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BEAUTY AND SCIENCE OF CAMELS

The Arab world has shown love and respect to camels which is evident by hosting a unique beauty competition for camels with strict norms for its judging. Twelve camels have been disqualified from Saudi Arabia's annual camel beauty contest after receiving botulinum toxin injections to make their pouts look more alluring. This year's event has been been mired in scandal after the lure of 20m Saudi riyals (£3.7m) in prize money for each category tempted some owners to cheat. On the other hand camel science is also rolling with the pace of time. A prion disease similar to that which causes "mad cow disease" in cattle has been discovered in camels, veterinarians confirmed the fatal degenerative disease in camels in Algeria. Laboratory tests positively confirmed Camel Prion Disease (CPD) in three animals, suggesting the disease was present in 3.1 per cent of the camel population presented for slaughter at the local abattoir. Attention is drawn towards nuclear techniques that would help diagnose camel disease in the Middle East. Veterinary diagnosticians from Bahrain, Iraq, Kuwait, Lebanon, Saudi Arabia, and the United Arab Emirates met at the FAO/IAEA laboratories in Austria. International experts trained them in modern molecular virus detection techniques (Genetic sequencing) to detect and control the virus. CVRL Dubai lab testing Mers vaccine in a bid to stop spread from camels to people. The UAE is among a handful of countries that have been most severely affected by Mers coronavirus. Dromedaries, or one-humped camels of the kind familiar in the Gulf, are a "reservoir" for MERS-CoV: they carry the virus but do not fall ill. According to Dr Wernery there is no need to vaccinate adult camels, but you vaccinate camels when they are four, five, six months old, then they cannot transmit to humans.

The contributing authors of JCPR are happy by making it triannual as the wait period is reduced and more articles are published. Current issue has good focus on two review articles, i.e. stenoxenous parasites and camel meat production. Sero-epidemiology and serological tests for the diagnosis of brucellosis in *Brucella melitensis* experimentally infected dromedary camels. Other studies include Immunohistochemical and molecular studies and physiological studies included flow cytometric analysis of phenotype peripheral blood leukocytes, impact of racing on serum concentrations of bone metabolism biomarkers in racing arabian camels, angiopoietin-like protein 3, expression and distribution of CYP2J gene in different part of the digestive system in bactrian camel and cerebrospinal fluid collection and its analysis in clinically healthy camels. Lymphoid tissue in the palatine tonsils, molecular identification of tick-borne zoonotic bacteria, parasitic profile of Saudi Arabian camels and concentrations of nutrients in six muscles of bactrian (*Camelus bacterianus*) camels. This issue has good papers on camel milk, i.e. machine milking parameters for an efficient and healthy milking, purification and thermal denaturation kinetics of serum albumin in bactrian camel milk, biochemical characterisation of camel milk from Punjab-Pakistan, determination of the bioactive potential (Antioxidant activity) during fermentation process.

I am sure that contributing authors would continue bestowing their trust in Journal of Camel Practice and Research which is a unique platform for strengthening the camelid literature on global basis.

(Dr. T.K. Gahlot)
Editor

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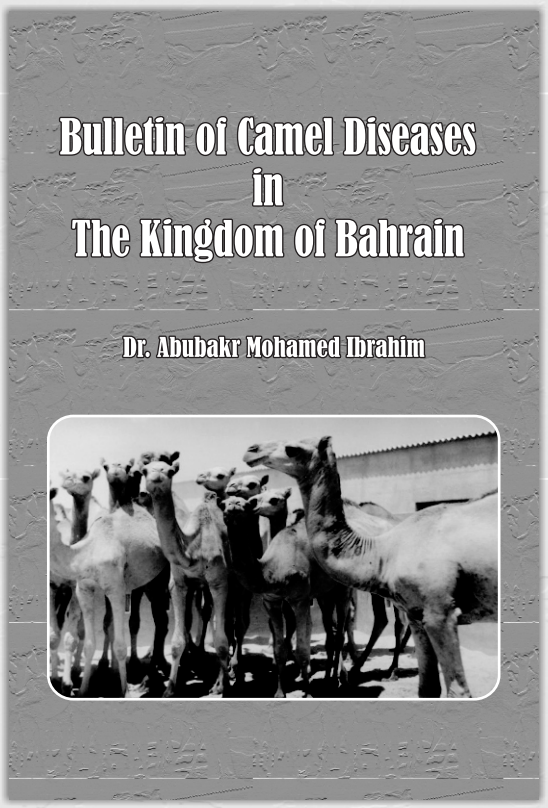
Bulletin of Camel Diseases in The Kingdom of Bahrain

This is a unique book which contains chapters on infectious and non-infectious diseases. The chapter on infectious diseases contains six sections. The section of bacterial diseases is subclassified as corynebacterium abscesses, paratuberculosis, hepatic necrobacillosis, mastitis, *Streptococcus zooepidemicus,* bacterial Infection in young camels, uterine Infection, infection of the vagina and vulva and other disorders. The section of protozoal diseases has narrations on trypanosomiasis, anaplasmosis and babesiosis. The section on parasitic infections is composed of gastrointestinal parasites in young camels, echinococcosis and mange. The section of mycotic diseases contains phycomycosis and ringworm. The section of viral diseases contains subsections on camel pox and contagious ecthyma. Edema Disease is described in miscellaneous section. The chapter on noninfectious diseases has three sections. Other section on poisoning describes pyrethroid, nitrate and toxic jaundice. The section describes zinc deficiency. The miscellaneous section describes foreign bodies, sand colic, bloat, caecal impaction, hydrocephalus, corneal opacity and osteochondroma.

About the Author

Dr. Abubakr Mohamed Ibrahim is a Veterinary Pathologist and worked for a long period as head of Royal Court Veterinary Laboratory. Kingdom of Bahrain which led to genesis of this publication out of his rich experience in diagnosing camel diseases in the Kingdom of Bahrain. This would be counted as his significant contribution and future researchers will find it easy to understand the pattern of camel diseases in this part of the world. Dr. Abubakr had majority of his publications based on camel diseases of Bahrain. Thus publication of this book would prove an important reference book for the camel practitioners and researchers.



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PARASITES OF DROMEDARIES AND BACTRIAN CAMELS – A REVIEW PART 1: STENOXENOUS PARASITES

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ABSTRACT

The parasite fauna of Old World Camelids consists of roughly 100 species of which 24 species are stenoxenous, occurring exclusively or mainly only in camels. To these species belong six coccideans (*Eimeria cameli, E. rajasthani, E. dromedarii, E. bactriani, E. pellerdyi, Cystoisospora orlovi*) at least three *Sarcocystis* species, the Balantidium-like *Infundibulorium cameli*, five gastro-intestinal nematodes (*Physocephalus dromedarii, Nematodiurus dromedarii, N. mauritanicus, Nematodirella dromedarii and N. cameli*), three extra-intestinal nematodes (*Dipetalonema, Onchocerca fasciata* and *Thelazia leesei*), the lung worm (*Dictyocaulus cameli*), the camel tick (*Hyalomma dromedarii*), the nasopharyngeal bot (*Cephalopina titillator*) and the camel lousefly (*Hippobosca equina*). Information about an unnamed *Demodex* mite found in dromedaries and about the camel sucking louse (*Microthoracius cameli*) is scanty. The existence of two other nematodes, *Trichuris cameli* and *Anthostrongylus somalilensis* is questionable.

Key words: Bactrian camel, dromedary, Stenoxenous parasites

Camels are fascinating animals adapted to desert conditions due to their ability to withstand dehydration, low protein diet, energetic and mineral deficiencies (Faye and Esenov, 2005).

Anatomical, physiological and behavior features (coprophagy) on one hand, environmental conditions and the occurrence of other hosts or vectors on the other hand determine the spectrum of parasites that can be found in camels.

Despite of the importance of dromedaries and Bactrian camels as source of meat, milk, hides and transportation, camel diseases take only relatively small space in bacteriological and parasitological textbooks. Camel specific books (Wernery and Kaden, 2002; Gahlot and Chhabra, 2009; Wernery *et al,* 2013) seem to be unavailable for experts in many places were camels are kept.

Wrongly identified parasites of camels leading to subsequent publications (Ibrahim *et al,* 2016; Kumar *et al,* 2016) showed how little is known about the parasites of camels. This gave reason to write this review.

Our own experience with camel parasites is based on the results of more than 1000 necropsies of dromedary carcasses and examination of more than 75,000 faecal samples and several thousand serum samples over the past 15 years as well as on the careful evaluation of reliable international literature.

Stenoxenous parasites of the genus *Camelus*

Relatively few parasites are camel specific. However, it is not exactly known whether or not they are species specific or genus specific because in most of the Russian literature there is no differentiation between dromedaries and Bactrian camels.

Without counting temporary parasites, such as mosquitoes, midges, blackflies, biting and nuisance flies more than 80 parasites can be found in camels. Out of these, 24 species have a narrow host spectrum with camels as the only or as the main hosts.

In connection with Old World Camelids there are five species of *Eimeria* coccidians named in the literature. Of these, *Eimeria cameli* is striking due to the large dark brown oocysts reaching 80 – 100 µm and 55 – 94 µm in length. *E. cameli* oocysts have a similar size as *E. macusaniensis* of New World Camelids and is lightly bigger and differ in shape compared to the equid species *E. leuckarti*. The second species, E. rajasthani with an ellipsoidal oocyst measuring 34-39 x 25 – 29 µm and a presence of a dom shaped pole cap is also easy to recognize. The subspherical to ellipsoid shaped oocysts of *E. dromedarii* measure 23 – 33 x 19 – 25 µm. Two

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further species, *E. pellerdyi* and *E. bactriani* that were mentioned in older references are doubtful and not considered valid anymore.

High *Eimeria* prevalence of up to 50% was reported from Bactrian camels in Inner Mongolia in China (Wei and Wang, 1990). Dromedaries in India's Rajasthan showed an overall prevalence of 25% (Partani *et al,* 1999) and subsequent prevalence in a survey in the Gassim region of Saudi Arabia reached only 13% (Mahmoud *et al,* 1998). Our own experience over the past 10 years for the Dubai Emirate of the UAE showed that the *E. cameli* prevalence usually rises from spring to summer where it can reach 20-25% and declines later on while *E. rajasthani* and *E. dromedarii* have a peak in spring only but the percentage of positive samples is less than 10% (Schuster *et al,* 2015a). All *Eimeria* of OWC were detected in Iran with total prevalence varying between 9.5 and 63% (Khodakaram-Tafti *et al,* 2000; Sazmand *et al,* 2012).

Contrary to other host species, *Eimeria* coccidians are less frequently found in camel calves and under extensive husbandry systems in the desert environments, their impact on camel health is low. When dromedaries kept in high concentrations and, especially under stress situations (camel racing) endogenous stages might cause catarrhal-haemorrhagic enteritis.

Oocysts of *Cystoisospora orlovi* (Syn. *Isospora orlovi*) was found for the first time in camel calves in Kazakhstan (Cygankov, 1950). In the following decades it was believed to be a pseudo-parasite of camels until Kinne *et al* (2002) found the endogenous stages and made a redescription of the parasite. Oocysts with the same morphological features were also found camel calves in India (Raisinghani *et al,* 1987) and Kenya (Younnan *et al,* 2002). Oocysts of *C. orlovi* are 27 – 33 x 15 – 20 µm and already in a stage of sporulation when excreted with faeces. Contrary to the genus *Eimeria* with 4 sporocysts sporulated *Cystoisospora* oocysts contain two sporocysts. *C. orlovi* is mainly found in young suckling calves and only in rare cases shedding was reported in adult camels. The life cycle of this parasite has not been completely disclosed. Experiments with *Cystoisospora suis* in piglets suggest the presence of extra-intestinal stages. Such dormant stages could be activated post-partum and could be excreted with the milk and thus transmitted to the calf. Such route of infection would explain why only single calves were infected while other animals of the same age kept in the same pen remained negative. Infected calves show diarrhoea over a period of up to 10 days with yellowish, watery to soft pasty faeces. Gross pathological alterations revealed severe diphtheroid to haemorrhagic colitis. The infection in camel calves is often fatal. Destruction and disorganisation of the mucosa together with haemorrhages and infiltration of inflammatory cells such as eosinophils and macrophages were seen in histological sections.

Sarcocystis spp. are cyst forming coccidia with a diheteroxenous life cycle. Already at the beginning of the 20th century Mason (1910) described sarcocysts in heart and oesophagus muscles of dromedaries in Egypt. There is still a confusion about validity and number of species. Final clarification can only be achieved by molecular investigations.

Five species have been described in camels of which four species, namely *Sarcocystis cameli*, *S. camelicanis*, *S. ippeni* and *S. camelocanis* form microcysts in dromedary camels. According to Fatani *et al* (1996) thin walled muscle cysts measure 141 – 400 x 70.5 – 188 µm and can be found in various muscles as well as in the oesophagus, whereas thick walled cysts with dimensions of 170 – 194 x 118 – 188 were detected only in the oesophagus. Based on material from two Egyptian camels, Dubey *et al* (2015) confirmed the validity of *S. cameli* (with *S. camelicanis* as its synonym) and *S. ippeni*. *S. camelocanis* was declared invalid because of inadequate description. *Sarcocystis* spp. that forms macroscopically visible, 5 – 15 mm long macrocysts were detected in camels in Kazakhstan by Kuraev (1981). Feeding experiments revealed that dogs act as final hosts for all these species. After a prepatent period of 8 – 13 days, dogs shed sporocysts for up to 57 days.

Several studies on muscle sarcocystiosis in dromedaries from African and Asian countries have been carried out and prevalences varied between 21.8 % in Jordan (Latif and Khamas, 2007) and more than 80% in Sudan (Hussein and Warrag, 1985), Somalia (Borrow *et al,* 1989), Iran (Valinezhad *et al,* 2008), Iraq (Lativ *et al,* 1999) and Saudi Arabia (Fatani *et al,* 1996). Fukuyo *et al* (2002) reported sarcocysts in Bactrian camels in Mongolia.

Sarcocystosis in intermediate hosts usually runs its course without major clinical signs. A generalised myositis is rarely found in connection with sarcocystosis and infected carcasses pass meat inspection without objection.

Infundibulorium cameli is a little known ciliate that had been described by Bozhenko (1925) in the alimentary tract of a camel. The genus *Infundibulorium*

is placed together with 10 other genera including Buxtonella in the family Pycnotrichidae (Lynn 2008). They are all endosymbionts and are characterised by an oral cavity as a long vestibular grove at least one half of the body length. Little is known about *I. cameli*. It was mentioned only in publication from India (Gill 1976) and it is suspected that *B. sulcata* that had been described from the caecum of cattle is a younger synonym for *I. cameli* (Levine 1985).

Trophozoites, the parasitic stage, are variable in size and have a cytostome to swallow food. Trophozoites are covered with ciliae and contain two nuclei. They multiply by dichotomy rather than by conjugation. When excreted with faeces, trophozoites become a greyish spheroid cyst of 40-60 µm in diameter without ciliae.

Pathogenesis and clinical picture for ciliate infection are best studied for *Balantidium coli* in pigs. Thus, *B. coli* is able to enter the intestinal mucosa and causes haemorrhages and multiple necrosis that later become ulcers. In histological cuts trophozoite with their characteristic macro-nuclei can be seen in the ulcer but also in the surrounding tissues of mucosa and sub mucosa. Ciliates in connection with diarrhoea in camels were mentioned from Sudan (Ali and Abdelaziz, 1982; Shommain and Osman, 1987) and from Bahrain (Abubakr *et al,* 2000). But there are also many apathogenic symbiotic ciliates that can be found in the fore stomachs of ruminants and camels (Levine, 1985). They belong to the normal microflora of the alimentary tract and cysts can be found at coproscopy. In a carbohydrate and protein rich diet, the ciliate density can reach 10^7/ml of fore stomach content and 24 ciliate species were counted in the compartments of 11 healthy dromedaries in Egypt (Selim *et al,* 1996).

Two species of the genus *Nematodirus*, namely *N. dromedarii* and *N. mauritanicus* have been found so far only in dromedairies. Both species differ from all other representatives of the genus *Nematodirus* by the length of the spicules of the males measuring more than 4.5 mm. Their eggs are 230 – 260 x 100 - 120 µm and contain 8 blastomeres. Contrary to many other trichostrongylids larvae of *Nematodirus* spp. do not leave the eggshell and infection of the host happens via coprophagy. The adult nematodes inhabit the small intestines and can cause catarrhal enteritis. Both species were found in camels in Turkmenistan (Badanin, 1969), Iran (Anvari-Tafti *et al,* 2013), Kuwait (Abdul-Salam and Farah, 1988) and Jordan (Al-Rawashdeh *et al,* 2000). Both *Nematodirus* species were the only representatives of the genus found at necropsy in dromedaries in Dubai. The prevalence of *Nematodirus* eggs in camel faecal samples in Dubai ranged between 2 and 6.5%.

Nematodirella dromedarii and *N. cameli* were described from camels in Central Asia (Rajevskaja and Badanin, 1933) and were also found in Iran (Borji *et al,* 2010, Rad *et al,* 2015). Both species were also recorded in gazelles and antelopes in western and central Asia. Having similar life cycles and appearances as *Nematodirus* spp. *Nematodirella* species differ in the location of the vulva in the anterior part of the body. Spicules of the male are extremely long measuring 13 - 14 mm.

Lungworms in camels are known from northern temperate zones with continental climate sufficient precipitations. Thus, outbreaks of dictyocaulosis in Bactrian camels with high mortality were seen in the southern Ural region of Russia, in Kalmykia and western Kazakhstan. Initially, lungworms in camels were attributed to *Dictyocaulus viviparus* but a description by Shumilina (1953) revealed differences in morphology. Adult *D. cameli* of both sexes are larger and males have longer spicules. *D. cameli* is a viviparous nematode. For their development *Dictyocaulus* larvae excreted with faeces of the infected camel need a moist environment. Infective 3rd stage larvae climb up grasses and are ingested by grazing. Experimental infections showed that cattle calves also can become infected but despite the high infection dosage calves produced only few larvae over a short period.

Anthostrongylus somalilensis was described from dromedaries in Somalia by Croveri (1929) and was also mentioned in a paper by Deli and Sobrero (1966). Croveri (1929) pictured and described a new parasite which he considered to be responsible in part, at least, for the disease known as Ber Cursu in Somaliland. In a tabular key he gives the distinguishing features of the 12 genera comprised in the Metastrongylidae. The following is taken from the key to *Anthostrongylus*: "Integument shows 12 longitudinal ridges, the mouth has 3 lips, the caudal pouch is campaniform and notched anteriorly to produce 3 lobes, the spicules are very long measuring 1,080 mm." Today, *Anthostrongylus* is considered a synonym of *Impalaia* and the species found in camels is most probably *I. tuberculata*.

Thelazia leesei was described from dromedaries in Africa by Railliet and Henry (2010). It is also found in Iran, Turkmenistan, Uzbekistan and India and in Bactrian camels in Kazakhstan. The adult nematode has a length of 10 – 12 mm (male) and 14 -15 mm

(female) and is located under the conjunctiva and in tear ducts of the upper eye lid. It is very host specific and differs from other species of the genus by an unequal length of spicules (1 : 3) of the male. The life cycle of *T. leesei* was disclosed by Dobrynin (1972, 1974) in Turkmenistan where flies of the species *Musca lucidula* act as intermediate host. First stage larvae of the parasite are ingested by flies when they feed on conjunctival excretions. Larvae penetrate the gut of the fly and develop in their abdomen within two to three weeks to the infective 3rd stage larva. These larvae leave the fly through their proboscis when the latter feeds around the eyes of the camel. While eye worms in most of the hosts cause severe conjunctivitis *T. leesei* seem to be well tolerated by camels. Apart from Turkmenistan *T. leesei* was found in Iran (Vosooghi-Afshar, 1976).

Trichuris cameli was described by Rudolphi (1819) from dromedary in India but description was that superficially that it could match also with *T. ovis*, *T. globulosus* and later authors considered it nomen nudum.

Physocephalus dromedarii inhabits the abomasum of dromedaries. It was formerly known as *Physocephalus sexalatus dromedarii* but scanning electron microscopical examination revealed morphological features that allowed to upgrade the former subspecies to species level (Schuster *et al,* 2012). Adult nematodes are 14 – 17 (males) to 22 – 29 mm long. In both sexes, the anterior half of the worm is equipped with three lateral pairs of cuticular alae. Females have a straight body with a striking swelling in the mid-body region. The posterior end of the males with caudal alae supported by papillae is cork-screw like twisted. The two spicules are of unequal length. *P. dromedarii* occurs in Turkmenistan (Mushkambarova and Dobrynin, 1972) and was recently found in high prevalence in Dubai on a farm that had imported camels from abroad. Local camels in Dubai had a much lower prevalence. Camels from other emirates of the UAE were negative for this nematode (Schuster *et al,* 2015b). Larval stages of *P. dromedarii* were found in *Scarabaeus cristatus* as well as in reptiles (Schuster *et al,* 2016a, 2016b). The pig stomach worm, *Physocephalus sexalatus* was described in the past in dromedaries in Iran (Mirzayans and Halim 1980; Anvari-Tafti *et al,* 2013), Kuwait (Abdul-Salam and Farah, 1988) and Algeria (Chauve *et al,* 1990) which are most likely cases of wrong identification. *Physocephalus cristatus* found in camels and donkeys in Algeria by Seurat (1912) and also mentioned in a paper on camel parasites of Iraq (Altaif, 1974) is a species inquirenda.

Of the four filaroid nematodes found in camels only *Dipetalonema evansi* (syn. *Dereiophoronema evansi*) and *Onchocerca fasciata* are camel specific. *O. armillata* and *O. gutterosa* can also infect camels but they are more often found in cattle. Filarial worms are biohelminths and blood sucking insects act as vectors.

Thread-like and pale coloured *D. evansi* reach a length of 9.5 – 10.5 mm (males) to 17.5 – 19.5 mm (females) and inhabit pulmonary and spermatic arteries, as well as mesenteric lymph nodes and lymph vessels, right heart atrium and ventricle and hepatic arteries. Males have spicules of uneaqual length and females have an opisthodelphic uterus with a genital opening situated in the anterior half of the body. They give birth to ensheathed microfilariae. These microfilariae are 200 – 315 µm in length and are circulating in the peripherial blood. *D. evansi* is transmitted by mosquitoes *Aedes* spp. The development time of *D. evansi* in the vector *Aedes caspius* was studied in Turkmenistan by Kataitseva (1968). Taken up with the bloodmeal, microfilariae leave the alimentary tract of the mosquito and undergo a development with two moults in the flight muscles of its vector. At 26 – 28°C it takes nine days for the formation of the infective larvae. *Aedes detritus* is the vector of *D. evansi* in Iran (Oryan *et al,* 2008). While mild infections are often unapparent acute disease can cause cardiac insufficiency and arteriosclerosis. A parasitic orchitis may occur, or aneurysms and haematomas may be present in the spermatic vessels. According to various sources, the disease is present in northern and eastern Africa, in Iran, Turkmenistan, India, Pakistan and Australia. In an abattoir study in eastern Iran Oryan *et al* (2008) detected *D. evansi* in testicular and epididymal blood vessels of 84% male dromedaries. Blood vessels of the lungs were less often affected. Microfilariae were found only in 21% of blood samples.

O. fasciata inhabit connective tissues and form palpable nodules in the fascial sheet of the ligamentum nuchae and in the subcutis of other body parts of the camel. The parasite was detected in Egypt, Ethiopia, Sudan, Mauritania, Saudi Arabia, Iran, Turkmenistan and India (Farjanikish *et al,* 2016; Moobedi *et al,* 2016).

Amongst ticks there are few species (*Hyalomma dromedarii*, *Hy. schulzei*, *Hy. punt* and *Hy. erythraeum*) that have camels as preferred hosts. Our own experience with *Hy. dromedarii* showed that it is seldomly found on other hosts such as horse, gazelle and oryx. It was never found on sheep and donkeys, although these hosts were kept close to camels that

were heavily infected with this tick. According to literature data *Hy. dromedarii* is the most frequent tick on dromedaries in Egypt, Sudan, Nigeria, Saudi Arabia, Jemen, Iran and India and is the only tick on camels in the UAE. *Hy. dromedarii* is well adapted to desert environments. In Sudan, it behaves as a two-host –tick with larval-nymphal feeding periods of 16 to 20 days whereas females feed for 6 – 9 days. Engorged stages drop off in early evening hours. This enables the tick to find a shelter during the cooler night hours. After a preoviposition period of 10 to 15 days, a female *H. dromedarii* can lay up to 7,000 eggs. Hatched larvae die within one week if they can't find a host (El Ghali and Hassan, 2010a, 2010b). Unengorged adults can survive up to three months in winter. Predilection site of preadult stages are head and hump while adults are most often found on udder, prepuce, between legs or under the tail. As other ticks, *Hy. dromedarii* harm camels only by its haematophagous nutrition. The role of *Hy. dromedarii* as vector is questionable. Although DNA of piroplasms that are transmitted by ticks was detected in camel blood samples (Quablan *et al,* 2012; El-Naga and Barghash, 2016) little is known about the significance of these findings.

Demodex mites are considered to be very host specific and infection of the host is via contact. Demodicosis commonly occurs in New World Camelids. In dromedaries there are only some reports of *Demodex* mite detection in Iran (Rak and Rahgozar, 1975), Iraq (Hussein *et al,* 2012) and Kenya (Wernery and Kaden, 2002).

The nasopharyngeal bot, *Cephalopina titillator* is an obligate parasite of dromedaries and Bactrian camels. While the parasitic larval stage can reach a length of up to 2 cm the adult fly is an inconspicuous greyish brown insect. Aphagous females with rudimentary mouthparts deposit small, 0.7 mm long larvae into the nostrils of the camels. These 1st stage larvae migrate into the labyrinth of the ethmoid bone and in the turbinates of the nasal cavity. The majority of the 2nd and 3rd instars are found in the pharyngeal cavity. Fully grown larvae are excreted through the nose into the environment where they pupate in the ground. Findings of 3rd instars in the compartments of the stomach suggest that at least some of the larvae leave the host though the alimentary tract. *C. titillator* is considered a palaearctic species that has extended its distribution into eastern, western and southern Africa and was brought with dromedaries to Australia (Spratt, 1984). Abattoir studies in Saudi Arabia showed that two generations of *C. titillator* occur with prevalence peaks of more than 90% from December to February and in September (Alahmad, 2002). Under conditions of continental climate in Central and East Asia the whole parasitic life cycle in the host can take up to 9 to 11 months (Erdenebileg, 2001). Prevalence in dromedaries can reach 50 to 80% or even up to 100% with average burdens of 28 to 35 larvae (Nwosu and Wachy, 1998; Bekele 2001; Mbaya *et al,* 2010). A maximum of 243 instars were counted in a camel in Sudan (Musa *et al,* 1989). Nasopharyngeal bots cause lacrimation, snorting, serous nasal secretion, sneezing, vigorous shaking of the head and gid like signs but due to the frequent use of avermectins mainly for nematode and mange control in camels cephalopinosis has lost its importance.

The camel louse, *Microthoracius cameli* was found on dromedaries in Algeria and India. Except some morphological descriptions and records in zoological collections no further data were available.

Hippobosca camelina is a lousefly that attacks camels and is occasionally also found on horses and donkeys. Contrary to sheep keds, these *Hippobosca* flies have wings and attack their hosts mainly on less hairy parts of the body (udder, prepuce, inner limbs). Female give birth to late larval stages that are ready to pupate when deposited at the ground. According to Higgins (1985) *H. camelina* occurs in northern and eastern African countries as well as on the Indian subcontinent. It has been found also in Nigeria (Lawal *et al,* 2007). As a bloodsucking insect it is also considered as vector for *Trypanosoma evansi* (Doutoum *et al,* 2002).

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CAMEL MEAT PRODUCTION AND QUALITY: A REVIEW

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ABSTRACT

Approximately 250,000 thousand camels are slaughtered annually in different countries. About 50% of the camels slaughtered are young males aged around 4 years. The camel meat is described as tough, coarse, watery and sweetish in taste compared to meats from other animals. However, evidence suggests that quality characteristics of camel meat are not much different from beef if animals are slaughtered at comparable ages. In some of the African and Asian countries, camel meat has been used for its medicinal properties. Based on recent FAOSTAT (2015) database, in 2013 global camel meat production reached 539,100 Tonnes. Region wise Africa was leading with 416,292 Tonnes produced, followed by Asia (122,608 Tonnes) and Europe (200 Tonnes). Camel meat is much better than beef in that it has lesser fat than all the other red meats such as beef and mutton. Camel lean meat contains about 78% water, 19% protein, 3% fat and 1.2% ash with a small amount of intramuscular fat. Camel meat has a comparable essential amino acid contents to beef, lamb and goat meat. The camel hump is important and commonly used for cooking in camel producing countries. On fresh weight basis, the camel hump contributes about 64.2-84.8% fat with very high content of saturated fatty acids of about 63.0%. The semitendinosus muscle in the dromedary and bactrian camels had more magnesium than infraspinatous, triceps brachii, longissimus thoraces and biceps femoris muscles. The semitendinosus and semimembranosus muscles had more iron than other muscles in dromedary. The male camels should be slaughtered between 1 to 3 years of age. This might be due to that less than 3 year of age, camels were not yet fully-grown (60-70% of full live weight), therefore, their meat is tender. A high ultimate pH in camel muscles is a consequence of low muscle glycogen as a result of pre-slaughter stress, including, poor nutrition, rough handling and long transportation. Muscle structure, glycogen concentration, collagen content, solubility and the activities of proteases and their inhibitors are the most important physiological parameters affecting meat tenderness. Water retention in meat is primarily caused by immobilisation of water within the myofibrillar system. The volume of the camel meat was reduced by 44.3% and weight by 48.2% after boiling in water for 40 min. The age of the camel has a significant effect on their meat colour (Kadim *et al,* 2006). Meat colour from 6-8 and 10-12 year old camels was darker (lower L^*), redder (higher a^*) and yellower (high b^*) than 1-3 year old camels because of higher concentrations of myoglobin. Camel meat is rich in many essential amino acids, minerals, vitamins, bioactives compounds such as carnosine, anserine, glutathione and essential fatty acids such as omega 3 fatty acids. Meat in general is considered a functional food for cures of many ailments and for improved performance in many cultures around the world. Camel meat has been processed into burgers, patties, sausages and shawarma to add value. The nutritional value of camel meat is similar to other red meats.

Key words: Camel, meat production, quality

Camels are multipurpose animals with females used primarily as milk producers, the males for transport or draught and both sexes providing meat as tertiary product. Camels are the most important animals domesticated by mankind in desert ecosystem and are considered environmentally friendly and very well adapted to desert harsh arid environments (Abrhaley and Leta, 2018). They are mainly held by nomads in arid regions, due to their high mobility, modest fodder requirement and water regulation. Camels are better suited than any other domestic animal to arid and semi-arid lands. In fact, camels can survive in times of extreme need for up to 30 days without water. According to Farah and Fischer (2004), most of the camels are raised by pastoralists in subsistence production systems. Camels are very versatile, in that, aside from being a source of food (milk and meat) and cash income, they can also be utilised for transportation, drawing water from wells, rivers and dams (Kadim *et al,* 2008; Abrhaley and Leta, 2018).

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Approximately 250,000 thousand camels are slaughtered annually in different countries. About 50% of the camels slaughtered are young males aged around 4 years, but their market has not yet well developed locally in spite of the existing regional camel market. A camel carcass can provide a substantial quantity of meat for human consumption with certain parts of the carcass such as the hump and liver considered a delicacy that is favoured in Middle eastern markets. Camel meat is mainly exported from the Horn of Africa to the Arabian Peninsula and in a 2nd extends from western Africa to northern countries of Africa. The role of the camel as a meat producer is becoming more important due to the versatile role it plays rather than as a symbol of social prestige, which was the major role it used to play and since greatly diminished (Dawood and Alkanhal, 1995). The camel meat valorisation through local or regional markets would be a strong opportunity for the integration of pastoralists into the market. However, the camel meat is described as tough, coarse, watery and sweetish in taste compared to meats from other animals. This may be partly attributed to the fact that camel meat is usually a by-product of primitive traditional systems of production where it is mainly obtained from old males and females that have become less effective in their primary roles of providing transportation, milk, or as breeding females (Kadim *et al,* 2008). However, evidence suggests that quality characteristics of camel meat are not much different from beef if animals are slaughtered at comparable ages (Khatami, 1970; Knoess, 1977; Elgasim *et al,* 1987; Tandon *et al,* 1988; Kadim *et al,* 2011). Although, the marketing systems for camel meat are not well organised, there is evidence of high demand for camel meat among societies herding camels (Kadim *et al,* 2008). However, camels are generally raised in less developed countries and research for improving their reproductive and productive characteristics has been limited (Skidmore, 2005). This chapter outlines the population, meat production, nutritional and health value, quality characteristics and the availability of muscle bioactive compounds in camel meats.

Camel Meat Production

One of the most important products from camels is meat and is the major commodity income under conventional camel production systems. That includes meat from male and female young camels, castrated or intact males and culled animals. The importance of the camel as a meat-producing animal is increasing due to healthy reasons in many societies that have never consumed camel meat before. Some of the factors that make the camels successful meat-producing animals are their ability to graze and utilise poor forages; ability to walk long distances; high turnover rates of investments, larger carcasses which are provided a good amount of high nutritive value meat, beside their ability to survive under harsh environments (Kadim *et al,* 2008). In some of the African and Asian countries, camel meat has been used for its medicinal properties (Bin Saeed *et al,* 2005; Kurtu, 2004; Bekhit and Farouk, 2013). Available statistics on camel meat production in the world are scarce due to limited information on number of animals slaughtered and hence estimated amount of meat produced (Faye, 2013). A significant number of camels are usually slaughtered out of official channels and they are unaccounted for suggesting that camel meat production is probably under-estimated (Faye, 2013). Therefore, camel meat production data reported here is reflective of that shortcoming. Based on recent FAOSTAT (2015) database, in 2013 global camel meat production reached 539,100 Tonnes. Region wise Africa was leading with 416,292 Tonnes produced, followed by Asia (122,608 Tonnes) and Europe (200 Tonnes). In terms of growth, camel meat production grew faster in Africa between 2000 and 2013 at 73.7%, followed by Asia at 13.4%. Camel meat production slowed down in Europe by -35.1%. Globally, however there was a 54.9% growth in camel meat production between 2000 and 2013. Share wise, this regional camel meat production distribution translates to 74.3%, 25.7% and 0.0%, respectively for Africa, Asia and Europe. In terms of top 10 camel meat producers in the world, Sudan tops the list whereby in 2013 the country produced 140,000 Tonnes, followed by Kenya (65,100 Tonnes), Egypt (47,300 Tonnes), Somalia (45,900 Tonnes), Saudi Arabia (44,000 Tonnes), UAE (30,200 Tonnes), Ethiopia (28,100 Tonnes), Niger (28,500 Tonnes), Mauritania (23,500 Tonnes) and Mali (15,000 Tonnes) FAOSTAT, 2015). Overall, between year 2000 and 2013 these top 10 camel meat producers, produced 467,600 Tonnes of camel meat out of the 539,100 Tonnes produced in the world during that period. In other words, the top 10 camel meat producers accounted for 87% of global Camel meat production during that period.

Camel Meat Consumption

It has been proved that camel meat is healthier than beef. In fact, camel meat has more protein, is richer in iron than both beef and lamb and doesn't contribute to heart disease. Camel meat is much better

than beef in that it has lesser fat than all the other red meats such as beef and mutton. Camel meat has been consumed for many years, especially, in the Arab world and it is said to be delicious, tasty and healthy. The popularity of camel meat is fast increasing with time (Kadim *et al,* 2008) because of its quality and comparability with regular beef meat. The camel brisket, ribs and loin are among the preferred parts and the hump is considered a delicacy. Camel 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 growing human populations. To add value, camel meat has been processed into a variety of mouthwatering and sumptuous products such as: sausage (Kargozari *et al,* 2014), burgers, patties and shawarma.

Composition of Camel Meat

The composition of camel meat is generally similar to meat from other species where an inverse relationship existed between the moisture and protein contents and the fat content (Table 1). Composition is an important indicator of meat functionality. For instance, moisture content plays an important role in keeping and eating qualities of camel meat (Kadim *et al,* 2008) whereas protein and fat contents dictate the manufacturing quality of meat.

Table 1. Chemical composition (%) of different camel muscles (Kadim *et al,* 2013).

Muscle Type	Moisture	Protein	Fat	Ash
Longissimus thoracic	73.8	19.0	6.2	0.85
Infraspinatus	73.2	18.2	5.3	0.96
Triceps brachii	77.7	17.1	1.9	1.00
Semitendinosus	75.4	18.5	3.1	0.91
Semimembranosus	63.0	22.1	2.5	0.93
Biceps femoris	74.3	20.8	2.5	1.00
Longissimus thoraces	65.7	19.5	2.1	1.20
Longissimus thoraces	73.8	23.7	3.6	-
Biceps femoris	73.0	22.8	1.1	0.75
Triceps brachii	72.0	21.2	1.4	0.81
Longissimus dorsi	68.3	21.5	1.6	0.69
Biceps femoris	71.4	22.2	1.6	0.98
Triceps brachii	70.5	20.3	2.4	1.06
Longissimus dorsi	67.8	20.5	2.5	0.95
Longissimus thoraces	74.8	21.1	2.8	1.34
Longissimus thoraces	71.7	22.7	4.4	1.10
Longissimus dorsi	75.9	21.6	1.4	1.05
Semitendinosus	75.8	21.4	1.4	1.38
Triceps brachii	75.2	22.1	1.4	1.22

Table 1 shows that moisture content widely varied in camel meat (63.0 to 78.9%). With exception of *longissimus thoraces* muscle, different muscles from the same animal appear to have similar moisture contents (Babiker and Yousif, 1990; Gheisari *et al,* 2009; Kadim *et al,* 2013; Abdelhadi *et al,* 2017). However, the range of moisture content of Biceps femoris (71.4-74.3%) and Triceps brachii (70.5-77.7%) muscles was higher than those from longissimus thoracis muscle (65.7-74.8%) due to the higher fat content in the longissimus thoraces muscle (Kadim *et al,* 2013). The higher moisture content of camel longissimus thoracis (77.9%) was reported by Abdelhadi *et al* (2017). Kadim *et al* (2006) stated that the moisture content of the camel meat decreases with increasing age. The differences between the maximum and minimum moisture contents of camel longissimus thoraces were 3.2%, 6.4% and 12.3% for 1-3, 3-5 and 6-8 years age groups, respectively (Kadim *et al,* 2006). However, Ibrahim *et al* (2015) reported small variation in moisture content between 3-4 and 6-7 year-old Sudanese camels across 4 muscle with the exception of *longissimus thoraces* muscle.

The protein content of camel meat is in the range of 17.1 to 23.7% (Table 1). Similar range was reported by Abdelhai *et al* (2017) for camel longissimus thoracic muscle. There are slight differences between various muscles and different age groups (El-Faer *et al,* 1991; Kadim *et al,* 2006, 2012; Raiymbek *et al,* 2012b). Meat from young camels has similar protein content to those found in young cattle, lamb and goat meats (Elgasim and Alkanhal, 1992; Kadim *et al,* 2009b). Protein contents of camel semitendinosus, infraspinatus, semimembranosus, biceps femoris, triceps brachii and longissimus thoracic muscles were studied by Kadim et al (2013) and found that longissimus thoracic muscle had the highest and the Triceps brachii muscle the lowest protein content. In contrast, Ibrahim *et al* (2015) found that semimembranosus muscle had the highest protein content (19.8%) and the longissimus thoraces had the lowest (17.1%). Total collagen content is higher in camel longissimus thoraces muscle than in semitendinosus or triceps brachii muscles possibly due to morphological requirement for stabilising the hump attached to the longissimus thoraces (Babiker and Yousif, 1990).

The fat content of camel meat ranged from 1.1 to 6.2.6% (Table 1). Similarly, Abdelhadi *et al* (2017) found that the range of intramuscular fat of longissimus thoraces was 2.33%. Animal's age have great effect on the fat content with camel meat

from older animals' containing higher fat compared with meat from younger animals (Kadim *et al,* 2006; Ibrahim *et al,* 2015). Other factors appear to affect the fat content of camel meat within similar age groups (El-Faer *et al,* 1991; Elgasim and Alkanhal, 1992; Kadim *et al,* 2006; 2008; 2009a,b; Gheisari *et al,* 2009).

The ash content in the camel meat has been reported in the range of 0.75 to 1.38% (Table 1). Ash content varies with muscles and between muscles (Kadim *et al,* 2008; 2013, Raiymek *et al,* 2012b). Gheisari *et al* (2009) found that age had a significant effect on ash content of camel meat, whereas others found no effect of age on ash content (Kadim *et al,* 2006, 2008). Abdelhadi *et al* (2017) found no significant difference in ash content between male and female dromedary camel. Camel meat has relatively lower ash content than beef, lamb and goat meat (Kadim *et al,* 2008).

Amino Acids

Camel meat has a comparable essential amino acid contents to beef, lamb and goat meat (Table 2). The amount of camel meat required to supply the daily requirements of essential amino acids for adult consumer is similar to that from lamb (based on methionine which has the lowest content in meat) but is less than the amount required from beef. Leucine (7.1 to 10.7% of protein) and lysine (8.3 to 10.9% of protein) are among the highest essential amino acids in camel meat (Table 2). The essential amino acid contents in longissimus thoraces and semitendinosus muscles differed by >2.1% with the exception of leucine, methionine and tryptophan, which differed by 18.5, 25.4 and 14.6 %, respectively (Al-Shabib and Abu-Tarboush, 2004). Similarly, essential amino acid contents in the Infraspinatus, longissimus thoraces and semitendinosus muscles differed by > 4.2% with the exception of isoleucine, methionine, threonine, tryptophan and valine which differed between 8 to 42% (Table 2). Tryptophan concentration in camel meat was lower than in other meats. Al-Shabib and Abu-Tarboush (2004) stated that tryptophan concentration was 1.76% of the total amino acids,

Table 2. Essential amino acid composition in camel meat (mg/100g) (Kadim *et al,* 2013).

	Amino acids[1]								
	His	**Ileu**	**Leu**	**Lys**	**Met**	**Phe**	**Thr**	**Trp**	**Val**
Longissimus thoracis	4.4	4.7	8.3	9.4	2.9	4.3	4.5	-	5.6
Semitendinosus	3.4	4.3	8.4	9.1	1.3	5.5	4.8	1.9	4.6
Infraspinatus	4.7	5.3	8.6	8.4	2.6	4.1	4.2	0.5	4.9
Longissimus thoracis	4.3	5.4	8.3	8.6	2.2	4.4	4.7	0.7	5.3
Semitendinosus	4.5	4.9	8.3	8.3	2.5	4.2	4.2	0.6	5.4
Camel	5.6	5.9	9.5	8.9	3.5	4.7	4.8	-	6.3
Beef	6.2	6.5	10.7	9.1	2.7	5.7	5.5	-	6.6
Lamb	5.9	5.8	9.6	8.5	3.3	4.9	4.2	-	5.9
Goat	4.7	6.0	7.9	10.9	3.9	6.5	4.4	-	6.8
Camel	3.4	4.3	7.7	9.1	1.4	5.5	4.8	1.8	4.7

[1]Amino acids: His Histidine, Ileu: Isoleucine, Leu: Leucine; Lys: Lysine, Met: Methionine; Phe: Phenylalanine; Thr: Threonine; Trp: Threonine and Val: Valine

Table 3. Non-essential amino acid composition in camel meat (mg/100g) (Kadim *et al,* 2013).

	Amino acid[1]							
	Ala	**Arg**	**Asp**	**Glu**	**Gly**	**Pro**	**Ser**	**Tyr**
Longissimus thoracis	6.5	6.6	9.3	15.9	4.3	3.9	3.6	3.5
Infraspinatus	6.3	7.5	9.3	17.1	6.0	5.4	3.5	3.0
Semitendinosus	6.3	7.5	8.6	16.4	5.9	5.9	3.6	3.3
Camel	3.9	7.1	10.8	18.6	6.1	3.9	3.2	3.8
Beef	7.7	7.1	10.8	16.5	6.2	4.5	4.2	4.1
Lamb	6.7	6.9	10.3	17.9	5.5	3.8	2.9	3.5
Goat	4.7	7.1	10.8	15.6	5.2	3.8	3.6	5.9
Camel	6.5	6.9	9.7	17.0	6.2	-	4.3	3.3

[1]Amino acid: Ala: Alanine; Arg: Arginine; Asp: Aspartic acid; Glu: Glutamic acid; Gly: Glycine; Ser: serine: Tyr: tyrosine

which was higher than the 1.28% reported for beef (Kadim *et al,* 2008). According to Dawood and Alkanhal (1995), the essential amino acid content of camel meat is not affected by the animal age or sex (Abdelhadi *et al,* 2017).

The glutamic and aspartic acids the major non-essential amino acids in camel meat ranged from 15.6 to 18.6% and from 8.6 to 10.8% of protein, respectively (Table 3). Similar to the essential amino acids, non-essential amino acids contents also slightly varied between muscles and larger variations are found between studies. In general, camel meat may be a better source of non-essential amino acids compared to beef, lamb and goat meats. Although, Elgasim and Alkanhal (1992) found low alanine level in camel meat compared to other red meats, Dawood and Alkanhal (1995); Al-Shabib and Abu-Tarboush (2004), Kadim *et al* (2011) and Abdelhadi *et al* (2017) found similar concentration of alanine in camel meats and other red meats.

Table 4. Fatty acid composition of the fatty acids in camel meat.

Fatty acids (%)	Rawdah *et al* (1994)	Al-Bachir and Zeinou (2009)	Kadim *et al* (2011)	Abdelhadi *et al* (2017)
Saturated (S)				
14:0	7.68	4.53	3.10	4.83
15:0	1.66	-	2.10	0.58
16:0	25.98	30.29	28.50	22.4
17:0	1.48	2.54	-	0.76
18:0	8.63	25.51	19.30	16.9
Monounsaturated (MUS)				
14:1	1.0	-	1.60	-
16:1	8.06	-	6.30	3.43
17:1	0.94	-	-	1.16
18:1	18.93	32.01	33.50	25.9
20:1	trace	-	-	-
Polyunsaturated (PS)				
18:2ω6	12.07	5.13	3.20	6.61
20:2ω6	0.11	-	-	-
18:3ω3	0.52	-	1.20	0.68
20:3ω9	0.37	-	-	0.47
20:3ω6	0.30	-	-	-
20:4ω6	2.84	-	1.20	2.59
22:4ω6	0.10	-	-	-
20:5ω3	0.32	-	-	-
22:5ω3	0.48	-	-	-
22:6ω3	0.10	-	-	0.14
P/S	0.36	-	0.11	0.28
Total saturated	51.54	-	53.00	48.2
Total MUSFA	29.90		41.40	36.9
Total PUSFA	18.55	-	5.60	13.6
ω3/ω6	0.092	-	-	-

Fatty Acids Composition

Fatty acid composition of meat is of great concern to consumers due to its important effects on human health. Reduction of saturated fat intake is very important to prevent obesity, hypercholesterolemia and to decrease the risk of cancer (Chizzolini *et al,* 1999). It has been shown that food containing high level of unsaturated fat decreased cholesterol levels (Mensink and Katan, 1989). Rawdah *et al* (1994) identified 22 fatty acids in camel meat (Table 4). Major fatty acids in camel meat were also reported by Al-Bachir and Zeinou (2009) and Kadim *et al* (2011). The composition of major fatty acids appears to be variable partially due to the number of fatty acids that affects the percentage of individual fatty acids (Table 5). Rawdah *et al* (1994) reported levels of 18.93% oleic (C18:1) and 12.07% linoleic acid (C18:2) in the camel meat. On the other hand, about twice the amount of oleic (C18:1) and less than half the amount of linoleic acid (C18:2) were reported by Al-Bachir and Zeinou (2009) and Kadim *et al* (2011). Linoleic acid is derived entirely from the diet (Wood *et al,* 2008) and such differences are not unexpected from studies from different regions. The major saturated, monounsaturated and polyunsaturated fatty acids in camel meat are (C16:0), (C18:1) and (C18:2), respectively (Table 5). While the total saturated fatty acids among the published reports (51.5 -53%) was closely reported; more variable for monounsaturated (29.9 and 41.4%) and polyunsaturated (5.6% and 18.6%) fatty acids have been reported (Rawdah *et al,* 1994; Kadim *et al,* 2011).

The fatty acid composition, total saturated, unsaturated, monounsaturated and polyunsaturated fatty acids of infraspinatus, triceps brachii, longissimus thoraces, semitendinosus, semimembranosus and biceps femoris muscles of camel are shown in table 5. The fatty acid composition of the 6 muscles were generally similar with the exception of palmetic (C16:0) and oleic (C18:1n9) fatty acids. The semitendinosus muscle had lower palmetic fatty acids (C16:0) than infraspinatus, triceps brachii, longissimus thoraces and semimembranosus

Table 5. Fatty acids composition (%) of the infraspinatous (IS), triceps brachii (TB), longissimus thoraces (LT), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) muscles of the dromedary camel (Kadim *et al,* 2013).

	Muscle						
	IS	**TB**	**LT**	**ST**	**SM**	**BF**	**SEM[1]**
Saturated fatty acid							
Lauric acid (C12:0)	1.71c	1.42	1.13a	1.66	1.53	1.44	0.186
Tridecanoic acid (13:0)	1.22	1.13	1.24	1.24	1.24	1.21	0.066
Myristic acid (C14:0)	7.62	7.78	7.16	7.24	7.48	7.83	0.544
Pentadecanic acid (C15:0)	2.32	2.14	2.39	2.40	2.35	2.12	0.095
Palmitic acid (C16:0)	27.64	27.26	26.92	25.09	26.45	26.16	2.378
Margaric acid (C17:0)	2.38	2.17	2.46	2.21	2.38	2.15	2.088
Stearic acid (C18:0)	8.79	8.90	9.82	8.71	8.37	8.02	2.277
Arachidic acid (C20:0)	0.08	0.03	0.09	0.02	0.04	0.03	0.022
Henicosanoic acid (21:0)	0.03	0.00	0.03	0.01	0.01	0.00	0.007
Docosanoic acid (C22:0)	0.02	0.01	0.02	0.01	0.00	0.02	0.004
Mono-unsaturated fatty acids							
Tetradecenoic acid (C14:1)	1.63	1.62	1.35	1.73	1.63	1.62	0.112
Ginkgolic acid (15:1)	1.04	1.03	1.01	1.01	1.03	1.02	0.051
Palmitoleic acid (C16:1)	8.88	8.56	8.25	8.79	8.66	8.57	2.233
Heptadecenoic acid (C17:1)	0.16	0.14	0.14	0.15	0.11	0.11	0.039
Oleic acid (C18:1n9)	25.04	26.26	26.21	26.42	26.80	26.88	2.182
Poly-unsaturated fatty acids							
Linoleic acid (C18:2n6)	7.14	7.83	7.11	7.79	7.98	7.94	0.207
α-Linolenic acid (C18:3n3)	0.64	0.43	0.59	0.62	0.54	0.54	0.122
Eicosadienoic acid (C20:2)	0.52	0.34	0.62	0.64	0.43	0.42	0.016
Eicosatetraenoic (C20:3n6)	0.33	0.23	0.34	0.43	0.42	0.41	0.009
Arachidonic acid (C20:4n6)	2.81	2.72	2.84	2.83	2.55	3.51	0.033

[1]SEM: standard error for the mean. Means on the same row with different superscripts are significantly different (P<0.05).

muscles. The infraspinatus muscle contained lower oleic acids (C18:1n9) than other muscles. Palmetic acid (C16:0) is the most abundant saturated fatty acid in camel intramuscular fat followed by stearic acid (18:0) and myristic acid (C14:0). The main monounsaturated fatty acids in camel muscles were oleic acid (C18:1n9c) followed by palmitoleic acid (C16:1). The main polyunsaturated fatty acids in the muscles were linoleic acid (C18:2n6c) and arachidonic acid (C20:4n6). The percentage of polyunsaturated fatty acids in camel meat (11.92%), which it is higher than beef (8.8%) and lower than buffalo (28.6%) and deer (31.4%) (Sinclair *et al,* 1982). The ratio of linoleic and linolenic acids in camel meat is about 13.9 whereas is much higher than that of the meat of cattle, sheep or goat (2.0, 2.4 and 2.8, respectively) (Sinclair *et al,* 1982).

The camel hump is important and commonly used for cooking in camel producing countries. On fresh weight basis, the camel hump contributes about 64.2-84.8% fat with very high content of saturated fatty acids of about 63.0% (Rawdah *et al,* 1994; Kadim *et al,* 2002). Researchers therefore, focused on the composition of the hump (Mirgani, 1977; Emmanuel and Nahapetian, 1980; Abu-Tarboush and Dawood, 1993; Kadim *et al,* 2002). Palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) are the most abundant fatty acids in the hump. The composition of the hump fatty acids is affected by the animal age. The highest percentage of unsaturated fatty acids and lowest percentage of saturated fatty acids were in animals of less than one year, whereas an opposite trend was in animals in the 1-3 years old age group (Kadim *et al,* 2002).

Mineral Composition

Minerals can be classified into required essential elements for growth and health or toxic elements. Deficiency or excess intake of essential elements can be hazardus to human health. Table 6 gives essential mineral contents of various cuts of camel meat.

