American-Eurasian J. Agric. & Environ. Sci., 10 (3): 354-360, 2011
ISSN 1818-6769

Seasonal Variations of Some Blood and Seminal Plasma Biochemical Parameters of Male Dromedary Camels

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Abstract: Blood samples were collected from eight Dromedary mature camel bulls of the Maghrabbi breed, for a period of one year. Samples were collected during pre-rut in November (autumn), rut in February (winter), post-rut in May (late spring) and after sexual activity had ceased in the non-rut in August (summer). The effect of season on some blood biochemical parameters was studied. The results showed no effect of season on total protein, while glucose and cholesterol level showed a significant increase during rut, while createnine level decreased during autumn and winter seasons. Testosterone levels reached the basal level during the non-rutting season (August) being 2.89 ± 0.26 ng/ml. In the pre-rut (November), significant increase ($P<0.05$) of testosterone level was detected reaching 5.8±0.74. This increase continued to reach a maximum of 7.95±1.85 during rut (February), then finally followed with a significant decrease during the post rut to reach 3.15±0.38 before reaching the basal level again. Total saturated fatty acids (SFAs) content was 47.85 ±2.79% in pre-rut, then showed significant high level being 65.57 ± 1.61% during winter. Contrarily a significant difference ($P<0.05$) was detected for unsaturated fatty acids (UFAs) content showing a decreased to reach 34.43 % in rut and a highest level of 53.15 ±3.69% in pre-rut. Electrophoretic pattern of serum proteins showed about 15 different fractions of peptides in each season with different molecular weights (Mwt.) ranged between 49 to 181 kDa with a difference in peptide intensity among the different seasons. In conclusion, during different seasons blood parameters of Magrabbi camel males are accompanied with endocrine changes in testosterone secretion levels; this is associated with some changes in the levels of glucose, cholesterol, createnine and fatty acid, while no significant fluctuations were observed in the protein pattern profile.

Key words: Dromedary · Camels · Seasonality · Blood parameters · Testosterone

INTRODUCTION

Male dromedary camels are early known as seasonal breeders [1], where the breeding season is confined to the cool winter months of the year. In the rut, the male exhibits morphological, behavioral and endocrinological changes [2, 3]. The rut stage of a male can last from 50 to 100 days [4]. However, Marie [5] mentioned that the marked peak in sexual activity (the rut) is during the breeding season and it is generally thought that the male is sexually quiescent for the remainder of the year, but it is capable of mating and fertilizing an estrous female at any time of the year. Azouz *et al*. [6] reported a significant decrease in testosterone level out of the breeding season as compared with the levels during rut. Hormonal concentration of blood samples collected from camels slaughtered at defined seasons (summer, autumn, winter and spring) was studied [7]. The results clearly differentiated the samples during the non-rutting and rutting seasons. Studying circulating testosterone profiles and correlation with sexual libido [8], concluded that seasonal changes in circulating testosterone governs sexual libido in male camels. Testosterone in dromedary bulls during camel rutting season was reported to be 4.8±0.7 ng/ml [9]. While, El-Bhrawi [10] reported a mean value of 5.5 ng/ml. The onset of rut activity is associated with significant rise in testosterone concentration (4213.94±278 ng/dl), which is maintained for 11-18 weeks followed by a decline to basal levels [11].

In different animal species the fatty acid composition was affected by different factors, among which is season. The season and lactation minimally

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affect fatty acids composition [12]. The effect of season and plasma polyunsaturated fatty acids and lipoprotein cholesterol was correlated with season effect [13]. While, Soppela and Nieminen [14] noted that there were only small differences in the fatty acid composition of adipose tissues between early winter and spring. The fatty acid composition of shot red and fallow deer males in various reproduction periods was studied by Zomborszky and Husvéth [15], who reported that lower significant percentages of polyunsaturated fatty acids were found in samples during rut as compared to those taken in out of breeding season. It was reported that the serum protein pattern exhibited 14 different fractions of peptides in rutting season (range of Mwt. 18.5 to 103 kDa) and 11 fractions in the non-breeding season in serum samples (range of Mwt. of 15 to 103 kDa) [10]. Blood parameters in male camels are always affected by different factors [16, 17]. Inter-relationships between blood parameters of the one-humped camel in relation to seasons were previously investigated by Sarwar and Majeed [18], Nazifi and Gheisari [19], Saeed *et al.* [20] and El Hag *et al.* [21].

