

Detection of Sex Steroid Hormones in Alfalfa and Some Rangeland Native Species in Saudi Arabia and Their Subsequent Effects on Camel Reproduction

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Abstract: The main objectives of the current study were to explore the existence of the three natural sex steroid hormones [i.e. testosterone (T), estradiol 17- β (E2) and progesterone (P4)] in the alfalfa and other 17 local natural rangeland native weeds that are predominant in the desert of the central region of Saudi Arabia and to advise the owners of camels that are grazing on such range species and suffering from some reproductive inefficiencies for the best method to avoid the harming weeds. In addition to alfalfa (*Medicago sativa*), seventeen samples of different rangeland species were collected from different locations in Al-Qassim region, central of Saudi Arabia. Plants were dried, ground and subjected to extraction. Commercial enzyme immunoassay kits were used for the hormone determinations. Data exhibited large variations among species in their sex hormone contents. The highest T concentrations were found in *Cakile arabica* (3.69 ng/g DM) and *Cyperus conglomerates* (2.97 ng/g DM). However, there were no detected levels of testosterone in six of the tested species, i.e. *Plantago boissieri*, *Rhanterium epapposum*, *Haloxylon salicornicum*, *Heliotropium bacciferum*, *Cenchrus ciliaris* and *Medicago sativa*. On the other hand, E2 concentration was found to be the highest in *Lactuca serriola* (378.89 pg/g DM), *Eruca sativa* (246.89 pg/g DM) and *H. bacciferum* (229.29 pg/g DM), while no E2 levels were detected in *Leptadenia pyrotechnica*, *R. epapposum* and *Stipagrostis pulmosa*. On the other hand, P4 concentrations were the highest in *L. serriola* (53.96 ng/g DM), *Neurada procumpens* (51.15 ng/g DM), *E. sativa* (51.15 ng/g DM), *Atriplex leucoclada* (46.93 ng/g DM) and *C. arabica* (44.13 ng/g DM). Contrariwise, the lowest P4 concentrations were detected in *P. boissieri* (17.43 ng/g DM) and *R. epapposum* (19.82 ng/g DM). Due to the presence of these hormones, some she camels suffer from cystic ovarian syndrome and delayed pregnancy.

Key words: Range plants • Alfalfa • Progesterone • Testosterone • Estradiol 17 β • Camel Reproduction

INTRODUCTION

In arid regions some rangeland species are highly nutritious and palatable forages. Studies have indicated that the quality of forage varies with the soil, season, rainfall, chemical nature and age of the plants that affects palatability and health of grazing animals [1-8]. Range animal productivity depends upon the amount and nutritive quality of vegetation available. Moreover, the health of livestock depends on the nutritional value of available forage [9].

Reproductive disturbances in animals grazing certain pasture species were first observed in Australia in connection with the increased use of subterranean clover (*Trifolium subterranean*) as a sheep pasture [10]. Since then, Moule [11] reviewed all the subsequent Australian research concerning infertility in sheep associated with estrogens in forage crops. Janeczko and Skoczowski [12] studied 128 species from over 50 families and stated that androsterone and progesterone were found in more than 80% of the investigated species androgens (testosterone and dihydrotestosterone) in 70%

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of species and estrogens (estrone and 17 β -estradiol) in 50% of species. Zhang *et al.* [13] estimated the content of total estrogens and 17 β -estradiol in pollen and in the style of *Ginkgo biloba* L., *Zea mays* L. and *Brassica campestris* L. Estradiol-17 β was present in the pollen of these plants in the range of 8-35 pg/g DM. In the style of *Lilium davidi* Duch., estradiol-17 β was found in the range concentration of 24-40 pg/g DM. Moreover, the mentioned authors showed changes of total estrogen and 17 β -estradiol concentration during flower development. Strain differences in plants must also be considered and may well explain some different estrogenic effects of these plants [14].

Early exposure to sex steroids permanently alters the sexual behavior of fish, amphibians, reptiles, birds and mammals [15], although the role of sex steroids in differentiation of gonads and gonadal ducts does appear to vary among classes of vertebrates.