Table 6. Mineral concentrations in camel meat (mg/100g fresh weight).

	Mineral[1]													
Factor	**Ca**	**Co**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Na**	**P**	**S**	**Zn**	
Rump	-	0.004	-	0.12	2.5	-	-	-	0.04	-	-	-	-	Badiei *et al* (2006)
Intercostal	8.5	0.29	0.42	0.13	51.0	515	29.5	0.19	-	300.5	-	-	74.0	Rashed (2002)
Scapula	10.0	0.35	0.32	0.21	54.5	670	51.0	0.22	-	225.0	-	-	58.0	
Sirloin	10.2	0.27	0.41	0.16	44.0	446	28.0	0.16	-	188.5	-	-	66.0	
Flank	8.4	0.32	0.33	0.12	49.0	811	49.5	0.19	-	223.0	-	-	69.5	
Front knuckle	8.4	0.26	0.42	0.25	44.5	630	37.0	0.17	-	299.5	-	-	73.5	
Front limb	9.8	0.19	0.37	0.26	50.5	548	42.5	0.19	-	312.5	-	-	85.5	
Chuck	11.5	-	-	-	3.2	249	17.4	-	-	73.5	-	-	3.7	Dawood and Alkanhal (1995)
Rib eye	8.1	-	-	-	2.9	231	16.3	-	-	67.1	-	-	3.7	
Leg	10.3	-	-	-	3.4	251	17.1	-	-	69.7	-	-	3.9	
Leg+loin	4.9	-	-	0.04	1.9	228	17.7	0.01	-	47.9	-	-	3.2	Elgasim and Alkanhal (1992)[2]
Shoulder	5.1	-	0.01	0.07	1.2	357	20.6	0.01	-	69.1	196	56.1	3.5	El-Faer *et al* (1991)
Thigh	5.4	-	0.01	0.09	1.4	361	21.0	0.01	-	70.4	199	55.0	3.1	
Ribs	4.7	-	0.01	0.07	1.2	324	18.5	0.01	-	84.1	181	58.0	3.9	
Neck	5.6	-	0.03	0.09	1.4	338	18.5	0.01	-	87.3	181	64.4	4.8	
Effect of species														
Camel	5.9	0.003	0.008	-	-	193	12.9	-	0.008	45.3	105	-	-	Kadim *et al* (2009a)
Beef	6.2	0.003	0.009	-	-	416	20.5	-	0.006	51.0	162	-	-	
Camel	4.9	-	-	0.04	1.94	228	17.7	0.01	-	47.9	-	-	3.2	Elgasim and Alkanhal (1992)
Beef	6.97	-	-	0.06	2.66	277	24.8	0.02	-	31.2	-	-	4.1	

[1]Mineral: Ca: Calcium; Co: cobalt; Cr: Chromium; Cu: Copper; Fe: Iron; K: Potassium; Mg: Magnesium; Mn. Manganese; Mo: Molybdenum; Na: Sodium; P: Phosphorus; S: Sulfate; Zn: Zinc

Calcium content (mg/100g fresh weight) were reported to be in the range of 1.33- 11.48 (Table 6). The level of variation reported by Kadim *et al* (2006; 2011), indicates that physiological status of camel plays a major role in determining the calcium contents in meat. The calcium content between different meat cuts ranged from 19 to 27% (Dawood and Alkanhal, 1995; Rashed, 2002). Although, there was up to 144% variation in calcium content between different meat cuts, cobalt and chromium contents were in the range of 0.003-0.004 and 0.008- 0.03 (mg/100g fresh weight) (Kadim *et al,* 2006). Copper contents in camel meat ranged between 0.04 to 0.12 mg/100g fresh weight (Table 6). The foreleg contains have higher copper content (Scapula, front knuckle and front limb) compared with other meat cuts (Rashed, 2002). Iron content in camel meat (1.16-3.39 mg/100 g fresh meat) varied among different meat cuts, which is most probably due to the different physiological requirements of myoglobin of different muscles. As with other red meat species, meat cuts containing oxidative muscles (e.g. leg and neck) has higher iron content than glycolytic muscles. Potassium is the major element in camel meat (193.4- 379.1 mg/100g fresh weight) and magnesium content in camel meat range between 10.41- 21.03 mg/100g fresh weight (Kadim *et al,* 2009a). Meat cuts from the limbs have higher potassium and magnesium contents compared with the loins and ribs (Table 6). Meat from camel contained similar manganese content (0.01 mg/100g fresh weight) across 4 different meat cuts (El-Faer *et al,* 1991; Elgasim and Alkanhal, 1992). However, Rashed (2002) found that camel meat contained higher manganese (mg/100g dry matter) and the concentration varied among different meat cuts. Sodium content in camel meat was in the range of 40.2-87.3 mg/100g. The loins cut had the lowest sodium content among the different meat cuts (Elgasim and Alkanhal, 1992; Rashed, 2002; Kadim *et al* 2006). Phosphorus is the second most abundant

Table 7. Macro and micro-element levels (mg/100g) in *Infraspinatus* (IS), *triceps brachii* (TB), *longissimus thoraces* (LT), *semitendinosus* (ST), *semimembranosus* (SM) and *biceps femoris* (BF) muscles of the dromedary (Kadim *et al,* 2013) and Bactrian camels (Raiymbek *et al,* 2012b).

	Species Species												
	Dromedary Muscle						Bactrian Muscle						SEM[1]
	IS	TB	LT	ST	SM	BF	IS	TB	LT	ST	SM	BF	
Phosphorus	6.49	7.76	5.23	6.39	7.96	6.79	3.32	3.72	2.29	3.97	3.66	3.74	0.233
Calcium	0.07	0.08	0.05	0.07	0.08	0.07	0.05	0.05	0.05	0.05	0.05	0.05	0.004
Magnesium	1.73	2.21	1.37	3.39	2.17	1.84	2.48	3.03	2.51	3.5	3.27	3.45	0.147
Sodium	6.33	5.98	5.18	7.38	5.78	6.93	5.01	4.57	3.59	5.78	4.93	5.16	0.285
Potassium	81.7	103	25.2	71.3	80.9	85.6	74.4	80.5	36.9	80.0	77.7	73.5	3.400
Zinc	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.001
Iron	0.02	0.06	0.03	2.42	2.52	0.05	0.06	0.08	0.05	0.08	0.12	0.08	0.318
Lead	0.001	0.01	0.01	0.01	0.03	0.002	0.03	0.03	0.03	0.03	0.03	0.02	0.014
Selenium	0.003	0.003	0.004	0.004	0.003	0.003	-	-	-	-	-	-	0.001
Copper	0.002	0.001	0.001	0.05	0.07	0.004	0.002	0.002	0.004	0.002	0.002	0.001	0.007

[1]SEM: standard error for the mean. Means on the same row with different superscripts are significantly different (P<0.05).

Table 8. Toxic/non-essential elements concentrations (mg/100g) of fresh weight.

	Mineral1 Mineral[1]							
Factor	Ag	Al	Au	Cd	Ni	Pb	Sr	
Effect of meat cut								
Intercostal	0.07	-	0.11	-	0.24	-	-	Rashed (2002)
Scapula	0.06	-	0.10	-	0.38	-	-	
Sirloin	0.11	-	0.19	-	0.05	-	-	
Flank	0.09	-	0.12	-	0.13	-	-	
Front knuckle	0.12	-	0.17	-	0.19	-	-	
Front limb	0.11	-	0.21	-	0.21	-	-	
Shoulder	-	0.51	-	-	-	-	0.02	El-Faer *et al* (1991)
Thigh	-	0.15	-	-	-	-	0.03	
Ribs	-	0.12	-	-	-	-	0.02	
Neck	-	0.58	-	-	-	-	0.03	
Effect of species								
Camel	-	-	-	0.003	0.025	0.015	-	Kadim *et al* (2009b)
Beef	-	-	-	0.003	0.044	0.006	-	

[1]Mineral: Ag: Silver; Al: Aluminum, Au: Gold; CD: Cadmium; Ni: Nickel; Pb: Lead; Sr: Strontium

element in camel meat (105.6-199.0 mg/100g fresh weight) and the leg and shoulder cuts have slightly higher phosphorus than ribs and neck cuts (El-Faer *et al,* 1991). Sulfur content in the range of 54.99-136.57 mg/100g fresh weight. The sulfur content in 4 meat cuts varied by 17% only (El-Faer *et al,* 1991). Red meat is an important source of zinc and camel meat contains about 3.07 to 4.80 mg/100g fresh weight (Table 6). The variation between different cuts was 7.6% (Dawood and Alkanhal, 1995) but higher percentage of variation (47-56%) has been reported in other studies (El-Faer *et al,* 1991; Rashed, 2002).

The mineral concentrations of infraspinatus, triceps brachii, Longissimus thoraces, semitendinosus, semimembranosus and biceps femoris muscles of dromedary and bactrian camel (Kadim *et al,* 2013; Raiymbek *et al,* 2012b) are presented in table 7. The phosphorus magnesium, sodium, potassium and iron contents of camel muscle samples varied between muscles. The triceps brachii and semimembranous muscles had the highest mean value of phosphorus, calcium, magnesium and potassium (Table 7). The semitendinosus muscle in the dromedary and bactrian camels had more magnesium than

infraspinatous, triceps brachii, longissimus thoraces and biceps femoris muscles. The semitendinosus and semimembranosus muscles had more iron than other muscles in dromedary. The longissimus thoraces muscle had a lower and the triceps brachii higher ($P<0.05$) potassium than other muscles (Table 7). For trace elements (zinc, iron, lead, selenium, copper), there was small variation between muscles of dromedary and bactrian camels (Table 7).

The concentrations of silver, gold and nickel in five camel meats have been reported at 0.06-0.12, 0.10-0.21 and 0.05-0.38 mg/100g dry matter, respectively (Rashed, 2002). The concentration of the three minerals varied among different muscles by 100%, 110% and 750% (Table 8). The concentrations of nickel, beryllium and vanadium increased in the dromedary camel longissimus thoraces with the increasing animal age (Kadim *et al,* 2006). The level of lead in camel longissimus thoraces was 2.5 times the concentration in beef longissimus thoraces (Kadim *et al,* 2009). Studies on the levels of trace and heavy elements in camel blood concluded that camel could be less efficient than other ruminants in detoxifying these elements in its body (Al-Qarawi and Ali, 2003). Therefore, monitoring of the toxic levels in biological materials from camel should get attention to (Faye *et al,* 2008). Monitoring the level of toxic compounds in the offal should be of priority since it is regularly consumed by low income groups as a source of animal protein in many developing countries.

Farming conditions (diet; desert *vs.* farm and soil composition) as well as the physiological conditions of the animals (breed, sex and age) seems to play an important role in determining the level of various elements in the meat and the camel blood (Faye *et al,* 2008). For instance, calcium content in the camel meat reported from the same laboratory (Kadim *et al,* 2006; 2011) or across different laboratories (Dawood and Alkanhal, 1995; Kadim *et al,* 2006) supports this contention. It is worth mentioning that the biological variation of elements content even within the same herd that has similar farming background is very high (Kadim *et al,* 2006).

Meat Quality Characteristics

Camel meat is often regarded as quality inferior to other red meat animals. This might be attributed to the strong reluctance of camel owners to sell their young stock and they usually slaughter older camels at the end of their productive life. Most camel meat trade is of meat from old camels with low quality, which has a direct bearing on the extent of demand for meat outside the camel herding societies. However, numerous studies reported that meat quality characteristics from young dromedary camel are comparable to those of beef (Leupold, 1968; Fischer, 1975; Knoess, 1977; Mukasa-Mugerwa, 1981; Kadim *et al,* 2006, 2009a,b; Shariatmadari and Kadivar, 2006). Camels 2-4 years-old and beef 2-3 years-old had similar meat quality characteristics (Kadim and Mahgoub, 2008). According to Kamoun (1995a,b), the camel meat lose more water during cooking than beef (48% vs. 37%) while no tenderness differences were observed between the 2 species. In contrast, Babiker and Tibin (1986) reported that camel meat have less cooking losses and higher water holding capacity than beef meat. Effect of age on meat quality is discussed in order to optimise the best age for slaughtering camel for high quality meat.Table 9 depicts the effect of camel age on meat quality parameters and shows that meat becomes less tender and of inferior quality with increasing animal age (Kadim *et al,* 2006). However, Kamoun (1995a,b) noted that age is not a predominant factor in meat quality in the case of dromedaries fed the same diet and slaughtered between 1 and 4 years of age. Kadim *et al* (2006) suggested that the male camels should be slaughtered between 1 to 3 years of age. Dina and Klintegerg (1977) reached a similar conclusion. This might be due to that less than 3 year of age, camels were not yet fully-grown (60-70% of full live weight), therefore, their meat is tender.

Table 9. Effect of age on some meat quality characteristics of the dromedary camel longissimus thoraces muscle.

	Kadim *et al* (2006)			**Kadim *et al* (2009b)**	
	Age group (year)			**Age group (year)**	
	1-3	**3-5**	**5-8**	**1-2**	**8-10**
Ultimate pH	5.91	5.84	5.71	5.68	5.65
WB- Shear force value (Newton)	68.4	79.5	131.9	6.74	8.90
Sarcomere length (µm)	1.85	1.24	1.06	1.66	1.60
Myofibrillar fragmentation Index%	80.99	73.3	60.4	72.2	67.3
Expressed juice (cm^2/g)	29.6	27.36	21.26	38.1	37.4
Cooking loss %	26.06	23.72	22.42	23.4	22.0
Colour parameters					
L* (lightness	37.74	34.03	31.69	39.1	38.1
a* (redness	13.37	13.82	16.18	16.5	15.6
b* (yellowness)	6.09	6.78	7.26	5.58	6.29

Table 10. Meat quality characteristics of six muscles of the dromedary and Bactrian camel carcasses.

	References											
	Kadim *et al* (2013)						Raiymbek *et al* (2012a)					
	Muscle[1]						Muscle[1]					
	IS	TB	LT	ST	SM	BF	IS	TB	LT	ST	SM	BF
Age (year)	1.5-2						2-3					
Ultimate pH	5.64	5.73	5.61	5.67	5.83	5.74	5.73	5.69	5.63	5.68	5.60	5.68
WB-Shear Force	34.8	42.1	41.8	36.8	42.4	40.2	10.8	8.9	6.0	10.0	9.8	8.6
Sarcomere Length (µm)	31.6	29.2	33.5	28.5	30.6	29.5	1.45	1.54	1.70	1.47	1.53	1.52
Myofibrillar Fragmentation Index%	6.3	6.7	6.5	9.0	12.9	10.3	76.8	76.9	73.9	77.7	76.7	78.4
Water-Holding Capacity	1.7	1.7	1.7	1.5	1.4	1.5	38.2	37.9	37.1	40.4	41.3	38.8
Cooking Loss %	75.8	74.0	74.2	70.3	65.3	70.5	32.7	32.6	25.1	3430	33.9	32.1
Colour												
L*	41.7	40.2	43.5	40.5	40.6	40.6	32.4	30.8	33.4	30.2	30.8	30.1
a*	12.7	12.6	14.0	10.5	13.6	13.3	13.2	13.1	13.8	12.8	13.8	13.5
b*	2.6	3.7	4.1	2.2	2.9	3.8	3.8	3.4	3.9	3.2	3.5	3.6

[1]Muscle: IS; Infraspinatos, TB; Triceps brachii, LT; Longissimus thoracis, ST; Semitendinosus, SM; Semimembranosus, BF; Biceps femoris

Ultimate Muscle pH

The ultimate pH of muscles is a consequence of lactic acid accumulation through glycolysis that affects meat quality characteristics (Simek *et al,* 2003). Laack *et al* (2001) noted that 40-50% of variation in muscle ultimate pH is controlled by glycogen concentration. On the other hand, Warris (1990) stated that it needs 0.81g/100g of glycogen to lower the pH of one kg of muscle from 7.2 to 5.5. In general, the ultimate pH of camel muscles is the outcome of many factors including pre-slaughter handling, postmortem treatment, glycogen storage and muscle physiology. A high ultimate pH in camel muscles is a consequence of low muscle glycogen as a result of pre-slaughter stress, including, poor nutrition, rough handling and long transportation. The declining and ultimate pH can affect muscle colour, tenderness, water-holding capacity, cooking time, flavor and drip loss. There is a variation in the ultimate pH values between different camel muscles (Kadim *et al,* 2013).

The ultimate pH of camel meat ranges between 5.5 and 6.6 (Babiker and Yousif, 1990; Kadim *et al,* 2006, 2009a,b; 2013; Abdelhadi *et al,* 2017), with the young animals tend to have meat with a higher pH than older camels due to lower levels of glycogen. In this respect, Kadim *et al* (2006) reported that 3 year old camel had 5.91 pH value while 6 year old one had 5.71 value. The ultimate pH of camel muscles varied between 5.53 and 5.75 and between 5.68 and 5.80 for electrically stimulated and non-stimulated camel carcasses, respectively (Kadim *et al,* 2009a). The breed of camels did not differ in terms of ultimate muscle pH (Suliman *et al,* 2011). However, Abdelhadi *et al* (2017) found high ultimate pH of dromedary camel (6.10-6.18), which was higher than other studies.

Tenderness

Tenderness is one of the most important organoleptic characteristics of meat quality (Koohmaraie, 1988). Muscle structure, glycogen concentration, collagen content, solubility and the activities of proteases and their inhibitors are the most important physiological parameters affecting meat tenderness (Hocquette *et al,* 2005). Major variation in meat tenderness is related to the variability of muscle structures and characteristics (Renand *et al,* 2001). Kamoun *et al* (1995b) stated that longissimus thoraces muscle had more soluble collagen than the semitendinosus and triceps brachii muscles. The triceps brachii muscle had the highest shear force values, maximum connective tissue strength and lowest collagen solubility than longissimus thoraces, semitendinosus, semimembranosus, psoas major and vastus lateralis in camel indicating that it is the toughest muscle in this group (Babiker and Youssif, 1990) The psoas major and longissimus thoraces muscles were the most tender and had less detectable connective tissue than other muscles. In another study, Kadim *et al* (2013) found that infraspinatus, triceps brachii and longissimus thoraces camel muscles had lower shear force values than semitendinosus, semimembranosus and

biceps femoris muscles, which might be due to less connective tissue (Table 12).

The most marked difference in meat quality characteristics between camel meat and other red meat animals is largely tenderness (Mukasa-Mugerwa, 1981). Camels are usually slaughtered at the end of their productive life (more than 10 year-old), which is classified as of low quality compared with other meat animals. Average shear force value of camel meat at 5-8 years was 48% and 40% higher than those of 1-3 and 3-5 year old, respectively (Kadim *et al,* 2006). Differences due to age may be related to changes in muscle structure and composition particularly in the nature and quantity of connective tissue (Asghar and Pearson, 1980). Significant differences ($P<0.05$) were found between the different ages (8, 16 and 26 months of age) and cuts for shear force values of camels (Dawood, 1995).

Water holding capacity (Expressed Juice)

Water retention in meat is primarily caused by immobilisation of water within the myofibrillar system. Applying compression on meat can move water from the intercellar to the extracellular space and then onto surface because of structural alterations at the level of the sarcomeres or of the myofilament structure. It affects the retention of minerals, vitamins and water contents (Beriain *et al,* 2000) and affected by ultimate pH (Hamm, 1975). Moreover, Dyer and Dingle (1967) stated that water content of meat is affected by ultimate pH, composition and denaturation of proteins by the ionic strength of the extracellular fluid and oxidation of lipids, which decreases the solubility of proteins. Kadim *et al* (2006) reported that meat from camels slaughtered at one to 3 years had higher expressed juice than those slaughtered at 5 to 8 years of age, probably due to variations in fat content and the binding ability of meat. The water-holding capacity decreases as fat levels increase due to an increase in the ratio of moisture to protein (Miller *et al,* 1968). Dawood (1995) reported that 8 month-old camel meat had significantly higher water-holding capacities than meat from 26 month-old camels.

The volume of the camel meat was reduced by 44.3% and weight by 48.2% after boiling in water for 40 min (Kamoun, 1995b). The camel longissimus thoraces and biceps femoris muscles had higher 37.9 and 37.1% cooking loss than the 33.2 % cooking loss in semitendinosus muscle (Babiker and Yousif, 1990). An increase in cooking loss was observed in the longissimus thoraces muscle (33.5%) when compared to the Infraspinatus (31.6%), Triceps brachii (29.2%), semitendinosus (28.5%), semimembranosus (30.6%) Biceps femoris (29.5%) with no significant differences between the last 5 muscles (Kadim *et al,* 2013). In the Bactrian camel, variation in expressed juice between 6 muscles ranged from 37.10cm^2/g (longissimus thoraces) to 41.27cm^2/g (semimembranosus) cm^2/g (Raiymbek *et al,* 2012a). The variation between muscles might be due to location, activity, proportion of muscle fiber types, pH, intramuscular fat and the ratio of water to protein of individual muscles. However, Suliman *et al* (2011) found that Biceps femoris muscles had higher cooking loss than longissimus thoraces muscles in 4 camel breeds. In accordance with the statement of Shehata (2005), young camels had higher cooking loss than old animals, Kadim *et al* (2009a,b) found that meat from 2 to 3 years old camels had significantly lower cooking loss (24.3%) than the values mentioned above due to age differences. Cooking loss is important because of its potential to change the level of nutrients in the meat once it is cooked. For example, while it generally regarded that the protein content of camel meat is similar to other red meats (Elgasim and Alkanhal, 1992; Gheisari *et al,* 2009), the higher cooking loss in camel meat (33-38%), compared to beef (24.6%), will generate a more nutritionally dense cooked meat (Kadim *et al,* 2009a,b).

Colour (L*, a*, b*)

Meat colour is one of the most important sensory characteristics according to which consumers make judgments on purchasing meats. The meat colour is related to the concentration and chemical structure of myoglobin content. Myoglobin concentration within a given muscle will differ according to the species or age and is dependent on muscle fibre type proportions, muscle pH, age, intramuscular fat and muscle texture (Gardner *et al,* 1999; Lawrie, 2006). There was a negative linear relationship between muscle colour and its pH (Menzies and Hopkines, 1996). Protein degradation after slaughter is related to the ultimate pH, which increases light scattering properties of meat and thereby increases *L**, *a** and *b** values (Offer, 1991). Low ultimate pH and high meat temperature might lead to more protein degradation resulting in higher colour values than the high ultimate pH meat samples. Postmortem glycolysis decreases muscle pH making muscle surfaces brighter and superficially wet. If the ultimate meat pH is high, the physical state of the proteins will be above their iso-electric point, proteins associate with more water in the

muscle and therefore, fibers will be tightly packed (Abril *et al,* 2001). Babiker and Yousif (1990) reported that camel longissimus dorsi muscles had higher lightness (*L**), redness (*a**) and yellowness (*b**) values than semitendinosus and triceps brachii muscles. On the other hand, Suliman *et al* (2011) reported that the colour of the *biceps femoris* muscle was not affected by breed. Abedlhadi *et al* (2017) found no differences in muscle colour between male and female dromedary camels. The lightness and yellowness colour values of dromedary camel meat reported by Abdelhadi *et al* (2017) were lower than those reported by others (Kadim *et al,* 2008) from dromedary camels. These differences between studies might be due to variation in muscle pH and intramuscular fat content. A high redness (*a**) colour component in the camel meat was associated with a lower lightness (*L**), while higher lightness associated with high fat content (Kadim *et al,* 2013). The same authors found that semitendinosus muscle had the darkest coloured lean compared to infraspinatus, longissimus thoraces, triceps brachii, semimembranosus and biceps femoris camel muscles. The longissimus thoraces, semimembranosus and biceps femoris camel muscles had higher redness (*a**) values than semitendinosus muscle, while *a** value for Infraspinatus and Triceps brachii muscles were in between. CIE *a** values were similar among longissimus thoraces, semimembranosus and biceps femoris muscles (Kadim *et al,* 2013). In camel, the highest average yellowness (*b**) value was recorded in the longissimus thoraces muscle with comparable values to the triceps brachii and biceps femoris muscles. The age of the camel has a significant effect on their meat colour (Kadim *et al,* 2006). Meat colour from 6-8 and 10-12 year old camels was darker (lower *L**), redder (higher *a**) and yellower (high *b**) than 1-3 year old camels because of higher concentrations of myoglobin (Kadim *et al,* 2006).

Health aspect of camel meat

Camel meat is rich in many essential amino acids, minerals, vitamins, bioactives compounds such as carnosine, anserine, glutathione and essential fatty acids such as omega 3 fatty acids (Williams, 2007; Schonfeldt and Gibson, 2008; Abrhaley and Leta, 2018). Apart from the nutritional value of meat, it provides several eating attributes and fulfilling experiences that normally are not achieved by other protein sources. In Africa, Middle East and some Asian countries, camel meat is regarded as a main source of animal protein that equal and in some cases surpasses other meats in commercial importance. Several epidemiological studies linked health problems such as obesity and high saturated fat and cholesterol intake to increased consumption of animal products (Biesalski, 2005; Chao *et al,* 2005). This has led to a concern that total dietary fat intake should be restricted by consuming smaller portions less frequently (Schonfeldt and Gibson, 2008) or replacing red meat consumption with white meat. The growing evidence of low cholesterol and fat contents with relatively high-unsaturated fatty acids in camel meat could potentially support its healthiness as a better alternative to the high fat and cholesterol meats such as mutton and beef (Kadim *et al,* 2008).

Meat in general is considered a functional food for cures of many ailments and for improved performance in many cultures around the world (Migdal and Živković, 2007). Camel meat and offals such as liver are believed to have medicinal properties (Bin Saeed *et al,* 2005). Kadim *et al* (2008) stated that Somalis and Indians particularly believe in the health benefits of consuming camel meat. Among many African and Asian countries, camel meat has traditionally been used to cure the following diseases in some countries: (1) seasonal fever, sciatica and shoulder pain, as well as for removing freckles (by placing hot camel meat slices on the freckled area); (2) camel meat soup was used to cure corneal opacity and to strengthen eyesight; (3) camel fat was used to ease haemorrhoidal pains and the hump fat was used to remove tapeworm; and (4) dried camel lungs used to be prescribed as a cure for asthma, especially if taken with honey. Kurtu (2004) pointed out that the majority of camel meat consumers believe it is a healthier option during the dry season in which cattle are infected with various zoonotic diseases. This believes probably originated from the historical use of animals' organs, including meat, in folklore and traditional medicine. Lev (2006) cited the use of camel meat in remedial formulation by Al-Tabari, al-Kindi and al-Qazwini which indicate the roots of some of the current believes.

Conclusion

Camels are good sources of high quality protein, especially in areas where the climate adversely affects the survival of other livestock. The camel has unique physiological characteristics, including a great tolerance to high and low temperatures, solar radiation, water scarcity, rough topography and poor vegetation. Camels are mostly produced under traditional systems on poor levels of nutrition and are mostly slaughtered at old ages after completing a career in work, racing or milk production. Camel lean

meat contains about 78% water, 19% protein, 3% fat and 1.2% ash with a small amount of intramuscular fat and cholesterol contents, which renders it a healthy food for growing human population. The amino acid and mineral contents of camel meat are often higher than other meat animals, probably due to lower intramuscular fat levels. Camel meat has been processed into burgers, patties, sausages and shawarma to add value. The nutritional value of camel meat is similar to other red meats. The quality characteristics of camel meat are similar to beef meat quality when they slaughtered at similar age. According to the composition and quality parameters of camel meat, it can be successfully marked alongside of cattle, dear, sheep and goat. Pre and post mortem factors should be carefully considered to improve meat quality characteristics. Future research efforts need to focus on exploiting the potential of the camel as a source of meat through multidisplinary research into efficient production systems and improved meat technology and marketing.

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EVALUATION OF SEROLOGICAL TESTS FOR THE DIAGNOSIS OF BRUCELLOSIS IN *Brucella melitensis* EXPERIMENTALLY INFECTED DROMEDARY CAMELS

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ABSTRACT

Fifteen non pregnant female dromedaries of different age were intra-tracheally and intra-nasally infected with a *Brucella melitensis* strain belonging to the genetic group East Mediterranean (former African group). The development of *Brucella* antibodies in the infected dromedaries was investigated over a period of 12 months by comparing 15 different serological tests. Three uninfected control dromedaries remained negative during the entire experiment. Our investigations revealed that only 2 serological tests were characterised by a high degree of sensitivity for the diagnosis of brucellosis in dromedaries. These two tests are a Rose Bengal Test (RBT) from Vircell, Spain and a competitive ELISA (cELISA) from Ingenasa, also from Spain. The blood culture revealed that all tested sodium citrate blood samples were negative. Results of the enriched citrate blood samples of the infected 15 dromedaries fluctuated and became positive for a short time, but at the end of the experiment only one remained positive. Direct examination by PCR did not show any evidence of the pathogen in the EDTA blood of the 15 infected dromedaries. Also, all nasal swabs taken up to a period of 3 months post infection remained negative in both PCR and microbial culture.

Key words: *B. melitensis,* brucellosis, dromedary, experimental infection, serological tests, UAE

Brucellosis remains one of the most common zoonosis worldwide with more than 500,000 new cases in humans reported annually. The World Health Organisation (WHO) classifies *Brucella* spp. in Risk Group III. The prevalence of brucellosis in the animal reservoir directly determines its incidence in humans.

Brucellosis has been reported in the dromedary camel (*Camelus dromedarius*), the two humped or Bactrian camel (*Camelus bactrianus*) as well as in the South American New World camels, the llama, alpaca, guanaco and vicuna. They contract their infection when intermingling with small and large ruminants infected with *B. abortus* and or *B. melitensis* (OIE, 2012).

It is known that no serological test is appropriate for animal brucellosis and all have limitations especially when it comes to screening individual animals (Nielsen *et al,* 2006). Most of the available serological tests are evaluated for bovine, caprine, ovine and porcine but none for camelids. The course of brucellosis is similar in species like buffaloes, bisons, yaks, elk, wapitis and camelids to that in cattle, but each test should be validated in the animal species under study (OIE, 2012). We, therefore infected 15 dromedaries with *B. melitensis* and studied the subsequent antibody development over a period of 12 months using 15 different serological tests.

Materials and Methods

Eighteen non-pregnant female dromedary camels were used for this experiment, of which 3 served as control. Four of these dromedaries were intra-nasally and 11 were intra- tracheally infected with a *B. melitensis* strain which was previously isolated from a dromedary placenta (Fig 1a, b).

The *B. melitensis* strain used for this experiment was genotyped with multiple-locus variable number tandem repeat (MLVA) and was grouped under the East Mediterranean subclade formerly located in African group (Gyuranecz *et al,* 2016).

The infected herd was kept in an isolated enclosure in the desert of Dubai, fed with Timothy

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hay *ad libitum*, 2 kg concentrate each and fresh water was readily available. Blood samples were first taken before the infection and then regularly at intervals, 1st every fortnight and then monthly for one year post infection. In total, 540 sera were tested over a period of 12 months with 15 different serological tests (Table 1), totaling 8,100 tests. Additionally, nasal swabs were regularly taken for *Brucella* culture during the first 3 months. EDTA and sodium citrate blood as well as sera were used for haematology, bacterial culture and the serological tests, totaling 540 EDTA and sodium citrate blood each. Rectal temperatures were regularly taken before infection and during each visit.

Results

All 15 infected dromedaries remained healthy during the entire experiment of 1 year except one which developed a swollen hind leg after 9 months. This animal was euthanazed on humane grounds 10 months *post infectionem* and pure *B. melitensis* bacteria were isolated from the tarsal joint of the right leg. None of the infected dromedaries developed fever and the haematological blood results remained in the reference range. From none of the 540 sodium citrate blood samples any *Brucella* bacteria were isolated when using direct method. However, when the same samples were enriched, the culture results of the infected 15 dromedaries fluctuated showing *Brucella* bacteria isolation from day 8 p.i. until the end of the trial. All 540 EDTA blood samples tested by PCR were negative and also all nasal swabs tested for 3 months at the beginning of the experiment.

Our serological investigation using 15 different serological tests gave conflicting results. Only 2 serological tests were suitable for the diagnosis of dromedary brucellosis while 13 others were not sensitive enough for many different reasons explained in the thesis by (Soellner, 2018, in press). The two serological tests, a Rose Bengal Test from Vircell, Spain and a competitive ELISA (cELISA) from Ingenasa, Spain, were a perfect combination for the diagnosis of brucellosis in camels. The RBT is a fast test and can even be performed in the field and the other one is a very simple laboratory ELISA that only requires an ELISA plate reader.

Table 1. Overview of all 15 serological tests used in the diagnosis of brucellosis in dromedaries.

Test	Name/Antigen	Conjugate	Species	Company	Country
RBT	Rose Bengal Antigen	–	Animals	APHA Scientific	UK
RBT	BENGATEST	–	Animals	Synbiotics Europe/Zoëtis	France
RBT	Pourquier® Rose Bengal Ag	–	Animals	IDEXX	USA
RBT	Rose Bengal	–	Human	Vircell	Spain
CFT	*Brucella abortus* antigen	–	Animals	APHA Scientific	UK
SAT	*Brucella abortus* antigen	–	Animals	APHA Scientific	UK
Lateral flow test	*B. melitensis/abortus/suis/* Antigen	Monoclonal anti-camel-IgG	Animals	MEDLINK, MSA™	UAE
Lateral flow test	*Brucella* sLPS (*B. melitensis/ abortus/suis*)	Protein G	Bovine, cattle, sheep, goat & human	Genomix	India
i-ELISA	*Brucella* abortus antigen	Anti-ruminant	Cattle	IDEXX	USA
i-ELISA	*Brucella* LPS	Anti-multi-species-IgG-HRP conjugate	Bovine, ovine, caprine, porcine	ID.vet	France
i-ELISA	Anti-*Brucella* ELISA Camel (IgG), *Brucella* LPS	No information	Camel	EUROIMMUN	Germany
i-ELISA	Argentina Antigen	Protein A	Animals	Inhouse	–
c-ELISA	*Brucella abortus* antigen	Goat anti-mouse IgG antibody	Bovine, ovine, caprine, porcine	Svanova Boehringer Ingelheim	Sweden
c-ELISA	*Brucella abortus* antigen	Monoclonal antibody specific to the epitope C of LPS of *Brucella*	Small ruminants, bovine, porcine	Ingenasa	Spain
c-ELISA	*Brucella melitensis* LPS extract	Monoclonal anti-*B. melitensis* LPS antibody	Cows, sheep & goats	APHA Scientific	UK

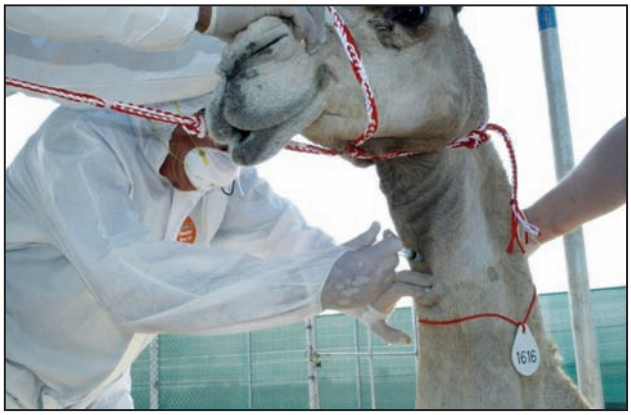

Fig 1a. Intratracheal infection with *B. melitensis*.

Fig 1b. Intranasal infection with *B. melitensis*.

Discussion

The increasing prevalence of brucellosis in Old World Camels (OWC) is of grave concern (Sprague *et al,* 2012) and is mainly caused by the uncontrolled trade of live animals (Wernery, 2016). As brucellosis infected animals do not show clinical signs, regular testing by laboratory test methods especially, serology is crucial for the diagnosis of brucellosis. This was also confirmed by our experiment. None of the 15 infected dromedaries showed fever and their blood count was in the normal range. Only one dromedary developed a swollen hind leg and became lame after 9 months. *B. melitensis* was isolated from the tarsal joint. In general, brucellosis is characterised by abortion, retained placenta and occasionally by orchitis and hygromas.

Innumerable serological tests are commercially available, but have not been evaluated for the use in camelids yet. Most of the serological tests use the S-LPS or OPS of *B. abortus* and only few of *B. melitensis*. It is known that LPS may cross-react with other Gram-negative bacteria than *Brucella*. Additionally, it is important to put into consideration that camelids possess a very special immune system. Their antibodies lack light chains, which is another reason to thoroughly evaluate existing serological tests for the use in camels. As proposed by Gall *et al* (2000 and 2001) each test should be systematically validated for the animal species under study. If this is done with the serological tests mentioned in Soellner's thesis, we are convinced that more serological tests will be suitable in future for the diagnosis of brucellosis in camels.

Our experiment made sure that dromedaries tested had brucellosis, because we had experimentally infected them with *B. melitensis*. The route of infection made no difference. All infected dromedaries became serologically positive and *B. melitensis* was isolated from all their blood samples, but only when the enrichment method was used. Interestingly, none of the PCR tested EDTA blood samples were positive. We can conclude that with Soellner`s research, the camel community has now 2 potent serological tests for the diagnosis of brucellosis in camels.

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FLOW CYTOMETRIC ANALYSIS OF PHENOTYPE AND COMPOSITION OF PERIPHERAL BLOOD LEUKOCYTES IN YOUNG AND OLD DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

The study of cellular immune system of dromedary camel has received less attention compared with immunoglobulins. The objective of the current study was to evaluate the impact of age on the phenotype and composition of leukocytes in peripheral blood of dromedary camels using flow cytometry. In the present study, old camels aged above 5 years showed lower percentages of lymphocytes and eosinophilic granulocytes but increased percentage of neutrophilic granulocytes in comparison to young camels aged under 5 years. While the expression of CD62L and MHC-II molecules on leukocyte populations did not differ between young and old camels, old camels showed lower expression of the adhesion molecules CD11b and CD18 on their myeloid cells but higher expression of CD11a on their lymphocytes when compared with young camels. In addition, the expression of the LPS receptor CD14 and the signal regulatory protein CD172a on monocytes was different in the 2 groups with lower expression of CD14 and higher expression of CD172a on monocytes from old camels in comparison to cells from young camels. In summary, the distribution and phenotype of leukocyte populations in the peripheral blood of dromedary camels was significantly influenced by age.

Key words: Blood dromedary camel, leukocytes, flow cytometry, adhesion molecules, phenotype

Leukocyte populations were phenotypically characterised for human, mouse and different animal species (Duvel *et al,* 2014; Gerner *et al,* 2015; Hopkins *et al,* 1993; Hussen *et al,* 2013; Lunn *et al,* 1998). However, the cellular immune system of dromedary camels has received less attention (Zidan *et al,* 2000a,b).

Flow cytometry represents a high effective method for performing phenotypic analysis of immune cells, enabling the characterisation of cell subpopulations. This tool allows the analysis of multiple immune parameters in a single measurement, which effectively helps in saving sample and time (Aydin *et al,* 2017; Kanegane *et al,* 2017; Keeney *et al,* 2017; Muchtar *et al,* 2017; Takashima *et al,* 2017).

Adhesion molecules are cell surface molecules with roles in cell adhesion and migration as well as the adhesion to bacterial surfaces and phagocytosis (Kourtzelis *et al,* 2017; Ley *et al,* 2007; Mitroulis *et al,* 2015; Muller, 2013).

CD11b antigen is the integrin α chain linked with CD18 to form CD11b/CD18, also known as Mac-1 or complement receptor type 3 (CR3). CR3 is mainly expressed on monocytes and granulocytes and binds to the iC3b complement fragment on opsonised targets and mediates the subsequent phagocytosis (Ley *et al,* 2007; Muller, 2013). CD11a dimerizes with CD18 to form the adhesion molecule lymphocyte function antigen-1 (LFA-1) expressed on all leukocytes (Roos and Law, 2001; van de Vijver *et al,* 2012). CD62L, also known as L selectin, is a cell adhesion molecule found on leukocytes including neutrophils, monocytes and lymphocytes and has been considered as marker for naïve T cells (Yang *et al,* 2011).

CD14 is membrane protein mainly expressed on monocytes and functions with TLR-4 as a bacterial pattern recognition receptor responsible for binding lipopolysaccharide (LPS), the cell wall component of gram-negative bacteria (Payne *et al,* 1993). CD172a, which is known as signal-regulatory protein alpha (SIRPa), is glycosylated cell surface receptor expressed on myeloid cells and functions as regulatory receptor that inhibits cell signaling (Hussen *et al,* 2013). Major

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histocompatibility (MHC) class II molecules are expressed on blood monocytes and B cells and function as antigen receptor that present antigens to T helper cells (Abeles *et al,* 2012).

Physiologic conditions have a strong impact on phenotype and composition of leukocytes (Elghetany and Lacombe, 2004). The objective of the current study was to evaluate the impact of age on the phenotype and composition of leukocytes in peripheral blood of dromedary camels.

Materials and Methods

Animals and blood sampling

Blood samples were collected between January and June 2017 from 15 apparently healthy dromedary camels (*Camelus dromedarius*) at Camel Research Centre, King Faisal University, Al-Ahsa, Saudi Arabia. Blood was obtained by venipuncture of the *vena jugularis externa* into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany).

Separation of whole leukocytes

Separation of whole camel leukocytes was performed by hypotonic lysis of erythrocytes according to an established separation method (Hussen *et al,* 2017). Briefly, blood was suspended in distilled water for 20 sec and double concentrated PBS was added to restore tonicity. This was repeated (usually twice) until complete erythrolysis. Separated cells were finally suspended in MIF buffer (PBS containing bovine serum albumin (5 g/L) and NaN_3 (0.1 g/L)) at 5 x 10^6 cells/ml. Cell purity of separated leukocytes was assessed by flow cytometry according to their FCS/SSC properties and always exceeded 90%. The mean viability of separated cells was evaluated by dye exclusion (propidium iodide; 2 µg/ml, Calbiochem, Germany) and it was above 90%.

Monoclonal antibodies

The full list of mAbs (commercially available antibodies) used in this study is shown in table 1.

Immunofluorescence and flow cytometry

Separated camel blood leukocytes (4 x 10^5) were incubated with monoclonal antibodies specific for MHC-II molecules, cell adhesion molecules CD18, CD11a, CD11b and CD62L (L-selectin) or myeloid markers CD14 and CD172a (Table 1) in PBS containing bovine serum albumin (5 g/l) and NaN_3 (0.1 g/l). After 30 minutes incubation (4°C), cells were washed twice and analysed on the flow cytometer. A Becton Dickinson FACS Calibur equipped with Cell Quest software (FACS Calibur; Becton Dickinson Biosciences, San Jose, California, USA) was used to collect the data. At least 100 000 cells were collected and analysed with the flowcytometric software FlowJo version 10 (FLOWJO LLC). Negative isotype controls for mouse IgG1, IgG2a, IgG2b (from BD) and IgM (from Beckmann Coulter) were also included as part of the study.

Statistical Analysis

Statistical analysis was performed with Prism (GraphPad). Results are presented as means ± S.E. of the mean (SEM). Differences between means were tested with one-factorial analysis of variance (ANOVA) and Bonferroni's correction for normally distributed data. Results were considered significant at a p-value of less than 0.05.

Results

Impact of age on the percentages of main populations of camel blood leukocyte

For the analysis of the impact of age on the distribution of main leukocyte populations in camel blood, the percentages of camel neutrophils, eosinophils, PBMC, lymphocytes and monocytes were evaluated under the whole leukocyte population in blood of young (1-4 years) and old (6-12 years) camels by flow cytometry (Fig 1). In blood of camels aged between 1 and 4 years, lower percentages of neutrophilic granulocytes (69 ± 1.8) but higher

Table 1. List of used primary monoclonal antibodies.

Antigen	Antibody clone	Labelling	Source	Isotype
CD62L	MEL14	PerCP	Biolegend	Rat IgG2a
CD11a	G43-25B	PE	BD	Mouse IgG2a
CD11b	ICRF44	PE-Cy7	BD	Mouse IgG1
CD18	6.7	FITC	BD	Mouse IgG1
MHC-II	TH81A5	-	WSU	Mouse IgG2a
CD14	M5E2	PE	BD	Mouse IgG2a
CD172a	DH59b	-	WSU	Mouse IgG1

Ig: Immunoglobulin; MHC-II: Major Histocompatibility Complex class II; WSU: Washington State University

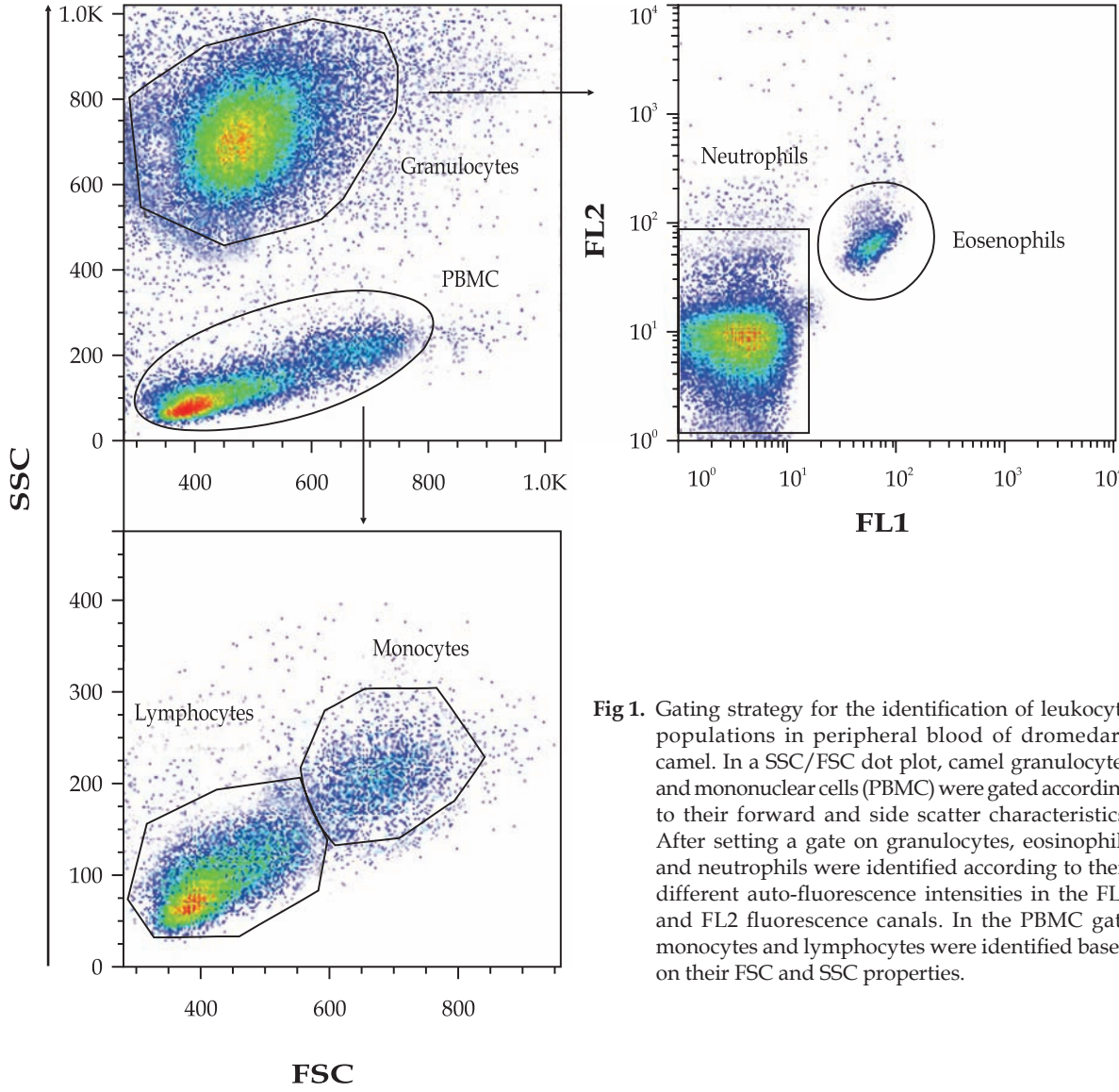

Fig 1. Gating strategy for the identification of leukocyte populations in peripheral blood of dromedary camel. In a SSC/FSC dot plot, camel granulocytes and mononuclear cells (PBMC) were gated according to their forward and side scatter characteristics. After setting a gate on granulocytes, eosinophils and neutrophils were identified according to their different auto-fluorescence intensities in the FL1 and FL2 fluorescence canals. In the PBMC gate monocytes and lymphocytes were identified based on their FSC and SSC properties.

percentages of eosinophilic granulocytes (10 ± 2.1) were seen when compared with the percentages of neutrophils (77 ± 2.6) and eosinophils (5 ± 0.6) in blood from older camels aged between 6 and 12 years. Although, the percentage of monocytes did not differ between young (7.5 ± 0.8) and old camels (6.1 ± 0.6), young camels showed significantly higher percentages of lymphocytes (24 ± 1.7) compared to old camels (17 ± 1.8). This was reflected in higher percentages of PBMC in young camels (31 ± 1.8) than in old camels (23 ± 2.6) (Fig 2).

Impact of age on adhesion molecules expression on the main populations of camel blood leukocyte

To evaluate the effect of animal age on the expression of adhesion molecules on the main population of camel blood leukocytes, the leukocytes separated from young (1-4 years) and old (6-11 years) camels were labelled with monoclonal antibodies specific for the cell adhesion molecules CD18, CD11a, CD11b and CD62L and analysed by flow cytometry. For blood neutrophils, although no difference could be seen in the expression of CD18, CD11a and CD62L, CD11b was significantly higher expressed on neutrophils from young (32 ± 3.5) animals than on neutrophils from old (15 ± 2.9) animals (Fig 3). Blood monocytes from young camels showed higher expression of CD18 (334 ± 38) and CD11b (44 ± 7.8) when compared with CD18 (234 ± 46) and CD11b (29 ± 2.4) expression on monocytes from old animals. The expression of CD11a and CD62L did not differ between the 2 animal groups. Only for CD11a, lower

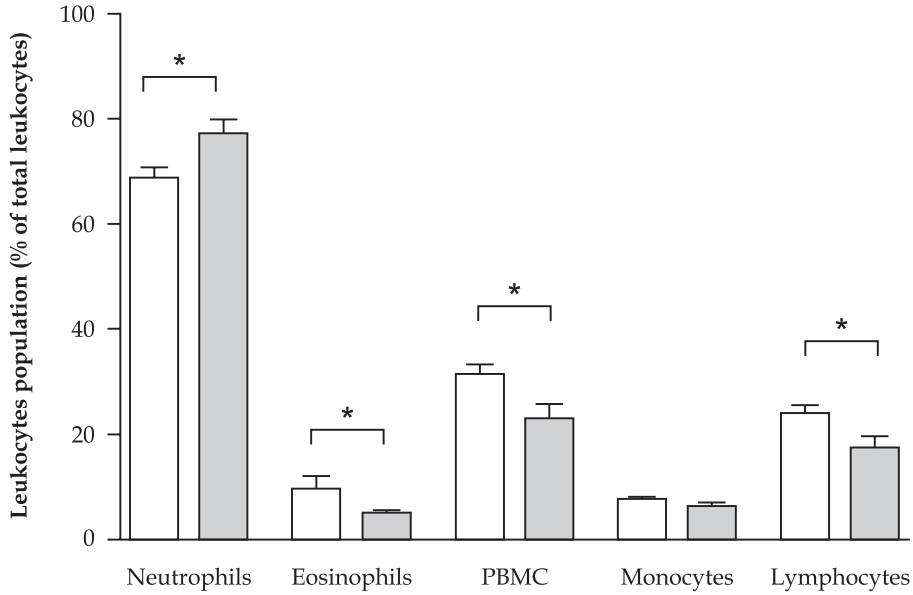

Fig 2. Impact of age on the percentage of main leukocyte populations in peripheral blood of dromedary camels. The percentages of gated leukocyte populations were calculated for camels aged between 1 and 4 years (white bars) and those aged between 6 and 12 years(gray bars) camels and data were presented graphically as mean ± SEM. (* = p<0.05).