Accordingly, the present study was designed to investigate serum profile of male reproductive hormone (testosterone) in addition to some blood parameters, namely; total protein, createnine, cholesterol, glucose, fatty acids profile and protein pattern.

MATERIALS AND METHODS

Experimental Animals: This investigation was carried out for 12 months starting from December 2007 until November 2008, in Maryout Research Station, belonging to the Desert Research Center, North West of Alexandria, Egypt. Eight dromedary camel bulls aged between 7 to 12 years old were used. Each animal was kept separately in its stable to avoid aggressive temperament during rutting season. The animals were daily fed at 9 am on a pelleted concentrates of 14% crude protein supplemented with barley as a source of energy in addition to straw and Berseem hay ad. lib. Animals were allowed to drink twice daily, with 3 - 4 hours free grazing period twice a week allover the year.

Blood Samples Collection and Serum Preparation: During each season, blood samples were collected in non-heparinized tubes from the neck jugular vein of the tested males. Blood samples were centrifuged at 3000 rpm for 15 minutes. Serum samples were collected and stored frozen at -20°C until analyzed.

Seminal Plasma Preparation: Semen was collected during winter representing the peak of the breeding season using El-Hassanein dummy technique [22], after liquefaction of semen samples from the gelatinous nature state. The semen was centrifuged at 18000 X g for 30 minutes and supernatant was aspirated in clean tubes and stored frozen at -20°C until analyzed.

Blood Serum Parameters: Testosterone levels in blood serum were determined by the ELISA method (Micro-Reader I, Hyperion, USA) at a wave length of 450 nm A kit (BIOSOURCE, Testo-ELISA, BioSource, Nivelles-Belgium) was used to measure testosterone levels. Testosterone labeled with horseradish peroxidase was used as a tracer [23]. Serum concentrations of total protein, cholesterol and createnine were analyzed using colorimetric kits (STANBIO LABORATORY), according to Henry *et al.* [24], Stein [25] and Faulkner and King [26], respectively, while glucose was determined by using colorimetric kits (Diamond Diagnostic, Egypt) [27].

Fatty Acid Analysis: Twelve pooled samples (three samples representing each season) of blood serum in addition to two pooled samples of seminal plasma collected during winter (rutting season) were analyzed for free fatty acid content using GC Model, Shimadzu-8A, equipped with FID detector and glass column 2.5 X 3mm [28].

Protein Pattern: Sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the discontinuous buffer system [29], Coomassie blue stain was prepared [30], molecular weights were detected using a Wide range SigmaMarker™ with wide range molecular weight 6.500-200.000 Da, by Sigma - Aldrich™, USA.

Statistical Analysis: Analysis of variance was detected using GLM procedure by SPSS (SPSS version 11.5 for Windows; SPSS Inc., Chicago, IL, USA).The differences between means were detected using Duncan's Multiple Range Test (DMRT) [31]. Results were quoted as arithmetic mean ± standard error of mean (S.E.M.) and significance was attributed ($p < 0.05$).

RESULTS AND DISCUSSION

Serum Parameters: Except for total protein, all examined parameters presented in Table 1 showed significant variation due to seasonal effect. Glucose level shows a peak of 116.86 ± 1.33(mg/dl) during rut (winter season)

Table 1: Effect of season on some serum blood parameters in male dromedary camels:

Parameter (mg/dl)	Rut (February)	Post- rut (May)	Non-rut (August)	Pre-rut (November)
Total Protein	6.24 ± 0.18 [a]	6.72 ± 0.33 [a]	6.47 ± 0.38 [a]	6.36 ± 0.21 [a]
Glucose	116.86 ± 1.33[a]	100.39 ± 2.94[c]	105.02 ± 2.56[bc]	112.26 ± 3.5[ab]
Createnine	1.46 ± 0.74[b]	1.68 ± 0.72[a]	1.64 ± 0.62[ab]	1.54 ± 0.47[ab]
Cholesterol	25.97 ± 1.97[a]	15.91 ± 1.36[c]	22.02 ± 1.18[b]	22.56 ± 0.61[ab]

a,b Means with different superscripts in the same raw are significantly different at P<0.05

