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REGULAR ARTICLE

Effects of dietary supplementation and parity of dam on plasma concentrations of Insulin-like growth factor (IGF)-I and IGF-binding protein-3 during post-partum period in dromedary camels

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Abstract

This study aimed to investigate the effects of concentrate supplement around postpartum period on plasma concentrations of insulin-like growth factor (IFG-I) and IGF-binding protein-3 in camels (Camelus dromedarius). Eighteen pregnant females were divided into supplemented (n = 9; S) and a non-supplemented (n = 9; C) experimental groups. During day, animals grazed 7 to 8 h on salty pasture and during night, they were kept in pen, where each female in group S received 4 kg/d of concentrate supplement during the last 3 months of pregnancy and 5 kg/d during the first 3 months postpartum. Plasma concentration of IGF-I was more dependent on the dietary level ($P < 0.01$) than on parity of dams ($P > 0.05$). Overall mean was more than threefold in group S (31.2 ± 12.0 ng/ml) than in group C (9.6±4.4 ng/ml). Relative plasma concentration of IGFBP-3 was affected by dietary and parity effects ($P < 0.01$). It was more important in supplemented and primiparous groups than in non-supplemented and multiparous groups. A positive correlation ($r = 0.45$; $P < 0.001$) was found between plasma IGFBP-3 and IGF-I concentrations of dromedary females in postpartum period. In conclusion, concordance of increasing between plasma concentrations of IGF-I and IGFBP-3, and energy balance was confirmed for dromedary.

Key words: Dromedary, Dietary supplementation, IGF-I, IGFBP-3

Introduction

In Tunisia, highest concentration of dromedaries is found in the arid and desert lands of the country where the pasture productivity is marginal and biomass highly variable by season and year. Nevertheless, this species could survive, produce and breed under these conditions. The influence of nutrition on performances and blood metabolites has been extensively investigated in cattle and others conventional livestock species (Cooke et al., 2008; Cappellozza et al., 2014 a; Cappellozza et al., 2014b; Moriel et al., 2012). Chronic restriction of dietary energy (Nugent et al., 1993) or postpartum negative energy balance (Laeger et al., 2014) decrease serum concentration of IGF-I in beef cows and, on the other hand, IGF-I increased with age and the onset of reproduction function in heifers and bulls (Brito et al., 2007).

In dairy cows, Grimard et al. (2013) reported that parity had an effect on plasma IGF-1 concentrations (PP: 61.65 ± 2.67 vs MP: 41.63 ± 5.81 ng/ml, $p < 0.001$),

In order to modulate IGF-I action and to prevent it from protease degradation in circulation, IGF-I associates predominantly with IGFBP-3 which is only depressed after prolonged periods of severe malnutrition (Ketelslegers et al., 1996). While effect of nutrition condition on somatotrope axis have been studied in many mammalian species, importance of IGF-I/II and IGFBPs in blood circulation and on productive and reproductive functions of dromedaries was not studied. However, there is few and fragmented information on the effects of nutritional deficiencies on the dromedary performances at critical periods including puberty and parturition (Moslah, 1990) and our studies (Hammadi et al., 2001a, 2002; 2004; 2005) were the first published papers on the metabolic factors IGF-I and IGFBP-3 in camels.

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Therefore, the objective of this study was to investigate the effects of dietary supplementation around postpartum and parity of dam on plasma concentrations of IGF-I and IGFBP-3 period in dromedary camels.

Materials and Methods

The experiment was conducted from September to June period at the Arid Lands Institute Experimental Station situated in the Southern of Tunisia (33° 30' N, 10° 40' E). This region is characterized by an arid climate with a mean annual rainfall of about 180 mm. Eighteen pregnant dromedary females, belonging to a 40-heads herd, were used for this experiment. The herd was kept in the pen during the night and moved to the range during the day to spend 7 to 8 hours grazing. The animals had access to water twice a day, in the morning before leaving to the range and in the evening when they come back to the camp. Pasture in which the animals graze covers approximately 500 ha which are dominated by salty native species (*Arthrocnemum indicum, Tamarix gallica, Limoniastrum gyunianum, Nitraria retusa, Suaeda mollis, Salsola tetrandra* and *Zygophyllum album*).

The experimental animals were randomly allotted, in equal number, to a supplemented (S) or non-supplemented (C: control) groups based on the body weight (S group: 359 ± 22 kg vs. C group: 362 ± 32 kg, P = 0.138), gestation days (S group: 252±20 days vs. C group: 241 ±30 days, P = 0.156) and age of the female (S group: 6.9±3.2 years vs. C group: 6.7±3.4 years, P = 0.455). There were 2 primiparous and 7 multiparous females in the S group and 3 primiparous and 6 multiparous females in the C group. At sun set, each female of group S received 4 kg/d of concentrated supplement (Table 1) during the last 3 months of gestation and 5 kg/d during the first 3 months postpartum. It was formulated and quantified to supply 70% of total daily requirement energy during the 3 pre and 3 postpartum months for a 360 kg dromedary female producing 5 liter of milk per day during the first three months of lactation (Hammadi et al., 2001).