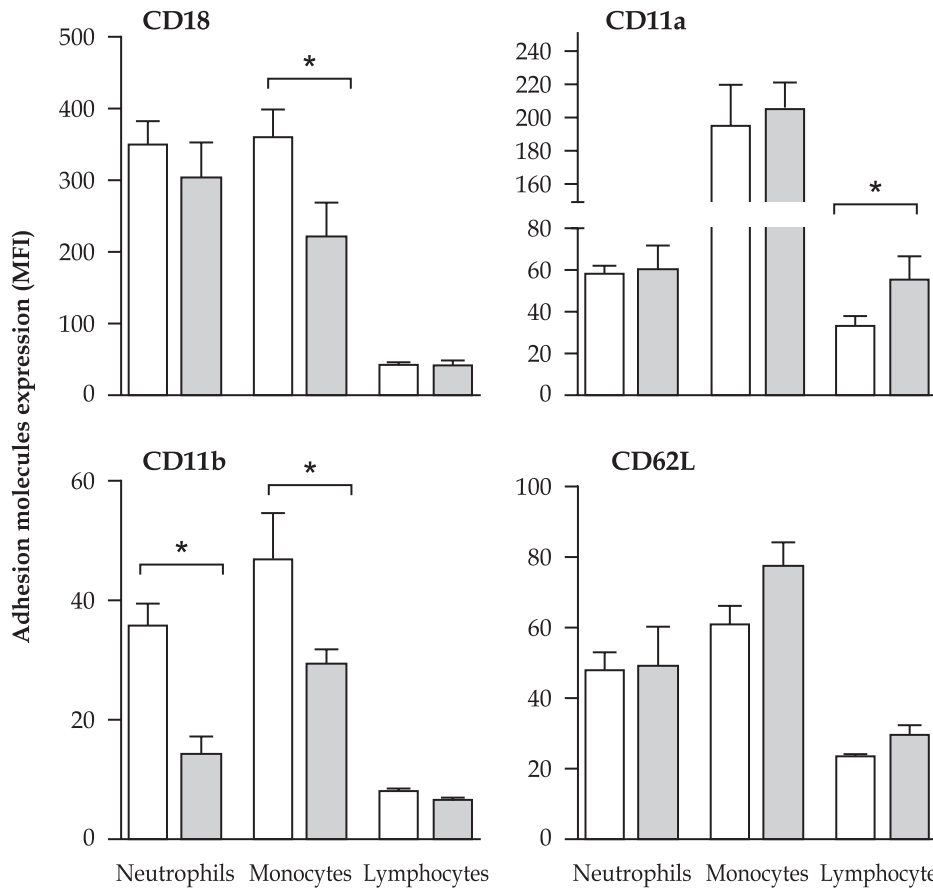

Fig 3. Impact of age on the adhesion molecules CD18, CD11a, CD11b and CD62L expression on leukocyte populations in peripheral blood of dromedary camel. After gating on neutrophils, monocytes or lymphocytes, the mean fluorescence intensity for each adhesion molecule and subset were calculated and presented graphically for both animal groups as mean +- SEM (* = p<0.05).

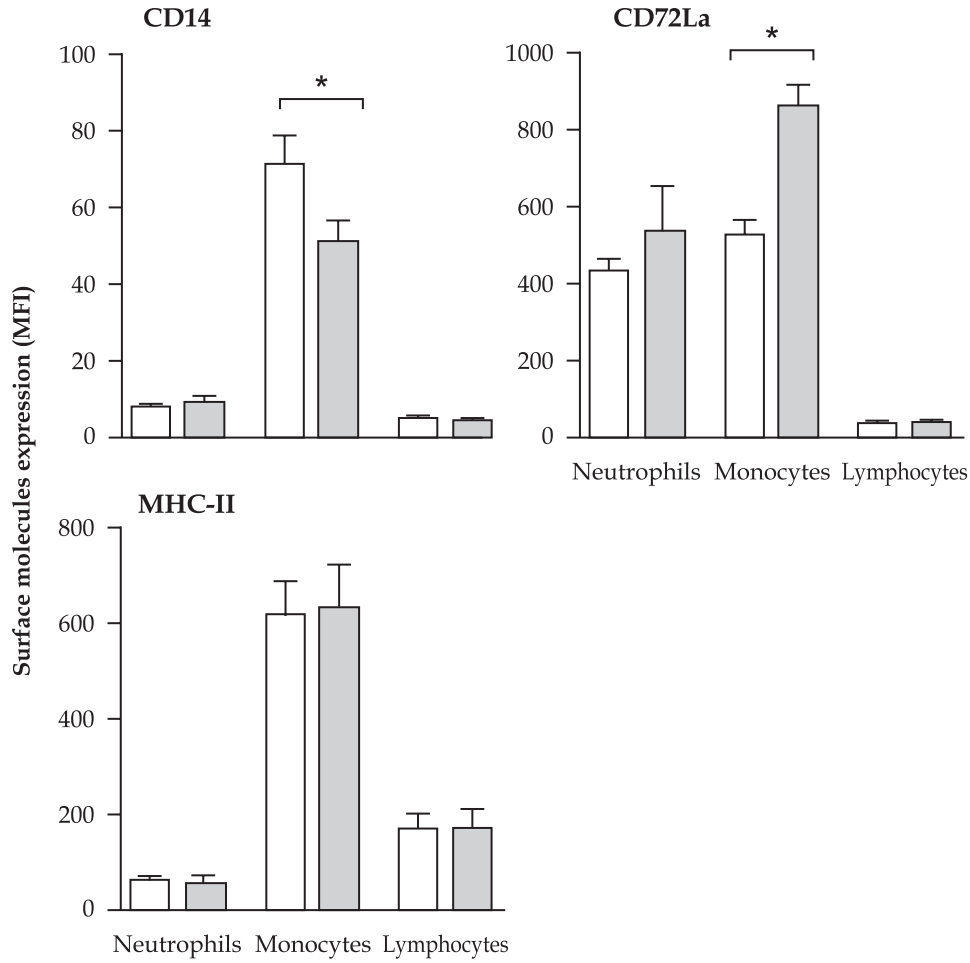

Fig 4. Impact of age on the expression of MHC-II molecules and the myeloid markers CD14 and CD172a molecules on leukocyte populations in peripheral blood of dromedary camel. Separated camel leukocytes were labelled with monoclonal antibodies specific for MHC-II molecules, CD14 and CD172a molecules and labelled cells were analysed by flow cytometry. After gating on neutrophils, monocytes or lymphocytes, the mean fluorescence intensity for each molecule and subset were calculated and presented graphically for both animal groups as mean +- SEM (* = $p<0.05$).

expression could be seen on lymphocytes from young (32 ± 5) animals in comparison to old (54 ± 12) ones (Fig 3).

Impact of age on the expression of MHC-II molecules and the myeloid markers CD14 and CD172a on the main populations of camel blood leukocytes

To evaluate the effect of animal age on the phenotype of camel myeloid cells, blood leukocytes separated from young (1-4 years) and old (6-11 years) camels were labelled with monoclonal antibodies specific for MHC-II molecules and the myeloid markers CD14 and CD172a molecules and analysed by flow cytometry. Although, the expression of studied molecules on blood neutrophils did not differ between young and old camels, monocytes from young animals showed higher expression of the monocytic marker CD14 (68 ± 7.4) but lower expression of the myeloid marker CD172a (522 ± 31) when compared with CD14 (19.5 ± 5.5) and CD172a (862 ± 51) expression on monocytes from old camels (Fig 4).

Discussion

The study of cellular immune system in dromedary camel has received less attention (Zidan *et al,* 2000a; Zidan *et al,* 2000b) when compared with the progress made by the investigation of camel immunoglobulins (Hamers-Casterman *et al,* 1993; Muyldermans, 2013). Earlier studies of camel blood leukocytes have focused on using haemocytometers to analyse the proportions of leukocyte populations in camel blood without analysing their phenotype (Ali *et al,* 1989; Azari *et al,* 2012; Chaudhary and Iqbal, 2000; Haroun *et al,* 1996; Mohamed and Hussein, 1999). Animal age has been shown to have a strong impact

on phenotype and composition of blood leukocytes (Elghetany and Lacombe, 2004). Two-parameter flow cytometry has been proven as a useful tool to perform single cell analysis aiming at the characterisation of immune cell phenotype (MacHugh *et al,* 1991; Maecker *et al,* 2012; Naessens *et al,* 1993).

According to earlier studies on the composition of camel blood leukocytes using the haemocytometer, lymphocytes represent the most predominant leukocyte type in camel blood whereas neutrophils presented the second commonest leukocyte (Mohamed and Hussein, 1999). In the current study, the higher proportion of neutrophils being the major fraction of camel blood leukocytes may be due to differences in the techniques used in sample preparation (cell separation) and analysis (haemocytometer, flow cytometer). However, recent haematologic studies have reported similar results with camel neutrophils being the dominant fraction of circulating leukocytes (Ali *et al,* 2010; Vap and Bohn, 2015; Zongping, 2003). Although, seasonal variation may be responsible for variations in camel leukogram (Amin *et al,* 2007).

The impact of age on the composition of camel blood leukocytes was investigated in the growing camel calves during the first year of life (Hussein *et al,* 1992). According to this study only counts of lymphocytes and neutrophils were affected by age with highest counts for both populations at 1 month age and decreased values with advanced ages. Age related changes in older camels were studied only for the whole leukocyte population (Saeed and Hussein, 2008). Based on the findings of this study the number of WBC decreased with advanced age with significant differences between animals of above 5 years and those of lower age groups. Studies in other animal species (Mohri *et al,* 2007; Yeom *et al,* 2012) reported a decrease in lymphocyte counts with advanced age. It has been reported that the eosinophils count, which plays a major role in parasitic immunity, increases with advanced age due to increased parasitic infestations (Roland *et al,* 2014). The results of the persent study, although agrees with the observations regarding the decrease in lymphocyte counts with advanced age, but seems to be in contrast to earlier studies regarding the increase in the percentage of neutrophils and reduced percentage of eosinophils in older camels.

There is a growing interest in using cell surface markers for the diagnoses of some inherited and acquired disorders in humans and animals (Roos and Law, 2001; Van de Vijver *et al,* 2012). Adhesion molecules are cell surface receptors that are characteristic for cell phenotype and function (Kourtzelis *et al,* 2017; Mitroulis *et al,* 2015). Age-related changes in the expression pattern of human leukocyte adhesion molecules have been reported in different studies (Crooks *et al,* 2010; Elghetany and Lacombe, 2004; Khalaji *et al,* 2017; Ponthieux *et al,* 2003). In the present study, the expression of CD11b and CD18 was lower on the myeloid cells, monocytes and neutrophils from old camels when compared with cells from young camels. CD11b and CD18 are the and integrins of the adhesion molecule Mac-1 or CR3 that play a role in cell adhesion to endothelial cells as well as in phagocytosis after adhesion to bacterial surfaces coated with complement factors (Imhof and Aurrand-Lions, 2004; Nicholson *et al,* 2007). The reduced expression of CR3 observed in this study may correlate with reduced functional capacities (migration and phagocytosis) of myeloid cells in old camels. This is also supported by the reduced expression of the LPS receptor CD14 (Hussen *et al,* 2012) and the increased expression of the regulatory molecule CD172a (Hussen *et al,* 2013) on monocytes of old camels in comparison to young camels observed in the current study. In addition, the present study showed an elevated expression of the adhesion molecule CD11a, which is expressed together with CD18 as a hetero-dimer termed as leukocyte function antigen 1 (LFA-1) (van Kooyk and Figdor, 2000), on lymphocytes from old camels in comparison to cells from young camels. This may indicate increased percentage of activated lymphocytes in blood of older camels, as CD11a is considered as activation marker of lymphocytes (Azeredo *et al,* 2006).

In conclusion, distribution and phenotype of leukocyte populations in the peripheral blood of dromedary camels are significantly influenced by age. The understanding of the impact of age on camel leukocytes composition and phenotype would help in the establishment of age-related reference values to be used in studying camel immune response as well as in the diagnosis of camel diseases.

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S100 EXPRESSION IN THE EPIDIDYMIS OF DROMEDARY CAMEL DURING BREEDING AND NON-BREEDING SEASONS: IMMUNOHISTOCHEMICAL AND MOLECULAR STUDIES

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ABSTRACT

The current study was undertaken to investigate the relationship between the expression of S100 gene in epididymal tissue and reproductive activity in dromedaries. Tissue specimens of both efferent ductules (ED) and of the three successive epididymal segments (caput, corpus and cauda epididymis) were obtained from 5 adult, clinically healthy dromedaries for each season (breeding and non-breeding). Specimens have been subjected to immunohistochemical and molecular investigations. The immunohistochemical findings showed that the S100-immunoreactivity (S100-IR) was the highest in the ciliated cells of ED and the principal cells in caput epididymis during breeding season. This S100-IR was reduced (ED) or even absent (caput epididymis) during non-breeding season. This reactivity was entirely absent from the epithelium lining the corpus and cauda regions (breeding season) or lining the whole length of the duct (non-breeding season). During breeding season, the peritubular smooth muscle cells (SMCs) exhibited weak (ED and corpus), moderate (caput) and strong (cauda) S100-IR. This reactivity was reduced (ED, caput and corpus) or even absent (cauda) during the non-breeding season. The blood vessels within the interstitium of the ED and the different regions of the epididymal duct failed to develop S100-IR throughout the year. The immunohistochemical findings were supported by the pattern of S100 mRNA expression, which was apparently higher in ED and caput epididymis obtained from camels in sexual activity compared to their counterparts from camels in non-breeding season. In conclusion, the seasonal variation in the gene expression in respect with the characteristic immunoreactivity and the sexual activity, proposes that S100 proteins could play a substantial role in regulation of the diverse epididymal functions in male dromedaries.

Key words: Dromedary, efferent ductules, epididymis, S100 proteins, seasonal changes

In spite of the economic value of dromedaries in Saudi Arabia, the literature about their reproduction biology displays remarkable shortage. Several studies were concerned with histochemistry of the epididymal duct in different mammalian species (Alkafafy and Rashed, 2008; Alkafafy, 2009; Schick *et al*, 2009; Schön and Blottner, 2009; Alkafafy *et al*, 2011a; Alkafafy and Sinowatz, 2012; Abd-Elmaksoud *et al*, 2014). However, the data available about the dromedary camel are still relatively limited (Alkafafy *et al*, 2011b; Alkafafy *et al*, 2012; Ahmed *et al*, 2013; Ibrahim *et al*, 2016).

As atypical seasonal breeders, camels may maintain their reproductive capacities throughout the year (Zayed *et al*, 1995). Furthermore, both morphometric and histological characteristics of camel epididymis show slight seasonal differences in breeding and non-breeding season (Zayed *et al*, 2012).

S100 proteins, named for their solubility in a 100% saturated solution of ammonium sulphate at neutral pH, are group of closely related, small, acidic, water-soluble proteins (Zimmer *et al*, 2013). S100 proteins belong to a multifunctional subfamily of Ca^{2+}-binding proteins that have a broad range of functions including motility, chemotaxis and secretion (Heizmann *et al*, 2002; Cruzana *et al*, 2003).

Several studies were concerned with histochemistry of the epididymis of dromedary bull (Alkafafy *et al*, 2011b; Alkafafy *et al*, 2012; Ahmed *et al*, 2013; Ibrahim *et al*, 2016). However, neither of these

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studies was concerned with the molecular aspects. The current research aims to use the molecular procedures for analysing the expression of S100 genes and to use the immunohistochemistry to underscore the spatial distribution of their proteins within the different regions of the camel epididymis during rut and non-rut season and attempt to highlight the potential structural-functional relationships.

Materials and Methods

Animals and tissues

For each season, tissue specimens of both ED and of the three successive epididymal segments (caput, corpus and cauda epididymis) were obtained from 5 adult (average age of 7 years), clinically healthy, dromedary camels (*Camelus dromedarius*) slaughtered at the local abattoir in Taif, Saudi Arabia. Specimens were taken immediately after slaughter.

Chemicals and methods

Bouin's-fixed specimens were used for routine histological and immunohistochemical staining (S-100). Tissue specimens were dehydrated in a graded series of ethanol, cleared in xylene, embedded in Paraplast wax (Sigma-Aldrich, St. Louis, MO, USA) and sectioned at 5 µm thicknesses. Tissue sections were mounted on positively charged and coated slides (Thermo Scientific, Menzel-Gläser GmbH, Braunschweig, Germany).

Conventional histological techniques

Tissue sections were stained with hematoxylin and eosin according to standard histological protocols (Bancroft *et al*, 1996) to investigate general histological structure.

Immunohistochemistry

Dewaxed and rehydrated sections were subjected to inactivation of endogenous peroxidases by incubation in 1% H_2O_2 for 15 minutes. Then, the sections were placed in 0.01 M citrate buffer (pH 6) and heated in a microwave oven (700 watts) for 10 minutes for antigen retrieval. The sections were blocked in phosphate buffered saline (PBS) containing 5% bovine serum albumin for 1 hr and then each section was incubated with its corresponding primary antibody [Polyclonal Rabbit anti-S100 (Z 0311) at a dilution of 1:400 , Dako Cytomation, Glostrup, Denmark] in a humidified chamber. The sections were washed three times in PBS for 5 minutes and incubated with biotinylated secondary antibodies [Polyclonal Swine Anti-Rabbit Biotinylated Igs (E 0353) at a dilution of 1:300 , Dako Cytomation, Glostrup, Denmark] for 30 minutes at room temperature. The sections were washed in PBS for 10 minutes. Then, the secondary antibody was detected using the Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA, USA). First, each section was covered with a 100× dilution of A and B reagent in PBS (1 µl reagent A, 1 µl reagent B and 98 µl PBS), washed three times in PBS for 10 minutes and colour was developed using DAB reagent (Sigma-Aldrich, St. Louis, MO, USA). Sections were counterstained with hematoxylin for 30 seconds, washed in water, dehydrated through graded ethanol, cleared in xylene and mounted with DPX permanent mounting media (Sigma-Aldrich).

Positive and negative controls

Immunohistochemical negative controls, in which the primary or secondary antisera or the ABC reagent was omitted, produced no positive staining. Positive controls were used according to the instructions provided by the manufacturers of the primary antibodies.

Labeling assessment and photomicrography

A semi-quantitative subjective scoring was used by three independent observers to assess the immunolabeling. Photomicrographs were taken using an imaging system consisting of a light microscope (Leica DM LB, Leica Microsystems, Wetzlar, Germany) and a digital camera (Leica EC3, Leica Microsystems Ltd., Heerbrugg, Switzerland).

Analysis of genes expression

RNA extraction and cDNA synthesis

Total RNA was extracted from 100 mg tissue sample of both ED and of the three successive epididymal segments (caput, corpus and cauda epididymis) using QIAzol lysis reagent (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions and as detailed previously (Ibrahim *et al*, 2016). Integrity of the prepared RNA was checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 nm and 280 nm. For cDNA synthesis, 2 µg RNA were reverse transcribed with oligo-dT primer and Moloney murine leukaemia virus (M-MuLV) reverse transcriptase (SibEnzyme Ltd. AK, Novosibirsk, Russia) as previously described (Ibrahim *et al*, 2016). The resultant cDNA was preserved at -20°C.

Semi-quantitative PCR

The used primers were designed using Oligo-4 computer program (Molecular Biology Insights,

Inc., Cascade, CO, USA) and nucleotide sequence published in Genbank (Table 1) and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu., Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 picomolar of each primer (forward and reverse) and 12.5 µl PCR master mix (Promega Corporation, Madison, WI, USA), the volume was brought up to 25 µl using sterilized, deionized water. PCR was carried out using a PeX 0.5 thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) with the cycle sequence of one cycle at 94°C for 5 minute, followed by 25 cycles each of which consisted of denaturation at 94°C for one minute, annealing at the specific temperature corresponding to each primer (see table 1) and extension at 72°C for one minute with an additional final extension for one cycle at 72°C for 5 minutes. As a reference, expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was detected using specific primers (see Table 1). PCR products were electrophoresed on 1% agarose A (Bio Basic Inc. Konrad Cres, Markham, Ontario, Canada) gel in 1.0 X- TAE (Tris-Acetate-EDTA) buffer (Sigma–Aldrich, St. Louis, MO, USA) at 100 volts for 30 minutes. The gel was stained with ethidium bromide and visualised under UV light then photographed using UVP gel documentation system (UVP, Upland, CA, USA).

Results

Immunohistochemical findings

The main immunohistochemical findings are summarised in table 2. The S100-immunostained sections from the ED showed that ciliated cells exhibit a strong (during the breeding season in winter) and weak (during the non-breeding season in summer) immunoreactivity (Fig 1a). Meanwhile, the non-ciliated cells within the same sections displayed negative reactivity throughout the year (Fig 1b).

The epithelium lining sections from the caput region of the epididymal duct exhibited negative to moderate S100-immunoreactivity (S100-IR) during the breeding season (Fig 1c). This reactivity disappeared in sections taken from animals in non-breeding season (Fig 1d). The S100-immunostaining was mainly confined to the principal cells and mostly absent from the other types of cells including basal ones (Figs 1c and 1d). Whereas the epithelium lining the epididymal duct at the corpus and cauda regions was entirely negative during breeding (winter) season, that lining the whole length of the duct was also totally negative during the non-breeding (summer) season.

During the winter (breeding) season, whereas the peritubular muscle coat (PMC) consisted of circularly arranged SMCs surrounding the epididymal duct displayed a moderate (caput)

Table 1. Primers and PCR conditions used for the tested dromedary genes.

Gene, accession number	Primer Sequence (5` -3`)	Annealing temperature & Cycles number	Product size
S100B (XM_010981945)	F- GGTGACAAGCACAAGCTGAA R- ACGCCTAACAGCACAGGACT	55°C, 28 cycles	423bP
GAPDH (XM_010990867)	F- CGACCACTTTGTCAAGCTCA R-TGACCTCCACACAGTTTCCA	54°C, 25 cycles	219 bP

Table 2. Effect of season on S100 immunoreactivity in the camel epididymis.

Season	Region	Epithelium				Interstitium	
		CC	NC	PC	BC	PMC	Bl.V
Winter (Rut)	ED	+++	–			+	–
	Caput			–/++	–	+/++	–
	Corpus			–	–	+	–
	Cauda			–	–	+++	–
Summer (Non-rut)	ED	+	–			±	–
	Caput			–	–	±	–
	Corpus			–	–	±	–
	Cauda			–	–	–	–

Efferent ductules (ED); Ciliated cell (CC); Non-ciliated cell (NC); Principal cell (PC); Basal cell (BC); Peritubular muscle coat (PMC); Blood vessels (Bl.V). Negative (–); negative to weak (±); weak (+); negative to moderate (–/++); weak to moderate (+/++) and strong (+++) reaction.

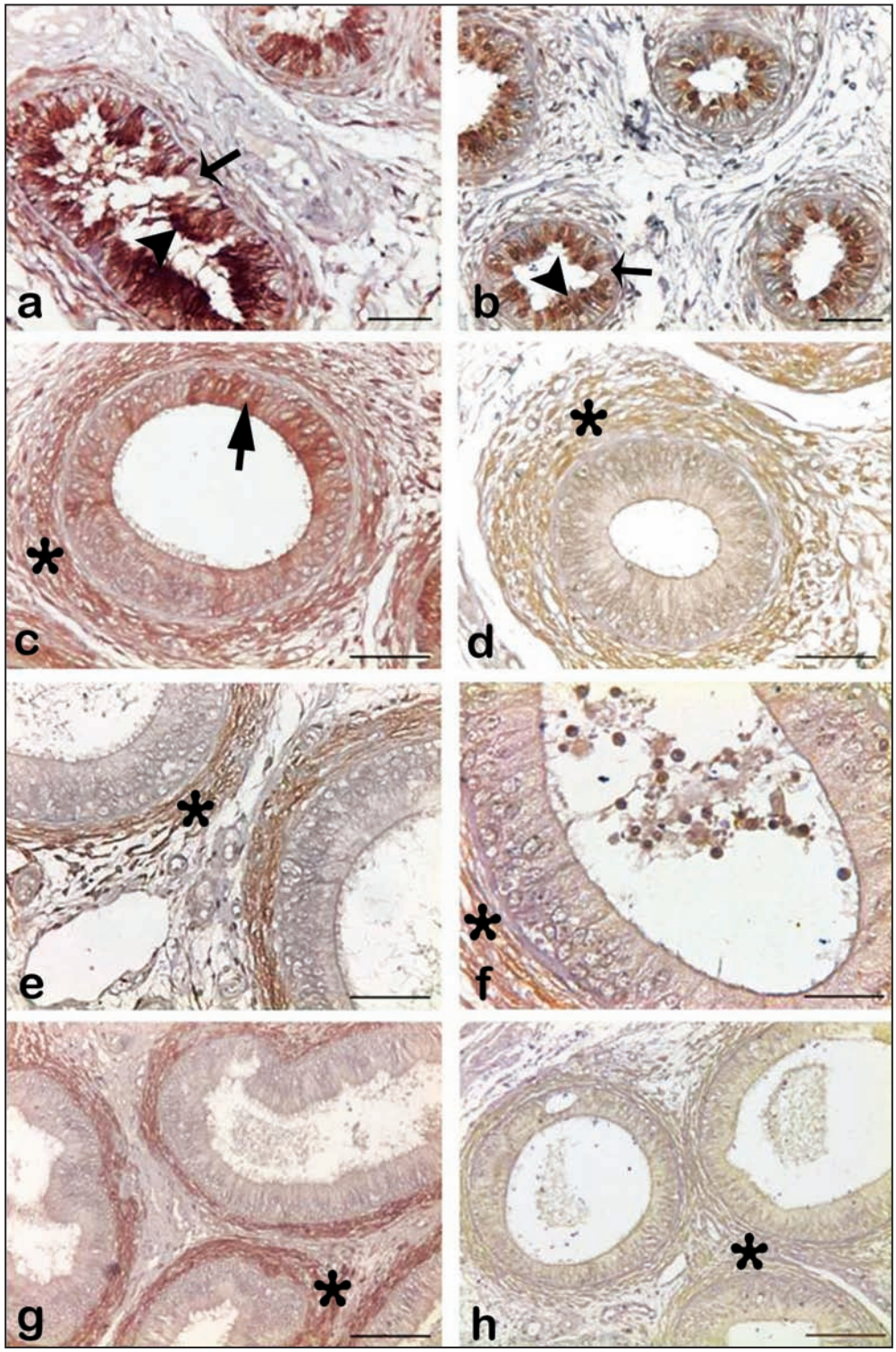

Fig 1. S100-immunostained epididymal sections from adult camels **a,** ED during winter showing strongly reactive ciliated cells (arrowheads) and negative non-ciliated cells (arrows); **b,** ED during summer displaying weakly reactive ciliated cells (arrowheads) and negative non-ciliated cells (arrows); **c,** Caput epididymis during winter showing moderately reactive principal cells (longhead arrow) and peritubular SMCs (asterisk); **d,** Caput epididymis during summer displaying negative epithelium and weakly reactive peritubular SMCs (asterisk); **e,** Corpus epididymis during winter exhibiting negative epithelium and weakly reactive peritubular SMCs (asterisk); **f,** Corpus epididymis during summer showing negative epithelium and weakly reactive peritubular SMCs (asterisk); **g,** Cauda epididymis during winter exhibiting negative epithelium and strongly reactive peritubular SMCs (asterisk); **h,** Cauda epididymis during summer displaying negative epithelium and negative peritubular SMCs (asterisk). Scale bars: 100 µm (a,b,c,d and e), 50 µm (f) and 200 µm (g and h).

to strong (cauda) S100-IR (Figs 1c and 1g), those surrounding the duct at corpus regions (Fig 1e) and those surrounding the ED (Fig 1a) showed a weak reactivity. On the other hand, the peritubular SMCs showed reduced S100-IR in the ED (Fig 1b) and in the regions of caput and corpus epididymis (Figs 1d and 1f), or even absent (cauda epididymis) during the non-breeding (summer) season (Fig 1h). The blood vessels within the interstitium of the ED and the different regions of the epididymal duct failed to develop S100-IR throughout the year (Figs 1a-1h).

Molecular findings

The mRNA expression of S100 was investigated in both the ED and the epididymal ducts collected from adult male dromedaries throughout the breeding and non-breeding seasons. S100 mRNA expression was apparently higher in the ED and caput epididymis obtained from camels in winter (season of sexual activity) compared to their counterparts from camels in summer (season of sexual inactivity) which showed weak expression. The expression was weak to moderate in the corpus and cauda epididymis obtained from camels in winter and very weak in their equivalents in summer. Meanwhile, the mRNA expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) displayed no differences between different epididymal regions and seasons (Fig 2).

Discussion

The S100-immunostained sections of the ED obtained from camels during breeding season showed an alternative pattern of reactivity between the negative non-ciliated and the distinctly positive ciliated cells. Similar findings have been previously reported in bovine ED (Alkafafy, 2005). In non-breeding season, the immunostaining pattern in the camel ED persisted but a weaker signal was displayed by ciliated cells. This may be attributed to seasonal and cyclic activity alterations (Helal *et al*, 2013; Ibrahim *et al*, 2016).

The variable regional S100-IR displayed by the epididymal epithelium during the breeding season agrees with previous studies in the ox (Alkafafy, 2005), donkey (Alkafafy, 2009), buffalo bull (Alkafafy *et al*, 2011a) and camel (Alkafafy *et al*, 2011b; Ibrahim *et al*, 2016) epididymis. On the contrary to the moderately S100-immunoreacting epithelium lining the caput epididymis during breeding season, the epithelium lining the whole length of the epididymal duct fails to develop any S100-IR during the non-breeding season. This reduced or even absent reactivity may be due to immaturity (Alkafafy and Sinowatz, 2012) or cyclic inactivity (Helal *et al*, 2013; Ibrahim *et al*, 2016).

During the breeding season, the peritubular SMCs showed a variable S100-IR ranged from weak (ED and corpus) to strong (cauda) reaction. Similar results were recorded in the epididymis from donkey (Alkafafy, 2009), rodents (Czykier *et al*, 2000) and camel (Alkafafy *et al*, 2011b). These findings go in line with those recorded for myoepithelial cells and periductal SMCs in the poll gland of male camels (Ebada *et al*, 2012). On the other hand, the reduced or even absent S100-IR displayed by the SMCs surrounding the ED and the entire length of the epididymal duct during the non-breeding (summer) season, may be a consequence of cyclic inactivity (Helal *et al*, 2013; Ibrahim *et al*, 2016).

The endothelial cells and vascular SMCs of the blood vessels within the interstitium of the efferent ductules and the different regions of the epididymal duct failed to develop S100-IR throughout the year. These findings agree with our previous work on camel epididymis during breeding season (Alkafafy *et*

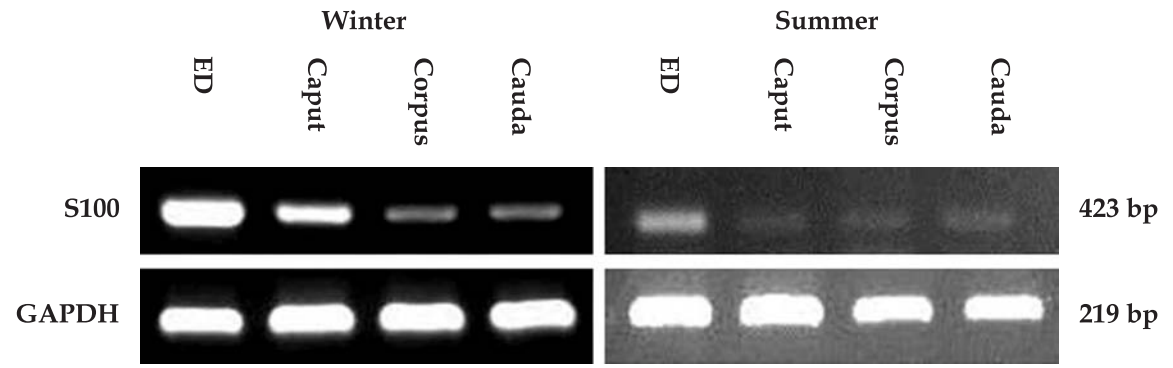

Fig 2. Representative blots for the effect of season on S100 mRNA expression in the epididymal tissues of camels. Efferent ductules (ED); Caput, Corpus and Cauda represent the camel epididymal regions of head, body and tail, respectively; glyceraldehyde-3-phosphate dehydrogenase (GAPDH); Base pairs (bp).

al, 2011b), but not with those reported by Czykier and coworkers (2010) in European bison who assumed that this reactivity is not correlated with age or maturity. The lack of S100 immunostaining in the epididymis of some species is not reasonable but might be due to species-specific difference (Abd-Elmaksoud *et al*, 2014).

Meanwhile, the current immunohistochemical findings were supported by the expression of S100 mRNA in the different regions of the epididymis collected from adult male dromedaries throughout the breeding and non-breeding seasons. Variable season-dependent S100-IR in adult camel epididymis agrees with previous works on camels (Ibrahim *et al*, 2016) and on prenatal bovine epididymis (Alkafafy and Sinowatz, 2012). Also, our current findings go in line with the differential expression of S100 proteins in the camel mammary gland during lactation (strong S100-IR) and non-lactation (weak S100-IR) periods (Helal *et al*, 2013). Therefore, a similar cyclic activity-dependent pattern of expression of S100 proteins could be seen in the dromedary epididymal tissues during breeding and non-breeding periods.

S100 proteins are multifunctional Ca^{2+}-binding proteins, so they have a wide range of diverse functions (Heizmann *et al*, 2002). Although the exact biological role of S100 in the epididymis is unknown, it is assumed to be involved in the absorptive and secretory functions of the intra-testicular excurrent duct system (Cruzana *et al*, 2003). Likewise, S100 may perform comparable roles in the extra-testicular excurrent duct system of the camel (Alkafafy *et al*, 2011b). As intracellular Ca^{2+}-binding proteins, S100 achieve several essential functions regulating Ca^{2+} homeostasis and are crucial molecules to transduce Ca^{2+} signaling via interaction with different types of target proteins in SMCs to promote their contractility (Czykier *et al*, 2000; Heizmann *et al*, 2002). The epididymal transport of spermatozoa is critically dependent on the contraction of peritubular SMCs (Hinton, 2010).

In conclusion, the seasonal variation in the gene expression in respect with the characteristic immunoreactivity and the sexual activity, proposes that S100 proteins could play a substantial role in regulation of the diverse epididymal functions in male dromedaries.

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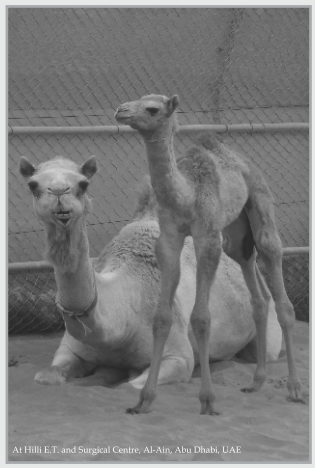

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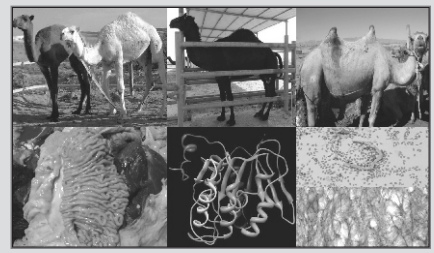

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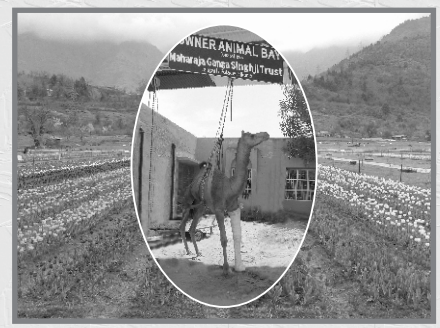

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LYMPHADENITIS CAUSED BY *Corynebacterium pseudotuberculosis* IN A DROMEDARY (*Camelus dromedarius*) HERD

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ABSTRACT

Lymphadenitis cases in a dromedary camel herd located at Bikaner, Rajasthan, India is reported. Enlargement and suppuration of one or more peripheral lymph nodes including parotid, maxillary, prescapular, inferior cervical and popleteal lymph nodes were observed. The overall morbidity rate was 11.37%. Morbidity rate was highest in female and male animals in the age group of 6 years and above. The male and female animals below 2 years of age were least affected. The genomic DNA extracted from pure bacterial culture was sequenced and the NCBI blast analysis of the 16s rDNA sequences revealed 98 per cent homology with *C. pseudotuberculosis*. *In vitro* antimicrobial sensitivity testing revealed that the isolate was sensitive to many antibacterial agents but resistant to cloxacillin. The surgical drainage of pus and parenteral therapy with enrofloxacin resulted into clinical recovery.

Key words: Camel, *Corynebacterium pseudotuberculosis*, lymphadenitis, lymph-nodes

Corynebacterium pseudotuberculosis is a Gram-positive, facultative intracellular actinomycete causing caseous lymphadenitis in sheep and goat, ulcerative lymphangitis in horses and diseases in cattle, camel and humans (Dorella *et al,* 2006). Enlargement and suppuration of peripheral and visceral lymph nodes in dromedary camel (*Camelus dromedarius*) caused by *C. pseudotuberculosis* infection has been reported from several countries across the globe (Tejedor *et al,* 2004; Wernery and Kinne, 2016). However, to our knowledge clinical disease caused by *C. pseudotuberculosis* infection in Indian dromedary camel has not been reported yet, except lymphangitis in camel in which isolation of causative organism was not undertaken (Purohit *et al,* 1985). Present report describes isolation and identification of *C. pseudotuberculosis* as the cause of lymphadenitis in a dromedary herd located at Bikaner, Rajasthan, India.

Materials and Methods

The animals of the present study belonged to a dromedary camel herd comprising 314 animals located at Bikaner, Rajasthan, India. The animals in the herd were kept in semi-intensive management.

Peripheral lymph nodes enlargement and abscessation was recorded in 37 animals during the winter season in November, 2016. The affected animals were examined carefully and their general appearance, behaviour and vital signs were recorded. Enlarged lymph nodes were examined for signs of inflammation, consistency and presence or absence of pain on palpation. Creamy to caseated and odorless pus samples collected aseptically from enlarged, suppurated lymph nodes were inoculated onto blood agar base (Himedia, Mumbai, India) supplemented with 5% defibrinated sheep blood and incubated aerobically at 37°C for 72 hours. The colony characteristics and bacterial morphology were recorded after Gram and Albert's staining (Quinn *et al,* 1994). Bacterial isolates were tested for antimicrobial sensitivity by disc diffusion method using blood agar. The antimicrobial discs (Himedia, Mumbai, India) used included Tetracycline, 30mcg, ceftriaxone 30 mcg, trimethoprim 5 mcg, cefotaxim 30 mcg, enrofloxacin 10 mcg, amoxycillin/ salbactam 30/15 mcg, gentamicin 10 mcg, penicillin G 10 IU, amoxycillin 10 mcg, cloxacillin 5 mcg, tobramycin 10 mcg, amikacin 30 mcg, rifampicin 5 mcg and doxycycline hydrochloride 30 mcg.

Bacterial genomic DNA was extracted using QIAGEN Dneasy blood and tissue kit as per the manufacturer's instructions (Qiagen GmbH, Strasse, Germany) after transferring few colonies from pure culture of *C. pseudotuberculosis* grown on blood agar plates. The DNA purity was checked on 0.8% agarose gel electrophoresis and stored at -20°C until used for

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sequencing. Bacterial 16S rRNA genes were PCR-amplified with primers of 27 F (5′-AGA GTT TGA TCM TGG CTC AG-3′) with 1492R (5′-CGG TTA CCT TGT TAC GAC TT-3′) (Lane, 1991). The PCR was carried out in 25 µl of reaction mixture containing 1× PCR buffer, 2 mM $MgCl_2$, 200 µM dNTP mix, 0.2 µlM each of forward and reverse primers, 1.25 units of Taq DNA polymerase (Sigma, UK) and 5 µl of DNA template in a thermal cycler (peqSTAR 96 Universal Gradient). After the initial denaturation for 5 min at 95°C, each PCR cycle (total 30 cycles) consisted of a 30s denaturation step at 94°C, a 45s annealing step at 54°C and a 2 min elongation step at 72°C. The final extension step was performed for 5 min at 72°C. The PCR products were resolved on 1% agarose gel electrophoresis with 0.5× Tris–borate–EDTA buffer and visualised with ethidium bromide staining. The specific sized bands were excised from gel under UV light and were subsequently purified using minielute gel extraction kit (Qiagen GmbH, Strasse, Germany). The purified products were sequenced by Xcelris genomics, Ahmedabad, Gujarat, India.

Animals with mild to moderate lymph node enlargement were treated with long acting enrofloxacin (Inj. Fortivir, M/s Virbac Animal Health Pvt. Ltd., Mumbai, India) given at the rate of 5 mg/kg body weight intramuscularly every 72 hours for 4 times. In animals with severe lymph node enlargement and suppuration, the pus material was drained out by surgical intervention and post operative antiseptic dressing was carried out along with antibiotic treatment.

Results and Discussion

Enlargement and suppuration of one or more superficial lymph nodes involving parotid, maxillary, prescapular, inferior cervical and popleteal lymph nodes were recorded in 37 animals (Fig 1 and 2). Inferior cervical lymph node enlargement was the most common, followed by enlargement of prescapular and popleteal lymph nodes. The mature females over 6 years of age appeared more susceptible, followed by male over 6 years old (Fig 3). Male below 2 years of age appeared least susceptible. Enlarged peripheral lymph nodes appeared cold, firm to doughy and painless on palpation. Signs of discomfort and dullness were recorded due to severe enlargement and abscess formation in maxillary (1 animal) and popleteal lymph nodes (2 animals), hence the abscess was opened by surgical intervention that revealed thick, creamy pus with occasional mixing of unclotted blood. The vital parameters, appetite, thirst and demeanour were normal in all affected animals.

The culture examination of pus on blood agar revealed growth of small white opaque colonies that were β-haemolytic after 48-72 hours incubation. Gram staining of the bacterial smears revealed presence of gram-positive, pleomorphic, curved rods with characteristic "Chinese letter" like arrangement. Metachromatic granules were also observed in Albert's staining. The 16s rDNA genome sequence obtained was compared with sequence available in the NCBI database. NCBI blast analysis revealed 98% homology with *Corynebacterium pseudotuberculosis*. The 16s rDNA sequence of the organism was submitted to NCBI genebank with accession no. MF537582. Based on the result of bacterial morphology and 16s r DNA sequencing and BLAST results, diagnosis of *C. pseudotuberculosis* induced lymphadenitis was given. The organism was resistant to cloxacillin, but sensitive to all other antibiotics tested.

The clinical recovery was achieved in all treated animals within a month. No mortality was recorded in affected animals, though their body condition score and productive potential was compromised during sickness.

C. pseudotuberculosis causes enlargement and suppuration of peripheral and visceral lymph nodes in dromedary camel (*Camelus dromedarius*). The organisms in the pus released from wound of infected animals contaminate the soil, water, feed and pasture. Low ambient temperature favours survival of the bacteria in the contaminated soil and eradication of *C. pseudotuberculosis* infection is very difficult due to longevity of the organism in soils and infected premises (Spier *et al,* 2012). Transmission occurs though direct or indirect contact or through wounds that come into contact with pus from the abscess of sick animals. In camel, wounds occurred during browsing on thorny plants are supposed to play an important role in pathogenesis (Wernery and Kinne, 2016). Isolation of *C. pseudotuberculosis* from engorged ticks from affected animal or herd has also been reported in several studies (Radwan *et al,* 1989; Hawari, 2008) suggesting their role in transmission of the infection.

In the present study, generalised lymphadenitis developed in a number of dromedaries over a short time period. Suppuration and caseation, particularly of inferior-cervical, prescapular, submandibular and popleteal lymph nodes and was a common sequelae in severe or chronic cases. In sheep and goat the abscessed lymph node often show laminated (onion ring) pattern, has not been recorded in dromedary camel. The Gram positive curved rods

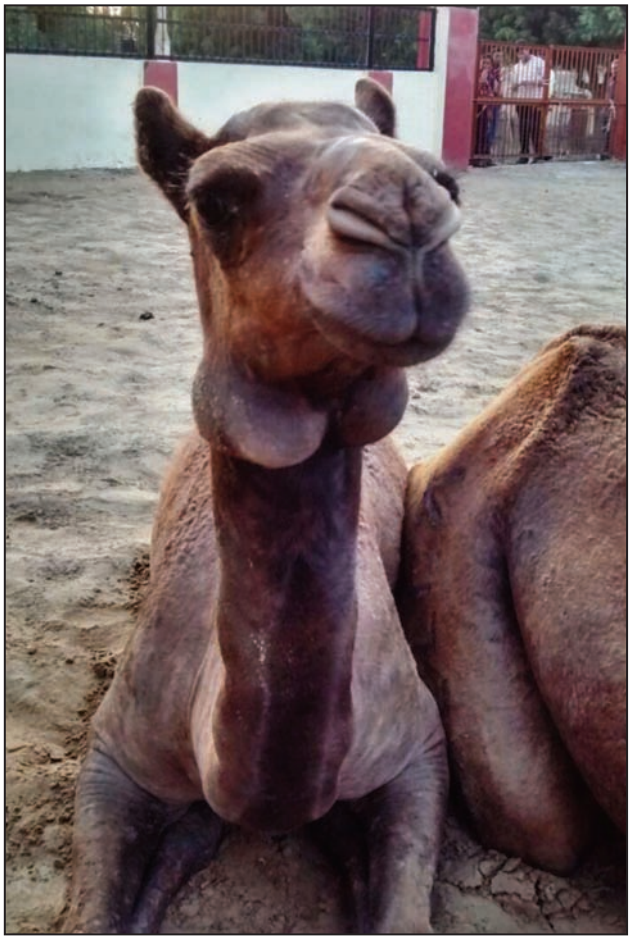

Fig 1. Enlargement and suppuration of submandibular lymph node.

Fig 2. Enlarged inferior cervical lymph nodes.

isolated from the pus sample was identified as *C. pseudotuberculosis* on the basis of morphological features and 16s rDNA sequencing results. In addition to *C. pseudotuberculosis*, isolation of other pathogenic bacteria like *Staphylococcus aureus*, *Streptoccous* spp, *C. equi, C. pyogenes, C. ulcerans* and *Salmonella enterica* from abscess in peripheral lymph nodes and visceral organs in camel is frequently reported (Tejedor *et al,* 2000; Hawari, 2008; Tejedor *et al,* 2009). Nevertheless, experimental production of typical suppurative lesions in peripheral lymph node following subcutaneous inoculation of the *C. pseudotuberculosis* isolated from clinical cases in camels indicate major role of the organism in pathogenesis of the disease (Afzal *et al,* 1996). The organism was found sensitive for a range of antibacterial agents except cloxacillin. The antimicrobial sensitivity pattern of the isolate in

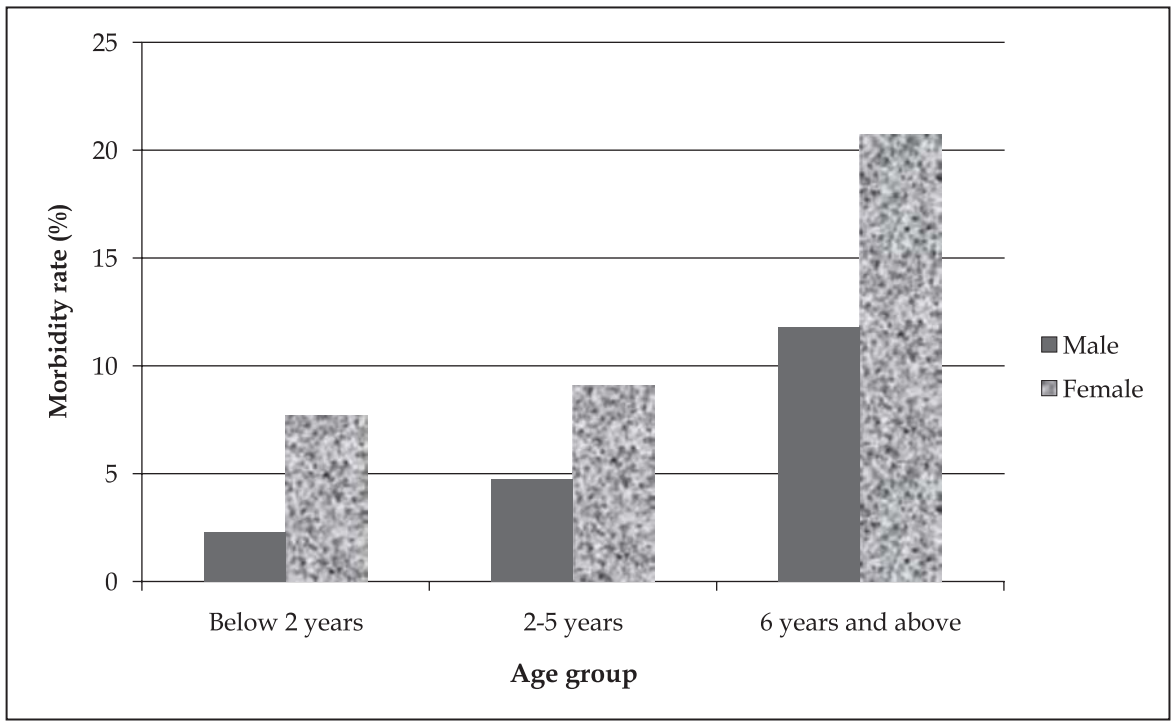

Fig 3. Age and sex wise morbidity rate in lymphadenitis in dromedary camel herd.

present study was largely in agreement with earlier reports (Costa *et al,* 1998; Tejedor *et al,* 2000). The local surgical drainage of abscess and intramuscular enrofloxacin administration gave good response as evident from clinical recovery of all treated animals. Treatment with enrofloxacin combined with dimethyl sulfoxide is reported to be effective in camel (Wernery and Kaaden, 2002).

The overall morbidity rate in present outbreak was 11.78%. In Saudi Arabia, overall morbidity rate of 15% was recorded in 2 dromedary camel herds comprising 2500 camels (Radwan *et al,* 1989). Likewise, in Egypt, lymphadenitis was detected in 10% camels in a herd comprising 339 animals (Abou-Zaid *et al,* 1994). Female appeared to suffer more than male animals and the morbidity rate vary inversely with the age group (Fig 3). Similar sex predisposition was also reported in *C. pseudotuberculosis* infection in goats (Tripathi *et al,* 2016). In an outbreak of *C. pseudotuberculosis* infection causing ulcerative granulomatous lesions in skin and muscle in an Israeli dairy cattle herd, morbidity rate was higher in older dairy cattle than that in young animals and it was concluded that age and perhaps breed are important risk factors for epidemic occurrence of *C. pseudotuberculosis* infection in cows (Yerhuam *et al,* 2004). However, to our knowledge effect of age and sex on distribution of *C. pseudotuberculosis* infection in dromedary has not been studied so far.

Conclusion

From the present report, it can be concluded that outbreaks of *C. pseudotuberculosis* infection may occur in dromedary camel herd during winter season. Female are more susceptible than male and the morbidity vary inversely with age of the animal.

Acknowledgements

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ANGIOPOIETIN-LIKE PROTEIN 3 AND HAEMATOLOGICAL PARAMETERS ASSOCIATED WITH VAGINAL ADHESIONS IN FEMALE CAMELS (*Camelus dromedarius*)

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ABSTRACT

Ten healthy fertile and eleven infertile female dromedary camels suffered from vaginal adhesions were used in this study during the breeding season. The life history was recorded and standard gynaecological examinations were performed for each infertile animal. Blood samples were collected from all females for haematological, serum angiopoietin-like protein 3 (ANGPTL3) and blood biochemical investigations. Results revealed no significant difference in serum ANGPTL3 concentrations between the infertile with vaginal adhesions and control camels (2.31±0.29 Vs 3.68±0.62 ng/mL, respectively). The haematological parameters white blood corpuscles (WBC x10^9/L), neutrophils (NEU x10^9/L), haemoglobin (HGB, g/dL), hematocrit test (HCT, %) and mean corpuscular haemoglobin concentration (MCHC, g/dL) were significantly ($P<0.05$ – $P<0.01$) higher in the infertile camels with vaginal adhesions than the control camels (21.94±2.47, 17.75±2.50, 17.93±0.73, 39.26±1.50 and 45.66±0.92 Vs. 11.00±0.91, 7.25±0.88, 10.78±0.71, 29.37±2.19 and 37.05±1.75, respectively). Serum alkaline phosphatase (ALP, IU/L), calcium (Ca, mg/DL), total protein (TP, g/dL), globulin (GLOB, g/dL), blood urea nitrogen (BUN, mg/dL) and magnesium (Mg, mg/dL) concentrations were significantly ($P<0.05$ – $P<0.01$) higher in the infertile than the control camels (56.27±16.00, 9.70±0.41, 7.25±0.40, 2.14±0.49, 15.73±5.10 and 2.44±0.22 Vs. 37.67±0.55, 8.87±0.46, 5.85±0.85, 0.98±0.82, 9.17±3.66 and 2.23±0.18, respectively). Serum gamma glutamyl transpeptidase (GGT, IU/L) and phosphorus (P, mg/dL) concentrations were significantly ($P<0.05$ – $P<0.01$) lower in the infertile than the control camels (4.00±1.18 and 4.38±0.86 Vs. 11.83±4.67 and 5.53±0.94, respectively). In conclusion, haematological parameters like WBS, NEU, HGB, HCT, MCHC and serum concentrations of ALP, Ca, GGT, TP, GLOB, BUN, P, Mg were differ between the infertile with vaginal adhesions and control camels.

Key words: Angiopoietin-like protein 3, camel, serum, vaginal adhesion

During the last decade, camels population in Saudi Arabia decreased from 183,894 in 2003 to 102,508 camels in 2013 (FAO, 2013). Reproductive disorders might be a factor in the reduction of number of camel population in this country (Ali *et al,* 2010a). Vaginal adhesion is the second major cause of infertility (16.1%) in female camels, usually ending with reproductive failure and the culling of animals with permanent infertility (Ali *et al,* 2010a; Ali *et al,* 2015; Derar *et al,* 2016). The pathogenesis of this affection is not clear. Chronic vaginitis, overbreeding, aggressive mating practice, injuries or excessive trauma during parturition, increasing parity and intrauterine infusion with caustic solutions could be suggested as factors which contribute to the problem (Tibary and Anouassi, 2000; Tibary *et al,* 2001; Ali *et al,* 2015). Compared to other domestic species, female camels seem to be reproductively unique in their susceptibility to severe secondary strictures and adhesions of the vaginal vault (Tan and Dascanio, 2008). The condition of vaginal adhesions in camels (Ali *et al,* 2010a and b; Tibary *et al,* 2006) and alpacas (Vaughan, 2008) has been reported in the literature; however, despite its importance, the condition has not been discussed separately and has been referred to only as a cause of infertility. Some blood parameters in relation to reproductive disorders have been described in female dromedaries (Ali *et al,* 2010b; Ghoneim *et al,* 2014). Angiopoietin-like protein 3 (Angptl3) is one of the angiogenic cytokines that stimulates endothelial cell adhesion, migration and neovascularisation (Hayashi *et al,* 2012). Therefore,

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the aim of the present study was the determination of angiopoietin-like protein 3 and blood profiles in female dromedary camels with normal vagina and those with vaginal adhesions to open a new window about the characters of this problem.

