

Dietary and Seasonal Effects on Body Weight, Ovarian Development and Blood Reproductive Hormone Levels in Peri-Pubertal Female Camels (*Camelus dromedarius*)

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Abstract: The aim of the present study was to investigate the effect of nutrition on body weight gain, ovarian development, blood components (total protein, albumen, globulin, cholesterol, glucose) and hormone (estradiol, progesterone, FSH, LH, GnRH and Leptin) levels in peri-pubertal female camels. Fourteen dromedary females (*Camelus dromedarius*) were divided into two groups (A and B) with respective average body weight and age of 381 kg, 24 months and 458 kg, 24 months at the start of the experiment. Group A received a diet with 13% Crude Protein (CP) and 2.9 Mcal Metabolizable Energy (ME). Group B received the traditional diet of the farm with 12.43 CP and 2.7 ME. Both diets contained 1:3 forage:concentrate ratio. Individual feed intake was calculated after a 14 days adaptation period. Feed offered andorts were recorded daily during the entire experimental period of 24 months. Blood samples were taken from the same 5 animals in each group at 15 day intervals throughout the experimental period. Hormone concentrations were measured using specific ELISA kits. Initial body weight, final body weight and average daily gain for the two treatment groups were not significantly different. Group B had greater ovarian size than group A but the difference was also non-significant. The size of the right ovary was less than the left ovary. Season had no significant effect on ovary's size. Group A camels tended to have higher blood estradiol, leptin, GnRh and LH levels than group B. However, the differences were not significant except in the case of estradiol. Progesterone and FSH levels were also comparable in the two groups. It was concluded that the feeding regimen used in this study did not significantly affect body weight, daily weight gain and blood progesterone level while a relative, though statistically non-significant, increase was recorded in blood estrogen, Leptin, GnRH and LH levels in group A.

Key words: *Camelus dromedarius*, body weight, ovary size, estradiol, progesterone, FSH, LH, GnRH, Leptin

INTRODUCTION

Despite the expansion of camel farming in Saudi Arabia where camel population is estimated at 800,000 (MOA, 2006) progress in improving camel productivity is poor. Reproductive efficiency is the primary factor affecting productivity and is hampered in the female camel by late puberty and long calving interval (Kaufmann, 2005; Musa *et al.*, 2006). Al-Qarawi *et al.* (2000) classified reproductive stages of camels according to age into: pre-pubertal (<3 years), peri-pubertal (3 to <5 years), mature (5 to <15) and aged (≥15). Age at puberty in the female camel was reported to range between 3-6 years this wide variation has been attributed to different environmental conditions under which these animals are kept (Matharu, 1966; Williamson and Payne,

1978; Khanna *et al.*, 1990; Shwartz, 1992; Musa *et al.*, 1993). Wilson (1989) reported that factors such as nutrition, body weight, photoperiod, temperature and water availability can influence the onset of sexual activity. Late puberty reduces the animal's productive lifetime. A high fertility level in the camel is therefore essential not only for profitable production but also to provide opportunities for selection and genetic improvement.

Tibary and Anouassi (1997) observed that well-fed and watered dromedary females show ovarian activity throughout the year and that the determining factors for observed seasonality in conception date (November to April) are related to a decrease in male libido during the Summer months. Nutrition also affects the overall growth, body weight and age at puberty. Animals on higher plain

of nutrition reach puberty earlier (Wilson, 1989) and the influence of body weight on puberty is more pronounced than the age of the animal (Marai *et al.*, 2009). Estrogen and progesterone are two important female sex steroid hormones. The secretion level of these hormones has a definite correlation with sexual behavior. Estradiol-17 β is responsible for the integrity of reproductive epithelia and development of secondary female sex characteristics (Sumar, 2000). Leptin is a 16 kDa hormone principally secreted by the adipose tissue (Zhang *et al.*, 1994). This hormone regulates the body fat mass by decreasing feed intake through interaction with hypothalamic leptin receptors (Tartaglia *et al.*, 1995). Amongst the neuroendocrine actions of leptin is its stimulatory effect on the hypothalamus-pituitary-gonadal axis this seems to be of particular physiological significance in consideration of the well-established causal link between nutritional status and reproduction in animals (Wade *et al.*, 1996; Ahima *et al.*, 2000) as well as in camel (Chilliard *et al.*, 2005; Delavaud *et al.*, 2013). It is thus, proposed that leptin may serve as a metabolic regulator of reproductive capability by signaling to the brain the amount of energy stored in the body (Smith *et al.*, 2002). Previous studies *in vitro* indicated that leptin acts directly on both the hypothalamus and the pituitary to stimulate the release of Gonadotropin-Releasing Hormone (GnRH) and Luteinizing Hormone (LH), respectively (Yu *et al.*, 1997a, b).

