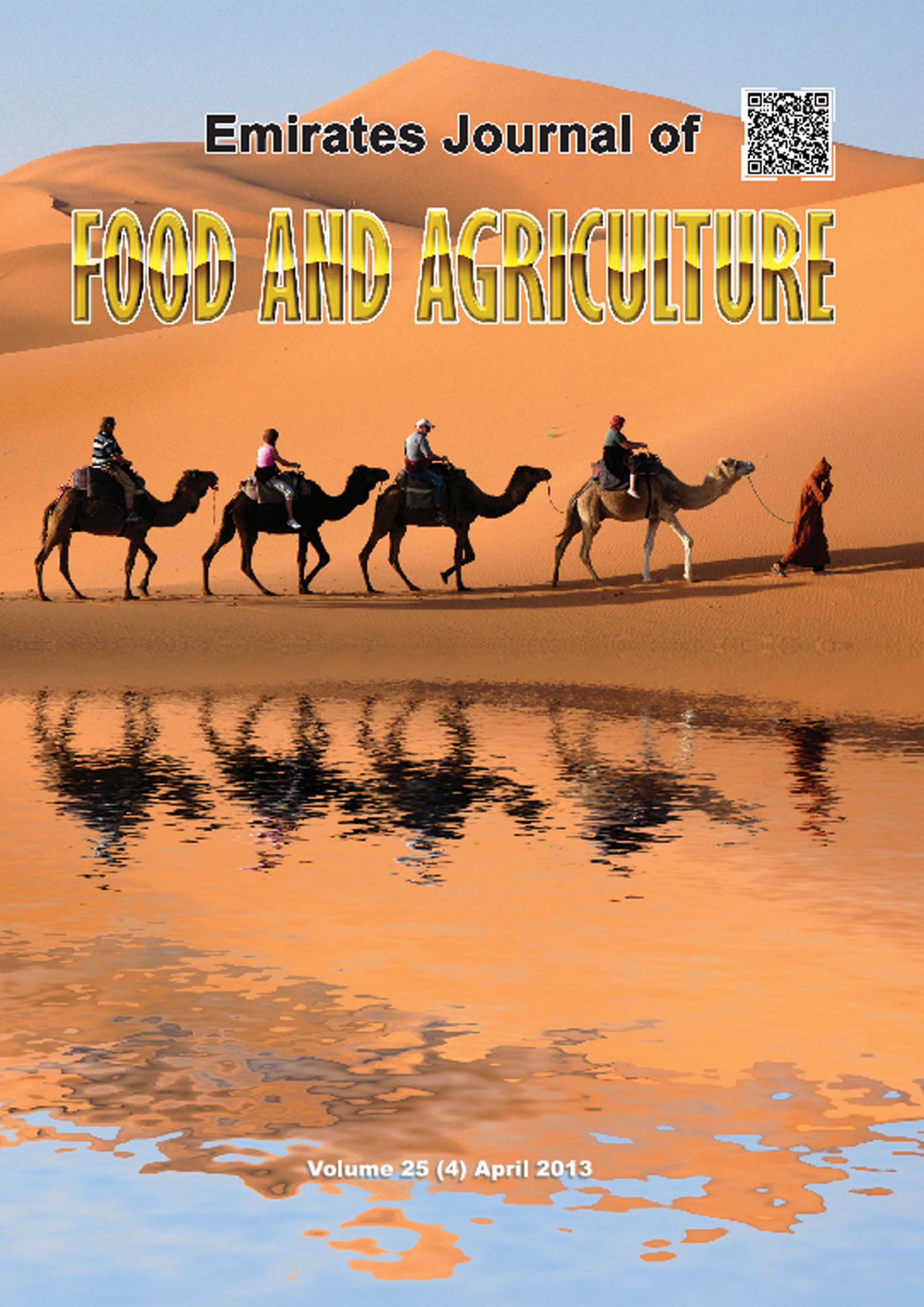
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About the Cover: A view of camel caravan going along the lake side of the Sahara desert in Morocco.

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EDITORIAL

Special issue on "Recent Trends in Camel Research"

Bernard Faye[1,2*]

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The camel (dromedary and Bactrian) is more and more a study object for scientists around the world with an increasing number of publications which are devoted to him either on its biology or on its performances, its health and its place in the desert ecosystems. The success of the last ISOCARD (International Society on Camelid Research and Development) conference at Muscat (Sultanate of Oman) testifies to the dynamism of the camel sciences and to the growing interest of the scientific world for a species remaining marginal in the research, including in the countries where the camel plays an undeniable economic and environmental part.

The "Emirates Journal of Food and Agriculture" (EJFA) has already published many papers on camel research in the past. As it is widely involved in research in arid regions, the EJFA is implied in the dissemination of the information regarding all agricultural and agro-food activities in such context. Thus, the proposal for a special issue on camel underlines the commitment of the journal for contributing to a better knowledge of camel biology and performances.

The present issue included 10 papers with wide variety of topics. In a first paper (Abdallah and Faye, 2013), presents changes in camel farming systems in an emblematic country (the Kingdom of Saudi Arabia) are analyzed. Emblematic because the Kingdom is probably the country of the Arabian Peninsula where the camel biodiversity is the most important and where the modernization of the camel farming is quite fast as we observed in the Emirates. The urbanization is pushing Bedouins to be settled around the town and to intensify the camel production. And consequently to propose a more adapted products to the urban consumers. For this reason, the quality of camel meat must be better known has it is proposed by the second paper on Bactrian camel meat (Raymbeck et al., 2013). The quality of camel meat is due to its gross composition widely described in this last paper, but also to its specific components as vitamin D (El-Khasmi et al., 2013).

Meat and milk are the main products issued from camel farming and the quality of milk proposed to the consumers deserves also to be better investigated. The milk quality concerns not only the hygienic aspects but also some of the virtues attributed to camel milk. For example, the capacity of the lactic bacteria strains isolated in fermented camel milk for absorbing the heavy metals and then, contributing to the detoxifying effect of the camel milk (Akhmetsadykova et al., 2013). In general, the milk composition, especially its protein part, has to be explored more deeply because it is only through a fine analysis that the scientific community will be able to understand the specific character of this milk (Si-Ahmed Z. Saliha et al., 2013). This composition as well the yield is varied according to very various factors. In their study, Attigui et al. (2013) explored the effect of the estrus status of lactating camel on the milk production and composition. Samara et al. (2013) showed that the infra-red thermographic technology could be a convenient method to assess the thermophysiological responses during lactating periodand thus to take the good decision under heat stress conditions.

The camel is seasonal breeder which represents a constraint for example for planning deliveries or for satisfying milk market demand. In consequence, methods for synchronization could be very helpful for answering to this constraint (Monaco et al., 2013). A method using the dosage of progesterone in fecal samples is also proposed especially by taking in account the storage temperature of those samples (Fatnassi et al., 2013). The camel is known for its ability to support long dehydration without negative effect on the kidney function. This property is studied by measuring the creatinine metabolism as the best indicator of the kidney function (Kamili et al., 2013).

Considering these contributions, it appears that the camel is arousing interest in many fields of research, from farming system up to physiology. It is encouraging also to see that the present special issue will contribute to deepen the knowledge regarding camel through the world with investigations and studies from Morocco, Algeria, Tunisia, Egypt, Saudi Arabia, Kazakhstan, France, Sultanate of Oman and Italy. The camel science is really entered in globalization.

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REGULAR ARTICLE

Typology of camel farming system in Saudi Arabia

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Abstract

A field survey involving 218 camel farmers from the northern, eastern and central part of Kingdom of Saudi Arabia was implemented in order to collect data on the status of the owner, herd composition and characteristics, feeding practices, moving strategies and disease prevention practices. The method used was Ascending Hierarchical Clustering, a well-adapted technique in case of exploratory approach. After automatic classification analysis of four groups of variables describing the farmer, its herd, some of its practices and the disease prevention practices, a final analysis regarding the clusters of these four items, allowed to identify 4 global types of farming systems with 2 sub-types in each main type. The explaining factors allowed distinguishing camel farms linked to the desert life in opposition to urban people having multi-activity. However, the integration to market could be variable whatever the opposition desert/city. A part of the people living in desert could improve their management and some of the urban owners have camel mainly for social aspect.

Key words: Camel, Farming system, Saudi Arabia, Typology

Introduction

The camel has a great importance in the local, social culture of the societies inhabiting the dry land. Because the camel has developed various adaptive mechanisms for living in the desert, its rearing is regarded like a constant resource for sustenance in the arid lands. Without camel, the rural life in these areas will be abandoned (Abbas et al., 2000). In the Kingdom of Saudi Arabia (KSA) where less than 1% of the lands are suitable for cultivation (Hussain and Al-Saati, 1999), the camel population is estimated to stand at more than 830000 heads (Ministry of Agriculture, 2006) and is considered as a national socio-cultural heritage. The camel population is regularly growing by 5.2%/year (source: FAOstat, 2010) since 1961, date of the first FAO official statistics. The camel population represented more than 50% of the total livestock unit in the country which is one of the highest in the world (source: FAOstat, 2010). Thus, the camel production is still central in the livestock economy of KSA. The life of Saudi Arabians still living in rural areas is effectively closely connected to the camel which was domesticated in the Arabian Peninsula thousands of years ago (Uerpman and Uerpman, 2002). The camel has played and will continue to play an effective and pivotal role in the history and society of KSA.

Nowadays, the camel farming systems are changing due to the urbanization, climatic changes and growth of the economy of KSA (Auty, 2001). Thus, traditional Bedouin way of life is probably changing. To understand how the camel farming systems are adapting and changing, the achievement of farm typology is a current tool used by the researchers and development institutes (Djurfeldt, 1996). A typology is a method to get an image of the diversity of farming systems in a determined zone. In the present study, a typological analysis of the current situation in some regions of KSA was achieved by focusing on structural aspects (herd composition) and functional aspects (moving strategies, feeding practices, diseases prevention practices, links to market). The objective of the present study was to determine the main current farming systems devoted to camel in different regions of KSA in order to underline the farm diversity and the probable changes in camel utilization.

Materials and Methods

To achieve the typology several methods could be used, by segmentation, by expert's knowledge or by automatic classification using a questionnaire

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with closed questions. The segmentation method is based on the hierarchy of identification keys as described by Breiman et al. (1984). The method by expert's knowledge is based on the aggregation of farms around poles defined by experts having a good *a priori* knowledge of the existing systems (Perrot, 1990). The method by automatic classification is based on statistical analysis of typological questionnaire using clustering method (Späth, 1980). The convenient identification keys being not known and in absence of identified experts on camel farming systems, the method based on automatic classification was used in the present study.

Data collection

The data were collected by interviews based on questionnaires with close questions. The interviews were conducted from February 2009 to July 2010. The farmers were chosen randomly by the Ministry of Agriculture from several regions. A number of 218 camel owners were interviewed. They belonged to different tribes coming from 7 regions: Skaka (n=70 farms), Arar (n=20), Tabuk (n=35), Al-Qaseem (n=25), Hail (n=30), Tabarjal (n=25) and Dammam (n=13).

The questionnaire included four page data form and was filled out for each camel farm. The interview took approximately 45 to 95 min per farmer. The questionnaire included 59 questions and was divided into the following five sections:

- Section 1 contained 8 questions regarding information on camel owner identity and its activities (status, place of living, number of herds)
- Section 2 contained 15 questions regarding the descriptions on the herd (number of camel, types and age of the camel, presence of other species…).
- Section 3 contained 20 questions focused on breed description (coat color, size…).
- Section 4 contained 4 questions only regarding informations on feeding and moving strategy (period and areas of moving, production system…)
- Section 5. Included 12 questions regarding informations on diseases (prevention for ticks, internal and external parasites, vaccinations…).

Statistical analysis

The questionnaire included quantitative and qualitative data. In order to get homogenous data for multivariate analysis, the data preparation involved five steps.

1. Univariate description of the variables (distribution of quantitative variables, number of modalities of qualitative variables). Variables with imbalanced modalities were discarded. The quantitative data were transformed into qualitative variable with modalities according to the distribution of the quantitative values (Snrka and Koeszegi, 2007).

2. Estimation of fecundity rate and mortality rate according to the known number of birth and dead animals within one year, reported to the number of adult females and the total number of animals respectively. For this estimation, we used the method proposed by Lesnoff et al. (2011).

3. Building synthetic variables describing similar topic. For example, herd composition included several questions on the presence or not of different species. After identifying the main combinations of data by cluster analysis, a new variable was created entitled "animals present in the herd" with two modalities: (i) pure camel herd or (ii) camel herd associated to small ruminants and/or cow. Similar approach was achieved for breed composition of the camel herd, camel diet (combination of different foods), camel marketing (selling and/or buying animals), and combination of practice diseases prevention. Finally, from the 59 questions in the previous questionnaire, a total of 31 variables were retained in the final multivariate analysis (Table 1).

4. Multivariate analysis of group of variables: four groups of variables were analyzed, i.e. (i) farmer's data, (ii) herd data, (iii) management practice data (feeding, fattening, moving, marketing) and (iv) disease prevention data. After automatic classification using the method of Ascending Hierarchical Classification (AHC) on Ward distance (Tuffery, 2010), types of farmer, herd, practice and disease prevention practices were identified. The principle of the method of automatic classification (or clustering) was based on the identification of homogeneous groups of individuals (clusters) in the population (here, camel herds). Two camel herds belonging to the same group were somehow close to each other (similar structures or practices). At reverse, two camel herds belonging to different groups are somehow far from each other (they have different structures and/or practices). The classification consists to build a partition of the population into homogenous clusters (having low within variability), different one from other (having high between variability). Each retained cluster, identified by the convenient cutting of a dendrogram (graphic expressing the dissimilarity between clusters or classes) would represent a "type". The convenience of the cutting was estimated when the gain in between-cluster variance is not significant. The retained clustering is expressed by the total between-cluster variance explained by the model. The interpretation of the types was achieved by analyzing the contribution of the different variables to the class. Only variables with significant contribution (assessed

by Chi square test) at $P > 0.05$ were retained for the final interpretation.

5. Final multivariate analysis of a data table including the types of farmers, types of herd, types of practices and types of diseases prevention practices, identified in the previous step. A final typology was proposed and interpreted, notably by calculating some quantitative data (number of camels, buying rate, selling rate, fecundity rate, and mortality rate) in each types and by testing the difference with variance analysis (ANOVA).

For all the statistical analysis used (ANOVA, AHC, chi square test), the software XLstat© was used (Addinsoft, 2007: http://www.xlstat.com).

Table 1. List of variables (acronym and signification) retained in the final multivariate model with their different modalities and the number (n) of each modality.

Item (acronym)	Signification	Modalities	n
1-Status	Status of owner	1- Owner	203
		2- supervisor	2
		3- shepherd	13
2-Assoc	associated with other owners	1- Yes	28
		2- No	190
3- Job	main job of the owner	1- Camel farmer	91
		2- Worker in security field (military, police, guard…)	63
		3- Retired owner	38
		4- Worker from Ministry or Educational field	10
		5- Workers from other field	16
4- Live	Place of living	1- In desert	16
		2 - In city	202
5- Nbherd	Number of herd of owner	1-One herd only	196
		2-Two or more herds	22
6- Animherd	Animals present in the herd	1- Camel only	161
		2- Camel, small ruminants and cattle	57
7- Nbcamel	Number of camels in herd	1-Small (less than 40)	89
		2-Medium (40-80)	68
		3-Big (more of 80)	61
8- Selbuy	Number of camel sold &bought since one year	1-Selling/not buying	73
		2-Buying/not selling	23
		3-Not selling/not buying	70
		4-Selling/buying	52
9-Herdcomp	Type of herd composition	1. Female with low fecundity rate	45
		2. Female with high fecundity rate	36
		3. Young herd	72
		4. Female herd with medium fecundity rate	28
		5. Male herd	34
10- Wean	Age of weaning (month)	1-less than 11 months	58
		2- 12 months	149
		3- More than 12 months	11
11-Typbreed	Types of herd composition	1. Herd with half Waddah, 40% Majahim and 10% Shual	26
		2. Herd with 3/4 Waddah and around 15% Homor	49
		3. Sofor mainly	25
		4. 100% Waddah	80
		5. Mainly Majahim and around 15-20% Waddah	38
12-Calvrate	Calving rate	1- Low calving rate less than 30%	30
		2- Medium calving rate 30-75%	98
		3- Large calving rate more than 75%	90
13- Brand	Age of branding young animals	1-At birth	45
		2- 0.3-11 months	53
		3- 12 months	112
		4- more than 12 months'	10
14- Pregmon	Monitoring of pregnant female	1- Yes	186
		2- No	32
15- Colint	Control of the colostrum intake	1- Yes	156
		2- No	62

16- Weantool	Weaning tools	1-Chmel	64
		2-Isolation of mother	79
		3- Khlel	50
		4- Chmel, Isolation of mother and khlel	23
		5- Nothing	2
17- Fatten	Camel fattening	1- Young males	69
		2-Adult males	16
		3-Adult females	11
		4- All kinds of camel	44
		5- Not fattening.	78
18- Fatdur	Duration of fattening	1-0 day (no fattening)	77
		2-15 days→3months	77
		3-4 months→8months	64
19- Fatsale	Place of sale fattened animals	1- Animal market	102
		2- Butcher	8
		3- Market and other	110
20-Typmov	Type of moving	1. Not moving	25
		2. Short moving at every season (less than 10 km)	73
		3. Medium moving every time (>10 to 100 km)	49
		4. High moving humid season, medium at dry season	44
		5. Long moving at every season (> 100 km)	27
21-Typseamv	Type of season moving	1. Moving in winter and summer	40
		2. Not moving in 2009 and moving in 2010	53
		3. Not moving except in winter 2009	35
		4. Not moving or moving short all over the year	90
22-Typdiet	Type of diet	1- Green fodder only	83
		2- Green fodder+hay	56
		3- High quantity green fodder+hay	26
		4- Barley+ green fodder+hay	34
		5- Barley+ green fodder+hay+vitamins+mineral	19
23- Quarant	Quarantine for new introduced animal	1- Yes	63
		2- No	155
24- Typmed	Type of medicine used for parasites	1- Drugs from market	183
		2- Traditional medicine	35
25- Intpar	Treatment against internal parasites	1- Never	55
		2- Occasionally	163
26- Poxvac	Vaccination against camel pox (jedari)	1- Against pox and other	27
		2- Against pox or other	23
		3- Not vaccinate	168
27- Eradpar	Eradication of ticks and mange	1- Against mange and ticks	8
		2- Against mange or ticks	15
		3- Not vaccinate	195
28-MaindisY	Main diseases in the last three years in young camel	1- No disease	12
		2- Mange and other parasites	35
		3- Digestive and respiratory syndrome	19
		4- All disease+ digestive and respiratory syndrom	152
29- Mortrat	Mortality rate	1- Low young mortality rate less than 8%	91
		2- Medium mortality rate 9-49%	102
		3- Large mortality rate more than 50%	25
30- Yngloss	Main causes of young losses	1- Diarrheas	94
		2- Natal mortality	17
		3- Feed deficiency	1
		4- Mixed diseases	50
		5- No declared disease	56
31- MaindisA	Main diseases reported in the last three years in adult camel	1- No disease	21
		2- Mange-Abcesses-ticks-ringworm	37
		3- Digestive and respiratory syndrome	12
		4- Several diseases+digestive and respiratory syndrome	63
		5- All type of diseases+tryps+skin disease	85

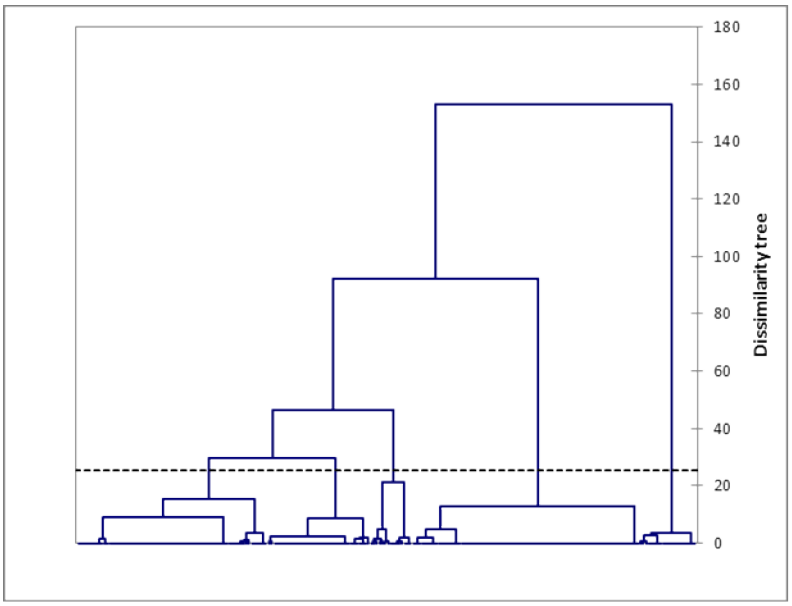

Figure 1. Dendrogram (dissimilarity tree) obtained after cluster analysis of the data table describing the camel farmers, and convenient level of cutting according to the optimal between cluster-variance (----) giving 5 types of farmer.

Results

The whole camel owners' population (n=218) corresponded to a camel population of 14394 camels, representing approximately 8% of the official number of camel in the involved regions of the country (based on FAO statistics). The herd size was 66 ± 89 on average. Among the present sampled camel population, the main breed was Waddah (55.6%) followed by Shual (17.1%), Majaheem (12.4%), Homor (10.5%) and Sofor (4.6%).

The marketing rate was on average 16.8% (sold and buy) with a selling rate (number of sold camel/total number of camels in the herd) of 10.1%, but with a high variability between farmers. The annual mortality rate was on average 16.6 ± 20.9% (all age classes) and birth rate (number of birth reported on number of expected pregnant females) was 96.4 ± 90.6%. For the last 12 months, the fecundity rate (number of birth reported on the total number of adult females) was estimated to 45.2 ± 32.7%. The four groups of variables (farmer, herd, management practices and disease prevention) were analyzed separately. At the end, 4 typologies were obtained.

Types of farmers

After classification of data describing the farmer (variables 1 to 5), 5 types of farmers were identified explaining 74.9% of the total variance between-classes (Figure 1). According to the contribution of the different variables to the clusters, the types of farmers could be summarized as follow: (1) urban camel owner having camel as hobby and working in security field (n=67); (2) pure camel farmer living in desert (n=80); (3) Urban camel farmer having camel as hobby and working in public sector (n=20); (4) retired farmer with one or several herds partly in desert (n=36); (5) shepherd or supervisor from different origin (n=15).

Types of herd

As for farmers, 5 types of herd were identified after classification of data (variables 6 to 12) describing the herds (Figure 2). The retained model into 5 clusters explained 46.2% of the between-cluster variance.

According to the significant variables contributing to the classes, the types of herds could be described as follow: (1) medium camel herd with different breeds, low fertility and commercial use (n=56); (2) camel herd of different size with more similar breeds widely involved in camel market and high turn-over (n=62); (3) small camel herd poorly market integrated (n=40); (4) Majahim small camel herd more or less market integrated (n=20); (5) Waddah small camel herd with good fertility rate (n=40).

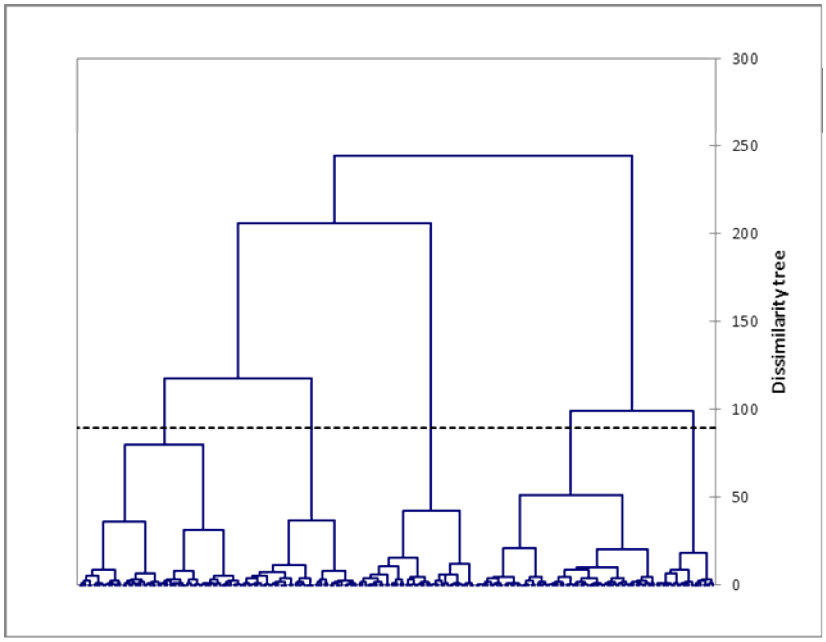

Figure 2. Dendrogram (dissimilarity tree) obtained after cluster analysis of the data table describing the camel herd, and convenient level of cutting according to the optimal between cluster-variance (----) giving five types of camel herd.

Types of management practices

With similar method involving data describing the farmer's practices (variables 13 to 22), 4 types of practices were identified explaining 40.7% of the total between-classes variance (Figure 3). The classes were interpreted according to the 8 significant variables as follow: (1) Farmer moving, no fattening animals and distributing green fodder without supplement (n=58); (2) Farmer moving, practicing late fattening and well integrated to market (n=42); (3) Farmer moving, practicing early fattening but well integrated to market (n=87); (4) No moving camel, no fattening, but branding young animals, distribution of barley and selling animal to market (n=31).

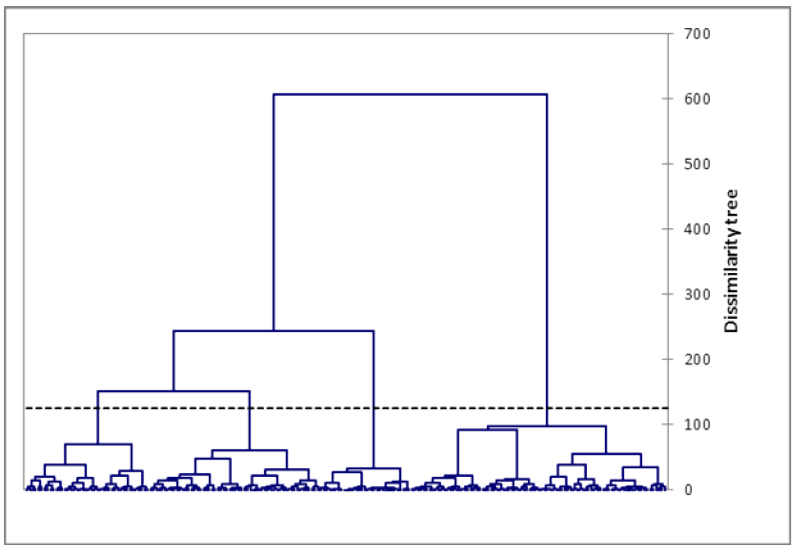

Figure 3. Dendrogram (dissimilarity tree) obtained after cluster analysis of the data table describing the management practices, and convenient level of cutting according to the optimal between cluster-variance (----) giving four types of practice.