Materials and Methods

This study was carried out during the breeding season (November to April; Arthur *et al,* 1985) in 11 infertile female dromedary camels suffered from vaginal and cervical adhesions, aged from 5 to 14 years admitted to the Veterinary Teaching Hospital of King Faisal University, Saudi Arabia. Ten fertile female dromedaries without vaginal and cervical adhesions were considered as control group, belonging to Camel Research Centre, King Faisal University. The infertile females mated before without failure of penile intromission. The animals with adhesions were continuously exposed to fertile males during the breeding season. The age, parity, duration of infertility, conditions of the last parturition were recorded for each infertile animal. Animals were subjected to gynaecological examinations using the standard transrectal, vaginal and ultrasonographic techniques via linear-array 5 MHz transducer (UST-588U-5, SSD-500V, ALOKA, Co., Ltd., Japan). All vaginal adhesions were intense and not easily separable. Two blood samples were collected from each female dromedary at the time of clinical examination, one in EDTA and the other in plain tubes for serum harvesting. Blood samples were centrifuged at 3000g and serum was stored at -20^{o}C until analysis. The whole blood samples with EDTA were used for haematological investigation. Using blood Analyzer (UDIHEM I, France), White blood corpuscles (WBC, x10^{9}/L), Lymphocytes (LYM, x10^{9}/L), Monocytes (MON, x10^{9}/L), Neutrophils (NEU, x10^{9}/L), Eosinophils (EOS, x10^{9}/L), Red Blood corpuscles (RBC, x10^{12}/L), Haemoglobin (HGB, g/dL), Hematocrit test (HCT, %), Mean corpuscular volume (MCV, fL), Mean corpuscular haemoglobin (MCH, pg), Mean corpuscular haemoglobin concentration (MCHC, g/dL), Red cell distribution width (RDW, %) were determined according to Feldman *et al* (2000). Serum angiopoietin-like protein 3 (ANGPTL3, ng/mL) concentrations were assayed using commercial kits (USCN Life Science Inc., Cloud-Clone Corp., Houston, USA) by ELISA (Absorbance Micro-plate Reader ELx 800 BioTek, USA; Micro-plate Strip Washer ELx 50 BioTek, USA). The coefficients of variance (CV's) of the intra- and inter-assay were 8.3% and 10.4%, respectively. Serum concentrations of Albumin (ALB, g/dL), Alkaline phosphatase (ALP, IU/L), Aspartate aminotransferase (AST, IU/L), Calcium (Ca, mg/dL), Gamma glutamyl transpeptidase (GGT, IU/L), Total protein (TP, g/dL), Globulin (GLOB, g/dL), Blood urea nitrogen (BUN, mg/dL), Creatine kinase (CK, IU/L), Inorganic phosphorus (P, mg/dL) and Magnesium (Mg, mg/dL) were assayed on an automated clinical chemistry analyser (ELLIPSE machine, Italy).

Statistical analysis

Data are presented as means ± SEM for haematological and blood biochemical parameters. These parameters were compared by Student's t-test (t) using SPSS program, version 24.0 (2016).

Results

As shown in Table 1, there was no significant difference in serum ANGPTL3 concentrations between camels in the infertile (female camels with vaginal adhesion) and control groups. Table 2 showed a highly significant (P<0.01) difference in HGB levels between the control and infertile female camels.

Table 1. Serum angiopoietin-like protein 3 (ANGPTL3) in female camels with vaginal adhesion and control ones.

Female cases	n	Serum ANGPTL3 (ng/mL)
Females with vaginal adhesion	11	2.31 ± 0.29
Control females	10	3.68 ± 0.62

P<0.088

Table 2. Haematological parameters in female camels with vaginal adhesion and control females.

Haematological parameters	Vaginal adhesion (n=11)	Normal vagina (n=10)	P values
WBC (x 10^{9}/L)	21.94^{a} ± 2.47	11.00b ± 0.91	P<0.05
LYM (x 10^{9}/L)	3.27 ± 0.49	2.79 ± 0.55	–
MON (x 10^{9}/L)	0.18 ± 0.05	0.09 ± 0.01	–
NEU (x 10^{9}/L)	17.75^{a} ± 2.50	7.25^{b} ± 0.88	P<0.05
EOS (x 10^{9}/L)	0.73 ± 0.15	0.88 ± 0.14	–
RBC (x 10^{12}/L)	13.89 ± 0.66	10.85 ± 1.66	–
HGB (g/dL)	17.93^{a} ± 0.73	10.78^{b} ± 0.71	P<0.01
HCT (%)	39.26^{a} ± 1.50	29.37^{b} ± 2.19	P<0.05
MCV (fL)	28.38 ± 0.50	30.33 ± 0.67	–
MCH (pg)	12.86 ± 0.45	11.27 ± 0.63	–
MCHC (g/dL)	45.66^{a} ± 0.92	37.05^{b} ± 1.75	P<0.05
RDW* (%)	25.99 ± 0.24	27.18 ± 1.12	–

Means with dissimilar superscripts in the same row are significantly different from P<0.05 to P<0.001.
n= number of female camels.
*RDW: Red cell distribution width % or fL

There were significant (P<0.05) differences in WBS, NEU, HCT and MCHC between the infertile and control female camels (Table 2). A highly significant (P<0.01) differences were found in serum ALP, GGT and GLOB concentrations between camels in the control and infertile groups (Table 3). A significant (P<0.05) differences were calculated in some blood biochemical parameters concentrations between the infertile and control female camels (Table 3). These parameters were Ca, TP, BUN, P and Mg. There were no significant differences in the concentrations of LYM, MON, EOS, RBC, MCV, MCH, RDW, ALB, AST and CK between the infertile and control female camels (Tables 2 and 3).

Table 3. Blood biochemical parameters in female camels with vaginal adhesion and control females.

Biochemical parameters	Vaginal adhesion (n=11)	Normal vagina (n=10)	P values
ALB (g/dL)	5.62 ± 1.05	4.90 ± 0.76	–
ALP (IU/L)	56.27^{a} ± 16.00	37.67^{b} ± 0.55	P<0.01
AST (IU/L)	91.4 ± 12.69	86.67 ± 28.70	–
Ca (mg/dL)	9.70^{a} ± 0.41	8.87b ± 0.46	P<0.05
GGT* (IU/L)	4.00^{a} ± 1.18	11.83^{b} ± 4.67	P<0.01
TP (g/dL)	7.25^{a} ± 0.40	5.85^{b} ± 0.85	P<0.05
GLOB (g/dL)	2.14^{a} ± 0.49	0.98^{b} ± 0.82	P<0.01
BUN (mg/dL)	15.73^{a} ± 5.10	9.17^{b} ± 3.66	P<0.05
CK (IU/L)	187.09 ± 40.47	128.17 ± 70.20	–
P (mg/dL)	4.38^{a} ± 0.86	5.53^{b} ± 0.94	P<0.05
Mg (mg/dL)	2.44^{a} ± 0.22	2.23^{b} ± 0.18	P<0.05

Means with dissimilar superscripts in the same row are significantly different from P<0.05 to P<0.01.

n= number of female camels.

*GGT: Gamma glutamyl transpeptidase

Discussion

The results of the present study revealed no significant difference in serum ANGPTL3 concentrations between camels in the infertile and control groups. This is indicated that ANGPTL3 has no role in the pathogenesis of vaginal adhesions in camels. Increasing WBC and NEU counts observed in this study is in agreement with a previous study (Ali *et al,* 2010b) suggests that this problem is an inflammatory process. Neutrophils are the first line of cellular defense against pathogens (Feldman *et al,* 2000). The levels of HGB, HCT and MCHC are significantly higher in the infertile than the control camels. However, chronic inflammation is accompanying with decreased levels of HGB (Fry, 2011; Markoulaki *et al,* 2011). In this study, it was noted that females with vaginal adhesions showed serum ALP, Ca, TP, GLOB, BUN and Mg concentrations higher than that of the control group. Similarly, elevated alkaline phosphate is associated with certain medical conditions, diseases and syndromes (Li-Fern and Rajasoorya, 1999). Nevertheless, there are no significant differences in serum Ca and Mg between female dromedaries with vaginal adhesions and control ones (Ali *et al,* 2010b). On the same direction of the present study, high TP and GLOB is accompanying many types of inflammation and certain infections including female camels with vaginal adhesions (Schreiber *et al,* 1982; Ali *et al,* 2010b; Koyama *et al,* 2016). On the contrary, TP and GLOB have no relationship with the rate of pregnancy in dairy cows (Forshell *et al,* 1991). Moreover, BUN is elevated with fatty liver disease and active inflammatory bowel disease (Lundsqaard *et al,* 1996; Liu *et al,* 2013). In the current study, serum GGT and P concentrations were significantly higher in the control than in female camels with vaginal adhesions. However, Serum GGT, a marker of oxidative stress, has been associated with cardiovascular disease events, metabolic abnormalities and inflammation in healthy subjects (Bo *et al,* 2005; Mistry and Stockley, 2010; Ali *et al,* 2016). Besides, serum P is direct and independently correlated with inflammatory parameters (Navarro-González *et al,* 2011; Izquierdo *et al,* 2012).

Conclusions

Haematological parameters like WBS, NEU, HGB, HCT, MCHC and serum concentrations of ALP, Ca, GGT, TP, GLOB, BUN, P, Mg differed between the infertile female camels with vaginal adhesions and the control camels.

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THE COMPARATIVE STUDY OF EXPRESSION AND DISTRIBUTION OF CYP2J GENE IN DIFFERENT PART OF THE DIGESTIVE SYSTEM IN BACTRIAN CAMEL

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ABSTRACT

This study was conducted to investigate the expression and distribution of CYP2J gene in different parts of the digestive system in Bactrian camel and to explore the related clues between the CYP2J genes and the characteristics of salt-sensitive hypertension of Bactrian camels. Firstly, the primers were designed according to predictive sequences (XM-006176094.1) of Bactrian camel CYP2J genes and ACTB of Bactrian camel, as well as Mongolian cattle CYP2J genes (NM-001077210.1) and ACTB Mongolian cattle, then the CYP2J genes were amplified by SYBR Green Real-Time PCR using ACTB as reference gene. Secondly, the relative quantitative method of $2^{-\Delta\Delta CT}$ was used to process the data and the expression level of CYP2J gene in 7 parts of digestive system including liver, spleen, caecum, colon, jejunum, ileum and duodenum of Bactrian camel and Mongolian cattle was comparatively analysed. The result showed that CYP2J gene was highly expressed in the liver of Bactrian camel and followed by colon, duodenum, ileum, jejunum and caecum. However, in Mongolian cattle, the relative expression of CYP2J gene was highest in pancreas, followed by liver, duodenum, ileum, jejunum, colon and caecum. Furthermore, the relative expression level of CYP2J gene in Bactrian camels' liver, pancreas, colon and duodenum was significantly higher than that of in corresponding parts of the digestive system in Mongolian cattle. Therefore, the expression and distribution of CYP2J gene in different parts of digestive system of Bactrian camel was significantly different from Mongolian cattle, however, these two species are closest in degree of evolution.

Key words: Bactrian camel, CYP2J gene expression, Mongolian cattle, RT-qPCR

CYP2J is one of the important member of CYP2 subfamily, unlike other cytochrome P450 enzymes, it is predominantly expressed in extrahepatic tissues including the heart, skeletal muscle, placenta, lung, pancreas, bladder, brain, small intestine and kidney (Zeldin *et al,* 1996, 1997; Enayetallah *et al,* 2004). Moreover, CYP2J2 is also highly expressed in various tumour tissues and promotes tumour growth and proliferation (Jiang *et al,* 2005, 2009; Chen *et al,* 2011). CYP2J subfamily enzymes are conserved among the various animals and they displayed similar enzymatic functions and activities (Wu *et al,* 1997; Ma *et al,* 1999; Messina *et al,* 2010). Specially, CYP2J drew more interest due to its function on metabolism of arachidonic acid (AA). It converts arachidonic acid to four different epoxyeicosatrienoic acids (EETs), which play significant roles in maintaining the homeostasis of the kidney, heart and lung by controlling crucial biological processes such as anti-inflammation, vasodilatation, relaxation of smooth muscle and angiogenesis (Kroetz and Zeldin, 2002; Spector *et al,* 2004). CYP2J2 can metabolise several drugs including Albendazole, Astemizole, Ebastine and Terfenadine etc (Matsumoto *et al,* 2003; Lee *et al,* 2010; Ghosal *et al,* 2011). It also has long been reported that there is a fairly close relationship between CYP-derived EET and salt-sensitive hypertension (Messina *et al,* 2010).

In this experiment, RT-qPCR SYBR Green I $2^{-\Delta\Delta CT}$ was used to detect the expression and distribution of CYP2J gene in the digestive system of Bactrian camels and Mongolian cattle and compared the relative expression of the two species.

Materials and Methods

Experimental Animals

The tissues were immediately collected from the three healthy Bactrian camels and three healthy Mongolian cattle at slaughterhouse in Alxa and Ordos, respectively. Samples were collected in liquid nitrogen firstly and then stored at −80°C in the lab until preparation of total RNA.

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Reagents and Chemicals

RNA loading buffer, DL500 DNA Marker, loading buffer, TaKaRa Ex Taq®, PCR machine and Premix Ex Taq (TAKARA, Dalian, China); HiScript Reverse Transcripase and AceQ® qPCR SYBR® Green Master Mix (Vazyme Biotech, Nanjing, China); RNeasy Plant Mini Kit (Axygen, Hangzhou, China); TAE Buffer, MOPS Buffer and free enzyme water(Tiangen Biotech, Beijing, China) and Agarose (USA).

Instruments

The primary instruments used throughout this study include, a high-speed refrigerated centrifuge (3-30k, sigma); PCR instrument (Germany); multi-mode microplate reader (Synergy H4); Electrophoresis apparatus (USA); and Applied Biosystems ViiATM 7(USA).

Primer design

All the primers used in this experiment were shown in Table 1.

Extraction of RNA and reverse transcription

RNA was extracted by instruction of kit (RNeasy Plant Mini Kit). The concentration of total RNA was detected by microplate reader and then all RNA was diluted down to less than 500 ng/μL in accord with the concentration of following cDNA. The preparation of components of the reverse transcription and the standard procedure of the reaction refer to the experimental instructions (Vazyme reverse transcription Kit).

Verification of primers and templates

Firstly, cDNA obtained in the previous step as template and four pairs of primers (CYP2J of Mongolian cattle and Bactrian camel and ACTB) were verified by conventional PCR. Polymerase chain reaction (PCR) was performed using 30 cycles including 10s at 98°C, 30s at 55°C and 1 min at 72°C. Then agarose gel electrophoresis was used to determine whether the PCR product is in line with expectations.

Note: due to premix including pigment marker, PCR products were not added to loading buffer and were observed directly as well as noted in Gel imaging system.

ReaI-Time qPCR

In the experiment, the two steps procedure was adopted. The RT-qPCR reaction components and reaction procedure were shown in table 2 and table 3, respectively.

Results

Quality and integrity detection of total RNA

The concentration of the total RNA extracted was measured by microplate reader and the ratio of OD260/OD280 was 1.8-2.2. This indicated that the extraction process of total RNA has no pollution and no degradation and meet all requirement of the reverse transcription in following step. The integrity of total RNA in each tissue was detected by agarose gel electrophoresis. Three specific bands of 28S, 18S and 5S were clear and no obvious tailing (Fig 1). It suggested that the total RNA extracted during the experiment is in good integrity, no pollution and no obvious degradation and it can be used for subsequent experiments.

Verification of primers and templates

Four pairs of designed primers were used to detect the specificity of the template cDNA and the specificity of the primers by the conventional PCR combined with agarose gel electrophoresis. As shown in Fig 2 (a, b), 4 pairs of primers can be located in the specific bands. Therefore, the test results prima facie prove that template cDNA and primers can be used for Real-Time qPCR detection.

The relative abundance of CYP2J in Bactrian camel

In the histogram and statistical analysis of relative abundance of CYP2J mRNA in seven parts

Table 1. The sequence of primers and the size of products.

Primer	Primer sequence (5′→3′)	Length of products
Bactrian camel CYP2J	F: GGTGTGGAAGGAGCAAAGAAGG R: CCCTACTGCCTGGGTGAGGT	118bp
Bactrian camel ACTB	F: GGACTTCGAGCAGGAGATGG R: AGGAAGGAGGGCTGGAAGAG	138bp
Mongolian cattle CYP2J	F: ATGTTTGTTGCCCGTATGATTG R: ATTGCCCTTATGCTTTTCTGTCC	108bp
Mongolian cattle ACTB	F: CGCAGAAAACGAGATGAGATTG R: GTTGCTAAGGGCAGGATTGG	230bp

of the digestive system of Bactrian camel: liver, pancreas, duodenum, jejunum, colon, ileum and caecum. Different letters represent the significant difference (P <0.05).

Table 2. RT-qPCR reaction components.

Components	Amount of added sample
AceQ®qPCR SYBR® Green Master Mix	10.0 μL
Primer 1 (10μM)	0.4 μL
Primer 2 (10μM)	0.4 μL
ROX Reference Dye 1	0.4 μL
Template DNA	2.0 μL
ddH_2O	Up to20.0 μL

As shown in Fig 3, the relative expression level of CYP2J mRNA in seven different parts of the Bactrian camel was significantly different and the relative expression level in the liver was the highest; and in the pancreas, colon, duodenum, ileum, jejunum and caecum is comparatively low, especially in the caecum.

The relative abundance of CYP2J mRNA in Mongolian cattle

In the column histogram and statistical analysis of CYP2J mRNA in 7 parts of digestive system of Mongolian cattle including liver, pancreas, duodenum, jejunum, colon, ileum and caecum (Fig 4), the same letters indicated that there was no significant difference (P> 0.05) between the relative expression of CYP2J mRNA in each part of Mongolian cattle digestive system, whereas different letters represent a significant difference (P <0.05).

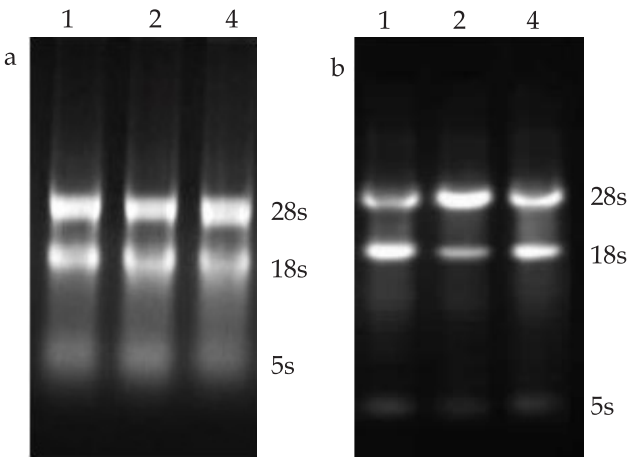

Fig 1. Agarose gel electrophoresis image of three kinds of tissues

a: the total RNA of Bactrian camel b: total RNA of Mongolian cattle. 1, 2, 3 refer to the liver, colon, duodenum of Bactrian camel and Mongolian cattle, respectively.

As shown in Fig 4, the relative abundance of CYP2J mRNA in Mongolian cattle is most abundant in the pancreas, followed by in the liver, duodenum, ileum and jejunum while in the colon and caecum are very low.

Table 3. RT-qPCR reaction procedures.

Steps	Temperature	Time
Stage 1 Pre-denaturation Reps: 1	95°C	5 min
Stage 2 Recycle Reps: 40	95°C	10 sec
	60°C	30*sec
Stage 3 Melting curve Reps: 1	95°C	15 sec
	60°C	60 sec

Analysis of differences in the relative abundance of CYP2J mRNA between Bactrian camel and Mongolian cattle

Fig 5 shows comparison results of the relative abundance of CYP2J mRNA between the digestive system of Bactrian camel and Mongolian cattle. The phase * in the Fig represents a significant difference (P < 0.05). As shown in Fig 5, the relative expression level of CYP2J mRNA in the liver, pancreas, colon and duodenum of Bactrian camels were significantly higher than that of in the corresponding tissues of

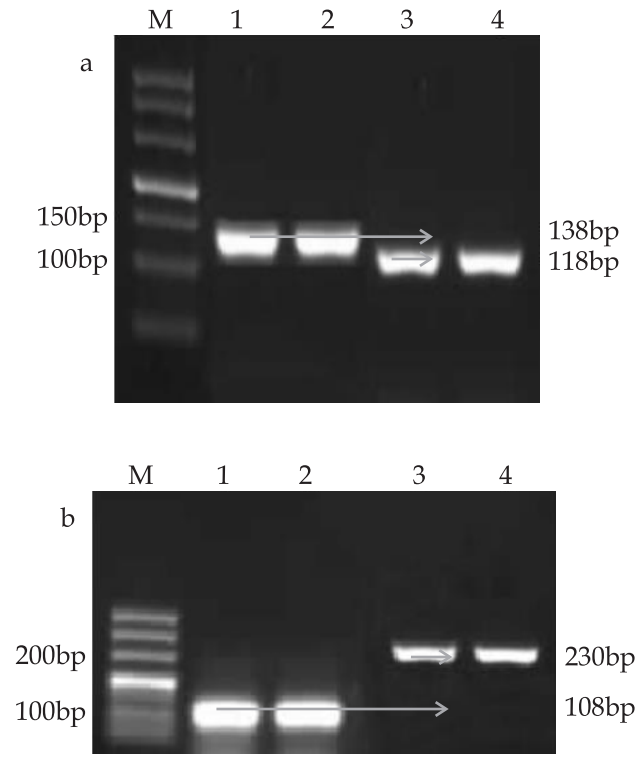

Fig 2. Conventional PCR combined with agarose gel electrophoresis Fig of four pairs of primers

a: verification of Bactrian camel b: verification of Mongolian cattle

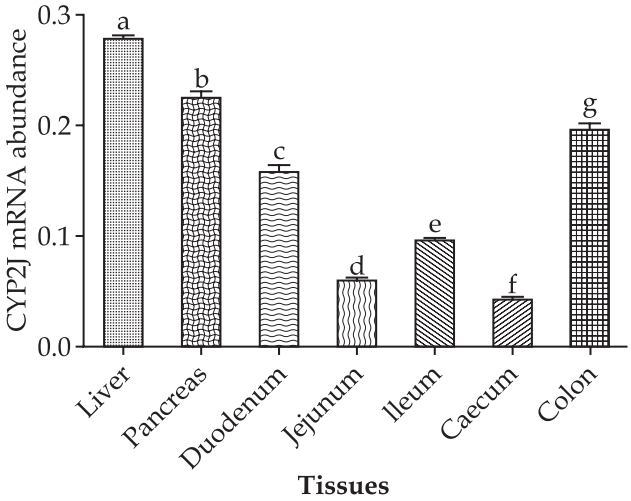

Fig 3. Abundance CYP2J mRNA in various tissues of Bactrian camel. The same letters indicate the difference is not significant(P>0.05); The different letters indicate the difference is significant(P<0.05).

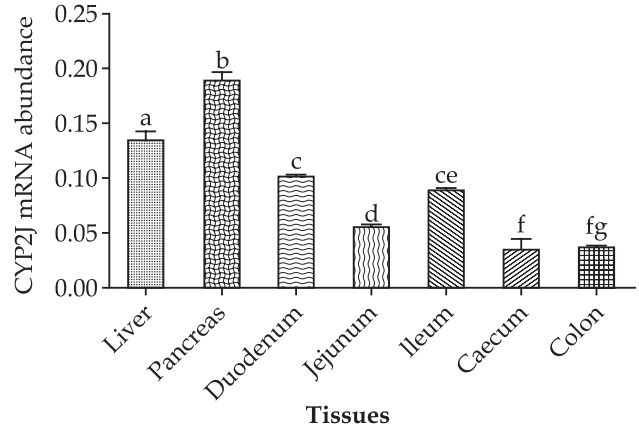

Fig 4. Abundance of CYP2J mRNA in various tissues of Mongolian cattle.

The same letters indicate the difference is not significant (P>0.05); The different letters indicate the difference is significant (P<0.05).

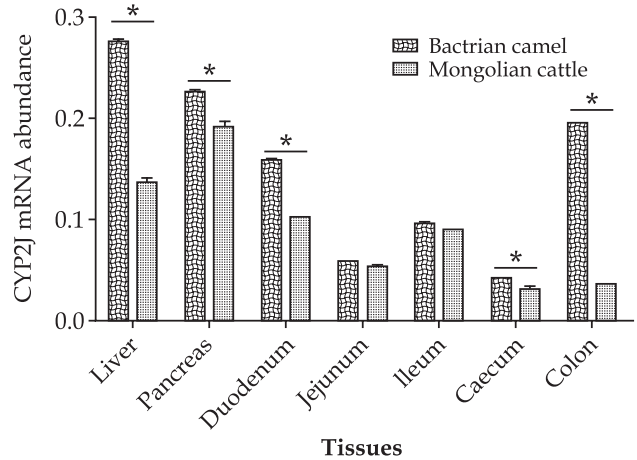

Fig 5. Comparison of the relative abundance of CYP2J mRNA in different part of digestive system in Bactrian camel and Mongolian cattle

The phase * in the Fig represents a significant difference (P <0.05).

Mongolian cattle, while the relative expression level of CYP2J mRNA in jejunum, ileum and caecum of Bactrian camels had no significant difference from that of Mongolian cattle.

Discussion

The unique biological characteristics of Bactrian camels has shown that they have the distinctive ability of metabolism on xenobiotics and possess different metabolic pathways compared with other mammals. Many members of CYP2J subfamilies are described in different mammals: one in rabbit (CYP2J1) (Kikuta *et al,* 1991), two (CYP2J3, CYP2J4) in rat (Wu *et al,* 1997; Zhang *et al,* 1997), four (CYP2J5, CYP2J6, CYP2J9, CYP2J11) in mouse (Ma *et al,* 1999; Scarborough *et al,* 1999; Qu *et al,* 2001) and only one (CYP2J2) in human, monkeys and dog. Totally 63 copies of the CYP gene were found in whole genome of Bactrian camel, which belong to 17 families and 38 subfamilies. Among them, there are 9 multigene families, namely CYP2, CYP3 and CYP4, which have 27, 6 and 7 members and account for 43%,10% and 11% of CYP gene, respectively. Moreover, compared with cattle, horse, chicken and human CYP gene, the distribution of CYP gene subfamily in Bactrian camel is obviously different, especially there are more CYP2J gene copies (Hasi *et al,* 2018). Therefore, there is speculation that the unique metabolic pathways, high salt tolerance and special detoxification ability of Bactrian camel is may be related to the high copies of the CYP2J gene.

After all cDNA templates were adjusted to the same concentration (134 ng/μL), the comparative expression level of CYP2J mRNA in various tissues of three Bactrian camels and three Mongolian cattle were obviously different. The results can corretate the role of CYP2J gene in anti-salt sensitive hypertension of the Bactrian camel.

Acknowledgements

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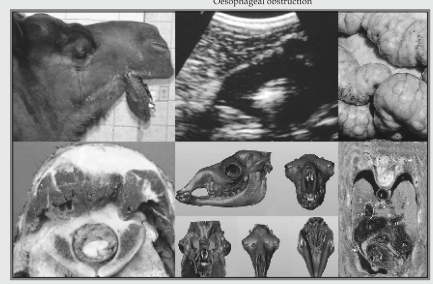

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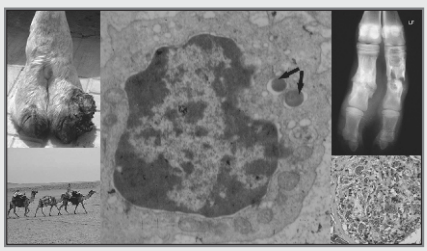

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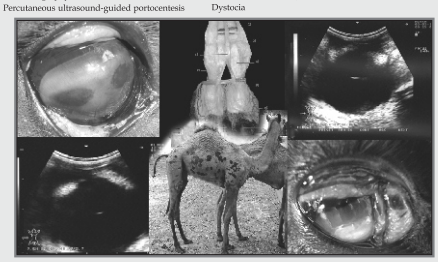

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THE IMPACT OF RACING ON SERUM CONCENTRATIONS OF BONE METABOLISM BIOMARKERS IN RACING ARABIAN CAMELS

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ABSTRACT

This study was carried out to investigate the effect of racing in Arabian camels on the serum concentrations of bone biomarkers osteocalcin (OC) and bone-specific alkaline phosphatase (b-ALP) and on bone resorption biomarker pyridinoline cross-links (PYD). Twenty-three female racing Arabian camels (*Camelus dromedarius*) that participated in a 5 km race were enrolled in this study. The concentrations of the bone biomarkers OC, b-ALP and PYD were determined in serum using commercial human immunoassay kits. The serum concentration of serum OC increased after race. However, there was no significant difference between the serum concentration of OC pre- and post-race. In a similar manner, the serum concentration of the bone formation b-ALP increased but not significantly post-race. On the contrary, the serum concentration of the bone resorption biomarker PYD increased significantly after racing. It is clear that the bone formation biomarkers are not influenced by the 5-km race. However, the bone resorption biomarker increased significantly. The influence of long-distance racing on these biomarkers is therefore, warranted. Understanding the effect of racing on stimulation of the bone remodelling is important for the development of strategies to increase and maintain bone mass.

Key words: Biomarkers, bone metabolism, camels, racing, stress

Camel racing is a very old traditional sport practised mainly in Arab countries. In recent years, there has been increasing interest in camel racing. In the Arabian Gulf countries and the United Arab Emirates, the sport of camel racing is developing more along the lines of thoroughbred horseracing. In general, the distance of an official race is between 4 and 20 km, based on the age, breed and gender of the camels and the decision of the organising committees. The average speed of a camel during a race is approximately 9.5 m/sec (Snow, 1992).

The common biomarkers of bone formation include osteocalcin (OC), bone-specific alkaline phosphatase (b-ALP) and amino and carboxy propeptides of collagen type I. The most common biomarkers of bone resorption on the other side include pyridinoline cross-links (PYD), deoxypyridinoline enzyme tartrate resistant acid phosphatase and amino and carboxy telopeptides of collagen type I. In human medicine clinical practice, biochemical markers of bone turnover are widely used, mainly for non-invasive monitoring of bone metabolism and response to therapy of certain musculoskeletal and bone disorders (Swaminathan, 2001; Watts *et al,* 2001; Kanakis *et al,* 2004; Sabour *et al,* 2014). In veterinary medicine, bone biomarkers are mostly used in preclinical and clinical studies as a rapid and sensitive method for assessment of bone response to medical treatment and surgical interventions and for the detection of musculoskeletal injuries (Allen, 2003; DeLaurier *et al,* 2004; Frisbie *et al,* 2008; Frisbie *et al,* 2010).

In humans as well as in animals, the considerably important role of bone biomarkers in relation to exercise has not been widely evaluated (Al-Sobayil, 2008; Cywinska *et al,* 2012; Shin *et al,* 2012; Casella *et al,* 2013; Sabour *et al,* 2014; Yazwinski *et al,* 2013). Recently, we have determined the changes in cardiac biomarkers (Tharwat *et al,* 2013) and inflammation biomarkers (Tharwat and Al-Sobayil, 2015) in racing dromedary camels. The aim of the current study was to investigate the effect of 5-km race in Arabian camels on the serum concentrations of the biomarkers for bone formation (OC, b-ALP) and bone resorption (PYD). Understanding the effects of physical exercise on stimulation of the bone remodelling is important for the development of strategies to increase and maintain bone mass.

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Materials and Methods

Animals and blood sampling

This study was approved by the Animal Ethical Committee, Deanship for Scientific Research, Qassim University, Saudi Arabia. The experimental design has been reported recently (Tharwat *et al,* 2013; Tharwat and Al-Sobayil, 2015). Twenty-three female racing camels (*Camelus dromedarius*) that participated in a 5 km race were enrolled in this study. All of the camels were appropriately screened and considered healthy. The mean age of the camels was 7.6±2.4 years with a mean body weight of 312±61 kg. All the racing camels received a full clinical examination on the day before the race, paying special attention to their cardiovascular systems.

Enrollment in the study was based on: (1) normal complete physical examination findings, (2) normal cardiac auscultation, (3) normal complete blood cell counts (VetScan HM5, Abaxis, CA, USA), (4) normal biochemistry panel (VS2, Abaxis, CA, USA), (5) a continuous electrocardiography (ECG) recording (Kenz-Cardio 302 Suzuken Co Ltd., Nagoya, Japan) and (6) echocardiography (SSD-500, Aloka, Tokyo, Japan) (Tharwat *et al,* 2012a). Blood samples (7 mL) were collected in plain vacutainer tubes from the jugular vein of each camel 24 h prior to the race and within 2 hours of completion of the race. Samples were centrifuged at 3,000 rpm for 10 minutes and the serum samples obtained were aliquotted in tubes and immediately stored at -20°C for 2 weeks.

Bone metabolism biomarkers assays

The bone biomarkers OC, b-ALP and PYD serum concentrations were determined using commercial human immunoassay kits (Metra Biosystems Inc., a division of Quidel Corp.). All 3 human assays had been proven to have a good cross-reactivity with canine OC, b-ALP and PYD (Allen *et al,* 2000; Breur *et al,* 2004; Belić *et al,* 2012). The limit of quantification of OC ranged from 2 to 32 ng/mL and precision CVs within and between runs were 5-10%. The dynamic range of BAP was 2-140 U/L and precision CVs within and between runs were 4-6% and 5-8%, respectively. The dynamic range of PYD was 15-750 nM/L and precision CVs within and between runs were 6-10% and 3-11%, respectively.

Statistical analysis

Data normality was examined using the Kolmogorov–Smirnov test. The data were presented as means ± SD and were analysed statistically using the SPSS statistical package (2009). A Student's t-test was used for comparisons between pre- and post- race values. Significance was set at $P<0.05$.

Results and Discussion

The serum concentration of serum OC increased after race. However, there was no significant difference ($P = 0.28$) between the serum concentration of OC pre- (49.5± 27.3 ng/mL) and post-race (69.0± 49.1 ng/mL) (Fig 1).

In a similar manner, the serum concentration of the bone formation b-ALP increased but not

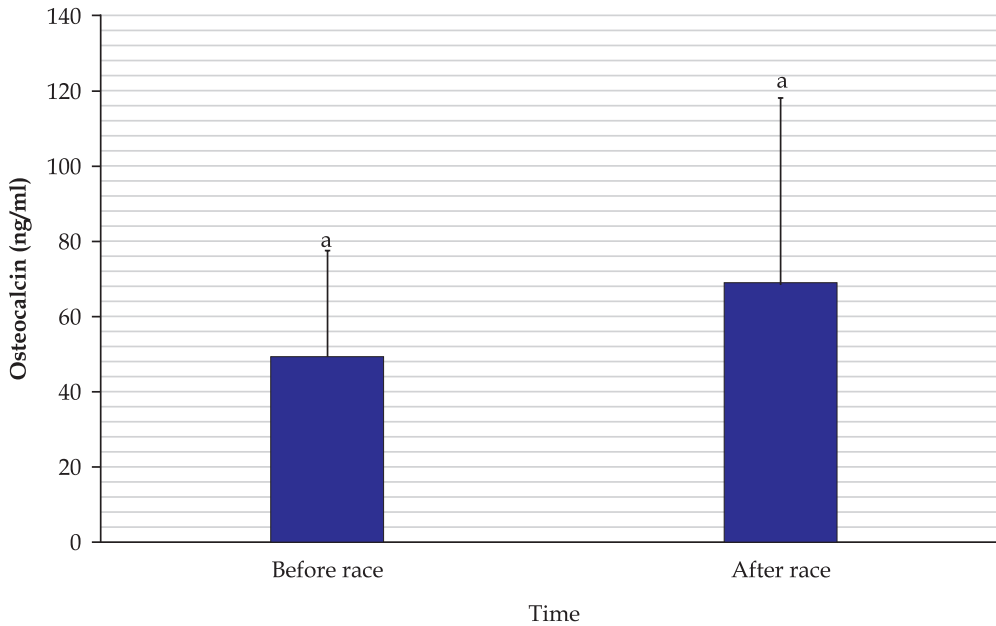

Fig 1. Serum concentration of osteocalcin in racing dromedary camels before and after race.

significantly (P = 0.0.09) post race (48.2± 26.2 ng/mL vs 67.9± 24.0 ng/mL) (Fig 2).

On the contrary, the serum concentration of the bone resorption biomarker PYD increased significantly (P=0.003) after racing (15.7± 7.4 ng/mL vs 7.5± 1.7 ng/mL) (Fig 3).

To the authors' knowledge, this is the first study to evaluate the influence of racing on the serum concentrations of bone formation (OC, b-ALP) and bone resorption (PYD) biomarkers in racing camels.

The non-collagenous protein OC, a product of the osteoblasts, is regarded as a sensitive indicator of bone formation (Pullig *et al,* 2000). In addition to its increase that accompany skeletal growth, weight-bearing exercise induces changes in serum concentrations of OC (Eliakim *et al,* 1997), probably reflecting exercise-induced bone deposition (Caron *et al,* 2002). In this study, we did not observe any significant changes in the serum concentration of OC post-race compared with baseline values; a result

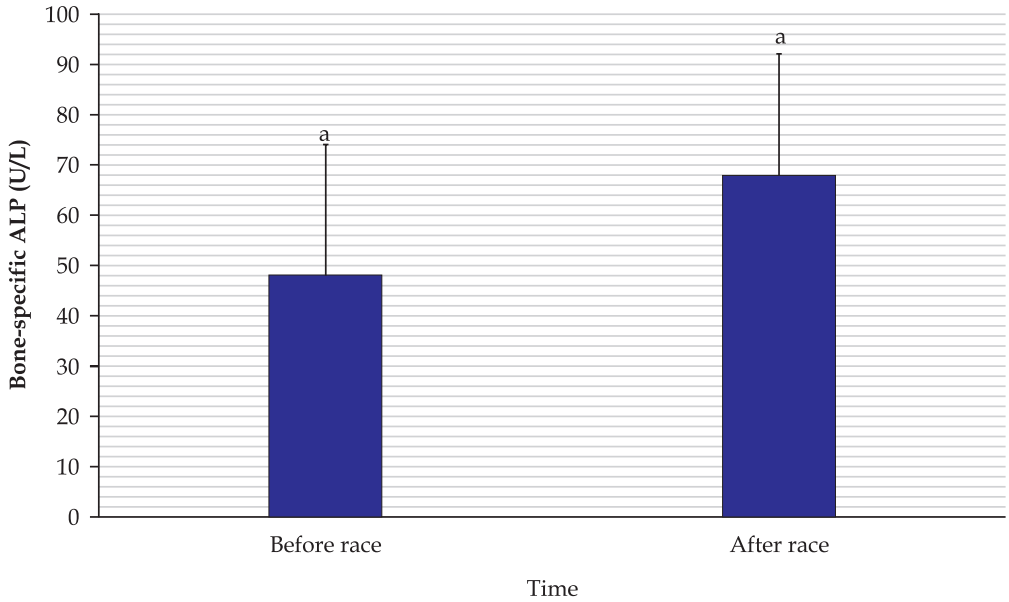

Fig 2. Serum concentration of bone-specific alkaline phosphatase (ALP) in racing dromedary camels before and after race.

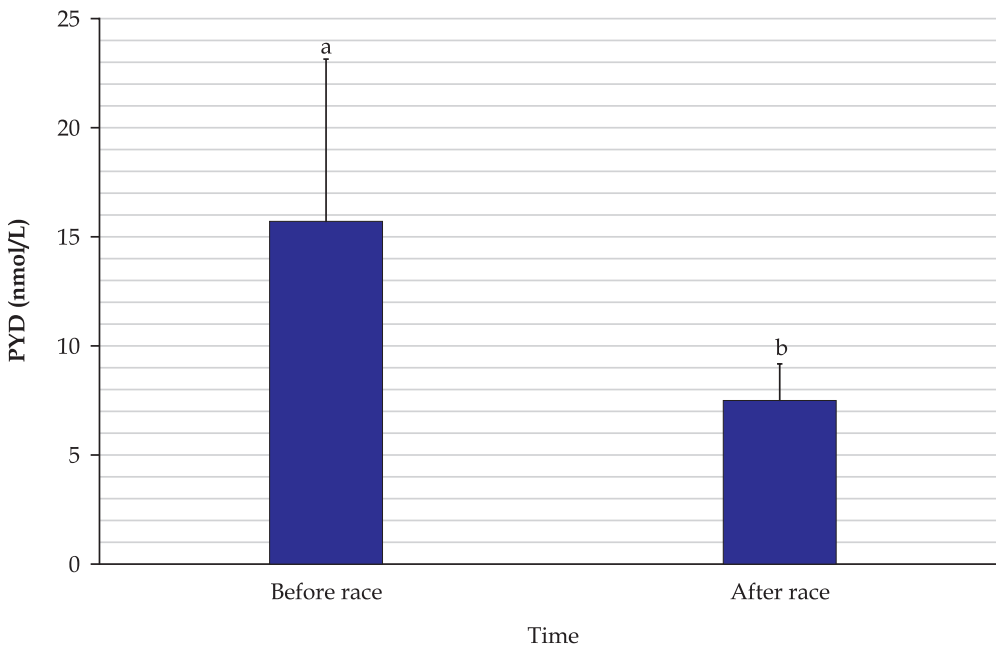

Fig 3. Serum concentration of pyridinoline cross-links (PYD) in racing dromedary camels before and after race.

consistent with findings of another exercise study involving highly conditioned Arabian horses (Porr *et al,* 1998). In addition, horses assigned to 48-week race training, the OC did not change during the early training period; however, it increased significantly 24 to 48 wk after onset of the study (Caron *et al,* 2002). In another study in horses exercised with a treadmill for 20 weeks, the OC was lower post-run (Jackson *et al,* 2003). Furthermore, training amplitude did not significantly influence serum OC values in horses assigned to 9 months of exercise (Carstanjen *et al,* 2005). Similarly, in racing camels, both moderate and full-speed exercise had no effect on the concentration of OC (Al-Sobayil, 2008). In human athletes performing in a 245 Km marathon, there was a transient suppression in osteoblast function during the marathon run which was attributed to the increase in cortisol concentration (Mouzopoulos *et al,* 2007). Other human studies have shown conflicting results. In one marathon, the OC decreased by 10-20% after the run, but 3 h thereafter rapidly returned to pre-run values (Malm *et al,* 1993). However, in athletes performing a 21 Km half-marathon run, there was a nearly 1.2-fold increase in OC concentration (Lippi *et al,* 2008).

The b-ALP, a glycoprotein found on the surface of osteoblasts, has been shown to be a sensitive and reliable indicator of bone metabolism (Kress, 1998). Similarily to the OC results, our findings showed a non-significant post-race elevation in the mean serum concentration of b-ALP compared with baseline values. In humans, in a marathon run over 245 Km, the serum activity of b-ALP decreased significantly post-run (Mouzopoulos *et al,* 2007). Rudberg *et al*. (2000) reported short-lasting increase in b-ALP after 4.7 Km of jogging. It has been suggested that the increased content of b-ALP could be released from the osteoblast membranes under local factors such as changes in the pH, which triggers the release of b-ALP from the osteoblasts (Anh *et al,* 1998).

The PYD cross-links, indicators of type I collagen resorption, are found in the mature collagen of bone. It is not only found in mature type I collagen, which is the major type of collagen in bone tissues (Von Der Mark, 1999), but also in collagen types II and III (Eyre *et al,* 1984). Increased concentrations of PYD in the blood or urine are most commonly considered as indicators of bone resorption (Thompson *et al,* 1992). In this study, we observed a significant decrease in the serum concentration of PYD post-race. Our results disagree with those of the study in horses assigned to 48-week race training (Caron *et al,* 2002), where no significant changes in serum PYD concentrations were detected post-race. It is clear from this study that the bone formation biomarkers are not influenced by the 5 km race. However, the bone resorption biomarker increased significantly. The influence of long-distance racing on these biomarkers is therfore warranted. Understanding the effect of racing on stimulation of the bone remodelling is important for the development of strategies to increase and maintain bone mass.

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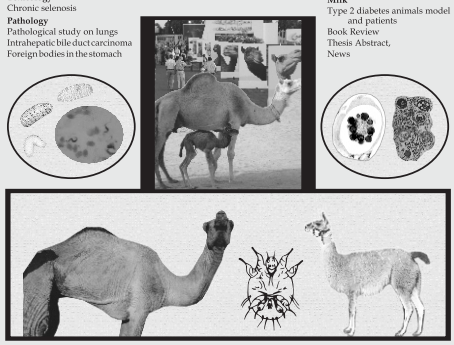

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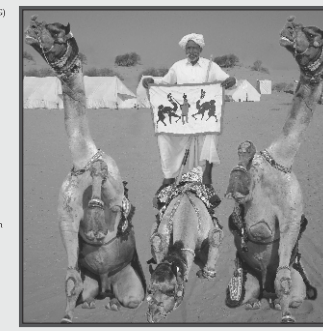

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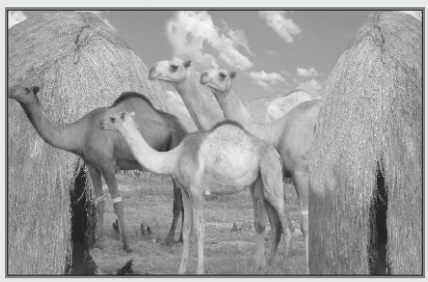

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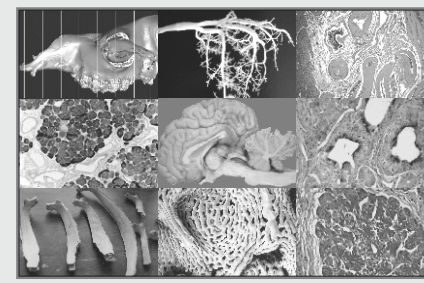

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THE LYMPHOID TISSUE IN THE PALATINE TONSILS OF THE DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

The current study was undertaken to investigate the microscopic features of the palatine tonsils of dromedary camel and to observe the distribution of some markers that might be related to the dendritic cells. Tonsils of 12 camels were processed for H&E stain and for IL-2, $CD8^+$ and TCRγδ immunohistochemistry. Our data showed that palatine tonsils formed of solitary or aggregates of more than one nodule in which the lymphocytes were distributed in the form of follicular and interfollicular areas. The mucosal epithelium of the tonsils was stratified squamous non-keratinied that was reflected into the crypts where it is called reticular epithelium, stratified squamous non-keratinised infiltrated with lymphocytes. The immunohistochemical study revealed that the IL-2 was highly expressed at the mucosal and reticular epithelium, strong at the vascular epithelium. The $CD8^+$ expression was strong at the reticular epithelium and moderate at mucosal epithelium and blood vessels. The expression of TCRγδ was strong at the mucosal epithelium without any expression at other tissues related to the palatine tonsils. Collectively, the present study provides indication that the mucosal and reticular epithelium of the camel could have certain role in the immune responses.

Key words: Dromedary camel, lymphoid tissue, palatine tonsils

The animal's body is connected with the external environment through the skin and mucosae. A well-equipped defense mechanism is present in the skin and along many mucosal linings, i.e. the skin associated lymphoid tissue (SALT) and mucosa-associated lymphoid tissue (MALT), respectively (Casteleyn *et al,* 2011). The MALT is situated along the surfaces of all mucosal tissues. Its most well-known representatives are gut-associated lymphoid tissue (GALT), nasopharynx associated lymphoid tissue (NALT), and bronchus-associated lymphoid tissue (BALT) (Cesta *et al* 2006; Casteleyn *et al* 2011). A common feature of MALT is the presence of a specialised epithelium overlying the mucosal lymphoid follicles, known as follicle-associated epithelium (FAE), which contains microfold or M-cells (Kimura, 2018). These cells are highly specialised in the endocytosis and transport of particulate antigens by trans-epithelial vesicular transport from the luminal mucosal surface to underlying abundant sub-epithelial dendritic cells (Neutra *et al,* 2001; McNeilly *et al,* 2008). Additionally, MALT characterised by the predominance of local IgA production and by the migration of activated lymphocytes from one mucosal surface to other mucosal sites via the integrated or common mucosal immune system (Rebelatto *et al,* 2000; Casteleyn *et al,* 2011).

Among MALT is the pharyngeal mucosa-associated lymphoid tissue, which is composed of 6 tonsils; paired tubal tonsils, paired palatine tonsils, lingual tonsils and tonsils of the soft palate (Palmer *et al,* 2009; Breugelmans *et al,* 2011; Pascual *et al,* 2016). The palatine tonsils are the most proximal along the digestive tract, therefore, they play a key role in initiating immune responses against many ingested antigens (Zidan and Pabst, 2011). It is well known that animal tonsils are the sites of entry and replication of several pathogens such as bovine viral diarrhoea (Palmer *et al,* 2009; Zidan and Pabst, 2011), foot-and-mouth disease (Ranjan *et al,* 2016; Topoets *et al,* 2011) and sheep scrapie (Cancedda *et al,* 2014). In camel, the Middle East respiratory syndrome corona virus (MERS-CoV) could find its way to the body through the tonsils (Andy *et al,* 2014). Moreover, palatine tonsils are relatively easily accessible, thus they could be used in the diagnosis of several diseases (Toppets *et al,* 2011).

In camel, palatine tonsil is located on both sides of the oropharynx wall within a tonsillar fossulae, extending between the palatogloss and palatopharyngeal archs. The corresponding tonsillar fossulae shows a deep and elongated depression, known as tonsillar sinus located just behind the

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fold of the soft palate diverticulum (Achaaban *et al,* 2016). Microscopically, palatine tonsils of camel had a unique form of several spherical macroscopic nodules protruding into the pharyngeal lumen. The tonsillar crypt is lined with stratified squamous non-keratinised epithelium (Zidan and Pabst, 2009). These 2 studies enrich the field of anatomy about the structural features of the palatine tonsils of the camel. However, to the best of the authors knowledge there was no work done on the palatine tonsils of the dromedary camel focusing on any of the immunohistochemical markers that could be important to the immunity of the camel. Therefore, the current study was aimed to revisit the histological structure and to study the immunohistochemical features of some markers namely; interleukin-2 (IL2), $CD8^+$ and of the T-cell receptor$\gamma\delta$ (TCR$\gamma\delta$).

Materials and Methods

Animals and tissue collection

Twelve adult (3 years-old and above) apparently healthy males (5) and females (7) dromedary camels were used in this study. Shortly after slaughtering the pharyngeal region was examined and animals with no apparent pathological changes were selected. The palatine tonsils were located according to Achaaban *et al* (2016) and the tonsillar tissues were excised completely. The tonsils were then placed in 10% neutral buffered paraformaldehyde solution for 48 hours. After fixation, tissues were processed by routine paraffin-embedment techniques, cut into 5µm and mounted on positively charged slides (Superfrost®, Thermo Scientific Portsmouth, New Hampshire, USA) and stored at 4C° until stained with different staining procedures.

Conventional histological staining

Standard hematoxylin and eosin (H&E) staining protocol (Bancroft and Cook, 1994) was used to study general histological structures of the palatine tonsils.

Immunohistochemistry

The expressions of IL-2, $CD8^+$ and TCR$\gamma\delta$ were detected in paraformaldehyde fixed, paraffin-embedded, palatine tonsils. The markers were detected with primary antibodies (types, sources, antibody dilutions, catalog number, host, reactivity and clonality) are shown in table 1. The primary antibodies were diluted in antibody dilution buffer, tris buffered saline (TBS; 0,25 mM TRis+125 nM NaCl +1% BSA, pH 7,5). Cross-sections of the paraffin embedded tissues (4-5 µm) were stained using procedures was described previously (Al-Ramadan, 2013). Briefly, sections were deparaffinised, rehydrated by decreasing strengths of alcohol until 100 PBS buffer. Sections were then incubated overnight with primary antibodies at 4°C. After overnight incubation sections were then washed several times with PBS followed by incubation with secondary biotinylated antibodies for 30 - 60 min at room temperatures washed with PBS, and incubated with streptavidin-HRP conjugate (HSS-HRP) for 30 min. Finally, sections were washed with distilled water and the visualisation of the reaction was achieved by immersing sections in freshly prepared AEC chromogen solution until desired stain intensity developed.

Staining were evaluated using a light microscope (Leica DM6000-B microscope) and histological images were obtained with Leica DEC-420 digital camera (Leica Microsystems, Germany). Primary antibodies were substituted with antibody-dilution buffer for negative controls which were carried out on sections adjacent to those used in normal immunostaining protocol to maintain similar treatments.