In a previous study, researchers investigated the effect of feed on body weight, ovaries development and serum estradiol and progesterone level in pre-pubertal female camel (Al-Saiady *et al.*, 2012). The following study was designed to evaluate the diet and seasonal effects on body weight gain, ovarian size and blood levels of some reproductive hormones of hypothalamic, pituitary and ovarian origins as reproductive indicators in peri-pubertal female camels.

MATERIALS AND METHODS

Animals and diets: This experiment was conducted at the Camel Breeding, Range Protection and Improvement Center in Al-Jouf Area, Saudi Arabia. Fourteen peri-pubertal dromedary females (*Camelus dromedarius*) were used. They were divided into two equal groups A and B, matched for body weight and age (381 kg, 24 months and 458 kg, 24 months, respectively). Group A received a diet with 13% Crude Protein (CP) and 2.9 Mcal Metabolizable Energy (ME). Group B received the traditional diet of the center (Table 1).

Animal's individual feed intake was calculated after allowing a 14 days adaptation period for group A camels.

Table 1: Diet ingredients and chemical composition (dry matter basis)

Items	Diet A	Diet B
Ingredients (%)		
Barley	60.22	62.23
Wheat bran	9.63	12.08
Soya Meal (48%)	4.25	-
Salt	0.47	-
Limestone	2.10	-
Acid buf	1.00	-
Molasses	3.00	-
Premix	0.30	-
Alfalfa	19.03	15.23
Wheat straw	-	10.46
Nutrients (%)		
Dry Mater (DM)	90.20	92.52
Crude Protein (CP)	13.08	12.43
Crude Fiber (CF)	10.19	15.35
Calcium	1.67	0.35
Phosphorus	0.42	0.27
ME (Mcal kg ⁻¹)*	2.90	2.70

ME: Metabolizable Energy, *Values reported should be Na, Cl, K, etc. (Salt is an ingredient and was already quoted). Acid buf-buffering agent

Feed offered and orts were recorded daily during the entire experimental period of 24 months. Diets contained 1:3 (roughage:concentrate, respectively). Diet A roughage and concentrates were combined in a pelleted form. Fresh water was freely available. Blood samples were collected from the jugular vein into vacutainer tubes with EDTA for plasma separation. Blood samples were taken in the morning from the same five animals from each group at 15 day intervals during the experimental period. Blood plasma was separated by centrifugation (1,500 g for 10 min) and frozen at -20°C until analyses. Estradiol, Progesterone, FSH, LH, GnRH and Leptin concentrations were determined using specific ELISA kits (Diagnostic Automation inc. CA. USA for Leptin and GnRH, GenxBio Health Sciences Pvt. Ltd. O.No. 6, S-553-554, Ground Floor, School Block, Shakarpur, Delhi-110092, India). The following parameters were also measured or calculated: bimonthly body weight in kg, the animals were weighed after 10 h. of fasting using a platform scale Mettler Toledo, 3000 kg capacity body weight gain in kg daily weight gain in kg/day. Ovary size and axes were measured by ultrasonography (SSD-500, Aloka Co., LMT Japan) and the size was calculated using the equation of ellipsoid volume: $4/3\pi a*b*c$ a.b.c. = axes of ellipsoid (<http://en.wikipedia.org/wiki/volume>).

Ambient temperature: Maximum and minimum ambient temperatures and dry and wet bulb temperatures were recorded daily and Temperature-Humidity Index (THI) was calculated:

$$THI = 0.72 (T_{db} + T_{wb}) + 40.6$$

Where:

T_{db} = Dry bulb temperature

T_{wb} = Wet bulb temperature

Statistical analysis: Data was subjected to statistical analysis using SAS Program (SAS, 2012). Data for changes in body weight were analyzed according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = The observation of the dependent variable obtained from jth animal of ith treatment

μ = The overall mean

T_i = The effect of ith treatment (i = A or B)

e_{ij} = The residual term

For ovary size and hormone levels the model was:

$$Y_{ijk} = \mu + T_i + S_j + e_{ijk}$$

Where:

Y_{ijk} = The observation of the dependent variable obtained from kth animal of ith treatment of jth season

μ = The overall mean

T_i = The effect of ith treatment (i = A or B)

S_j = Effect of jth season (j = Winter, Spring, Summer or Autumn)

e_{ijk} = The residual term

The General Linear Model (GLM), Least Squares Means (LSMEANS, correlations between hormones were determined using Pearson coefficient. The PROC MIXED procedure was used for repeated measures.