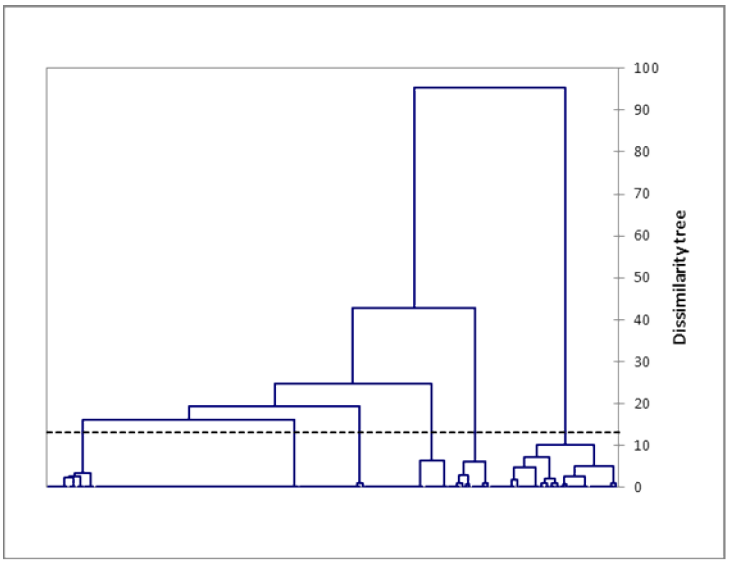

Figure 4. Dendrogram (dissimilarity tree) obtained after cluster analysis of the data table describing the disease prevention practices, and convenient level of cutting according to the optimal between cluster-variance (----) giving six types of prevention practice.

Types of prevention practices

After classification of data describing the practice of disease prevention (variables 23 to 31), 6 types of practices were identified explaining 75.1% of the total between-classes variance (Figure 4). Only 6 variables contributed significantly to the classes and could be interpreted as follow: (1) Low health management (n=78); (2) High health management (n=49); (3) Traditional health treatment (n=16); (4) No health management (n=32); (5) Parasite management mainly (n=25); (6) Traditional prevention against parasite (n=18). The use of traditional medicine or ethnoveterinary medicine (mainly by using desert plant) rather than medicine from the market was more common for treating camel parasites as mange or ringworm.

Camel farm types

In the last step, a global classification of the camel farms described by their 4 different clusters identified in the previous steps was achieved, and gave a balanced dendrogram including four main classes divided each into two sub-types explaining 70.2% of the total between-classes variance. Those types could be interpreted as follows (Figure 5):

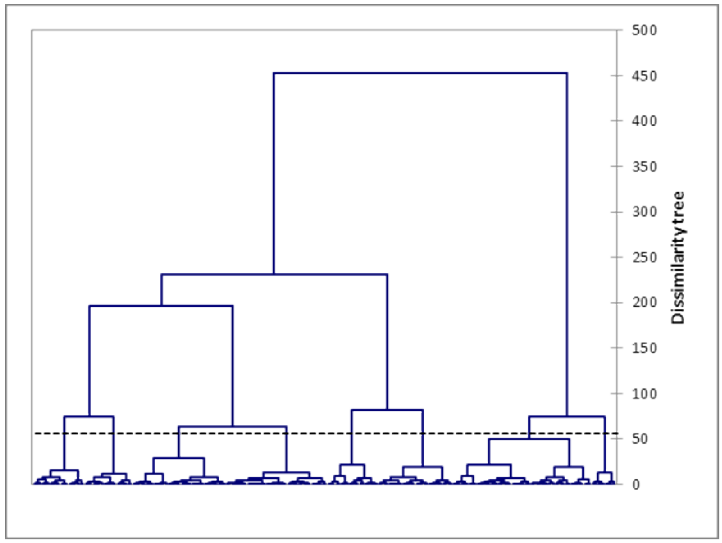

Figure 5. Dendrogram (dissimilarity tree) obtained after from final cluster analysis of the data table where the camel owners are described by their types of farmer, herd, practice and disease prevention, and convenient level of cutting according to the optimal between cluster-variance (----) giving eight types of camel farms.

The first type contained pure camel farmers living more or less exclusively by their camel rearing. They are divided into 2 sub-types:

- Type 1a (16.1% of the camel farms): Pure camel farmers having big herd, living in desert and regularly moving, using green fodder sometimes added with mineral and vitamin, no fattening practice (2/3 of the cases) or late fattening (1/3 of the cases), more or less integrated to market, with low health management. They can be regarded as pure camel farmers having big herd with traditional way of life more or less integrated to market.
- Type 1b (17.4%): Moving pure camel farmers with homogeneous herd (only one breed), high turn-over to camel market, practicing early fattening for camel market, but low (55% of the cases) or high (45%) health management. They could be defined as pure camel farmer with higher integration to market and modernized management.

The second type was retired people still keeping camel for different purposes. They can be divided into 2 sub-types again:

- Type 2a (8.3% of the sample): retired people or shepherd with low reproductive performances, variable health management, given supplementation in diet with barley, weak integration to market. They could be defined as retired people keeping camel for hobby.
- Type 2b (23.9% of the sample): retired people with low or traditional health management but with very high market integration, adding mineral and vitamin in the diet. They are clearly retired people having camel for market activity.

The third type included pure camel farmer or multi-active people with small herd but good integration to market, and health management focused on parasite prevention. The 2 sub-types could be described as follows:

- Type 3a (12.8%): Pure camel farmer living in desert with small or medium camel herd composed of one breed only, low reproductive performance but commercial use with more or less good health management. They are moving herds. They can be defined as small pure camel farmer well integrated to market but with low management
- Type 3b (4.1%): Multi-active farmers (security work) with small camel herd (mainly Waddah), good reproductive performance but only parasite prevention, management with traditional drugs, moving or not moving herd, well integrated to market: They are Multi-active farmer with small herd using camel for market but with traditional management.

The fourth type was mainly multi-active owners practicing camel rearing as hobby but looking for proper management. They could be divided into

- Type 4a (8.7%): Camel farmer living in city, multi-active or sometimes retired, herd having one breed only with good reproductive performance, but rather low market integration and low health management in spite of a good calf mortality control. They are multi-active retired farmer using camel for hobby with low commercial objective and health management.
- Type 4b (8.7%): Multi-active owner (mainly from security field), herd having one breed only with high reproductive performance but with moving animals and better commercial objective than 4a. They could be defined as multi-active owner looking for proper commercial management.

In order to interpret the identified types, some quantitative variables were estimated: number of camels per herd, buying rate, selling rate, fecundity rate, and young mortality rate (Table 2). The number of camels was significantly higher in type 1a while the marketing rate was significantly higher in type 2b. No significant difference was observed for annual fecundity rate (from 30.4 to 53.0%) and mortality rate (from 10.5 to 20.6%) in spite of a high observed variability.

Table 2. Some characteristics of the 8 camel farming types identified in KSA.

	1a	1b	2a	2b	3a	3b	4a	4b
Number of camels	100.2[a]	66.0[b]	71.8[b]	56.1[b]	66.3[b]	72.8[b]	59.8[b]	63.3[b]
Buying rate (%)	4.0[b]	22.6[a,b]	4.0[b]	68.9[a]	6.7[b]	2.5[b]	1.1[b]	3.0[b]
Sold rate (%)	6.5[b]	22.8[a,b]	7.2[b]	54.3[a]	7.7[b]	6.2[b]	14.4[a,b]	16.5[a,b]
Fecundity rate (%)	41.0[a]	45.4[a]	30.4[a]	37.1[a]	43.5[a]	47.2[a]	42.1[a]	53.0[a]
Mortality rate (%)	18.2[a]	15.0[a]	23.1[a]	13.9[a]	17.2[a]	25.6[a]	10.4[a]	13.6[a]

a,b Different letter in one row is significant at $P < 0.05$

Discussion

The typology of camel farming is a common tool to understand the organization and/or the strategies of the camel owners. It is generally helpful for the people in charge of the rural development, because the recommendations could change according to the type of farmers and of their activities. Camel farming typologies were already achieved in Morocco (Michel et al., 1997), in Niger (Chaibou and Faye, 2005), in Mauritania (Correra et al., 2009) and in India (Laval et al., 1998; Benard et al., 2008). A description regarding dairy camel systems was also available in Sudan (Eisa and Mustafa, 2011).

In Saudi Arabia, livestock farming systems were described according to the ecosystem where livestock is reared and according to the link with agriculture (Boum, 2003). Some references on the herding strategies and health performances in Saudi camel farms were available but limited to restricted area (Abbas et al., 2000). Few data published in scientific papers were available for specific description of camel farming system, notably according to the husbandry practices (Gaili et al., 2000).

Traditionally, livestock systems in Saudi Arabia like in most of the arid countries are divided into nomad (or transhumant) systems and settled systems (Jasra and Mirza, 2005). But this distribution did not underline the differences between the farmers' strategies and practices. At the national level, a recent report (Mahmoudi, 2010) had identified 6 types of camel farms including commercial farm, racing farm, camel farm for leisure ("week-end farm"), camel farm for renting, traditional farm, and camel for prestige. This typology was based on a simplified questionnaire including essentially data on farmer and herd composition, but little information on practices. In the survey published by Abbas et al. (2000), four types were described in Qassim region as commercial dairies, prestige herders, pastoralist and agropastoralist herders and periurban feedlots, but this typology was based on *a priori* classification.

In the present study, the identification of the farming systems were focused on four items: (i) the farmer status, (ii) the herd composition, (iii) the main practices which are the classical parameters used to describe livestock system (Lhoste et al., 1993) defined as the interaction between the farmer, the herd and the farming conditions. This approach was applied both in cattle production (Lhoste, 1984) and camel production (Saini et al., 2006). Prevention practices (iv) were added in order to take in consideration the importance of this item in camel management.

Of course, some items were lacking for an exhaustive understanding of the owner's strategies as education level, age pyramid of the camel herd, reasons for culling, disease prevalence, individual reproductive performances, etc. However, a questionnaire is a compromise with the acceptable time for the interview, and some data could be reliable by monitoring only (longitudinal study). A typological questionnaire is applied in a cross-sectional survey with limiting time of interview in order to give the priority to the number of interviewed owners.

In Saudi Arabia, the place of camel in the social life is very central because it is a heritage of the Bedouin culture. This explains the importance of camel rearing without economical purpose and the fact that many people having another activities or being retired wanted to have camels. Among the eight types described in the present study, there was also a clear distinction between farmers living in desert with moving animals and those living in cities, having other professional activities but keeping camel around the city or in desert. The role of camel in that sense was fundamentally a matter of social satisfaction rather than economical target. However, this distinction is not always strict as the present typology showed. Even urban owners having multi-activities could be highly integrated into market, could search proper management of disease and production performance. For example, the type 2b gathering mainly retired people, has on average a significant higher selling rate and buying rate (54.3 and 68.9% respectively) compared to the other types. However, owners keeping their animal mainly for hobby (type 2a) had a lower fecundity performance (around 30%) and a high mortality rate (more than 20%).

It is obvious that camel farming systems in the Kingdom are changing progressively, the traditional way of life in desert having to face to urbanization and improvement of the incomes. The tendency to be settled around the town and the highest integration to milk and meat market is increasing as in other camel country (Faye et al., 2002). Since the sixties, urban population in Saudi Arabia changed from 20 to 87% whereas in the same time the camel population increased according to FAO statistics (http://www.faostat.org) from 80 thousand to 260 thousand heads (which is widely lower than the probable population estimated to more than 800 thousand by

the Ministry of Agriculture, 2006) i.e. an annual growth of 5.2%. For the same period (from 1961 and 2008) the official growth of camel milk and meat production was 6.4 and 6.6% respectively indicating an increase of the productivity. This increasing productivity was possible with the intensification of the production (Gaili et al., 2000; Al-Mutairi et al., 2010) as it is observed in periurban farming system (improving of the diet, better health management) described in other part of the world (Faye et al., 2003). Furthermore, economical survey would be necessary for a better understanding of the economic importance of camel, not only at national level, but also in the incomes for the camel owners (Al-Khamis and Young, 2006).

The camel farm types in Saudi Arabia are characterized by a wide variability of the total number of camels in the herd. The herd size varied from 1 to more than 800 heads. The camel herd size was higher in farms where owner was living in desert (the mean camel herd size was more than 100 camels in type 1a *vs* between 56 and 72 in all other types). On average, the camel farms had fewer camels in number within the herd but probably with a higher productivity. The settlement, especially around the town was facilitating the access to health and other services, to inputs and to higher quality food.

Conclusion

The present typology gave overview of the current situation of camel farming system in some part of KSA (Northern, eastern and Central regions). Without previous similar studies, it is difficult to assess the trajectories of these production systems and to know how the different types of camel farms are still changing. However, the country has known very strong changes since the last decades (improvement of the life level, very high urbanization growth). The national authorities have to pay attention to the current changes in camel farming. The requirements of the camel farmers (in high quality food, health protection, camel product processing, and improvement of the camel product marketing…) are increasing. The services to the farmers have to be adapted to these trends. In our sample, more than the half of the farmers appeared to be multi-active or retired. Further surveys have to be envisaged in order to make a clear diagnosis of the change in the farming system and to deepen also the knowledge on the camel market sector in the country. Even if the camel industry is probably not dominant in the national economy, the investment of many people living in urban areas shows the still high interest of Saudi population to the camel farming. It appears clearly that camel farming is compatible with the modern way of life.

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REGULAR ARTICLE

Chemical composition of *Infraspinatus, Triceps brachii, Longissimus thoraces, Biceps femoris, Semitendinosus,* and *Semimembranosus* of Bactrian (*Camelus bactrianus*) camel muscles

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Abstract

The objective of this study was to determine chemical composition of *Infraspinatus, Triceps brachii, Longissimus thoraces, Biceps femoris, Semitendinosus,* and *Semimembranosus* muscles from nine Bactrian carcasses (2-3 years of age). The left side muscles were collected and kept in a chiller (3-4ºC) for 48 hrs then stored at –20ºC. Chemical analyses were carried out to determine moisture, crude protein, fat (ether extract), ash, essential and non-essential element contents. The *Infraspinatus, Triceps brachii, Biceps femoris, Semitendinosus* and *Semimembranosus* muscles had significantly higher moisture content than *Longissimus thoraces* muscle. The range of variation in protein content among the muscles was from 17% (*Longissimus thoraces*) to 18.8% (*Semitendinosus*). The *Longissimus thoraces* muscle had significantly higher fat content than other muscles. The *Longissimus thoraces* muscle had significantly lower phosphorus, magnesium, sodium and potassium contents than *Infraspinatus, Triceps brachii, Biceps femoris, Semitendinosus* and *Semimembranosus* muscles. Small variation in iron, zinc, lead, cadmium, copper, cobalt and magnesium contents were found among selected muscles. This study indicated that muscle location of the Bactrian camel may have an effect on its chemical composition.

Key words: Bactrian camel, Chemical composition, Essential minerals

Introduction

Camel has been well recognized as an important meat animal in less developed parts of the world but its meat is gaining importance due to its low fat content and it is relatively rich in polyunsaturated fatty acids (Kadim et al., 2008). Meat is an essential source of protein, energy, vitamins and minerals for human nutrition. However, recently there has been a concern about the health hazards of diets containing high levels of animal fat and cholesterol. These have been identified as a cause of a wide range of health problems including obesity, cardiovascular diseases, cancer, etc. Consequently, low fat diets have been attaining more acceptability. Camel meat is considered leaner as they produce less proportions of carcass fat than other meat animals. Camel meat has a good market potential, as it could become an ideal choice for health conscious consumers (Kadim et al., 2008). The chemical composition of camel meat is similar to meats from other species where an inverse relationship existed between the moisture and protein contents and the fat content. The chemical composition of camel meat is an important indicator of meat functionality. Moisture content of camel meat plays an important role in the keeping and eating qualities of meat (Kadim et al., 2006) whereas protein and fat contents dictate the manufacturing quality of meat.

In Kazakhstan, camel meat is consumed and preferred by the local population and camels are slaughtered regularly for social, special occasions and certain time of the year. Kazakhstan has 168,000 heads with 80% Bactrian camels (*Camelus bactrianus*). Although, the nutritive value of camel meat has recently become a growing aspect in the marketing of meat products in Kazakhstan, there is no information on its meat composition. Therefore, it is time to establish better criteria of Bactrian camel meat composition. This will be practically applicable for every region raising Bactrian camels for meat production. An efficient marketing system

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for the Kazakhstan Meat Industry needs more information on meat composition in relation to consumers. The aim of this study was to investigate the chemical composition of *Infraspinatus*, *Triceps brachii*, *Longissimus thoraces*, *Biceps femoris*, *Semitendinosus*, and *Semimembranosus* of Bactrian camel muscles in Kazakhstan.

Materials and Methods

Animals and meat samples

Nine Bactrian camels (2 to 3 years of age) were slaughtered at Zhengis Sharua Kozhalygy camel farm, Kyzylorda, Kazakhstan. The *Infraspinatus*, *Triceps brachii*, *Longissimus thoraces*, *Biceps femoris*, *Semitendinosus*, and *Semimembranosus* muscle samples were dissected within 20 min postmortem. Each muscle was trimmed off external fat and transported in an insulated cool box and kept in a chiller (3-4^{o}C) for 48 hrs for proximate analysis.

Proximate composition

All visible fat was removed from each muscle before they were cut into small pieces. Two-hundred grams of meat sample were placed in plastic containers and then dried in a thermo freeze dryer (Modulyol-230, Milford-UK) for five days under 100-mbar pressure at –50ºC. The frozen dry samples were ground using Pansonic-Mixgrinder, Model MX119N-Japan grinder in order to obtain a homogenous mass for chemical analyses. The proximate chemical composition of the muscle tissue was determined as described by Kadim et al. (2009). In brief, protein was determined using a Foss Kjeltec 2300 Nitrogen/Protein Analyzer. Fat (ether extract) was determined by Soxhlet extraction method, using petroleum ether. Ash content was determined by ashing samples in a muffle furnace at 500ºC for overnight.

Mineral composition

Evaluation of mineral levels in camel meat was carried out in two phases, digestion of samples and analyses. Stock of Co, Zn, Cu, Mn, Pb, Mg, Ca, Cd, and P standard (1000mg/L) solution were purchased from Sigma-Aldrich (Chemie GmbH. Riedstrasse 2. D-89555. Steinheim Germany), while K and Na standards (1000mg/L) solutions were obtained from Sherwood (The Paddocks, Cherry Hinton Road, Cambridge, UK). Working standard was prepared by suitable serial dilutions of stock (1000mg/L) of all standard in deionized water and in house standard reference materials used for validation of the method. Complete digestion was achieved using a CEM microwave system Model MARS 907511(CEM Cooperation, Mathews, North Carolina, USA) with a maximum temperature of 200°C in closed polytetrafluoroethylene (PTFE) vessels. Concentrated HNO_3 was used for the digestion of samples. In brief 10 ml of conc. HNO_3 were added to each digestion vessels. They were then heated to 200°C over a 15 minutes period, and then held at 200°C for another 15 minutes. The digest obtained was collected in 100-ml volumetric flasks and made up to volume. Measurements of Co, Zn, Cu, Mn, Pb, Mg, Cd and Ca were carried out on Atomic Absorption Spectrophotometer (AAS) system type Shimadzu Model AA-6800, equipped with GFA-EX7 240V CE Graphite Furnace, HVG-1 Hydride Vapor Generator, MVU-1A Mercury Vaporizer and ASC-6100 Auto Sampler (Japan). Whereas K and Na were analysis by Sherwood Flame photometer, model 420 equipped with Auto sampler model 860 (The Paddocks, Cherry Hinton Road, Cambridge, UK) and P were analysis by Helios UV Visible Spectrophotometer, model Helios Beta (Thermo Electron Corporation, UK).

Statistical analysis

The data were analyzed using General Linear Model's procedure (SAS, 1993) to compare the effect of muscle type (*Infraspinatus*, *Triceps brachii*, *Longissimus thoraces*, *Biceps femoris*, *Semitendinosus*, and *Semimembranosus* muscles) on proximate composition essential and no-essential elements of Bactrian camel. Significant differences between means were assessed using the least-significant-difference procedure.

Table 1. Chemical composition of Bactrian camel *Infraspinatus* (IS), *Triceps brachii* (TB), *Longissimus thoracis* (LT), *Semitendinosus* (ST), *Semimembranosus (SM)*, and *Biceps femoris* (BF).

	Muscles						
	IS	TB	LT	ST	SM	BF	SEM[1]
Moisture (%)	78.5^{b}	78.4^{b}	72.1^{a}	78.0^{b}	79.0^{b}	78.5^{b}	0.64
Protein (%DM)	18.0	17.5	17.0	18.8	18.2	18.3	0.52
Fat (%DM)	2.5^{a}	3.0^{a}	10.0^{b}	2.2^{a}	2.0^{a}	2.1^{a}	0.57
Ash (%DM)	1.0	1.0	0.9	1.0	1.0	1.1	0.03

[1]SEM: standard error for the mean.

Results and Discussion

The *Infraspinatus, Triceps brachii, Biceps femoris, Semitendinosus,* and *Semimembranosus* muscles had significantly (($P < 0.05$) higher moisture content than *Longissimus thoraces* muscle (Table 1). With the exception of *Longissimus thoraces* muscle, no differences in moisture content between muscles in the present study is agreement with findings of Babiker and Yousif (1990), El-Faer et al. (1991), Al-Shabib and Abu-Tarboush (2004) and Alfawaz (2004). They reported that different muscles from the same animal appear to have similar moisture contents. In the present study, the difference between *Longissimus thoraces* and other muscles might be due to higher fat content of the *Longissimus thoraces* muscle. Similarly, Shehata (2005) reported that *Biceps femoris* muscle had higher moisture content (74.2%) compared with *Longissimus thoraces* muscle (69.2%) due to the higher fat content in the *Longissimus thoraces* muscle. However, the average value for moisture of *Longissimus thoraces* muscle was within the range reported for moisture (70-77%) for *Longissimus thoraces* muscle of dromedary camel (Kilgour, 1986; Babiker and Yousif, 1990; El-Faer et al., 1991; Elgasim and Alkanhal, 1992; Al-Ani, 2004; Cristofaneli et al., 2004; Kadim et al., 2009). The moisture contents of *Triceps brachii, Semitendinosus and Biceps femoris* muscles were higher than reported by Bebiker et al. (1990) and Gheisari et al. (2009). Moisture is important as far as its pronounced effects on meat shelf life, processing potential and sensory characteristics (Kadim et al., 2009). The current study showed that moisture content of Bactrian camel meat was higher than dromedary camel meat.

The protein content of Bactrian camel muscles was in the range 17.0 to 18.8% (Table 1). No significant differences in protein content between the *Infraspinatus, Triceps brachii, Longissimus thoracis, Semitendinosus, Semimembranosus,* and *Biceps femoris* muscles in the present study is on line with the conclusions of others (El-Faer et al., 1991; Dawood and Alkanhal, 1995; Kadim et al., 2006). Protein contents of Bactrian camel muscles were slightly lower than those reported by Bebiker and Yousif (1990) and Gheisari et al. (2009) and Kadim et al. (2006) for dromedary camel's muscles. Breed may cause slight differences in camel meat composition. Studies from Saudi Arabia (El-Faer et al., 1991; Elgasim and Alkanhal, 1992; Al-Shabib and Abu-Tarboush, 2004) reported lower protein content than those from United Arab Emirates, Iran, Sudan and Syria (Babiker and Yousif, 1990; Kadim et al., 2006, 2009; Gheisari et al., 2009; Al-Bachir and Zeinou, 2009). However, content of *Longissimus thoracis* muscle in Bactrian camel is similar to those of dromedary *Longissimus thoracis* (16.8%) muscle (Abdelhadi et al., 2012). The difference of protein content between this study and other studies may be due to breed and age differences.

The fat content of Bactrian camel muscles ranged from 2.0 to 10.0% (Table 1). The *Longissimus thoracis* muscle had significantly ($P < 0.05$) higher fat content (10.0%) than *Infraspinatus* (2.5%), *Triceps brachii* (3.0%), *Semitendinosus* (2.2%), *Semimembranosus* (2.0%) and *Biceps femoris* (2.1%) muscles. The nature of the connective tissue matrix also affects the accumulation of fat. Loosely arranged muscles such as the *Longissimus thoracis*, having parallel connective tissue strands, contained more fat than tightly compacted muscles such as *Semimembranosus, Semitendinosus,* or *Biceps femoris* muscles. The latter's connective tissue strands are thicker and more tightly structured, thus physically preventing excess fat accumulation. Such difference between muscles can be explained by location of muscles and nature of connective tissue. No significant differences in the fat content between *Infraspinatus, Triceps brachii, Semitendinosus, Semimembranosus* and *Biceps femoris* muscles were supported by findings of Kadim et al. (2006, 2009, 2011), Ghaisari (2011), Gheisari and Motamedi (2010), Al-Bachir and Zeinou (2009), Gheisari et al. (2009), Sallam and Morshedy (2008), Afawaz (2004), Al-Sheddy et al. (1999) and Al-Shabib and Abu-Tabboush (2004). They concluded that slight differences in the fat content were found in different cuts and muscles with significant variation in fat content between *Longissimus thoracis* muscles and other meat cuts. Fat contents of Bactrian camel meat were higher than dromedary camel meat (Bebiker et al., 1990; Gheisari et al., 2009). Camel meat contains less fat than beef, lamb and goat meat (Kadim et al., 2008). This makes the camel meat a healthy option and advantageous in special diets.

