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INFLUENCE OF FEVER BARK STEM BARK EXTRACTS ON ESTRUS SYNCHRONIZATION AND REPRODUCTIVE PERFORMANCE OF BUNAJI HEIFERS IN ADAMAWA STATE

Efusiauten, O. N., Bobbo, A. G., and Ardo, M. B.
Department of Animal Science and Range Management, Modibbo Adama University, Yola, Adamawa State Nigeria
Corresponding author's E-mail: obadiahnoah@yahoo.co.uk

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ABSTRACT

This research conducted to assess the efficacy of Crossopteryx febrifuga (fever bark) stem bark extracts on estrus synchronization of Bunaji heifers. Fifteen Bunaji heifers aged between three and four years and two bulls were used to determine the onset of estrus and conception rates. The heifers allotted to five different treatment groups, T_1, T_2, T_3, T_4, and T_5, for ethyl acetate, ethanol, aqueous extracts, $PGF_2\alpha$ and placebo as positive and negative controls respectively. Each treatment was replicated three times. Of the three heifers administered with ethyl acetate, a heifer displayed heat within 24-46hrs, one heifer in 48-72hrs and the remaining heifer greater than 96 hrs. Similarly, animals administered with Ethanol, one showed heat within 24 -48hrs, one in 48-72hrs and one above 96hrs. Furthermore, one heifer came on heat in 48-72hrs and the remaining two greater than 96hrs after the administration of aqueous extracts. Two heifers showed heat in 24-48 hrs, while the remaining in 48-72hrs when administered with $PGF_2\alpha$. All the heifers in control group were negative. The presence of squamous epithelial cells, cornified epithelial cells, and spermatozoa in the heifers administered with the extracts and $PGF_2\alpha$ is a confirmation that the heifers were on heat. The estrus and conception rates were 100,100,100 and 66.67% for Ethyl acetate and Ethanol, $PGF_2\alpha$ and Aqueous extracts respectively. $PGF_2\alpha$, ethyl acetate, and ethanolic extracts were better synchronizing agents compared to the aqueous extract indicating that the extracts contained phytochemicals and hormones of veterinary importance. Ethanol and ethyl acetate extracts recommended as alternative to synthetic synchronizing agents.

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INTRODUCTION

Estrus synchronization involves manipulating the estrous cycle within a herd to express estrus approximately at the same time, or the manipulation of estrus to bring a large percentage of group of females into estrus at a short-predetermined time (Odde, 1990). It is very useful in large herd size where individual animal monitoring is difficult and often subjective or because small intensively managed herds are milked in robotic systems that minimize animal staff interactions (Macmillan, 2010). The term "estrus" refers to the phase of the estrus cycle in which a sexually mature non- pregnant female is receptive to mating. This period commonly often referred to as "heat period". This periodic pattern of sexual receptivity is the result of organized and complex hormonal changes that occurs in the reproductive system of cattle (Xu, 2011). Ovulation occurs at approximately this time, depending on the specie of animal. It is the process of targeting female mammals to come on to heat within a short time from (36 to 96 hours). This is achieved through the use of one or more hormones. It optimizes labour and time and improves the ease of using artificial insemination (AI) **(**Lamb *et al.,* 2010**)**. Furthermore, estrus synchronization is a labour saving breeding management tool effective in artificial insemination (AI) and embryo transfer program. Groups of females benefited in perspective of parturition by decrease labour, decrease calving period; reduce calving season

and more uniform weaning weights. It synchronized by a large group to ovulate at same time, helps in scheduling the parturition time at most favourable season when the new-borns can be reared in suitable environment with ample food for better survivability and at the same time also facilitates short calving season, reduce labour required for AI breeding, marketing of uniform calf crop (same age) and improves management practices (cattle grouped - closer observation, better feeding practice etc.). Estrus synchronization has a number of advantages but still possess few problems in its practical use like low conception rates, expensiveness, skilled labour etc. (Prajapati *et al.,* 2019).