The labeling intensity of cells was scored on a subjective scale, i.e. negative (-); weak (+); moderate (++); strong (+++) and very strong (++++).

Results and Discussion

Histology

The H&E staining of the palatine tonsils of camel showed massive number of lymphatic cells arranged into ovoid or spherical nodules. These nodules either existed as solitary or aggregates of more than one nodules (Fig 1 A). Within each nodule, the cellular components were arranged into heavily cellular follicles and less populated interfollicular areas (Fig 1 B).

The tonsils were covered with thick layer of stratified squamous epithelium non-keratinised, that is reflected inside the tonsil forming a blind ended crypt but the high stratified squamous mucosal epithelium changed to thinner epithelium and usually infiltrated with lymphocytes forming reticular epithelium (Fig 1 C&D). Moreover, 4 layers of the mucosal epithelium on the palatine tonsils can be described, namely from the bottom up; basal layer, spinous layer, intermediate layer and superficial layer (Fig 4 C). It is also noticed that the reticular epithelium is desquamated and the lumen of the

crypt is populated with these epithelial cells and many lymphocytes. The thickness of the reticular epithelium is not uniform where it could be formed of several layers in some areas and of a single layer in others. The collagen fibres of the lamina propria were arranged into a hemicapsule separating the lymphoid tissue of the tonsil from the surrounding pharyngeal structures (Fig 4 B). The venules of lymphatic nodules were lined with higher endothelium than those outside the tonsils (Fig 4 D).

Immunohistochemistry

The scoring of immunohistochemical staining of the camel palatine tonsils is presented in tables 2 and 3.

IL-2: Very strong immunoreactivity was detected at the mucosal and reticular epithelia of palatine tonsils of the dromedary camel (Fig 2 B). On the other hand, moderate staining was detected at the follicular lymphoid tissue, while the interfollicular tissue were weak. The vascular epithelium showed strong reactivity. The stain of intermediate layer of the mucosal epithelium was the strongest in comparison to the rest of the layers (Fig 3).

$CD8^+$: The palatine tonsil showed strong reactivity at the mucosal epithelium and moderate to strong reactivity at the reticular epithelium (Fig 2 C). The follicular lymphoid tissues were negative to the staining while the interfollicular tissue was weakly stained. However, few $CD8^+$ cells with strong reactivity were detected at the subepithelial connective tissue. Moderate reactivity was detected at the blood vessels. Within the mucosal epithelium the intermediate at the intermediate layer and strong at superficial layer in comparison to basal and spinosum layers (Fig 4).

TCRγδ: The only tissue reacted to TCRγδ antibody was the mucosal epithelium, whereas the rest of the tonsillar tissues were negative to the stain. The staining was strong at the spinosum and superficial layers with week at the basal and intermediate layer (Fig 2 D).

When the primary antibodies were substituted with antibody-dilution buffer as a negative control, only faint background was detected (Fig 5).

In camel, the palatine tonsils could be classified as tonsils with crypts, tonsillar folliculate aggregate *disjuncta impressa*. This type of tonsil is similar to the tonsils of horse, bovine, ovine, caprine, buffalo, and swine (Kumar and Timoney, 2005a; Casteleyn *et al,* 2011a; Breugelmans *et al*, 2011; Zidan and Pabst, 2011; Liu *et al,* 2012); but those without crypts are seen in the carnivores (Belz and Heath, 1995; Casteleyn *et al,* 2011). In agreement with previous studies, the palatine tonsils of camel are formed of nodules which exist as solitary or aggregates of more than one nodules (Zidan and Pabst, 2009; Achaaban *et al,* 2016). The variation in the number of the lymphoid nodules might be related to the age. In this respect, the age related changes in the density and distribution of mucosal lymphoid tissues (MALT) has been profoundly investigated (Brandtzaeg, 2010). All animals used in the persent study were adult

Table 1. Information of the antibodies used in the study.

	Antibody	Company	Dilution	Cat. No.	Host	Reactivity	Clonality
1	Anti-IL-2 alpha	abcam	1:200	Ab8235	Mouse	Human	Monoclonal
2	Anti-$CD8^+$	abcam	1: 200	Ab4055	Rabbit	Human, monkey	Polyclonal
3	TCRγδ	abcam	1:200	Ab114983	Mouse	Rat	Monoclonal

Table 2. The patterns of immunohistochemical staining of different antibodies to the palatine tissues.

	Antibody	Mucosal epithelium	Reticular epithelium	Follicles	Interfollicular tissue	Blood vessels
1	Anti-IL-2 alpha	++++	++++	++	+	+++
2	Anti-$CD8^+$	++	+++	–	+	++
3	TCRγδ	+++	–	–	–	–

Table 3. The patterns observed in immunohistochemical staining of different markers at different layers of mucosal epithelium of the palatine tonsils.

	Antibody	Basal layer	Spinous layer	Intermediate layer	Superficial layer
1	Anti-IL-2 alpha	+	+	++++	+++
2	Anti-$CD8^+$	++	++	+++	++++
3	TCRγδ	+	+++	+	+++

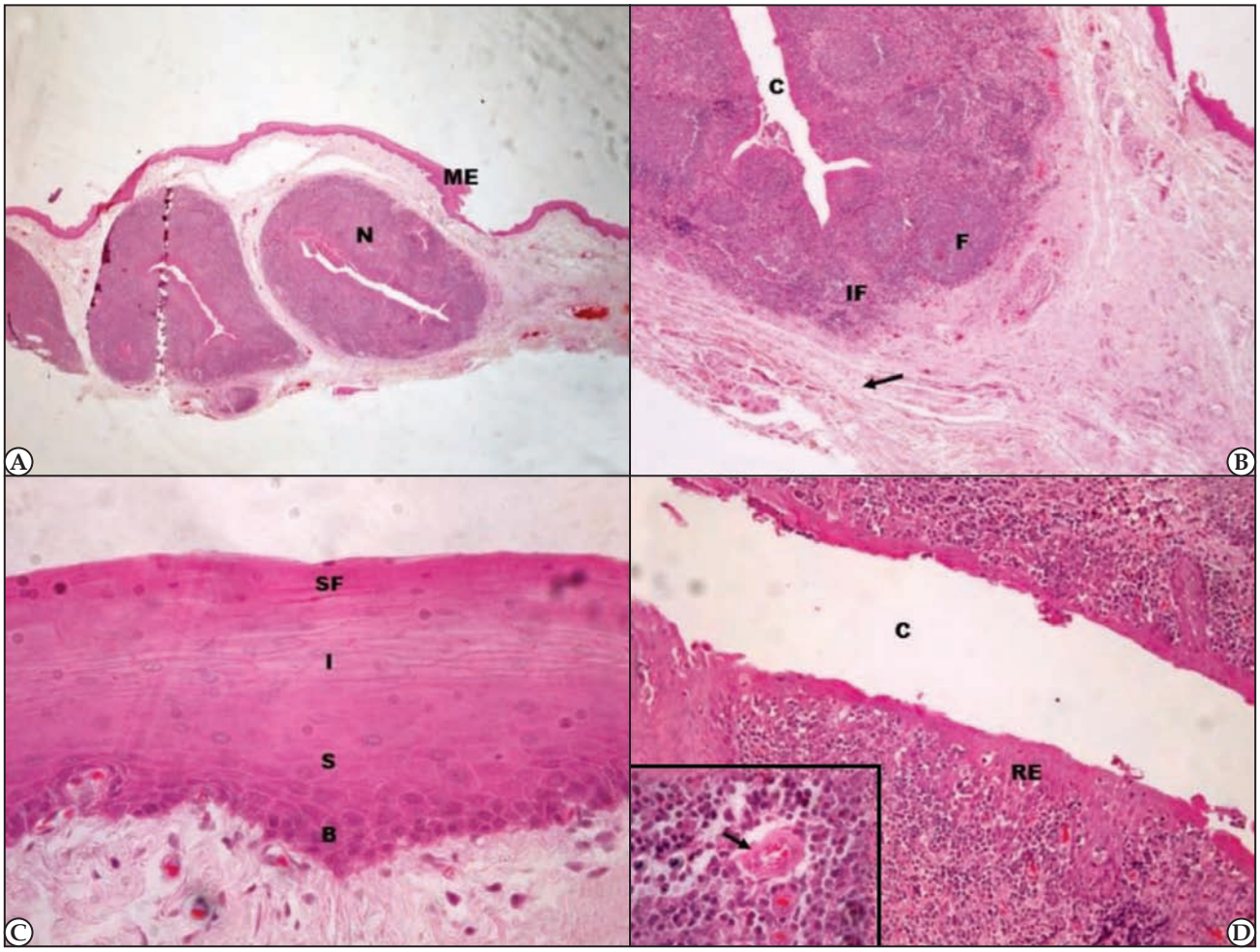

Fig 1. Histological features of the palatine tonsils of dromedary camel. (A) Three nodules appear in the section, N; the mucosal epithelium, ME (H&E, X1.25). (B) The crypt of the nodule C, and arrangement of lymphocytes within the lymphoid nodule which are distributed into follicular F, and interfollicular areas IF (H&E, X5). (C) The four layers of mucosal epithelium of the tonsils; the basal B, spinous S, intermediate I and superficial SF (H&E, X40). (D) Crypt of the palatine nodule C, lined with reticular epithelium RE (H&E, X20); insertion is for venules lined by high endothelium arrow (H&E, X100).

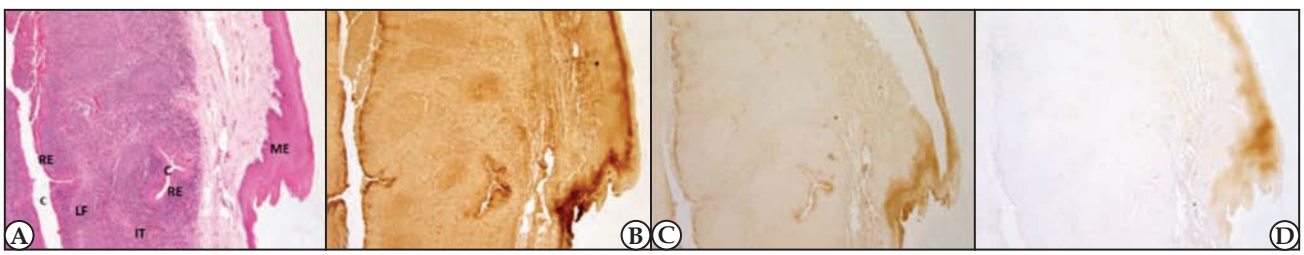

Fig 2. A micro photograph of stained serial sections from palatine tonsil of camel X5. (A) Hematoxylin and eosin stained section showing different tissue components of the lymphoid tissue of palatine tonsil, ME mucosal epithelium, RE reticular epithelium, LF lymphoid follicle, IT interfollicular tissue, and C crypt. Sections (B, C & D) are stained by Anti-IL2, CD8 and TCRγδ antibodies, respectively. Note the differences in the staining intensity at different tissue locations.

aged 3 years and above (some of the animals were more than 16 years old), which were also similar to the age range that have been used in the 2 previous studies done on the palatine tonsils of camel by Achaaban *et al* (2016) and Zidan and Pabst (2009). However, Achaaban *et al* (2016) noticed that density of the nodules is reduced in the elderly animals. Such variation was not a specific character of the palatine tonsils. This variation apparently is common feature for the mucosal associated lymphoid tissue (MALT). Similar age-related variation has been also reported in the lymphoid tissue of the stomach of the bactrian camel (*Camelus bactianus*) where the highest nodular population of the abomasum were reported in the

pubertal age group in comparison to the young, middle aged or old groups (Zhang *et al,* 2012).

The persent study showed that tonsils of the camel is covered by stratified squamous non-keratinised epithelium. This finding contradict the previous reports of Zidan and Pabst (2009) and Achaaban *et al* (2016) where they described the mucosal epithelium of the palatine tonsils in the camel as stratified squamous non-keratinised. Similar observation about the mucosal epithelium, non-keratinised stratified squamous epithelium were also reported in the palatine tonsils of dog (Belz and Heath, 1995), horse (Kumar and Timonet, 2005a), sheep (Casteleyn *et al,* 2007), cattle (Palmer *et al,* 2009), buffalo (Zidan and Pabst, 2011) and pig (Liu *et al,* 2012). Different areas of the buccal cavity were lined with different types of mucosa. Since the palatine tonsils are located at an area of less mechanical compression and frictional forces, it is reasonable to be covered with non-keratinised epithelium.

The epithelium that lined the crypts was reticular epithelium is stratified squamous non-keratinised, heavily infiltrated with lymphocytes and did not show uniformity in thickness. Similar type of epithelium was also reported within the crypts of the palatine tonsils of cattle (Palmer *et al,* 2009), buffalo (Zidan and Pabst, 2011), sheep (Casteleyn *et al,* 2007; Breugelmans *et al*, 2011) and horse (Kumar and Timoney, 2005a). The number of the layer in the reticular epithelium in the palatine tonsils of sheep varied from one layer up to ten layers (Casteleyn *et al,* 2010). Similar to the camel, the latter authors also reported the desquamated epithelium and lymphocytes in the lumen of the crypts (Casteleyn *et al,* 2010).

The arrangement of the collagen fibres in the camel tonsils seen in the persent study was also

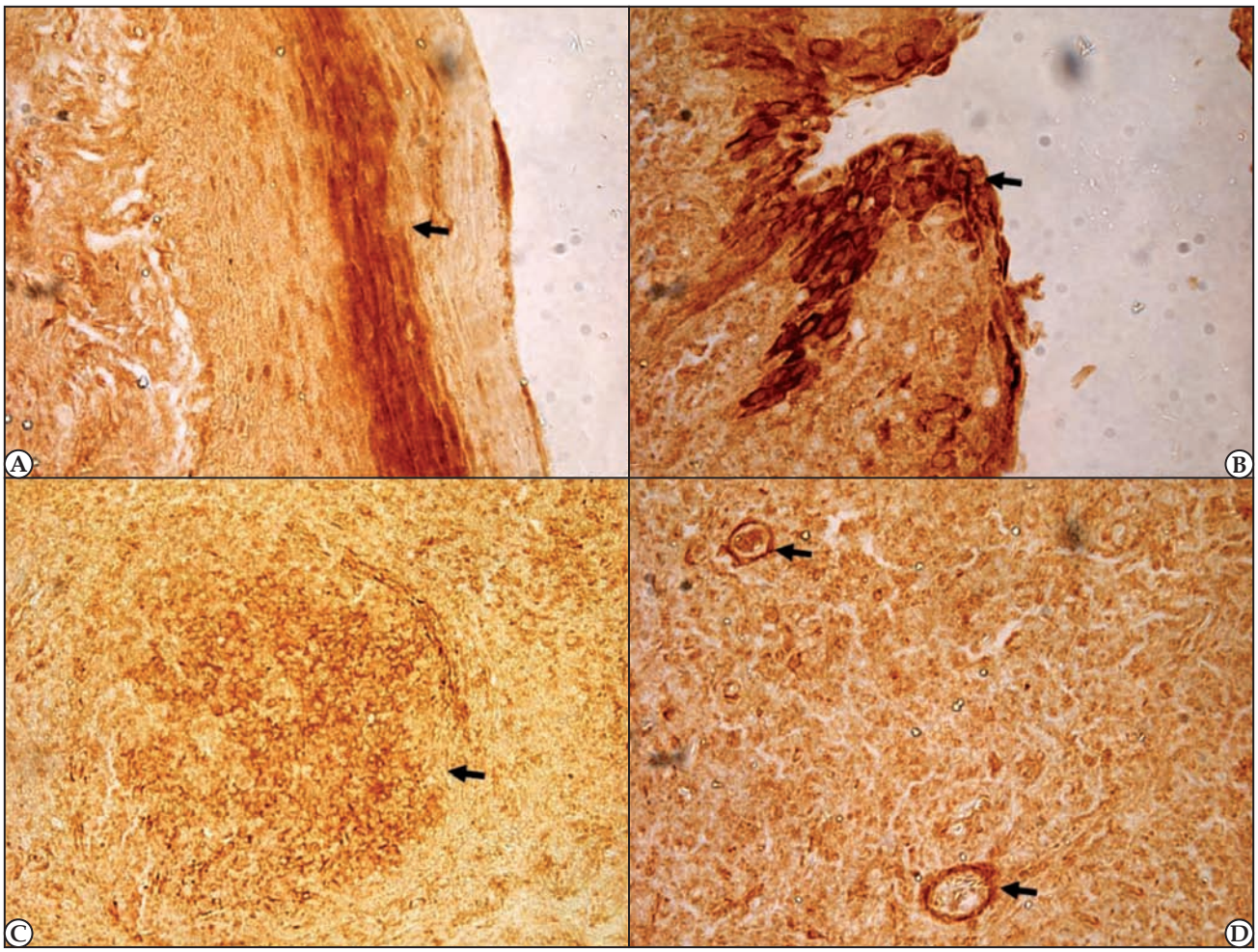

Fig 3. A micro photograph of palatine tonsil of camel stained by anti-IL-2 antibody showing: (A) the mucosal epithelium with very strong immunoreactivity at intermediate layer and strong reactivity at superficial layer (arrow) X40. (B) the reticular epithelium (arrow) showing very strong reactivity X40. (C) the lymphoid follicle (arrow) showing moderate staining in comparison to weak reactivity to the surrounding inte follicular tissue X20. (D) strong reactivity detected at the vascular endothelium X40.

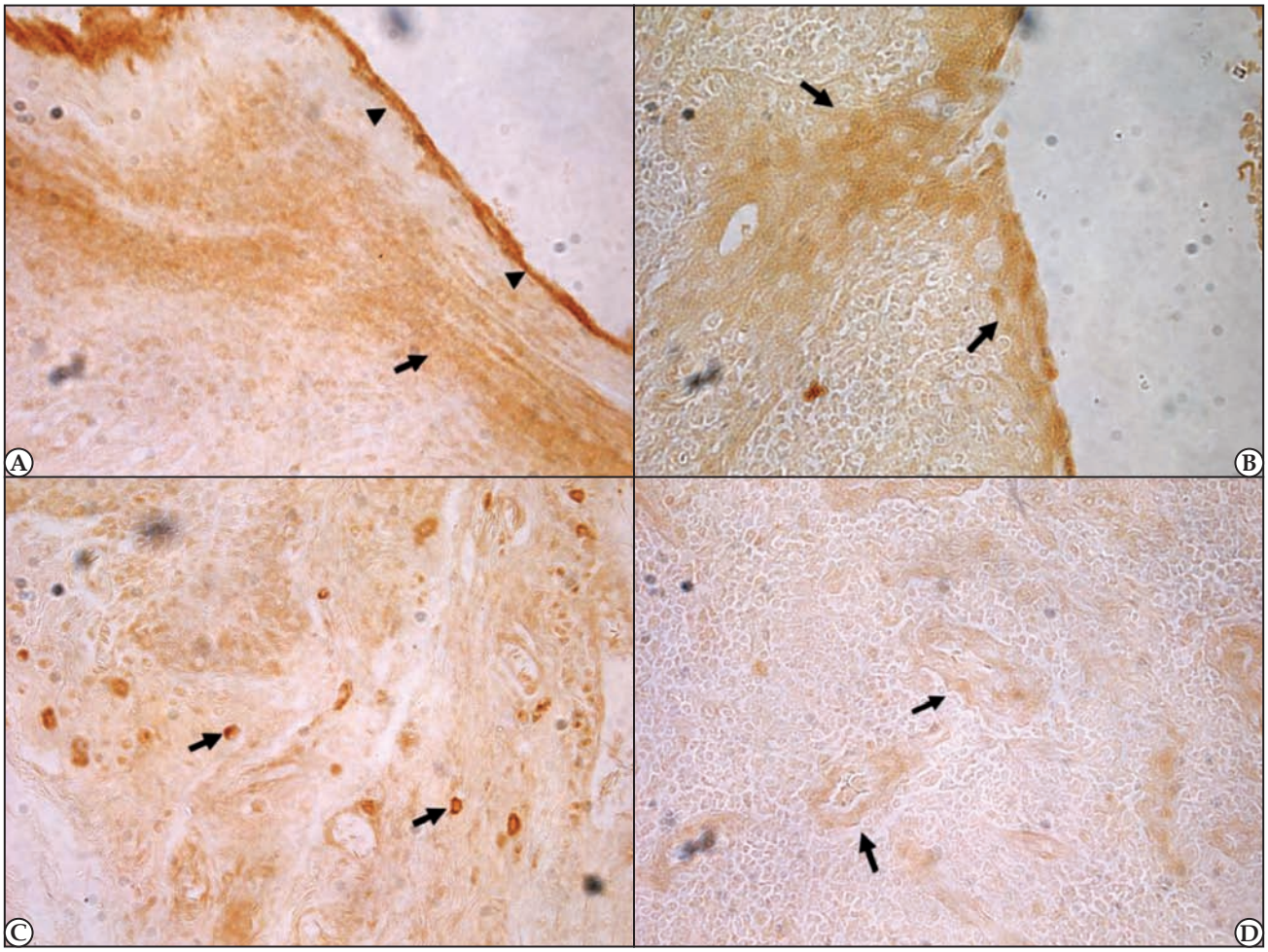

Fig 4. A micro photograph of immunohistochemical stain palatine tonsil by anti-CD8+ antibody. (A) showing the staining intensities of mucosal epithelium, moderate staining at the intermediate (arrow) and strong at superficial layers (arrow heads) of mucosal epithelium X40. (B) moderate staining at the reticular epithelium (arrows) X40. (C) some positive lymphocytes at the subepithelial connective tissues (arrows) X40. (D) the blood vessels are moderately stained (arrows) X40.

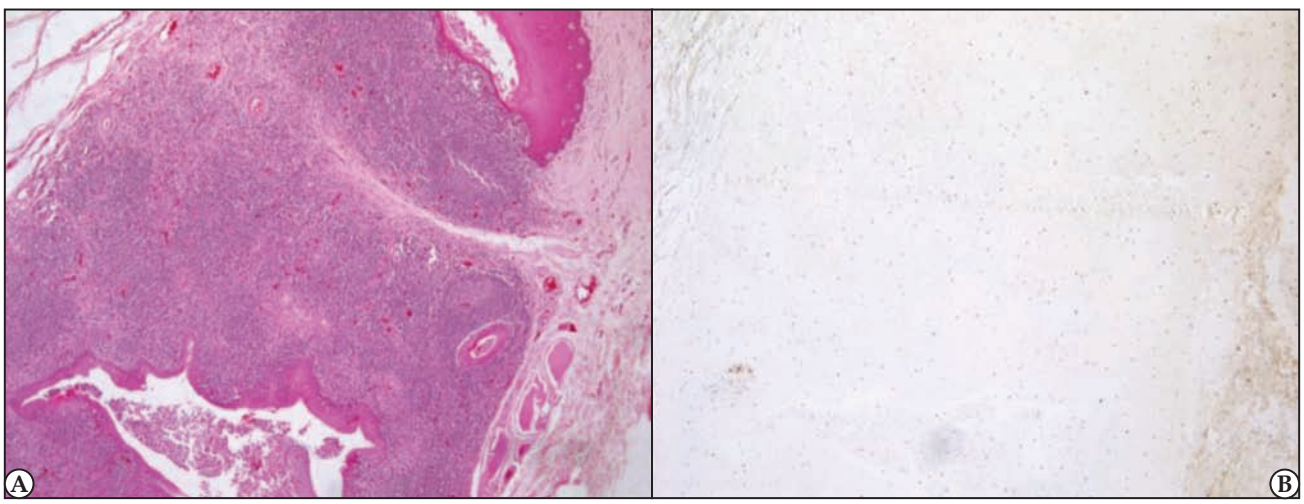

Fig 5. A micro photograph of serial sections stained by (A) H&E and (B) a representative to the control stain where primary antibody replaced by antibody dilution buffer X5.

reported previously (Zidan and Pabst, 2009). This arrangement resembled to that of the horse (Kumar and Timoney, 2005a). The endothelium of the venules, which drains the lymphoid follicles, is higher than the regular endothelium. The high endothelium was reported in the venules of the palatine tonsil of camel but was previously reported in dromedary camel by Zidan and Pabst (2009). This type of endothelium was also detected in the palatine and lingual tonsils of horse (Kumar and Timoney, 2005a,b). The endothelium that lines the blood vessels of lymphoid tissues plays very important role in the trafficking and recruitment of the lymphocytes, hence, the type of the lymphocyte and the site of entry into lymphoid tissues is controlled by the endothelium (Ager, 2017).

Very strong immunoreactivity toward IL-2 was detected at the mucosal and reticular epithelia. IL-2 signal is crucial for differentiation of lymphocyte subsets. IL-2 is found in either soluble state or binds to dendritic cells (DCs) or to the extracellular matrix (Boyman and Sprent, 2012). DCs migrate from the site of generation to be resided at the non-lymphoid tissues like the respiratory mucosa, where they capture and transfer information from peripheral tissue and outside environment to the cells of immune system by releasing cytokines and stimulate immune responses (Bonavida, 2015). Therefore, the positive signals that have been detected at the mucosal and crypt epithelium of the tonsils most probably were due to the dendritic-bound IL-2 molecules. It is also noteworthy that DCs could be classified into two majors groups; the plasmacytoid DCs and conventional DCs. The latter group was also subdivided into migratory and lymphoid tissue-resident subsets (Segura *et al,* 2013). Among lymphoid tissue-resident DCs are the $CD8^+$ subset which is superior to CD8- DCs for presentation of exogenous antigens (Segura *et al,* 2013). Moreover, the presentation of exogenous antigens, known as cross-presentation, is essential for the initiation of $CD8^+$ T-cells responses (Joffre *et al,* 2012). Given the relevance of $CD8^+$ of DCs subset, it is intriguing that we detected $CD8^+$ immunoreactivity in the same location as that of IL-2. Thus it is reasonable, to assume that the co-localisation of IL-2 and $CD8^+$ expressions are of the same cellular population. In bovine platelets, however, $CD8^+$ cells were localised in the epithelium of tonsils besides the intefollicular and paracortix (Rebelatto *et al,* 2000).

A study on the expression of CD markers in normal camel mammary glands indicated high expression of the TCRγδ (Al Mohammed Salem *et al,* 2012). Two γδ phenotypes were identified with respect to their CD8, CD2, and WC+1+ expression and their cytokines profile (Chien and Bonneville, 2006; Wilson *et al,* 2002). The γδ cells that are CD8CD2+ but WC+1- have anti-inflammatory property while γδ cells that lack CD8 and CD2, but express WC+1 have proinflammatory property (Wilson *et al,* 2002). The CD8CD2+WC+1- γδ cells are widely distributed in spleen, gut, lamina propria and mesenteric lymph nodes, whereas CD8CD2-WC+1+ γδ cells are found in blood, peripheral lymph nodes and skin, the proinflammatory activity of the CD8CD2-WC+1+ γδ cells is greatly related to their copious production of IFN-γ and IL-2 (Cassidy *et al,* 2001).

T cell receptor (TCRγδ) was detected at the mucosal epithelium of the palatine tonsils. The high expression of $CD8^+$ at the mucosal epithelium most probably indicate that the cells expressing this marker are γδ-cells which also highly expressed at the same position. The evidences came from *in vitro* studies which indicated a relationship between dendritic cell, γδ cells and the IL-2 secretion. Price and Hope (2009) investigated the interaction between γδ T-cells co-cultured with *Mycobacterium bovis* infected DCs. The authors concluded that there is signal between the DCs and γδ cells, which lead to the synthesis of IL-2 that is dependent upon cell-cell contact. In human, however, the early *in vitro* study showed that activated γδ T-cells cause partial maturation of DCs (Shrestha *et al,* 2005). The overall role of the γδ T-cells is to efficiently clear the infection in the tonsillar surfaces and to limit the inflammatory responses in the recurrent tonsilitis (Olofsson *et al,* 1998).

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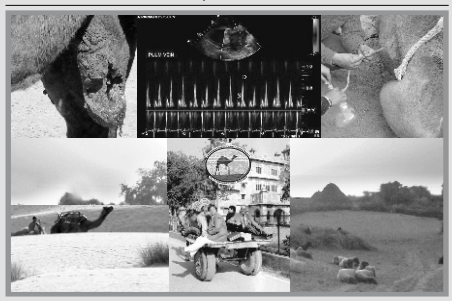

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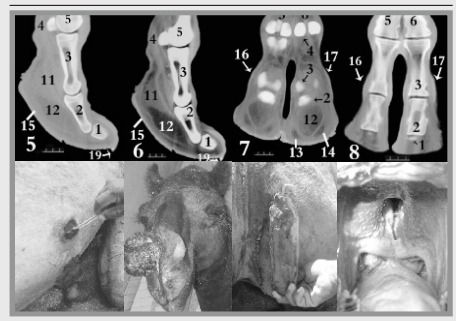

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CEREBROSPINAL FLUID COLLECTION AND ITS ANALYSIS IN CLINICALLY HEALTHY DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

Seven apparently healthy camels (4 males and 3 females) aging 4-9 yrs. old were investigated in the study. Blood samples for serum biochemical analysis were collected from the jugular vein under aspetic condition after animals being clinically examined. Each camel was then sedated with an intravenous injection of 2% xylazine hydrochloride. CSF samples were withdrawn from the atlanto-occipital articulation. Lateral radiographs of the neck were obtained while the needle was *in situ*. Eighteen biochemical parameters were determined both from the serum and CSF of each camel. Most of CSF tested parameters were extremely significant in comparison to serum parameters. CSF parameters had lower concentration of all the studied parameters than the serum, except for the sodium and chloride. The mean value of sodium (150.3 ± 1.70 mmol/l) and chloride (114.5 ± 1.49 mmol/l) in CSF were significantly ($P<0.03$) higher than their mean values in serum; 154.5 ±0.92 mmol/l and 131.3 ± 2.5 mmol/l, respectively. Gender had no relevant effect on the most CSF measured parameters.

Key words: Atlanto occipital, camel, cerebrospinal fluid, CSF sampling, serum

CSF is frequently examined for diagnostic purpose and for understanding the severity and nature of the disease process involving the CNS (Kulkarni *et al,* 2009). Chemical analysis of cerebrospinal fluid can provide evidence of information in the brain metabolism, evaluate disruption of CNS, and identify biomarkers for diagnosis of CNS diseases (Johanson *et al,* 2008). There is a wide range of biochemical and cellular parameters in cerebrospinal fluid, which can provide an important information on health, physiological conditions and neurological disorders of animals (AI-Sagair *et al,* 2005). Composition of cerebrospinal fluid depends strongly on blood composition and it reflects the blood plasma constituents (Ahmed *et al,* 2009). The significance of cerebrospinal fluid analysis has been compared to that of complete blood counts for systemic diseases moreover, CSF changes generally follow specific patterns depending on the etiopathogenesis (inflammatory, metabolic, neoplastic) of neurological diseases, thus indicating which further diagnostic modalities should be applied.

There is lack of information on standard values in CSF of Saudi camels. Thus, the aims of this paper illustrate a new welfare method for a aspirating CSF fluid from dromedary camels and to identify the normal range of some chemical parameters in the CSF and its corresponding values in the serum in healthy dromedaries.

Material and Methods

Seven apparently healthy dromedary camels (four male, three female) aging 4-9 years were used in the present study.

These were fasted overnight and blood samples (5 mL aliquots) were collected from the jugular vein into serum and EDTA vacuum tubes. Serum was separated for biochemistry analysis by centrifugation. Each animal received an intravenous injection of xylazine (Seton 2%., Sweden) at the dose of 0.2 mg/kg body weight. The animals were later taken into right lateral recumbency. The head was pulled where by the frontal bone at right angle to the spine. A 15 cm long 14G needle, L 80 mm was pushed between the conjoined line of the ligamentum nuchae (a distance

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approximately 10 cm away from the external occipital protuberance) (Fig1C). While the thumb and middle fingers were kept on occipital condyles, the index finger pointed to the location of foramen magnum (Fig 1A, B) in form of a depression between the occipital condyle and the atlas (C1). The needle was gently pushed with in an angle of about 60 degrees from the vertical line till an empty cavity was felt. Then, the needle was kept perpendicular to the dorsal laminae of the vertebral column, at the level of the atlanto-occipital space, and advanced very slowly through the skin, the atlantooccipital ligament, the meninges (dura mater and arachnoid) and into the cisterna magna. This enabled a free flow of clear colourless cerebrospinal fluid spontaneously, which was collected into plain sterile collection tubes for CSF biochemical analysis (Fig1d). Radiographs were done in some cases to show the depth, direction and the exact position of the head of the needle (Fig 1D). Once collection was completed, the needle was gently removed from the collection site.

CSF and blood serum biochemistry

CSF and blood serum were immediately transferred to the laboratory for estimation of albumin (ALB); alkaline phosphatase (ALP); aspartate aminotransferase (AST); gamma glutamyl transferase (GGT); total serum protein (TP); blood urea nitrogen (BUN); creatine kinase (CK); inorganic phosphorus (PHOS); magnesium (MG); calcium (Ca); Sodium (Na); chloride (Cl); potassium (K); alanine aminotransferase (ALT); amylase (AMY); total bilirubin (TBIL); creatinine (CRE) and glucose (GLU) using Vet scan vs 2 analyser (ABAXIS, USA).

Statistical analysis

Data were recorded in Excel spreadsheets and imported into Stata version 14 (Stata Corp., TX, USA)

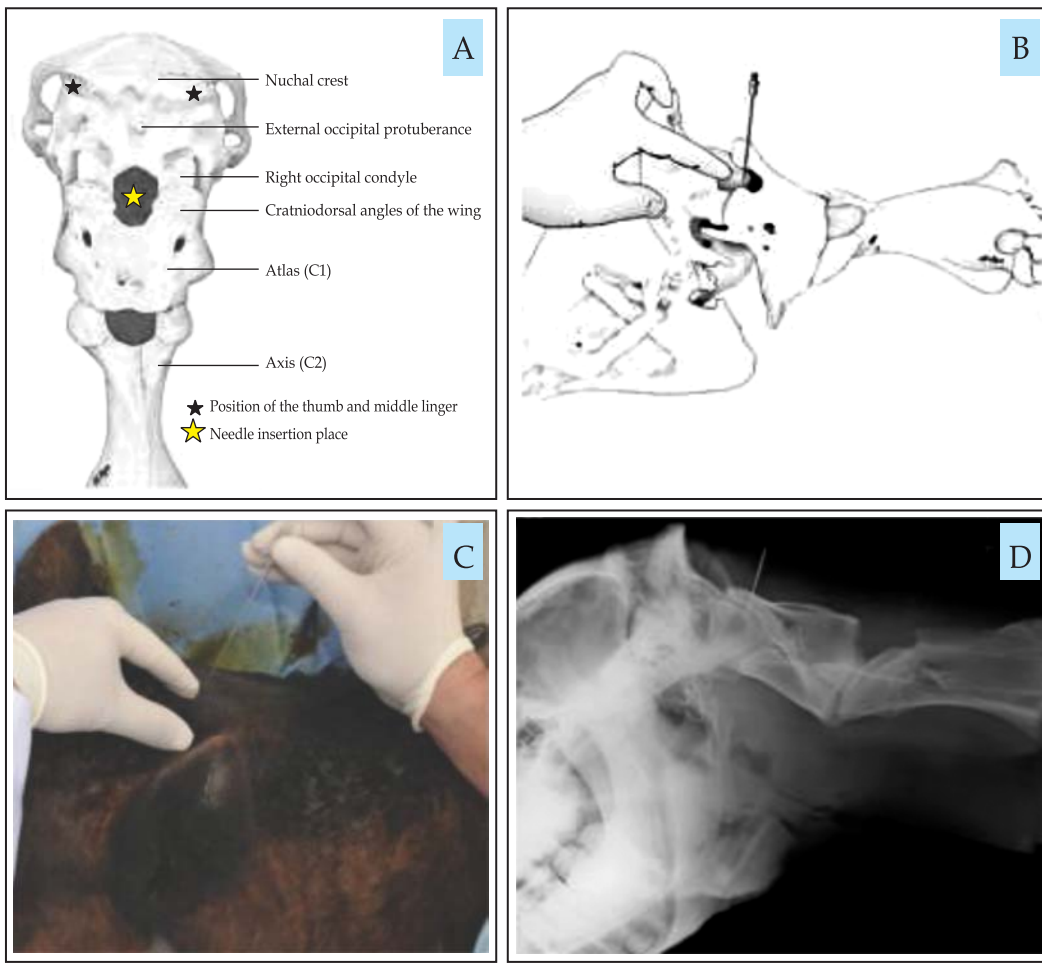

Fig 1. (A) Dorsal view of the composite occipito-atlas-axis bones; (B)The thumb and middle finger of one hand hold the (the external acoustic meatus) occipital condyles and the index finger used to palpate place of foramen magnum; (C) Insertion of the needle at the level of the atlanto-occipital space; (D) Lateral radiography during insertion of the needle to view where the collection is actually taking place.

for further analyses. Descriptive statistics (mean, SEM) were calculated for each parameter. Variation within each parameter was evaluated using coefficient of variation (CV). Effect of sex on each parameter was evaluated using linear regression model. Effects were considered significant at $P < 0.05$.

Results

The results are presented in Table 1 and 2 and recorded as mean±SEM in both CSF and blood serum. Most of CSF tested parameters were extremely significant in comparison to serum parameters. CSF parameters had lower concentration of all the studied parameters than the serum, except for the sodium and chloride (Tabel 1). The mean value of sodium (150.3 ± 1.70 mmol/l) and chloride (114.5 ± 1.49 mmol/l) in CSF were significantly ($P<0.03$) higher than their mean values in serum; 154.5 ±0.92 mmol/l and 131.3 ± 2.5 mmol/l, respectively.

There is no significant differences in concentration of Mg and TBIL in serum and CSF.

Table 2 showed mean, SEM and P value of CSF parameters in males and females of dromedary camels. Most chemical parameters had no significant value regarding sex except TP and AMY, which have slightly significant difference.

The values of ALB, ALP, GGT, TP, BUN, CK, Ca, Na, Cl, K, AMY and TBIL were slightly higher in female than male camels. However, concentration of AST, ALT, CRE and glucose were slightly higher in male than female camels (Table 2).

Discussion

The analysis of cerebrospinal fluid and its comparison with blood is frequently examined for diagnostic purpose and for understanding the severity and nature of the disease process involving the CNS (Kulkarni *et al,* 2009). In previous studies, CSF was collected from slaughtered camel (Ahmed *et al,* 2009; AI-Sagair *et al,* 2005) and from living animals without sedation using 18 guage needle after flexing the head (Nazifi & Maleki, 1998). The advantages of our CSF collection method were that the animals were sedated and save manipulation was obtained considered the animal welfare. Moreover, the lateral recumbency and head position allowed easy and gentle flow of CSF into the tubes and considerable amount was obtained. Use of radiography during sampling as a test in some cases helped in finding the depth and direction of the needle.

Chemical analysis of cerebrospinal fluid can provide evidence of information in the brain

Table 1. Mean, SEM, range and Serum/CSF ratio of biochemical value of blood serum and cerebrospinal fluid in the clinically normal dromedary camel.

Items	Serum		CSF		P value	Serum/CSF Ratio
	Mean±SEM	Range	Mean±SEM	Range		
ALB (g/dl)	3.43 ± 0.47	2.1-4.2	0.13 ± 0.02	0.1-0.2	<0.001	19.8
ALP (IU/l)	179.8 ± 8.51	156-196	52.17 ± 1.3	49-57	<0.001	3.3
AST (IU/l)	107 ± 2.52	100-112	26.17 ± 1.42	22-30	<0.001	4.0
GGT (IU/l)	12.75 ± 2.06	9-18	6 ± 0.89	3-9	0.017	1.9
TP (g/dl)	6.78 ± 0.33	6-7.5	0.33 ± 0.05	0.1-0.5	<0.001	8.3
BUN (mg/dl)	14.1 ± 0.83	12.5-16.1	9.68 ± 0.75	7.8-12.9	<0.001	1.4
CK (IU/l)	134.8 ± 17.9	105-185	23.2 ± 3.39	12-32	0.013	4.6
PHOS (mmol/l)	6.18 ± 1.30	2.3-7.9	0.65 ± 0.07	0.5-0.9	0.003	10.3
MG (mmol/l)	2.53 ± 0.15	2.2-2.9	2.25 ± 0.09	2-2.5	0.35	1.0
Ca (mmol/l)	9.88 ± 0.35	9-10.7	4.97 ± 0.18	4.3-5.4	<0.001	1.9
Na (mmol/l)	150.3 ± 1.70	147-155	154.5 ± 0.92	152-158	0.09	0.96
Cl (mmol/l)	114.5 ± 1.49	110-119	131.3 ± 2.5	122-138	<0.001	0.9
K (mmol/l)	5.7 ± 0.30	4.8-6.1	4.28 ± 0.05	4.1-4.4	0.019	1.3
ALT (IU/l)	13.25 ± 1.11	11-16	10.67 ± 0.33	10-12	0.025	1.2
AMY (IU/l)	492.3 ± 23.9	451-556	10.33 ± 1.23	8-16	<0.001	45.6
TBIL (µmol/l)	0.2 ± 0.04	0.1-0.3	0.15 ± 0.03	0.1-0.3	0.071	1.6
CRE (mg/dl)	1.48 ± 0.22	1-1.9	0.6 ± 0.03	0.5-0.7	0.006	2.3
GLU (mg/dl)	101.8 ± 15.61	71-143	61.5 ± 1.69	56-68	0.014	1.7

metabolism, evaluate disruption of CNS, and identify biomarkers for diagnosis of CNS diseases (Johanson *et al,* 2008). Most of CSF tested parameters were extremely significant in comparison to serum parameters. Similar results were reported by (Nazifi & Maleki, 1998; Guyton and Hall, 2005; Saladin, 2012). On the other hand, the results of most CSF parameters in the present study were extremely lower than those previoussly reported in CSF of camels (Ahmed *et al,* 2009; AI-Sagair *et al,* 2005).

Tabel 2. Sex-related changes in biochemical value of cerebrospinal fluid in the clinically normal dromedary camel.

Items	Mean±SEM (Male (n=4)	Mean±SEM Female (n=3)	P-Value
ALB (g/dl)	0.12 ± 0.02	0.15 ± 0.05	0.24
ALP (IU/l)	52 ± 1.78	52.5 ± 2.5	0.36
AST (IU/l)	27.25 ± 1.8	24 ± 2	0.09
GGT (IU/l)	4.75 ± 0.62	8.5 ± 0.5	0.09
TP (g/dl)	0.32 ± 0.09	0.35 ± 0.05	0.01
BUN (mg/dl)	9.12 ± 0.59	10.8 ± 2.1	0.43
CK (IU/l)	19.33 ± 4.05	29 ± 3	0.06
PHOS (mmol/l)	0.65 ± 0.09	0.65 ± 0.15	0.32
MG (mmol/l)	2.25 ± 0.09	2.25 ± 0.25	0.36
Ca (mmol/l)	4.92 ± 0.26	5.05 ± 0.25	0.51
Na (mmol/l)	154 ± 0.91	155.5 ± 2.5	0.21
Cl (mmol/l)	129.5 ± 3.28	135 ± 3	0.36
K (mmol/l)	4.27 ± 0.07	4.3 ± 0.1	0.33
ALT (IU/l)	10.75 ± 0.48	10.5 ± 0.5	0.21
AMY (IU/l)	9 ± 0.71	13 ± 3	0.05
TBIL (µmol/l)	0.12 ± 0.02	0.2 ± 0.1	0.12
CRE (mg/dl)	0.6 ± 0.04	0.15 ± 0.05	0.50
GLU (mg/dl)	61.75 ± 2.53	52.5 ± 2.5	0.09

Almost all of the proteins normally present in CSF are derived from plasma (Reiber, 2003). In normal CSF, protein levels consist almost entirely of albumin which is in agreement with previous studies (Ahmed *et al,* 2009; Di Terlizzi & Platt, 2006; Nazifi & Maleki, 1998; Reiber, 2003). The CSF protein values in present study were significantly lower as compared to serum. Usually, presence of high protein in CSF animals indicated neurological disorders where blood-brain barriers were disrupted (Scott, 2010). As our examined animals were apparently healthy assuming that the blood-brain barrier were intact that prevents large size molecules from entering the subarachnoid space (Di Terlizzi & Platt, 2006).

The function of magnesium (Mg) is essential for transport of calcium and potassium ions, and modulates signal transduction, energy metabolism, and cell proliferation (Sun *et al,* 2009). Interestingly in our study there was higher value of Mg (not significant) in CSF compared to serum. Similar results was reported in mice (Sun *et al,* 2009); the reverse is reported in CSF of camel (Ahmed *et al,* 2009; Nazifi & Maleki, 1998) and in CSF of buffalo (Khadjeh *et al,* 2004). The reason for the difference in CSF Mg values between species is unclear.

The mean value of sodium (150.3 ± 1.70 mmol/l) and chloride (114.5 ± 1.49 mmol/l) in CSF were significantly (P<0.03) higher than their mean values in serum; 154.5 ±0.92 mmol/l and 131.3 ± 2.5 mmol/l, respectively. The concentration of sodium, chloride and potassium in CSF in present study were higher than that reported about camels in previous studies (Ahmed *et al,* 2009; AI-Sagair *et al,* 2005; Nazifi & Maleki, 1998). The differences of electrolytes in CSF in camels with previous studies could be due to the feed typing and environmental changes.

As glucose is actively transported across the blood brain barrier the CSF glucose levels are directly proportional to the plasma levels and therefore simultaneous measurement in CSF and blood is required (Deisenhammer *et al,* 2011). Concentration of glucose in CSF in the present study was similar to that reported for camel (Nazifi & Maleki, 1998) and sheep (Patra *et al,* 1993) and cattle (Welles *et al,* 1992) and human (Twijnstra *et al,* 1989), where the normal CSF glucose was 40-60% of serum glucose; the reverse was reported by (Ahmed *et al,* 2009; AI-Sagair *et al,* 2005). CSF glucose takes several hours to equilibrate with plasma glucose therefore, glucose in CSF had no specific diagnostic importance and is related to an elevated blood glucose concentration during physiological and pathological disorders (Deisenhammer *et al,* 2011).

Most chemical parameters had no significant value regarding sex except TP and AMY, which have slightly significant difference. The values of ALB, ALP, GGT, TP, BUN, CK, Ca, Na, Cl, K, AMY and TBIL were slightly higher in female than male camels. In contrast to our results, Khadjeh reported higher values of K, Ca and Mg in CSF of male buffalo than female (Khadjeh *et al,* 2004).

In conclusion, CSF can safely be collected from camels and need further studies in diagnost is of neurological disorders.

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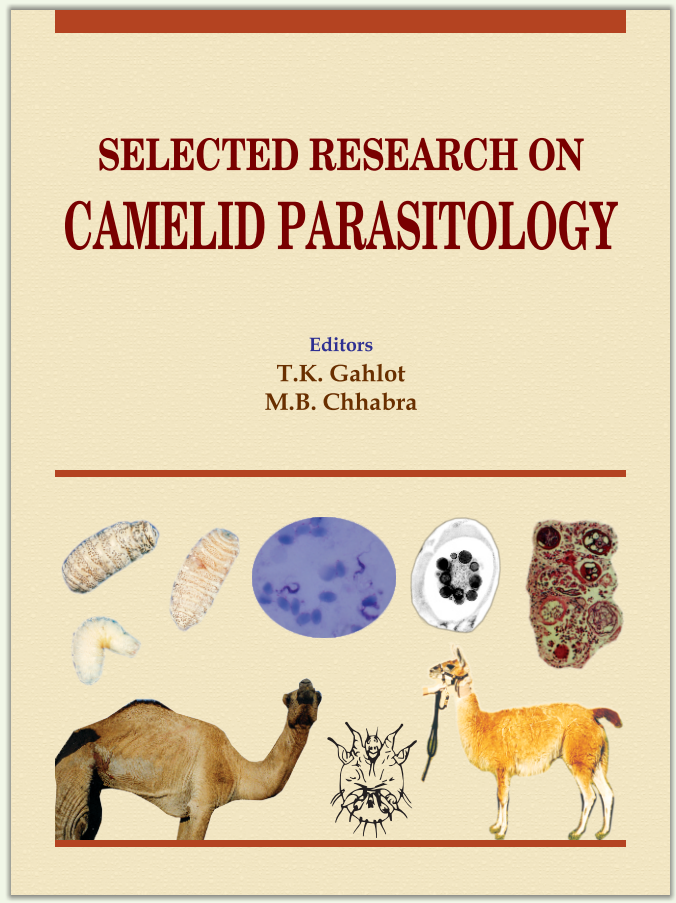

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MACHINE MILKING PARAMETERS FOR AN EFFICIENT AND HEALTHY MILKING IN DAIRY CAMELS (*Camelus dromedarius*)

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ABSTRACT

The effect of vacuum level and pulsation rate on machine milking efficiency in lactating dromedary camels were studied in 2 separate experiments. In the first experiment, a total of 14 multiparous camels in early (n=7) and late (n=7) lactation were used to study the effects of vacuum level (45 and 50 kPa) and pulsation rate (52 and 60 pulsations/min) on milk fractionation and flows traits. At the morning milking, volumes of machine milk (MM), machine stripping milk (MSM) and residual milk (RM) were recorded at two different days. For the second experiment, another 10 multiparous dromedary camels at mid stage of lactation were used to evaluate teat condition and udder health during 10 weeks. Milk samples were collected weekly at each milking and analysed for major milk constitutes and udder health. The 50 kPa vacuum level and 60 pulsations/min decreased ($P<0.05$) the MSM (from 15.2 to 5.9%) and RM (from 44.1 to 29.8%) compared to 45 kPa and 52 pulsation rate. Moreover, milking camels at high vacuum level resulted in significantly more milk in a shorter time with higher milk flows rate at early stage of lactation. No subclinical mastitis was detected in any of udders quarters as indicated by the CMT (<1), SCC ($387\times10^3\pm86$ cells/mL) and bacteriology (coliform=0 and total flora=32097±396 ufc/mL) test. There were no differences in teat diameters, texture and color before and after milking. In conclusion, dromedary camels are readily to milk efficiently at 50 kPa and 60 pulsations/min without affecting negatively teat condition or udder health.

Key words: Dromedary camel, machine milking, milk ejection kinetic, milk fraction, udder health

The interest for camel's milk in human nutrition is increasing because its functional properties (Agrawal *et al,* 2003; Konuspayeva *et al,* 2006; Sboui *et al,* 2009). Under the pressure of market demand for camel milk, the camel farming systems tend to become more intensive. The intensification process includes the use of modern machine milking more or less adapted to the physiology of lactation of camels (Wernery *et al,* 2004; Hammadi *et al,* 2010; Musaad *et al,* 2013; Ayadi *et al,* 2009, 2016). Indeed, similar parameters than for dairy cows are generally used for dairy camels. Yet, little information is available on machine milking ability for this species (Atigui *et al,* 2014).

Machine milking efficiency can be defined as harvesting the maximum amount of milk that is rich in total solids in the shortest possible time with the least amount of physical intervention of the milkers, without any deleterious effects on udder health (Labussiere, 1988; Lee and Choundhary, 2006). The assessment of machine milkability is based on the milk partitioning, residual milk measurement and milk flow traits during milking (Labussiere, 1988). Teat and udder morphology, animal temperament, milking machine parameters, milking routine and characteristics of milking cluster are among the factors affecting machine milking ability of the dairy species (Labussiere, 1988) including camel (Atigui *et al,* 2015). Data regarding the effect of vacuum levels on milk flow traits in dairy camels are scarce (Atigui *et al,* 2015).

It is generally admitted that machine milking could affect the udder health of animals. During milking, the teats are under stress which leads to

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change their conditions (Hillerton *et al,* 2002). Among the factors influencing such condition, vacuum level, pulsation rate and teat cups are the more important. For example, a too high working vacuum level can provoke irritation of the mammary gland and lead to congestion and oedema of the teat tissue, especially at the teat end. It can also influence the teat diameter (Hamann *et al,* 1993; Rasmussen and Madsen, 2000; Mein *et al,* 2001) as it was observed in dairy cows milked by 50 kPa (Hamann *et al,* 1993). Elsewhere, in dairy cow, high vacuum level is positively correlated with milk somatic cell counts (SCC) in dairy cow, (Rasmussen and Madsen, 2000) as well as in buffalo (Pazzona and Murgia, 1992). Such parameters were not assessed in camel.

The objective of the present study was (i) to investigate in dairy camel (*Camelus dromedarius*) the effect of different vacuum levels and pulsation rates on machine milking efficiency at early and late stage of lactation and (ii) to evaluate the teat condition and udder health in camel maintained under intensive milking condition.

Material and Methods

Animals and their management

Twenty-four multiparous dromedary camels (8-12 years old) from Conservation and Genetic Improvement Centre (Al-Kharj district, Riyadh, Kingdom of Saudi Arabia) were included in two experiments described below. Each lactating camel received daily *ad libitum* alfalfa hay +3 kg of commercial pellets (Wafi®, ARASCO, Riyadh, Saudi Arabia). Fresh water was available in continue. For the first month of lactation lactating dromedary camels suckled their calves freely all over the day. After the month, the dams were introduced to milking parlour and trained to accept the milking machine. For the first two weeks, milking was achieved once a day, then twice a day and in all the cases, in presence of the calves, even after weaning at 12 month of age, leaving the dams to continue their lactation still for 6 months.

