9$  hours) compared with the control group ( $2^{\text{nd}}$  -  $27.16 \pm 5.66$ ;  $3^{\text{rd}}$  -  $30.00 \pm 1.4$  hours)

## DISCUSSION AND CONCLUSION

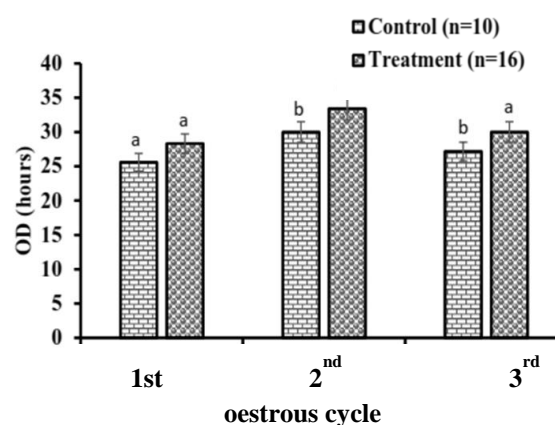
The oestrous cycle is the cyclical pattern of ovarian activity that enables female animals to move from one period of reproductive receptivity to another (Crowe, 2022). There was no difference in the time of onset of oestrus in the first oestrous cycle. This may be attributed to the fact that supplementation with hesperidin was yet to take full effect in terms of its therapeutic and biological properties, and thus, resulted in insignificant difference. There was a reduction in the time of onset of oestrus from the second and third oestrous cycles, suggesting shorter oestrous cycle length in the Yankasa ewes in the current study. This is similar to the reports of Idris *et al.* (2023) in rats treated with glyphosate-based herbicides (GBH) but contradicts the reports of Essiet *et al.* (2018) and Schimpf *et al.* (2022) in rats treated with *Salacia lehmbachi* and GBH, respectively, where there was an increment in the length of oestrus. Vaadala *et al.* (2019) also reported prolonged oestrous and suppressed fertility following the use of flavonoid and baicalein, in female mice. The findings of the current study is also contrary to the reports of Resum *et al.* (2017) who found no significant difference in TOO in buffaloes synchronized using CIDR protocol alone or in combination with antioxidants (vitamin E and selenium). It is also contrary to the reports of



**Figure I: Time of onset of oestrus (TOO) (days) of Yankasa ewes fed hesperidin**



**Figure II: Oestrous response rate (ORR) (%) of Yankasa ewes fed hesperidin. <sup>ab</sup>Bar charts paired together with different superscripts differ significantly ( $P < 0.05$ )**



**Figure III: Oestrus duration (OD) in Yankasa ewes fed hesperidin. <sup>ab</sup>Bar charts paired together with different superscripts differ significantly ( $P < 0.05$ )**

Abubakar *et al.* (2015) in *Trypanosoma congolense* infected ewes where there was no significant effect. A shorter oestrous cycle length is common during the anoestrus period in ewes with a seasonal breeding pattern (Brown *et al.*, 2014). The differences between all these reports with the current study may be associated with the cold-dry season when this current study was carried out. This period is characterized by lowered environmental temperatures that lead to malfunctioning of the hypothalamus-pituitary-gonads axis (Romo-Barron *et al.*, 2019). In the present study, the oestrus response rate (ORR) increased in the second and third oestrous cycles of the hesperidin-fed group. This agrees with the findings of Musa *et al.* (2018), who found higher ORR in Yankasa ewes supplemented with Vitamin E and selenium. However, it disagrees with the report of Sejian *et al.* (2014) in Malpura ewes administered mineral and antioxidants as well as the report of Gore & Lehloeny (2020) in Saanen goats supplemented with  $\beta$ -carotene, where there was no effect. Oxidative stress is associated with the different stages of the oestrous cycle during which excess reactive oxygen species (ROS) are produced, resulting in deleterious effects on ewes, particularly poor oestrus outcomes. With hesperidin supplementation, excess ROS were scavenged and destroyed, thus resulting in enhanced ORR.

The oestrus duration OD obtained in this study was longer in the hesperidin-fed group, similar to the findings of Sejian *et al.* (2014) in Malpura ewes administered mineral and antioxidants. It is also similar to the findings of Abu El-Ella *et al.* (2016) in four groups (n=15 each) of Rahami ewes given different synchronization protocols of hormonal treatment with CIDR + PMSG, MAP + PMSG, gonadotrophic releasing hormone-prostaglandin F<sub>2</sub> $\alpha$  (GnRH+GnRH+PGF<sub>2</sub> $\alpha$ ) protocol and gonadotrophic releasing hormone (GnRH-PGF<sub>2</sub> $\alpha$ -GnRH) protocol, respectively. Hesperidin is an antioxidant obtained mostly from citrus fruits and has biological and therapeutic properties such as scavenging free radical species (Sa'Ayinzat *et al.*, 2021). Contrary to the findings of this current study, Gore (2017) found no effect of  $\beta$ -carotene on OD in Saanen goats. The discrepancies could be attributed to differences in the species of animals used, location and the season during which the study was carried out. Season, genotype, breed, location, diseases and health status, management style and nutrition is believed to influence OD (Omontese *et al.*, 2017). This current study was carried out during the cold dry harmattan season, but Gore (2017) conducted his study on goats indicating species variations under sub-tropical conditions. Cold stress has been reported to shorten oestrus duration in West Bengal goats (Kumar *et al.*, 2015), which is contrary to the findings in this current study. In addition, cold stress may result in total loss of the

oestrous cycle (Tumenbayar *et al.*, 2019). Extreme cold stress is associated with the depletion of energy reserves and increased metabolic activity in ewes and other farm animals, which adversely influence homeostasis and cause overall poor reproductive performance (Gebregeziabhear *et al.*, 2015).

In conclusion, the study shows that hesperidin administration had a beneficial effect on Yankasa ewes by improving the oestrous cycle parameters. The parameters evaluated in Yankasa ewes given hesperidin were impressive. This was characterized by reduced TOO, higher ORR and OR. It is recommended that similar studies be carried out in thermally-stressed environments like the Sahelian regions of northern Nigeria where temperatures range between 30 and 46°C. It is also recommended that farmers in cold-stressed environments use hesperidin for their sheep to improve their reproductive performance.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with the publication of the outcomes of this scientific study.

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