The ash content in the Bactrian camel muscles was reported in the range 0.90 to 1.10% (Table 1). Many studies found that the ash content vary with muscles and meat cuts (Babiker and Yousif, 1990; Dawood and Alkanhal, 1995; Gheisari et al., 2009. Ash contents for *Longissimus thoracis, Triceps brachii, Semitendinosus,* and *Biceps femoris* Bactrian muscles were slightly lower than dromedary camel meat (Bebiker et al., 1990; Kadim et al., 2006, 2009; Abdelhadi et al., 2012). Camel meat has relatively lower ash content than beef, lamb and goat meat (Elgasim and Alkanhal, 1992; Gheisari et al., 2009).

Table 2. Essential and non-essential element levels (g/100g DM) of Bactrian camel *Infraspinatus* (IS), *Triceps brachii* (TB), *Longissimus thoracis* (LT), *Semitendinosus* (ST), *Semimembranosus (SM)*, and *Biceps femoris* (BF).

	Muscles						
	IS	TB	LT	ST	SM	BF	SEM[1]
Essential/nutritional elements							
Phosphorus	3.32[b]	3.72[b]	2.29[a]	3.97[b]	3.66[b]	3.74[b]	0.131
Calcium	0.05	0.05	0.05	0.05	0.05	0.05	0.002
Magnesium	2.48[a]	3.03[ab]	2.51[a]	3.5[b]	3.27[b]	3.45[b]	0.143
Sodium	5.01[b]	4.57[b]	3.59[a]	5.78[c]	4.93[b]	5.16[b]	0.154
Potassium	74.4[b]	80.5[b]	36.9[a]	80.0[b]	77.7[b]	73.5[b]	1.53
Iron	0.06	0.08	0.05	0.08	0.12	0.08	0.009
Zinc	0.02	0.02	0.01	0.02	0.02	0.02	0.001
Copper	0.002	0.002	0.004	0.002	0.002	0.001	0.0005
Cobalt	0.002	0.002	0.004	0.003	0.003	0.003	0.0006
Magnesium	0.003	0.002	0.003	0.003	0.003	0.003	0.0007
Toxic elements							
Lead	0.03	0.03	0.03	0.03	0.03	0.02	0.004
Cadmium	0.03	0.05	0.03	0.03	0.03	0.03	0.008

[1]SEM: standard error for the mean.

The present study indicated that no significant differences among the six muscles on essential or non-essential element contents (Table 2). Minerals are generally classified as either that are required for growth and optimal health or toxic elements, which poses health risk to organism. Both the deficiency and excess intake of essential elements as well as exceeding the safe limits of toxic elements can be detrimental to human health.

Muscles contained high levels of potassium, followed by sodium, phosphorus, magnesium and calcium. Comparison of phosphorus, magnesium, sodium and potassium contents of Bactrian camel muscle samples showed significant differences between selected muscles (Table 2). Phosphorus is the third most abundant element in Bactrian camel meat (2.29-3.97 mg/100g). The *Longissimus thoracis* muscle had significantly ($P<0.05$) lower phosphorus content than other muscles, which it may be due to biological role of each element in muscle physiology. Calcium content (mg/100g) for dromedary camel meat cuts were reported to be in the range of 1.33- 11.48 (Faer et al., 1991; Elgasim and Alkanhal, 1992; Dawood and Alkanhal, 1995; Rashed, 2002; Badiei et al., 2006; El- Kadim et al., 2009). However, the level of variation between the current study and the previous studies may be due to physiological factors, which play a major role in determining the calcium contents in camel meat. Small variation in calcium content was reported among different meat cuts. The variation between four to six different meat cuts in dromedary camel were 19-27% (El-Faer et al., 1991; Dawood and Alkanhal, 1995; Rashed, 2002) whereas up to 144% variation in calcium content can be observed among different meat cuts from different studies (Kadim et al., 2008). Magnesium is another essential mineral for the normal contractions of muscles. The present study showed that *Infraspinatus* (2.48 g/100g) and *Longissimus thoracis* (2.51 g/100g) muscles contained significantly lower magnesium than *Triceps brachii* (3.03 g/100g), *Semitendinosus* (3.5 g/1oog), *Semimembranosus* (3.27 g/100g) and *Biceps femoris* (3.45 g/100g) muscles. Meat from dromedary camels appears to have lower magnesium content (0.01 mg/100g) across four different meat cuts (El-Faer et al., 1991; Elgasim and Alkanhal, 1992). However, meat from camels in dromedary camel appears to have higher magnesium content and the concentration varied among different meat cuts (Rashed, 2002). Sodium content in camel meat was in the range of 3.59 – 5.78 mg/100g (Table 2). The *Longissimus thoracis* (3.59 g/100g) muscle had significantly lowest sodium content while the Semitendinosus (5.78 g/100g) muscle had the highest content comparing to other muscles. Similar conclusion was reported by Elgasim and Alkanhal (1992); Rashed (2002) and Kadim et al. (2006). They found that loins region had the lowest sodium content among the different camel meat cuts tested. Potassium has a bioactive role in muscle function, particularly energy metabolism and neuromuscular excitability (Allen, 1989). The *Longissimus thoracis* muscle had significantly ($P<0.05$) lower potassium content than other five muscles tested. Although, no significant difference for micro elements among the selected muscles in the present study, zinc, lead

cadmium, copper, cobalt and magnesium levels were within the range of dromedary camels (El-Faer et al., 1991; Dawood and Alkanhal, 1995; Rashed, 2002; Kadim et al., 2006). Iron content in camel meat (1.16-3.39 mg/100g) varied among different meat cuts (El-Faer et al., 1991; Dawood and Alkanhal, 1995; Rashed, 2002) which is expected due to the different physiological requirements of myoglobin of different muscles. As with other red meat species, meat cut containing oxidative muscles (*Semitendinosus, semimembranosus* and *biceps femoris*) has higher iron content than glycolytic muscles (*Longissimus thoracis*). However, the range of iron content in the present study was low compare to other camel study, which may be due to different methods of determination, age and location of meat samples.

Conclusion

The composition of Bactrian camel meat is similar to other red meats, but it can be considered as a healthy option due to the low fat content of the meat. A better understanding of chemical composition of individual camel muscles may benefit the meat industry to maximize potential marketable by improving nutritive value of camel meat.

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REGULAR ARTICLE

Meat levels of 25-hydroxyvitamin D3 in Moroccan one-humped dromedary camels (*Camelus dromedarius*)

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Abstract

The aim of this study was to determine the 25-hydroxyvitamin D3 (25-OH-D3) amounts in meat and serum of the Moroccan one-humped camels. The obtained results showed that the 25-OH-D3 amounts in serum (ng/mL), liver, kidney and muscle (ng/g) were 390±45; 7.071±1.003; 6.154±1.067 and 4.241±1.045 respectively. Values in liver were significantly higher ($p< 0.05$) than those of muscle. Serum 25-OH-D3 levels in camels were very higher than those of ruminant species. Meat of camel was rich in water and ash, and the amounts of 25-OH-D3 in it's various analyzed tissues were similar to those reported for this constituent in the corresponding tissues of bovine species and slightly higher than those measured in other domestic animals.

Key words: 25-hydroxyvitamin D3, Dromedary camel, Ash, Meat, Morocco

Introduction

Meat is a food eaten by man since time immemorial. It stands for strength, health and wealth (Williams, 2007). The camel is an important source of red meat production especially in arid and semi-arid areas which adversely affect the performance of other meat animals. Any time, this source is limited by modest growth rates of the species and traditional extensive livestock systems. In the one-humped camel muscles, bones and fat represent respectively 57%, 26% and 17% of the mass of the carcass of the animal and lean meat contains about 78% water, 19% protein, 3% fat, and 1.2% ash with a small amount of intramuscular fat, which renders it a healthy food for humans (Kadim et al., 2008). Camel meat is also a good source of potassium, phosphorus, sodium, magnesium and calcium, and is therefore a high quality mineral intake in arid and semi-arid areas and contains less cholesterol compared to beef or lamb (Kadim et al., 2008), which could play an important role in preventing atherosclerosis, controlling obesity and cholesterol, and reduce the risk of cancer in human. In addition, the camel meat is regarded in many countries and regions have significant effects of fight against several diseases, including hyperacidity, hypertension, pneumonia and respiratory dysfunctions (Kurtu, 2004).

In man, diet is an important determinant of circulating vitamin D concentrations which are lower in vegetarians than in meat and fish eaters (Crowe et al., 2011). One of the most important roles of vitamin D is to maintain skeletal calcium balance by promoting calcium absorption in the intestines (Holick, 2007) and a lack of vitamin D causes rickets in children and exacerbation of osteoporosis and the development of osteomalacia in adults. Moreover, several research showed that vitamin D plays an important role as an agent preventing or delaying the onset of certain autoimmune (diabetes type I) and proliferative diseases (solid cancers, leukemia, psoriasis) (Tissandié et al., 2006). Thus, highlighting physiological role of this hormone justifies its growing interest. However, the information available on nutritional value of camel meat is very limited, and to our best knowledge, there is no report evaluating the vitamin D3 amount in meat of camels. Therefore, this study was undertaken to

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determine the 25, hydroxyvitamin D3 levels in serum and tissues of camel. In addition, water and ash levels in muscle were analyzed.

Materials and Methods

Blood and tissues collection

In municipal slaughterhouse of Casablanca, samples of blood, muscle (*longissimus thoracis* between the 10th and the 13th rib of the left side), liver and kidney were collected from ten 4–5-year-old male Moroccan dromedary camels *(Camelus dromedarius)* weighing 300–350 kg.

During the slaughter blood was taken around 5 to 7 am in dry tubes of 5 ml. After the veterinary inspection, samples of tissues were performed at about 10 am using a sharp knife at a depth of 2 to 3 cm. The blood and tissue samples were taken aseptically at 4°C in a cooler to the laboratory of Molecular Genetics and physiopathology in faculty of Sciences Ben Sik in Casablanca. The blood was centrifuged at 4000 rpm for 10 min, and the serum was divided into aliquots and stored at - 20°C until assay of 25-OH-D3. Tissues were divided into 2 parts: one to measure the water content and ash and the other to extract and analyze the 25-OH-D3.

Humidity and Ash

Moisture tissue was determined by desiccation of a test sample in an oven at 105 °C for 24h until a constant weight. The rate of moisture (% water) or solids (%S) was determined by the difference of weight.

S% = (sample mass / dry mass of fresh sample) x 100.

Water % = 100 - S%.

The rate of total ash was obtained by incineration. After baking at 105 °C for 24h the meat samples were incinerated in a muffle furnace (1h at 600°C). The ashes were evaluated by the difference in weight.

Total ash % = (ash mass / mass of dry sample) x 100.

25-hydroxyvitamin D3 analysis

Meat samples (1.5 g) were cut into thin slices with a scalpel and extracted with 2.5 ml of acetonitrile diluted with distilled water (10v/4v) for 3h. The samples were shaken vigorously every 30 min to facilitate extraction. The extracts obtained were subsequently centrifuged for 5 min at 4000 rpm and the supernatant was aliquoted and stored at -20°C until analysis of the 25-OH-D3.

Serum and meat levels of 25-OH-D3 were analyzed by radioimmunoassay method in the National Center of Science and Nuclear Technical Energy in Maamoura, Morocco, using kits marketed by DIAsource Immunoassays SA (Nivelles-Belgium). Validation for 25-OH-D3 assays included limits of detection, and precision in standard curve following sample dilution, inter- and intra-assays.

Values were expressed as mean and standard error (X ± SEM) and analyzed by the Student test for comparison between samples, and $P< 0.05$ was regarded as statistically significant.

Results and Discussion

Levels of Water, dry matter and ash in meat

In our animals, the muscle content (%) of water, dry matter and ash was respectively 78.58 ± 7.1, 21.42 ± 2.4 and 1.12 ± 0.10 (table I). These results were similar to those reported by numerous studies in camelidae species and higher than those found in sheep and beef (Table 1).

The rate of water and ash in meat can judge it's richness or it's poverty in minerals. In the work reported here, camel meat is richer in water compared to that of Sheep (Sen et al., 2004) and Beef (Mills et al., 1992). Moreover, the outcome of Elkady and Fahmy (1984) confirmed that camel meat contains more water than the buffalo. This richness is due to moisture changes in several parameters, including: race, gender, individual, age, health status, diet and slaughter conditions (Craplet, 1966) and preslaughter water deprivation (Vogel et al., 2011). The ash rates in muscle of our camels were comparable to those found in several species but lower than those measured in Lama (Table 1).

Table 1. Muscle content (%) in water and ash in some species.

Species	Water	Ashes	Muscle	Reference
Camel	74.07	1.10	*Longissimus*	Salvá et al., 2009
	78.58 ± 7.1	1.12 ± 0.10	*Longissimus*	Our study
Lama	73.9	2.43	*Longissimus*	Cristofanelli et al., 2005
Alpaga	73.6	2.5	*Longissimus*	Cristofanelli et al., 2005
Beef	71.5	0.9	*Longissimus*	Mills et al., 1992
Sheep	68.9	1.2	*Longissimus*	Sen et al., 2004
Goat	76.5	0.87	*Longissimus*	Marinova et al., 2001
Duck	76.8	1.0	*Pectoralis*	Baeza et al., 2002

Table 2. Levels of circulating vitamin D3, 25-hydroxyvitamin D3 (25-OH-D3) and 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] in domestic animals.

Compound	Value	Source	Reference
Vitamine D3 (ng/mL)	4.015 ± 0.79	Cow serum	Cho et al., 2006
	3.34 ± 1.43	Calf plasma	Foote et al., 2004
	88 ± 7.1	Cow serum	Cho et al., 2006
	62.66 ± 16.74	Calf plasma	Foote et al., 2004
25-OH-D3 (ng/mL)	35.2 ± 7.8	Beef serum	Rivera et al., 2005
	40 – 50	Heifer plasma	Carnagey et al., 2006
	10 ± 6.7	Cow serum	Cho et al., 2006
	390 ± 45	Camel serum	Our study
1,25(OH)2D3 (pg/mL)	143.14 ± 20.08	Calf plasma	Foote et al., 2004
	33.3 ± 8.83	Beef serum	Rivera et al., 2005
	65 ± 14	Heifer plasma	Carnagey et al., 2006

Ould El Hadj et al. (2002) showed that the muscle solid content of camel increases with the age. It was an average of 22.93; 23.92 and 25.20% respectively for the three age groups studied: under 2 years, 2 to 5 years and over 5 years to 20 years. According to these authors, the rate of the dry matter depends on the water content of meat, which was inversely proportional to the dry matter. The average water content of muscle was: 77.07, 76.08 and 74.8% respectively for age groups: under 2 years, 2 to 5 years and over 5 years to 20 years.

Serum 25-hydroxyvitamin D3

Serum levels of 25-OH-D3 in camels used in this experimentation were 390 ± 45 (ng/mL). Compared to other domestic ruminants, these values are 10 to 15 times higher (table 2).

In previous studies we have reported in camel, that circulating levels of 25-OH-D were higher in summer than those measured in winter (El Khasmi et al., 2011) and didn't show any variation under preslaughter stress conditions such as road transportation (El Khasmi et al., 2010) nor with age (El Khasmi et al., 2009).

Meat levels of 25-hydroxyvitamin D3

In our camels, the content of 25-OH-D3 (ng/g) in muscle, liver and kidney were respectively 4.241 ± 1.045; 7.071 ± 1.003 and 6.154 ± 1.067 respectively (Table 3).

Our values were close to those reported for meat of cattle (Foote et al., 2004; Cho et al., 2006) but slightly higher than those reported in other domestics species (Table 4).

The content of vitamin D in meat was generally low, difficult to measure and had not been indicated at the beginning of food composition in any meat. However, recent studies have been conducted in New Zealand, reported that the values (mg/100g) of vitamin D3 and 25-OH-D3 were respectively about 0.10 and 0.45 in beef and 0.04 and 0.93 in sheep (Cali et al., 1991). These studies have estimated that for an individual aged 51 to 70 years including adequate vitamin D was 10 mg/d, 12% and 25% of this vitamin intake could be covered by 100 g of cooked beef after respectively or lamb (Windaus et al., 1936). According to Heaney et al. (2009), in an adult woman of 70 kg total vitamin D is 14,665 IU, 65% in the form of vitamin D and 35% as 25-OH-D. Nearly three-quarters of vitamin D found in fats, while the 25-OH-D in the body was divided as follows: 20% in muscle, 30% in serum, 35% fat and 15% in other tissues. Circulating 25-OH-D was considered as a biomarker of vitamin D status of man and it was closely linked with the consumption of oily fish, margarine and foods with added vitamin D and exposure to sunlight (Zerwekh, 2008). The prevalence of vitamin D deficiency (serum 25-OH-D < 25 nmol/L) was 36.5% in Morocco, Turkey 41.3% and 19.3% in Nederland (Van der Meer et al., 2008).

Table 3. Serum and meat levels of 25-hydroxyvitamin D3 in Moroccan dromedary camel.

Serum	Muscle	Liver	Kidney
390 ± 45 ng/mL	4.241 ± 1.045 ng/g	7.071 ± 1.003 ng/g	6.154 ± 1.067 ng/g

Table 4. Meat levels of 25-hydroxyvitamin D3 (ng/g) in domestic animals.

Value	Source	Reference
0.27-0.53	Cow's liver	Koshy et VanDer Slik, 1977
4.5±2.6	Cow's liver	Cho et al., 2006
2.59±0.73	Calf's liver	Foote et al., 2004
0.44	Pig's liver	Mattila et al., 1995
0.51-0.98	Cow's Kidney	Koshy et VanDer Slik, 1977
4.2±2.0	Cow's Kidney	Cho et al., 2006
3.02±1.13	Calf's Kidney	Foote et al., 2004
0.15-0.34	Cow's muscle	Koshy et VanDer Slik, 1977
1.83±0.24	Cow's muscle	Cho et al., 2006
0.6±0.1	Heifer's muscle	Carnagey et al., 2006
1.68±0.37	Calf's muscle	Foote et al., 2004
0.9-0.10	Beef's muscle	Wertz et al., 2004
4.24±1.04	Camel's muscle	
7.071±1.003	Camel's liver	Our study
6.154±1.067	Camel's kidney	

It is largely known that vitamin D plays a major role in bone mineralization and Ca balance and a deficit in vitamin D is associated with rickets in children and osteomalacia in adults, osteoporosis and fractures, often synonyms in elderly loss of autonomy. The vitamin D may also play a protective role against hypertension, cardiovascular disease and some cancers and be an important modulator of the immune system (Bell et al., 2010).

Vitamin D is mainly produced endogenously by the action of ultraviolet light at wavelengths between 270 and 300 nm on the epidermal strata of the skin. Vitamin D3 production is greatest in the stratum basal and stratum spinosum of most vertebrate animals, including humans and the peak synthesis occurs between 295 and 297 nm (Crissey et al., 2003).

In the liver, vitamin D is converted into 25-OH-D which may then be converted into 1,25-dihydroxyvitamin D or calcitriol, the biologically active form of vitamin D, either in the kidneys or by monocyte-macrophages (Adams and Hewison, 2010; Courbebaisse et al., 2010). After the final converting step in the kidneys, calcitriol is released into the circulation as a hormone, then transported to various target organs by binding to vitamin D-binding protein (VDBP), a carrier protein in the plasma (Tissandié et al., 2006). This hormone regulates the Ca and P levels in the bloodstream and promotes the healthy growth and bone turnover. It also affects neuromuscular function and inflammation, and regulates the proliferation, differentiation and apoptosis of cells by modulating the action of many genes (Tissandié et al., 2006).

The physiological effects of calcitriol are mediated by the Vitamin D Receptor (VDR), which is principally located in the nuclei of target cells. When the calcitriol binds to the VDR, this later acts as a transcription factor by modulating the gene expression of transport proteins (such as TRPV6 and Calbindin), which are able enhance the intestinal Ca absorption (Bouillon et al., 2003).

It has been observed that low levels of serum 25-OH-D seem to be associated with rickets, osteoporosis, heart diseases, cancers, diabetes, immune deficiency, depression, neuro-degeneration and chronic pain (Holick and Chen, 2008). However, a few number of foods (fish, meat and offal, milk, eggs and dairy products) may naturally contain vitamin D, and the circulating levels of 25-OH-D are more influenced by several other factors (vitamin D supplementation, degree of skin pigmentation, and amount and intensity of sun exposure) than diet (Holick and Chen, 2008). According to an (U.S.) Institute of Medicine Committee, a serum 25-OH-D level of 20 ng/mL is required for normal bone metabolism and overall health (DRI, 1997). In desert areas, the camel meat may contribute with no negligible exposure to sun to provide 25-OH-D in order to satisfy the demand of normal phosphocalcic metabolism in individuals living in these environments.

Conclusion

The camel is an important source of red meat production especially in arid and semi-arid areas, characterized by high temperatures, solar radiation, lack of water, rugged terrain and vegetation very poor which adversely affects the performance of other meat animals. Its meat rich in water and minerals may have nutritional benefits to human health. Despite the circulating levels of 25-OH-D3 very higher in camels than those of domestic animals, the amounts of 25-OH-D3 in the various tissues of the camel are similar to those reported for this constituent in their corresponding tissues. Our

findings show that camel meat may replace that from other animals and may be implicated in dietary source of vitamin D in man, and considered as indicators of an osteoblast activity very adapted in camel to survive in arid and semi-arid conditions.

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REGULAR ARTICLE

Protection against lead contamination by strains of lactic acid bacteria from fermented camel milk

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Abstract

The effectiveness of the fermented milk product for decreasing the absorption of Lead (Pb) in vivo by testing several combinations of different strains and individual strains of LAB isolated from camel milk and shubat (fermented camel milk) was determined. During 4 weeks 0.5 ppm of Pb was given to cavies in fermented milk product and water. Control group and groups treated only by fermented milk products, also, were observed. Faeces, Blood, Heart, Lungs, Liver, Kidneys, and Spleen were analyzed. The lead concentrations in faeces of Control group and lead nitrate treated group were nearly the same. The quantity of Pb in faeces of fermented milk treated groups was higher than in Control and Water Pb groups. In the different cavies' organs of Water Pb group, the higher concentration of heavy metal (ppm) was observed in spleen (1.04), heart (0.65), kidneys (0.58), and blood (0.46) to be compared to 0.82, 0.2, 0.58 and 0.31 respectively in control group. In groups treated with fermented milk without/with Pb, the lead concentration decreased in target organs. Quantity of lead in blood samples of Control group and groups treated fermented milk products without/with Pb is nearly same. Highest concentration of blood Pb was observed for Water Pb group.

Key words: Cavies, Fermented milk product, Lactic acid bacteria, Lead, Target organs

Introduction

Heavy metals are widely responsible of environmental contamination in the world. The quality analysis of drinking water and soil from some areas of Kazakhstan, as mineshafts and nuclear sites, has shown the presence of heavy metals and radionuclides with a content exceeding the permissible value (Kenesariev et al., 2008; Sarsenbayev et al., 2002). This situation should have various adverse effects on a human body (Tuhvatshin et al., 2008). The pollution by lead (Pb) is a health hazard for consumers of dairy products because this metal is concentrated throughout the food chain (Tajkarimi et al., 2007; Dallak, 2009; Kan and Meijer, 2007). High level of lead was observed in the blood samples of individuals inhabiting the polluted areas. This is due to the high content of lead in the soil, drinking water and in the local product (meat, cow milk). The quantity of lead in the blood depends of the age of individuals (Kenesariev et al., 2008; Hallen et al., 1995; Bhagwat et al., 2008).

For long time, it was shown that injected lead, penetrates the cells quickly, being present in all fractions after one hour (Castellino and Aloij (1969).

Lead toxicity results in neurotically disorders; it damages the kidney, affects cardiovascular system and reproduction (Babalola et al., 2005). To avoid a penetration of lead in the food chain, nowadays, there are many methods to remove Pb from different biological matrix. A vast array of biological materials, especially bacteria, algae, yeasts and fungi have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and large available quantities. Biosorbents are cheaper, more effective alternatives for the removal of metallic elements, especially heavy metals from aqueous solution. The capability of some living microorganisms to accumulate metallic elements has been observed at first from toxicological point of view (Volesky and Holan, 1995). Bacteria were used as biosorbents

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because of their small size, their ubiquity, their ability to grow under controlled conditions, and their resilience to a wide range of environmental situations.

Halttunen et al. (2006) showed that bacteria could have characteristics which would allow them to fix the toxins in food and water. And to be an efficient and economical alternative compared to methods of classical detoxification. Lactic acid bacteria are capable to eliminate the Cd and Pb from the water. Elimination was quick, influenced strongly by the pH, pointing out a mechanism of ionic exchange.

Fandi et al. (2006) assessed the correlation of probiotic bacteria of camel's milk with the cadmium and the lead. In vitro study is an initial stage of riddling to identify the useful strains for the decontamination of food and to develop the intestinal model.

Camel milk and fermented *shubat*, its derivative product could be contaminated (Konuspayeva et al., 2008, 2009, 2011). The lactic fermentation of *shubat* could reduce the availability of lead in the digestive tract of consumers because lactic acid bacteria (LAB) are able to absorb this metal which is then excreted in the faeces (Dallak, 2009; Al-Hashem, 2009; Akhmetsadykova et al., 2009; Loiseau et al., 2009).

Therefore, the present study was carried out to determine *in vivo* the effectiveness of the fermented milk for decreasing the absorption of Lead Nitrate ($PB_2(NO_3)$) by testing several combinations of different strains of LAB isolated from camel milk and *shubat*.

Materials and Methods

Animals

Female cavies (250-300 g) were purchased from Antigen Ltd, Almaty, Kazakhstan. They were housed in standard metal cages (10 cavies / cage). They were divided into eight treatment groups:

(1) Control (n=10): cavies not receiving lead and used as Control group,

(2) Water Pb (n=10): group with 2 ml of water solution containing Lead Nitrate (0.5 ppm),

(3) 4SF (n=10): cavies treated with 2 ml of milk product fermented by 4 different LAB strains having proved capacity to absorb Pb,

(4) 4SFPb (n=10): cavies treated with 2 ml of milk product fermented by 4 different LAB strains in which the same concentration of Lead Nitrate than group 2 was dissolved.

(5) SF (n=10): cavies treated with 2 ml of milk product fermented by 1 LAB strain having proved capacity to absorb Pb,

(6) SFPb (n=10): cavies treated with 2 ml of milk product fermented by 1 LAB strain in which the same concentration of Lead Nitrate than group 2 was dissolved,

(7) SNF (n=10): cavies treated with 2 ml of milk product fermented by 1 LAB strain without capacity to absorb Pb,

(8) SNFPb (n=10): cavies treated with 2 ml of milk product fermented by 1 LAB strain without capacity to absorb Pb in which the same concentration of Lead Nitrate than group 2 was dissolved.