In adult female mammals of different species, it is proven that estrous behavior is critically dependent upon estrogen production/exposure [16]. The prerequisite of raised plasma estradiol results in an ovulatory surge of luteinizing hormone in spontaneously ovulating mammals such as rodents, ruminants, canines and primates, whereas reflex ovulating mammals such as felines, rabbits and camels have essentially continuous estrogen production from overlapping waves of maturing ovarian follicles [16]. Therefore, it was the goal of the present study to highlight the sex steroid hormone contents in alfalfa (*Medicago sativa*) and some rangeland species grazed by ruminants in the central region desert of Saudi Arabia and their subsequent effects on camel reproduction.

MATERIALS AND METHODS

Plant Materials Collections: Beside alfalfa (*Medicago sativa*), seventeen species of rangeland plants (listed below) were collected from several rangeland locations in Al-Qassim region.

Plant Species:

- *Cakile arabica*
- *Bassia eriophora*
- *Cyperus conglomerates*
- *Plantago boissieri*,
- *Rhanterium epapposum*,
- *Rumex vesicarius*
- *Halexylon salicornicum*,

- *Heliotropium bacciferum*,
- *Lyceum shawii*
- *Cenchrus ciliaris*
- *Lactuca serriola*
- *Leptadenia pyrotechnica*
- *Eruca sativa*
- *Ochradenus baccatus*
- *Stipagrostis pulmosa*.
- *Neurada procumpens*
- *Atriplex leucoclada*

Plant Sample Preparation: Plant samples were washed in distilled water and then dried in an oven at 105°C for two hours. Samples were cut in small pieces of 2-3cm length and grinded in a mill. Duplicate of 1 gram each was taken for each species and exposed to steroid extraction.

Sex Steroid Hormones Extraction: Duplicates of one gram of ground samples were taken in 15-ml glass tubes. Four milliliters of methanol (90%) were added to the sample and centrifuged (3000 rpm /20min). The supernatant was taken and exposed to a nitrogen stream until all solvent was dried. The precipitate was then dissolved in one milliliter phosphate buffered saline. This preparation was ready to the determination of the three hormones.

Progesterone, Estradiol 17 β and Testosterone Determinations: The three hormones were determined by commercial kits. Progesterone concentration was measured by a simple solid phase enzyme immunoassay according to Radwanska *et al.* [17]. Intra and interassay coefficient of variation (C.V) were 3.4% and 4.6%, respectively. Testosterone was measured according to Chen *et al.* [18] with intra-and interassay C.V of 7.4 and 5.5% respectively. Estradiol 17 β was determined according to Peckham and McElwain [19] with intra and interassay C.V of 4.6 and 6.7%, respectively.

Camel Female Reproductive Traits: Thirty two adult multiparous she camels belong to several bedouins who leave their animals to graze on natural rangeland vegetation. The above tested plants were the predominant species in the area. The animals owners main complain was the delayed occurrence of pregnancy with frequent mating.

Genital Tract Ultrasonography: Animals were subjected for ultrasound diagnoses (Aloka SSD 500, Japan) using 5-10 MHz transrectal probe.

RESULTS

As illustrated in Figure 1, progesterone (P4) concentration per gram dry matter of the plant samples was high in *L. serriola* (53.96 ng/g), *N. procumpens* (51.15 ng/g), *E. sativa* (51.15 ng/g), *C. conglomerates* (49.74 ng/g), *A. leuoclada* (46.93ng/g), *L. shawii* (44.83 ng/g) *Cakile arabica* (44.13 ng/g). However, the lowest P4 concentration was found in *P. boissieri* (17.43 ng/g), *R. epapposum* (19.82ng/g) and *S. pulmosa* (23.05 ng/g). Other plants contain intermediate P4 concentrations ranging 23.9- 35.98 ng/g.

Figure 2 depicts estradiol 17β (E2) concentrations. The high P4 concentration was found in *L. serriola* (378.89 pg/g), *E. sativa* (246.89 pg/g) and *H. bacciferum* (229.29 pg/g). On the contrary, the low non detected E2 concentration (0 pg/g) was found in *R. epapposum*, *S. plumosa* and *L. pyrotechnica*. Other plant species contained intermediate E2 concentrations ranging 50.58- 172.43 pg/g DM.

