

Research Article



Comparative Study of Hormonal Treatment and the 'Male Effect' on Estrus Induction and Fertility in Local African Ewe Breeds

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Abstract | In many African countries, sheep farming plays a significant role in the socio-economic life of the population, although the practice remains relatively unmodernized. The aim of the present study is to optimize the reproductive performance of local sheep breeds in order to enhance the meat production potential of small ruminants in northern Nigeria and southern Niger. A total of forty-two (42) adult ewes of local African breeds were divided into seven equal groups, each subjected to a specific estrus induction treatment. The first two groups (A and B) received vaginal sponges containing progestagen and injections of PMSG (Pregnant Mare Serum Gonadotropin). Additionally, Group A received an injection of prostaglandin on day 12. Groups C and D were treated with oestradiol at doses of 1 ml and 2 ml, respectively. Rams were introduced into these groups on the twelfth day following treatment. Group E was exposed solely to the "male effect," following a 45-day separation between ewes and rams. Groups F and G also received oestradiol at doses of 1 ml and 2 ml, respectively; however, rams were introduced on the first day of hormonal treatment. Results showed that the onset of estrus within 48 hours of treatment ranged from 20% to 40%, while pregnancy rates varied between 40% and 60%, depending on the treatment group. Notably, only the ewes treated with progestagen and PMSG conceived during the first induced estrus cycle. However, prolificacy was highest among the groups treated solely with exogenous oestradiol. Although further studies with a larger sample size are needed, these findings suggest that the efficacy of estrus induction protocols in local sheep breeds varies significantly depending on the type and combination of hormones used.

Keywords | Heat induction, Sheep, Breeding, Hormones, Reproduction

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INTRODUCTION

Sheep farming plays a vital role in the national economies of many countries worldwide. It contributes to the production of meat, milk, wool, and hides, thereby providing a significant source of income for rural populations (Yabrir *et al.*, 2013). In terms of sheep population, China leads globally with 25% of the total, followed by Australia

(14%), India (12%), Iran (10%), Sudan (9%), and Nigeria, New Zealand, and the United Kingdom, each contributing around 6% (FAO, 2010). In recent decades, many of these major sheep-producing countries have enhanced their productivity through artificial estrus induction and heat synchronization. These reproductive technologies represent some of the earliest biotechnological advances to have significantly transformed livestock production. In

sheep, estrus synchronization has been practiced since the 1960s and is now widely adopted by farmers in developed countries. In contrast, this technique remains relatively unknown and underutilized in sub-Saharan Africa, despite the region's substantial potential for sheep production, estimated at over 160 million small ruminants (Mulumba *et al.*, 2008).

Several methods are used to induce estrus in ewes, including the administration of progestagens, prostaglandins, the "male effect," and other exogenous hormones. The effectiveness of these methods varies considerably depending on the treatment protocol and the physiological condition of the animals. For instance, when comparing the efficacy of progestagens on fertility outcomes, estrus induced by fluorogestone acetate (FGA) resulted in significantly higher lambing rates than medroxyprogesterone acetate (MAP) (Eppleston and Roberts, 1986). Similarly, ewes synchronized with FGA exhibited higher birth rates than those treated with controlled internal drug release (CIDR) devices, although the difference was not statistically significant during the spring season (Salamon and Maxwell, 2000; as cited in Salamon and Maxwell, 2000). Furthermore, the percentage of ewes exhibiting estrus within the first 24 hours post-treatment was significantly higher ($P < 0.05$) in the group treated with MAP combined with PMSG (72.2%) compared to those treated only with MAP (5.6%) or with MAP and PGF2 α (7.6%) (Dogan and Nur, 2006).

To be effective, any estrus induction and/or synchronization protocol must be contextualized, taking into account both endogenous and exogenous factors that influence the reproductive physiology of the females. The objective of the present study is to optimize the reproductive performance of local sheep breeds, with the broader goal of enhancing meat production from small ruminants in the northern Nigeria and southern Niger regions. Specifically, the study aims to evaluate the effectiveness of different estrus induction methods including the ram effect, prostaglandin administration, and the use of vaginal sponges on estrus response, pregnancy rate, lambing rate, and prolificacy in local ewes.