Fermented cow milk product was achieved by utilization LAB strains isolated from fermented camel milk. $Pb_2(NO_3)$ was used as a source of lead (0.5 ppm) for fermented milk product and water solution for Water Pb group. Cavies were orally administered (2 ml) their respective doses every day for 28 days. Water and standard pellets were provided *ad libitum.*

Sampling

Faeces were collected every 7 days. On the 28th day blood was collected by cardiac puncture (Rader et al., 1981), and following were obtained (a) Heart, (b) Lungs, (c) Liver, (d) Kidneys, (e) Spleen (Schroeder et al., 1964, 1965; Babalola et al., 2010; Chun-Yan et al., 2011). All samples were stored in a freezer at - 20ºC until analysis.

Determination of lead concentration

Samples were mixed by type and by group. At the first step the samples (blood, faeces, and organs) were dried in an oven at 150ºC for 1h and homogenized. Then 1 g of each sample was mineralized by wet mineralization using pure nitric acid 65% of "Carlo Erba Reagents" Ltd. (Italy) by Kjeldahl method "DK6 VELP SCIENTIFICA" (Italy). Lead concentration in the mineralized samples was measured by Atomic Absorption Spectrometry 30 "Carl Zeiss" (Germany) at the Laboratory "KazMekhanObr" (Kazakhstan). Standard solutions of lead were aspirated to calibrate the AAS before the aspiration of the samples.

Statistical analysis

The effect of treatment on the concentration of lead in different organs was assessed by Kruskal-Wallis test. The matrix of correlation for the table treatment x concentration of Pb in the organs was analyzed by Principal Component Analysis (PCA). ANOVA procedure was used to observe the effect of the week on the concentration of lead in faeces. The software used was XLSTAT© (Addinsoft, 2010).

Results

Content of Pb in the faeces

Diet was slightly contaminated: in milk 0.32, water 0.12, and fodder 0.32, HNO_3 0.1 ppm of lead was found. Quantity of Pb in the samples of faeces after 7 days of lead treatment (ppm): Control Gr. 0.43, 4SF 0.54, 4SFPb 0.63, SF 0.4, SFPb 0.5, SNF 0.47, SNFPb 0.68, Water Pb 0.43. The quantity of Pb in faeces of fermented milk treated groups was higher than in Control and Water Pb groups. There was no difference between Control group and Water Pb group for the Pb content in the faeces of cavies, except for 14th day where higher concentration (1.57 ppm) was observed. These results need to be confirmed (Figure 1). The lead concentration in total faeces samples was higher in the groups 4SF and 4SFPb compared to Control group (Figure 2). However, in the 4SF group which was not treated by lead, the quantity of this metal was also higher than in control group. The highest quantity of Pb was observed in 4SFPb group. But the fecal content of lead in those groups changed during the study.

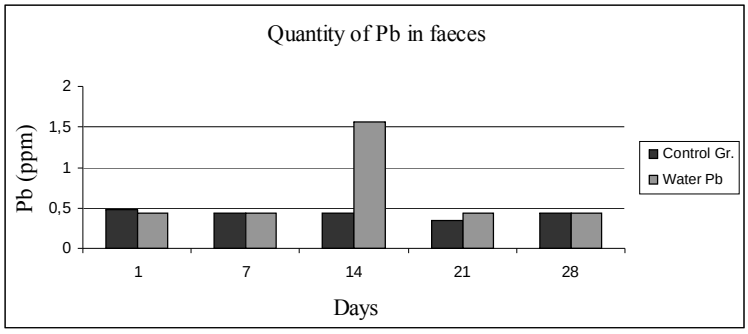

Figure 1. Quantity of lead in faeces of control and water Pb groups during of study.

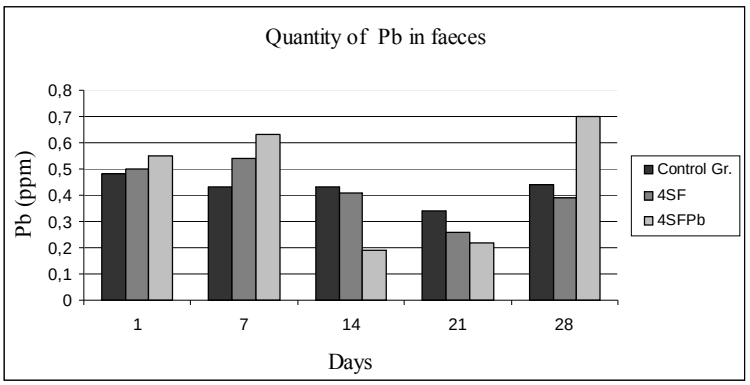

Figure 2. Quantity of lead in faeces of control, 4SF and 4 SFPb groups during of study.

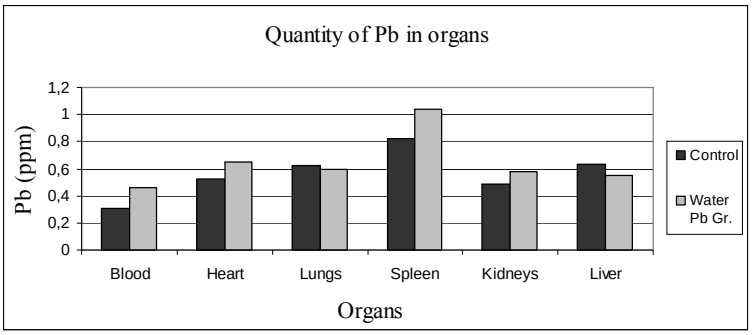

Figure 3. Quantity of Pb in blood and different organs of control and water Pb groups.

Content of Pb in the different organs

In the different cavies' organs of Water Pb group, the high concentration of heavy metal (ppm) was observed in spleen (1.04), heart (0.65), kidneys (0.58) and blood (0.46) to be compared to 0.82, 0.2, 0.58 and 0.31 respectively in control group (Figure 3). Quantity of kidney lead is the same in Control and Water Pb groups. In groups treated with fermented milk without or with Pb, the lead concentration decreased in target organs (spleen, kidneys, liver and lungs). Mainly, for the SFNPb and 4SFPb groups, quantity of lead in the heart samples was less than in Water Pb group. Also, in the spleen samples of 4SFPb group, concentration of Pb was lower compared to Control and Water Pb groups. The Pb concentration in blood and heart was similar in Control, 4SF and 4SFPb groups (Figure 4) in spite of the lead treatment of the 4SFPb group.

Compared to Control group, in groups treated with fermented milk product diminution of Pb concentration (ppm) in lungs (4SF 0.52; SF 0.41; SNF 0.56 to Control gr. 0.62) and in liver (0.53; 0.49; 0.5 to 0.63) was observed. In the samples of spleen of 4SF and SNF groups the quantity of lead was lower than in Control group, 0.7, 0.6 and 0.82 ppm, respectively. For other tissues Pb quantity was nearly the same.

Quantity of lead in blood samples Control, 4SF, SF groups and in the lead treated group 4SFPb was nearly same. In the blood sample of SFPb group the lowest concentration of lead was observed. On the contrary, in the groups of the strains without capacity to fix Pb the quantity of lead increased and the highest concentration was observed for Water Pb group (Figure 5).

Correlations between lead concentrations in the different organs of different groups

The concentration of Pb in the heart was significantly correlated with that of the spleen. There was no significant correlation between the other organs. However, according to the circle of correlations obtained by PCA, the levels of heart Pb and spleen Pb appeared much higher when the levels in the kidneys and especially in the blood were low. The levels in the lungs and liver were independent from the concentrations observed in other organs. Regarding the correlations with the groups, the high levels in the heart and spleen were closed to the group SFPb. The high levels in the blood were associated with 4SFPb and SNF groups. Control and Water Pb groups were associated by high levels in the liver and lungs. SF group was characterized by a low content of Pb in all organs, SNFPb and 4SF groups by average values in all organs.

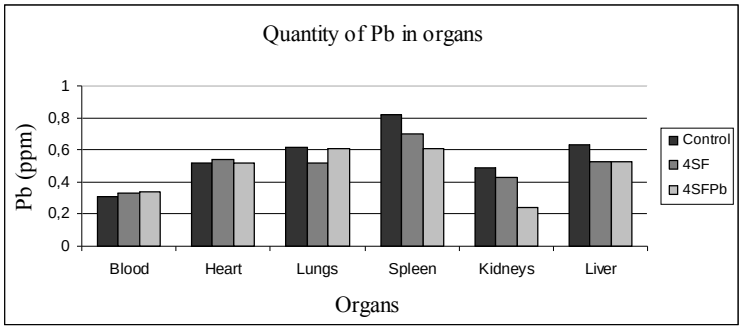

Figure 4. Quantity of Pb in blood and different organs of control, 4SF and 4SFPb groups.

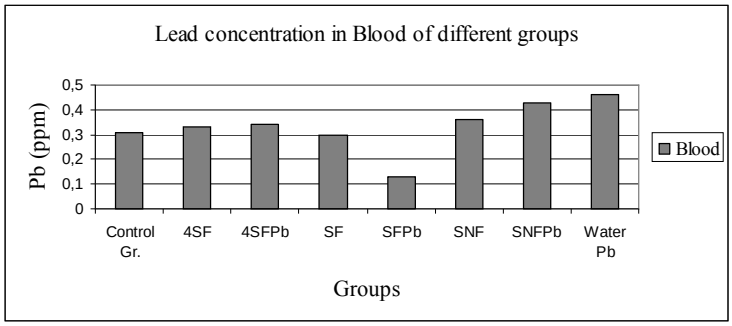

Figure 5. Quantity of Pb in blood of different groups.

Discussion

Quantity of lead in faeces

According to the literature, several LAB strains are capable of surviving the passage through the gastrointestinal conditions (Corcoran et al., 2007; Guerra et al., 2007; Lambert and Hull, 1996; Takahashi et al., 2004). Many studies about detoxification of water and food by utilization of different microorganisms are available (Haltttunen et al., 2006, 2007; Fandi et al., 2006). Many experiences were done with laboratory animals to study lead distribution *in vivo* by treating orally, inhalation or injection (Dallak, 2009; Castellino and Aloij, 1969; Rader et al., 1981; Smith et al., 1992). But, there are no many studies about interaction of LAB and heavy metals *in vivo* and influence for retention or elimination of Pb.

The quantity of faeces lead in groups treated by fermented milk product containing or not lead nitrate was high relatively to Control and Water Pb groups. Such observation means that strains of LAB could increase lead elimination. But, results showed, that elimination of lead from organism was not constant. More lead was excreted in the faeces (35%) than in the urine (15%) (Castellino and Aloij, 1969). Blood lead concentrations were more stable than those in urine (Rabinowitz, 1998).

If the results of fixation test on the nutritive culture and the theory about fixation of Pb by LAB strains during fermentation are right (Akhmetsadykova, 2008), many references show that LAB have capacity to survive and transit through the gastrointestinal tract (Duez et al., 2000; Rochet et al., 2008; Su et al., 2007). Berrada et al. (1991) showed that *Bifidobacterium* strains contained in two different fermented milk behave very differently when exposed to *in vitro* simulated gastric environment. One strain survives very well for 90 min at least (greater than 10^7/g), but the second strain studied was quite less resistant. The results *in vitro*, with slight differences, were confirmed by *in vivo* study in human.

A significant increase in the number of *bifidobacteria* (DN-173010) in faeces was observed during ingestion of fermented milk, and decreased when the ingestion stopped (Collado et al., 2006). Strains which were utilized for fermentation of milk have a good opportunity to survive because some probiotics survives better when investigated in fermented milk (Pochart et al., 1992). Survival rates of *Lactobacillus casei* DN-114 001 ingested in fermented milk were up to 51.2% in the ileum and 28.4% in the feces (Oozeer et al., 2006). Consumption of yogurt and fermented milk products containing LAB/probiotics influence the increase of their number in fecal samples (Rochet et al., 2000; Yuki et al., 1999). *Streptococcus thermophilus, L. bulgaricus* and *L. casei, Bifidobacteria* were found in the faeces of rats treated with yogurt fermented milk (Djouzi et al., 1997). All these references show that strains can transit in gastrointestinal tract and be eliminated in faeces. Even in groups treated only by fermented milk product, the quantity of Pb in tissues was lower and quantity of Pb in faeces was slightly higher. The presence of lead in the faeces, blood and tissues of the cavies not receiving lead, can be attributed to the metal present in the food given to the animals (Schroeder et al., 1965).

Further, the treatment by strains of LAB decreased total lead quantity in organism. This can be due to beneficial effects of fermented milk product which was as a barrier against lead retention in tissues or because LAB strains absorbed Pb in organism.

Distribution of lead in the different tissues

The half-life of lead in the some tissues was regarded as about 3 months (Rabinowitz et al., 1976). But, the latest data shows that the half-life of lead in these tissues ranges from 40 - 50 days (Babalola et al, 2010).

In the present study, lead was expected to accumulate in the soft tissues within 28 days of exposure. In the Control group spleen Pb and liver Pb were high. Based on the results of Pb quantity in different matrixs of Water Pb group, lead distribution in cavies organism of Water Pb group was achieved: spleen>> blood> heart> lungs> liver> kidney. Many experiments were done with different laboratory animals in this direction. Schroeder et al. (1964) determined lead quantity in the organs of 700 mice after given them 5 ppm Cd, Pb, Cr, Ni or Ti in drinking water. High concentration of lead was noted for spleen and kidneys. These results are nearly same for spleen Pb results, but not for kidney Pb. This may be partly due to a common problem usually encountered in oral exposure method. It is almost impossible to determine accurately the quantity of materials ingested by the animal. Quantity of food and water taken by the animals also affect the amount of the material that will be absorbed by the animal. This observed discrepancy was also true for the concentration of lead in the various organs of the animal ((Rabinowitz et al., 1976). The relatively low quantity of lead (0.5ppm) didn't give clear results about lead distribution. Furthermore, in such

study more high concentration of lead could be taken without fatal effect for animals (O'Tuama et al., 1976; Sierra and Tiffany-Castiglioni, 1992). Only female cavies were used for this study. Thus, according to some references, sex difference was observed for quantity of lead in tissues. Female rats and mice showing significantly higher levels than males (Schroeder et al., 1964; Donald et al., 1987). Also, age influence was described. The young monkeys retained 64.5 and 69.8% of the orally administered $_{210}$Pb at 70 and 150 days of age, respectively, while adult monkeys retained 3.2% of the 210Pb dose (Castellino and Aloj, 1964; Wiles et al., 1977). In general, daily exposure to lead via drinking water resulted in the highest lead content in blood, brain, kidney, and femur for both weanling and adult rats (Rader et al., 1981). Experiments with rats showed the main storage organs: kidneys and bone. Initially, 20% of the dose could be accounted for in the kidneys and the biological half-life was about 100 hours. The level in bone built up rapidly at first and then more slowly. After a week, between 25 and 30% of the dose was present in bone (Castellino and Aloj, 1964; Morgan et al., 1977).

The skeletal lead level is commonly regarded as the best indicator of the cumulative exposure to this element, as more than 90% of the body burden of lead is stored in this tissue. The half-time of lead in finger-bone of humans is about 5 years. However, lead in rats is eliminated faster than in humans (Hac and Krechniak, 1996).

Lead accumulates permanently in bones; consequently, estimation of lead in the teeth can provide a good index of the body burden (Rabinowitz et al., 1976).

The remaining 10% is stored in soft tissues like kidney, liver and brain (Chun-Yan, 2011). Thus, the duration of our experiment being 28 days, Pb had time to be stored in tissues. Castellino and Aloj (1964) studied rats for 14 days after single intravenous injections of 100 μg of lead per rat. ^{210}Pb was rapidly distributed in the tissues, the highest concentrations being in the kidneys, liver, and bones (Castellino and Aloj, 1964).

Chun-yan et al. (2011) exposed male mice to lead nitrate solution (0.1mg/ml). For other target organs, the accumulation of lead was in the order: kidney, liver, spleen, lung, brain and heart. The accumulation of lead in brain gradually increased with lead exposure while the accumulation of lead in the other target organs reached balance after 15-20 days (Chun-Yan et al., 2011).

Usually, the level of lead was determined in blood (as index of current exposure) and in hair, nails (as indices of long-term exposure) (Babalola et al., 2005; Kello and Kostial, 1978). Hair fixes easily elements such as lead and thus provides an accurate and permanent record of exposure of some minutes' duration (Babalola et al., 2005).

It's possible that data of Pb quantity in different matrix weren't clear and difficult to explain because bone, hair simples, brain weren't analyzed.

LAB strain of SNF group determined like strain without capacity to fix Pb by test of fixation *in vitro* (Akhmetsadykova et al., 2009). But diminution of Pb in heart samples was observed. It's possible that this strain fixed better in the fermentation process than on the surface of nutritive culture due to the difference of conditions.

Blood lead quantity

As blood flows through the soft tissues, lead is deposited and bio-accumulated. Some references report that lead concentration in the blood is transient and only represents recent exposure of some days (Rabinowitz et al., 1976). On the contrary, Hilts (2003) showed that lead in whole blood was considered to provide to be the best measure of exposure for 25-35 days. In our experiment, samples of blood were collected for 28 days. Total blood volumes, based on the information given by Schroeder et al. (1965) a rat of 300 g weight contain 18 ml of blood. If this estimation is correct then, on average, 0.7% of the total blood volume was collected. Its means that in Water Pb group having lead concentration of 0.46 ppm in 0.7%, it would be about 66 ppm of lead in total blood volume. Blood becomes a matrix with the highest concentration of lead. On the contrary, Rabinowitz (1998) showed that only 10 mg of 60 mg absorbed lead would remain in the blood, the other 50 mg was thought to be elsewhere in the body, only to leave slowly over a period of many months.

Regarding Pb contained in blood, 98–99% was found in blood cells and 1–2% in blood plasma; 5–8% of the Pb in blood cells was bound to blood cell membranes. None of these parameters varied significantly with age (Willes et al., 1977).

Chun-yan et al. (2011) exposed male mice to lead nitrate solution (0.1mg/ml). The resulting data showed that 98% of lead exposed in blood was accumulated in red blood cells (Rabinowitz, 1998).

But, Schroeder et al. (1965) didn't note lead in red blood cells except for a trace in one female.

Lead is submitted to a rapid elimination from the blood and slow elimination from the bones. Lead bounds reversibly to tissues. The longer term accumulation of lead in the skeleton is regarded as

a future source of blood lead. This release of stored lead is the rate-limiting factor in its clearance of lead from the blood (Rabinowitz et al., 1981).

Correlations between lead concentrations in different tissues

In cavies, significant correlations between the heart lead and spleen lead were found. The lead concentration in kidney was relative to levels in blood. They depend from each other. Smith et al. (1992) showed that elevated concentrations of lead in kidney (fresh weight) relative to levels in blood were consistent with the presence of specific lead-binding sites in the kidney at very low levels of exposure. Rader et al. (1981) observed correlations between blood lead and kidney lead, and correlations between blood lead and femur lead were found only in the rats receiving lead steadily in drinking water.

Conclusion

Effects of fermented milk product containing LAB strains on the retention of lead by cavies' organisms were observed. But, to explain the way of detoxification in the future, a large study should be realized. The main question is in which conditions LAB strains release fixed Pb. This could be achieved by making a test *in vitro* with conditions of gastrointestinal tract, in consideration of difference between human and cavy organisms. In the environment contaminated by heavy metals, it will be more useful to take higher concentration of Pb to see the exact way of the distribution in organism. To have a complete idea of Pb distribution, more possible matrix should be taken to analysis as bone, brain, hair samples which weren't taken in the current study.

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REGULAR ARTICLE

Separation and characterization of major milk proteins from Algerian Dromedary (*Camelus dromedarius*)

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Abstract

To characterize major protein fraction in Algerian dromedary's milk, two samples from Sahraoui population collected from two different regions were analyzed using electrophoretic and chromatographic techniques. Casein components and whey proteins were separated by DEAE-cellulose ion exchange chromatography and Sephacryl S200 permeation gel chromatography respectively, and then identified by polyacrylamide gel electrophoresis which looked different from the corresponding patterns of the caseins and whey proteins from cow milk. Differences in casein composition between camel and bovine milk may influence their digestibility, hydrolysis of camel milk caseins using four different proteases (trypsin, chymotrypsin, pepsin and papain) was studied. Caseins were more rapidly hydrolyzed by pepsin because of the greater number of potential pepsin cleavage sites present in the primary structures of camel caseins.

Key words: Camel milk, Enzymatic hydrolysis, Protein characterization

Introduction

The camel is one of the most important domesticated animals in the arid and semiarid zones of tropical and sub-tropical countries. Not only can camels survive under conditions of severe water and heat stress, but they also provide an important source of nutrients in desert communities, especially important during periods of prolonged drought (Farah, 1993). Available information concerning dromedary milk (Farah and Farah-Riesen, 1985; Beg et al., 1984, 1986a, b, 1987; Mehaia, 1987; Mohammed and Larsson-Raznikiewicz, 1989, 1991; Farah, 1993; Alim et al., 2005; Zhang et al., 2005; Konuspayeva et al., 2009; Al-Haj et al., 2010; Ereifej et al., 2011) is related mainly to the Arabian dromedary Camelus dromedaries species. The present work has been carried out in order to present a more description of the major milk proteins from Algerain dromedary's milk. This paper describes the separation of the caseins and the whey proteins by different chromatography and characterized by polyacrylamide gel electrophoresis. The sensitivity of camel's casein to the action of four proteases (trypsin, chymotrypsin, pepsin and papain) has been reported in the present work.

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Materials and Methods

Preparation of milk samples

Two samples of dromedary milk from *Sahraoui* type were collected in Ouargla and Ghardaia regions. They were defatted by centrifugation 4000g at 4°C for 15 min.

Separation of the protein fraction into caseins and whey proteins was conducted according to a modified method from Ochirkhuyag et al. (2000). Specifically, whole casein was obtained from skimmed milk by isoelectric precipitation (pH 4.3) at 22°C using 1N HCl. The precipitate was washed twice with distilled water at pH 4.3, solubilized at pH 7 by addition of 1M NaOH, precipitated again at pH 4.3 with 1N HCl and washed three times with distilled water. Finally, the whole casein was solubilized at pH 7, freeze–dried and stored at -20°C. The supernatant, containing the whey proteins was dialyzed against distilled water and then freeze dried and kept at -20°C until used.

Fractionation of caseins and whey proteins

The individual caseins were separated by ion-exchange chromatography on DEAE-Cellulose (DE52, Watman, France) column (26 mm i.d x 26 cm) equilibrated with 10 mM imidazole/HCl buffer, pH 7.0, containing 3.3 mM urea and 10 mM 2-mercaptoethanol, and the bound proteins were eluted from the column with a linear gradient of 0-1M NaCl, at room temperature, at a flow of 0.3 mL min^{-1} (Larsson-Raznikiewicz and Mohamed, 1986). The ion exchange chromatography was carried out on a low-pressure chromatography system (Bio-Rad, France). Fractionation of the whey proteins was performed by gel permeation chromatography on Sephacryl S200 (Amersham Biosciences) equilibrated with 0.02M Tris-HCl buffer pH 8.6 at room temperature, at a flow of 0.3 mL min^{-1}. The fraction size of collected eluate was about 1 mL. The absorbance of the fractions was determined at 280 nm. The fraction absorbance was plotted against elution volume using the LP Data View software.

Electrophoresis

Native PAGE with the vertical slab gel unit SE-250 series (Hoefer Scientific Instruments, San. Francisco) according to Hillier (1976) with a 12% (w/v) polyacrylamide gel in 0.75M Tris-HCl buffer, pH 8.9. Samples (2 mg mL^{-1}) were solubilised in 75 mM Tris-HCl buffer, pH 8.9, containing 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue.

Urea-PAGE was performed according to Andrews (1983) with an 8.2% (v/v) polyacrylamide gel in 75 mM Tris-HCl buffer, pH 8.9, in the presence of 4M urea. Samples (2 mg mL^{-1}) were solubilised in 75mM Tris-HCl buffer, pH 8.9, containing 4M urea, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue.

SDS-PAGE was performed on a 4.9% (w/v) polyacrylamide in 0.125M Tris-HCl buffer, pH 6.8 stacking gel and a 15.4% (w/v) polyacrylamide in 0.38M Tris-HCl buffer, pH 8.8 containing 0.1% (w/v) SDS separation gel (Laemmli and Favre, 1973). Samples were dissolved at 2 mg mL^{-1} in 0.125M Tris-HCl buffer, pH 6.8, containing 0.1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue. After heating at 100°C for 3 min, 20 µL of sample was loaded in the gel. The molecular mass standards (Sigma chemical CO, Missouri, USA) were Urease (270.0 kDa), bovine serum albumin (66.0 kDa), egg albumin (45.0 kDa), carbonic anhydrase (29.0 kDa) and bovine lactalbumin (14.0 kDa). For both electrophoretic methods, volumes of 20 µL of samples were loaded in the gel,

proteins were fixed with 12% (w/v) trichloroacetic acid (TCA) for 30 min and then, stained for 60 min with 0.5% (w/v) R-250 Coomassie blue dissolved in a mixture of 50% (v/v) ethanol and 12% (w/v) TCA, followed by an overnight destaining in a solution containing 30% (v/v) ethanol, 7.5% (v/v) acetic acid, and 5% (w/v) TCA.

***In vitro* proteolysis of camel whole caseins**

The in vitro hydrolysis were performed as follows: a) chymotrypsin (EC 3.4.21.1; activity 45 U mg^{-1} protein) and trypsin (EC 3.4.21.4; activity 13500 U mg^{-1} protein): enzyme/protein ration 1/200 (w/w) in 0.1M sodium phosphate buffer (pH 8) at 40°C; b) pepsin (EC 3.4.23.1; activity 400-800 U mg^{-1}): enzyme/protein ration 1/250 (w/w) in 0.01N HCl (pH 2) at 37°C; c) papain (EC 3.4.22.2; activity 12 U mg^{-1}): enzyme/protein ration 1/800 (w/w) in 0.5M Tris-HCl buffer (pH 7) at 37°C and the final concentration of caseins was always 10 mg mL^{-1}. The reaction was stopped at different times by diluting the digestion mixture with the same volume of sample buffer (0.125M Tris-HCl buffer, pH 6.8 containing 0.1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue, and then heating for 10 min at 100°C. Controls containing whole casein but without addition of enzymes, was also sampled.