RESULTS AND DISCUSSION

Meteorological conditions: The meteorological data were recorded during the experimental period with average THI of 81 (Fig. 1). The hottest months of the year were July,

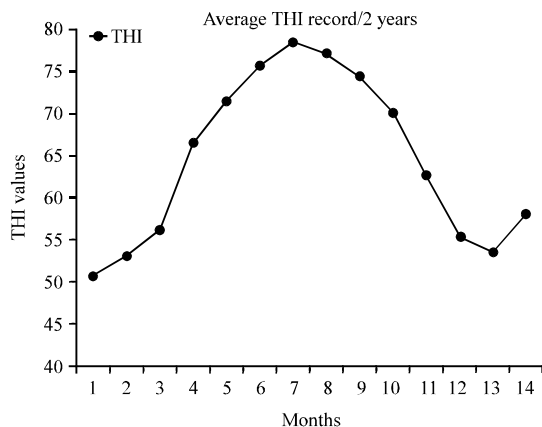


Fig. 1: Temperature-Humidity Index (THI) curve

August and September. The maximum temperature during these months ranged from 45.6-46.6°C while THI ranged from 78.9-83.1. It is documented that in the central region of Saudi Arabia, animals suffer from heat stress (Al-Saiady *et al.*, 2012).

Changes in live body weight and feed intake: The differences within treatment in initial and final body weight, body weight gain and daily weight gain were non-significant (Table 2).

Animals in group A consumed less feed than those in group B. This agreed with the increase in leptin level in group A (Table 3). Season of the year significantly affected feed consumption which was higher in Autumn and Winter compared to Spring and Summer (Table 4).

These results agreed with those by Mohamed (2006) who observed a clear variation in camel performance when fed different types of rations. Numerical values of daily weight gain were higher in group A than group B. The encouragement of rapid growth during the peri-pubertal period by a good plain of nutrition can assist early sexual development and breeding maturity in she-camel (Marai *et al.*, 2009).

Changes in ovaries size: No interaction between treatment and season was detected. The size of the left ovary was numerically higher than the right ovary

Table 2: Treatment effects on changes in body weight and body weight gain

Items	Initial weight	Final weight	Body weight gain	Daily weight gain
Treatments				
A	381.83±36.34	498.17±19.77	116.33±33.06	0.144±0.04
B	458.00±33.64	518.43±18.30	60.43±30.61	0.075±0.04

Table 3: Treatment and season effects on Leptin, GnRH, FSH and LH hormones

Items	Leptin (H. ng L ⁻¹)	GnRH (pg L ⁻¹)	FSH (IU L ⁻¹)	LH (IU L ⁻¹)
Treatments				
A	6.11±0.20 ^{ns}	268.65±5.17 ^{ns}	3.62±0.60 ^{ns}	1.28±0.14 ^{ns}
B	5.98±0.20 ^{ns}	261.58±5.17 ^{ns}	3.95±0.61 ^{ns}	0.99±0.14 ^{ns}
Seasons				
Autumn	5.88±0.31 ^b	282.84±7.71 ^a	4.53±1.01 ^a	1.90±0.24 ^a
Spring	5.95±0.31 ^b	256.60±7.71 ^b	4.43±0.69 ^a	0.85±0.16 ^b
Summer	5.53±0.31 ^b	236.91±7.71 ^b	4.43±0.69 ^a	1.04±0.16 ^b
Winter	6.82±0.24 ^a	284.12±5.98 ^a	1.72±0.98 ^b	0.74±0.23 ^b

Different letters within the same column indicates significant differences (p≤0.05)

Table 4: Treatment and season effects on feed intake

Items	Feed intake
Treatments	
A	6.44±0.04 ^b
B	6.55±0.04 ^a
Seasons	
Autumn	6.93±0.06 ^a
Spring	5.98±0.07 ^c
Summer	6.46±0.06 ^c
Winter	6.61±0.05 ^b

Different letters within the column indicates significant difference (p≤0.05)

Table 5: Treatment and season effects on ovaries size and blood estradiol and progesterone levels

Items	R. ovary (cm ²)	L. ovary (cm ²)	Estradiol (pg mL ⁻¹)	Progesterone (ng mL ⁻¹)
Treatments				
A	32.65±1.85	32.82±1.60 ^b	25.83±1.66 ^a	0.77±0.01
B	35.70±1.86	39.53±1.61 ^a	18.33±1.69 ^b	0.77±0.01
Seasons				
Autumn	32.85±2.39 ^b	37.00±2.07	21.82±2.79 ^b	0.71±0.02 ^b
Spring	31.30±2.39 ^b	34.18±2.07	22.92±1.92 ^b	0.80±0.01 ^a
Summer	33.14±2.39 ^b	37.12±2.07	14.72±1.92 ^c	0.75±0.01 ^b
Winter	38.38±2.00 ^a	37.34±1.73	28.89±2.71 ^a	0.81±0.02 ^a