WHO (2023) has recognized the value and imperative need for adopting traditional herbal practices in global health care and recommended all member countries to promote native herbs of their country as well as to initiate steps to conserve and/or cultivate herbal plants, so that genuine raw materials become readily available to large section of the population. Ethno Veterinary Practices (EVPs) have significant contribution in maintenance of animal health and regarded as sustainable veterinary medicine in the new era (Lin *et al*., 2003). The increase in the use of herbal products is due to their cultural acceptability, availability, affordability, efficacy and safety claims. Herbal products are considered today to be the symbol of safety in contrast to the synthetic drugs that are regarded as unsafe to human beings, farm animals and environment. The blind dependence on synthetics is over and people are returning to the natural with hope of safety and security. It is time to promote them globally (Khan, 2016; UNESCO, 1998).

Therapies involving herbal products like extracts derived from *C. febrifuga* have shown promising potential but many of such products remain untested and their use are either poorly monitored or not even monitored at all. Medicinal plants have been utilized for centuries across various cultures for treating both human and animal diseases. The increasing interest in natural product research necessitates systematic studies of traditionally used plants like *Crossopteryx febrifuga* which has been employed in West African ethno veterinary medicine but remains insufficiently investigated scientifically (Ajaiyeoba *et al*., 2006). *Crossopteryx febrifuga* commonly known as “fever bark” is a small tree widely distributed across tropical Africa. It is commonly found in all parts of Nigeria and known as Ayeye among Yorubas in Nigeria (Ojewale, 2014). Hausa people of Northern Nigeria call it Kasfiya, Hashin Awaki or Giyayyata (Halilu *et al*., 2012). Traditional healers have utilized various parts of this plant to treat fever, dysentery, pain and reproductive disorders in livestock (Halilu *et al.,* 2012). It is widely used in the management of malaria, fever and painful inflammatory disorder (Adeola *et al.,* 2011). It is used traditionally for symptomatic relief of dry cough and for treatment of respiratory infections, fever, dysentery and pain. The decoction of the stem bark is used in central Africa as an antipruritic lotion. The infusion of the root bark is used in treatment of fever, malaria, diarrhoea, intestinal worm and opthalmia and for application to wounds (Edeoga *et al.,* 2005). It has been reported that the crude methanolic extract contains biologically active substances with potential values in the treatment of Trypanosomiasis, Malaria, *Staphylococcus aureus* infection (Yusuf *et al.,* 2006; Hostettmann *et al.,* 2000). Salawu *et al.* (2009) have reported that the extract possesses analgesic, antipyretic and anti-inflammatory activities. There are claims by some herbal practitioners that the aqueous roots extract of *C. febrifuga* is effective in the management of Diabetes and diabetic complications (Ojewale *et al.,* 2014).

So far, there is dearth of information and very limited research findings on the use of *C. febrifuga* as synchronizing agents and the management of infertility in cattle in this environment.

MATERIALS AND METHODS

The Study Area

The experiment carried out in Duware, 2km away from Yola south metropolis. Yola South Local Government created in 1996 and has an area of 719km^2. The Local Government bounded to the North by Yola North and Girei, East and South by Fufore and to the West by Demsa Local Government area of Adamawa. It lies at Latitude 9^0 and 14^1 North and Longitude 12^0 and 28^1 East and altitude of about 152 Metres above sea level and it cover an area of

about 54 hectares. The climatic conditions of the area are constant. The temperature of the area is high throughout the year, with the maximum temperature of about 42^0C observed with minimum temperature range of between 26.9^0C and 27.8^0C. The rainfall shows a variable element of tropical climate to which most of the characteristics such as amount, frequency and intensity vary widely with time (season), it usually starts during the month of April to October with an annual range of 1500 – 2000 per annum and this is accompanied by high relative humidity (Alexander, 2015). The relative humidity is very low between January and March about (20-30%) which will starts increasing from April to reach its peak in August and late September, while the cool dry harmattan starts in November to February (Adebayo, 2010). The study area falls within the North Guinea Savannah vegetation zone and has a tropical wet and dry climate. The zone is characterized by high grassland with shrubs and fewer trees. Yola South has a population density of 386, with Yola town, Namtari, Ngurore, Njoboli and Yolde pate as the major settlements (Adebayo *et al*., 2020)

Plant Collection and Identification

The stem bark of *C. febrifuga* plant collected from Sebore farm, Mayobelwa Local Government Area of Adamawa State. The plant identified by the staff of the Department of Forestry and Wildlife of Modibbo Adama University Yola with voucher number 1576 (*Crossopteryx febrifuga*). The stem bark was shade-dried at room temperature for 14 days in a clean environment to avoid contaminations. The sample was grinded into fine powder using pestle and mortar. The powdered plant sample was stored in appropriate container until required for use.