Protein assay

The protein concentration was measured using Lowry's method with bovine serum albumin (BSA) as standard (Lowry et al., 1951); each measurement was done three times.

Results and Discussion

Electrophoretic and chromatographic separation of camel whey proteins

Samples of camel milk whey proteins from two different regions as well as cow milk were examined to determine whether they present differences or have similar composition as proteins from bovine milk. In order to identify the different whey proteins in camel and bovine milk, native-PAGE electrophoresis of whey camel samples from the two regions were compared to bovine whey proteins. In Figure 1 lane 1 (bovine whey) Ig, BSA, α-lactalbumin and β-lactoglobulin were observed. Several faint bands probably correspond to α-lactalbumin dimers and β-lactoglobulin octamers (Merin et al., 2001). Lane 2 and 3 are camel whey. Similar band to BSA and α-lactalbumin was observed. This result showed that α-lactalbumin

can exist in two forms, as seen previously in milk of Camelus dromedarius (Conti et al., 1985), with a slight difference in their amino acid composition and isoelectric point (5.1 and 5.3 respectively). Conti et al. (1985) reported that the two forms of α-lactalbumin in Camelus dromedarius whey differed at the first N-terminal position. The presence of small amount of a third α-lactalbumin was also reported by Ochirkhuyag et al (1998). β-lactoglobulin appears only in bovine milk, which is in agreement with published data (Farah, 1986; Ochirkhuyag et al., 1998). Lack of β-lactoglobulin is also reported for milk of other species including human milk (Jenness, 1985) and is not due to preparation artifact, science in the SDS-PAGE electrophoresis (Figure 2) no band in the vicinity of 18 kDa was detected in camel whey.

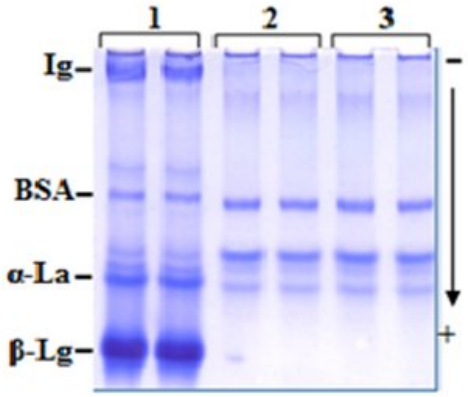

Figure 1. Native-PAGE of camel and bovine whey proteins.
(1) Bovine whey, (2), (3) camel whey from Ouargla and Ghardaia respectively.

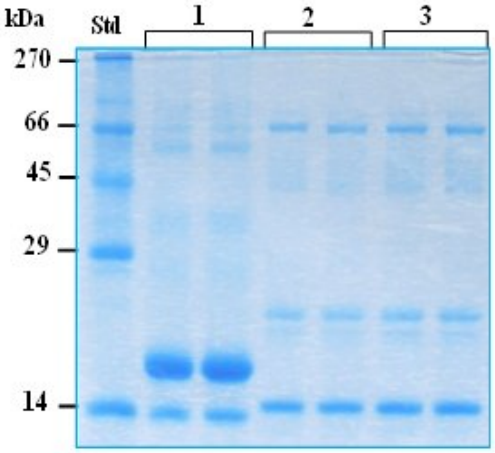

Figure 2. SDS-PAGE of camel and bovine whey proteins.
(1) Bovine whey, (2), (3) camel whey from Ouargla and Ghardaia respectively, std: molecular weight standard.

SDS-PAGE of cow and camel milk whey proteins (Figure 2) showed four bands in the two camel milk samples. Their MWs were estimated at 66.0, 43.0, 29.0 and 14.0. Two major bands (66.0 and 14.0) were identified as serum albumin and α-lactalbumin by comparing the observed electrophoretic profiles with those reported by Ochirkhuyag et al. (1998). The two forms of α-lactalbumin have identical molecular weight according to their mobility in SDS-PAGE at pH 8.0.

A chromatogram of camel whey is presented in Figure 3. Camel whey proteins were separated into 3 fractions. As observed by native-PAGE (Figure 4), serum albumin was eluted in fraction1, the two forms of α-lactalbumin were eluted in fraction 2 and the third peak contained no identified proteins which could correspond to heterogeneous camel milk whey proteins (Beg et al., 1987). It is assumed that other whey components such as lactoferrine (75-76 kDa), lactoperoxydase (69 kDa) and the 43 kDa fraction (Kappeler, 1998) will not be separated on the columns used in this work. It would be reasonable to assume that the whey proteins of camel and bovine that appear at similar elution times are actually identical as was presented by sequencing of the different camel whey proteins by Ochirkhuyag et al. (1998).

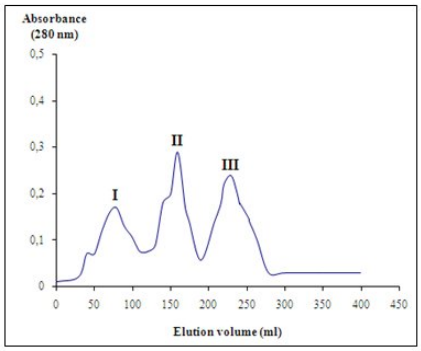

Figure 3. Elution pattern of permeation chromatography on sephacryl S200 of camel whey proteins. The column was equilibrated in 0.02M Tris-HCl buffer pH 8.6. the flow rate was of 0.3 mL min^{-1}. The fraction size was 1 mL.

Electrophoretic and chromatographic separation of camel milk caseins

Characterization of cow and camel milk caseins were performed by urea-PAGE (Figure 5) and the electrophoretic patterns show the same main bands of equal intensity and mobility for the two camel milk samples from different regions. The electrophoretic pattern showed two sharp and distinguishable main bands in camel milk.

According to their increasing electrophoretic mobility, in comparison with cow milk casein, the two bands can be regarded as a possible homologue to bovine. The last band of cow milk sample which corresponding to α_S doesn't appear in the whole camel casein fraction. No protein bands homologous to bovine k-casein could clearly be detected in the electrophoretic pattern. Compared with cow milk caseins, camel's casein presented a lower mobility, than that of their bovine counterparts. This is probably depending on the degree of their phosphorylation (Mohamed and Larsson-Raznikiewicz, 1991). Mohamed and Larsson-Raznikiewicz (1991) and Ochirkhuyag et al. (1997) have obtained dromedary β-like casein band with a migration similar to that of cow k-casein. Neither a band corresponding to κ–casein, nor proteins with mobility similar to bovine casein fractions could be detected. SDS dissociates proteins into their constituent polypeptide chains and has been used for the separation of the proteins according to their molecular weight. SDS-PAGE patterns of camel caseins are presented in Figure 6. The marker proteins with molecular weight between 14 200 and 270 000 were excellently separated in the selected acrylamide gel concentration (15.4%). the molecular masses of the camel casein bands estimated from calibration curve, are 32 000 and 35 500. This is considerably higher than the possible homologous bovine caseins which are estimated at 24 000 for β-casein and 22 000 to 27 000 for α_S-casein.

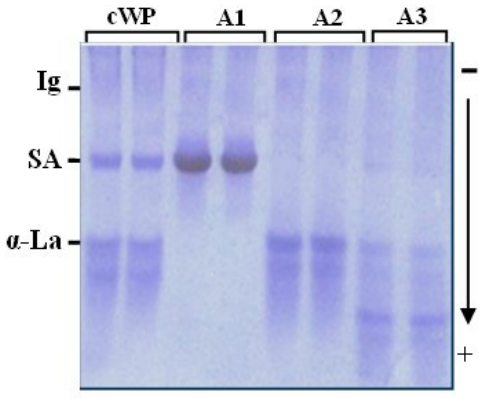

Figure 4. Native-PAGE patterns of fractions issued from permeation chromatography on Sephacryl S200 of camel milk whey proteins.
cWP : camel whey proteins ; A1, A2 and A3: whey protein fractions.

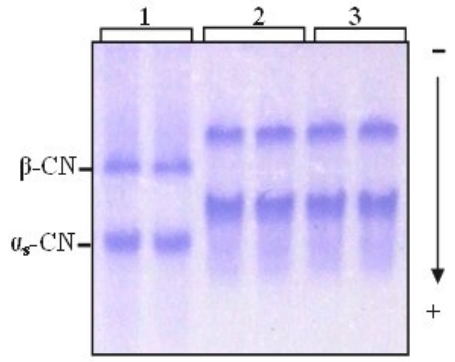

Figure 5. Urea-PAGE of camel and bovine casein.
(1) Bovine casein, (2), (3) casein from Ouargla and Ghardaia camel milks.

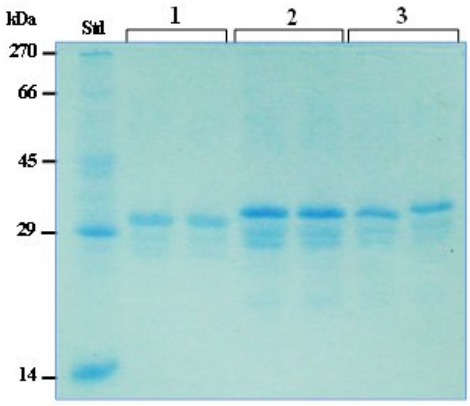

Figure 6. SDS-PAGE of camel bovine casein.
(1) Bovine casein, (2), (3) casein from Ouargla and Ghardaia camel milks, std: molecular weight standard.

Acid-precipitated of whole casein from dromedary milk were separated by anion-exchange chromatography on DEAE-cellulose column (Figure 7). As already know, classical anion exchange chromatography on DEAE-cellulose column resolved whole bovine casein into different fractions containing γ, k, β, α_{S2} α_{S1} respectively (Mercier et al., 1968). As shown in figure 7 caseins were eluted in four peaks at 0.08, 0.16, 0.23 and 0.26 mol L^{-1} NaCl respectively. The electrophoretic pattern of each peak obtained by anion exchange chromatography (Figure 8) suggest that peak 1 contained β-, peak 2 and 3 contained α_{S1}- and peak 4 contained α_{S2}-casein which was co-eluted with α_{S1}-caseins. In comparison to the results from Kappeler et al. (1998), in which κ-casein was eluted faster than other caseins, the most prominent finding is the absence of a chromatographic peak referable to κ-casein. It may therefore be difficult to fractionate them in a single run of anion-exchange in which proteins are fractionated based on their charge.

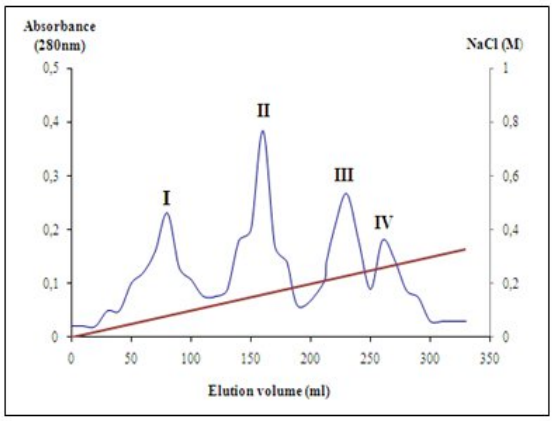

Figure 7. Elution pattern of ion exchange chromatography on DEAE cellulose of camel casein. The column was equilibrated in 10mM imidazole/HCl buffer, pH 7.0, containing 3.3 mM urea and 10mM 2-mercaptoethanol, eluted with a linear gradient of 0-1 M NaCl, at room temperature, at a flow of 0.3mL min^{-1}.

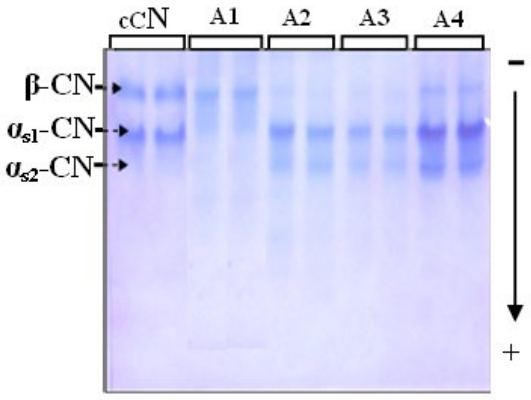

Figure 8. Urea-PAGE patterns of fractions issued from ion exchange chromatography on DEAE cellulose of camel milk caseins.
cCN: camel whole casein; A1, A2, A3 and A4: casein fractions eluted at 0.08, 0.16. 0.23 and 0.26 mol L-1 NaCl respectively.

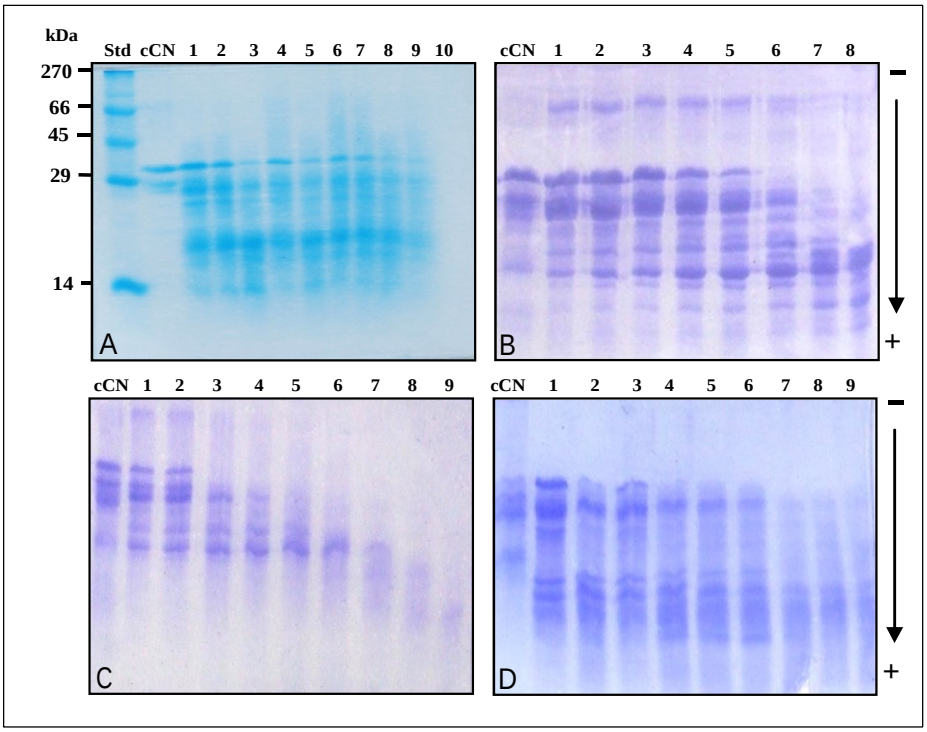

Figure 9. SDS-PAGE analysis of the kinetics of chymotrypsin (A), trypsin (B), pepsin (C) and papain (D) hydrolysis of camel whole casein. Std: molecular weight standard; cCN : camel whole casein, 1,2,3,4,5,6,7,8,9: Hydrolysis times at 5, 10, 15, 20, 30, 45,60, 120, 180 min.

Enzymatic hydrolysis of whole camel casein

In order to study the degree of hydrolysis of camel milk caseins, the enzyme-treated and untreated protein samples of whole CNs were analyzed by SDS-PAGE for pepsin, trypsin, chymotrypsin and papain assays (Figure 9). The α_{S1}-CN was almost fully degraded by both enzymes after 10 min of incubation; it appears like sharp and diffuse band; whereas hydrolysis of β-CN was complete after 5 min of hydrolysis by pepsin, 30 min by trypsin and papain and 48h by chymotrypsin. β-CN from camel milk were more

resistant to trypsin, chymotrypsin and papain digestion, it's very quickly hydrolyzed by pepsin. After 5 min of hydrolysis of camel CNs by chymotrypsin, trypsin and papain, some peptide fragments were still detected on SDS-PAGE, which were stable up to 4h of incubation with chymotrypsin, trypsin and papain, but whith pepsin, peptide fragments were disappeared completely after 60 min of incubation. Similar peptide fragments were not obtained when CNs were treated with different proteases. The major protein components of camel milk, α_{S1}- and β-CNs, contain different numbers of covalently attached phosphate groups bound to residues of serine and threonine (Dickson and Perkins, 1971; Medina et al., 1992). The bound phosphate groups influence many functional properties of these proteins, including their digestibility, bioavailability of divalent cations and immunogenicity (Tezcucano et al., 2007). As reported previously, covalently bound phosphate groups of CNs are supposed to be one of the factors reducing the digestibility of CNs (Li Chan and Nakai, 1989). Taking into account the number of phosphoseryl and phosphothreonyl residues as one of the possible factors reducing the hydrolysis of CNs, β-CN should be hydrolyzed to a greater extent than α_{S1}-CN in both animal species. The results obtained on SDS-PAGE (Figure 9) showed that this was not the case when trypsin or chymotrypsin was used. At least, some portion of β-CN of both species treated by trypsin or chymotrypsin remained uncleaved even after 15 min of digestion. It seems that this protein, which contains four phosphoseryl residues, could better resist digestion by trypsin or chymotrypsin than bovine α_{S1}-CN containing eight phosphate groups (Salami et al., 2008). Thus, another factor must be taken into account to explain the greater hydrolysis of α_{S1}-CN by trypsin, chymotrypsin or papain compared with β-CN, which could be the number of target peptide bonds available for attack by the proteases. Although the greater susceptibility of α_{S1}-CN to trypsin, chymotrypsin and papain hydrolysis compared with β-CN could arise from the number of enzymatic cleavage sites, the accessibility of these sites is another important factor.

Conclusion

Results of this study performed on Algerian dromedary's milk proteins showed homogeneity between samples under both quantitative and qualitative aspects. Results indicate that the whey of Algerian camel milk contains a major protein, α-lactalbumin, existing in two different forms, with identical molecular weight, which are eluted together during Sephacryl S200 permeation gel chromatography and migrated in the same region on SDS-PAGE. They could be separated by isoelectric focusing and/or by anion exchange chromatography, using column with high resolving power. β-lactoglobulin is responsible for some of the observed allergies to cow's milk. Since Algerian camel milk is devoid of β-lactoglobulin, it could be interesting as a new raw material for infant diet and for alleviating some allergic reactions, especially in children. Algerian camel milk samples contain three casein fractions (α_{S1}-, α_{S2}-, β-caseins). Interestingly, analytical results suggested the absence of κ-casein in two samples. The observed differences of their molecular masses, in comparison with cow milk casein, are either due to few variations in their primary sequences or to the divergence of their primary glycosylation and/or phosphorylation.

Whole camel milk caseins were used as substrate for different proteases such as trypsin, chymotrypsin, pepsin and papain. These digestive enzymes, which have different specificities towards the protein substrates, have generated different peptides during proteolysis. The kinetic degradation patterns of camel caseins were visualized by SDS-PAGE. The extent of proteolysis of camel milk caseins by different enzymes depended on the number of target sites available for each enzyme and perhaps on how well these target peptide bonds are accessible to the proteases. The accessibility of the target sites to the digestive enzymes finally depends on fine tertiary structure of milk proteins. Nonetheless, investigations at DNA level are necessary in order to better characterize dromedary's genetic structure.

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REGULAR ARTICLE

Effects of oestrus on milk yield and composition in Tunisian Maghrebi camels (*Camelus dromedarius*)

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Abstract

In order to investigate the effects of oestrus on milk yield and composition in camel (Camelus dromedarius) reared in oasis intensive system in southern Tunisia, 8 healthy females Maghrebi camels (age: 10.6 ± 2.9 years, body weight: 505 ± 39 kg and day in milk: 275 ± 18 days) were equitably divided in two groups. Each female in group 1 received 5 ml of Receptal® (20 µg Buserelin; GnRH analogue) to induce oestrus while dams in group 2 were not injected and served as control. Females were monitored during morning milking for the following 15 days, to record oestrus behaviour, œstradiol-17β levels, milk yield, estimated daily milk, lag-time, time of milking, titrable acidity and density of raw and 24 h conserved milk, somatic cell count (SCC) and milk's major components (dry matter, fat, protein and ashes). Our results suggest that oestrus did not affect (P>0.05) production and physicochemical parameters in milk and did not alter milk conserved in 4°C during 24 h. These data indicate that changes in physiological status of dairy camels during the breeding season do not require alternative measures to guarantee milk quality.

Key words: Dromedary camel, Milk yield and composition, Oestrus, SCC

Introduction

In the last few years, Tunisian farmers became more interested in dairy camels breeding due to the increasing consumption of camel's milk in the market. Therefore a number of dairy camels' intensive farms rose in the oasis of southern Tunisia. The integrity and persistence of these farms rely on succeeding both reproduction and milking management of the she-camel.

Among these milking units, it has been recorded a change of milk physical characteristics and its aptitude to conservation that coincide with the onset of the seasonal mating period and associate with some changes in social and sexual behaviours of the she-camel. These signs suppose that the reproductive status, especially oestrus, during the breeding season influence the milk characteristics. The influence of the oestrus on milk characteristics has been reported in many dairy species. In dairy goat, McDougall and Voermans (2002) reported a decrease in milk yield associate to the induction of oestrus. Increases in SCC have been reported to coincide with the onset of the seasonal mating period (Calderini et al., 1994; Moroni et al., 2007) and associated with induction of oestrus in dairy goats (McDougall and Voermans, 2002; Christodoulopoulos et al., 2008). Likewise, increases in protein content have been recorded during oestrus (Moroni et al., 2007) and associate to synchronization treatments; though, fat and lactose were not affected by oestrus (Christodoulopoulos et al., 2008).

In dairy cattle, several studies reported a decrease in milk yield and an increase in SCC and fat content during oestrus (King, 1977; Horrell et al., 1986; Lopez et al., 2005). Whereas, other studies state that oestrus have no effect neither on SCC (Anderson et al., 1983) nor milk yield and composition (Cowan and Larson, 1979). Therefore, data from cattle are equivocal about the effect of oestrus on milk yield and composition.

The aim of this study was to investigate the effects of oestrus in dairy camels on milk yield, milk composition and SCC.

Materials and Methods

Animals' management

The study was conducted on eight multiparous Maghrebi dairy camel (age: 10.6±2.9 years, body weight: 505±39 kg and day in milk (DIM): 275±18 days) from the experimental farm of the Arid

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Regions Institute (IRA, Medenine, Tunisia). Camels were housed in loose stalls and exercise area was 25 m^2/ camel. Each animal was identified by an ear tag. Daily ration per animal was 10 kg of oat hay (DM, 86.9%; CP, 4.1%; NDF, 66.1%; on DM basis), supplemented with 2 kg of a commercial concentrate (DM, 87.9%; CP, 15.2%; NDF, 22.6%; on DM basis). Animals had a*d-libitum* access to clean water.

Experimental design

The experiment was carried out at the onset of the breeding season (mid-September till mid-December). The she-camels were daily monitored to investigate oestrus behaviour in absence and in presence of sire. At the end of November, females were equitably divided in two groups; each female in group 1 (#240, #229, #237, #250) received intravenous (i.v.) injection of 5 ml of Receptal® (20 µg of Buserelin; GnRH analogue) to induce oestrus (Skidmore, 2004), while dams in group 2 (#228, #232, #238, #243) were not injected and served as control. Females were monitored for signs of oestrus during 15 days following injection. Oestrus was considered when these signs had occurred: the female is repeatedly chasing and biting other females, restlessness, swelling of the vulva, vaginal mucus discharge, straddling the hind legs, raising the tail and urinating frequently, and most important seeking the male.

Milk and blood sampling

Dams were machine milked once a day (8:00 h) in restraining stall and using a portable milking machine. The milking machine was set on 48 kPa, 60 pulses/min, and 60:40 pulsation ratio. Milk ejection was enhanced by tactile stimulation of udders. Lag-time from start of teat stimulation until onset of milk ejection and milking time from attachment of milking unit until milk flow ceased, were recorded. A commercially available iodine disinfectant was applied by dipping each teat after milking.

Milk yield was measured and a milk sample (~ 250 ml) was collected from each camel. The samples were processed immediately after milking and 24h later for physical parameters (titrable acidity and density). Two other samples for chemical analysis (~ 60 ml, -20°C) and SCC determination (+pinch of $K_2Cr_2O_7$, 4°C) were stored.

A blood sample was taken from the jugular vein of each camel after milking using a venoject tubes. Blood samples were centrifuged and sera were stored at -20°C until analysis.

Analyses

Milk density was assessed using a thermolactodensimeter (Funke-Gerber, Berlin, Germany). The titrable acidity (°D) was obtained by titrating 10 ml of milk with N/9 NaOH, using phenolphtalein as an indicator. Total milk solids and ashes were analyzed by gravimetry. Milk protein content was determined by spectrophotometer using Bradford method (Bradford, 1976) and fat was determined by butyrometer using the Neusal method (Wangoh and Farah, 2004). SCC was determined using a Fossomatic 5000.

Oestradiol-17β was determined by direct RIA kits. Gamma counter was used for counting and the produced number was converted by the way of calibration curve for measuring the hormone in unknown samples. Sensitivity was 6 pg/ml and intra-and inter assay coefficients of variation were 12.1 and 11.2%, respectively.

Statistical analysis

Data were statically analysed by the GLM procedure of SAS (SAS version 9.0, SAS Inst. Inc., Cary, NC). The model included the general mean, the fixed effect of physiological state and the residual terms. Results are presented in least squares means ± standard error.

Results and Discussion

Confirmation of oestrus

Three out of four dams injected with Buserelin did exhibit oestrus signs that lasted 4 to 5 days. Two camels of the untreated group have shown spontaneous oestrus signs. These signs lasted 2 to 4 days. The occurrence of oestrus was confirmed by the oestradiol-17β profiles (Figures 1 and 2). These profiles show that maximum levels did not exceed 20 pg/ml for camels which did not exhibit signs of heat, while, for camels in heat, these levels reach 52 pg/ml. The peak of oestradiol-17β lasted 2 to 4 days and the average level was mainly 32.8 ± 13.9 pg/mL.