MATERIALS AND METHODS

ETHICAL REGULATION

Due to the absence of an institutional Animal Care Committee at the time of the study at Dan Dicko Dankoulodo University and The Farm, Katsina LTD, the research was conducted under the direct supervision of the principal investigator. All procedures involving animals were carried out in accordance with the guidelines of the Canadian Council on Animal Care (2009), as reformatted in March 2011.

STUDY SITE

The present study was conducted from 05 May 2022 to 07 March 2023 at The Farm, Katsina Ltd, located in the Shinkafi area of Katsina City, in northern Nigeria. Katsina State encompasses two main climatic zones: the tropical continental zone and the semi-continental zone. Rainfall patterns vary significantly throughout the year. The climate is characterized by four distinct seasons: a cold, dry season from December to February; a hot, dry season from March to May; a hot, humid season from June to September; and a transitional season from October to November, marked by decreasing rainfall and gradually declining temperatures (Ibrahim and Abdullahi, 2022). Shinkafi is geographically situated at latitude 13°1'37" N and longitude 7°38'33" E. The natural vegetation in the area consists predominantly of scattered trees and shrubs, typical of the Sahelian zone. The average annual rainfall ranges between 400 and 600 mm, with the rainy season typically occurring between April and October (Bello *et al.*, 2020).

ANIMALS AND EXPERIMENTAL DESIGN

The ewes used in this study were all adult females of African breeds, specifically Ladoum, Balami, and their cross-breeds. They were housed in individual stalls and received a balanced diet consisting of cowpea pods, wheat bran, and groundnut and cowpea hay. In addition to their regular diet, the animals were given vitamin supplements and underwent routine deworming and treatment for external parasites every four months. All animals on the farm were also regularly vaccinated against pasteurellosis and sheep pox in accordance with local veterinary guidelines.

The experimental protocol involved seven treatment groups, each comprising six (6) ewes, assigned to different estrus induction strategies involving vaginal sponges, prostaglandin, PMSG, oestradiol, and/or the male effect. The treatment schedule was organized into seven groups, each receiving a specific estrus induction protocol.

Group A, (P4 + Prost) received vaginal sponges on day 1, which were kept in place for 14 days. On day 12, each ewe was injected with 2 ml of prostaglandin. On day 14, the sponges were removed, and an injection of 500 IU of PMSG was administered. Rams were introduced on day 15 and remained with the ewes for one month.

Group B, (P4 - Prost) also received vaginal sponges on day 1, maintained for 14 days. On day 14, the sponges were removed, and 500 IU of PMSG was administered. Rams were introduced on day 15 and remained with the ewes for one month.

Group C, (1 ml E₂) received an injection of 1 ml oestradiol (E₂) on day 1, followed by the introduction of the ram on day 12. Rams remained with the ewes for two months.

Group D, (2 ml E₂) was treated with 2 ml of oestradiol (E₂) on day 1, and rams were introduced on day 12, remaining for two months.

Group E, (EM – Male Effect) was managed by introducing the ram into the flock on day 1, for a duration of two months, following a prior 45-day period of complete sexual isolation between rams and ewes.

Group F, (1 ml E₂ + EM) received 1 ml of oestradiol (E₂) on day 1, with the ram introduced simultaneously for a period of two months.

Group G, (2 ml E₂ + EM) was treated with 2 ml of oestradiol (E₂) on day 1, and the ram was also introduced on the same day for two months.

HORMONAL TREATMENTS AND DATA COLLECTION

The hormonal products used in this study had the following specifications: Vaginal sponges were of the Chronogest type, containing 20 mg of fluorogestone acetate. They were from batch A366A01, manufactured by Intervet Productions S.A., Rue de Lyons, France. Equine serum gonadotropin (PMSG) was sourced from Laboratory SERIGAN (Lot: 572N001A), produced by OVEJERO, León, Spain. Prostaglandin used was D-Cloprostenol (Veteglan), Lot: 215034AS03, manufactured by CALIER S.A., Barcelona, Spain. Estradiol (E₂) was estradiol benzoate 0.2%, from batch 20071607, produced by J.A.P., India.

Regular monitoring of the animals was carried out to assess several reproductive parameters, including the percentage of females in estrus, pregnancy rate, birth rate, and prolificacy.

The percentage of females in estrus was determined by observing which ewes were mounted by rams during the first two days following ram introduction. The pregnancy rate was assessed using transabdominal ultrasonography performed 40–50 days after ram introduction.