Testosterone concentration (Figure 3) was found to be high in *C. arabica* (3.69 ng/g) and *C. conglomerates* (2.97ng/g), *R. vesicarius* (1.66 ng/g) and *O. baccatus* (1.22 ng/g), low in *B. eriphora* (0.1 ng/g), *L. pyrotechnica*

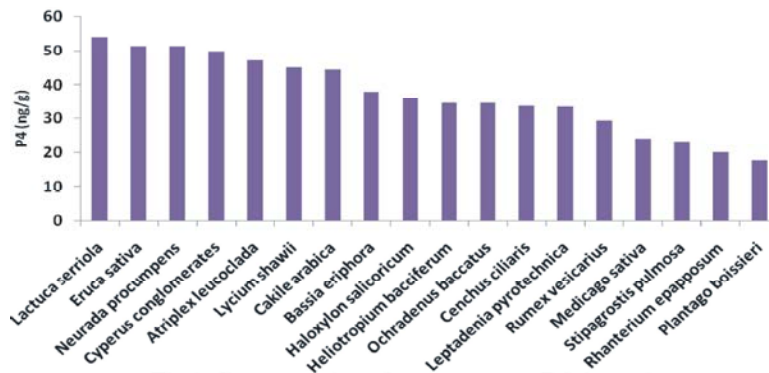


Fig 1: Concentration of Progesterone (P4) in Various Range Plants

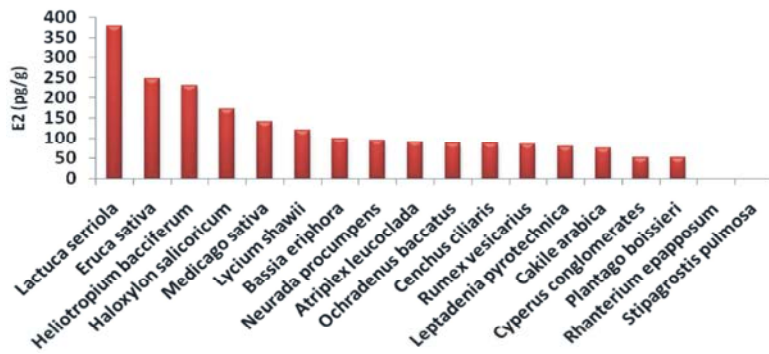


Fig 2: Concentration of Estradiol 17 B (E2) in Various Range Plants

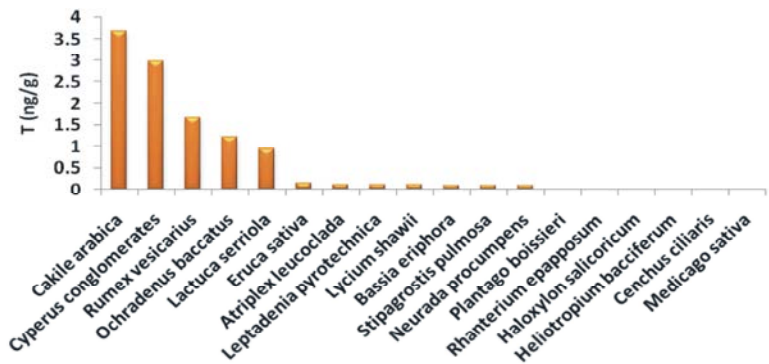


Fig 3: Concentration of Testosterone (T) in Verious Range Plants

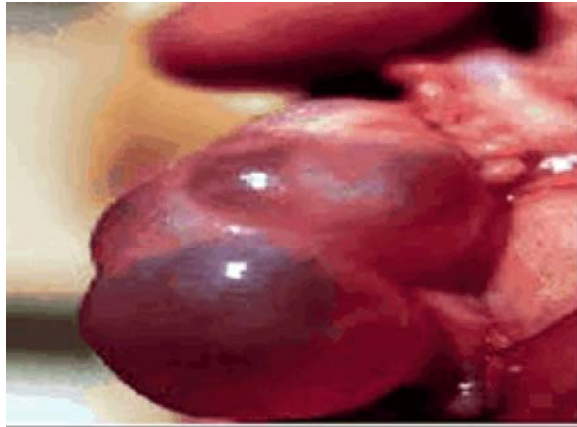


Photo 1: Cystic ovarian follicles in camel female grazed on desert range plants rich in estrogens

