EFFECT OF DIETARY INCLUSION OF DRIED OKRA (*ABELMOSCHUS ESCULENTUS*) FRUIT AND LEAVES ON THE REPRODUCTIVE HORMONES OF WEST AFRICAN DWARF DOES

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ABSTRACT
Okra fruit is highly consumed in Nigeria and had been proposed to be responsible for modulating hormones that are responsible for sterility in males as a result of gossypol or a gossypol-like compound that stimulates infertility. However, there was no information available on the effects of the fruits and leaves on fertility in female goats. This study was carried out to evaluate the effects of okra fruits and leaves on the reproductive hormones of West African Dwarf Does. A total of sixteen (16) does with mean body weight of 17.5±5 kg were allotted by weight to four dietary treatments formulated to contain 0 % dried okra fruits and leaves (D), 3 % dried okra leaves (DL), 3% dried okra fruits (DF), and 3 % dried okra fruits and leaves (DLF) in ratio 1:1 for a period of twenty-eight weeks. Does were synchronized using PGF2 alpha injection (Lutalyse®) and then mated using four Kalahari red bucks (one buck per group). Blood samples were collected before synchronization and at the end of each trimester for hormonal analysis and data on performance and reproductive indices taken were subjected to One-Way Analysis of Variance using in a Completely Randomized Design. The results obtained from the study revealed that Does fed DL had significantly highest ($p<0.05$) estradiol concentration (7.93 pg/mL) in the second trimester while DF (3.69 ng/mL) and DLF (3.63 ng/mL) had highest ($p<0.05$) progesterone concentration and D (3.16 ng/mL) had lowest progesterone concentration prior to parturition. The study concluded the inclusion of okra fruits with or without the leaves increased progesterone concentration prior to kidding.
Keywords: Okra, Estradiol, Progesterone, Follicle stimulating hormone, West African dwarf does,

INTRODUCTION

The nutritional and pharmaceutical uses of plants are as old as humans. The usefulness of these plants' constituents has become more validated by the animal models replicating their proposed or confirmatory activities in humans (Shafaei *et al*., 2011). Okra (*Abelmoschus esculentus* Moench L.) leaf is the vegetable in the diet responsible for multiple births among the natives of Igbo-Ora, Oyo State. Nigeria. Igbo-Ora is known for having the highest incidence of multiple births in the World. Okra is a seasonal crop of hot regions from the family of Malvaceae that grows well in high temperatures, especially in regions with hot nights (20°C) (Mousavi *et al*., 2021). Okras are a rich source of oil, proteins, carbohydrates, fibres, vitamin C, minerals, and antioxidants (Mousavi *et al*., 2021). Decoction of the leaf is demulcent, diuretic, and emollient, and against catarrhal infections, dysuria, and gonorrhoea (Kumar *et al*., 2013). The fruit had fertility potentials in males (Bello *et al*., 2017), the effects of the seed on human cancer cell lines (Chaemsawang *et al.,* 2019), and its nutritional qualities and health benefits (Gemede *et al*., 2014). The root worked against syphilis, cuts, wounds, and boils (Kumar *et al.,* 2013). The seed has antispasmodic and sudorific properties (Kumar *et al.,* 2013). The female reproductive system is coordinated by oestrogen, progesterone, prolactin, follicle stimulating hormone, and luteinizing hormone (Onyegeme-Okerenta and Essien, 2015), while the fertility hormone in males is testosterone. These hormones maintain the stages of development and function of the female reproductive system. Several studies (Lyngso *et al.,* 2014) have revealed that phytochemicals from plant extract could alter the availability and functioning of female reproductive hormones.

This study evaluated the effects of including dried okra fruits and leaves as additive in does' diet on the reproductive hormones.

MATERIALS AND METHODS

Description of the experimental site

The experiment was carried out at the Kalahari Red Goat Unit of Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The climate is humid with an annual rainfall of 1,112.7mm and average temperature of 34.7°C. This area is located in the derived savannah vegetation zone of South Western Nigeria with latitude 7°10'30.14" N, longitude 3°25'41.93" E, and relative humidity of 82 % (FUNAAB AGROMET, 2023).