The following formulae were used to calculate reproductive performance indicators:

$$\text{Birth rate (\%)} = \frac{\text{Number of females that lambed} \times 100}{\text{Number of females bred}}$$

$$\text{Prolificacy rate (\%)} = \frac{\text{Total number of lambs born} \times 100}{\text{Number of females that bred}}$$

STATISTICAL ANALYSIS

The data collected were analyzed using R software version 4.3.0 (2023-04-21). One-way ANOVA was used to calculate the means of the parameters tested. To compare the rates (%) of reproductive parameters (heat, pregnan-

cy, farrowing and prolificacy) obtained in the ewes tested, Tukey's post hoc test was used. Means were compared at 5% level of significance ($P < 0.05$). Results were presented as the mean $\pm$ standard error of the mean.

RESULTS

The rate of estrus expression was similar across most groups, with 40% of the ewes showing signs of heat within the first two days following the introduction of the ram. The exception was Group A (treated with progestagen and prostaglandin), which exhibited the lowest heat response rate at 20% (Figure 1).

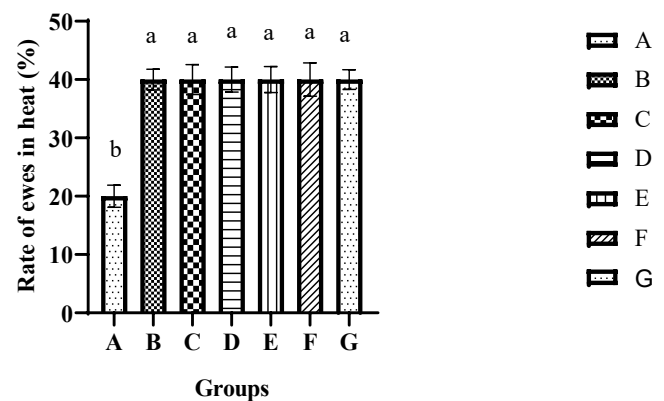


Figure 1: Estrus response rate by treatment group of ewes ($P < 0.0001$).

In terms of conception, ultrasound examination revealed that the pregnancy rate reached 60% in most groups. However, Groups D and E recorded a lower pregnancy rate of only 40% (Figure 2).

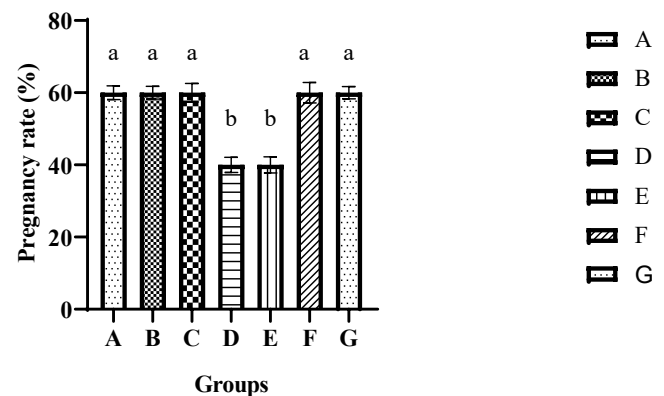


Figure 2: Pregnancy rate by treatment group of ewes ($P < 0.0001$).

As for lambing, 80% of the bred ewes successfully gave birth in all groups, except in Groups B and E, where the lambing rate was reduced to 60% (Figure 3).

Among the ewes that lambed, only those in Group B conceived during the first heat induced by the treatment. They

were followed by Groups A and C, where 50% of the lambings resulted from matings during the first estrus cycle. In contrast, Groups D, E, F, and G showed no lambings from first-cycle matings. All lambs born in these groups were the result of matings during the second or third estrus cycles presented by the ewes (Table 1).

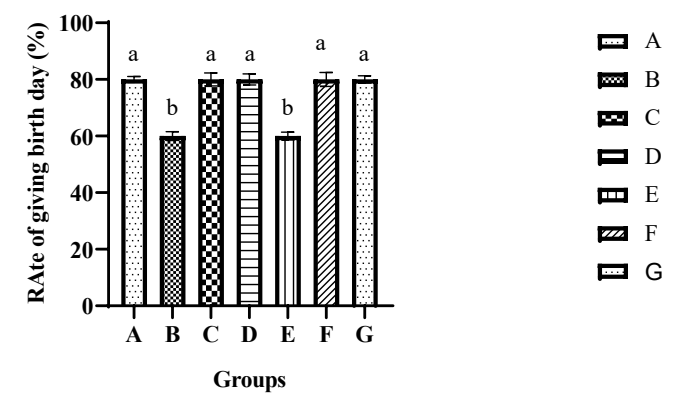


Figure 3: Lambing rate by treatment group of ewes ($P < 0.0001$).