Extraction Methods

Three extraction methods were used and these are: Solvent extraction method using ethanol, solvent extraction method using ethyl acetate and solvent extraction method using aqueous

Solvent Extraction Method Using Ethanol.

Extract of the stem bark was made by maceration method using 95% Ethanol. After vigorous shaking to ensure thorough mixing, it was allowed to settle for 48 hours. The solution was filtered using cheese cloth and allowed to evaporate in an oven at a temperature 50^0 C. A dark green residue obtained after the evaporation process is the ethanol extract.

Solvent Extraction Method Using Ethyl Acetate

Extract of the plant was made by maceration method using ethyl acetate as a solvent. Two hundred (200) grams of shade dried stem bark powder was extracted with ethyl acetate for 72 hours at room temperature. The extract was filtered through cheese cloth and through Whatman filter paper No 1. The filtrate was evaporated to 500ml at room temperature and then in a vacuum concentrator. The dried powder obtained was stored in a sterile glass bottle and used for the research as described by Javarappa *et al*. (2016).

Solvent Extraction Method Using Aqueous

A standard method was used to obtain the aqueous extract by boiling the powder of the stem bark in water at a temperature range of 60-80^0 C for approximately 20-30 minutes and the mixture filtered through Whatman filter paper to obtain the aqueous extract used for the research.

Experimental Animals

Fifteen (15) Bunaji (White Fulani) heifers aged between three and four years and two (2) bulls with average body condition score (BCS) of 6 were used in the study. The animals were managed semi - intensively throughout the study period which lasted for over three (3) months.

Routine Management

The heifers and bulls were treated with Oxytetracycline for promoting and boosting the general health condition of the animals, Multivitamins to enhance or improve the appetite and libido of the animals, Ivermectin for control of both internal and external parasites. The animals were adequately fed with maize bran, cotton seed cake, fresh grasses/shrubs, salt lick and watered ad libitum.

Heat Detection

Heat is a short period of sexual receptivity of open cows and heifers, normally occurring in every 18-24 days. Each synchronized heifer was observed for primary and secondary signs for heat detection. The primary sign is standing still for mounting, while the secondary signs are, mount other heifers, friendly disposition, clear mucus from vagina and swollen vulva. A minimum of 30 minutes three times in a day

was spent watching the heifers for standing heat detection as described by Smith (1994).

Kamar heat mount detector was also used to detect heat. Kamar heat mount detector is a pressure sensitive device with in-built timing mechanism that is activated by standing heat behaviour. When glued onto the tail head of the heifer, pressure from the brisket of a mounting bull turns the detector from white to red in about three seconds (M'Barek, 2003).

Experimental Design

Fifteen (15) bunaji heifers aged between three (3) and four (4) years and two (2) bulls with good body condition score were used in the study. The heifers allotted to five (5) different treatments. Each treatment was replicated three times. Heifers were administered the following - ethanol, ethyl acetate and aqueous extracts, prostaglandin ($PGF_{2\alpha}$) and distilled water (placebo) as positive and negative control for groups 1,2,3,4, and 5 respectively. Kamar heat mount detector was glued onto the tail head of each of the heifers which were ear tagged for heat detection and easy identification respectively. Two bulls were introduced for natural breeding immediately after the administration of the extracts with uniform concentration of 20% in 2mls intramuscularly per heifer, $PGF_{2\alpha}$ – 2mls (standard dose) and placebo (2mls) per heifer through intramuscular injection (IM). Vaginal swabs of each of the heifers with positive signs of estrus as indicated by Kamar heat mount detector were collected in transport media (neutral formalin) and analysed for vaginal cytology in the laboratory as confirmatory test for estrus.

Statistical Analysis

Simple descriptive statistics (percentage) was used to determine the onset of estrus, estrus response rate and conception rate.