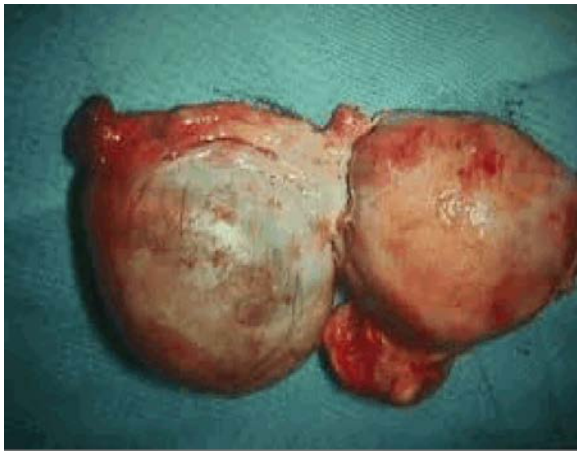


Photo 2: Cystic ovarian corpus luteum in camel female grazed on some desert range plants rich in progesterone.

(0.12 ng/g), *A. leuoclada* (0.12 ng/g), *S. pulmosa* (0.1 ng/g), *L. shawii* (0.12 ng/g), *N. procumpens* (0.1 ng/g) and *E. sativa* (0.15 ng/g). No testosterone (0 ng/g) was detected in *P. boissieri*, *R. epapposum*, *H. salicornicum*, *H. bacciferum*, *C. ciliaris* and *M. sativa*.

The ingestion of large amounts of *L. serriola* in addition to *M. sativa*, *H. bacciferum* and *H. salicornicum* might cause irregularities of estrous cycles and cystic ovaries (Photo 1 and 2) in grazing animals.

DISCUSSION

Similar results for the current study were reported earlier by Janeczko and Skoczowski [12] in 128 species from over 50 families. They found androsterone and progesterone in more than 80% of the investigated species. Moreover, they also reported differences in E2 concentration between investigated species. They found estrogens (estrone and 17 β -estradiol) in 50% of 128 species from over 50 families. They also reported the

presence of androgens (testosterone and dihydrotestosterone) in 70% of plant species investigated.

Moreover, Stob *et al.* [14] stated that strain differences in plants must also be considered and may well explain some different estrogenic effects of these plants.

Different stages of growth during sampling period, could also explain the differences in hormones concentration in the investigated plant species. Zhang *et al.* [13] showed changes of total estrogens and 17 β -estradiol concentration during flower development.

Eruca sativa is traditionally considered as a general tonic and potent aphrodisiac. It is known generally as a food, in which the leaves are eaten as part of salads. It has been known as a garden vegetable since Biblical Times. Moreover, there are many records of its household usage from the Hellenistic period onwards. It has been found to promote fertility, sperm production and treat men impotence [20]. Also, *L. serriola* is known as wild lettuce

and was mentioned to have potent antioxidants [21]. To date no report has indicted the steroidal content in this wild lettuce. The current study revealed the existence of high E2 contents in this plant. Most reproductive disorders in camel females admitted to the university clinic were mainly due to either cystic ovarian syndrome or smooth ovaries. The cystic ovarian follicles and corpora lutea in camel grazing on desert rangeland in Saudi Arabia represent about 45% of the reproductive inefficiencies in this animal. About 12% of the examined females were of smooth inactive ovaries. The most potent steroid deteriorating the function of the hypothalamo-pituitary-ovarian axis is estrogens, mainly estradiol. Excess external ingestion of estradiol might cause a block on the axis, resulting in impediment for the release of luteinizing hormone with ultimate ovarian follicular cyst [22].

Raised testosterone above the normal level in females was also found to be a cause of ovarian corpus luteum cyst [23]. Increased progesterone in blood circulation might also impede the release of gonadotrophin hormones from anterior pituitary resulting in extended estrous cycles with non ovulation [24]. Deficiency of LH surge is considered the main cause of cystic ovaries and the changes of the pituitary are related to the feedback mechanism from the higher levels of the ovarian steroids [25].

CONCLUSION

Although some of the rangeland species are notorious and palatable to the animals, there still are lacks of knowledge in the effects of each individual specie upon the productive as well as the reproductive performances of the native animals which should be a future focus to a multidisciplinary study in this area. Also, the stage of ripening of the plant might constitute an important factor to be taken in consideration at evaluation for the steroidal contents.

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