Table 1: Distribution of lambings (%) recorded during the first three estrus cycles by treatment group of ewes.

Estrus Cycle	Group A	Group B	Group C	Group D	Group E	Group F	Group G
1st estrus	50%	100%	50%	0%	0%	0%	0%
2nd estrus	25%	0%	50%	50%	33,33%	25%	25%
3rd estrus	25%	0%	0%	50%	66.66%	75%	75%

In addition, birth records showed the highest prolificacy (175%) in groups C and D and the lowest (100%) in groups A, E and G (Figure 4).

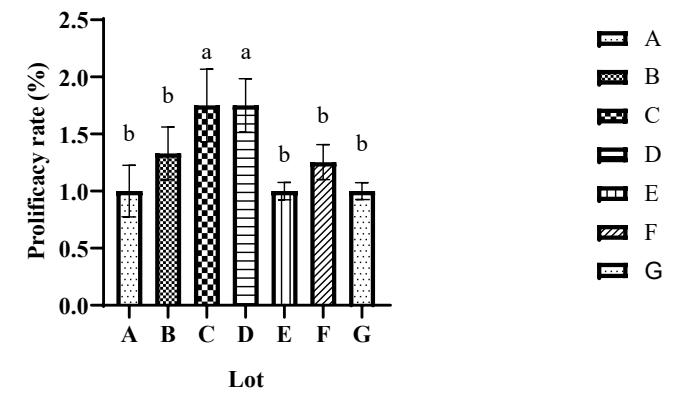


Figure 4: Prolificacy rate by treatment group of ewes ($P < 0.0001$).

DISCUSSION

In the present study, the use of six heat and fertility induction protocols in ewes of African breeds (Ladoum, Bala-

mi and their crosses) was compared with that of the ‘male effect’ (Group E). These protocols differ according to the type of hormone used, their combination and their dose. Different results have also been obtained depending on the induction and fertility protocol used. According to Skliarov *et al.* (2021), the effect of hormone treatment on the reproductive system of ewes may manifest itself differently depending on the initial functional state of the reproductive organs and their hormonal status. According to the same authors, seasonality and the status of the female are important factors in choosing the most appropriate hormone treatment.

In the present study, the rate of estrus occurrence during the first two days of ram introduction in the different groups of ewes was similar (40%) except for group A (20%), which consisted of ewes treated with progestagen and prostaglandin.

To synchronize oestrus in ewes during the non-breeding season, the use of progestin in intravaginal devices can be combined with the administration of gonadotropins. This allows treated animals to enter oestrus simultaneously after hormone treatment has stopped (Skliarov *et al.*, 2021). Oestrus synchronization occurs in 90% of treated animals within 24 to 48 hours of removal of vaginal devices (Erohin, 2011) cited by Skliarov *et al.* (2021). The use of intravaginal sponges impregnated with synthetic progesterone analogues makes it possible to extend oestrus synchronization during and after the breeding season (Skliarov *et al.*, 2021). The administration of equine serum gonadotropin (PMSG) in addition to the introduction of vaginal sponges in ewes precipitates the onset of heat and the peak of LH (Touré *et al.*, 1995).

In group A, it is difficult to explain the lowest heat rate (20%) obtained during the first two days of ram introduction. This is because, in addition to the progestin-based treatment, the ewes were given a luteolytic substance and a follicular growth stimulator. It is clear that the administration of prostaglandin certainly had an effect on the corpus luteum, as the corpus luteum of ewes are sensitive to the luteolytic action of prostaglandins between days 4 and 14 of the sexual cycle (Skliarov *et al.*, 2021). The development of the corpus luteum is necessary for the production of progesterone (Shabankareh *et al.*, 2009). According to Fierro *et al.* (2013), the use of prostaglandin for heat induction has a luteolytic effect and the decrease in plasma progesterone (P4) concentrations is more pronounced after prostaglandin (PG)-induced luteolysis than after natural luteolysis. The administration of prostaglandin to synchronize oestrus is only recommended for the breeding season, due to its luteolytic effect (Fierro *et al.*, 2013; Skliarov *et al.*, 2021). However, administration of PMSG twice at an interval of 7 days favours more synchronous oestrus induc-