Blood samples for IGF-I and IGFBP-3 evaluation were taken in EDTA vacuum tubes at 21, 45, 60, 75 and 90 days postpartum. They were immediately centrifuged and stored at – 20°C until they were analyzed. Plasma IGF-I concentrations were determined by radioimmunoassay using the method described for camel species (Hammadi et al., 2005). Briefly, after acid-ethanol extraction (ethanol and 2 mol/l HCl (0.875: 0.125 v/v)), an aliquot of the supernatant was neutralized with 0.855 mol/l Tris base solution in a ratio of 5:2. The samples were stored at – 20°C for 1 h, then immediately centrifuged at 3000 x g for 30 min at 4°C. The supernatant was used in radioimmunoassay. Recombinant bovine IGF-I was used as standard and as an iodinated tracer. Anti-rbIGF-I rabbit antiserum (batch: Rab3) was used at final dilution of 1/1250. The antibody showed no cross-reactivity against recombinant bovine IGF-II (rbIGF-II; batch GTS-2, Monsanto St louis, MO) or bovine insulin (Sigma, Chemical Co., St louis, MO). Recovery of IGF-I added to the dromedary plasma was 94.4 ± 3.2% (no. = 9), the minimum detectable dose of IGF-I was 1 ng/ml, and the intra- and inter-assay coefficients of variation were 9% and 13%, respectively.

Evaluation of IGFBP-3 was carried out by Western ligand blotting following the procedure described by Renaville et al. (1996). Briefly, 5 µl samples were denatured with 45 µl of denaturing solution in boiling water for 4 min. Denatured sample (15 µl) was applied to the stacking gel and electrophoresis was carried out on a 12.5% acrylamide gel using the MINI-PROTEAN II system (Bio-Rad, California). Standards molecular weight markers (^{14}C RAINBOW MARKERS, Amersham) and bull plasma pool used as reference were run in parallel lanes. Blotting sub-unit of the MINI-PROTEAN II system was used to transfer proteins from the acrylamide gel to nitrocellulose membrane (Hybond-C extra, Amersham Belgium). After blotting, the membranes were saturated and separately incubated (overnight, 4°C) in sealed plastic bags with 10 ml of tracer containing about 300,000 cpm of I^{125} labeled rbIGF-I. Nitrocellulose membranes were washed, dried and exposed to autoradiography films (Kodak X-omat) for 5 to 7 days at –70°C. The developed film was scanned with a SHARP JX-325 scanner and the bands were quantified with ImageMaster Software (Amersham Pharmacia Biotech, Uppsala, Suede).

Statistical analysis of data was performed with SAS computer software. Effects of of dietary supplementation and dam parity on the IGF-I and IGFPB-3 were analysed using GLM model. Values are presented as means ±Standard Deviation (SD). Regression analyses were performed using PROC REG of SAS.

Table 1. Ingredients and chemical composition of the concentrate; CMV: Mineral and vitamin supplement; OM: Organic matter; CP: Crude protein; ADF: Acid detergent fiber; NDF: Neutral detergent fiber.

Ingredients % full matter basis		Chemical composition % dry matter basis	
- Barley	60.0	- DM (% full matter basis)	90.9
- Wheat bran	17.5	OM	91.9
- Olive cake	17.5	CP	11.4
- CMV	5.0	ADF	13.2
		NDF	31.6

Results

Evolution of the body weight of dams during 100 days postaprtum is shown in Figure 1. After calving, females in group S were heavier ($P < 0.001$) than females in group C and the difference of the average body weight between the two groups was 55 kg, 72 kg and 73 kg at 30, 60 and 90 postpartum days respectively. Average daily body gain from day 15 to day 90 postpartum was 116±130 g and – 203±135 g in group S and group C, respectively. During this period, the primiparous females lost daily 117±169 g while the multiparous females lost only 39±226 g but the difference was not significant.

Regarding the plasma concentrations of IGF-I in dromedary female during the 90 days postpartum (Table 2), animals in group S had elevated plasma IGF-I concentrations as compared to those in group C. At day 45 postpartum, the difference between the two groups was maximal and was evaluated at 23.4 ng/ml. Overall mean was more than threefold in group S (31.2±12.0 ng/ml) than in group C (9.6 ± 4.4 ng/ml).