RESULTS

Confirmation of Estrus by Vaginal Cytology

The result of vaginal cytology revealed that two of the heifers treated with Ethyl Acetate extract of *C. febrifuga* (stem bark) showed the presence of moderate positive (++) squamous epithelial cells while one heifer showed positive (+) presence of squamous epithelial cells. Two heifers treated with the same extract showed positive (+) presence of cornified epithelial cells while one heifer showed moderate positive (++) presence of spermatozoa and two heifers showed positive (+) presence of spermatozoa.

Three of the heifers treated with Ethanolic extract of *C. febrifuga* (stem bark) showed positive (+) presence of squamous epithelial cells and spermatozoa while one heifer shows positive (+) presence of cornified epithelial cells.

One of the heifers treated with Aqueous extract of *C. febrifuga* (stem bark) showed strong positive (+++) presence of squamous epithelial cells and two heifers showed positive (+) presence of squamous epithelial cells while two heifers treated with the same extract showed moderate positive (++) and one heifer showed positive (+) presence of spermatozoa respectively. The result also revealed that one of the heifers treated with prostaglandin (PGf_2 alpha) showed moderate positive (++) presence of squamous epithelial cells and two heifers treated with same synchronizing agent showed positive (+) presence of squamous epithelial cells while two heifers showed strong positive (+++) presence of spermatozoa and one heifer showed moderate positive (++) presence of spermatozoa.

None of the heifers treated with Placebo (Negative control) showed the presence of all parameters as seen in Table 1.

Onset of Estrus and Estrus Response Rate

There were variations in the onset of heat when the bunaji heifers administered with different plant extracts and $PGf_2\alpha$. Of the three heifers administered with ethyl acetate, a heifer displayed heat within 24-48hrs, one heifer in 48-72hrs and the remaining heifer greater than 96 hrs. Similarly, animals administered with Ethanolic extract, one heifer showed heat between 24 -48hrs, one in 48-72hrs and one above 96hrs. Furthermore, one heifer came on heat in 48-72hrs and the remaining two heifers showed heat greater than 96hrs after the administration of aqueous extracts. Two heifers showed heat in 24-48 hrs, while the remaining in 48-72 hrs when administered with $PGf_{2\alpha}$, All the heifers in control group were negative. The estrus and conception rates were 100,100,100 and 66.67% for Ethyl acetate, Ethanol, $PGf_2\alpha$ and Aqueous extracts respectively.

Table 1: Confirmation of Estrus by Vaginal Cytology

S/N	Test	Features of vaginal cytology	Outcome of vaginal cytology at the time of estrus											
			24 – 48 hours			72 – 96 hours					>96 hours			
			A	B	C		A	B	C			A	B	C
1	T_1	Squamous epithelial cells	++	-	-		-	+		-		-	-	++
		Cornified epithelial cells	+	-	-		-	+		-		-	-	-
		Spermatozoa	++	-	-		-	+		-		-	-	+
		Pus cells	+	-	-		-	-		-		-	-	-
2	T_2	Squamous epithelial cells	+	-	-		-		+	-		-	-	+
		Cornified epithelial cells	-	-	-		-		+	-		-	-	-
		Spermatozoa	+	-	-		-		+	-		-	-	+
3	T_3	Squamous epithelial cells	-	-	-		+		-	-		-	+++	+
		Cornified epithelial cells	-	-	-		-		-	-		-	++	-
		Spermatozoa	-	-	-		++		-	-		-	++	+
4	T_4	Squamous epithelial cells	++	+	-	-			-	+	-		-	-
		Cornified epithelial cells	-	-	-		-		-	-	-		-	-
		Spermatozoa	+++	++	-	-			-	+++	-		-	-
5	T_5	Squamous epithelial cells	-	-	-	-			-	-	-		-	-
		Cornified epithelial cells	-	-	-	-			-	-	-		-	-
		Spermatozoa	-	-	-	-			-	-	-		-	-

Keys

T_1 = Ethyl acetate extract T_2 = Ethanol extract

T_3 = Aqueous extract T_4 = PGf2α (Prostaglandin)

T_5 = Placebo ++ = Moderate positive

+++ = Strong positive - = Nil/Negative

> = Greater than + = Positive

Table 2: Onset of Estrus, Estrus and Conception Rates of Bunaji Heifers Administered with different extracts, $PGf_2\alpha$ and Placebo