The time of machine milking was 06:00 and 16:00 h in a single-tunnel milking parlour equipped with medium-pipeline (1.8 m) milking stalls and electronic pulsator (BouMatic, Itak Company, Riyadh, Saudi Arabia) as described in a previous paper (Ayadi *et al,* 2013). The parameters of the milking machine were (i) weight of the cluster: 1.9 kg, (ii) diameter of the mouthpiece liners: 25 mm, (iii) vacuum level: 45 kPa, (iv) pulsations: 52/min and (v) pulsation ratio: 60:40. After milk let-down by calves (without suckling), the milking routine included udder preparation (teat and udder washing and drying), machine milking and final stripping by the calf.

Experiment 1: Milk fraction and milk flow traits

For the first trial, 7 multiparous dromedary camels at early lactation stage (68 ± 6 days) producing on average 5.2 ± 1.0 kg/d and 7 at late lactation stage (353 ± 8 days) producing 3.6 ± 1.2 kg/d, were used. Two vacuum levels (45 and 50 kPa) and two pulsations rates (52 and 60 pulsation/min) were tested during 4 consecutive weeks (1 week for each treatment). Milk fractions were recorded at the morning milking for two different days. The fractions included (i) machine milk (MM) = the quantity of milk obtained after the setting of teat cups until milk flow dropped below 0.1 L/min; (ii) machine stripped milk (MSM) = the amount of milk taken by udder stripping with hands without removing the teat cups; and (iii) residual milk (RM) = the milk amount taken by machine milking after an IM injection of synthetic oxytocin (20 IU/camel; Biocytocine, Laboratoires Biove, Arques, France)). Based on the above data, it was possible to calculate the total machine milk produced (TMM = MM + MSM) and the udder volume (UV = TMM + RM).

Milk flows rates were recorded in the same days of milk fractions by using two electronic mobile milk flow meters (Lactocorder®, WMB, Balgach, Swizerland) calibrated for low milk flow rate (<0.05 kg/min). The milking traits provided by this equipment were: (i) milk yield (kg) = total milk yield per head from the beginning to the end of the morning milking), (ii) total milking time (min) = total milking time from attachment of the cluster till their removal, (iii) milk flow latency (min) = time between the attachment of teat cups and milk flow of 0.250 kg/min, (iv) milk ejection time (min) = time from milking cluster attachment till milk ejection occurs, (v) average milk flow rate (kg/min) = average milk flow rate during milk ejection time and (vi) peak milk flow rate (kg/min) = peak milk flow rate during milk ejection time). The measurement of milk flow traits was performed by the associated software lactopro® (version, 6.0.28). The lag time (sec), i.e. the time from cup attachment to the first observed drops of milk was also visually determined.

Experiment 2: Teat condition and udder health

For this second trial, 10 multiparous dromedary camels at mid stage of lactation (256 ± 8 days) and

producing on average 4.0 ± 0.9 kg/d were used to evaluate teat condition and udder health under machine milking for 10 weeks. Camels were milked twice a day (06:00 and 16:00) by milking machine set at 50 kPa, 60 pulsations/min and 60:40 pulsation ratio. California mastitis test (CMT) was used to check the mastitis status of the camels and to discard eventual positive animals. Individual milk yield was measured during morning and evening milking by using the electronic milk flow meter (Lactocorder®). The length of front and rear teat (TL), i.e. the distance from the teat insertion base to the teat orifice and teat diameter (TD), measured in the middle of the teat using a Vernier caliper (ASAHIT, Hamburg, Germany) were measured before and after each milking. To assess the teat condition, two parameters were taking in account: the teat texture (soft/firm) by manual palpation and, teat color (normal/red) by visual assessment before and after each milking.

Milk samples (100 mL) were collected weekly at each milking and analysed for major milk components. Udder health was evaluated by CMT, SCC and bacteriology (coliform and total flora). Chemical analyses including fat, protein, lactose and total solids percentages were determined by milk analyser Milkoscan (type FT1, FOSS Electric, Denmark). The CMT was performed using Bovivet CMT kit (Bovi Vet, Kruuse, Germany). The SCC was determined as cells/mL using Fossomatic Minor somatic cell counter (Fossomatic 90, FOSS Electric, Denmark). Regarding microbiological analysis, milk samples (5 ml) were obtained aseptically by the following method: teats cleaned with 70% ethanol, discarding the first three streams of foremilk and placed into sterile tubes. Thereafter, 0.01 ml of milk was streaked on Plate Count Agar (IDF 100B, 1991) for total flora and VRBL Agar; (IDF 73B, 1998) for coliforms, then incubated for 24-48 h at 37°C. The plates were then examined for colonies counting. Cultures with five or more identical colonies area considered positive for intramammary infection.

Statistical analysis

The data were analysed by least square means method using the Proc Mixed procedure of Statistical Analysis System (SAS version 9.1, SAS Inst. Inc., Cary, NC). The model included the general mean and the fixed effects of vacuum level (45 and 50 kPa), pulsation rate (52 and 60 pulsation/min) and lactation stage (early and late), their interaction and the residual error. Correlations between traits were also calculated (Pearson correlation). The level for statistical significance was set at $p<0.05$.

Results

The results regarding milk partitioning and milk flow traits at different levels of vacuum and pulsation rate were presented in Table 1 and 2. The highest milk yields recorded for high vacuum level were 3.15 ± 0.41kg at early lactation and 2.53 ± 0.42 kg at late one. The combination of 50 kPa vacuum levels and 60 pulsations rate for milking decreased significantly ($p<0.05$) the MSM (from 15.2 to 5.9%)

Table 1. Milk partitioning and milk flow traits at different levels of vacuum (kPa) and pulsation (pulse per minute) in dairy camels at early stage of lactation.

Vacuum level	**45**		**50**	
Pulsation rate	**52**	**60**	**52**	**60**
Milk fraction (kg)[1]				
MM	1.68 ± 0.39^{b}	1.89 ± 0.39^{b}	1.90 ± 0.40^{b}	3.15 ± 0.41^{a}
MSM	0.37 ± 0.09^{a}	0.30 ± 0.08^{a}	0.30 ± 0.05^{a}	0.19 ± 0.05^{b}
RM	1.63 ± 0.34^{a}	1.60 ± 0.34^{a}	1.47 ± 0.34^{ab}	1.16 ± 0.35^{b}
Milk emission[2]				
LT (s)	1.93 ± 0.30	2.14 ± 0.32	1.82 ± 0.40	2.06 ± 0.40
MFL (min)	0.84 ± 0.10	0.76 ± 0.09	0.77 ± 0.09	0.68 ± 0.10
MET (min)	2.23 ± 0.28	2.63 ± 0.30	2.03 ± 0.37	2.33 ± 0.40
TMT (min)	4.53 ± 0.42^{a}	4.16 ± 0.42^{ab}	3.94 ± 0.21^{ab}	3.70 ± 0.21^{b}
AFR (kg/min)	0.71 ± 0.21	0.86 ± 0.18	0.86 ± 0.17	0.96 ± 0.17
PFR (kg/min)	1.91 ± 0.28^{ab}	1.78 ± 0.31^{b}	1.61 ± 0.27^{b}	2.31 ± 0.28^{a}

[1]MM: machine milk. MSM: machine striping milk. RM: residual milk.

[2]LT: lag time. MFL: milk flow latency. MET: milk ejection time. TMT: total milking time. AFR: average flow rate. PFR: peak flow rate.

^{abcd}Means in the same line with different letters were significantly different ($p<0.05$).

Table 2. Milk partitioning and milk flow traits at different levels of vacuum (kPa) and pulsation (pulse per minute) in dairy camels at late stage of lactation.

Vacuum level	45		50	
Pulsation rate	**52**	**60**	**52**	**60**
Milk fraction (kg)[1]				
MM	1.92 ± 0.40	2.04 ± 0.40	2.10 ± 0.42	2.53 ± 0.42
MSM	0.27 ± 0.08^{a}	0.17 ± 0.08^{b}	0.19 ± 0.08^{b}	0.15 ± 0.07^{b}
RM	1.93 ± 0.33^{a}	1.43 ± 0.34^{ab}	1.36 ± 0.44^{ab}	1.14 ± 0.44^{b}
Milk emission[2]				
LT (s)	2.93 ± 0.42	3.34 ± 0.42	3.42 ± 0.57	3.68 ± 0.57
MFL (min)	0.64 ± 0.08	0.51 ± 0.07	0.61 ± 0.08	0.56 ± 0.09
MET (min)	2.74 ± 0.26	2.52 ± 0.29	2.46 ± 0.32	2.16 ± 0.40
TMT (min)	4.97 ± 0.42^{a}	4.29 ± 0.41^{ab}	4.19 ± 0.22^{ab}	3.84 ± 0.23^{b}
AFR (kg/min)	0.59 ± 0.21^{b}	0.81 ± 0.21^{b}	0.81 ± 0.20^{b}	1.29 ± 0.19^{a}
PFR (kg/min)	1.59 ± 0.30	1.34 ± 0.37	1.44 ± 0.32	1.91 ± 0.29

[1]MM: machine milk. MSM: machine stripping milk. RM: residual milk.

[2]LT: lag time. MFL: milk flow latency. MET: milk ejection time. TMT: total milking time. AFR: average flow rate. PFR: peak flow rate.

[abcd]Means in the same line with different letters were significantly different ($p<0.05$)

and RM proportions (from 43.2 to 29.8%) during lactation compared to the combination 45 kPa vacuum and 52 pulsations rate. The camels milked with these parameters extracted significantly more milk (+ 40%) in a shorter time (-46 sec) with higher milk flows rate (+23%) at early stage of lactation.

Vacuum level did not modify the lag time (1.9 ± 0.4 s) nor at early, neither at late stage of lactation, suggesting that 45 kPa is sufficient to open the teat sphincter and drain cisternal milk. However, the vacuum level could affect the milk flow rate at early and late lactation period. With high vacuum level, the peak flow rate (PFR) reached 2.31 ± 0.28 kg/min and the average flow rate (AFR) was 1.29 ± 0.19 kg/min. Daily milk yield and milk flow characteristics were positively correlated (r=0.28 to 0.53; $p<0.05$) during lactation.

In our study, 68 milk flow curves were recorded (Fig 1a and b) and characterised according to the typology reported by Atigui *et al* (2015). The vacuum level lead to different milk emission kinetic patterns: at 50 kPa, 72% of milk flow curves were characterised by sharp peak flow curve followed by a declining phase without plateau phase vs 28% only at 45 kPa. While at 45 kPa, most of the curves were characterised by a low milk flow rate and a longer milking duration, this proportion was only 21% at 50 kPa.

No subclinical mastitis was detected in any of the udder quarters as indicated by the CMT (<1), SCC (387×103 ± 86 cells/mL) and bacteriology (coliform=0 and total flora=32097±396 cfu/mL) test. The overall mean of log10SCC was 5.55±0.05. Daily milk yield was 6.25 ± 1.05 kg/day and milk components (in %) were 3.87± 0.62 fat, 3.14± 0.45 protein, 4.10 ± 0.65 lactose and 11.10 ± 1.15 total solids. The observed high fat/protein ratio (>1) indicated a complete milk let-down during machine milking.

The teat measurements before milking were presented in Table 3. Before each milking, front and rear teat length and diameter were positively correlated (r=0.42 and 0.56, $p<0.05$), suggesting that only one single teat measurement could be included in the selection schemes. Front and rear teats length increased ($p<0.05$) after morning and evening milking by 23.2%, 23.3%, 19.4% and 20.9%, respectively. There were no differences in teat diameters, texture and colour before and after milking.

Table 3. Front and rear teats measurements (cm) in dairy camels (means) before and after morning and evening machine milking at mid stage of lactation.

	Morning milking		Evening milking	
	Before	**After**	**Before**	**After**
Front teat				
Length	6.3±1.7^{b}	8.2±1.5^{a}	5.8±1.8^{b}	7.2±1.7^{a}
Diameter	3.3±0.8	3.5±1.0	2.9±0.9	3.1±0.9
Rear teat				
Length	5.6±1.8^{b}	7.3±1.7^{a}	5.3±1.7^{b}	6.9±1.8^{a}
Diameter	3.2±0.7	3.3±0.9	3.0±0.8	3.1±0.8

[abc]Means in the same line with different letters were significantly different ($p<0.05$)

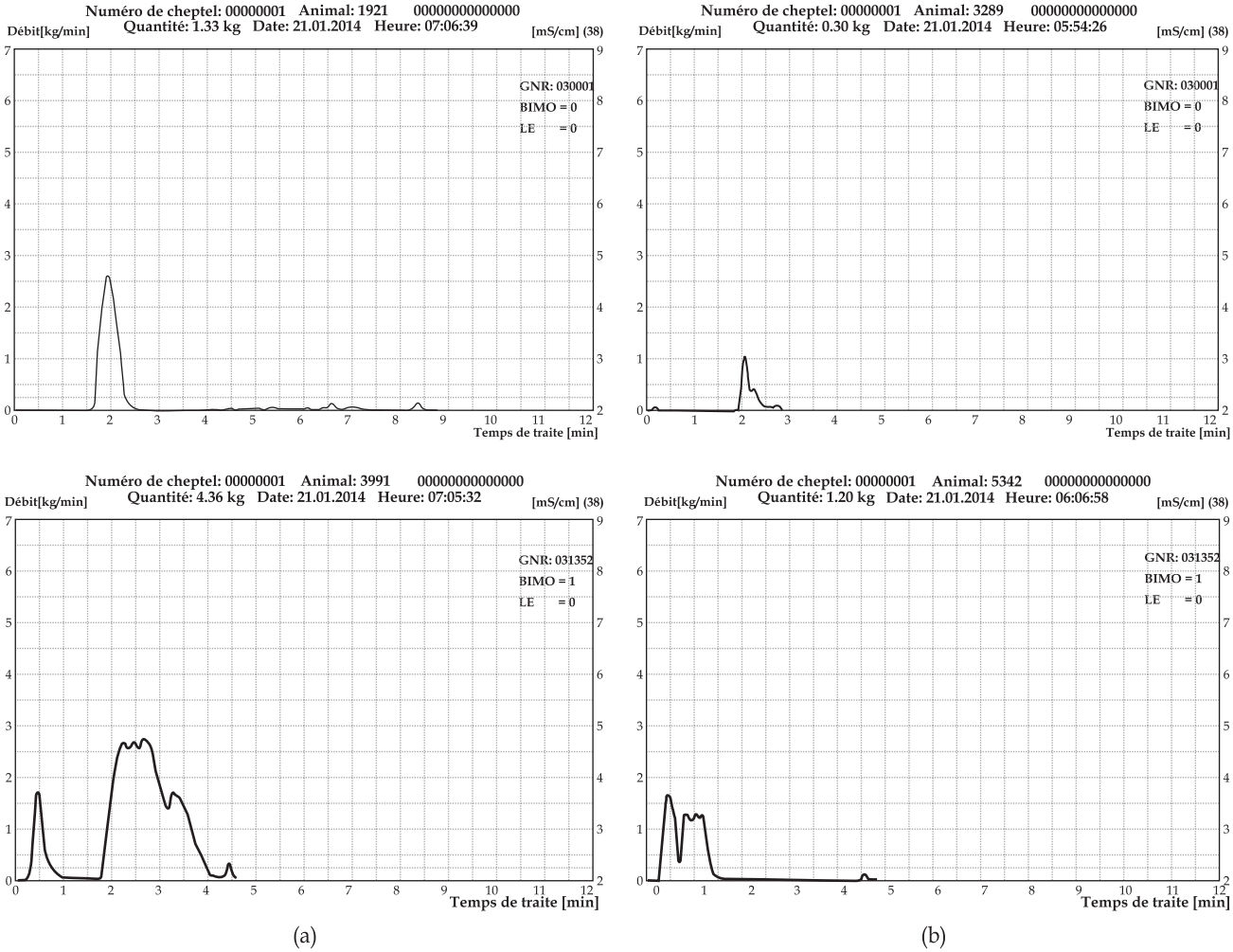

Fig 1. Examples of bimodal and normal milk flow curves for dromedary camel at early (a) and late (b) stage of lactation.

Discussion

Milking machine parameters effect

The optimum vacuum level and pulsation rate in dairy camels are not well determined (Atigui *et al,* 2015). Milking camels at 50 kPa led to the extraction of more milk in a shorter time than 45 kPa at early lactation. These results are similar to those recently reported in dairy camels milked at 48 kPa compared to 38 kPa, the highest vacuum level increasing milk yield and milk flow rate and decreasing milking time (Atigui *et al,* 2015). Our finding agree with those previously obtained in dairy cows (Rasmussen and Madsen, 2000) and dairy goats (Sinapis *et al,* 2000). At reverse, however, in dairy buffaloes, high vacuum level did not significantly affect the milk production (Caria *et al,* 2012). The average percentage of MSM was 9% in the present study that was higher than the results reported in dairy camels from Tunisia by Atigui *et al* (2014) (1% only). In all cases, those values are lower than those recorded in dairy ewes (19%; Such *et al,* 1999) and dairy goats (18%; Sinapis *et al,* 2000). Furthermore, the RM (30%) recorded in the present study was higher than those previously reported in dairy camels hand milked (20%; Kamoun, 1995) and dairy cows (15%; Tancin and Bruckmaier, 2001). Recently, Ayadi *et al* (2016) observe a large variation in the proportion of RM between camels according to the duration of manual udder preparation.

Milk ejection in our study was observed 2 min after teat-cup attachment whatever the vacuum levels and lactation stage as it was stated by Wernery *et al* (2004) in dairy camels. However, lower delay (1min only) was reported in Tunisian dairy camels (Atigui *et al,* 2015). Among dairy animals, a higher milk ejection time (3 min.) was necessary for buffalo, characterised by very tight and resistant teat sphincter (Caria *et al,* 2012) that requires higher vacuum level

to open it. Bimodal curves occurred in 47.2% of total recorded milk flow patterns, but this type was more common at early stage of lactation: 63% vs 32% in late lactation stage. Such results confirmed those previously reported in dairy camels by Atigui *et al* (2015).

Udder health

Somatic cell count (SCC) and bacteriology test in milk are the common indicators used for detecting subclinical mastitis in camels (Guliye *et al,* 2002; Wernery *et al,* 2004; Eberlein, 2007; Aljumaah *et al,* 2011; Nagy *et al,* 2013). No subclinical mastitis was detected during the experimental period. Those results were in the same range than those reported previously in camel milk under intensive system with good hygiene practices (Eberlein, 2007; Hammadi *et al,* 2010; Saleh and Faye, 2011).

Teat morphology and changes

The teat measurements (average front and rear teat length and diameter) before milking (table 3) were in the same range than those observed by Atigui *et al* (2015) in Maghrebi camels, but higher of those observed by Ayadi *et al* (2013) in the same management conditions. Our dromedary camels had larger teats diameters (3.3±0.8 cm) than values reported for dairy cows (2.5 cm; Rogers and Spenser, 1991) and Buffalos (2.7 cm; Caria *et al*, 2012). The huge variation in size of teats between camels might cause difficulties during machine milking and require special practice. Recently, Atigui *et al* (2015) emphasised the need of high milking vacuum during milking for camels with large size teats.

The use of high machine milking vacuum can cause short-term changes in teat measurement and condition (Hamann *et al,* 1993; Mein *et al,* 2001). In our experiment, front and rear teats length increased after machine milking. Eisa *et al* (2010) reported in camels an increase of teat length by 12.8% immediately after hand milking. Contrary to dairy cows in which teats diameters increased by 20% immediately after milking (Hamann *et al,* 1993), in our study, the diameters did not change after each milking, confirming the absence of congestion. However, further investigations using cutimeter or ultrasound are required to confirm the impact of high machine milking vacuum on the thickness of the camel teats. To compare the performance of commercial milking machine, the parameters generally used are the change of teat colour and texture. In our study, no differences in these parameters were observed before and after milking when high vacuum level was used. The percentage of teats classified as soft-normal and firm-normal were 80 and 20%, respectively. According to Hillerton *et al* (2002) the change of teat color immediately after milking was probably more related to different levels of over-milking rather than the parameters of machine milking used.

Conclusion

Milking camels at 50 kPa and 60 pulsations/min resulted in milking greater milk amount in shorter time without affecting negatively teat condition and udder health in dairy dromedary camels at least for the duration of our observations. These results have to be confirmed by taking in account the entire lactation period. Moreover, the milk productivity in this species could be improved by a better understanding of the milking physiology. Indeed, because the high retained amount of milk in the udder (30%), innovative methods to induce a better milk ejection during milking are required.

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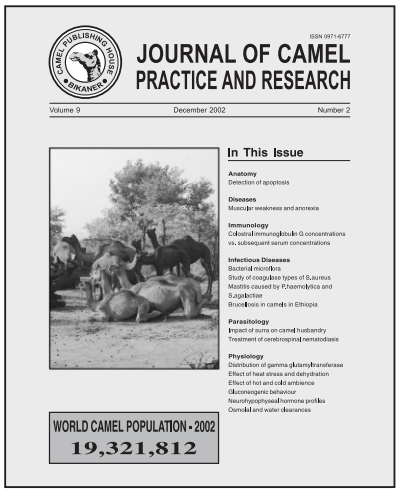

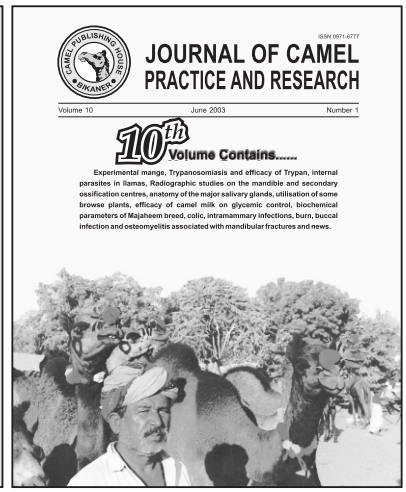

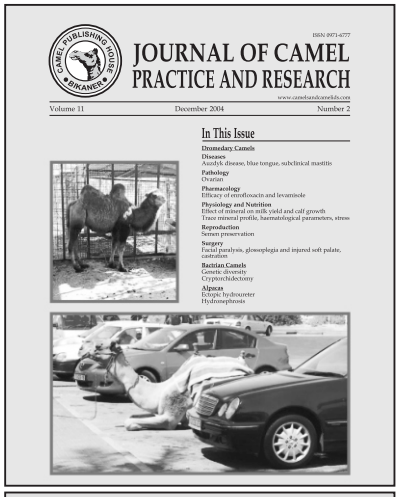

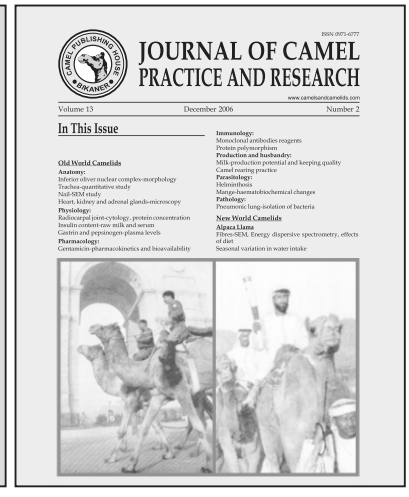

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MOLECULAR IDENTIFICATION OF TICK-BORNE ZOONOTIC BACTERIA IN ONE HUMPED CAMEL (*Camelus dromedarius*)

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ABSTRACT

In this study, the presence of tick-borne zoonotic bacteria (*Borrelia* spp., *Coxiella burnetii* and *Rickettsiae* spp.) in camel blood samples were examined using by PCR. A total of 50 blood samples were collected from one humped dromedary camels found in western side of Aegean region which had tick infestation in their anamnesis. Three pathogens, *viz Borrelia burgdorferi*, *C. burnetii* and *Rickettsiae* spp. were detected in camel blood samples. A single PCR was performed for the *C. burnetii* and multiplex PCR for the *Rickettsiae* spp. and *Borrelia burgdorferi*. At the end of PCR study, *C. burnetii* was identified at the rate of 4% out of 50 blood samples. *Borrelia burgdorferi* and *Rickettsiae* spp. were not detected from all blood samples. The results showed that tick-borne zoonotic pathogens may come up with risk factors for *Camelus dromedarius* population. For this reason, it is important to increase microbiological studies for *Camelus dromedarius* populations.

Key words: Bacteria, *Camelus dromedarius*, PCR, Tick-borne, zoonotic diseases

Bacterial tick-borne diseases (BTBDs) alter the outcome of livestock animals in different geographical places of the world leading to a significant adverse impact on the production of resource-poor farming communities (Kırkan *et al,* 2017). *Amblyomma* spp., *Hyalomma* spp., *Boophilus decoloratus*, *Rhipicephalus* spp. and *Ornithodoros savignyi* were recorded from camels with tick infestations in earlier studies (Banaja and Ghandour, 1994; Kaufman *et al,* 1996; Anwar and Khan, 1998). Since there is no long tail and superficial back muscles in the camels, they are extremely sensitive to bite and stings of ticks.

Among tick-borne bacteria, extracellular spirochetes of the genus *Borrelia* are widely seen in various geographical locations and the vast majority of researches of tick-borne bacteria have been carried out about this microorganism (Korenberg *et al,* 2002). Some of these species belong to the *Borrelia burgdorferi sensu lato complex,* are causative agents of Lyme borreliosis (Raoult *et al,* 2001). In addition, intracellular alpha-proteobacteria, which includes the families *Anaplasmataceae, Bartonellaceae* and *Rickettsiaceae* could be transmitted by ticks (Dumler *et al,* 2001). Q Fever has been presented in blood materials of infected camels in Iran (Doosti *et al,* 2014). Another previous study reported that transmission of *C. burnetii* in camel appeared to be through the faeces followed by urine in Saudi Arabia (Mohammed *et al,* 2014). Camels may present an intensive infestation of ixodid ticks and *Hyalomma dromedarii* is the most frequent tick species reported to infest camels (Abdel-Shafy *et al,* 2012; Fard *et al,* 2012). The presence of *R. aeschlimannii* and *Rickettsia africae* in Hyalomma ticks in camels from Africa and the Sinai Peninsula was previously reported (Morita *et al,* 2004; Abdel-Shafy *et al,* 2012).

The purpose of this study was to reveal the presence of tick-borne diseases, Rickettsiosis, Borreliosis and Q Fever infections, from camels by the Polymerase Chain Reaction (PCR).

Materials and Methods

This study was conducted in Aydin province of Aegean region of Turkey. One third of Turkey's camel population is located and extensively raised in this area. The samples were collected from 50 dromedary male camels which were produced for trophies and folkloric wrestling. Tick infestations were observed majorly on gluteal region of the camels. A total 50 blood samples were collected from jugular vein of the tick infested camels. Samples were immediately transported to Department of Microbiology laboratory and kept at −20°C until laboratory diagnosis.

This research was carried out in accordance with the International Guiding Principles for

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Biomedical Research Involving Animals developed by the Council for International Organisations of Medical Sciences (CIOMS) of 1985 and in accordance with local laws and regulations.

DNA Extraction

A DNA extraction kit was used for extraction. Blood samples were treated with proteinase K enzyme for 1 hour prior to extraction and then DNA extractions were performed from the samples using a DNA extraction kit (Fermentas®) designed for the isolation of genomic DNA. Extraction procedure was carried out in accordance with manufacturer's recommendation. DNA extracts were stored in cryo-tubes at -20°C until PCR studies.

Primers

In this study, *Coxiella burnetii* (Trans 1-Trans 2), SFG Rickettsiae (Rr190.70p-Rr190.602n), *Borrelia* spp. (BORF-16S) and *B. burgdorferi sensu lato* (23SN2-5SCB) primers were designated to manufacturer as previously reported (Barandika *et al,* 2007).

Extracted DNA (100–200 ng) was subjected to one single and the other multiplex PCR amplifications, one for the detection of *C. burnetii* and a 2nd for SFG *Rickettsiae* and *Borrelia* spp. PCR conditions are detailed in previous studies (Berri *et al,* 2000; Barandika *et al,* 2007).

Detection of C. burnetii

The PCR was performed in a thermocycler (Eppendorf – Mastercycler personal) in a total reaction volume of 50 µl, containing 5 µl of 10X PCR buffer (10 mMTris-HCl, pH 9.0, 50 mM potassium chloride, 0.1 per cent Triton X-100), 5 µl 25 mM magnesium chloride, 250 µM of each deoxynucleotide triphosphate, 2 U of Taq DNA polymerase (MBI Fermentas), 1 µM of each primer and 5 µl of template DNA. The PCR amplification was carried out under the reaction conditions described (Berri *et al,* 2000). The 'touchdown' PCR assay was performed under the following conditions: 5 cycles consisting of denaturation at 94°C for 30 s, annealing at 66±61°C (the temperature was decreased by 1°C between consecutive steps) for 1 min and the extension at 72°C for 1 min and then 40 cycles consisting of denaturation at 94°C for 30s, annealing at 61°C for 30 s and extension at 72°C for 1 min. The reference strain of *C. burnetii* Nine Mile Strain Phase I ATCC® RSA 411 was used as positive control in the PCR; for negative control, *Escherichia coli* ATCC® 25922 was also used to ensure that contamination did not take place during the assays. (Kırkan *et al,* 2008).

Detection of SFG Rickettsiae and Borrelia spp.

The multiplex PCR was examined to the 100 to 200 ng template DNA. Multiplex PCR was performed in a thermocycler (Eppendorf – Mastercycler personal) in a total reaction volume of 30µl with 10x PCR buffer (10 mMTris-HCl, pH 9.0, 50 mM potassium chloride, 0.1 per cent Triton X-100), 25 mM magnesium chloride, 330 µM of each deoxynucleotide triphosphate, 2 U of Taq DNA polymerase (MBI Fermentas). BORF-16Sand Rr190.70p-Rr190.602n primers were used at a concentration of 30 pmol/ reaction mixture for multiplex PCR and 23SN2-5SCB primer was used at 5 pmol/reaction mixture for PCR. Cycling conditions for multiplex PCR were 3 min at 94°C; 50 cycles each of 10s at 94°C, 1 min at 46°C and 1.5 min at 72°C; and a final elongation of 7 min at 72°C. For 23SN2-5SCBPCR, the cycles consisted of 1 min at 94°C; 50 cycles each of 30 s at 94°C, 30 s at 57°C and 1 min at 72°C; and a final elongation at 72°C for 5 min (Gil *et al,* 2005).

Detection of the Amplified Products

The 10 µl amplified products were detected by staining with 0.5 µg/ml ethidium bromide after electrophoresis at 80 V for 40 min in 2 % agarose gel.

Results and Discussion

Positive amplification was obtained, using the primers which amplify the repetitive transposon-like regions of *C. burnetii*, from blood of the camels investigated in this study. The screening of electrophoresis gel was carried out via UV illuminator imaging system. PCR results revealed the positive bands 687 bp in 2 out of 50 samples for *C. burnetii*. *C. burnetii* positivity was detected in 4% of the blood samples (Fig 1).

However, none of the samples collected from camels revealed positive amplification for *Rickettsiae* spp. and *Borrelia* spp.

The present study is the first report on the direct detection of *Borrelia* spp., *Coxiella burnetii* and *Rickettsiae* in camels found in Aegean region of Turkey. Given the geographical and climatic conditions in which Turkey is located, various ticks within borders continue to exist. The ticks complete the part of the life cycle in camels, especially in the materials that make up study material of this research. In addition, camels are natural hosts for *Hyalomma* spp. In this process, it is evaluated that the camels may transmit the tick-borne bacterial zoonotic infections and infects people with various ways (Kırkan *et al,* 2017).

C. burnetii has previously been described by PCR in faeces, urine, milk and blood samples in Saudi Arabia and Iran (Doosti *et al,* 2014; Hussein *et al,* 2015; Pirouz *et al,* 2015). It is estimated that positivity rates were 20-30% in these studies and regarded as high when compared to our study and it is predicted that the density of the camel populations was high and the hygienic quality was poor in the subjected studies.

The dromedary camels that are produced in our region is fed individually and it is determined that the hygiene procedures are given importance according to anamnesis from animal owners and the condition of animals. Disease transmissions from animal to animal have been eliminated in this sense and it has come to the conclusion that the ticks infesting the camels have also transmitted Coxiellosis.

In previous studies (Pirouz *et al,* 2015), it has been reported that seropositive animals for coxiellosis do not show clinical signs and sporadic abortion cases may occur in female animals. In our study, pneumonia and cardiac arrhythmias were diagnosed as a result of the general examination in the animals that were positive for the end of coxiellosis in the PCR. It was concluded that the coxiellosis disease could be detected by PCR in the acute phase according to the clinical findings and positive blood samples.

C. burnetii has been detected using different PCR methods in blood samples of infected camels in Iran previously (Doosti *et al,* 2012), where partial 16S ribosomal RNA gene was amplified at the rate of 10.8%.

Positive amplification was detected using the primers which amplify the repetitive transposon-like repetitive regions of *C. burnetii*, from blood

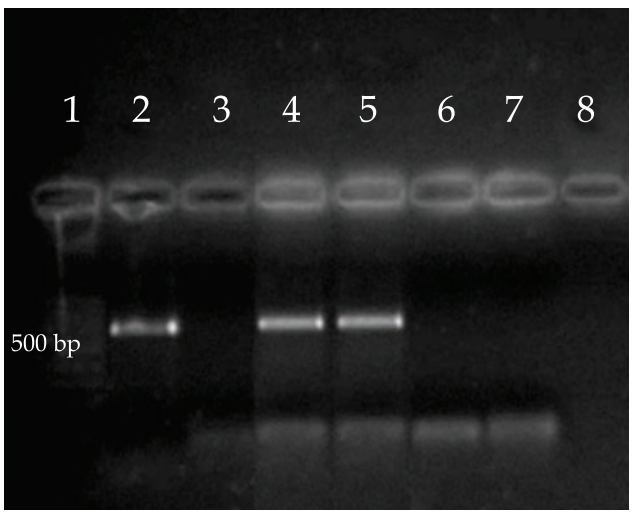

Fig 1. *Coxiella burnetii* electrophoresis gel image.

of the camels investigated in the present study. Similar to our results, a research conducted in Saudi Arabia (Mohammed *et al,* 2014), has reported positive amplification using repetitive transposon-like and transposase regions of *C. burnetii* in the ratio of 15.9%.

Coxiellosis in camels has been detected in animals older than 7 years of age in Saudi Arabia, Iran and Afghanistan. Considering the age range in this study, it was determined that the age of animals positive for Coxiellosis was 9 and 10 and it is shown that adult animals are more susceptible to acute infection.

In our study, the camels were produced for wrestling and agro touristic purposes, so the group of the subjects was male animals. Therefore, an evaluation of the effect of gender determinant on the distribution of infectious diseases was not made.

Given the population density of tick populations in our country, the importance of tick-borne bacterial zoonoses and the adaptation of methods for diagnosing these diseases to the site conditions are also gaining importance. In addition to conventional methods, the development of fast and safe methods will ensure the detection of diseases in a short period of time and will contribute to the country's economy through treatment and prophylactic measures to be made as an early diagnosis. In addition, infection of tick-borne bacterial zoonoses will be prevented and community health will be preserved.

It is obvious that the tick control strategies are important in preventing the spread of Coxiellosis disease, which is a zoonotic disease in Turkey, especially in the Aegean Region. It is also recommended to take necessary measures to prevent the spread of tick-borne diseases in camel breeding, which has its place in terms of agro tourism. In conclusion, the current study reports significant findings suggesting that ticks may infect camels with Coxiellosis.

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PARASITIC PROFILE OF SAUDI ARABIAN CAMELS

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ABSTRACT

The prevalence and biology of *Toxoplasma, Neospora,* mange, ticks and gastrointestinal parasites among Saudi Arabian camels (*Camelus dromedarius*) were investigated. The results of the study revealed that 35.8% (N=190) were *Toxoplasma* seropositive and 40% (N=169) were *Neospora* seropositive. Examination of 2449 camels showed that 61.7% (N=1512) were infested with hard ticks of *H. dromedarii* and *H. anatolicum* and 15.6% (N=382) were infected with Sarcoptic scabiei. Out of 203 camels 31.5% (N=64) were found to be infested with gastrointestinal parasites. The parasites encountered were *Haemonchus, Camelostrogylus, Trichostrongylus, Nematodirs* and coccidian spp.

Key words: Camels, parasites, Saudi Arabian

There is increasing evidence that parasitic infections are the most common wide spread diseases in camels. Evidence come from many publications on prevalence, distribution and harmful effect of parasites from different parts of the world. Parasitism, too is a limiting factor in the production of milk and meat (Al-Ani and Vestweber, 2004). In a previous study (Al-Afaleq *et al,* 2015), it was shown that an overall prevalence of *T. evansi* in Kingdom of Saudi Arabia (KSA) was 0.8% based on parasitological examination and 39.4% based on serological examination. This study continues the previous one and addresses further, the parasitic profile of camels of KSA.

Materials and Methods

A total of 190 samples from different regions of Saudi Arabia were collected for *Toxoplasma* investigation. The number of samples from eastern, central and southern regions was 50 each and 45 from the western region. For Neospora study, 169 camels were examined. The number of samples taken from eastern, central, western and southern regions were 50, 40, 29 and 50, respectively. Those camels comprised 4 different categories, *viz.* pastoral camels, camels presented to veterinary clinics, animal market places or slaughterhouses. All of the camels were bled by jugular venipuncture into plain vacutainer tubes (Becton-Dickson, NJ, USA). Sera were kept at -20°C until used.

The ELI Tex Toxo agglutination kit (ELI Tech Group, Puteaux, France) was used to detect IgG antibodies to toxoplasmosis. The CIVTEST *Bovis Neospora* kit (HIPRA-CIVTEST, Girona, Spain) containing sonicate lysate of tachyzoites was used to detect antibodies to neosporosis. The tests were performed as described by the manufacturers.

Individual faecal samples collected from 203 camels distributed equally among the 4 regions of KSA were examined for gastrointestinal parasites by floatation, sedimentation and Baerman-Wetzel method (Maff, 1986).

Samples for tick and mange were collected from 2449 camels distributed equally among the 4 regions of KSA. Camels infested with ticks were investigated by visual examination. The ticks from infested animals were collected in labeled vials containing ethyl alcohol (70%). The adult male ticks were identified under stereomicroscope according to standard identification keys (Walker *et al,* 2003). Skin scrapings from clinically suspected cases of mange were collected and preserved in 10% formalin. Potassium hydroxide (KOH) 10% was then added to the sediment to digest or clean the scraped material of skin, hair and other debris, so that mites were released from scabs. Finally, the specimens were carefully placed on slides for microscopic examination. Identification of the mange mite species was based on the morphological characteristics described by Urquhart *et al* (1996).

Results and Discussion

Seroprevalence of Toxoplasma infection

The results of this study shows that the overall seroprevalence of *Toxoplasma* infection among the

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survey population of camels in the KSA was 35.3% (N=190). Highest infection rate (78%) was reported among camels of the southern region while lowest infection rate (11.1%) occurred amongst camels of the western region. Infection rate varied from 51% in camels tested in veterinary clinics to 20% in camels presented at the market places. The details of these results are depicted in table 1. It can be noted that the lowermost infection rate (4%) was detected in slaughter camels of the eastern region whereas, the highest infection rate (85%) was recorded for camels attended at the veterinary clinics in the southern region.

Seroprevalence of Neospora infection:

The overall prevalence of *Neospora* infection in the survey population was 40% (N=169). The infection rate varied with category and location of the examined camels (Table 2). Camels of the eastern region exhibited the lowest infection rate (26%) while the camels of the southern had the highest (64%) rate of infection with this parasite. It is also shown that camels sampled at the veterinary clinics had the highest (55%) infection rate and those bled at marketplaces presented the lowest (28%) infection rate. Highest prevalence (65%) of Neospora infection occurred in camels visiting the veterinary clinics in the southern region while lowest (6%) prevalence was reared in the fields or farms in the western region.

Tick Infestation:

Table 3 shows the number of camels infested with hard ticks. About 61.7% of total number of camels were infested with ticks. About 38% of camels were infested in western region whereas, 58.9 to 70.3% of camels were infested with ticks in other region. The highest percentage of infestation (66.5%) being in slaughter house and the lowest value was reported to be in farm camels. The predominant types were *H. dromedarii* and *H. anatolicum*.

Mange infection:

The percentage mange infection in 2449 camels is given in table 4. It shows that 15.6% of camels were infected with mange. The distribution ranged from 9.5% in eastern region to 22.2% in western region. Based on the category, mange infection was shown to be 30.5% in clinics and 10.5 to 17.7% in other categories. The causative agent being *Sarcoptes scabiei.*

Worm burden:

Frequency distribution of gastrointestinal parasites in 203 camels is shown in Table 5. About 31.5% of animals were infected with parasites; 26.6% were round worms and 4.9% were coccidian parasite. The round worm included *Haemonchus, Camelostrongylus, Trichostrongylus* and *Nematodirus* species and the coccidian was *Eimeria cameli*.

An earlier survey was conducted mainly on epidemiology of trypanosomosis in camels in KSA (Al-Afaleq *et al,* 2015). This study continues the previous study and focuses on the prevalence of *Toxoplasma*, *Neospora,* ticks, mange and gastrointestinal parasites in camels.

The present study demonstrated an overall prevalence of 35.3% toxoplasmosis of camels ranged

Table 1. Frequency distribution of seropositive camels tested for anti-*Toxoplasma* antibodies according to category and location in the Kingdom of Saudi Arabia.

Region / Category	Eastern	Central	Western	Southern	Total
Field & farms	2/11 (18%)	4/11(36%)	1/10 (10%)	16/22 (73%)	23/54 (43%)
Slaughterhouses	1/24 (4%)	6/12 (50%)	1/18 (6%)	6/8 (75%)	14/62 (23%)
Veterinary clinics	2/10 (20%)	4/11 (36%)	2/8 (25%)	17/20 (85%)	25/49 (51%)
Marketplaces	3/5 (60%)	1/16 (6%)	1/4 (25%)	Not done	5/25 (20%)
Total	8/50 (16%)	15/50 (30%)	5/45 (11.1%)	39/50 (78%)	67/190 (35.3%)

Table 2. Seroprevalence of Neospora antibodies in camels by category and location in the Kingdom of Saudi Arabia.

Region / Category	Eastern	Central	Western	Southern	Total
Field & farms	5/17 (29%)	3/13 (23%)	1/16 (6%)	14/22 (64%)	23/58 (40%)
Slaughterhouses	3/15 (20%)	2/10 (20%)	4/9 (44%)	5/8 (63%)	14/42 (33%)
Veterinary clinics	4/9 (44%)	4/9 (44%)	3/6 (50%)	13/20 (65%)	24/44 (55%)
Marketplaces	1/9 (11%)	2/8 (25%)	4/8 (50%)	Not done	7/25 (28%)
Total	13/50 (26%)	11/40 (28%)	12/29(41%)	32/50 (64%)	68/169 (40%)

between 4 to 85% in different categories and regions of KSA. This reflects the wide spread of the parasite. Reports refer to the wide spread of Toxoplasmosis among camels with 13.6% in Saudi Arabia (Al-Anazi, 2012), 25.2% in Iraq (Mahmoud, 2014), 4% in Iran (Sadrebazzaz *et al,* 2006), 17.4% in Eygpt (Hilali *et al,* 1998), 8.3% in Ethiopia (Gebremedhin *et al,* 2016), 10% in Pakistan (Chaudhry *et al,* 2014a) and 25.9% in Sudan (Elamin *et al,* 1992) are available. The infection is thought to occur through ingestion or inhalation of sporulated oocysts that are shed by cats in the environment (Elamin *et al,* 1992). The infection has been demonstrated in many domestic livestock, wild life, dogs and cats in most areas of the world (Tenter *et al,* 2000). Interestingly, in Sudan where a 49.7% prevalence was reported, tachyzoites were excreted in milk which may pose a possible source for human infection (Medani and Mohamed, 2016).

The infection rate of Neospora was 40% which was varied with category and location. Antibodies to neospora in our study were much higher than those found in 3.7% of Egyptian camels (Hilali *et al,* 1998), 3.3% of Iranian camels (Sadrebazzaz *et al,* 2006) and 5.6% of Saudi camels in Central Region of KSA (Al-Anazi, 2011) which require further research probably in the line of experimental neosporosis. Neosporosis is the major disease of cattle causing abortion and dogs are the only known definitive hosts (Dubey, 2003). Furthermore, in Pakistan an overall 11.1% prevalence with 26.1% infection rate in females with abortion history was reported (Nazir *et al,* 2017), that suggests a thorough investigation of the role of Neospora as pathogen in the camel.

Mange in camels through its high morbidity is rated 2nd only to trypanosomosis in importance (Chhabra and Gahlot, 2010). The overall prevalence of camel mange mite infestation studied here was 15.6%. This value is higher than the values given by Dinka *et al* (2010), Lawal *et al* (2007) and Chaudhry *et al* (2004b) who reported 10.7, 3.5 and 3.14% prevalence in Ethiopia, Nigeria and Pakistan, respectively and lower than a prevalence of 83% (Al-Ani *et al,* 1998) in Jordon and 31.5% (Feyera *et al,* 2015) in Ethiopia. Variation in genetics, environment and husbandry practices could justify these differences. The herd size, too may contribute to spread of infection as animals living in larger herds tend to come into contact with infested animals. The identified species in this case was *S. scabiei* as reported elsewhere (Dinka *et al,* 2010; Awol *et al,* 2014; Lawal *et al,* 2007).

Table 3. Per cent tick infestation in 2449 camels according to category and location in the Kingdom of Saudi Arabia.

Region / Category	Eastern	Central	Western	Southern	Total
Field & farms	20	22.2	19.4	31.9	25.7(39/152)
Slaughterhouses	60.2	70	81.8	61.9	66.5(1404/2110)
Veterinary clinics	28.6	58.1	23.3	30	37.6(32/85)
Marketplaces	36.8	31.4	39.6	Not done	36.3(37/102)
Total	58.9	64.7	70.3	38	61.7(1512/2449)

Table 4. Per cent mange infection in 2449 camels according to category and location in the Kingdom of Saudi Arabia.

Region / Category	Eastern	Central	Western	Southern	Total
Field & farms	4	7.4	6.5	15.9	10.5(16/152)
Slaughterhouses	9.4	15	17.7	33	15.2(322/2110)
Veterinary clinics	21.4	29	30	50	30.5(26/85)
Marketplaces	21.1	17.1	16.7	Not done	17.7(18/102)
Total	9.5	15.5	17.6	22.2	15.6(382/2449)

Table 5. Frequency distribution of gastrointestinal parasites in camels by type and location in the Kingdom of Saudi Arabia.

Region / Type of Parasite	Eastern (N=50)	Central (N=55)	Western (N=45)	Southern (N=53)	Total (N=203)
Round Worms	8(16%)	20(36.4%)	15(33.3%)	11(20.8%)	54(26.6%)
Coccidia	2(4%)	3(5.5%)	3(6.7%)	2(3.8%)	10(4.9%)
Total	10(20%)	23(31.5%)	18(40%)	13(24.5%)	64(31.5%)

Regarding hard ticks, this survey showed a 61.7% prevalence of tick infestation in camels and together with sarcoptic mange may represent the main external parasites of camels in KSA. Ticks cause anaemia, skin injury and can act as vectors for some pathogenic agents. A rate of 97% was found in Somalia with the largest dromedary population (Isse, 2017). Even in smaller population of camels like in Namibia, South Africa ticks are found to be the main external parasite (Horak *et al,* 2018). In this study *H. dromedarii* and *H. anatolicum* were the predominant tick species found on infested camels. A frequency of 70.7% of *H. dromedarii* and 4.8% of *H. anatolicum* was found in Iranian camels (Fard *et al,* 2012; Moshaverinia and Moghaddas, 2015). In a cross-sectional study in Ethiopia, 98.6% of camels were found infested with ticks, *Hyalomma* species contributed 23.3% (Klros *et al,* 2014). *Hyalomma dromedarii* is a thermophilic tick species which is capable of surviving in climate similar to hot dry regions of KSA. The species has a role in transmission of *Theleria annulata* (Chisholm *et al,* 2012), that may pose a potential threat to other animals as the tick is not host-specific.

The results of gastrointestinal parasites show 31.5% prevalence. Al-Megrin (2015) reported 59.6% prevalence in KSA depending on geographical area. Camels may acquire such infections by grazing on infected pastures, by ingestion of infective larvae in drinking water or by arthropod vector. Several investigators have reported occurrence of different helminths in camels in different parts of the world (Bekele, 2002; Dia, 2006; Schuster and Wernery, 2004; Cirak *et al,* 2011; Ibrahim *et al,* 2016; Magan *et al,* 2017). Of 31%, 26.6% were round worm and 4.6% coccidian. Al-Megrin (2015) reported prevalence rate of coccidian parasite 7.1% in Central Region of KSA. The results indicated that all camels were found carrying mixed infections with different parasite genera. The parasites encountered being *Haemonchus, Camelostrongylus, Trichostrongulus* and *Nematodirus* spp. Similar pattern of parasitic occurrence in KSA was reported by Magzoub *et al* (2000), who suggested a seasonality factor contributing to frequency of incidence of parasites.

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SCIENTISTS CONFIRM 'MAD COW DISEASE' IN CAMELS SPARKING FEARS IT COULD BE PASSED TO HUMANS

A prion disease similar to that which causes "mad cow disease" in cattle has been discovered in camels, raising fears that it could be passed to humans. A study published in the Journal of Emerging Infectious Disease by an international team of food safety scientists and veterinarians confirmed the fatal degenerative disease in camels in Algeria and called for urgent action to safeguard both animal and human health. "Our identification of this prion disease in a geographically widespread livestock species requires urgent enforcement of surveillance and assessment of the potential risks to human and animal health," say the authors. Laboratory tests positively confirmed Camel Prion Disease (CPD) in three animals, suggesting the disease was present in 3.1 per cent of the camel population presented for slaughter at the local abattoir.

(Source: The Telegraph News)

NUCLEAR TECHNIQUES HELP DIAGNOSE CAMEL DISEASE IN THE MIDDLE EAST

An emerging disease affecting camels and people is threatening the Middle East and its neighbours. Last month, the IAEA in partnership with the Food and Agriculture Organisation of the United Nations (FAO) trained veterinary laboratory staff from this region on how to detect and diagnose the disease using nuclear and nuclear-derived techniques. Veterinary diagnosticians from Bahrain, Iraq, Kuwait, Lebanon, Saudi Arabia and the United Arab Emirates met at the FAO/IAEA laboratories in Austria. International experts trained them in modern molecular virus detection techniques (see Genetic sequencing) to detect and control the virus. The techniques that these veterinary workers learned will help them accurately distinguish this virus in a matter of hours. By contrast, when using traditional techniques, the disease takes several days or even weeks to spot.

(Source: www.iaea.org)

RESEARCHERS TURN TO UAE CAMELS TO HELP FIND HIV CURE

The Central Veterinary Research Laboratory (CVRL) in Dubai has played an important role in research looking at whether antibodies produced by camels could be used to combat HIV. It was thought that camels might prove useful in the fight against HIV because part of the antibodies they produce are much smaller than those generated by most other animals, including humans. These "nanobodies", are derived from antibodies consisting solely of two heavy chains of amino acids – the protein building blocks – while typically antibodies are made of two heavy chains and two light chains. They also have some features typically found in broadly neutralising human antibodies, which are effective against multiple HIV strains.

Dr Dietrich's laboratory provided the CVRL with "spike"-like proteins from the HIV "envelope", which is located on the surface of the virus and responsible for its infectivity. These envelope proteins are exposed to the immune system and are the target of neutralising antibodies. The proteins were derived from HIV subtype C, the virus's most common form. At the CVRL, these envelope proteins were injected once a week for six weeks into four camels to stimulate an anti-HIV immune response. "The camel recognises it as a foreign body and wants to get rid of it and it produces antibodies," said Dr Ulrich Wernery who took blood samples from the camels and these were analysed by the researchers, who were interested in the type of nanobodies that had been produced.

More than two dozen nanobodies were identified by Dr Dietrich's group and tested in the laboratory against various "subtypes" of HIV. When the actions of two nanobodies were added together, VHH-A6 and VHH-28, they were effective against 19 out of 21 strains of the most common and harmful type of HIV, known as HIV-1. The other main type of the virus, HIV-2, is largely restricted to West Africa and is less potent in its ability to cause illness.

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PURIFICATION AND THERMAL DENATURATION KINETICS OF SERUM ALBUMIN IN BACTRIAN CAMEL MILK

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ABSTRACT

Serum albumin is a non-specific transporter protein. It is the 2nd most abundant whey protein present in camel milk, with significantly higher content than in milk from other animals. In this study, we isolated and purified camel serum albumin (CSA) from milk by DEAE-Sepharose FF and Sephacryl S-100 gel filtration chromatography and checked its purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and ultra-performance liquid chromatography (UPLC). The main band of the purified product on SDS-PAGE corresponded with the location of a bovine serum albumin standard. In UPLC, the purified product presented a single sharp peak, which indicated it was a homogenous preparation. Amino acid sequence analysis of the purified protein confirmed it was CSA. Subsequently, we analysed the thermal denaturation of CSA from 70 to 95°C for 10, 20, 30, 40 and 50 min. CSA had upper stability under 75°C, the residual rate range over 70.25% to 87.12%. The degree of denaturation increased with temperature and time, but small range of fluctuations between denaturation rate. The residual rate of CSA, heated 10 min at 95°C, was about 58.34%. After CSA was heated 50 min at 95°C, it was not entirely denatured. These results suggested that CSA could maintain high stability in dairy products treated by low-temperature long-time or high-temperature short-time heating treatment and the reaction order of heat denaturation was 1.9. This study provides reference and application data for further research on CSA.