Parity did not have significant effect (P=0.95) on the plasma IGF-I concentrations. However, at day 21 postpartum, primiparous females had 10.1 ng/ml IGF-I more than multiparous females, but after day 45 postpartum, concentrations were very similar in the two parity group. Plasma IGF-I concentrations were positively correlated to the female body weight (r=0.69; P<0.001, unpublished data).

Western ligand blotting of dromedary plasma gave bands for IGF-binding proteins slightly different to that observed in bovine plasma (Figure 2). However, at positions between 40 and 50 kDa a doublet band was observed which, by analogy with the IGF binding protein described in bovine (Cohick et al., 1992) could be associated to IGFBP-3. Overall mean was affected by dietary (5.3 ± 3.9 vs. 2.8 ± 2.0, $P < 0.01$) and parity (5.9 ± 3.0 vs. 2.9 ± 2.3, $P < 0.001$) effects.

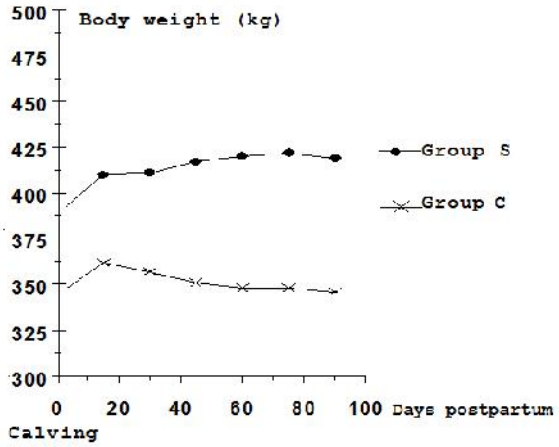

Figure 1. Evolution of the body weight of dams during 100 days postpartum.

Table 2. Effects of dietary level and parity of dam on the change in plasma IGF-I concentrations (means ± SD, ng/ml).

	Dietary level (DL)		Parity (PA)		*P*-value	
	Group S	Group C	Primi.	Multi.	DL	PA
Days postpartum						
21	34.7 ± 17.4	11.5 ± 5.9	29.2 ± 23.3	19.1 ± 12.9	0.023	0.259
45	30.7 ± 14.2	7.3 ± 4.0	16.8 ± 14.2	17.5 ± 16.3	0.001	0.616
60	28.6 ± 8.2	10.7 ± 4.6	15.6 ± 10.2	19.9 ± 12.0	0.003	0.772
75	31.1± 12.2	9.5 ± 4.3	13.9 ± 3.0	18.9 ± 17.6	0.009	0.751
90	31.0 ± 9.4	10.7 ± 1.5	15.0 ± 5.6	25.2 ± 14.0	0.016	0.213

At day 21 postpartum, relative plasma concentration of IGFBP-3 was influenced ($P < 0.01$) by dietary and parity groups (Table 3). For all the other dates, concentration showed no significant difference between the two dietary groups. At days 60 and 75 postpartum, primiparous females had more ($P < 0.05$) IGFBP-3 than multiparous females. Finally, a positive correlation ($r = 0.45$; $P < 0.001$) was found between plasma IGFBP-3 and IGF-I concentrations of dromedary females in postpartum period.

Discussion

It has been documented that the IGF system plays an important role in cell growth, differentiation, and mediation of growth hormone (Daughaday et al., 1989, Wu et al., 2010). Circulating IGF-I is linked to four IGF binding proteins (IGFBP-2, 3, 4 and 5; Silva et al. 2009) that regulate its bioavailability. The significant difference in plasma IGF-I levels found in our study between S and C groups agrees with previous reports in early underfeeding cows (Moriel et al., 2012) indicating that nutrient supplementation is associated with increased concentrations of IGF-I in plasma. In their study, Hodgkinson et al. (1991) found that IGF-I concentration in sheep submitted to high and low nutrition, was lower in the low nutrition group. In ewes, Meza-Herrera et al. (2007) concluded that body condition and protein supplementation increased the secretion of GH, insulin, IGF-1 and LH on day 10 of the oestrous cycle of ewes. Similar findings were obtained by Yung et al. (1996) in heifers which were fed to be in negative energy balance or positive energy balance. These researchers found that deprived heifers synthesize less GH (-60.7%) but more (+37%) IGF-I than well-fed heifers. Bossaert et al. (2014) found that, cows displayed lower IGF-I concentrations than heifers during the 1st month after conception. In our study, start of lactation tended to be associated with a reduction in plasma IGF-I concentration. This observation is more illustrated in primiparous females in which plasma concentration of IGF-I decreased by more than 14 ng/ml between day 21 and day 90 postpartum and multiparous females tended to have more stable concentrations. In the same conditions, primiparous dairy cows had more plasmatic IGF-1 concentrations than Multiparous (Grimard et al., 2013). It has been suggested that the decrease in systemic concentration of IGF-I after prolonged undernutrition is a result of an important decrease of hepatic growth hormone receptors (Counts et al., 1992).