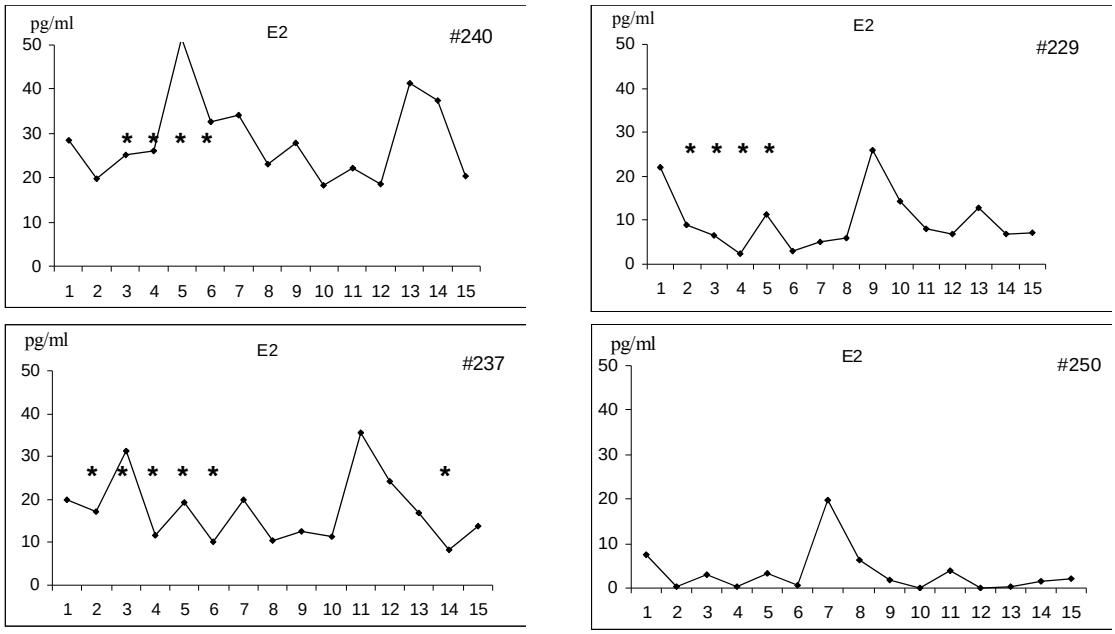

Figure 1. Oestradiol-17β concentration and oestrus days (*) in females treated by Buserelin.

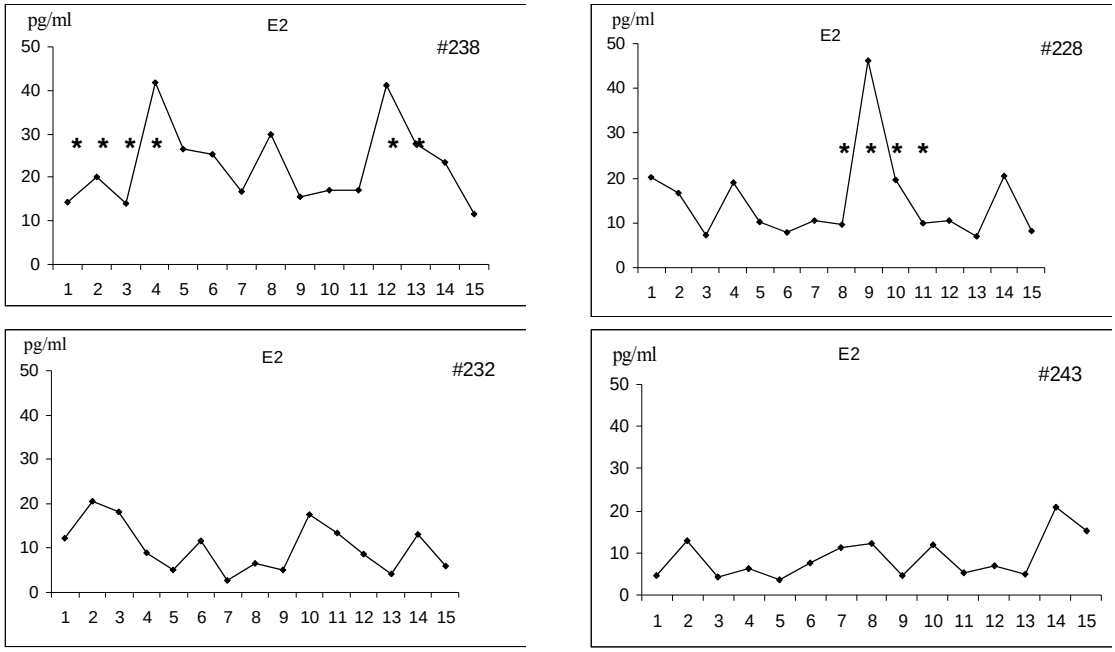

Figure 2. Oestradiol-17β concentration and oestrus days (*) in untreated females.

Milking parameters

Regarding milk yield, lag-time and milking time during oestrus (Table 1), our data suggested that oestrus had no effect neither on lag-time nor on milking time ($P>0.05$). This indicates that oestrus does not alter the milk ejection reflex of the she-camel. In fact, during milking, dams entered the milking parlour in the usual order and they had shown only minor signs of stress. These findings support an earlier study in dairy cattle, Horrell et al. (1984) stated that readiness to enter the parlour, restlessness and elimination in the parlour were not found to be significantly affected by oestrus.

Table 1. Lag time, milking time and milk yield in camels during and out of oestrus.

	oestrus	Out of oestrus	*P*-value
Lag-time (s)	68 ± 4	74 ± 5	0.619
Milking time (s)	210 ± 6	211 ± 6	0.925
Milk yield (ml)	3698 ± 155	3714 ± 84	0.935
EDMP* (ml)	5547 ± 233	5571 ± 126	0.935

*Estimated daily milk production

Milk yield and estimated daily milk production of the she-camel seem to be irrelevant of oestrus. There were no reports on the effect of oestrus on the she-camel milk that we know about till the day of the study. However, several previous studies in dairy cattle and dairy goat provided evidence of a decline in milk volume associated with oestrous (King, 1977; Cowan et Larson, 1979; Mc Dougall et Voermans, 2002; Lopez et al., 2005). Erb et al. (1952) reported that this decline was more apparent as stage of lactation increased. Although camels were in late lactation (275 DIM), the effect of oestrus was not obvious.

Milk conservation ability

As camel milk is usually consumed raw or conserved at 4°C for few days, we aimed to study the potential effect of oestrus on conservation ability of camel milk. Therefore, we estimated the following parameters:

*d (density) = (Milk density 24h later) – (raw milk density)

*d (acidity) = (Milk acidity 24h later) – (raw milk acidity)

Our results regarding the variations in physical parameters of raw and milk conserved at 4°C for 24h associated with oestrus are presented in table 2.

Our results indicated that oestrus had no significant (P>0.05) effect on raw milk physical parameters and it didn't cause a change after 24 h at 4°C (table 2). Hammadi et al. (2007) had studied the effect of breeding system on camel's milk conservation ability. They reported that milk acidity did not change after 24 h of conservation at 4°C.

Milk composition and SCC

Regarding the effect of oestrus on chemical composition and SCC (table 3), our findings suggested that oestrus had no significant (P>0.05) effect on gross components of camel's milk, except for ash that seemed to decrease (P<0.05) during oestrus.

These results were in accordance with those reported by Cowen and Larson (1979) in dairy cattle at early lactation. Nevertheless, for cows at late lactation, oestrus caused an increase of fat content. As for the other major content, Erb et al. (1952) found that they are perturbed during oestrus. In dairy goat, some studies reported an increase of protein content and a decrease of fat content associated with oestrus (Christodoulopoulos et al., 2008; Moroni et al., 2007).

The present study indicates that oestrus is not linked to SCC of camel's milk. These results agreed with those in dairy cattle (Cowan and Larson, 1979, Anderson et al., 1983), yet, disagreed with those found in dairy goat and dairy ewe in which oestrus appeared to provoke a significant increase in SCC (Aleandri et al., 1994; Mc dougall et Voermans, 2002; Christodoulopoulos et al., 2008; Talafha et al., 2009).

Table 2. Effect of oestrus on physical parameters of raw milk and milk conserved for 24h.

		Oestrus	Out of oestrus	*P*-value
Density (g/l)	Raw milk	1.0311 ± 0.0003	1.0303 ± 0.0002	0.163
	Milk 24 h later	1.0325 ± 0.0003	1.0314 ± 0.0003	0.059
	d (density)	0.0015 ± 0.00017	0.0011 ± 0.00009	
Acidity (°D)	Raw milk	17.6 ± 0.3	17.6 ± 0.1	0.869
	Milk 24 h later	17.8 ± 0.3	17.9 ± 0.1	0.774
	d (acidity)	0.2 ± 0.2	0.4 ± 0.1	

Table 3. Effect of oestrus on gross components and SCC of milk.

	Oestrus	Out of oestrus	*P*-value
Total solid (g/l)	121 ± 2.6	118.2 ± 1.35	0.384
Fat (g/l)	44.2 ± 2.2	41.7 ± 0.95	0.281
Protein (g/l)	28.1 ± 0.9	27.5 ± 0.46	0.607
Ash (g/l)	8.6 ± 0.06	8.9 ± 0.04	0.013
SCC (cell/ml)	299750 ± 58744	312362 ± 27516	0.922

Conclusion

Oestrus had no significant effect on physical and chemical camel's milk parameters and it did not alter milk conserved 24h at 4°C. This suggests that no major procedure is needed to ensure milk quality during the breeding season of the she camel. Other studies are required to investigate hygienic practices during milking and milk storage to reveal the reasons of changing milk physical characteristics and its aptitude to conservation in private farms.

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REGULAR ARTICLE

Ovarian monitoring and effects of Controlled Intravaginal Drug Releaser (CIDR) on vaginal environment and follicular activity in dromedary camels, during non-breeding season in Egypt

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Abstract

The study was carried out to monitor ovarian activity in female dromedary camels reared in semi-intensive system in Egypt, during non-breeding season, and to assess the effects of Controlled Intravaginal Drug Releaser (CIDR 1,38g) on vaginal environment and on follicular number and diameters. Twenty females were monitored through vaginal inspection and ultrasonographic examination. Group I (n=10) was monitored in July, while Group II (n=10) in September 2010. Follicle number and diameter were recorded before CIDR's insertion (T0). In Group I CIDR's were inserted after cleaning of perineum with dry paper while, in Group II, after washing of perineum and vaginal flushing with water-5% iodopovidone solution. After 10 days CIDR's were removed, the vaginal status observed and follicles again counted and measured (T1). Results revealed that ovaries were active in July and even if in less measure, in September, which are considered non breeding season month in Egypt. CIDR's treatment caused vaginitis in almost all Group I camels but a better vaginal environment. On the day 10, CIDR were removed in Group II. Statistical analysis revealed that the CIDR's treatment significantly reduced mean follicular diameter in the two months ($P<0.01$; $P<0.05$ respectively) but did not affect follicular number, thus demonstrating its inefficacy in synchronize follicular wave in camels. Both ultrasonographic and hormonal studies will be necessary, simultaneously with CIDR treatment, for better understand effects of exogenous progesterone administration on ovarian activity and follicular wave pattern in female dromedary camels.

Key words: Dromedary, Semi-intensive, Season, CIDR, Vaginal environment, Ovary

Introduction

Dromedary camels (*Camelus dromedarius*) are seasonal breeders and induced ovulators (Tibary and Anouassi, 1997) but factors that affect seasonality are not well documented and information about the breeding season are rather conflicting; in Egypt, it extends from December to May (Al-Eknah, 2000; Shalash, 1987). Camels could be fertile as any other domestic species if appropriate reproductive management is applied (Nagy and Juhasz, 2008). Methods for follicular wave and A.I. synchronization could be very helpful for programming deliveries, for satisfying milk market demand as well as to provide adequate assistance to dam and calves. Nevertheless, research and development in estrus synchronization and controlled breeding have been extensively achieved in ruminants but not enough in camels (Al-Eknah, 2000). Progesterone Releasing Intravaginal Devices (PRID's) with 1.55 g of Progesterone have been tested, in camels, but seems to be of unsatisfactory use (Skidmore et al., 1992) causing vaginal discharges, and also a high proportion of recipients ovulated whilst carrying the device *in situ* (Cooper et al., 1992; Skidmore et al., 1992). Controlled Intravaginal Drug Releasers (CIDR, 330 mg) however, have been proved to control ovarian activity in the related species Lama (*Lama glama*) (Chaves et al., 2002). The present work was performed during two periods of the non-breeding season in Egypt, mid-late of July and mid-late of September, respectively, in female dromedary camels reared in semi intensive system. Aim of the study was to monitor ovarian activity and to understand if CIDR can be effective in

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control and synchronize follicular waves. In addition, to observe clinical effects of CIDR on vaginal environment trying to overcome, possible side effects.

Materials and Methods

Animals, management and feeding

The study was performed at Maryout Research Station of the Desert Research Center (34 Km North West of Alexandria, Egypt). Housing management and experimental procedures were carried out according to requirement of the Council for International Organization of Medical Sciences (CIOMS).

Twenty, healthy, non-pregnant and non-lactating dromedary, aged between 6 and 15 years, were selected. Mean animal weight was 415kg ± 55 and body condition score, measured according to Faye et al. (2001), was 2.5 ± 0.5. Animals were daily watered and left free to graze from 10:00 a.m. to 16:00 p.m. After grazing they received supplement nutrition to cover their requirement as reported from Wilson (1989). Gynecological examination was performed for excluding disease or genital abnormalities, including anovulatory/ hemorrhagic follicles and vaginal septum (Tinson and McKinnon 1992; Tibary and Anouassi, 1996).

Ovarian monitoring, CIDR insertion and removal

Animals were randomly divided in two groups and monitored in different periods: Group I (n=10) from 15th to 26th of July; Group II (n=10) from 15th to 26th of September. The two periods were investigated as corresponding to the beginning and to the middle of non-breeding season, respectively.

Gynecological examination was performed, with camels in standing position, through vaginal inspection, transrectal palpation and ultrasonography. A Dynamic Imaging, concept MLV scanner ultrasound device (PieMedical 100, Falco) integrated with a 6.0 and 8.0 MHz linear dual frequency probe was used; follicles were counted and measured by the electronic caliper.

After ultrasonographic examination (T0), Controlled Intra-vaginal Drug Releasers (CIDR 1.38 g, Pfizer®, Italy), were inserted. The stage of the follicle development at the time of CIDR insertion was random. In Group I, CIDR were inserted after cleaning the perineum with dry paper. In Group II, instead, after washing the perineum with water and povidone–iodine-based detergent solution (Betadine®, Italy); moreover a vaginal flushing with 20 ml of water and 5% Betadine solution, was performed through a syringe and an insemination catheter. Particular care was taken at the moment of insertion, in both group, gently but firmly pushing the applicator against the cervix during pressing its plug for releasing the device; polyester tails were cut to avoid biting from other females. Devices were left in vagina for 10 days and every three days animals were controlled in case of CIDR lost. On day 9, 500 µg of $PgF_{2\alpha}$ (Estrumate®, Ontario, Canada), were administered (Skidmore et al., 1998). On the day 10, CIDER were removed, vaginal cavity observed trough a speculum and the vaginal discharge evaluated. According to the amount of the debris associated with the device and in the vagina a score was assigned: 0, no debris; 1, small flecks of purulent debris in the vagina and on the device; 2, copious amount of purulent debris in the vagina and on the device (Walsh et al., 2008). Moreover follicles were again counted and measured, by ultrasonographic scanning, the day of CIDR removal (T1).

Statistical analysis

Number and follicle dimensions were subjected to a repeated measures analysis of variance (ANOVA) using the general linear model procedure (SAS, 1999). Independent variables were the period of the year (July and September) the time (T0 and T1) and the interaction between those variables. Data were normally distributed. Turkeys post hoc test was used to perform statistical multiple comparison. P level was set at 0.5. All data were expressed as quadratic mean and mean standard error (SEM).

Results

Mean follicle number was found not to be statistically different between July and September in T0 (Table 1). Mean follicle diameter was found to be higher in July rather than September: 1.05 Vs. 0.61 cm ($P<0.05$) respectively, at T0 (Table 2). None of the animal lost the CIDR except one in group I where the tip pf the device was observed out of the vagina after six days; CIDR was then removed, washed and reinserted. At the time of CIDR removal 6 females of Group I revealed vaginitis vaginal score 2 and 4 females had vaginal score 1; foul smell of vaginal discharge, was noticed in some of those animals but any sign of metritis was detected by ultrasonography. All Group II animals, instead, presented an overall vaginal score of 0-1. In those females, a small amount of white creamy mucus was found on CIDR surface and in vaginal cavity but such white mucus lacked of purulent and foul smell and vaginal walls were not hyperaemic. In two animals of Group I ultrasonography at the time of CIDR

removal revealed round body hyperechoic structures, embebbed in ovaries and differentiated from ovarian stroma. Those animals were supposed to spontaneously ovulated while retaining the CIDR, and excluded from statistical analysis regarding comparison in T1. Progesterone treatment did not affect follicle number (T1, Table 1) in the periods of the study but, on the other side, statistically affected follicle diameters that regressed from 1.05 cm to 0.51 cm ($P<0.01$) in July and from 0.61 cm to 0.41 cm ($P<0.05$) in September, respectively (T1 Table 2).

Table 1. Mean follicle numbers (n) in female camels during July and September before and after CIDR treatment.

Period	T0[1]	T1[2]	SE
July	2.50	2.30	0.42
September	2.70	1.70	

1CIDR insertion 2CIDR removal

Table 2. Mean follicle diameters (cm) in female camels during July and September before and after CIDR treatment.

Period	T0[1]	T1[2]	SE
July	$1.05^{A;x}$	0.51^{B}	0.10
September	$0.61^{a;y}$	0.41^{b}	

1CIDR insertion 2CIDR removal

Different letters in the same line show statistical differences (A, B: **P<0.01; a, b: *P< 0.05)

Different letters in the same column show statistical difference (x, y: *P< 0.05).

Discussion

Results revealed that, camel bred in semi-intensive system and in optimal condition, showed prolonged ovarian activity in July as well as, although with lower intensity, in September. This is contrast with Shalash (1987) that defined camel's seasonal anoestrus from June to November. Indeed, references concerning camel breeding season in Egypt are scanty and there aren't specific data concerning seasonality in extensive, semi-intensive or intensive breeding system. Arthur et al. (1985) claimed that camels can be truly polyestrous with a continuous supply of sufficient food. Adequate management and nutrition of the camel reared in Maryout Research Station, probably helped in overriding environmental effect on ovarian activity extending ovarian activity until July-September. Similar observations reported in India by Vyas et al. (2004), who found pre-ovulatory follicles ≥1.0 cm during non-breeding season (June–August), and obtained pregnancy with programmed mating in dromedary camels, bred in a semi-intensive system. CIDR have been well retained from almost all camels. Good results in term of PRID retention were reported also from Cooper et al. (1992), who observed only four devices expelled once, and one animal completely failing in retaining the device, out of 66 camels. PRID retention relies on the resistance associated with the broad flat surface of the device against the vaginal mucosa, due to its outside diameter (4.5 cm) while, CIDR fastening is due to the pressure of the 2 wings against the vaginal walls. Both mechanisms seem to be effective for retaining of such devices in dromedary camels vagina. CIDR treatment, however, caused bacterial vaginitis in almost camels of Group I. The distinction between of devices on the vaginal environment, and the introduction of bacteria during insertion is difficult, because in this study, we performed only a clinical evaluation of CIDR effects. However, as a significant improvement of the vaginal environment was obtained in group II with washing of perineum and vaginal flushing, we could reasonably suppose that the contamination of vagina at the time of insertion was the main responsible of the observed vaginitis and vaginal discharge. Al-Sobayil (2008) treated 10 female camels with PRID for estrus synchronization. Devices were inserted after washing, disinfection and lubrication of vulva, and left in vagina for 17 days, but no data about vaginal environment at the time of their removal was reported (Al-Sobayil, 2008). Walsh et al. (2008), observed an 11% of vaginitis in cows when Intravaginal devices (with or without progesterone impregnation) were inserted, after washing of perineum with water, and lubricated with 1% w/w chlorhexidine cream. Further analysis would be necessary for evaluating the effect of vaginal flushing, compared to clorexidine cream, on bacterial growth during CIDR retention as well as the effect of 5% water-betadine solution on vaginal mucosa. According to ultrasonography in T1, two spontaneous ovulations (10%), were supposed to happens. Cooper et al. (1992), by monitoring plasma progesterone after the retrieval of PRID devices, found 33% of spontaneous ovulation in 36 camels during the treatment. Marie and Anouassi (1987) reported some spontaneous ovulation in female camels exposed to a male after a prolonged period of separation and after the regression of an induced luteal phase. They also reported that, in one of four camels, progesterone secretion was observed after the removal of PRID device. Mechanism operating in spontaneous ovulators can be effective in certain

situations in dromedary camel (Marie and Anouassi, 1987; Nagy et al., 2005) but is not possible to distinguish such spontaneous ovulation from a follicular luteinization (Tibary and Anouassi, 1996). Thus, for clarifying the occurrence and mechanism of "spontaneous ovulation" and/or "follicular luteinization" induced by progesterone intravaginal devices (CIDR or PRID), it would be useful to monitor follicular dynamics by ovarian ultrasonography, estradiol and progesterone determination, during and after the treatment. It would be also worthwhile to examine mechanical effect of "unloaded" or progesterone free CIDR (or PRID) on ovarian activity, as well as on vaginal environment, as suggested from Cooper et al. (1992). The progesterone administered through CIDR was effective in reducing mean follicular diameter in both periods of this study. Statistical differences concerning the efficacy of the treatment in those periods, probably was related to the different diameters of follicles at the time of insertion in July and September. Nevertheless, the treatment did not affect mean follicular number, demonstrating its failure in controlling the emergence of new follicular waves. This result is in agreement with Skidmore et al. (2009) that, treating camels with a daily dose of 150 mg progesterone-in-oil for 14 days induced a reduction in follicular size at the end of treatment, but only a small reduction of its number. Latest authors stated that, although exogenous progesterone will hasten the regression of large follicles it does not completely inhibit follicular growth in camels (Skidmore et al., 2009). Indeed, endogenous progesterone does not completely control ovarian activity in dromedary camel females; it is reported that mature follicles (>10 mm) can be found until 105 days of pregnancy (El-Wishy et al., 1981; Musa and Abusineina 1978). However, in the related South American Camelids, Vaughan (2001) stated that 100 or 200 mg of progesterone, administered IM every two days, were effective in inducing regression of the existing dominant follicle and preventing new wave emergence. Whereas, Chaves et al. (2002), by evaluating follicular diameters and sexual hormones concentrations during treatment, stated that CIDR (0.33 g) could be effective in completely preventing follicular development for a period of up to 7 day (Chaves et al., 2002). Those result let us suppose that a higher dose of progesterone (CIDR 1.9 g), probably should be investigated as a mean for control and synchronize follicular waves in dromedary camel females.

Conclusion

Ovarian activity in female dromedary camels, bred in semi intensive system could be found in July and, even if with lower intensity, in September, yet considered non breeding season periods in Egypt. Cleaning of perineum and flushing of vagina with a iodopovidone solution before CIDR insertion clinically improved vaginal environment at the end of the treatment. Progesterone administration through CIDR (1.38 g) for 10 days, was effective in reducing follicular diameter in the beginning and in the middle of the non-breeding season, but did not reduced follicular number, revealing its inefficacy for synchronizing follicular waves. More comprehensive studies will be necessary for better understand effects of exogenous progesterone administration through CIDR, on ovarian activity and follicular wave patterns in dromedary camels.

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REGULAR ARTICLE

Effects of storage temperature and time on fecal progesterone concentration in camel (*Camelus dromedarius*)

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Abstract

This study aimed to evaluate the effect of temperature (-20°C, + 4°C, room temperature) and storage time (7, 15, 107 and 173 days) of feces on progesterone concentration in camel (*Camelus dromedarius*). Plasma and feces were collected from 10 pregnant and one non-pregnant camels. Extraction of fecal metabolites of progesterone was performed with methanol and petroleum ether. The analytical validation was provided by internal quality control (IQC) and the success of the validation tests (sensitivity, precision, recovery and parallelism). In comparison to the value found in the day of collection, the mean concentration of progesterone in feces which was frozen or dried at room temperature showed no significant difference after 7 and 15 days. A significant increase was found for fecal samples stored at + 4°C. After 107 and 173 days, freezing is inadequate condition of storage because the fecal progestagen concentrations vary significantly. However, drying feces at ambient temperature maintained stable progestagen concentrations. Therefore, results indicated that drying feces is a reliable method, independent from an electric power source and the freezing equipment.

Key words: Camel, Feces, Progesterone metabolites, RIA, Storage

Introduction

Sexual hormone measurements traditionally involve invasive techniques such as blood collecting. This technique is stressful for animal and requiring a strict conservation of samples. As an alternative, the measure of steroid metabolites in feces is widely suggested to monitor reproductive hormones in wildlife species (Graham, 2004; Freeman et al., 2010) as well as in domestic animals (Cebulj-Kadunc et al., 2000; Kornmatitsuk et al., 2007). It is a non-invasive method, since it avoids the stress effects related to blood sampling and fecal samples are easily collected and stored. However, the reliability of the results based on a rigorous analytical and physiological valiadation for each species is primordial (Capezzuto et al., 2008).

The storage of fecal samples is a critical concern because fecal bacteria metabolize fecal steroids within hours after deposit. For this reason, several studies were made to control for any variation due to storage procedures, which to avoid misinterpretation of the results.

In order to minimize degradation of the fecal steroid, many authors were recommended to store fecal samples at -20°C until analysis (Whitten et al., 1998; Mostl et al., 2005). Other studies showed that ethanol has been used as a preservative for short-term ambient temperature storage of fecal samples (Khan et al., 2002; Lynch et al., 2003). Furthermore, Terio et al. (2002) suggested that storage of fecal samples at room temperature in ethanol was the best alternative to freezing for subsequent analysis of steroid hormone concentrations because it stabilized the concentrations of metabolites for progestogen and estrogen in cheetah for a period of up to 2 weeks. Other ambient temperature field storage techniques included drying samples. In this case, Pettitt et al. (2007) showed that drying feces provides a reliable method for long-term preservation of fecal steroid concentrations and was optimal alternative when freezing was not a viable option.

The present study was designed to compare different methods of preserving (frozen at -20°C, stored at + 4°C, dried and stored at ambient temperature) fecal samples over several time periods (after 7, 15, 107, 173 days) in order to determine which method provided the most accurate and reliable technique for measuring fecal progesterone in camel.