Source of experimental animals and ingredients

The Kalahari Red bucks and West African Dwarf does used for the study were sourced from IFSERAR, Federal University of Agriculture Abeokuta, Nigeria. while Okra fruits and leaves were purchased from markets within Abeokuta in Ogun state and Igbo-ora in Oyo state, Nigeria, respectively.

Preparation of okra fruits and leaves

Okra fruits were sun-dried to constant weight in order to reduce spoilage and increase the shelf-life, then milled while the leaves were air dried to constant weight and milled. The test ingredients were incorporated into the experimental diet at 3 % level of inclusion. Maize stover was fed *ad libitum*.

Experimental animals and management

The experiment was conducted using sixteen Does weighing between 17.5±5 kg were allotted by weight equalization into four groups (four Does each) and managed intensively. Throughout the experiment, Does were fed the experimental diets at 3 % of their body weight on as fed basis in the morning while maize stover was fed in the evening.

The animals were fed the experimental diets for six weeks after which they were synchronized using prostaglandin F2alpha (PGF2α) (Lutalyse® Injection) with a dosage of 2 mL of injection per doe. Mating was done immediately by introduction of four Kalahari Red bucks into the pen houses (i.e. a buck per pen). Proper observation was done to ensure that the bucks mate the Does before data collection began. Fresh and clean water was available to the Does *ad libitum*; animals were observed till parturition.

Blood sample collection

Blood samples were collected from the experimental animals just before mating (before synchronization) and at the end of each trimester. This was collected via the jugular vein punctured with new hypodermic needle fitted on a new 5 mL calibrated syringe in the morning. It was deposited into a plain bottle without anticoagulant after which it was spun with a centrifuge and the serum collected and stored at -2 for hormonal analysis. The reproductive hormones analysed were progesterone, estradiol and follicle stimulating hormones.

Table 1: Gross composition (%) of experimental diet and maize stover

Feed ingredients	Quantity(%)	Maize Stover
Maize	16.00	
Soya bean meal	6.00	
Wheat offal	52.00	
Palm Kernel cake	24.00	
Bone meal	1.00	
Common salt	1.00	
Total	**100**	
Calculated analysis (%)		
Crude protein	16.69	3.60
Crude fibre	10.22	33.20
Ether extract	5.20	0.80
Ash	5.05	10.90
TDN	69.81	
ME (kcal/kg)		

*Nutrient composition of maize stover according to Aduku (2005)

Treatment 1: 100 g of experimental diet with 0 g of okra fruit and leaves (D)
Treatment 2: 100 g of experimental diet with 3 g of okra leaves (DL)
Treatment 3: 100 g of experimental diet with 3 g of okra fruits (DF)
Treatment 4: 100 g of experimental diet with 1.5 g of okra leaves and 1.5 g of okra fruits (DLF)

Serum hormonal analysis
Hormonal analysis was performed base on the principle of Enzyme-Linked Immunosorbent Assay (ELISA) technique using the commercial kits (Bio-Inteco®) from Inteco diagnostics Ltd, UK and ELISA Reader were used to analyse for the absorbance of progesterone, estradiol and follicle stimulating hormones in the serum sample at a wavelength of 450 nm according to the specification of the manufacturer.

Data analysis
Data obtained were subjected to One way analysis of variance in a completely randomised design by following the procedure of SAS (SAS, 2002). Level of significance was taken at 5 % probability, while significant means were separated using Duncan's Multiple Range test method (Duncan,1955).
Statistical model
$Yij = \mu+Ti+\sum ij$
Where:
Yi is the dependent variable
μ is the population mean
Ti is the effect of the i^{th} factor (i=concentrate diets)
Σij is the random error term

RESULTS
Table 2: Concentration of estradiol, progesterone and follicle stimulating hormones of does before mating and at the end of each trimester