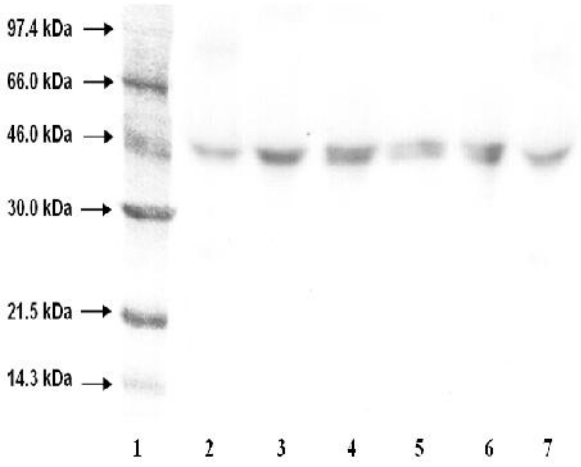

Figure 2. Autoradiogram of a Western ligand blotting of plasma from dromedaries on day 21 postpartum (group S: lanes 3 and 4; group C: 5 and 6). Line 1 is molecular weight markers. Lanes 2 and 7 are bull reference plasma.

Table 3. Effects of dietary level and parity of dam on the change in plasma IGFBP-3 concentrations (means ± SD, arbitrary densitomer unit).

Days postpartum	Dietary level (DL)		Parity (PA)		*P*-value	
	Group S	Group C	Primi.	Multi.	DL	PA
21	9.7 ± 4.5	3.7 ± 3.0	10.3 ± 4.6	4.3 ± 3.3	0.0030	0.0031
45	3.3 ± 2.0	3.0 ± 2.2	4.2 ± 2.0	2.9 ± 2.0	0.6162	0.3708
60	5.7 ± 4.6	2.3 ± 1.7	6.7 ± 5.2	2.8 ± 2.6	0.0738	0.0422
75	4.0 ± 2.3	2.6 ± 1.8	4.4 ± 1.5	2.0 ± 1.6	0.2128	0.0258
90	2.8 ± 1.2	1.8 ± 0.7	2.3 ± 0.6	2.4 ± 1.3	0.3607	0.9975

Blood concentrations of IGF-I have been linked to many economically important traits such as BW and carcass weight, carcass fatness, and the feed efficiency measurements of ADG and residual feed intake in beef cattle (Johnston et al., 2001). This favorable link between IGF-I and feed efficiency has led to promotion of the concept that concentration of IGF-I can be used as an indirect predictor of residual feed intake (Wood et al., 2004). In this study, plasma IGF-I concentrations were positively correlated to the female body weight.

Contrary to the IGF-I concentrations, IGFBP-3 seems to be stable towards dietary supplementation but is probably age-dependent. In ewes, Snyder et al. (1999) found that plasma IGFBP-3 levels were increased in high than in low body condition score groups. In human, plasma IGFBP-3 concentrations are known to be influenced by growth hormone secretion (Cohen and Nissley, 1976), age, pubertal development, nutrition and hepatic function (Blum et al., 1991). Western ligand blot technique used in this study is a semi-quantitative method for determination of IGFBPs and development of quantitative methods (RIA, ELISA) for each IGFs-binding protein could improve sensibility between studied treatments (Portetelle et al., 1998).

Blood IGF-I and IGFBP-3 concentrations were investigated during the 3 postpartum months in camels; a positive correlation was found between these two metabolic parameters. In some in vivo studies, IGFBP-3, which is the most abundant IGFBP in blood (McGuire et al., 1995; Roberts et al., 1997; White et al., 2008), stimulated the endocrine effects of IGF-I. Likelihood of fertility in dairy cows increased when plasma IGFPB-3 concentrations decreased: this supports an inhibitory effect of IGFBP-3 on IGF-I's biological activity (Grimard et al., 2013). So, it is important to elucided the relationships between blood concentrations of IGFBPs and fertility in camels which are characterise by long postpartum period.

In conclusion, plasma concentrations of IGF-I and IGFBP-3 concord with energy balance of animal. The determination of this parameter could serve as a good indicator for veterinary or physiologist in laboratory to appreciate body condition of animals. IGFBP-3 seems to be more stable towards dietary supplementation but is probably age-dependent. Other investigations on male and female dromedary camels could be very important to understand the role of the energy balance on somatotrope axis compounds.

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Author contributions

M. H. designed and conducted the work and wrote the paper, M. C. did statistical analysis, T. K. and R. R. supervised the work.

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