Key words: Bactrian camel milk, purification, serum albumin, thermal denaturation, thermal kinetic analysis

Camel milk is of great interest in the dairy industry because of its unique nutritional properties. In many countries and regions, camel milk is applied as an adjuvant therapy (Korish *et al,* 2015) or to prevent hepatitis (El-Fakharany *et al,* 2017), allergy (Rubino *et al,* 2014), diabetes (Kebir *et al,* 2017) and hepatitis (El Miniawy *et al,* 2014). These medicinal properties are attributed to the special components of the milk. In addition, compared with milk from other mammals, camel milk contains more protein, less fat and casein N and markedly higher whey protein nitrogen (WPN) (Zhang *et al,* 2005). It also contains many protective proteins such as α-lactalbumin, IgG, lactoferrin and SA at higher levels than other milk (Kosa *et al,* 1998; Gollopa *et al,* 2001). Camel serum albumin (CSA) is the 2nd highest component in camel milk whey, ranging from 0.3 to 0.46 mg/mL of whey protein (Farah, 1986; Zhang *et al,* 2005; Levieux *et al,* 2006; Ereifej *et al,* 2011; Omar *et al,* 2016). In recent years, the purification, characterisation and immunochemical quantification of CSA have been reported by independent studies (Levieux *et al,* 2006; Malik *et al,* 2013; Felfoul *et al,* 2015). However, most focused on CSA in camel serum; studies on camel milk are still limited.

The stability of proteins is of vital importance in biotechnology; the maintenance of their functional characteristics *in vitro* is an issue of both fundamental and practical significance. The stability of most proteins is affected strongly by temperature, pH and ionic strength. In the presence of denaturants and high temperatures, native proteins can unfold and lose biochemical function (Kopito, 2000; Serio *et al,* 2000; Villaverde and Mar Carrió, 2003; Markossian and Kurganov, 2004; Oldfield *et al,* 2005). However, heat treatment is an essential process in dairy production, e.g. for yogurt, milk powder and ultra-high temperature milk, to render milk safe for consumption and prolong its shelf life (Oldfiel *et al,* 2005; Felfoul *et al,* 2015b). Excessive heating will inevitably denature the major protein components of milk such as α-lactalbumin, lactoferrin, SA and so on, so the thermal stability, as a basis for protein

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processing application, which has importantly theoretical and practical significance. It has been reported that SA has high thermal stability, second only to α-lactalbumin in camel milk (Felfoul *et al,* 2015a). However, the protein denaturation was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and no data was provided to support this result (Elagamy, 2000; Felfoul *et al,* 2015b).

In this study, an effective method was developed to isolate and purify CSA from camel whey and then analysed the thermotolerance of CSA using the thermal denaturation kinetics.

Materials and Methods

Materials and samples

Milk samples were collected from 6 Alxa Bactrian camels on a local farm in Alxa right banner, Inner Mongolia, China. An ÄKTA purifier, DEAE-FF column (25 × 16 mm) and Sephacryl S-100 column (60 × 1.6 cm) were obtained from GE Healthcare (Uppsala, Sweden). SDS-PAGE assembly and an ultra-performance liquid chromatography system (UPLC) were from Bio-Rad (CA, USA) and AB SCIEX (USA), respectively. Protein molecular weight markers (10 to 170 kDa) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were purchased from Thermo Fisher Scientific (NY, USA). Amicon Ultrafree-MC centrifugal filter devices with a Biomax®50 membrane (size exclusion 50,000 Da) were from Millipore (Bradford, MA, USA). All chemicals used were of analytical grade.

Preparation of milk samples

Camel milk was defatted by centrifugation at 5000 × g for 30 min. Casein was precipitated and isolated by decreasing the pH to 4.3 with 1 M HCl (Yang *et al,* 2013). After centrifugation at 4000 × g for 30 min at 4°C (Omar *et al,* 2016), the supernatant (whey) was dialysed against 20 mM Tris-HCl buffer (pH 8.2), freeze-dried and stored at −20°C until use (El-Hatmi, 2015).

Purification and identification of CSA in Bactrian camel milk

Ion-exchange chromatography

CSA was purified by anion exchange chromatography on a DEAE-FF column equilibrated in 20 mM Tris-HCl buffer, pH 8.2 (El-Hatmi *et al,* 2006). The volume of the packed gel was about 5 ml. Frozen whey was thawed at 4°C, then filtered through a 0.22-µm filter and injected onto the DEAE-FF column (Levieux *et al,* 2006). The bound proteins were eluted from the column with a linear gradient of 0.1–0.4 M NaCl in the same buffer, at a flow rate of 1 ml/min, collecting fractions of 2.0 ml (Neyestani *et al,* 2003). The absorbance of the eluate was monitored at 280 nm. Fractions were by concentrated ultrafiltration.

Gel filtration chromatography

CSA was purified using a Sephacryl S-100 gel filtration column equilibrated and eluted with 20 mM Tris-HCl, pH 8.2. The flow rate was 1 ml/min. The fraction size collected was about 2 ml and detection was at 280 nm. Fractions were concentrated by ultrafiltration.

Purity of CSA analysed by SDS-PAGE and UPLC

The purity of CSA was checked by SDS-PAGE and UPLC (Salihabr *et al,* 2013; Halima *et al,* 2014). After each purification step, the CSA was checked by SDS-PAGE. Electrophoresis was run with a 5% stacking gel and a 15% separating gel. Protein samples (40 µL) were mixed with 10 µL 5× SDS-PAGE loading buffer and, after heating for 3 min at 95°C, 20 µL sample was loaded onto the gel. SDS-PAGE was run at 90 V for about 30 min until the bromophenol blue reached the separating gel and then run at a constant 120 V until the bromophenol blue dye was about 0.5 cm from the bottom of the gel. After electrophoresis, gels were stained with Coomassie Brilliant Blue G250. Destaining was done with a mixture of ethanol (5% v/v), glacial acetic acid (10% v/v) and distilled water (85% v/v) until the background colour was totally removed. Protein bands were analysed using a gel imaging system.

Homogeneity of the purified CSA was determined by UPLC and a Mabpac RP analytical column (Thermo Fisher). Mobile phase A was water containing 0.1% formic acid and mobile phase B was acetonitrile containing 0.1% formic acid (Malik *et al,* 2013). CSA was bound to the column and eluted with a linear gradient of mobile phase B. The elution was performed at a flow rate of 0.2 µL/min with detection at 280 nm.

Amino acid sequence analysis

With reference to the method of Nagaraj *et al* (2012) and Thakur *et al* (2011), the amino acid sequence of CSA was analysed by LC-MS/MS. Trypsin was added to 200 µg CSA at a mass ratio of 1:50 (trypsin:CSA) for overnight enzymolysis at 37°C. The mixture was desalted using a Sep-Pak C18

solid phase extraction column. Desalinated peptides were loaded on to a C18 column. For analysis of CSA by LC-MS/MS, mobile phase A was 97.9% water containing 0.1% formic acid and 2% acetonitrile and mobile phase B was 98% acetonitrile containing 0.1% formic acid and 1.9% water. The peptides were separated with a segmented gradient containing mobile phase B at 2% (v/v) for 0–1 min, 6% (v/v) for 1–65 min, 25% (v/v) for 65–75 min, 80% (v/v) for 75–85 min and 5% for 80–90 min, with a flow rate of 5 µL/min. The purified protein was identified by the peptide mapping using LC-MS/MS analysis and NCBI database searching (Zhao *et al,* 2013; Gromova *et al,* 2017).

Heat treatment

The CSA was dissolved in phosphate-buffered saline (pH 7.4, 0.01 M) to prepare a 1.50 mg/mL solution (Laleye *et al,* 2008). Then, sample was added to a test tube and incubated at 70, 75, 80, 85, 90 or 95°C in a water bath (± 0.1°C) (Felfoul *et al,* 2015a). Heated samples, in triplicate, were taken out of the bath at different intervals and immediately cooled by immersion in an ice-water bath. According to Salami *et al* (2008) method and also an improved, a BCA protein assay kit with bovine serum albumin (BSA) as the standard was used for the determination of protein concentration. The residual amount of CSA was calculated by the formula:

$$\text{Protein residual rate (\%)} = C/C_0 \times 100\% \quad \text{(Eq. 1)}$$

where C is the concentration of protein after heating (Levieux *et al,* 2006; Sanchez *et al,* 2006) and C_0 is 1.50 mg/mL (the initial protein concentration).

Kinetic analysis

Order of reaction and denaturation rate constant

The rate equation for thermal denaturation of CSA can be expressed as:

$$-dC/dt = k \cdot C^n \quad \text{(Eq. 2)}$$

where –dC/dt is the rate of protein denaturation, k is the denaturation rate constant, C is the concentration of protein at each holding time and n is the order of the reaction.

When n = 1:

$$-dC/dt = k \cdot C;\ -dC/C = k \cdot dt$$

by integrating, we obtain

$$\ln(C_t/C_0) = -kt \quad \text{(Eq. 3)}$$

When n≠1, integrating the general rate Eq. 2, we obtain:

$$(C_t/C_0)1-n = 1 + (n-1)k't \quad \text{(Eq. 4)}$$

$$\text{where } k' = kC_0^{n-1}$$

Then, for n = 0, 1.1, 1.2, …, 1.8, 1.9 and 2, respectively, Eq. 4 yields straight lines, from which the coefficients of correlation R2 and the rate constants of denaturation can be calculated. The order of the reaction, n, can be calculated from the means of the coefficients of correlation R2 (Sanchez *et al,* 2006).

Calculation of thermodynamic parameters

D values, *i.e.* the time required for 90% protein denaturation, were calculated for each temperature by regression analysis, as the inverse of the slope of lines obtained by plotting the logarithm of residual native protein (%) as a function of heating time. The Z value is a temperature change value that reduces the D value by 90%. This value was calculated by regression analysis as the reciprocal of the slope of the line obtained from the natural logarithm of D values vs. the corresponding temperatures (Mainer *et al,* 1997; Levieux *et al,* 2006).

Calculation of Kinetic Parameters

In the process of thermal denaturation, the denaturation rate constant and the temperature of treatment are related according to the Arrhenius equation (Skelte and Anthony, 1996; Oldfiel *et al,* 1998):

$$k = Ae^{-Ea/RT} \quad \text{(Eq. 5)}$$

where k is the rate constant for the denaturation process, A is the Arrhenius constant, E_a is the apparent activation energy of the reaction, R is the ideal gas constant and T is the absolute temperature.

Eq. 5 takes the logarithm:

$$\ln k = \ln A - (E_a/R)(1/T) \quad \text{(Eq. 6)}$$

When the natural logarithm of the denaturation rate constant is plotted *vs.* the reciprocal of the absolute temperature according to Eq. 6, a straight line is obtained by linear regression. The Ea value may be obtained from the slope and the lnA value from the ordinate intercept. These values can be used to determine the thermodynamic parameters enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) for the protein denaturation by using the following equations:

$$\Delta H = E_a - RT \quad \text{(Eq. 7)}$$

$$\Delta S = R[\ln A - \ln(k_a/h_p) - \ln T] \quad \text{(Eq. 8)}$$

$$\Delta G = \Delta H - T\Delta S \quad \text{(Eq. 9)}$$

where k_a is the Boltzmann constant and h_p is the Planck constant.

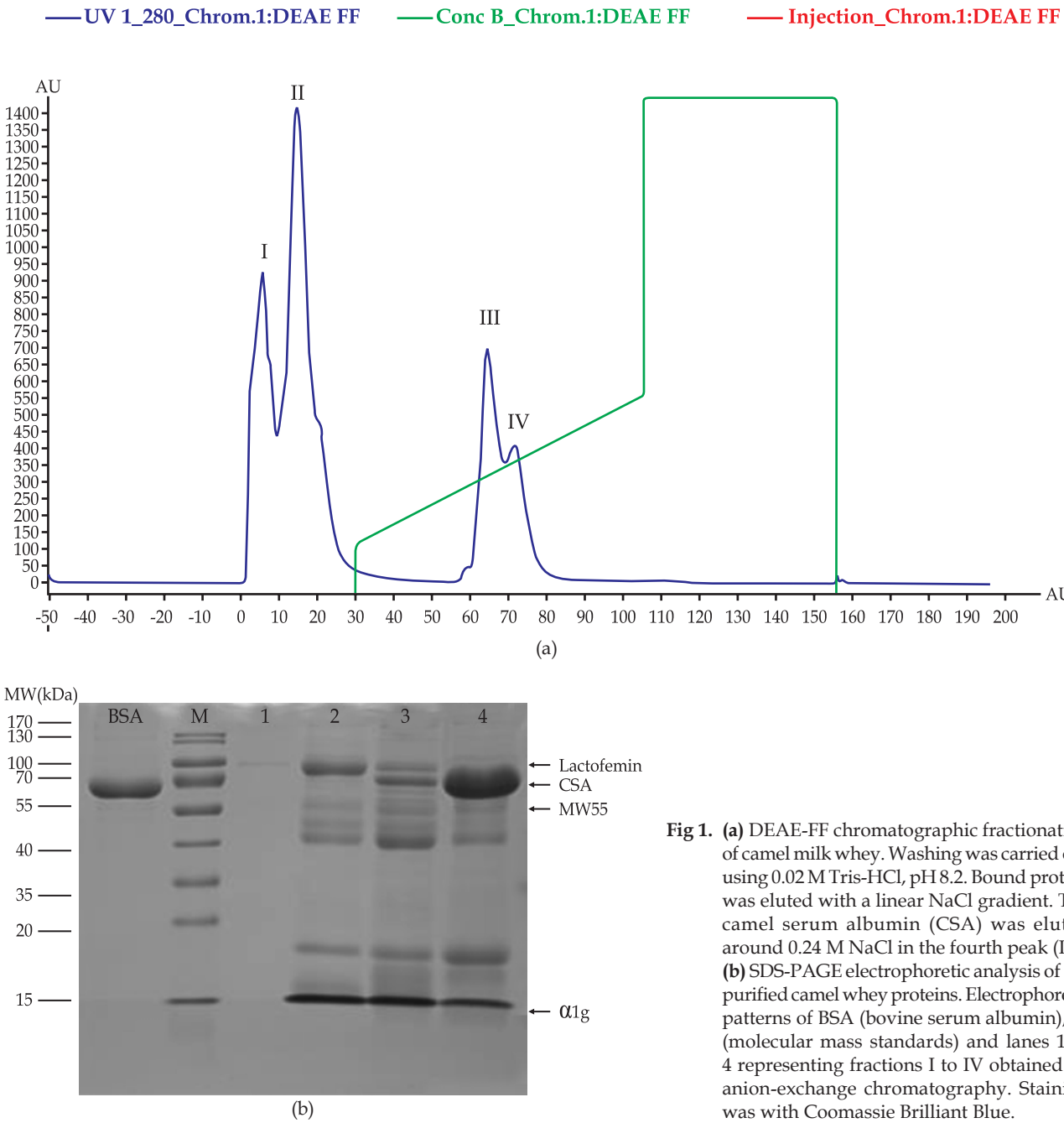

Fig 1. **(a)** DEAE-FF chromatographic fractionation of camel milk whey. Washing was carried out using 0.02 M Tris-HCl, pH 8.2. Bound protein was eluted with a linear NaCl gradient. The camel serum albumin (CSA) was eluted around 0.24 M NaCl in the fourth peak (IV). **(b)** SDS-PAGE electrophoretic analysis of the purified camel whey proteins. Electrophoretic patterns of BSA (bovine serum albumin), M (molecular mass standards) and lanes 1 to 4 representing fractions I to IV obtained by anion-exchange chromatography. Staining was with Coomassie Brilliant Blue.

Results and Discussion

Purification and detection of CSA from Bactrian camel milk

CSA from Bactrian camel milk was purified by DEAE-FF anion-exchange chromatography on an ÄKTA-PURE system (Fig 1a). The anion exchange chromatography method was based on linear-gradient elution. Four peaks were obtained in the anion-exchange chromatograph of camel whey resolved by DEAE-FF. The SDS-PAGE patterns of the 4 fractions are shown in Fig 1b. In lanes 1 to 4 of the SDS-PAGE, it was possible to observe protein bands with apparent molecular weights (MW) of 14, 20, 42, 50, 66 and 80 kDa, respectively. From the literature, the bands of 14, 66 and 80 kDa were assigned as α-lactalbumin, CSA and lactoferrin, respectively (Farah, 1986; Levieux *et al,* 2006; Malik *et al,* 2013; El-Hatmi *et al,* 2015; Omar *et al,* 2016). As can be seen from the SDS-PAGE pattern, most of the CSA eluted around 0.24 M NaCl-in the peak IV of the chromatogram-but the CSA was not pure (*i.e.,* it remained contaminated with other proteins).

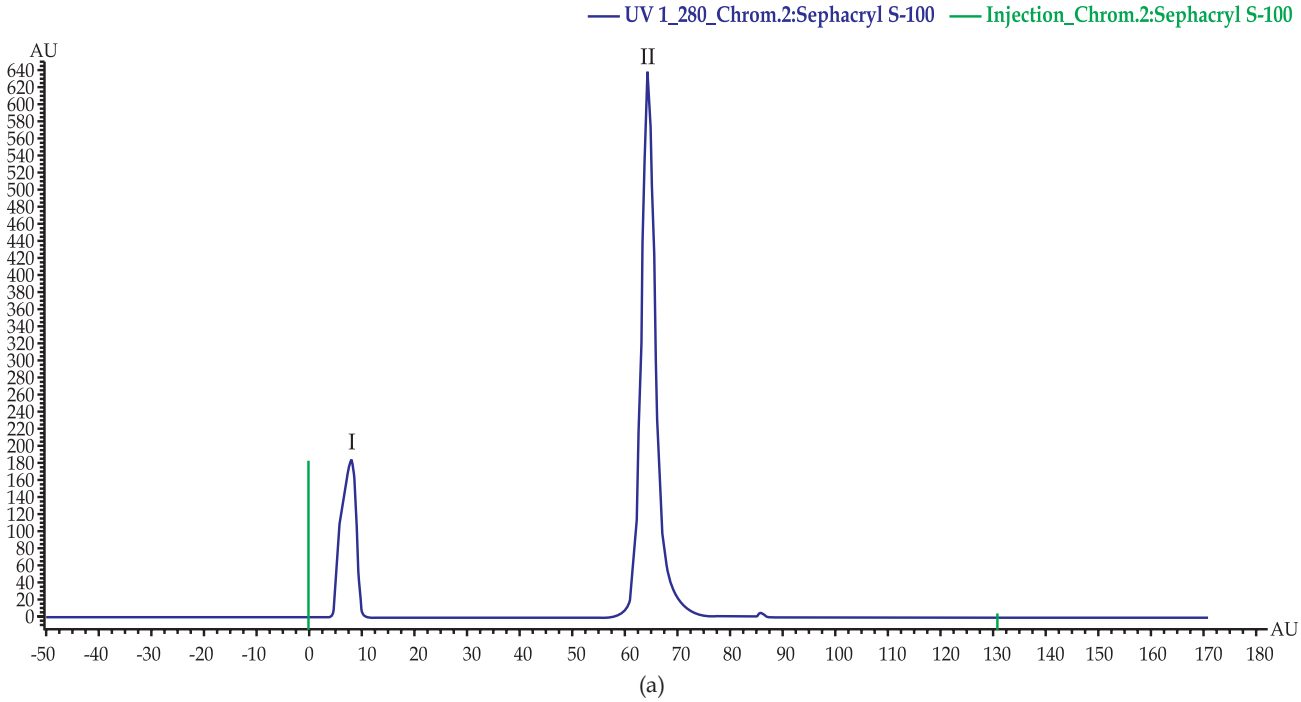

Fig 2. **(a)** Sephacryl S-100 chromatographic fractionation. The sample obtained from the fourth peak (IV) of anion-exchange chromatography was loaded to the column. The column was washed with 20 mM Tris-HCl, pH 8.2. CSA was eluted in the second peak (II). **(b)** SDS-PAGE electrophoretic analysis of the purified CSA. Electrophoretic patterns of BSA (bovine serum albumin), M (molecular mass standards) and fraction II from the Sephacryl S-100 column.

Therefore, the fractions of the 4th DEAE-FF peak (peak IV) were pooled and loaded onto a Sephacryl S-100 gel filtration column. Two major fractions were obtained on elution of this column (Fig 2a). By SDS-PAGE, the second peak (peak II) showed a relatively pure band with apparent molecular weight around 66 kDa (Fig 2b). The results agreed with a previous report about purified albumin from dromedary serum (Farah, 1986; Malik *et al,* 2013). We concluded that we had purified the target protein, CSA.

To ensure the reliability of subsequent experiments, the homogeneity and purity of the CSA were tested by UPLC. The purity of the protein was quantitatively determined by the area normalisation method in which the larger the ratio of the target peak area observed, the purer the protein. As Fig 3 shows, the purified CSA presented a single, sharp peak. The peak retention time for purified CSA was 9.145 min. Empower data processing software analysis showed that the purity of the CSA was >95% after the DEAE-

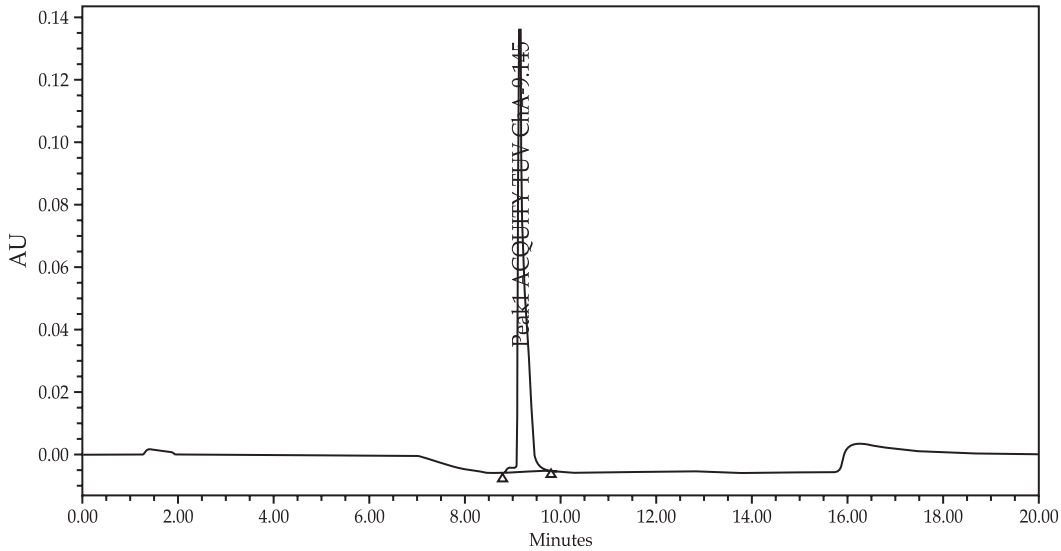

Fig 3. Ultra-performance liquid chromatography (UPLC) analysis of CSA. Purified CSA was loaded onto MAbPac RP analytical columns. CSA showed a single peak.

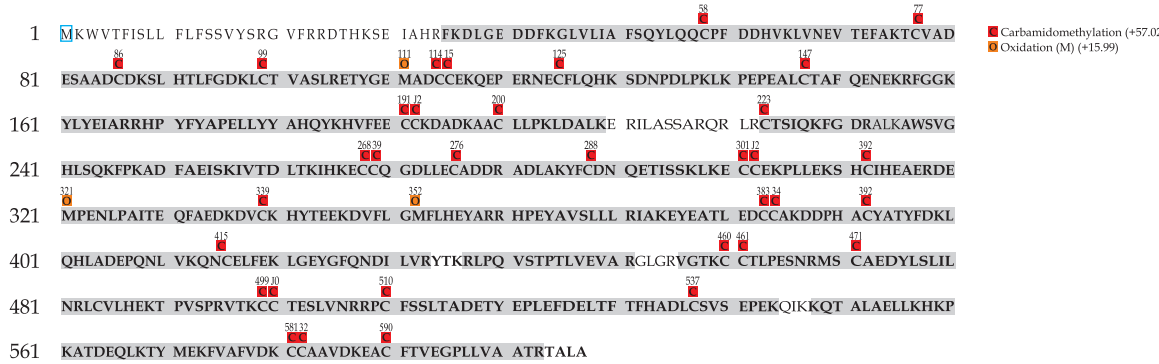

Fig 4. The database amino acid sequence of CSA (NCBI accession no. XP_006188754) was compared with that determined experimentally by liquid chromatography-tandem mass spectrometry from purified protein. Bold indicates matched peptides. The sequence coverage was 89%. Carbamidomethylation of cysteine residues was considered a fixed modification, oxidation of methionine.

FF and Sephacryl S-100 gel filtration chromatographic steps. Thus, the purification protocol was feasible. Moreover, the purified CSA was not subjected to high concentrations of salt that may cause conformational changes.

Confirmation of CSA by LC-MS/MS

The purified CSA was treated with trypsin and its amino acid sequence was then analysed by LC-MS/MS. As Fig 4 shows, multiple fragments of CSA were identified. PEAKS studio 8.5 database search software was used to analyse the homology of the amino acid sequences compared with the *Camelus ferus* SA sequence in the NCBI database (NCBI: XP_006188754). The sequence coverage of CSA by LC-MS/MS was 89%. This confirmed that the purified protein was CSA.

Effect of heat treatment on CSA

We studied the thermal stability and thermal denaturation kinetics of CSA. We heated CSA at 70, 75, 80, 85, 90 and 95°C for different time periods. The degree of CSA thermal denaturation increased significant with time and temperature of treatment (Fig 5). As shown in Fig 5, CSA denatured slowly and was little affected by heating at 70°C, with the residual amount of intact protein at 10, 20, 30, 40 and 50 min being 87.12%, 86.84%, 84.87%, 84.04% and 82.66%, respectively. This was in accordance with data from Felfoul *et al* (2015a), who reported no significant changes in WP gel patterns on heating camel milk at 70°C for 30, 60, 90 or 120 min. Denaturation of CSA was markedly higher at 80°C than at 70°C, but there was no significant change

between 80°C and 95°C on heating for 10 to 50 min. These results were consistent with those of Levieux *et al* (2006).

The D values and the Z value were calculated and are presented in table 1. The D values decreased with increasing temperature (Fig 6). CSA denatured approximately 5 times faster at 80°C than at 70°C. When the temperature was increased to 95°C, the D value was 111 min. Levieux *et al* (2006) indicated that the residual rate of α-lactalbumin and camel serum CSA, heated 10 min at 90°C, were about 9% and 55%, respectively. These results suggested that camel milk CSA has relative good thermotolerance.

Table 1. D values (min) at different temperature and Z value (°C) for CSA.

Temp (°C)	70	75	80	85	90	95
D value (min)	1000	500	200	167	143	111
Z value (°C)	27.03					

Kinetic analysis - order of reaction and degeneration rate constant

Eqs. 3 and 4 were used to analyse denaturation as a function of time at each temperature to determine the order of reaction (n) for the thermal denaturation of CSA (Table 2). The mean value of the correlation coefficient was highest when n was 1.9, so the order of reaction of the thermal degradation of CSA from Bactrian camel milk was determined to be 1.9. According to Levieux *et al* (2006), the reaction order for the denaturation of CSA in camel serum was 2, whereas Xiao *et al* (2010) showed that the reaction order for BSA was 1.8. Through this research and based on previous studies, we abused that the thermal denaturation reaction order is different for SA from different sources. In addition, because of the complexity of the heating reactions occurring in milk, the reaction order is also dependent on the composition of the reaction medium (Oldfiel *et al*, 1998).

Table 2. The correlation coefficients of reaction progression for CSA thermal denaturation (R^2) at different temperatures.

n	70°C	75°C	80°C	85°C	90°C	95°C	Mean
0	0.687	0.741	0.783	0.787	0.813	0.824	0.773
1.0	0.707	0.779	0.850	0.866	0.875	0.909	0.831
1.1	0.709	0.783	0.856	0.872	0.877	0.912	0.835
1.2	0.711	0.787	0.861	0.878	0.879	0.913	0.838
1.3	0.713	0.791	0.866	0.884	0.879	0.913	0.841
1.4	0.715	0.795	0.871	0.889	0.879	0.912	0.844
1.5	0.717	0.799	0.876	0.894	0.877	0.909	0.845
1.6	0.719	0.803	0.880	0.899	0.875	0.905	0.847
1.7	0.710	0.806	0.884	0.903	0.872	0.900	0.846
1.8	0.722	0.810	0.887	0.906	0.868	0.894	0.847
1.9	0.724	0.814	0.890	0.909	0.864	0.887	0.848
2.0	0.726	0.818	0.893	0.912	0.859	0.600	0.801

Table 3. The rate constant (k) and the ordinate intercept (b) for CSA and correlation coefficient (R^2) when plotting ln (C_t/ C_0) versus holding time (n = 1.9).

Temp (°C)	70	75	80	85	90	95
k (10^{-3})	2.208	4.417	11.042	13.251	19.140	27.974
b	1.054	1.085	1.117	1.123	1.066	1.028

Table 4. Energy of activation (E_a) and changes in enthalpy of activation (ΔH), entropy of activation (ΔS) and free energy of activation (ΔG) of CSA at different temperatures (n = 1.9).

Temp (°C)	70	75	80	85	90	95
Ea (kJ/mol)	104.091					
ΔH (kJ/mol)	101.238	101.196	101.155	101.113	101.072	101.030
ΔS (kJ/mol·K)	0.00808	0.00796	0.00784	0.00772	0.00761	0.00750
ΔG (kJ/mol)	98.465	98.425	98.386	98.347	98.309	98.271

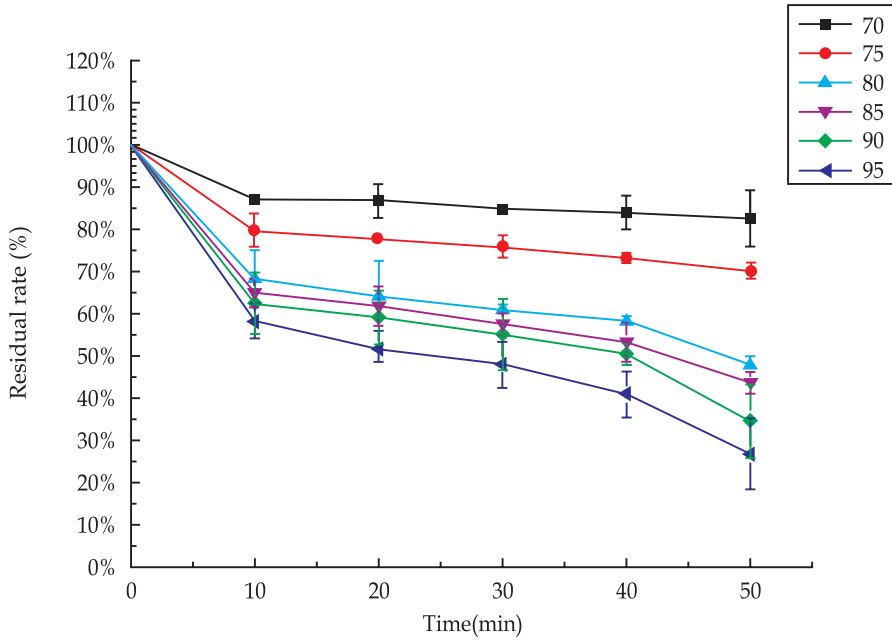

Fig 5. Thermal denaturation of CSA in the range 70–95°C after heating for 10, 20, 30, 40, or 50 min. Unheated CSA was used as a blank control (residual rate of 100%).

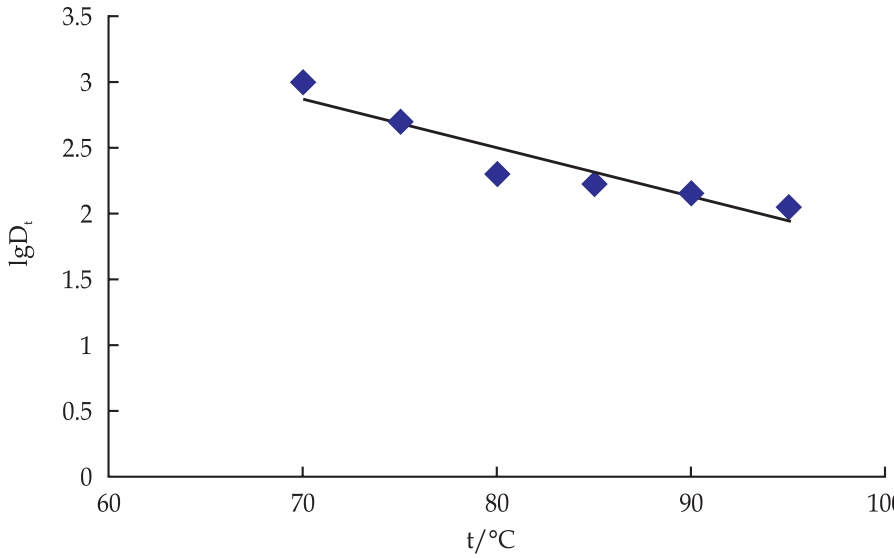

Fig 6. Effect of temperature on D values (time for 90% denaturation) in denaturation of CSA.

For n = 1.9, the values of the rate constant (k) and the ordinate intercept (b) for the denaturation of CSA at each temperature were determined (Table 3). It can be seen from these data that the denaturation rate constant increased with increasing temperature, which was consistent with previous studies (Kundu *et al,* 2016).

Thermodynamic parameters

Thermodynamic parameters of the denaturation were obtained using the Arrhenius equation (Eq. 5). A regression equation was obtained when plotting ln(k) *versus* the reciprocal of the absolute temperature for n = 1.9 (Fig 7). A linear relationship was observed, which allowed the activation energy (Ea) to be calculated, as well as enthalpy of activation (ΔH), entropy of activation (ΔS) and free energy of activation (ΔG) for the thermal denaturation of CSA, calculated using Eqns. 6, 7 and 8 (Table 4). The values obtained were typical for heat induced protein denaturation reactions (Felfoul *et al,* 2015a). In our experiments, ΔH, ΔS and ΔG decreased with increasing temperature. This result was consistent

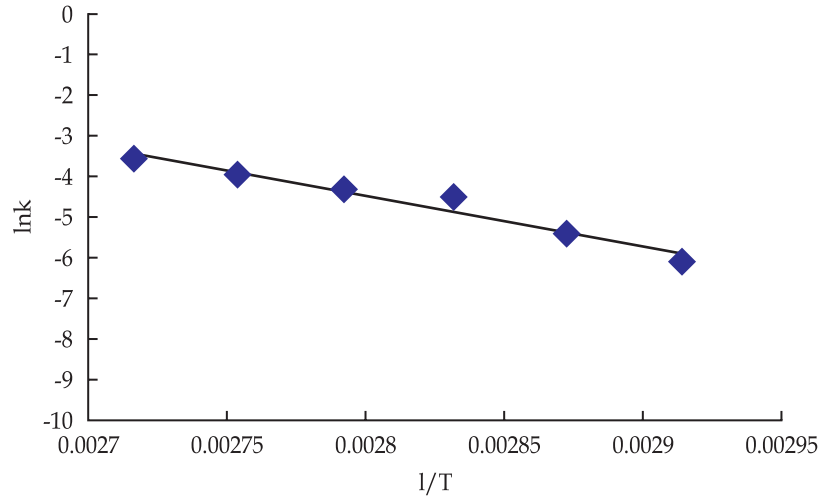

Fig 7. Arrhenius plot for denaturation of CSA from camel milk. 1/T represents the reciprocal of the absolute temperature (unit K^{-1}).

with the research of Levieux *et al* (2006) and Mainer *et al* (1997). However, our values were much smaller than those previously reported (Mainer *et al,* 1997; Levieux *et al,* 2006). In the heat treatment temperature range, *i.e.,* 70 to 95°C, the ΔH values were 101.238 to 101.030 kJ/mol and the ΔS values were 0.00808 to 0.00746 kJ/mol•K. The low ΔH and ΔS values reflect an aggregation process in which intermolecular bonds were formed and the order of the system was thus increased (Skelte and Anthony, 1996). Recent research also showed that CSA denaturation reactions were involved in the formation of insoluble protein aggregates after heating camel milk (Felfoul *et al,* 2015a).

This is the first study on the isolation, purification and thermal stability of CSA in Bactrian camel milk.

Which provides an efficient purification protocol for CSA and also provides a theoretical basis for further research into the structure and structure-function relationship of the protein. However, there are still some aspects which need to be clarified. We should analyse the mechanism of the thermal denaturation and compare the protein with the SA in camel serum. Based on its unique characteristics, CSA might be an alternative to human and bovine serum albumin in the future.

Acknowledgements

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CONCENTRATIONS OF NUTRIENTS IN SIX MUSCLES OF BACTRIAN (*Camelus bacterianus*) CAMELS

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ABSTRACT

Differences between muscles in concentrations of proximate composition, minerals, cholesterol, amino acids, fatty acids and vitamins for the *Infraspinatus* (IS), *Triceps brachii* (TB), *Longissimus thoraces* (LT), *Biceps femoris* (BF), *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles of 9 bactrian camels (2-3 years of age) were investigated. The composition of lean bactrian camel was shown to be highly desirable with a high nutrient density for many nutrients. Although lean meat samples from six muscles were similar in most nutrients detected, several significant differences were found. LT muscle had significantly higher dry matter and fat% than other muscles. The IS and LT muscles had significantly ($P<0.05$) higher cholesterol levels than TB, BF, ST and SM muscles. Concentrations of Myristic (C14:0), Palmitic (C16:0), Palmitoleic (C16:1) and Oleic acids (C18:1n9) were significantly ($P<0.05$) different between muscles. The LT muscle contained a significantly lower proportion of mono-unsaturated fatty acids than other muscles. The ratio of polyunsaturated to saturated fatty acids, which ranged from 0.40 to 0.50, was ≥ the minimum ratio of 0.40 recommended to reduce the risk of coronary diseases in humans. The amino acids and vitamin composition were similar for meat sample from six muscles. Consuming 150 to 200 g of camel meat will cover the daily requirement for an adult man weighing 70 kg for essential amino acids. This information on the nutritional value of camel meat is of great importance for promotion of the product.

Key words: *Camelus bacterianus,* camel, meat composition, meat quality, nutritive value, vitamins

Camel farming for meat production is growing due to its nutritional and health aspects. Camel meat can be considered as a new alternative healthy meat for human consumption (Bekhit and Farouk, 2013; Abrhaley and Leta, 2018). This may lead to an increase in camel meat consumption but the level of consumption is currently not comparable to that of other meats (Kadim *et al,* 2008). Meat is generally considered as a major source of fat in human diets, which is associated with various cancers and coronary heart diseases. Recently, there has been a lot of interest in camel meat because it contains relatively higher concentrations of long chain n-6 and n-3 polyunsaturated fatty acids than cattle and sheep meats (Kadim *et al,* 2008). Moreover, camel meat is believed to have medicinal properties (Bin Saeed *et al,* 2005; Kurtu, 2004; Abrhaley and Leta, 2018). Published evidence suggests that quality characteristics and nutritive value of camel meat are not much different from beef when slaughtered at comparable ages (Elgasim *et al,* 1987; Tandon *et al,* 1988; Kadim *et al,* 2011; 2013). However, utilisation of camel meat is hampered by a lack of knowledge about its nutritive value overall and within individual muscles. Few studies have been carried out on this aspect (Kadim *et al,* 2006, Kadim *et al,* 2008, 2011, 2013). The available information is mainly related to just a few camel muscles (Rawdah *et al,* 1994; Al-Bachir and Zeinou, 2009; Kadim *et al,* 2011; 2013).

Characteristics of individual muscles in beef, pork and sheep have identified certain muscles that can be marketed more successfully on an individual basis (Jones *et al,* 2000; 2001; Tschirhart-Hoelscher *et al,* 2006). Marketing on an individual-muscle basis may increase the demand for camel products, but such a marketing system requires more information

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on the nutritive value of individual muscles. Such information should permit more efficient marketing of camel meat and may encourage camel farmers to produce more attractive cuts with known quality characteristics.

In Kazakhstan, camel meat is one of the most important animal protein sources and is preferred in some parts of the country over meat from other animal species due to its quality and availability at affordable prices. However, there are no detailed studies in the nutritive value of the bactrian camel meat. The aim of this study was to determine the concentrations of minerals, cholesterol, fatty acids, amino acids and vitamins of *infraspinatus* (IS), *triceps brachii* (TB), *longissimus thoraces* (LT), *biceps femoris* (BF), *semitendinosus* (ST) and semimembranosus (SM) muscles of bactrian camels in Kazakhstan, with the aim to improve camel meat acceptability to consumers by providing more information about it.

Materials and Methods

Animals and Meat Samples

Nine intact male bactrian camels (2-3 years of age) were slaughtered at Kyzylorda camel farm in Kazakhstan. The IS, TB, LT, BF, ST and SM muscles were excised from the left side of each carcass within 20 min of slaughter. Each muscle was trimmed of external fat and the loose connective tissue and kept in a chiller (3-4°C) for 48 hrs and then stored at -20°C for subsequent nutritional composition analysis. Two-hundred grams from each muscle were cut up into small pieces and placed in plastic containers and dried in a Thermo freeze dryer (Thermo-Model Modulyo-23, Milford-UK) for 5 days under 80-mbar pressures at -60°C. They were then ground in a Cyclotech 1093 sample mill with a 0.5-mm screen and were placed in sealed plastic bags until analysed.

Proximate analysis

The chemical composition of the muscle tissue was determined by proximate analysis according to the standard methods of the AOAC (2000). The moisture was determined by weighing 200 g meat sample before and after drying in a thermo freeze dryer for 5 days (AOAC 950.46). Protein was determined using a Foss Kjeltec 2300 nitrogen/ protein analyser (method 976.95). Fat was determined by Soxhlet extraction method using petroleum ether (method 920.39). Ash content was determined by ashing samples in a muffle furnace at 500°C for 24 h (method 942.05).

Mineral Composition

Macro and micro mineral profiles of bactrian camel meat samples were estimated following 2 phases, digestion and analyses. Standard (1000 mg/L) solutions (Sigma-Aldrich; Chemie GmbH. Steinheim Germany and Sherwood: Paddocks, Cambridge, UK) were used to determine Ca, P, Mg, Na, K, Fe, Zn and Cu of the muscles. Digestion of 1 g freeze dried meat samples was completed 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. Ten ml of concentrated. HNO_3 was added to each digestion vessels and heated to 200°C over 30 minutes period. The digest obtained was collected in 100-ml volumetric flasks and made up to volume. Measurements of minerals (g/100g DM) were carried out on an 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).

Cholesterol Content

The cholesterol contents of muscles were determined according to a modified method of Bohac *et al* (1984). Briefly, 2 g of extracted fat sample from each muscle were saponified with 2 ml of 50% potassium hydroxide and 3 ml of 95% absolute alcohol and heated for complete solubilisation at 60°C for 15 min. When the mixture cooled, 5 ml of distil water were added and shaken for 1 min using vortex mixture. The non-saponifiable fraction was extracted 3 times using 5 ml of hexane. Three ml aliquots of hexane extracts were dried under a nitrogen flow at 50°C. The dried extracts was re-suspended in 3ml glacial acetic acid and thoroughly mixed then 2ml of $FeCl_3$ (Colouring solution) was added and the solution were allowed to stand for 30 min and the resultant colour was read at 565 nm using spectrophotometer model, Helions BETA, Thermo Spectronic, Cambridge, UK.

Fatty acid composition

The intramuscular fat content of each muscle sample was extracted following the method 991.36 (AOAC, 2000) using petroleum ether for 8 hrs. The fatty acid profiles were quantified following the method described by Ayerza *et al* (2002). Briefly, 0.2 g of this extracted fat sample was mixed with 4 ml of chloroform: methanol (2:1), 1 ml of internal standard

[henecosanoic acid (C21)] was added and the mixture was left overnight at -20°C. The mixture was then dried in a rotary evaporator at 40°C, suspended in 6 ml of diethyl ether, transferred to a test tube, dried under a stream of nitrogen, reconstituted with 1 ml of NaOH (0.5M), heated for 15 min at 100°C and then cooled in water. Two ml of BF_3/CH_3OH was added, mixed thoroughly, heated for 5 min at 100°C and cooled. One ml of hexane and 2 ml of distilled water were added, mixed for 15 seconds and centrifuged at 3000rpm for 5 min. The upper hexane layer was collected and extracted with 1 ml of hexane. Hexane extracts were passed over anhydrous Na_2SO_4 and transferred into a 2 ml GC vial, GC Agilent 6890N with flame ionisation detector were used to quantify fatty acids. Fatty acids were separated with a SUPELCO SP-2560 (100 m length x 0.250 mm I.D. x 0.200 um film thickness). Helium was used as a carrier gas at a constant flow of 1.0 ml/min. The injection and detector temperatures were 250°C and 255°C, respectively. The oven temperature programme was 80°C at a rate of 4°C / min–240°C held for 15 minutes. Fatty acids were identified by comparison of their retention times with that of the Heneicosanoic acid internal standard (ISTD). The total fatty acid content was calculated as mg/g = (area of sample/area of ISTD) × (amount of ISTD (mg) /sample weight (g) = mg/g. Sigma-Aldrich, CH9471, Buches, 081/755-25-11 (Germany) was used as standards to identify individual fatty acids. The long-chain n-3 PUFAs (viz EPA, DPA and DHA) have not been measured.

Amino acid analysis

Total amino acid composition of meat samples was determined using modified procedures from 3 methods (Maria and Toldra, 1991; White *et al,* 1986 and Wu *et al,* 2009. One gram of freeze-dried muscle sample was transferred to a 250 mL screw-cap bottle, then 25 µL of 2.5 mM Nle (L-Norlencine TLc rad, Sigma Aldrich, Biotech GmbH, 82024 Tantkirchen, Germany) and 10 mL of 6M hydrochloric acid phenol reagent were added to each bottle. The contents of each bottle were lightly vortexed and then placed in an oven (Oven 300 plus series, Gallenkamp, Midlands, Betton Road, Leicester) at 110°C for the 1st hour, the bottle cap was opened and then closed for the next 23 hr in the oven. After hydrolysis, the bottle was cooled down to room temperature and the cap carefully opened. The content was filtered into 50 mL volumetric flask, made up to 50 mL with HPLC water (Water Chromasolv for HPLC, Sigma Aldrich, Biotech GmbH, 82024 Taufkirchen, Germany) and 2 ml of the extract was poured into a 2 ml vial. Then 20 µL of methanol-0.5% sodium acetate triethanolamine (TEA, T58300 Sigma-Aldrich) (2:2:1) was added to each sample and dried again under vacuum. Twenty µL of methanol-water TEA-phenylisothiocyanate (PITC,139742 Sigma-Aldrich) (7:1:1:1) was added to each sample, the bottle was sealed, then vortexed and left to stand for 20 min at room temperature and again dried under vacuum. Finally, 500 µL of 5 mM sodium phosphate, pH 7.6, containing 5% acetonitrile were added to each sample to dilute and the liquid sample was filtered through a 0.22 µm membrane (Sartorius stedim Biotech GmbH, 37070 Gottingen, Germany) before injection. Total amino acids profile were analysed using a Dionex UltiMate 3000 High Performance Liquid Chromatography (HPLC) System with Diode Array Detector, equipped with a Dual Gradient Pump DGP-3600SD, an Inline-3000TSL Split Loop Auto-sampler, Thermostatted Column Compartment TCC-3000RS, Solvent Rack with Degasser SRD-3600, Thermostatted Column Compartment TCC-3000SD and controlled with Chromoleon 7, version 7.1. A Dionex Acclaim, 120 - C18 (3 µm particle size) column (3×150mm) (Thermo Scientific, Waltham, MA, USA). The mobile phase A was 0.14 M sodium acetate containing 0.5 mL/L of TEA adjusted to pH 6.4 with glacial acetic acid. The mobile phase B was 60:40 acetonitrile-water and filtered through a 0.45µm membrane. The temperature of the column oven was 40°C. The flow rate was 0.8 mL/min; the gradient program was as follows: the initial flow rate was 10% B; at 6 min, linear change to 12.5% B; at 32 min, linear to 58% B; at 33 min, step to 100% B; wash for 8 min and re-equilibrate at 10% B over 20 min before a new injection. The amount of individual amino acid in the sample was calculated by dividing the peak area of each amino acid by L-Norleucine butyric acid (internal standard), which was used to correct for losses during the hydrolysis analysis steps.

Vitamin determination

The water and fat soluble vitamin contents of muscle samples were determined using HPLC. All the chemicals and reagents used were of the highest purity available and purchased from Sigma-Aldrich (Chemie Gm6H Steinheim, Germany). Forty grams of fresh meat sample were mixed with 20 ml of hot water (100°C and blended (Black and Decker blender, model SC300,UK) to obtain an homogeneous sample and transferred to 100 mL amber glass bottle that was sealed. The bottle were placed in a boiling water bath

(100°C) for 30 min. Eight g of boiled sample were placed into a 50 ml centrifuge tube and 1 g of TCA was added and mixed thoroughly and centrifuged at 3000 rpm for 10 min to separate the two phases. Then 3 ml of 4% TCA were added to the upper layer (acid extracts) mixed and centrifuged at 3000 rpm for 10 min. The solid phase was discarded and the two acid extracts were combined and placed at −20°C for 10 min. The acid extracts were centrifuged at 4000 rpm for 5 min and placed at −20°C for 5 min. The layer of the fat was removed with a spatula and the acid extract was centrifuged again. Then the extract was filtered through a 0.45 µm filter before being injected into the HPLC. The standard solutions L-methionine 200 mg/L, ascorbic acid 600 mg/L, vitamin B_6 200 mg/L, vitamin B 200 mg/L and Riboflavin 2mg/L were prepared using eluent A which consisted of Potassium dihydrogen phosphate (0.005 M) and 5% v/v acetonitrile (HPLC grade) and adjusted to 5.6. pH Sonication and heating was used to prepare folic acid 2 mg/L and Riboflavin 2 mg/L, then both sets of standards were mixed and filtered through a 0.2 µm membrane filter prior to injection into the HPLC column. The vitamins were separated as described by Lebiedzinska *et al* (2007) except that the Acclaim C18 column 3 × 150 mm (µm particle size) was used. The mobile phase consisted of potassium dihydrogen phosphate (0.005M)-acetonitrile (5%) and potassium dihydrogenphosphate (0.005M)- all to 50%.

Statistical Analysis

Statistical analysis was carried out using the analysis of variance procedure (Ott, 1993) to evaluate the effect of muscle type (*infraspinatus, triceps brachii, longissimus thoraces, biceps femoris, semitendinosus* and *semimembranosus*) on proximate analysis, minerals, cholesterol, fatty acids, amino acids and vitamin concentrations. A General Linear Model procedure (PROC GLM; SAS, Institute, Inc., Cary, NC, USA, 1993) was used. A nested ANOVA model was used in which concentration of nutrients was nested within each muscle within each animal. Animal was used as the main plots and muscles as the subplots in split plot design. All statistical tests of LSM were performed for a significance level P<0.05. Significant differences between means were assessed using the least-significant-difference procedure.

Results and Discussion

Effect of type of muscle on chemical composition

This study revealed that variation between 6 muscles in certain proximate composition may be due to muscle physiological function and locations (Table 1). The moisture percentage of the IS, TB, ST, BF and SM muscles were significantly (P<0.05) higher than the value of LT muscle. The importance of moisture content in camel meat is in its marked effects on its shelf-life, processing potential and quality characteristics. Usually consumers prefer juicy over dry meat. There were no significant differences in moisture: protein ratio between the muscles. This study indicated that camel muscles content high dry matter to protein ratio, which reflects the suitability of camel meat for processing (Forrest *et al,* 1975). Similarly, Abdelhadi *et al* (2017) found dromedary camel LT contained high protein ratio. The LT muscle had significantly (P<0.05) higher fat percentage than other muscles possibly because of morphological attachment of LT to the hump (Babiker and Yousif, 1990). Kadim *et al* (2013) reported similar conclusion in dromedary camel muscles. These findings favour marketing of certain individual camel muscle due to its lower fat content. The ash content ranged from 0.9% for LT muscle to 1.1% for BF muscle with no significant differences between muscles.

Effect of type of muscle on mineral composition

The mineral composition of IS, TB, LT, ST, SM and BF muscles of bactrian camel are grouped into macro- and micro-mineral contents in table 2. There were no significant differences in calcium content between the 6 muscles. Similarly, Kadim *et al* (2013) reported no differences in calcium levels between 6 dromedary camel muscles. Insignificant variations in calcium content were also reported between different dromedary meat types (Badiei *et al,* 2006; Rashid, 2002; Elgasim and Alkanhal, 1992). However, calcium level in bactrian meat appears to be markedly higher than that in the Dromedary (Badiei *et al,* 2006; Rashed, 2002; Kadim *et al,* 2009).The level of variation between the bactrian and dromedary meats may be due to physiological factors such as adaptation to two different extreme low and high environmental temperatures with variation in type of feed intake, which may play a major role in determining the calcium contents in camel meat.