	D	DL	DF	DLF	SEM	P-VALUE
Estradiol (pg/mL)						
Before mating	9.80	10.67	10.25	10.04	0.20	0.53
1st trimester	6.33	6.38	6.26	8.99	0.56	0.25
2nd trimester	4.59[b]	7.93	5.36[ab]	5.06[ab]	0.56	0.12
3rd trimester	3.44	5.16	5.5	4.25	0.43	0.33
Progesterone (ng/mL)						
Before mating	3.15	2.99	3.09	3.4	0.09	0.45
1st trimester	3.03[ab]	2.99	3.02[ab]	3.23[a]	0.04	0.11
2nd trimester	3.11	3.07	3.13	3.28	0.05	0.47
3rd trimester	3.16b	3.30	3.69[a]	3.63[a]	0.08	0.01
FSH (mIU/mL)						
Before mating	1.40	1.56	1.54	1.58	0.05	0.62
1st trimester	1.37	1.31	1.37	1.31	0.02	0.29
2nd trimester	1.36	1.67	1.36	1.50	0.07	0.42
3rd trimester	1.36	1.34	1.40[ab]	1.54[a]	0.03	0.12

[ab] Means on the same row having different superscripts are significantly different (P<0.05)

Keys for acronym
D Experimental diet without okra fruit and leaf
DL Experimental diet with 3% okra leaves
DF Experimental diet with 3% okra fruits
DLF Experimental diet with 1.5% okra fruits and 1.5% leaves

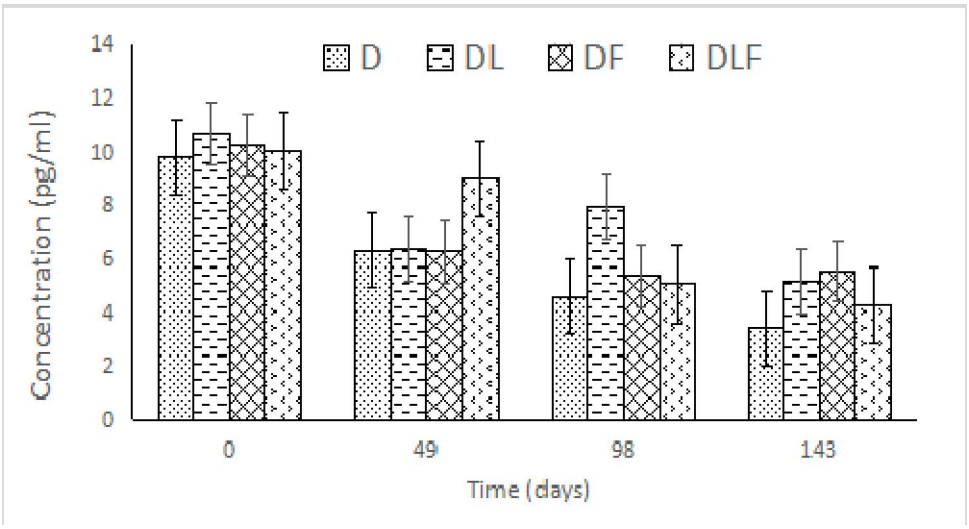

Fig 1: Estradiol concentration (pg/mL) of WAD Does fed dried okra fruit and leaves.