tion in ewes (Skliarov *et al.*, 2021). Dogan and Nur, (2006) showed in their work that the use of medroxyprogesterone acetate sponges combined with PMSG was more effective in achieving early and clustered induction and synchronization of oestrus in Kivircik ewes during the non-breeding period compared with the use of medroxyprogesterone acetate sponges combined with Prostaglandin. According to several authors, the use of PG-600 consisting of 400 IU of equine chorionic gonadotropin (eCG) and 200 IU of human chorionic gonadotropin (HCG) gives good results for oestrus stimulation in sheep, unlike that of a single dose of eCG (Safranski *et al.*, 1992; Windorski *et al.*, 2008). However, the use of hCG in ewe oestrus synchronization protocols as an alternative to eCG to induce ovulation is controversial, due to the low fertility rates reported (38.5%) linked to a high occurrence of abnormal follicular growth patterns, disturbances and delays in ovulation and the concomitant formation of follicular cysts in treated females (Bruno-Galarraga *et al.*, 2021).

The present results contrast with those of a previous study comparing five oestrus synchronization protocols in ewes where the authors used an intra-vaginal sponge device of 45 mg fluorogestone acetate for 11 or 13 days depending on the protocol, followed by an injection of 0.1 mg prostaglandin on day 9 or 11 as appropriate and an injection of 6 µg GnRH or 330 IU PMSG or their simultaneous injection after removal of the sponge. The protocol in which an 11-day vaginal sponge device was used, followed by an injection of prostaglandin on day 9 and an injection of 330 IU of PMSG after removal of the sponge is the most recommended, as the rate of ewes in oestrus (83%), the conception rate (80%), the lambing rate (192%) and the twinning rate (70%) were among the best (Yu *et al.*, 2022).

In the present study, ultrasound scans detected the maximum number of pregnant females (60%) in most groups (A, B, C, F, G) with the exception of groups D and E where only 40% of ewes were able to conceive. Group D consisted of ewes that had received a 2 ml injection of oestrogen (E2), while group E consisted of ewes that had benefited from the 'ram effect' without any other hormonal treatment. It should be noted that the ewes in group C, 60% of which were found to be pregnant, also received a treatment based on oestrogen (E2), but with a smaller quantity (1 ml) than that received by group D (2 ml). Fertility after oestrus synchronization depended on species, breed, treatment and insemination method (Skliarov *et al.*, 2021). The lowest fertility rate obtained in group D can be explained by an excessively high plasma oestradiol (E2) level (Evans and Robinson, 1980).

The oestrous cycle in ewes is regulated by interrelationships between hormones from the hypothalamus (GnRH),

pituitary (LH and FSH), follicle (oestradiol and inhibin), corpus luteum (progesterone and oxytocin) and uterus (prostaglandin) (Scaramuzzi *et al.*, 1993). Follicular development, maturation, ovulation and corpus luteum formation are mainly controlled by pituitary gonadotropins. The regulation of pituitary hormone secretion and availability depends on a complex interaction between several internal and external factors (Bartlewski *et al.*, 2011). Oestrogens are involved in the physiological control of ovulation rate in ewes. In an experiment conducted by Adams *et al.* (1989), supra-physiological doses of oestradiol were shown to induce anovulation in ewes, whereas lower doses only reduced the proportion of twin ovulations. Seekallu *et al.* (2010) reported that supra-physiological concentrations of estradiol completely eliminate the serum LH pulse and suppress the emergence of follicular waves, while the FSH secretion peaks that preceded the follicular waves are not affected. Atkinso *et al.* (1989) reported in their study that regulation of LH pulse frequency is not a critical factor in determining the rate of ovulation in ewes. According to Bartlewski *et al.* (2011), only supra-physiological doses of oestrogen administered during the luteal phase in ewes are capable of modifying FSH secretion and delaying the emergence of follicular waves.