Different letters within the same column indicates significant differences ($p \leq 0.05$). R = right; L = left

(Table 5) this finding agreed with Zeidan *et al.* (2011) who reported that the weight of the left ovary was greater than right one. The right ovary size was not significantly different between the two groups while the left ovary size in group B was significantly bigger than group A. This suggests that the increase in ovarian size in group B may not necessarily be due to increased activity of secretory cells because of the decrease estradiol level in this group. Season of the year had also a positive effect on the size of both right and left ovaries. This agrees with Zeidan *et al.* (2011) who reported that the highest weight of ovary was observed during Winter and the lowest during Summer. the results also agreed with Shujait *et al.* (2007) who reported that ovarian length, width and weight were significantly affected by season being greater in Winter and Spring compared to Summer and Autumn. Group A camels had significantly higher blood level of estradiol-17 β compared to group B ($p < 0.05$). Season also modified blood estradiol levels. The highest levels of estradiol-17 β were observed in winter and Spring when compared to Autumn and Summer. This result agreed with Zeidan *et al.* (2011), El-Hariary *et al.* (2010), Abd El-Azim (1996) and Agarwal *et al.* (1987) progesterone level in treatment A did not differ from treatment B. Season affected blood progesterone levels significantly. Higher level of progesterone was observed in Winter and Spring. Progesterone level in the present study was > 1.00 ng mL⁻¹. This result agreed with Homeida *et al.* (1988) and Skidmore *et al.* (1994) who reported that plasma progesterone level remained under 1.00 ng mL⁻¹ throughout the follicular wave.

FSH and LH secretion is regulated by GnRH from the hypothalamus. There was no significant difference between groups A and B in their plasma levels of Leptin, GnRH, FSH and LH hormones (Table 3). Higher levels of these hormones were reported in Winter and Autumn. The level of leptin negatively affected feed intake (Table 4). There was a highly positive correlation between Leptin and GnRH ($r = 0.56$) indicating that the former acts directly on the hypothalamus to release GnRH. This confirms earlier findings by Yu *et al.* (1997 a, b),

Table 6: Treatment effects on mean blood total protein, albumin, globulin, glucose and cholesterol

Items	Treatment A	Treatment B
Total Protein (TP) (g L ⁻¹)	62.32±1.29	59.97±1.29
Albumin (Alb) (g L ⁻¹)	45.21±1.19	42.37±1.19
Globulin (Glo) (g L ⁻¹)	17.11±0.99	17.60±0.98
Alb/Glo	3.11±0.18	2.91±0.18
Glucose (Glu) (Mmol L ⁻¹)	8.33±0.21	8.88±0.21
Cholesterol (Chol) (Mmol L ⁻¹)	66.04±3.22	60.82±3.22

Carro *et al.* (1997) and Smith *et al.* (2002). This correlation was higher in group A ($r = 0.63$) compared to group B ($r = 0.51$; $p < 0.0001$) suggesting that diet could have affected Leptin to stimulate the hypothalamus to secrete GnRH which in turn, stimulated the pituitary gland to secrete FSH and LH as reproductive regulators.

Physiological status: Blood parameters (Table 6) are good indicators of the physiological status of camels. All parameters measured in the present study were within normal physiological levels (El-Hariary *et al.* (2010) indicating that animals in both treatment groups had no health problems.

Albumin level was relatively but not significantly, higher in treatment A compared to treatment B. Albumin is one of the major transport proteins in the body and is the primary regulator of plasma osmotic pressure. Its normal level also reflects adequate protein intake.

CONCLUSION

It is concluded that under the conditions of this experiment, a complete, balanced ration with 13% crude protein, 2.9 Mcal ME affects reproductive hormones. Plasma estradiol concentration was significantly higher in the group fed this ration (group A) than that given traditional feed (group B). Leptin, GnRH and LH were also higher, albeit lack of significance in peri-pubertal females fed on the complete diet (group A) compared to group B receiving traditional camel diet (alfalfa+barley+wheat bran). The lack of significance in this case could be attributed to the small number of animals used. Further, studies using a larger number of animals are needed to compare the effects of the two diets in term of protein and energy on reproductive hormonal changes and the development of reproductive organs.

ACKNOWLEDGEMENTS

This study has been undertaken within FAO project UTF/SAU/021/SAU with the support of Camel and Range Research Center (CRRC). Researchers thank all the local staff in the camel farm for their assistance. Special gratitude is extended to Professor Mansour F. Hussein for editing and valuable suggestions. Researchers are

also grateful to ARASCO for providing the feed for this trial and for their R&D team especially those of the data processing and statistical division for the time and efforts invested in discussing the results of this research.

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