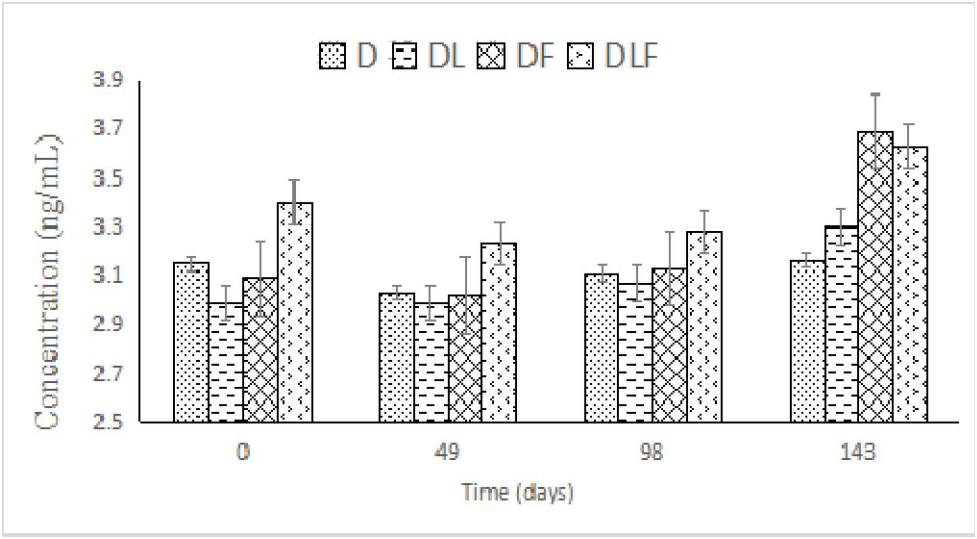

Fig 2: Progesterone concentration (ng/mL) of WAD Does fed dried okra fruit and leaves.

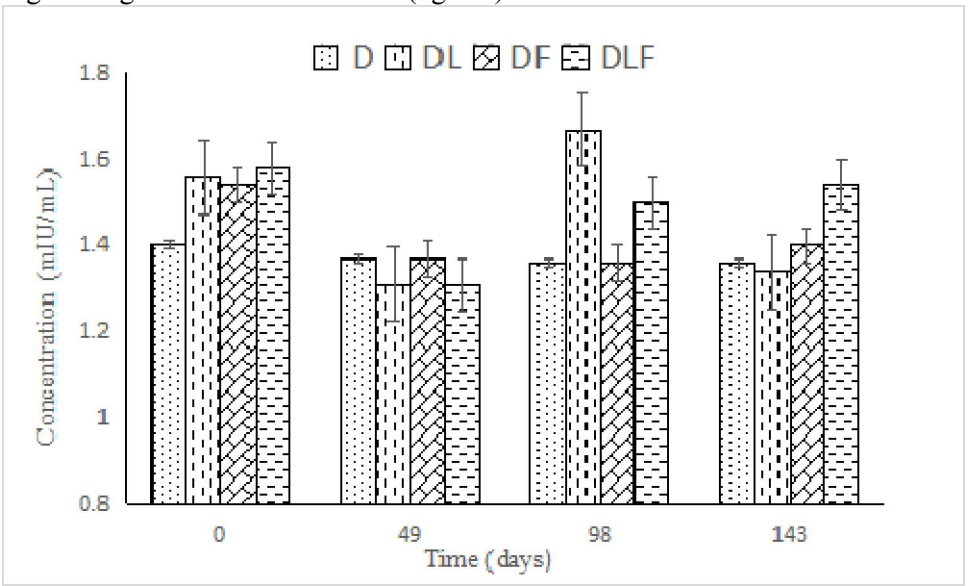

Fig 3: Follicle stimulating hormone concentration (mIU/mL) of WAD Does fed dried okra fruit and leaves

Discussion

The okra leaf extract modulates hormonal parameters-oestrogen and progesterone. From this study, estradiol concentration of DL varied significantly from D at the end of the second trimester. There was no significant difference observed in the concentration of the reproductive hormones across treatments before mating which indicates a common ground for the does.

The antagonistic effects on the trend of oestrogen and progesterone concentrations was observed due to the physiological state of the animals. A high concentration of oestrogen is necessary for the surge of luteinizing hormone, which induces ovulation. The corpus luteum secretes progesterone hormone that facilitates the implantation and establishment of pregnancy. The decrease in the concentration of oestrogen inhibits ovulation (El An-daloussi *et al*., 2018).

The concentration of progesterone increased in Does fed okra fruits and Does fed both okra fruit and leaf. Progesterone concentration in the study varied significantly towards parturition with Does fed okra fruit having the highest concentration (3.69 ng/mL). This result is similar to that of Mondal *et al*. (2014) who reported the plasma progesterone concentration of black Bengal goats to be 3.41±0.14 ng/mL on day 5 prior to parturition.

During gestation, fig 2 shows there was little evidence of an increase in serum progesterone concentration above levels attained during the luteal phase of the oestrus cycle. De Montigny *et al.* (1982) also reported > 1.5 ng/mL as being compatible with pregnancy.

CONCLUSION

Based on the result of the study, it can be concluded that okra (*Abelmoschus esculentus* L.) fruits with or without the inclusion of okra leaves increases the concentration of serum progesterone prior to parturition in WAD does.

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