			Time of onset of estrus (hrs)					Response rate (%)	
Synchronizing agent	No of heifers	Heifers age (years)	0-24 hrs	24-48hrs	48-72hrs	72-96hrs	>96hrs	Estrus	Conception
Ethyl acetate extract	3	3-4	0	1(33.33)	1(33.33)	0	1(33.33)	3(100)	3(100)
Ethanol extract	3	3-4	0	1(33.33)	1(33.33)	0	1(33.33)	3(100)	3(100)
Aqueous extract	3	3-4	0	0	1(33.33)	0	1(33.33)	2(66.67)	2(66.67)
$PGF_2\alpha$	3	3-4	0	2(66.67)	1(33.33)	0	0	3(100)	3(100)
Control	3	3-4	0	0	0	0	0	0	0

DISCUSSION

The presence of spermatozoa, squamous epithelial cells and cornified epithelial cells as confirmed by vaginal cytology has shown that the heifers treated with the various extracts of *C. febrifuga* and prostaglandin ($PGf_2\alpha$) were at the various stages of estrus. This is in agreement with Suebkhampet and Chaikhun-Marcou (2024); Siregar *et al*. (2016) who observed that there are histological changes in the vaginal mucosa of the cow during oestrus cycle and following exogenous hormone such as estraidiol benzoate and progesterone treatment. The positive (+) presence of pus cells in one of the heifers treated with ethyl acetate extract of *C. febrifuga* could be as result of contaminants during the time the vaginal sample was taken for cytology.

Eight (66.67%) heifers administered with synchronizing agents in this study exhibited signs of estrus within 48-72hrs and this is partly in agreement with Lemaster *et al.* (2001) who reported that 60% of cross bred cows used in their study showed estrus 48-72hrs after $PGF_2\alpha$ injection. All the remaining heifers displayed heat within 14 to 21 days of administration and is in in agreement with Kebede *et al.* (2013) who reported that heat detection period should be extended to 7 to 10 days or more as most of the cows studied came to standing estrus after mass synchronization operation was concluded.

The variation in the onset of estrus exhibited by the animals may be due to the stage of follicular development at the time $PGF_2\alpha$ in T_4 and other test substances in T_1, T_2, and T_3 were administered which after affected the interval from ovulation to standing heat. Another reason for the variation in the onset of estrus could be due to the physiological response of the animal at the time of injection of the test substances (Repasi *et al.,* 2003).

Estrous synchronization will not substitute for lack of nutrition, herd health or poor herd management and it is not effective in non – cycling females (Tom, 2012). The development of methods to control estrous cycles of the cow has occurred in various distinct phases. Regulation of estrous cycle was believed to be associated with control of the corpus luteum whose life span and secretory activity are regulated by trophic and lytic mechanisms (Patterson *et al.,* 2003). Numerous estrous synchronization protocols developed that use a combination of different drugs and products to alter hormonal changes in the female's estrous cycle. Since different reproductive hormones are used in estrous synchronization, developing a basic understanding of several reproductive hormones is helpful for determining which protocol will work best (Ted and William, 2017).

The results also indicated that the heifers responded positively to the stem bark extracts of *Crossopteryx febrifuga* and the plant contained the bioactive compound that has ability to regulate female reproductive cycle by ensuring the regression of corpus luteum (luteolysis), initiating the development of new follicles, guarantee ovulation and reinitiate the releasing of progesterone to maintain the pregnancy as reported by Bobbo *et al.* (2021).

CONCLUSION

The study revealed that extracts of *Crossopteryx febrifuga* (stem bark) are effective in synchronization and conception of our indigenous heifers aged between three (3) and four (4) years. Comparison of the extracts and $PGf_{2\alpha}$ in terms of estrus synchronization and conception rates showed that ethyl acetate, ethanol extracts and $PGf_{2\alpha}$ are better synchronizing agents than the aqueous extract.

RECOMMENDATIONS

Extracts of *C. febrifuga* (stem bark) can serve as alternative to synthetic synchronizing agents like $PGF_2\alpha$ which are scarce, costly and less acceptable to our local livestock farmers. Further studies on the pharmacological activities of *C. febrifuga* should be investigated in animals.

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