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Materials and Methods

Animals and samples collection

Ten adults, fertile and pregnant camels were used. They weighed 502.4 ± 57.4 kg and were 13.2 ± 3.8 years old. Another non pregnant camel (#814) was used in the test of accuracy. They were moved during 6 to 7 hours grazing salty species. Animals had access to water once a day.

Blood and feces samples were collected in the morning for one day. The blood samples (5 ml) were withdrawn from the jugular vein into heparinized tubes, centrifuged immediately for 30 min, and the plasma were stored at -20°C until assayed. Fecal samples were collected directly from the rectum. They were taken in plastic bags clearly identified (number of camel, collection day).

Fecal sample processing

Storage conditions of feces

The initial concentration of metabolites of progesterone was determined by extraction immediately just after collection (day zero). In this study, we examined the effects of different storage treatments and periods on the fecal progestagen concentration. Each sample was thoroughly mixed. The aliquots were equally divided into three sub-samples and subjected to the various storage conditions described below (-20°C, 4°C, or room temperature) for a short (7 and 15 days) and medium (107 and 173 days) storage period. The extraction and radioactivity counting by a gamma counter were performed after each storage period.

Fecal extraction

All fecal samples were extracted following Korndorfer et al. (1998) with some modifications. Briefly, each sample was weighed out to exactly 0.25 g and placed in glass tubes. Distilled water (0.25ml) and methanol (2.0 ml) were added. The tubes were shaken for 30 min (200 rpm). Petroleum ether (1.5 ml) was added. They were again shaken on a vortex for 15 sec, and centrifuged for 30 min (1500g, 4°C). The tubes were frozen for 5 min. The methanolic phase (1.0 ml) was transferred carefully into other tubes.

Radioimmunoassay

Fecal and plasma progestin concentrations from each sample were analyzed in duplicate by RIA using six Kits (Immunotech, France). The standards curve ranged from 0 to 49 ng/ml. Two internal controls (0.91 to 1.63 ng /ml) were run in every assay.

We validated the radioimmunoassay by serial dilutions from a fecal extract of pregnant camels (1, 1:4, 1:8, 1:16, 1:32 and 1:64) in phosphate buffer (0.01M; pH 7.4; 0.01% BSA). The intra- and inter-assays coefficients of variation for plasma and fecal was obtained using three levels of progesterone (low, medium, high). All samples were tested in triplicate in the same and other immunoassays, respectively. The recovery rate was determined by adding 250µl of I^{125}- Progesterone (approximately 47452 cpm) to 9 samples before extraction.

Linearity was estimated by a serial dilution (1:32, 1:64, 1:128) in phosphate buffer for a fecal sample #9810 rich in progesterone metabolites (2885 ± 381 ng/g DM fecal).

Statistical analysis

We calculated the concentration of plasma hormones (ng/ml) or fecal metabolites (ng/g MD of feces) in every sample. The concentrations of fecal progesterone metabolites were assessed using the GLM-Procedure of SAS (version 9.0). We used Dunnett multiple comparison test ($P<0.05$) to compare the mean concentrations of metabolites of progesterone after each storage period with the control. Results are expressed using mean ± standard error.

Results and Discussion

Analytical validation

For fecal assays, the average maximum binding (B0/T) was 52.4%. The average of nonspecific binding (NSB) of the reagents was 1.37%, and a total count (TC) of about 23957 cpm/500 µl. A standard curve is characterized by a concentration of progesterone corresponding to 20, 50 and 80% were 6.7, 1.12 and 0.2 ng/ml.

The intra and inter-assays coefficients of variation for internal quality controls were 6.5% and 6.1%, respectively. In pregnant camels, B/B0 of non-diluted samples was lower than 5%. Therefore, the methanol phase was diluted 1:64 in phosphate buffer prior to radioimmunoassay with B/B0 values ranging between 37.8 and 46.2%. In our study, the dilution ratio was higher than that found by Capezzuto et al. (2008) in the goat, which was 1:40. In addition, Ben Mohammed et al. (2011) used a dilution which was equal to 1:50 for cycling females and 1:160 for pregnant gazelles (Gazella gazella). To assay the metabolites of progesterone in the Cape ground squirrel (*Xerus inauris*), Pettitt et al. (2007) used a dilution that ranged from 1:51 - 1:150.

The intra-assay coefficient variation for a sample having high fecal P4 concentration was equal to 11.9%. This value was similar to that reported by Cebulj-Kadunc et al. (2000) in sheep (11.4%). It was slightly higher than that published by Isobe et al. (2005) in bovine, which was about

10.9%. For this same sample, the inter-assay coefficient variation was equal to 17.3%. This coefficient was similar to that found by Dantzer et al. (2010) for cortisol (17.9%). However, this coefficient was higher than that obtained by Korndörfer et al. (1998) in rabbit (15.7%) and Cebulj-Kadunc et al. (2000) in sheep (13.8%) for progesterone. The intra and inter-assay coefficient variation for a sample having high concentration plasmatic of P4 were, respectively, equal to 17.3% and 17.6%.

The recovery rate of progesterone metabolite from feces was 70.4 ± 0.9%. It was lower than that found by Kornmatitsuk et al. (2007) in cows (89.7%). The parallelism between the theoretical and observed concentrations of metabolites of progesterone in the feces was estimated by the linear regression analysis which gave the following equation with a correlation coefficient of 0.991: $y = 0.743\ x + 0.046$, where x = the theorical concentration and y = the observed concentration.

Plasmatic progesterone concentration in pregnant camels

In the third month of gestation, level of the plasmatic progesterone concentration varied from 3.0 ± 0.2 ng / ml to 5.2 ± 0.2 ng / ml, and averaged 4.4 ± 0.7 ng / ml (Table 1). The mean plasmatic progesterone concentration confirmed pregnancies.

Table 1. Mean concentrations of plasma progesterone in pregnant camels.

Camels	Mean ± SD (ng/ml)	Min (ng/ml)	Max (ng/ml)
#9703	4.6 ± 0.3	4.3	4.9
#0413	3.3 ± 0.3	3.1	3.6
#9606	4.3 ± 0.6	3.8	5.0
#9402	3.0 ± 0.2	2.8	3.3
#0013	5.2 ± 0.2	5.0	5.5
#9810	4.2 ± 0.4	3.7	4.6
#9408	4.6 ± 0.3	4.7	4.9
#9108	5.0 ± 0.3	4.6	5.4
#0019	5.2 ± 0.01	5.2	5.2
#9403	4.7 ± 0.2	4.5	4.9

In this study, plasmatic concentrations of progesterone were consistent with concentrations reported by Al Eknah (2000) in camels and by Adams (2007) in llamas and alpacas. Both authors proclaimed that the plasmatic concentration of progesterone was more than 2 ng/ml during pregnancy.

Effect of temperatures of short-term storage on the concentration of progesterone metabolites in feces

The initial concentration of metabolites of progesterone (day 0) varied from 313.3 to 3154.3 ng/g DM feces, and averaged 1441.9 ± 177.9 ng/g DM feces.

There were no significant differences in fecal progestagen concentrations between the control group (day 0) and after 7 to 15 days of storage at -20°C (Figure 1). The mean concentration of progesterone decreased slightly. It was equal to 1054.7 ± 122.2 ng/g DM feces and 1169.1 ± 135.5 ng/g DM feces, respectively, after 7 and 15 days of freezing. Our result were consistent with those obtained in baboons (*Papio cynocephalus*) fecal samples stored in 95% at -20°C for two weeks prior to lyophilization, as described by Lynch et al. (2003). In addition, this study showed that freezing immediately after defecation did not affect the concentration of progestagens. This result was an agreement with those reported by Galama et al. (2004) and Pettitt et al. (2007). Furthermore, a study conducted by Khan et al. (2002) showed that storing fecal samples at sub-zero temperatures, alcohol may prevent deterioration of steroid metabolites.

Following 7 and 15 days of storage at 4°C, the mean concentrations of metabolites of progesterone (±SE) showed a significant increase ($P<0.05$). It became equal to 3119.2 ± 325.6 ng /g DM feces at day 7 and 2126.6 ± 278.3 ng /g DM feces at day 15. Our results were in agreement with that found by Lynch et al. (2003). These authors reported that storage of feces in 95% ethanol for two weeks in a charcoal refrigerator caused a significant increase in fecal progestogen concentrations. However these samples still maintained the ability to distinguish reproductive states.

The significant increase in concentration of progesterone was due to the transformation of steroid metabolites of the conjugate form to the unconjugated form through the activity's effect of fecal bacteria and microorganisms (Ziegler et al., 1996; Khan et al., 2002). Thus, the increase of the steroid concentration could be the result of the antibody cross-reactivity with other hormonal metabolites created by microbial transformation during storage (Terio et al., 2002).

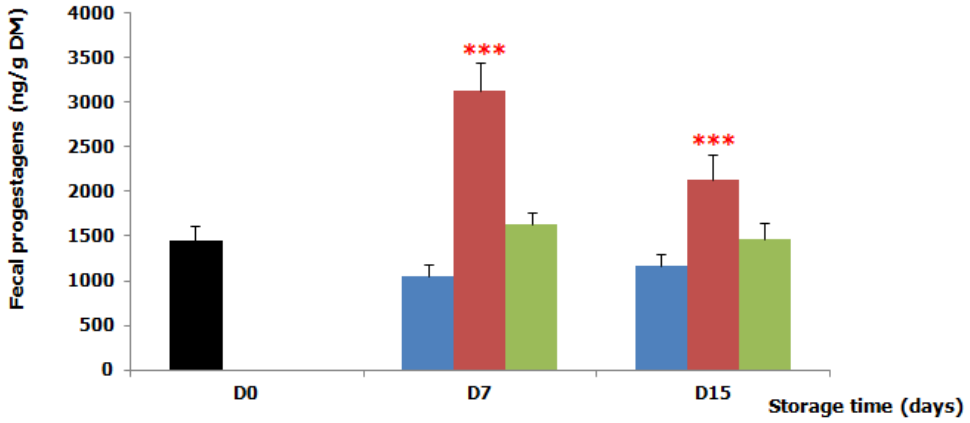

Figure 1. Effect of temperature of short-term storage on the mean fecal progestagen concentrations ±SE for a pregnant female (*Camelus dromedarius*).
Fecal samples stored at -20°C; fecal samples stored at 4°C; and fecal samples dried and stored in ambient temperature. The *** indicates statistical significance (P<0.05) with the initial concentration.

However, samples that were dried and stored in room temperature did not have significantly different fecal progestagen from initial concentration at 7 and 15 days. In fact, the level of metabolites of progesterone varied from 449.0 ng/g DM feces to 2759.8 ng/g DM feces and averaged 1626.3 ± 144.8 ng/g DM feces after 7 days of storage at room temperature. It becomes equal to 1467.4 ± 188.2 ng/g DM feces with a range from 492.3 ng/g DM to 3683.7 ng/g DM feces after 15 dayes. This study showed that storing fecal samples in room temperature did not affect progesterone metabolites concentration for 15 days. Hence, our result was similar with that published by Pappano et al. (2010) for both glucocorticoid and testosterone metabolite levels until 4 weeks of storage. Furthermore, this study was consistent with that reported by Brockman and Whitten (1996) in a primate (*Propithecus verreauxi*) for which the concentration of fecal progesterone remained stable after 3 weeks of drying and storing at room temperature. Also, Galama et al. (2004) showed that stability in concentration of fecal progesterone in rhinoceros was observed even after one month of storage at room temperature. Then, Khan et al. (2002) suggested that storing of fecal samples at room temperature for no longer than 30 days for glucocorticoids and for a shorter period for estrogen was preferable, if the freezing was not feasible.

In the present study, stability in the concentration of fecal progesterone may be explained by a high level of dry matter (45%) in comparison with feces in cows (15% DM) or a lack of moisture which can slow down bacteria growth and may prevent the transformation or the deterioration of steroids. The form of feces (pellets) which are easier to dry should be also considered.

Effect of temperatures of medium-term storage on the concentration of progesterone metabolites in feces

In comparison with the initial concentration (day 0), the mean concentration of metabolites progestagen decreased significantly (P< 0.05) after 107 and 173 days of freezing (Fig 2). It was respectively equal to 932.8 ± 108.4 ng/g DM feces and 949.7 ± 121.1 ng/g DM feces.

Our finding was comparable to that published by Khan et al. (2002) who reported that the concentration of some fecal steroid metabolites varies after months of storage at -20 °C. Similarly, Neumann et al. (2002) showed that keeping samples after one and three months at -20°C caused a significant decrease in fecal progesterone concentration of giraffe in comparison with the initial concentration. In contrast, a study by Pettitt et al. (2007) concluded that the level of fecal progesterone remained stable during three months of freezing. Neumann et al. (2002) showed also no significant changes of progesterone concentration, when samples were stored at -20°C after one and three months, for both rhinoceros and gazelle. In order to have a strong conclusion, it was interesting to validate the effect of freezing on fecal progesterone concentration after one month of storage.

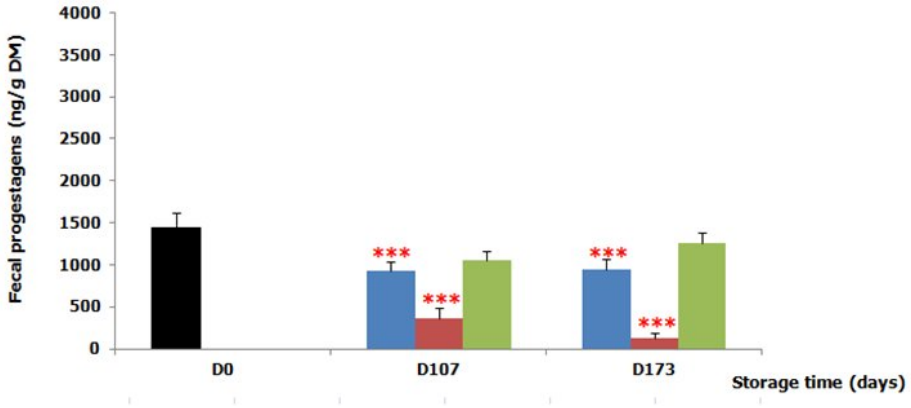

Figure 2. Effect of temperature of medium-term storage on the mean fecal progestagen concentrations ±SE for a pregnant female (*Camelus dromedarius*).
Fecal samples stored at -20°C; fecal samples stored at 4°C; and fecal samples dried and stored in ambient temperature. The *** indicates statistical significance (P<0.05) with the initial concentration.

After 107 and 173 days of storage at 4°C, the mean concentration of metabolites progestagen droped dramatically and significantly (Fig 2). It became respectively equal to 366.3 ± 125.2 ng/g DM feces (with a rate of reduction is equal to 75.5%) and 133.2 ± 48.9 ng/g DM feces (with a rate of reduction is equal to 90.8%).

In camel, the mean progesterone concentration decreased significantly, when fecal samples stored at 4°C, compared with those obtained in feces dried and stored at room temperature. Contrary, Schlenker et al. (1999) reported that concentrations of progesterone and estrogen in fecal cow samples decreased more rapidly after 13 weeks of storage at room temperature (30°C) than at 5°C.

This sudden and significant drop in progesterone concentration in fecal camel samples was explained by the presence of moisture in storage environment, which causes growth and multiplication of bacteria and detritus. Moreover, this study showed the presence of fungus on all fecal samples stored after 107 and 173 days at 4°C, which could be correlated with the level of the progestagen concentration.

The increases or decreases in fecal hormone concentrations during long-term storage may be influenced by a combination of variables including storage treatment, storage time and antibody specificity (Pettitt et al., 2007). Other Studies suggested that changes in hormone concentrations could be due to the influence of storage treatment and time on the activity of fecal bacteria and microorganisms (Khan et al., 2002).

After 107 and 173 day of keeping at room temperature, the mean concentration of metabolites progestagen decreased slightly (Figure 2). It averaged 1059.5 ± 108.3 ng/g DM feces and 1253.9 ± 136.0 ng/g DM feces, respectively, after 107 and 173 days. Consequently, drying and storage feces at room temperature did not affect the level of fecal progesterone for 6 months.

Our result was similar with that reported by Galama et al. (2004), who have showed that progesterone concentration remained stable for at least 180 days when fecal rhinoceros samples were stored at room temperature after drying in a solar box cooker (45°C) or mixing feces in methanol (80%). Moreover, Pettitt et al. (2007) showed that drying and storing feces at room temperature for 330 days was a best alternative of storage in field.

While Khan et al. (2002) showed that metabolites concentrations of estrogen (122%) and glucocorticoid (92%) increased after 90 and 120 days, respectively, when fecal samples stored in a 95% ethanol solution at room temperature. After 180 days, concentrations of both hormones metabolites declined to near initial concentrations. Similarly, Galama et al. (2004) reported that metabolites concentrations of progesterone increased significantly in feces stored at room temperature without heat or chemical treatment.

Conclusion

In our study the measure of metabolite progesterone concentrations in fecal camel by radioimmunoassay was validated. In addition, freezing samples immediately after collection was an optimal means of preventing bacterial metabolism of steroid metabolites and maintain the stability of the fecal progesterone concentration in camels for a short term of storage. Then, we proved

that storing fecal samples at 4°C causes significant changes in progesterone concentrations. Therefore, it was an inappropriate method for short and medium terms of storage. However, we showed that drying and storing camel fecal samples at room temperature for 180 days was advisable. Consequently, it is a field method of preservation fecal, independent of freezing equipment and it is a reliable method to monitoring the physiology and behavior of camels.

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REGULAR ARTICLE

Thermophysiological study in lactating and dry camels (*Camelus dromedarius*) under summer conditions

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Abstract

A comparative study on thermophysiological responses of 24 lactating and non-lactating (dry) camels maintained under natural summer conditions was carried out with 2 Arabian native breeds. Study parameters (meteorology, thermophysiology, infrared thermography) were measured in both breeds at the evening milking (17:00h) throughout 3 consecutive days. It appears evident that lactating camels are more thermally labile than their dry counterparts under such environmental conditions. This fact was proven by higher body temperatures of lactating camels than their dry counterparts (rectal temperature: 38.01 ± 0.07°C VS 37.80 ± 0.06°C and vaginal temperature: 38.20 ± 0.08°C VS 37.79 ± 0.07°C, P< 0.05). Additionally, this was further emphasized by the noticeable increases of several thermophysiological parameters in lactating camels including their respiratory rate (6.57%), heart rate (9.36%), as well as body (11.06%), udder (4.74%), teat (5.52%), and milk vein (4.51%) surface temperatures. In conclusion, lactating camels expressed higher thermophysiological responses over dry camels. Infra-red thermography can be a suitable tool for non-invasive method that detects surface heat radiation in dromedary camels.

Key words: Dromedary camel, Infrared thermography, Lactation, Thermoregulation

Introduction

Dairy camels are quite distinct from other dairy animals. This is mainly attributable to their ability to continue lactating even under severe conditions confronted in their natural environmental habitat (Saleh and Faye, 2011; Al-Saiady et al., 2012). Beside the environmental heat load, body heat production and the resultant increase in heat storage associated with lactation in dairy cattle were considered another impingement factors (Shearer and Beede, 1990; Ben Younes et al., 2008). Thus, due to the greater body heat storage, lactating cattle showed higher body temperatures compared to the non-lactating cattle (Araki et al., 1984; El-Nouty et al., 1990). This fact indicates that lactating animals may have greater susceptibility to environmental changes than dry animals. It is definitive, therefore, that lactating animals must dissipate both the heat gained from the environment together with their own metabolic heat to maintain a constant body temperature. In Saudi Arabia, camel population is estimated to be approximately 830,000 heads distributed in different parts of the country (Agriculture Statistical Year Book, 2009). Majahiem (black coat) and Maghatier (white coat) camels are the predominant dairy breeds of camels in Saudi Arabia, where their milk yield under favorable climatic conditions ranged between 6 to 18 liters per day (Aljumaah et al., 2011). Excessive solar heat stress in livestock at the geographical region of Saudi Arabia has been previously recognized (Ali et al., 1999; Al-Haidary, 2006). However, the extent of thermotolerance to such stress by these indigenous dairy camel breeds has not been investigated.

Infra-red thermographic technology (IRT) is a non-contact and non-invasive method that detects surface heat emitted as infrared radiation. This technology has previously been used to study temperature patterns of udder and teat surface temperature in dairy cows (Kunc et al., 2002), dairy ewes (Stelletta et al., 2007), dairy goats (Caruolo et al., 1990), and in lactating camels (Ayadi et al., 2012). Therefore, the IRT technology beside other thermophysiological measurements were adapted to conduct a comparative study between lactating and dry camels belonged to 2 breeds and maintained under the natural summer conditions.

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Materials and Methods

Twenty four dairy dromedary camels of 2 Arabian native breeds (12 Majahiem and 12 Maghatier) were allotted into 2 groups of similar size according to their lactation status (lactating and dry). Dry camels had a mean parity of 2.8 ± 0.1 (means ± SD). Meanwhile, lactating camels (3.1 ± 0.2 parities, 134.2 ± 19.0 days in milk, 9.3 ± 1.1 L/day milk; means ± SD) were regularly milked twice daily (05:00 and 17:00h) using a down pipeline machine milking system (DELAVAL, Riyadh, KSA) at Al-Watania agricultural farm stock intensive industrial system, Al-Jouf, Saudi Arabia. All camels were identified by electronic ear tags (Shearwell Data Ltd, Minehead, Somerset, UK), fed twice a day, and had free access to clean tap water. Daily ration per animal consisted of 13.5 kg of alfalfa hay (DM, 92.2%; CP, 21.8%; NFD, 26.7%; GE, 4.36 Mcal/kg; DM basis) and supplemented with 2 kg of a commercial concentrate (DM, 90.2%; ME, 2.9 Mcal/kg). All camels used in the study were free from clinical mastitis and pregnancy.

Ambient temperature (T_a) and relative humidity (RH) were concurrently and continuously recorded at 15 min interval throughout study period using 2 data loggers (HOBO Pro Series data logger, Model H08-032-08, ONSET Co., Wareham, MA, USA) mounted in the barn at a height of approximately 2 m from the ground, and placed away from direct sources of heat, sunlight and water. Special data logging software (BoxCar Pro 4, ONSET Co., Wareham, MA, USA) was applied for programming the loggers and for data analysis.

Before the evening milking (17:00h), a variety of thermophysiological measurements were quantified for each lactating and dry camel throughout 3 consecutive days. Rectal (T_r) and vaginal (T_v) temperatures in addition to respiratory (RR) and heart (HR) rates were determined. Measurements were recorded using a digital thermometer (ARTSANA digital thermometer, Grandate Co, Italy) measure to the nearest 0.1ºC for T_r and T_v. Meanwhile, camels' RR and HR were recorded by placing the diaphragm of the stethoscope (3M Littmann Classic II S.E. Stethoscope, UK) in the space between the 9th and 11th ribs or between the 3rd and 6th intercostals spaces, counting 10 breath/beat, and then expressing the recorded time as number of breathes/beats per minute, respectively.

For dry surfaces, body, udder, teat and milk vein surface temperatures (Ts) were recorded simultaneously with thermophysiological measurements. Left side thermograms (infrared thermographic images) for body, udder, teat (front and rear) as well as milk vein surfaces were obtained using a forward-looking and automatically calibrating infrared camera (VisIR-Ti200 infrared vision camera, Thermoteknix Systems ltd, Cambridge, UK) placed perpendicular and approximately 150 cm (for body thermograms) or 50 cm (for udder, teat and milk vein thermograms) away from camel's surfaces. This camera was equipped with 25° lens, 1.3 M pixel visible camera, and LCD touch screen. The spectral range and precision of the camera were 7.5–13 μm and ± 0.1°C, respectively.

After capturing, thermograms were stored inside a 250 MB internal memory, readout and analyzed using a special thermograms analysis program (TherMonitor, Thermoteknix Systems ltd, Cambridge, UK). For all thermograms, the rainbow color scheme was chosen. A total of 143 thermograms were analyzed by defining areas circumscribed by hand with the software functions. The software then calculated the average, minimum, and maximum T_s within the defined areas. Additionally, the distance between the camels and the camera as well as emissivity of animal body was supplied for the camera to compensate for the effects of different radiation sources. It's worth mentioning that the recording time between camels was kept to minimum, and similar body emissivity (0.97; Monteith and Unsworth, 1990) was used for all thermograms. Illustrations of body, udder, teat and milk vein thermograms of lactating and dry dairy camel obtained under the same T_a and within the same barn are presented in Figure 1.

Study data were analyzed using the random model of the general linear model procedure of the statistical analysis system (Statistical analysis systems, 2003). To determine the differences between lactating and dry camels; the influence of animal, breed (Majaheim and Magateir), lactation status (lactating and dry) and their respective interactions were included in the model. Statistically significant differences between means were determined by Fischer's least significant difference. The overall level for statistical significance was set at $P< 0.05$.