As for group E, which was made up of ewes subjected to the ram effect and also recorded the lowest pregnancy rate (40%), it should be remembered that the ram effect is a biological method used by breeders to stimulate oestrus in ewes during the non-breeding season through multisensory contact with mature rams. Although rams are capable of mating all year round, there are seasonal variations in testicular weight, seminal vesicle fructose concentration, sexual behaviour and semen characteristics. These seasonal variations in males and females are linked to hormonal levels. Concentrations of FSH and LH in the pituitary gland are reduced by 50% outside the breeding season. Concentrations of LH in peripheral blood vary throughout the year, while those of prolactin follow the rhythm of day length (Abecia *et al.*, 2012). The stimulation of oestrus in ewes via the ram effect is a consequence of a change in GnRH pulse frequency and an increase in tonic LH secretion. The stimulatory effect of introducing males depends on their sexual maturity, sexual activity and body weight (Skliarov *et al.*, 2021).

The first ovulation occurs 2 to 3 days after the introduction of the males, but it often takes place without any signs of heat and is accompanied by low fertility in the ewes. The second ovulation occurs on average 5 days after the first, accompanied by the usual signs of oestrus and the normal duration of the luteal phase (Skliarov *et al.*, 2021). One of the limiting factors in using the ram effect is the reduction in ewe fertility during the first cycle (Skliarov *et al.*

al., 2021). To increase the effectiveness of the ram effect, it could be useful to artificially extend the light period in ewes over the previous two months (Pellicer-Rubio *et al.*, 2007; Abecia *et al.*, 2012). In females of low live weight, the response to the introduction of males into the flock is significantly lower than in animals of medium and high live weight (Pellicer-Rubio *et al.*, 2007). Feeding can also influence reproductive performance, as a study of the effect of feeding during induced oestrus on hormonal and reproductive performance showed that a 60-80% increase in concentrate level had a positive effect on fertility, twin rate and blood metabolites in ewes (Laghrou *et al.*, 2020).

In the present work, the conception results obtained during the first induced heat are very low in the majority of ewe groups. This observation has also been made in previous work, in particular that of Skliarov *et al.* (2021) who reported that, in general, the fertility of sheep after the first insemination in synchronized heat is slightly lower (0 to 15%) than in spontaneous heat.

And among farrowings, only ewes in group B treated with intra-vaginal progesterone sponges combined with gonadotropin administration all conceived during the first induced heat, while 50% of ewes in group A treated with Prostaglandin and group C treated with oestradiol (1ml) conceived during the first heat.

The present results are partially in agreement with the observations made by Wei *et al.* (2016) on the oestrus synchronization pattern and its application efficiency in Lanzhou fat-tailed ewes in oestrus. For they played estrus synchronization rates of 85.72% and 57.14%, respectively in progesterone and prostaglandin treated ewe groups within 72 hours of treatment application.

CONCLUSIONS AND RECOMMENDATIONS

Sheep farming plays a significant role in the economies of many countries worldwide. In developed nations, reproductive efficiency in sheep production is often enhanced through the use of estrus induction and synchronization techniques. However, these methods remain relatively unknown and underutilized among farmers in sub-Saharan Africa. To support the improvement of reproductive performance in local sheep breeds, this study compared seven estrus induction protocols. The outcomes varied depending on the hormonal treatment applied. Higher conception rates were observed in ewes treated with progesterone and PMSG, while better prolificacy was recorded in those treated with exogenous oestradiol. In contrast, ewes treated with prostaglandin showed a lower estrus response. These findings offer useful insights for selecting estrus induction

protocols based on specific reproductive goals. Nevertheless, further studies involving larger sample sizes are recommended to validate these preliminary results.

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NOVELTY STATEMENTS

Most previous studies have focused on evaluating oestrus induction protocols using vaginal sponges and prostaglandins. However, the present study is novel as it evaluated the efficacy of different oestrus induction methods including the use of progestagen-based vaginal sponges, prostaglandin administration, PMSG, variable dose oestradiol and ram effect on oestrus response, pregnancy rate, lambing rate and prolificacy of local African ewes.

AUTHORS'S CONTRIBUTIONS

This work was carried out by the contribution of all authors. Akourki Adamou designed the protocol, collected and analyzed data, and draft the manuscript. Moustapha Issoufou Ahamidou analyzed data and revised the manuscript. Abdullahi Aliyu Dahiru and Yakub Ladan collected the data. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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