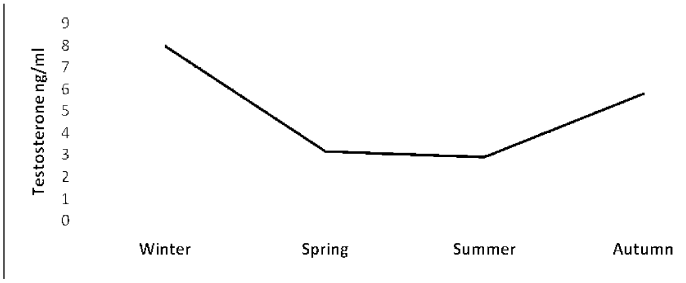

Fig. 1: Testosterone levels (ng/ml.) fluctuations during different seasons

a level slightly higher than that of the pre-rut season as compared with glucose concentration during post-rut (late spring) or out of rut (summer season), in the same trend, Cholesterol also showed significant increase during rut, with significant (P<0.05) decline in post-rut. On the other hand, only createnine showed decrease during autumn and winter seasons with concentrations of 1.54 ± 0.4 and 1.46 ± 0.74 (mg/dl), respectively, as compared to other seasons of the year.

Total protein and glucose contents were in range of other results reported by Patodkar *et al.* [32]. The same findings were previously reported by El-Bhrawi [10], who revealed that no difference was detected between winter (6.9 mg/dl) and summer (6.1 mg/dl) serum content of total protein. The results showed that glucose levels were significantly higher starting from the beginning of rut and during rut as compared to other seasons. These results are in agreement with those obtained by Roy [33] who reported the values during non-rutting season in comparison to the rutting season being 116 and 126 mg/dl., respectively in young dromedary camels. While, Amin *et al.* [34] noted that total protein rose during the dry season, contrarily, glucose levels raised during the green season. It was believed that this may be attributed to seasonal effect with relation to nutritional effect for difference of roughs while grazing during different seasons.

Createnine levels found in the present work were low as compared to other levels reported by Saeed *et al.* [20]. Generally Createnine level did not tremendously decrease during the winter as compared to other seasons. This suggests that the dromedary camels do not demonstrate a winter physiological state of protein conservation; this agrees with the findings previously reported by Harlow and Nelson [35]. Cholesterol levels estimated in the present work were generally in agreement with the normal levels reported by Saeed *et al.* [20] and El Hag *et al.* [21].

Testosterone: During the non-rutting season (August) testosterone reached the basal level (2.89 ± 0.26 ng/ml), during the pre-rut (November), significant increase (P<0.05) of testosterone level reaching 5.8±0.74. This increase continued to reach the peak of 7.95±1.85 during rut (February) and finally followed with significant decrease during the post rut to reach 3.15±0.38 before reaching the base level again during the summer (non-breeding season), (Fig. 1) illustrates the testosterone level fluctuations among different seasons and reproductive physiological stages.

In Morocco, it was noted that camel serum testosterone level increases from 2 ng/ml out of breeding season to reach 24 ng/ml in the breeding season and declines again during May at the end of the breeding season [2]. These results were similar to those obtained in Negev and Judian desert camels [3].The major endocrinological change in the male camel during the rutting season is the increased secretions of androgens especially testosterone [4]. Higher testosterone levels during breeding season may be attributed to an increase in sensitivity of leydig cells to LH or enhanced secretions of LH from the pituitary gland or both [36]. Variations were observed by Bono *et al.* [37] in androgen secretion by the testes which increased as pasture condition improves during rainy season. It was assumed that plasma concentration of testosterone is likely to be the main cause of sexual activity in male camels [38].

Table 2: Effect of season on fatty acid profile of serum male dromedary camels:

Fatty acids	Rut (February)	Post- rut (May)	Non-rut (August)	Pre-rut (November)
Caproic C6:0	0.69 ± 0.19	0.55 ±0.10	0.53 ± 0.07	0.56 ±0.11
Caprylic C8:0	2.13 ± 0.36^{a}	1.26 ± 0.03^{b}	0.96 ±0.27^{b}	1.22 ±0.15^{b}
Capric C10:0	1.22 ± 0.13^{a}	0.65 ± 0.10^{b}	0.75 ±0.05^{b}	1.1 ±0.13^{a}
Lauric C12:0	3.36 ± 0.29^{a}	2.11 ± 0.36^{b}	1.45 ±0.24^{b}	1.84 ±0.25^{b}
Myristic C14:0	5.9 ± 0.33^{a}	4.92 ± 0.33^{ab}	4.23 ±0.25^{b}	5.64 ±0.24^{a}
Pentadecanoic C15:0	0.83 ± 1.15^{a}	0.36 ± 0.07^{b}	0.74 ±0.15^{a}	0.2 ±0.33^{b}
Palmitic C16:0	35.37 ± 0.62^{a}	23.41±1.05^{c}	31.93 ±1.01b	16.77 ±2.09^{d}
Heptadecanoic C17:0	2.04 ± 0.18^{a}	1.78 ± 0.09^{a}	0.94 ±0.02^{b}	0.87 ±0.22^{b}
Arachidic C20:0	1.03 ±0.30^{ab}	0.72 ±0.11^{b}	1.02 ±0.24^{ab}	1.36 ±0.17^{a}
Total saturated %	65.57 ± 1.61^{a}	52.68 ±1.44^{bc}	56.71 ±2.61^{b}	47.85 ±2.79^{c}
Palmitoleic C16:1	3.10 ± 0.11^{b}	5.41 ± 0.13^{a}	2.96 ±0.28^{b}	4.85 ±0.14^{a}
Heptadecanoic C17:1	0.78 ± 0.17	0.56 ± 0.05	0.53 ±0.08	0.55 ±0.13
Oleic C18:1	20.53 ± 0.71	22.34±1.47	23.47 ±0.67	21.86±0.82
Linoleic C18:2	7.99 ± 0.79^{d}	17.62 ±1.28^{b}	13.65 ±1.34^{c}	21.77 ±1.06^{a}
Linolenoic C18:3	2.03 ± 0.71^{ab}	1.4 ±0.05^{b}	2.72 ±0.92^{ab}	3.63 ±0.37^{a}
Total unsaturated %	34.43 ± 1.61^{c}	47.32 ±1.43^{b}	43.29 ±2.61^{bc}	53.15 ±3.69^{a}

a,b,c,d Means with different superscripts in the same raw are significantly different (P<0.05)

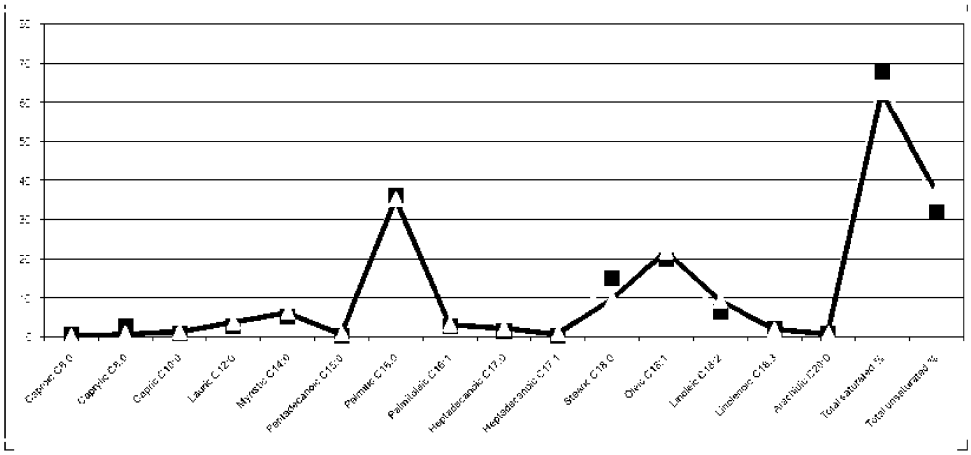

Fig. 2: Effect of season on fatty acid profile of serum and seminal plasma during winter (rutting season) in male dromedary camels:

Fatty Acids: Fatty acids profile during different seasons is presented in Table 2. Total saturated fatty acids (SFAs) was found to be low (47.85 ±2.79%) in pre-rut, then showed a significant high level (65.57 ± 1.61%) in rut during the winter season in February. In general, most of the saturated fatty acid showed the same significance for increased values during rut as compared to other environmental and physiological conditions, with no significant difference in Caproic (C6:0). On contrary a significant difference (P<0.05) was detected for unsaturated fatty acids content (UFAs) showing a decreased to reach 34.43 % in rut while the highest level (53.15 ±3.69%) was demonstrated in pre-rut as shown in Table 2.