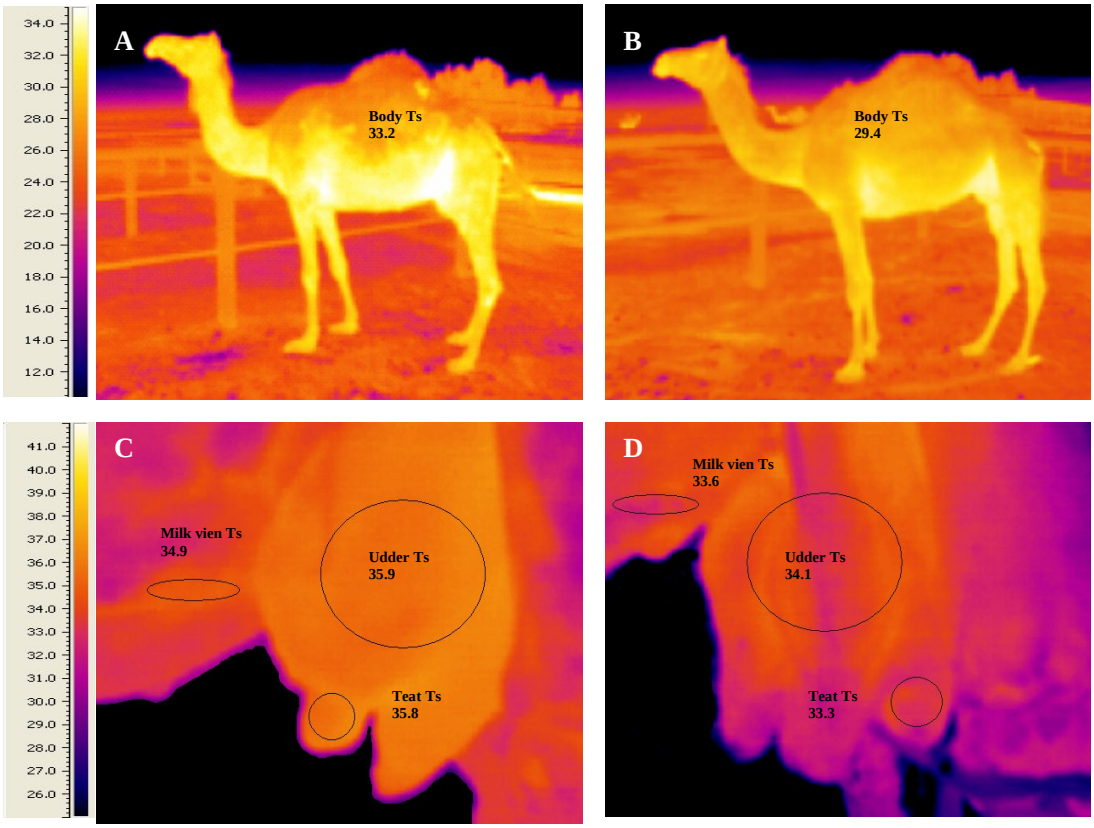

Figure 1. Body and udder thermograms of lactating (A and C) and dry (B and D) dairy camels obtained under the same ambient temperature (*Ta* = 34.93 °C) and within the same barn. In these thermograms, mean body and udder surface temperatures are clearly higher in lactating camel than dry counterpart.

Results

Meteorological measurements

Overall means of daily recorded barn T_a and RH were 37.7 ± 0.4°C and 15.2 ± 0.9%, (means ± SD) respectively. Moreover, recorded T_a showed a circadian rhythm, with minimum values (20-21°C) were recorded at early morning (05:00-06:00 h) and maximum values (40-41°C) at middle of the day (14:00 - 15:00 h). Meanwhile, overall mean of RH showed the reverse trend, where relative humidity of 34% and 10% were associated with minimum and maximum T_a, respectively.

Thermophysiological and IRT measurements

In the current study, no differences ($P>0.05$) in the measured thermophysiological and IRT measurements were observed between both camels breeds. Alternatively, these measurements were influenced by animal's lactation status where lactating camels had higher values than their dry counterparts (Table 1). The overall means of thermophysiological measurements (T_r, T_v, RR, and HR) revealed higher values in lactating camels compared to dry camels ($P<0.05$) (Table 1). Furthermore, overall means of different body parts T_s measured by IRT showed higher values in lactating camels compared to their dry counterparts ($P<0.05$) (Table 1).

Table 1. Least squares means of thermophysiological and infrared thermographic measurements of lactating and dry camels belonging to 2 breeds (n= 24).

Parameters	Lactation Status		Difference %
	Lactating (n= 12)	Dry (n= 12)	
Thermophysiology			
T_r (^oC)	38.01 ± 0.07 [a]	37.80 ± 0.06 [b]	0.53
T_v (^oC)	38.02 ± 0.08 [a]	37.79 ± 0.07 [b]	0.58
RR (breath/min)	15.82 ± 0.28 [a]	14.78 ± 0.24 [b]	6.57
HR (beat/min)	55.01 ± 1.81 [a]	49.86 ± 1.73 [b]	9.36
Infrared thermography			
Body T_s (^oC)	33.12 ± 0.21 [a]	29.46 ± 0.20 [b]	11.06
Udder T_s (^oC)	36.07 ± 0.15 [a]	34.36 ± 0.14 [b]	4.74
Teats T_s (^oC)	35.51 ± 0.11 [a]	33.55 ± 0.11 [b]	5.52
Milk vein T_s (^oC)	35.33 ± 0.19 [a]	33.74 ± 0.18 [b]	4.51

a-b Mean values within the same row bearing different superscripts are significantly different at $P<0.05$.

Tr: rectal temperature, *Tv*: vaginal temperature, *Ts*: surface temperature, RR: respiratory rate, and HR; heart rate.

Discussion

Homeothermic body can be described as an open thermodynamic system; that continuously exchanges heat with its external environment using several thermoregulatory mechanisms (Al-Haidary, 2000). Under heat stress conditions, physiological thermolytic responses are dominated to assist for more body heat dissipation. They incorporate an external shift of blood distribution (increase in peripheral to splanchnic blood flow ratio) (da Silva and Maia, 2011; Abdoun et al., 2012), inhibition of thermogenic hormone production (Al-Haidary et al., 2001, 2002), and recruitment of evaporative mechanisms (de Lamo et al., 2001; Scharf et al., 2008; Abdoun et al., 2012). Most mammals employ panting and/or sweating for evaporative heat dissipation (Willmer et al., 2000). Dromedary camel, in particular, is mainly a sweating animal with respiratory water loss of only 3% of the total evaporative water loss (Schroter et al., 1987; de Lamo et al., 2001).

When lactating camels were compared to their dry counterparts in the current study under the natural summer conditions, lactating camels exhibited higher body temperatures (T_r and T_v). These differences were expected due to greater body heat storage of the lactating camels. Thermal homeokinesis is a steady state where body temperature is relatively maintained constant despite any fluctuating of the external environment (International union of physiological sciences, 2001). According to this definition, it appears that lactating camels are more thermally labile than dry camels. This finding is consistent with the previous studies on dairy cattle which reported that body temperatures of early and/or late lactating dairy cattle are higher than dry cattle (Araki et al., 1984; El-Nouty et al., 1990; Vickers et al., 2010).

The higher body temperatures of lactating camels reinforce the basic axis that lactating animals have greater susceptibility to environmental changes than dry animals. It is definitive, therefore, that lactating camels must dissipate both the heat that gained from the environment together with its own metabolic heat to maintain a constant body temperature. In response to the increased thermal load, noticeable divergences in several thermophysiological measurements including the RR, HR, as well as all T_s parameters were occurred in lactating camels compared to their dry counterparts. The slight tachypnea (approximately 7%) in RR of lactating camels indicated an increase in the respiratory evaporative cooling mechanism in order to counterbalance to some extent the elevated thermal load (Schroter et al., 1987). The possibility of consuming more drinking water by lactating camels than their dry twins to compensate for the potential rise in evaporative water loss is of further interest. Moreover, particular attention should be given to the significant differences (approximately 10%) in HR between lactating and dry camels. This manifests a clear evident that an extensive change in hemodynamics was initiated in lactating camels over their dry counterparts (Table 1). This response is mainly attributed to the increased cutaneous blood flow due to blood redistribution from deep splanchnic to peripheral body regions (Abdoun et al., 2012). This fact was actually observed using IRT, where body, udder, teat and milk vein T_s of lactating camels exhibited noticeable percentage alterations (5-11%; Table 1) over the dry dairy camels.

In case of lactating animals, however, milking can be a special heat dissipation avenue (Araki et

al., 1984). Because more than 85% of most mammals' milk is water (Mepham, 1987), milk would possess a high heat capacity and therefore a considerable part of body heat can be stored in the milk. Consequently, milk removal from the animals may reduce the amount of heat that must be dissipated to lower body temperature. This fact was actually observed in dairy cattle maintained under heat stressed conditions. Araki et al. (1984) reported a decrease in the body temperatures of dairy cattle immediately to several hours after milking when animals were sent back to their pens. Additionally, Ben Younes et al. (2008) demonstrated that milking had resulted in a decrease in udder as well as body (rectal and vaginal) temperatures of dairy cattle. These observation emphasize that milking can alleviate the environmental heat stress in dairy cattle. Numerous studies have monitored the circadian rhythm of body temperature in lactating and dry dairy cattle in their natural habitat (Araki et al., 1984; Al-Haidary, 2000; Vickers et al., 2010). Unfortunately, however, the circadian rhythm of body temperature in dairy camels has not been defined. Thus, it seems very demanding to characterize the circadian rhythm of core body temperature for dry, early, and late lactating camels with the advance of telemetry equipments nowadays to accurately measure the difference between lactating and dry camels.

Conclusions and recommendations

Current study sheds, for the first time, some basic light upon the thermophysiological responses of lactating and dry camels. It appears evident that lactating camels are more thermally labile than dry camels, as emphasized by the noticeable increases of several thermophysiological and infrared thermographic parameters in lactating camels over their dry counterparts. Management can significantly alter body temperatures of lactating camels. Offering shade as well as cooling during the hottest time of the day can affect favorably body heat storage in subsequent periods by rectifying heat stress conditions. This may improve milk production and reproductive performance of dairy camels. The economic importance of such possibility can have far reaching consequences.

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REGULAR ARTICLE

Assessment of glomerular filtration rate in normally hydrated and dehydrated dromedary camel by plasma exogenous creatinine clearance test

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Abstract

The main objective of this study was to assess glomerular filtration rate (GFR) in the camels (*Camelus dromedarius*) under free water access and dehydration conditions (after a 34 days-period of water deprivation) using plasma exogenous creatinine clearance without urine collection. Trials were carried out on six non-pregnant, non-lactating and healthy female camels. Creatinine was administered as an IV bolus at a dose of 16 mg/kg body weight. Blood samples were collected at predetermined times over 24 h post-injection. Plasma creatinine concentration was analysed using Jaffé method. Creatinine clearance was calculated by pharmacokinetic analysis using a non-compartmental approach. Water deprivation induced a significant 15%-decrease in body weight but did not affect haematocrit and total plasma proteins. Mean corpuscular volume increased and red blood cells number decreased in dehydrated conditions. Dehydration produced a significant 30%-increase in plasma creatinine and mean residence time and a significant 20%-decrease in GFR. In conclusion, water deprivation decreased glomerular filtration and plasma exogenous creatinine clearance test could be used as a practical method for GFR assessment in dromedary camel in field conditions.

Key words: *Camelus dromedarius*, Creatinine, Dehydration, Glomerular filtration rate, Kidney

Introduction

Dromedary camel is well adapted to extreme ambient temperature and associated plasma osmotic fluctuations. Indeed, this animal species can endure long periods without water in desert conditions. When water is available, it is often brackish due to high evaporation rate. Moreover, food available in such arid environments is frequently very salty and considered as unpalatable by other domestic species (Yagil, 1986).

Adaptation of the dromedary camel to dehydration results from unique anatomical and physiological features. When long term water deprived, this animal is able to ensure homeostasis and maintain water balance by specific mechanisms reducing water losses (reduced urine production with increased urine concentration, limited sweating, decreased basal metabolism, changes in body temperature, etc). The urinary excretion of metabolites that need large quantities of water (e.g., glucose, urea, phosphorus) for their elimination is also decreased (Bengoumi and Faye, 2002). Kidney function by regulating water balance and urine excretion therefore plays a major role in the camel's adaptation to extreme environmental conditions (Yagil, 1993; Bengoumi et al., 1993).

The best overall indicator of kidney function is the glomerular filtration rate (GFR) which can be assessed using an appropriate marker like creatinine. Plasma creatinine is indeed used for indirect GFR assessment. GFR can be also directly estimated by urinary clearance of creatinine. However, this approach is tedious and time consuming and requires collection of urine. For these reasons, alternative plasma clearance methods based only on repeated blood sampling after

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administration of the marker has been proposed. The plasma exogenous creatinine clearance test has been used for GFR assessment in dogs (Watson et al., 2002) and cats (Le Garreres et al., 2007), but has never been performed in dromedary camel.

The present study aims i) to assess GFR in dromedary camel under normal and dehydrated conditions using plasma exogenous creatinine clearance as an estimate and ii) to evaluate the practicability of this method in dromedary camel for GFR testing.

Materials and Methods

The study was performed over 2 periods. During the first period, GFR was assessed under normal watering conditions. In the second period, dehydration was induced by total water deprivation and GFR was measured after 34 days of dehydration.

Animals

This trial was carried out in Institut Agronomique et Vétérinaire Hassan II in Rabat-Morocco on six non-lactating female dromedary camels older than 7 years. Animals were fed before and during this experiment with 2 kg/animal/day concentrated feed and 3 kg/animal/day wheat straw which contains few quantity of water (10%). Water was given *ad libitum* during normal hydration period. Body weight was assessed using barymetric measurements on the day of GFR testing (Schwartz and Dioli, 1992).

Induction of dehydration by water deprivation

Water was removed the day after completion of blood sampling for the GFR testing performed in normal conditions. Animals were water deprived for 34 days. Throughout the study, the animals were housed in a barn where the ambient temperature was maintained between 20°C and 23°C. For animal welfare reasons, animals were examined every day to collect body temperature and to observe their behaviour, to detect any adverse effect and discomfort (eg, apathy and pain).

GFR testing

The injectable creatinine solution was prepared as follow: 40 g of anhydrous creatinine (creatinine free base anhydrous-crystalli, C2455, Sigma-Aldrich, France) was progressively dissolved in 500 ml of distilled water, and sterilized by filtration using 0.22 µm paper filter. The prepared solution (at a final concentration of 8 g/100 ml) was intravenously injected at a nominal dose of 16 mg/kg of body weight corresponding to 20 ml of the solution/100 kg of body weight. This dose was chosen according to results from a pilot study. The volume to be injected to each animal was calculated according to the body weight measured on the same day. The solution was injected *via* a catheter inserted in the left jugular vein. Immediately after the end of administration, the catheter was rinsed with distilled water (around 100 ml) and removed. An aliquot of the solution was kept for measurement of the creatinine concentration in the solution.

For injection and first blood samples, animals were kept in sternal recumbency position. Animals were fed until creatinine administration but no food was offered during the day of the GFR testing.

Blood (8-10 ml) was collected from the right jugular vein in vacuum tubes with anticoagulant (heparin) at 0 (just before injection for determination of basal plasma creatinine concentration), and then at 2, 6, 10, 20, and 40 min and 1, 1.5, 2, 4, 6, 8, 12, 18 and 24 h after administration.

Blood was centrifuged (3000g/min during 15 min) within 30-45 min after sampling. Plasma was stored at -20°C until laboratory analyses. After the last blood sampling, animals were untied and food was given; water was distributed *ad libitum* for each animal.

Other tested variables

Blood was collected before administration of creatinine and every week for measurement of total plasma proteins, haematocrit, red cells number (RCN) and mean corpuscular volume (MCV) which were used as indicators of dehydration.

Assays

Plasma creatinine concentration was analyzed using Jaffe method. Haematocrit was determined using haematocrit tubes after centrifugation at 3000g during 10 min. Total plasma proteins concentration was determined using refractometer. RCN was counted on total blood using Neubauer cells counter and MCV was calculated as the ratio of haematocrit and RCN.

Pharmacokinetic analysis

Pharmacokinetic analyses were performed using WinNonlin Software (Version 5.2, Build 200701231637 Core version 18 Sept 2006) by a non-compartmental approach. The basal concentration of creatinine determined just before administration (time 0) was subtracted from the plasma creatinine concentration observed after administration of creatinine. The area under the curve (AUC) of plasma creatinine concentration versus time was determined using trapezoidal rule with extrapolation to infinity (Watson et al., 2002).

The AUC was calculated by adding area of each trapeze defined by successive time points (T_n and T_{n+1}) and corresponding to plasma creatinine concentrations (C_n and C_{n+1}) (Creton, 2008).

$$\text{Aire}_{\text{trapèze}} = \frac{[C_n] + [C_{n+1}]}{2} \times (T_{n+1} - T_n)$$

$$\text{Aire}_{\text{totale}} = \sum_{i=1}^{n} \text{Trapèze}_i$$

Linear extrapolation from last points to infinity was done. Extrapolated AUC was calculated as follow:

AUC extrapolated = Clast/ λz and so:

$$\text{AUC} = \sum_{t=0}^{t_{last}} \frac{[C_n] + [C_{n+1}]}{2} \times (T_{n+1} - T_n) + \frac{[C_{last}]}{\lambda_z}$$

Clast is the last observed concentration (at time Tlast), and λz is the slope of the elimination phase determined from last points of the plasma creatinine concentration *vs* time curve.

Plasma creatinine clearance was calculated by dividing administered dose by AUC (Watson et al., 2002). Steady state volume (Vss) of distribution and mean residence time (MRT) were obtained by standard equations (Watson et al., 2002). These two parameters are linked together and to GFR by the following formula (Creton, 2008): Vss = DFG x MRT

Statistical analysis

Statistical analysis was performed using Excell software. Comparisons of tested variables between normal and dehydrated conditions were performed using Student's t test. $P<0.05$ was considered for the difference to be significant and results are expressed as mean ± standard error.

Results

Effect of water deprivation on body weight, haematocrit, total proteins, RCN and MCV

The 34-day water deprivation induced a significant decrease in the mean body weight by 15% ($p<0.001$), RCN by 33% ($p<0.01$) and MCV by 34% ($p<0.05$) (Table 1). Haematocrit and total proteins were not significantly affected by dehydration.

Table 1. Body weight, hematological and plasma variables 6 adult camels dromedaries before and after 34 days of water deprivation.

(data are expressed as mean ± SD)

	Before	After
Weight (kg)	391±51	333±52***
Haematocrit (%)	26.5±1.517	27.5±2.429
Total Proteins (g/100ml)	42.667±2.658	45.167±1.169
RCN (10^6/mm3)	8.617±1.402	5.783±0.739**

In bold are indicated the statistically significant differences induced by dehydration.

** : p<0.01 *** : p<0.001

Effect of water deprivation on plasma basal concentration and kinetics of creatinine

Water deprivation induced a significant ($p<0.05$) increase in mean plasma creatinine concentrations by 30%. Two camels (B and F) however did not show any increase for this parameter (Table 2).

Table 2. Plasma creatinine in 6 adult camels dromedaries before and after 34 days of water deprivation.

Animals	Before		After	
	mg/dl	µmol/l	mg/dl	µmol/l
A	0.69	61	1.59	140
B	1.45	128	1.33	118
C	1.17	104	1.72	152
D	1.15	102	1.56	138
E	1.15	102	1.59	140
F	1.46	129	1.41	124
Mean	1.18	104	1.53*	135*
SD	0.28	25	0.14	12

In bold are indicated the statistically significant differences induced by dehydration.

*: p<0.05

Table 3. Glomerular Filtration Rate in 6 adult camels dromedaries before and after 34 days of water deprivation.

Animals	GFR mL/min/kg Before	After	% change
A	1.23	0.90	-26.8
B	1.64	1.20	-26.8
C	1.18	1.25	5.9
D	1.58	1.30	-17.7
E	1.08	0.81	-25.0
F	1.27	0.91	-28.3
Mean	1.33	1.06*	-19.8
SD	0.23	0.21	5.4

In bold are indicated the statistically significant differences induced by dehydration.

* : $p<0.05$

The extrapolated part of the AUC to infinity represented 9.3 ± 6.3% and 11.2 ± 4.9% of the total AUC in normal and dehydrated conditions, respectively. Plasma exogenous creatinine clearance (i.e. GFR estimate) was significantly ($p<0.05$) lower by 20% following dehydration. Changes in GFR showed large inter-individual variability from -28% to 6% (table 3). MRT increased significantly by 53% following water deprivation from 317 ± 73 min to 484 ± 131 min whereas the Vss remained unchanged (409 ± 65 ml/kg before vs 496 ± 79 ml/kg after water deprivation).

Discussion

The present study was performed under controlled ambient conditions (temperature, feeding, water intake) using the same animals before and after induction of dehydration.

Dehydration is generally reported to induce changes in body weight, haematocrit, and total proteins according to its severity. The 34-day water deprivation period induced a 15%-decrease of body weight. These results were different from those previously published by Bengoumi et al. (1993) who reported that a 14-day water restriction reduced body weight by 35%. However, in this latter study, the ambient temperature was higher (45°C) and thus a more severe dehydration was expected. In other animal species, body weight decreased also in response to dehydration. Only a 3-day water deprivation period causes a decrease in body weight by 21% in indigenous male goats in Saudi Arabia, 18% in Sudanese male goats and 20% in Bedouin non lactation female goats (Alamer, 2006).The loss in body weight in dromedary camel was supposed to be due only to water loss and not to tissue substance losses, in contrast to what was reported in most animal species which stop eating once water is no more available (Schmidt Nielsen et al., 1956). According to Djegham and Belhadj (1986), dromedary camel resistance to water deprivation was due to its ability to mobilise its water storage and to transfer it from one to another compartment. Thus, the dromedary camel is able to loss until 25% of total body water without any dehydration-associated clinical sign.

In the present study, haematocrit values remained within the usual values [20-33%] as described by Yagil et al. (1974a). They were lower than those reported by Bengoumi (1993) (30% in hydration state and 38% in dehydration state), but again the dehydration conditions were different. In the present study, the 34-day water deprivation period did not affect significantly the haematocrit. This variable appears therefore to be a relatively insensitive indicator of moderate dehydration in the dromedary camel. It cannot be excluded however that haematocrit could increase in other conditions (higher ambient temperature, prolonged water deprivation period) leading to severe dehydration, as previously observed for goats (Alamer, 2006). Bengoumi et al. (1993) showed in the dromedary camel that haematocrit did not change during the first dehydration week, then increased significantly from 30% to 38% after 14 days water deprivation with ambient temperature of 40.1°C. Rehydration induced a progressive return to pre-dehydration values from 32% to 30%, respectively, after 12 hours and 4 days. In contrast, a decrease has been reported also in camels between 7 and 12 days of water deprivation. Haematocrit then returned to normal value (Mahmud et al., 1984). Similar results were also observed by Yagil et al. (1974b) who suggested that haematocrit decrease was explained by the decrease in red cells size resulting from increased blood tonicity.

RCN remained within previously described usual values, i.e. between $3.8\times10^6/mm^3$ and 12.6×10^6 $/mm^3$ (Yagil et al., 1974a), but dehydration caused a significant decrease in RCN. Similar findings were reported by Yagil et al. (1974a). The increase in MCV after dehydration results from the decrease in RCN as the haematocrit remained unchanged.

The plasma creatinine values observed in normally watered animals were similar to those previously published by Ben Romdhane et al. (2003). Water deprivation induced a significant 30%-increase in plasma creatinine concentration. The values observed in the present study were close to those reported by Bengoumi (1993) (116 µmol/l) in normally hydrated but lower than those in dehydrated animals (437 µmol/l). Again, the dehydration conditions were more drastic in this latter study. Different results about the effect of dehydration on plasma creatinine in the dromedary camel have been previously published. Yagil and Berlyne (1977) stated that moderate dehydration did not affect plasma creatinine concentrations, while severe dehydration induced a significant increase by 61%. In fact as in other mammals, Sulivan (1974) noticed increase of plasma creatinine resulting from decreased renal elimination in dehydrated camels (Yagil et Berlyne, 1977).

The moderate increase of creatinine observed in this study after 34 days of water deprivation was in contrast with what has been reported in other species living in arid environment after short period of water deprivation. A 72 h-dehydration in indigenous goats in Saudi Arabia induced an 81%-increase in plasma creatinine concentration. Also, an 88%-increase was reported in Awassi sheep after only 5 days of water deprivation. In Barki sheep, a 13%-increase was observed but only after 3 days of water deprivation (Alamer, 2006). These comparisons could lead to the conclusions that the kidney function in the dromedary camel is less sensitive to dehydration than the other species. Nevertheless, the basal plasma concentration of creatinine is a hybrid parameter depending not only on the renal excretion of creatinine but also on its production and volume of distribution (Watson et al, 2002). Therefore, interpretation of changes in basal plasma creatinine concentration in terms of renal function alteration should be done cautiously.

One of the distinct advantages of this study is that direct assessment of GFR has been performed in both conditions. The plasma exogenous creatinine clearance test was here selected for measuring GFR for the following reasons: i) it requires only repeated blood sampling without any urine collection, ii) plasma creatinine can be easily measured, iii) creatinine is stable in blood and plasma allowing delays for centrifugation and storage (Braun et al., 2003), iv) it is a cost-effective method as the injectable solution and assay for creatinine are not expensive compared to those for other markers (e.g., inulin, iohexol), v) creatinine is an endogenous compound which was shown in dogs to have an excellent tolerance when injected as an IV bolus at 160 mg/kg (Watson et al., 2002), and vi) the creatinine clearance can be, if needed, manually calculated without any specific pharmacokinetic software (Watson et al., 2002). Although no commercially available formulation of creatinine exists, the major advantage of the plasma exogenous creatinine clearance test therefore is that it is an easily practicable method in field conditions, as illustrated here for the dromedary camel. No adverse effect was observed following creatinine administration in any animals in normal and dehydrated conditions, and tolerance of repeated blood sampling in this animal species appears to be good. The extrapolated part of the AUC observed in normally hydrated and dehydrated camel was less than 15% of the total AUC, as previously recommended (Watson et al., 2002), indicating that performing the last blood sample at 24h post-injection is appropriate. From λz, the mean elimination half-life (data not shown) was estimated to 4.4 h in normally hydrated and 6.3 h after induction of dehydration. Approximately 4 times the elimination half-life is required to clear 94% of the exogenous creatinine injected. Therefore, in order to minimize the proportion of the AUC extrapolated, the last blood sampling should be performed between 16 and 24 h post-injection according to the hydration status.

In our study, 34 days of water deprivation in camels induced a significant but moderate decrease (20%) in GFR (1.33±0.22 ml/min/Kg in hydration status and 1.06±0.21 ml/min/Kg in dehydration status). Bengoumi (1993) observed a 60%-decrease in endogenous creatinine urinary clearance after 13 days of water deprivation. Yagil and Berlyne (1977) reported as well that severe dehydration (T°= 36.9°C, 10 days) induced a decrease in urinary creatinine clearance by 72%. These results were in agreement with those of Alamer in sheep and goats (2006). GFR decrease is less important in our study compared to these previous data. This discrepancy could be explained by the dehydration conditions (less severe in the present study) and the method used for GFR assessment. Urine collection in dromedary camel is indeed tedious with risk of inaccurate estimation of the urine volume collected traduced by a lower value of GFR and therefore an underestimation of renal clearance.

Conclusion

In conclusion, GFR decreased moderately after a 34-day water deprivation period confirming the

adaptation of dromedary camel to water scarcity. This study demonstrated practicability and good tolerance of plasma exogenous creatinine clearance testing in the dromedary camel which could be used for improving knowledge about GFR in various physiological and clinical settings in this animal species.

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