These changes in the UFAs content are mainly due to the changes in Palmitoleic (C16:1), Oleic (C18:1) and Linoleic (C18:2) acids. Mainly the Linoleic (C18:2) showed a significant decrease during rut as compared with other seasons. Limited publications and investigations were conducted on camels' serum lipids [39] or concerned with the effect of environmental or physiological conditions in animal species on fatty acid content, however, in reindeer calves, the proportions of the principal C18-polyunsaturated fatty acids, Linoleic and á-Linolenic acid, were significantly lower in all adipose tissues in poor environmental conditions than in good conditions [14].

Fig. 2 shows the fatty acid profile of both serum and seminal plasma in male camels during rut. It could be observed that there is a matching profile between serum and seminal plasma, with limited variations between values for different fatty acids with an increased value of total saturated fatty acid of serum (65.57%) as compared to 62.5 % for seminal plasma content. Differences in the concentrations of blood lipids and blood lipoproteins

Table 3: Effect of season on protein pattern profile; number of bands and molecular weight (Mwt.) in kDa of serum in male dromedary camels:

Band No and Mwt with kDa	Rut (February)	Post- rut (May)	Non-rut (August)	Pre-rut (November)
Band1	181.373	181.937	177.748	181.937
Band2	171.245	171.245	171.245	171.245
Band3	158.699	159.192	157.961	159.192
Band4	148.218	148.218	148.218	148.218
Band5	137.785	141.471	134.404	142.574
Band6	129.286	131.923	125.722	130.902
Band7	119.628	124.750	118.151	121.878
Band8	110.863	114.894	109.155	112.773
Band9	104.025	104.025	104.025	104.025
Band10	94.770	99.444	94.770	97.004
Band11	88.649	91.872	87.963	90.036
Band12	82.026	86.877	82.665	85.273
Band13	75.194	79.394	74.613	78.536
Band14	69.793	72.895	69.793	70.556
Band15	Non detectable	52.938	49.136	50.923

have been found between various breeds of ruminants such as dairy cows, sheep and beef cows [40]. Changes in the FFA concentrations may be due to the release of FFA from adipose tissue under the influence of insulin and changes in blood glucose levels [41].

Electrophoretic Protein Pattern: The electrophoretic peptide pattern of serum during pre-rut in November (autumn), rut in February (winter), post- rut in May (late spring) and after sexual activity had ceased in the non-rut in August (summer) exhibited 15 different fractions of peptides in rutting season with different molecular weights (Mwt.) as listed in Table 3. The Mwt. ranged between 49 to 181 kDa (regardless to season) in the serum samples during the different seasons. Different molecular weights were detected within every season. Not only the difference in the Mwt within seasons, but there was also difference in the peptide intensity as appeared in the deep dark intensities of peptide bands with almost the same Mwt in different seasons.

The results were in agreement with those previously reported by Asadi *et al.* [42, 43] assuming that the reason for the variations in serum analytes is likely to be seasonal effects. Further disciplinary investigations are needed to high lighten the chemical and immunological role(s) of each of the separated fractions of camel serum and their relation to fertility and seasonality of male camel.

In general, Marai [2] reported that globally the breeding season of the camels begins at different dates beginning in September and ends on different dates until June in the different parts of the northern world and from June to September in the southern parts of the world, which are the mildest periods of the year. The severe environmental conditions usually disturb the physiological functions that affect the sexual activity. Yet there is some evidence suggesting that the suprachiasmatic nucleus (SCN) may be sensitive to changes in ambient temperature, with some cells being more responsive to cold and others more responsive to heat.

In conclusion, during different seasons blood parameters of Magrabbi camel males are accompanied with endocrine changes in testosterone secretion levels; this is associated with some changes in glucose, cholesterol, createnine and fatty acid while on significant fluctuations were observed in the protein pattern profile. However, additional verification of these results in the future studies is necessary to understand the effects of season on the serum analytes of the camel

ACKNOWLEDGMENTS

Sincere thanks and gratitude are due to Dr. Nagwa Hossein in protein pattern analysis, Mr. Ibrahim El Hareiry for assisting laboratory work and finally for Mr. Demian Gali for his help and assistance in the field work activities.

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