The LT muscle had significantly (P<0.05) lower phosphorus contents than other muscles, which might be due to biological role of each element in muscle physiology and function. El-Faer *et al* (1991) reported that the leg and shoulder dromedary muscles have slightly higher phosphorus than ribs muscles. The range levels of phosphorus content in bactrian muscles (272 to 393 mg/100g) were higher

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

Parameter	Muscle						
	IS	TB	LT	ST	SM	BF	SEM
Number of animals	9	9	9	9	9	9	
Moisture%	78.5[b]	78.4[b]	72.1[b]	78.0[b]	78.8[b]	78.5[b]	0.08
Protein%	18.0	17.6	17.0	18.8	18.2	18.3	1.49
Intramuscular fat%	2.5[a]	3.0[a]	10.0[b]	2.2[a]	2.0[a]	2.1[a]	1.41
Ash%	1.0	1.0	0.9	1.0	1.0	1.1	0.09
Moisture: protein	4.36	4.45	4.24	4.15	4.33	4.29	0.49

SEM: standard error for the mean. Means in the same row with different superscripts are significantly different (P<0.05).

Table 2. Macro- and micro-mineral contents of Bactrian camel *Infraspinatus* (IS), *Triceps brachii* (TB), *Longissimus thoracis* (LT), *Semitendinosus* (ST), *Semimembranosus* (SM) and *Biceps femoris* (BF).

	Muscle						
	IS	TB	LT	ST	SM	BF	SEM
Macro-mineral (mg/100g fresh tissue)							
Phosphorus	373[b]	375[b]	272[a]	390[b]	389[b]	393[b]	43.5
Calcium	13.7	13.4	14.5	14.02	14.7	13.9	9.87
Magnesium	37[a]	41a[b]	34[a]	44[b]	44[b]	45[b]	4.25
Sodium	149[b]	146[b]	135[a]	157[c]	151[b]	150[b]	19.2
Potassium	797[b]	803[b]	651[a]	804[b]	800[b]	799[b]	32.1
Micro-mineral (mg/100g fresh tissue)							
Iron	3.25	3.95	2.85	3.89	3.95	3.84	1.16
Zinc	5.72	5.61	4.88	5.74	5.69	5.78	1.59
Copper	4.11	4.01	4.41	3.89	3.95	3.91	0.95

SEM: standard error for the mean. Means in the same row with different superscripts are significantly different (P<0.05).

than the levels in the similar dromedary muscles (Bekhit and Farouk, 2013). The LT and IS muscles contained significantly (P<0.05) lower magnesium contents than ST, SM and BF muscles. In general, bactrian muscles appear to contain higher magnesium levels in comparison to dromedary camel muscles (Bekhit and Farouk, 2013). The sodium contents were significantly (P<0.05) higher in ST muscle and lower in LT within the muscles studied. The average of the sodium content in the bactrian muscles (148 mg/100 g) appears to be lower than the values found in dromedary muscles (Bekhit and Farouk, 2013). Similarly, Elgasim and Alkanhal (1992), Rashed (2002) and Kadim *et al* (2006) found that the loin region had the lowest sodium content among the different dromedary meat cuts studied. The potassium content was significantly (P<0.05) lower in the LT muscle (651 mg/100 g) than in other muscles. However, there were no differences between IS, TB, ST, SM and BF muscles, with values ranged between 797 to 804 mg/100 g. The potassium content in Bactrian LT muscle was greatly higher compared with value reported in dromedary muscle (Bekhit and Farouk, 2013).

There were no significant differences in micro elements between the muscles in the present study. Iron, zinc and copper levels were within the range of dromedary camels (Bekhit and Farouk, 2013). Similarly, Dawood and Alkanhal (1995) found small variations in zinc contents between different dromedary muscles. On the other hand, El-Faer *et al* (1991) and Rashed (2002) found large variations in between dromedary muscles. Copper contents in the dromedary meat ranged between 0.04 to 0.26 mg/100g (Bekhit and Farouk, 2013), which was lower than in the Bactrian meat. The foreleg of dromedary camel contained higher copper concentrate than other meat cuts (Rashed, 2002). Small variations in iron content in the Bactrian muscles might be due to the different physiological requirements of myoglobin of different muscle functions. Similar conclusions were reported by Rashed (2002), Kadim *et al* (2006, 2008; 2013) for dromedary muscles. However, the range of iron content in the present study was lower

that reported for the dromedary camel (Bekhit and Farouk, 2013). This may be due to different methods of analysis, age or location of meat samples. As with other red meat species, meat cuts containing oxidative muscles (e.g. leg and neck) has higher iron content than glycolytic muscles. Iron deficiency is the most prevalent nutritional disorder in the developing countries. The level of iron (haeme and non-haeme iron) in camel meat is of great importance due to the variation in iron bioavailability for human nutrition (Lombardi-Boccia *et al,* 2002).

Table 3. Cholesterol content (mg/100g) of the *Infraspinatus, Triceps brachii, Longissimus thoraces, Semitendinosus, Semimembranosus* and *Biceps femoris* muscles of bactrian camels (n = 9).

Muscle	Cholesterol level (mg/100)	SEM
Semimembranosus	43.2[a]	4.18
Infraspinatus	60.0[b]	3.94
Semitendinosus	49.0[a]	4.18
Triceps brachii	53.0[a]	3.88
Longissimus thoraces	59.1[ab]	4.23
Biceps femoris	49.7[a]	3.94
Average	52.3	

SEM: standard error for the mean. Means in the same column with no superscripts or with a common superscript letter are not significantly different (P < 0.05).

Effect of type of muscle on cholesterol content

Statistical analysis showed that the IS muscle had significantly (P<0.05) higher cholesterol contents than LT, TB, BF, ST and SM muscles (Table 3). The cholesterol concentration of the muscles was in the following order: IS >, LT > TB > BF > ST > SM. The small variation in cholesterol contents between muscles might be due to the amount of intramuscular fat and/or muscle fibre types. There is a variation between the 6 muscles in the amount of lipid and proportion of muscle fibre types (data not presented). Differences in muscle fibre types and intramuscular fat content have been reported to cause differences in cholesterol content of meat collected from different anatomical locations (Dinh *et al,* 2011). Oxidative muscle fibres (Type I, red muscle fibre types), smaller in diameter and contained high lipid tend to have more cholesterol (Alasnier *et al,* 1996). The range of cholesterol contents in the present study were similar to those reported by El-Magoli *et al* (1973) were 0.50 mg/100 g in the dromedary *longissimus dorsi* muscle. The current study supports the earlier finding that camel meat contained lower cholesterol levels than beef and lamb (Abu-Tarboush and Dawood, 1993; Elgasim and Elhag, 1992). The cholesterol contents in different Bactrian muscles in the current study were lower than those in lamb (Rowe *et al,* 1999), goat (Pratiwi *et al,* 2006), beef (Costa *et al,* 2006; Costa *et al,* 2009), chicken (Piironen *et al,* 2002; Rule *et al,* 2002) and deer (Polak *et al,* 2008) muscles. On the basis of a daily consumption of a 200 g steak, trimmed of all visible fats, except for intramuscular fat, camel meat provides 116 mg of cholesterol which represents 38% of the maximum daily cholesterol recommendations (<300 mg/day) (USDA, 2012).

Differences between muscles for intramuscular fatty acid composition

This study presented the total fatty acid content of muscles with a number of non-significant variables showing the relative amounts of different classes of fatty acids and individual fatty acids (Table 4). Palmitic acid (C16:0) was the most abundant saturated fatty acid in bactrian meat intramuscular fat with values of 55.6% followed by stearic acid (18:0) with values of 21.0% and myristic acid (C14:0) with values of 17.8% of total intramuscular saturated fatty acids. Similar conclusions were reported by Kadim *et al* (2011; 2013), Sajid *et al* (2015) and Abdelhadi *et al* (2017). While oleic acid (C18:1n9c) was the main monounsaturated fatty acids followed by linoleic acid (C18:2n6). The highest Palmitic acid (C16:0) values were found in TB and BF muscles, with values of 56.7 and 57.7%, respectively. Similar results were reported by Rawdah *et al* (1994); Al-Bachir and Zeinou (2009); Kadim *et al* (2011, 2013) and Abdelhadi *et al* (2017) for dromedary camel muscles.

The predominant monounsaturated fatty acids was oleic acid (C18:1n9), at levels around 83.6% of the total intramuscular monounsaturated fatty acids. Similar proportions were reported in the dromedary meat (Rawdah *et al,* 1994; Al-Bachir and Zeinou, 2009; Kadim *et al,* 2011, 2013; Abdelhadi *et al,* 2017). The lowest values were found in ST (82.1%) and TB (82.7%) muscles and the highest in LT (85.6%) and SM (84.3%). Kadim *et al* (2013) also found that the level of oleic acid was higher in LT muscle than the current study. Correlation test indicated that MUFA content was positively related (P<0.01) to C18:1n−9 (r=0.99).

Linoleic acid (C18:2n6) was the predominant polyunsaturated fatty acid in bactrian meat with an average of 69.2% of total intramuscular polyunsaturated fatty acids. The highest values were in BF (71.1%), IS (70.4%), TB (69.3%) and LT (69.3%) muscles. In agreement with the present findings, Rawdah *et al* (1994); Kadim *et al* (2013) and Abdelhadi *et al* (2017) found that more than 50% of

Table 4. Fatty acids composition of the *Infraspinatus* (IS), *Triceps brachii* (TB), *Longissimus thoraces* (LT), *Semitendinosus* (ST), *Semimembranosus* (SM) and *Biceps femoris* (BF) muscles of the Bactrian camel.

Fatty acid (% of total fatty acids)	Muscle						
	SM	IS	ST	TB	LT	BF	SEM
Saturated fatty acids (SFA)							
Lauric acid (12:0)	0.22	0.22	0.22	0.24	0.25	0.21	0.037
Myristic acid (14:0)	7.40	8.59	8.42	8.49	8.30	7.85	0.334
Pentadecanic acid (15:0)	0.53	0.62	0.52	0.58	0.55	0.53	0.182
Palmitic acid (16:0)	25.1	24.0	25.8	26.1	25.8	26.9	2.754
Margaric acid (17:0)	0.68	0.76	0.74	0.86	1.02	0.66	0.828
Stearic acid (18:0)	8.99	8.74	7.19	9.30	13.1	10.1	3.892
Arachidic acid (20:0)	0.19	0.17	0.17	0.19	0.22	0.18	0.078
Docosanoic acid (22:0)	0.04	0.04	0.04	0.04	0.04	0.04	0.002
Mono-unsaturated fatty acids (MUFA)							
Tetradecenoic acid (14:1)	0.24	0.25	0.24	0.20	0.12	0.20	0.112
Palmitoleic acid (16:1)	4.65	5.17	5.36	5.02	3.69	4.72	2.115
Heptadecenoic acid (17:1)	0.63	0.73	0.65	0.65	0.61	0.55	0.212
Oleic acid (C18:1n9)	29.4	30.2	28.9	28.4	26.0	28.2	3.112
Poly-unsaturated fatty acids (PUFA)							
Linoleic acid (C18:2n6)	14.7	13.9	14.4	13.6	14.1	13.2	1.783
α-Linolenic acid (C18:3n3)	1.22	1.26	1.19	1.25	1.19	1.10	0.412
Eicosadienoic acid (C20:2)	0.49	0.57	0.48	0.57	0.60	0.56	0.121
Eicosatetraenoic (C20:3n6)	0.75	0.46	0.52	0.69	0.75	0.68	0.102
Arachidonic acid (C20:4n6)	4.60	3.50	3.32	3.41	3.69	3.06	0.895
Total SFA	43.4	44.0	43.3	46.0	49.3	47.8	5.639
Total MUFA	34.8	36.3	35.2	34.3	30.3	33.6	3.271
Total PUFA	21.8	19.7	21.5	19.7	20.3	18.6	2.523
PUFA:SFA	0.50	0.45	0.50	0.43	0.41	0.40	0.055

SEM: standard error for the mean.

the polyunsaturated fatty acids in the dromedary meat was linoleic acid. The second most important polyunsaturated fatty acid in dromedary camel muscles is α-linolenic acid (18:3n-3). The present α-linolenic acid (18:3n-3) values were lower than those in the dromedary muscles reported by Kadim *et al* (2011) but higher than those reported by Kadim *et al* (2013) and Rawdah *et al* (1994). Daeau and Ferlay (1994) stated that a variable proportion of dietary 18:3n-3 to ruminant is bio-hydrogenated (85-100%), so less is available for incorporation into tissues. Bactrian muscles contained lower amounts of polyunsaturated fatty acids (Eicosadienoic acid C20:2 and Eicosatetraenoic acid C20:3n6). Important products are arachidonic acid (C20:4n-6), which have various metabolic roles. The bactrian muscles contained descent levels of arachidonic acid (C20:4n-6) with the highest level in ST muscle and lower level in BF muscle. In line with the present results, Kadim *et al* (2013) found similar range of arachidonic acid (C20:4n-6) in similar muscles in the dromedary camel. Correlation test indicated that total PUFA contents were positively related to C18:2n−6 (r=0.88).

The ratio of polyunsaturated to saturated fatty acids in bactrian camel muscles ranged from 0.40 to 0.50 (Table 4) were similar or slightly above the minimum ratio of 0.40 recommended by the British Department of Health (1994) to contribute to a reduction in the risk in coronary diseases in human. A possible explanation for these results may be due to slightly higher proportion of phospholipid in Bactrian muscle, which is associated with the 'redder' muscle fibre type profile compared with dromedary species. The present study showed that the SM and ST muscles contained the highest ratio of polyunsaturated to saturated fatty acids, while BF contained the lowest ratio. On the other hand, the ratio of all polyunsaturated to saturated fatty acids, the target for which is 0.45 or above, is much higher,

beneficially so, in Bactrian meat compared with cattle, sheep and goat meats.

Effect of type of muscle on amino acid profile of protein

There were no significant differences between Bactrian muscles on amino acid composition (Table 5). Similar results were reported by Al-Shabib and Abu-Tarboush (2004), Dawood and Alkanhal, (1995) and Elgasim and Alkanhal (1992) in the dromedary, who found that amino acid composition of the protein, remained constant in different camel commercial cuts. The most abundant essential amino acid in bactrian meat was lysine, followed by leucine, methionine, isoleucine, threonine and phenylalanine. Similar values of amino acid contents reported by Urbisinov (1992) in Bactrian camel meat and dromedary meat (Kadim *et al,* 2011 and Abdelhadi *et al,* 2017). The Bactrian muscle has a comparable essential amino acid contents to dromedary, beef, lamb and goat muscles (Al-Shabib and abu-Tarboush, 2004; Dawood and Alkanhal, 1995; Elgasim and Alkanhal, 1992; Kadim *et al,* 2011; Abdelhadi *et al,* 2017). In the essential fraction, there were small non-significant differences between muscles studied in the current study for lysine. Urbisinov (1992) found that bactrian meat contained 16.35 mg/100g of lysine, which is almost more than twice of that in the present study. The difference in lysine content between the present study and Urbisinov's study might be due to age, nutrition status, location and method of determination. The mean values obtained for lysine, leucine and methionine were higher than those obtained by Al-Shabib and Abu-Tarboush (2004), Dawood and Alkanhal (1995), Elgasim and Alkanhal (1992); Kadim *et al* (2011) and Abdelhadi *et al,* (2017) for dromedary camel muscles. Kadim *et al* (2013) reported values ranging from 7.1 to 8.6 g/100 g leucine and from 8.4 to 9.4 g/100 g for lysine in the dromedary muscles, which were similar to the values in the present study. In the present study, the essential amino acid concentrations differed between the highest and lowest values by 18.3, 31.0, 7.3, 32.4, 13.2, 11.5, 9.3, 17.6 and 10.4 between the 6 muscles for lysine, phenylalanine, leucine, histidine, methionine, isoleucine, threonine, tryptophan and valine, respectively. In the study of Al-Shabib and Abu-Tarboush (2004), the essential amino acid contents in dromedary LT and ST muscles differed by >2.1% with the exception of leucine, methionine and tryptophan, which differed by 18.5, 25.4 and 14.6%, respectively. Similarly, essential amino acid contents in the IS, LT and ST muscles differed by > 4.2% with the exception of isoleucine, methionine, threonine, tryptophan and valine which differed between 8 to 42% (Dawood and Alkanhal, 1995). On the other hand, differences in essential amino acids reported across different camel muscles ranged between 0.5 to 9.5% (Elgasim and Alkanhal, 1992; Dawood and Alkanhal, 1995; Al-Shabib and Abu-Tarboush, 2004). Tryptophan concentration in bactrian and dromedary meats (Dawood and Alkanhal, 1995) were lower than in other meats. Al-Shabib and Abu-Tarboush (2004) stated that tryptophan concentration was 1.76% of the total amino acids which was higher than the 1.28% reported for beef (Kadim *et al,* 2008). According to Casey (1993), the quality of muscle protein lies in the extent of the availability of essential amino acids such as lysine and leucine in proportions required by human. The amount of camel meat required to supply the daily requirements of essential amino acids for adults is similar to that from lamb (based on methionine which has the lowest content in meat) but is less than the amount required from beef. The lysine and leucine requirements for an adult human weighing 70 kg are 2.1 and 2.7 g/day (FAO/WHO/UNU, 2007), respectively. One hundred and fifty grams of lean bactrian meat will cover the daily requirement for lysine and leucine. The value of tryptophan in the present study was in agreement with report by Dawood and Alkanhal (1995) who found low tryptophan content in the loin and leg muscles of dromedary camel. The present study also showed that isoleucine is one of the abundant essential amino acids in the bactrian camel meat. According to the amino acid requirements for adults (Institute of Medicine, Food and Nutrition, 2002), 100 to 200 g edible portion of bactrian meat would be an excellent source of high quality proteins because it contains the major essential amino acids in an appropriate ratio. The essential amino acid requirement for an adult person weighing 70 kg is about 12.90 g/day (FAO/WHO/UNU, 2007).

Muscle type had no significant effect on non-essential amino acid composition (Table 5). Similar to the essential amino acids, non-essential amino acids contents also slightly varied between bactrian muscles. Glutamic acid (15.23-17.01 g/100 g protein), aspartic acid (9.83-10.31 g/100 g protein), arginine (6.67-7.82 g/100 g protein) and proline (4.01-5.88 g/100 g protein) were the most abundant amino acids in the non-essential fraction. The lowest mean values were in serine (3.12-4.11 g/100 g protein), tyrosine (3.45-4.15 g/100 g protein) and alanine (3.89-4.22 g/100 g protein). However,

Table 5. Amino acid compositions of Bactrian camel *Infraspinatus* (IS), *Triceps brachii* (TB), *Longissimus thoracis* (LT), *Semitendinosus* (ST), *Semimembranosus* (SM) and *Biceps femoris* (BF).

Gms	Muscle						
	IS	SM	TB	ST	BF	LT	SEM
Essential amino acids (EAA)							
Lysine	9.74	9.71	8.07	8.65	8.81	9.88	0.722
Phenylalanine	6.33	6.35	5.51	4.38	4.74	4.79	1.278
Leucine	7.43	7.25	6.89	6.91	6.82	7.08	1.325
Histidine	4.23	5.13	4.54	4.24	3.47	4.23	0.532
Methionine	6.62	7.28	6.56	7.58	6.74	7.03	0.101
Isoleucine	6.79	6.76	6.67	6.97	6.13	6.01	0.337
Threonine	5.85	5.45	5.75	6.01	5.99	5.67	0.664
Tryptophan	0.75	0.81	0.77	0.83	0.87	0.91	0.073
Valine	5.18	5.31	5.23	5.64	5.78	5.66	0.632
Total EAA	52.92	54.05	49.99	51.21	49.35	51.26	5.664
Non-essential amino acids (NEAA)							
Aspartic	9.83	10.11	10.09	10.31	10.13	9.91	1.024
Glutamic	15.23	15.94	15.67	16.34	17.01	15.99	0.893
Serine	3.12	3.81	3.78	4.11	3.87	3.89	0.217
Tyrosine	3.52	3.45	3.67	4.01	4.15	3.52	0.207
Arginine	6.76	7.14	7.11	7.82	6.88	6.67	0.647
Alanine	4.22	3.95	3.89	3.99	3.98	3.89	0.237
Proline	5.22	5.88	5.67	4.99	4.86	4.01	0.234
Total NEAA	47.9	50.28	49.88	51.57	50.88	47.88	3.459
EAA:NEAA	1.10	1.07	1.00	0.99	0.97	1.07	0.201

SEM: standard error for the mean.

lower values for bactrian muscles were reported by Urbisinov (1992). Similarly, in dromedary muscles, the glutamic and aspartic acids, the major non-essential amino acids in camel meat, ranged from 15.95 to 18.60% and from 9.30 to 10.80% of protein, respectively (Dawood and Alkanhal, 1995; Elgasim and Alkanhal, 1992; Al-shabib and Abu-Tarboush, 2004; Kadim *et al,* 2011). In general, camel meat may be a better source of non-essential amino acids than beef, lamb and goat meats (Kadim *et al,* 2011; Dawood and Alkanhal, 1995; Elgasim and Alkanhal, 1992; Al-Shabib and Abu-Tarboush, 2004). Although, Elgasim and Alkanhal (1992) found low alanine levels in camel meat compared to other red meats. Dawood and Alkanhal (1995); Al-Shabib and Abu-Tarboush (2004) and Kadim *et al* (2011) found similar concentration of alanine in the dromedary muscles and other red meats. Finally, a particularly high essential amino acid/non-essential amino acid ratio was also recorded with SM and LT muscles having the highest ratios, while the BF muscle had the lowest ratios (Table 5).

Effect of type of muscle on Vitamins

Water and fat soluble vitamins concentration (mg/100 g fresh meat) in bactrian camel IS, TB, LT, ST, SM and BF muscles are presented in table 6. The water-soluble vitamins in meat varied in quantities from a few micrograms to several milligrams per 100 g. There were no significant differences in thiamine (B1) concentration between individual muscles, ranging from 0.08 mg/100 g determined in bactrian camel TB, LT and BF muscles to 0.09 mg/100g determined in both IS, ST and SM muscles. In contrast, Lombardi-Boccia *et al* (2005) showed significant variation in thiamine concentration among the cuts of the same species. In beef, there were significant differences (P<0:05) in thiamine content between the loin muscles (0.2 mg/100g) and leg muscles (0.8 mg/100g) (Lombardi-Boccia *et al,* 2005). The latter authors found that chicken and turkey's breast had low thiamine concentration ranging between 0.2 and 0.4 mg/100. The thiamine concentration in bactrian camel muscles (0.09 mg/100g) was higher than beef (0.5 mg/100g),

lamb (0.06 mg/100g), rabbit meats (0.05 mg/100g), chicken (0.04 mg/100g) and Turkey (0.02 mg/100g) and lower than veal (0.11 mg/100g), horse (0.18 mg/100g), ostrich (0.16 mg/100g) and similar to pork (0.8 mg/100g) (Lombardi-Boccia *et al* (2005). Vitamin B6 is related to protein content of the diet. It is also necessary for the formation of haemoglobin (Henderson *et al,* 2003). The vitamin B_6 concentration in the present study ranged from 0.61 to 0.67 mg/100g (Table 6). The current values are higher than reported by Moss *et al* (1983), 0.35 to 0.49 mg/100 g for pork meat, turkey meat (0.42 mg/100g), chicken meat (0.53 mg/100g) and fish (0.34 mg/100g) (Sauberlich *et al,* 1982). The daily recommended dietary allowance for vitamin B_6 is 1.6 mg for women and 2.0 mg for men (Food and Nutrition Board, 1989). Meats, along with dairy products and eggs are the major providers of vitamin B_6. An average serving of bactrian camel meat (200 g) provides 80% of the RDA for vitamin B_6 for the young adult male. Pantothenic acid plays a key role in energy metabolism. The variations in pantothenic acid (B_5) between the selected muscles were not high enough to reach significant level. The TB muscle had the highest B_5 level (0.89 mg/100g) and the SM muscle (0.82 mg/100g) had the lowest. Vitamin B_{12} is required by rapidly dividing cells such as those in the bone marrow which form blood cells (Henderson *et al,* 2003). Small variations were found between bactrian camel muscles for vitamin B_{12} concentrations, with the range value from 4.53 to 4.98 µg/100 g. Meat and meat products serve as the main source of vitamin B_{12} in the food supply and about 35% of vitamin B_{12} intake comes from meat and meat products (Henderson *et al,* 2003). According to Karmas (1988), meat contributes in general 77% (7.5 µg of the 9.7 µg) of the vitamin B_{12} in the diet. The RDA for vitamin B_{12} is 2.0 µg for men and women. Fifty gram of bactrian camel meat will contain 2.38g/100 g vitamin B_{12}, which represent 118% of the RDA for vitamin B_{12}. The average bactrian camel meat contained 4.75µg/100 g vitamin B_{12}, which provides ample amounts of this vitamin. The vitamin B_{12} concentration (0.75–0.92 mg/100 g) in pork reported by Moss *et al* (1983) was higher than the value found in the bactrian camel meat. The bactrian camel meat had higher vitamin B_{12} than sheep (0.25 mg/100g) and veal meats (0.18 mg/100g) (Ono *et al,* 1984; Ono *et al,* 1986) The SM muscle (4.98 µg/100 g) had the highest vitamin B_{12} content and the BF muscle (4.53 µg/100 g) among the muscles studied. Riboflavin is necessary for normal growth and helps maintain the integrity of mucous membranes, skin, eyes and nervous system (Henderson *et al,* 2003). Riboflavin is found in red meat and 15% of the average daily intake in human is derived from meat and meat products. Riboflavin content varied between bactrian camel muscles from 0.20 to 0.29 mg/100g) with BF muscle had the highest value (0.29 mg/100g) while the ST (0.20 mg/100g) the lowest value (Table 5). Similarly, Lombardi-Boccia *et al* (2005) reported that among beef meat cuts, riboflavin concentration varied from 0.09 to 0.17 mg/100 g, with fillet showing the highest concentration. Beef (0.13 mg/100g), veal (0.08 mg/100g), lamb (0.195 mg/100g), ostrich (0.10 mg/100g), pork (0.13 mg/100g), chicken (0.03 mg/100g), turkey (0.06 mg/100g) and rabbit (0.11 mg/100g) meats showed a riboflavin concentration lower than in bactrian camel muscles (Lombardi-Boccia *et al,* 2005; Purchas *et al,* 2014). Horse meat had similar concentration of riboflavin (0.20 mg/100 g) among all the species (Lombardi-Boccia *et al,* 2005).

Table 6. Effect of type of muscle on water and fat soluble vitamins levels of *Infraspinatus, Triceps brachii, Longissimus thoracis, Semitendinosus, Semimembranosus, Biceps femoris* muscles of bactrian camel.

Vitamin	Muscle						
	IS	TB	LT	ST	SM	BF	SEM
Water soluble vitamins							
Thiamine (B_1) (mg/100g)	0.09	0.08	0.08	0.09	0.09	0.08	0.009
Pyridoxine (B_6) (mg/100g)	0.64	0.62	0.66	0.65	0.61	0.67	0.081
Pantothenic acid (B_5) (mg/100g)	0.88	0.89	0.86	0.87	0.82	0.84	0.135
Cyanocobalamin (B_{12}) (µg/100g)	4.64	4.74	4.83	4.86	4.98	4.53	0.033
Riboflavin (B_2) (mg/100g)	0.23	0.21	0.22	0.20	0.26	0.29	0.011
Fat soluble vitamins							
Retinol (A) (µg/100g)	10.5	10.3	11.2	9.99	10.1	9.97	0.153
Alpha-Tocopherol (E) (mg/100g)	0.85	0.89	0.92	0.82	0.86	0.87	0.032

SEM: standard error for the mean.

Vitamin A is present in small amounts in meat and it depends in the amount of intramuscular fat of the meat. The camel meat has a low intramuscular fat (Kadim *et al,* 2008). Therefore, vitamin A will be low compared to other species of red meat animals with high intramuscular fat content. In the present study, vitamin A content (9.97 -10.5 µg/100g) was very low and similar between the individual muscles (Table 6). Vitamin E is a powerful antioxidant and has been shown to improve the colour stability and shelf-life of red meat (Pearce and Jacob, 2004). Although, vitamin E content was similar between the bactrian camel muscles, the LT muscle (0.92 mg/100g) and TB muscle (0.89 mg/100 g) among the highest, while ST muscle (0.82 mg/100g) among the lowest muscles. The small variations between muscles for vitamins studied may be due to small differences in muscle fiber types and intramuscular fat content between muscles studied. bactrian camel meat is reputed to be healthier than other red meats such as beef or lamb. It is leaner and a good source of protein and vitamins.

In persent study, a comparison of the nutrient content of six muscles from bactrian camels revealed that camel meat is rich in a wide range of essential nutrients for human. Type of muscle had a significant effect on the concentrations of several nutrients. Small differences in fatty acid composition and cholesterol were detected between muscles. The amino acid composition and vitamin content in muscles were similar and can match with recommended requirements for human nutrition. bactrian camel meat can compete well with other red meats for the fatty acid profile as it contains high levels of PUFAs with a high UFA:SFA ratio. In general, the bactrian camel meat would be a healthy alternative to traditional red meat and can be competitively marketed alongside meat from cattle, deer, sheep and goats.

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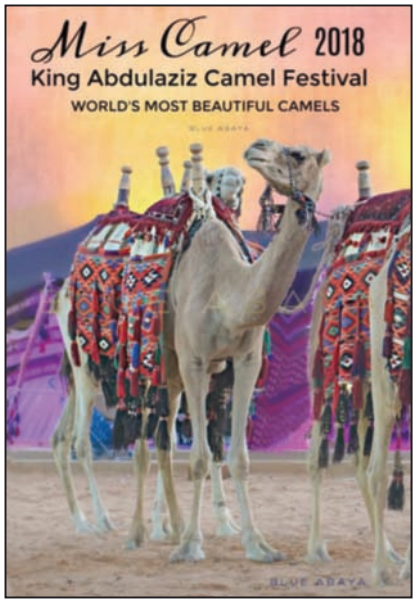
12 CAMELS DISQUALIFIED FROM SAUDI BEAUTY CONTEST IN 'BOTOX' ROW



Twelve camels were disqualified from Saudi Arabia's annual camel beauty contest after receiving botulinum toxin injections to make their pouts look more alluring. Saudi authorities have raised the profile of the King Abdulaziz camel festival by relocating it from the desert to the outskirts of the capital, Riyadh. This year's event has been been mired in scandal after the lure of 20m Saudi riyals (£3.7m) in prize money for each category tempted some owners to cheat. The key attributes in camel beauty are considered to be delicate ears and big nose. But there are strict rules against the use of drugs in the lips, or shaved or clipped body parts. This year, a dozen camels were banned after a vet was caught performing plastic surgery on them. Camels were also given Botox-type injections at his clinic, according to Saudi media. "They use Botox for the lips, the nose, the upper lips, the lower lips and even the jaw," Ali al-Mazrouei, the son of an Emirati camel breeder, told the UAE daily ."It makes the head more inflated so when the camel comes it's like, 'Oh, look at how big that head is. It has big lips, a big nose.'" After the ban was imposed, the chief judge of the show, Fawzan al-Madi, told Reuters: "The camel is a symbol of Saudi Arabia. We used to preserve it out of necessity, now we preserve it as a pastime." The month-long festival is the biggest in the Gulf and involves up 30,000 camels.

UNITED STATES CAMEL CORPS

In 1836, Major George H. Crosman, United States Army, who was convinced from his experiences in the Indian wars in Florida that camels would be useful as beasts of burden, encouraged the War Department to use camels for transportation. In 1848 or earlier, Major Henry C. Wayne conducted a more detailed study and recommended importation of camels to the War Department. United States Camel Corps was active in 1856–1866 at USA and was a branch of US Army. Its role was experimental and post was Camp Verde, Texas. Its first commander was Major Henry C. Wayne. Newly appointed as Secretary of War by President Franklin Pierce, Davis found the Army needed to improve transportation in the southwestern US, which he and most observers thought a great desert. In his annual report for 1854, Davis wrote, "I again invite attention to the advantages to be anticipated from the use of camels and dromedaries for military and other purposes...."[2]:10 On March 3, 1855, the US Congress appropriated $30,000 for the project.[1]:393–394.

The United States Camel Corps was a mid-19th century experiment by the United States Army in using camels as pack animals in the Southwestern United States. While the camels proved to be hardy and well suited to travel through the region, the Army declined to adopt them for military use. The Civil War interfered with the experiment and it was eventually abandoned; the animals were sold at auction.

(Source: Wikipedia, the free encyclopedia)

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BIOCHEMICAL CHARACTERISATION OF CAMEL MILK FROM DIFFERENT REGIONS OF PUNJAB-PAKISTAN

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ABSTRACT

The objective of this present study was to explore the attributes of camel milk (Marecha breed) collected from different regions of the Punjab Province, Pakistan during the months of January to April. These were MLP, MCP and MUP meaning milk from lower, cental and upper Punjab. Variations were observed in gross chemical composition, physical attributes and insulin concentration of camel milk, i.e. 3.84±0.09% (MLP3) protein content, 3.13±0.12% (MLP1) fat contents, 4.25±0.17% (MLP2) lactose content, 0.84±0.03% (MLP1) ash content, 47.46 ±1.01 IU (MLP2) insulin content, 6.69±0.09 (MUP4) pH and 0.162±0.03% acidity (MLP5). The SNF values ranged between 8.13±0.16% to 6.64±0.14% among all regions. Total solids were found maximum in (MLP3) 11.21±0.4% and minimum in (MCP1) 9.64±0.5%. Most prevalent minerals were Ca (115mg/100g), Fe (0.46 mg/100g), Zn (0.57 mg/100g), Na (65.32 mg/100g), K (160 mg/100g) and Mg (9.56 mg/100g). In fatty acids profile, oleic acid (18.5±1.40g/100g), Palmitic (10.17±0.60g/100g), stearic (6.22±0.10g/100g) and Palmitoleic (6.13±0.04g/100g) were found in significant amount. Camel milk with best compositional and physicochemical attributes, was further subjected to rheological (rheometer) and thermal characterisation (Differential scanning calorimetry) to predict and optimise conditions for pasteurisation of camel milk.

Key words: AAS, camel milk, DSC, GC-FID, physicochemical characterisation, Punjab, rheological and thermal properties, rheometer

Pakistan has camel population of 1.2 million camel heads, which offers a huge market for milk, meat and live trade of camels. Balochistan owns highest number (41.22 %) of camels followed by Sindh (30.23 %), Punjab (21.61 %) and Khyber Pakhtunkhwa KPK (6.94 %) (Faraz *et al,* 2013; Khan *et al,* 2016).

Marecha breed of Pakistani camel particularly produces 3.5 to 35 kg per animal per day with a lactation yield of 4575 to 20675 kg/year, which is probably the best yield all over the world camel rearing countries (Khaskheli *et al,* 2005). The major proportion of this milk is used to fulfill the nomads owns need by consuming it freshly or to feed the young ones and rest of the milk is sold to big cities by mixing with buffalo milk to earn maximum profit (Khaskheli *et al,* 2005).

Present study was aimed to explore the biochemical attributes of milk of Marecha breed of dromedory camel in Punjab province of Pakistan.

Materials and Methods

Collection of Milk samples

The fifteen fresh camel milk samples from Marecha breed during the months of January to April from three ecological zones of Punjab i.e. upper, central and lower Punjab. The collected milk samples were kept in airtight, sterilised plastic bottles and were well preserved at refrigerated temperature in Food Microbiology and Biotechnology lab, University of Agriculture, Faisalabad (UAF) during analyses.

Gross Chemical Composition

The milk samples were subjected to biochemical analysis including crude protein, crude fat, ash and lactose according to the methods as described below. All analysis were conducted in triplicates.

Crude Protein

Protein in milk sample was determined by International Dairy Federation method, IDF 20-1 (2014).

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Protein % = 6.38 x % Nitrogen

Ash, crude fat and lactose contents

These were assessed by following the guidelines of AOAC (2006).

Physical properties of milk samples

pH

pH of camel milk was determined by using calibrated digital pH-meter (Ong *et al,* 2012).

Titratable Acidity

Acidity (expressed as lactic acid) was determined by direct titration method of AOAC (2006) No. 947.05.

Total solids and SNF

Total solids and SNF contents were determined by method mentioned in AOAC (2006).

Mineral profiling

Calcium (Ca), Sodium (Na) and Potassium (K) were determined by using flame photometer and Copper (Cu), iron (Fe), Zinc (Zn), Manganese (Mn), Magnesium (Mg) were estimated by using Atomic absorption spectrophotometer (AAS) as described in the AOAC (2006). 2ml of milk sample was taken in flask and subjected to wet digestion in 7 ml HNO_3 and 3ml of $HClO_4$. The mixture was heated until the light green or clear solution was attained. After that the samples was filtered and diluted up to 50 mL by using distilled water for the further analysis on flame photometer and AAS.

Fatty acid profiling of camel milk

The fatty acid analysis of camel milk samples were done by using Gas Chromatography as (Sheraz Ahmed *et al,* 2013). Camel milk fat was extracted by following method of Feng *et al* (2004).

Determination of Insulin in camel milk samples

Insulin content in milk samples was measured by using UV-Vis spectrophotometer (RayLight, UV-1600, China) and maximum wavelength was recorded at 260-350 nm (Royatvand *et al,* 2013).

Rheological and thermal characterisation of camel milk

The best camel milk characterised from three ecological regions in terms of composition, physicochemical properties, minerals and fatty acid profile was subjected to rheological (rheometer) and thermal characterisation (Differential scanning calorimetry) for determination of optimising conditions for camel milk pasteurisation.

Rheological behaviour of camel milk

Rheological behaviour of camel milk from lower Punjab region was observed under different temperature and shear rate using TA-ARG2 rheometer, Geometry DIN concentric cylinders, Peltier steel-105606 for the development of rheological profile of camel milk. Camel milk viscosity was assessed from 30-95°C with 1 and 2-degree temperature ramp and keeping the shear rate (5-150 1/s) at 80, 85, 90 and 95°C (Hattem *et al,* 2011). All samples were run in triplicates.

Thermal characterisation of camel milk

Camel milk was subjected to thermal analysis using differential scanning calorimetry (TA Discovery series) that measures temperature and heat flows associated with thermal transition in food matrix. Camel milk sample (18 mg) was taken in T-zero pans and subjected to heat with 5°C ramp from 30- 95°C temperature range (Rahman *et al,* 2012). The results were obtained after running three samples.

Statistical Analysis

All the parameters results were subjected to statistical analysis to determine the level of significance (Montgomery, 2017).

Results and Discussion

Chemical composition

The chemical composition of collected camel milk samples from three different ecological zones of Punjab are shown in Table 1. The protein contents in camel milk from lower Punjab desert region were found higher than other two regions and differences were found highly significant ($P<0.01$). These results were in accordance with those of Abbas *et al* (2013) and Alwan *et al* (2014) who studied the composition of camel milk under different rearing conditions and reported protein variations in milk. The disparity in proteins contents was due to miscellaneous factors like age, health status, feeding regime and rearing conditions of animals. Milk fat content differed possibly due to breed and also from animal to animal due to the difference in their fatty acids profiling and the feeding pattern. Statistical results showed that the fat content in camel milk from lower Punjab region was significantly ($P<0.05$) different from the other two regions camel milk. Fat contents varied between three ecological regions with highest fat

contents found in MLP1 (3.13±0.12%) from the desert region while lowest fat contents were observed in MUP2 (2.81±0.13%) in plain region. These results were comparable with the outcomes reported by Abbas *et al* (2013), Gizachew *et al* (2014) and Panwar *et al* (2015) who documented variation in fat contents while studying camel milk properties under different husbandry practices. These variations in fat content among three ecological regions could be due to animal breed and feed variation as nomads do not provide sustained diet to their animals that ultimately reflects on fatty acid pool, in the same lactation period.

Table 1. Mean values of compositional analysis in raw milk from different regions of Punjab.

Regions	Protein%	Fat%	Lactose%	Ash%
MLP1	3.28±0.13^a	3.13±0.12^a	4.16±0.19^a	0.84±0.03^a
MLP2	3.51±0.14^a	3.09±0.12^a	4.25±0.17^a	0.79±0.06^a
MLP3	3.84±0.09^a	3.08±0.08^a	4.06±0.19^a	0.76±0.03^a
MLP4	3.52±0.11^a	3.09±0.09^a	4.15±0.2^a	0.78±0.02^a
MLP5	3.27±0.15^a	3.11±0.13^a	4.01±0.19^a	0.81±0.01^a
MCP1	3.09±0.13^b	2.89±0.11^b	3.4±0.15^b	0.72±0.05^b
MCP2	2.89±0.14^b	2.86±0.12^b	3.81±0.16^b	0.71±0.04^b
MCP3	3.11±0.16^b	2.84±0.14^b	3.99±0.18^b	0.72±0.01^b
MCP4	2.98±0.15^b	2.85±0.11^b	3.72±0.17^b	0.69±0.04^b
MCP5	3.04±0.12^b	2.87±0.08^b	3.85±0.18^b	0.71±0.06^b
MUP1	3.29±0.14^b	2.86±0.11^b	3.41±0.14^b	0.72±0.04^b
MUP2	3.16±0.16^b	2.81±0.13^b	3.81±0.16^b	0.75±0.05^b
MUP3	3.01±0.14^b	2.82±0.12^b	3.99±0.17^b	0.71±0.02^b
MUP4	3.09±0.12^b	2.87±0.09^b	3.72±0.16^b	0.72±0.06^b
MUP5	3.32±0.13^b	2.83±0.11^b	3.85±0.18^b	0.74±0.01^b

Means carrying different letter are significantly different from each other, whereas means without letters are non-significant.

MLP: Milk from lower Punjab; **MCP:** Milk from central Punjab; **MUP:** Milk from upper Punjab; The number (1-5) indicates location in each region.

Lactose analysis depicted significant ($P<0.05$) difference in camel MLP while the difference between other two regions (MUP & MCP) was non-significant. Lactose variation within regions was maximum in MLP2 (4.25±0.17%) and minimum (3.4±0.15%) in MCP1. The current findings were in accordance with previous studies who determined the chemical composition of camel milk under different rearing and environmental condition of camel farming (Abbas *et al,* 2013). The variation in lactose may also be attributed to consumption of natural vegetation by animals in desert area where camels prefer to eat natural halophilic desert plants like acacia, artilples and salosa to fulfil their physiological needs. Lactation period and parity were the other variables that contribute towards the lactose contents not only in camel milk but in other milch animals as well. By advancing lactation, negative correlation of lactose content with camel breeds has been observed (Fouzia *et al,* 2013; Alwan *et al,* 2014).

Maximum ash content was recorded in MLP2 (0.84±0.03%) whereas, minimum ash content was recorded in MCP4 (0.69±0.04%). Fluctuations in ash content in milk samples has been attributed to differences in feed, parity, water intake, rearing practices and analytical procedures. The results for ash content are comparable with the findings of Alu datt *et al* (2010), who reported variation in camel milk properties from eight different locations in North and South areas of Jordan. Furthermore, variations in ash contents have also been reported by different researchers who explored camel milk for its physicochemical properties (Abbas *et al,* 2013). Gross chemical composition variations were due to the difference in individual animal's health, lactation periods, rearing conditions and experimental methods that were considered as major factors in composition of camel milk.

Physical attributes:

The physical attributes of camel milk samples from different regions have been given in Table 2. The variations in pH of camel milk ranged from (6.69±0.09) in MUP4 to (6.51±0.05) in MLP2, MLP5

Table 2. Mean values for physicochemical properties in raw camel milk from different regions of Punjab.

Regions	pH	Acidity%	SNF%	Total solids%
LP1	6.55±0.03	0.154±0.001	7.71±0.13^a	10.84±0.46^a
MLP2	6.51±0.04	0.161±0.004	7.98±0.15^a	11.07±0.52^a
MLP3	6.57±0.07	0.153±0.002	8.13±0.16^a	11.21±0.41^a
MLP4	6.54±0.01	0.158±0.005	7.86±0.12^a	10.95±0.55^a
MLP5	6.51±0.05	0.162±0.003	7.75±0.11^a	10.86±0.49^a
MCP1	6.61±0.03	0.151±0.002	6.66±0.15^c	9.64±0.47^b
MCP2	6.54±0.04	0.156±0.006	6.87±0.14^c	9.88±0.41^b
MCP3	6.57±0.06	0.153±0.003	7.21±0.15^c	10.26±0.51^b
MCP4	6.56±0.05	0.154±0.003	6.78±0.16^c	9.78±0.39^b
MCP5	6.53±0.02	0.157±0.005	7.00±0.14^c	9.98±0.53^b
MUP1	6.51±0.04	0.161±0.005	6.64±0.14^b	9.76±0.49^b
MUP2	6.55±0.06	0.159±0.007	7.06±0.16^b	10.01±0.35^b
MUP3	6.54±0.05	0.157±0.005	6.94±0.11^b	9.96±0.55^b
MUP4	6.69±0.09	0.142±0.006	6.78±0.18^b	9.85±0.43^b
MUP5	6.64±0.07	0.148±0.004	6.97±0.21^b	10.18±0.51^b

Means carrying different letter are significantly different from each other, whereas means without letters are non-significant.

and MUP1. These results are in accordance with the findings of Alwan *et al* (2014) and Abbas *et al* (2013). The fluctuations in pH values depends on water and feed. The acidity in camel milk samples ranged from (0.162±0.003% to 0.142±0.006%) in all regions. The acidity results were comparable with the findings of Aludatt *et al* (2010); Khaskheli *et al* (2005) and Abbas *et al* (2013). These variation in acidity can be ascribed to lactation stage of animal. The SNF values were ranged between (8.13±0.16% to 6.64±0.14%) among all regions. Maximum value for total solids was found as (11.21±0.41%) in MLP3 and minimum was observed to be (9.64±0.47%) in MCP1.

Mineral profile of Camel Milk

The mineral profile including Ca, Cu, Fe, K, Mg, Mn, Na and Zn of camel milk from different regions have been presented in Table 3. Calcium contents differed in regions with maximum Ca content (115.41±2.88mg/100g) found in MLP4 while minimum (91.11±4.55mg/100g) in MCP4. The results were in harmony with the findings of Haddadin *et al* (2008) who concluded that calcium variations were observed in different camel milk samples while carrying different experiment accordingly. The Fe content in camel milk ranged between (0.46±0.12mg/100g) to (0.21±0.12mg/100g) in MLP2 and MUP1, respectively. The variation in Fe content in different camel milk samples was attributed to different ecology, breed and feed of animals. The amount of Fe content was relatively higher as compared to other milking animals, therefore camel milk can be a superb alternate to mother milk in anaemic communities. Additionally, camel milk is blessed with iron having relatively lower molecular weight fractions which facilitates the intestinal absorption. The concentration of Zn was found maximum in MLP2 (0.57±0.08 mg/100g) while minimum was in MCP4 (0.29±0.08 mg/100g). The concentration of potassium was maximum in MLP3 (160.15±4.32mg/100g) and minimum in MLP5 (141.31±4.75mg/100g) and findings were comparable with the results of Kula and Tegene (2016). Magnesium contents in all camel milk samples ranged from (9.71±0.31mg/100g) in MLP1 to (8.04±0.21mg/100g) in MUP5. The results were comparable with the findings of Abbas *et al* (2013) who conducted research on composition and physicochemical analysis of camel milk. Concentration of Na in camel milk was calculated in such a way that minimum amount was found in MCP4 (50.99±3.44mg/100g) while maximum found in MLP1 (65.32±2.77mg/100g). The variations in Na content among different regions might be attributed to the availability of water and temperature difference in environment of camel rearing regions. Higher Na content in MLP may be due to higher concentration of sodium in halophilic plants of desert region (lower Punjab) consumed by camels during grazing. Lactation period can also be the reason for Na contents difference in milk samples. The results were in accordance with the findings of Alwan *et al* (2014), who determined effect of farm management of camel raising on mineral contents of camel milk.

Table 3. Mean values for Mineral contents (mg/100g) in raw camel milk from different regions of the Punjab.

Region	Ca	Fe	Zn	Na	K	Mg
MLP1	113.05±2.04^{a}	0.44±0.14^{a}	0.51±0.13^{a}	65.32±2.77^{a}	143.67±4.84	9.71±0.31^{a}
MLP2	108.34±3.06^{a}	0.46±0.12^{a}	0.57±0.11^{a}	62.55±2.01^{a}	153.09±4.02	9.58±0.18^{a}
MLP3	114.06±3.15^{a}	0.41±0.08^{a}	0.55±0.07^{a}	59.87±3.21^{a}	160.15±4.32	9.42±0.26^{a}
MLP4	115.41±2.88^{a}	0.41±0.07^{a}	0.53±0.09^{a}	61.22±3.43^{a}	156.09±4.44	9.11±0.32^{a}
MLP5	110.69±3.74^{a}	0.39±0.11^{a}	0.55±0.08^{a}	60.09±3.74^{a}	141.31±4.75	9.56±0.22^{a}
MCP1	92.56±4.46^{b}	0.22±0.13^{b}	0.34±0.09^{b}	52.56±3.35^{a}	149.534.46	8.75±0.39^{b}
MCP2	91.67±4.17^{b}	0.24±0.09^{b}	0.36±0.11^{b}	51.89±3.06^{b}	153.54±4.07	8.67±0.41^{b}
MCP3	93.56±3.96^{b}	0.26±0.1^{b}	0.31±0.09^{b}	52.12±2.85^{b}	146.61±4.74	8.52±0.52^{b}
MCP4	91.11±4.55^{b}	0.25±0.08^{b}	0.29±0.09^{b}	50.99±3.44^{b}	145.06±4.55	8.71±0.61^{b}
MCP5	93.88±3.66^{b}	0.25±0.09^{b}	0.31±0.06^{b}	52.06±2.55^{b}	144.82±4.15	8.49±0.42^{b}
MUP1	97.63±4.01^{b}	0.21±0.12^{b}	0.33±0.08^{b}	52.43±3.99^{b}	143.73±4.02	8.34±0.41^{c}
MUP2	94.92±4.72^{b}	0.26±0.06^{b}	0.34±0.16^{b}	52.34±3.61^{b}	147.73±4.73	8.23±0.48^{c}
MUP3	93.84±4.68^{b}	0.23±0.15^{b}	0.31±0.05^{b}	51.66±3.57^{b}	147.18±4.46	8.08±0.24^{c}
MUP4	95.69±4.32^{b}	0.25±0.08^{b}	0.36±0.07^{b}	52.06±3.21^{b}	142.47±4.11	8.19±0.34^{c}
MUP5	95.64±3.99^{b}	0.22±0.09^{b}	0.36±0.08^{b}	51.52±2.88^{b}	148.29±4.74	8.04±0.21^{c}

Means carrying different letter are significantly different from each other, whereas means without letters are non-significant.

Aludatt *et al* (2010) also reported variation in Na content in camel milk due to different location in south and north areas of Jordan. Concentration of Zn was recorded as (0.58mg/100g) in MLP3 and lower was observed as (0.28 mg/100g) in MUP2. The concentration of Zn founded in camel milk among all regions were comparable with the findings of Sawaya *et al* (1984). The variation in mineral content in camel milk from all regions may be attributed to the environmental conditions, rearing practices, feed, stage of lactation period, breed and availability of water and different analytical procedure.

Fatty Acids profile of camel milk

The fatty acids composition of camel milk from three different regions of Punjab is elucidated in Table 4. The results indicated that fatty acid content in camel milk from lower region were relatively higher than samples from central and upper Punjab. Fatty acid compositional analysis from MLP exhibited minimal amount of Linolenic acid (1.06±0.06g/100g) however Oleic acid (18.5±1.40g/100g) was found to be maximum followed by Palmitic (10.17±0.60g/100g), Stearic acid (6.22±0.10g/100g), palmitoleic (6.13±0.04g/100g) and Lauric acid (6.09±0.10g/100g).

Table 4. Mean values of Fatty acids composition in raw camel milk from different regions of Punjab.

Fatty acid	MLP Region	MCP Region	MUP Region
Caprylic acid	1.76±0.02	1.58±0.04	1.56±0.06
Capric acid	3.25±0.12	2.84±0.09	2.76±0.07
Lauric acid	6.09±0.10	5.86±0.09	5.85±0.06
Tri-decanoic acid	3.17±0.05	2.69±0.30	2.70±0.24
Myristic acid	2.94±0.05	2.78±0.09	2.69±0.05
Myristoleic acid	1.72±0.05	1.74±0.08	1.69±0.09
Pentadecanoic acid	1.72±0.03	1.68±0.04	2.56±1.97
Palmitic acid	10.71±0.60	10.42±0.45	10.34±0.19
Palmitoleic acid	6.13±0.049	6.13±0.09	6.14±0.05
Heptadecanoic acid	3.24±0.17	2.91±0.39	3.12±0.06
Stearic acid	6.22±0.10	5.83±0.51	5.93±0.08
Oleic acid	18.5±1.40	17.79±0.73	15.90±3.02
Linoleic acid	1.53±0.35	0.98±0.11	0.91±0.07
Arachidic acid	1.75±0.07	1.67±0.04	1.68±0.06
Eicosaenoic acid	1.77±0.09	1.75±0.04	1.70±0.06
Linolenic acid	1.06±0.06	0.75±0.11	0.78±0.11
Behenic acid	1.74±0.03	1.71±0.08	1.71±0.04
Erucic acid	1.76±0.06	1.72±0.05	1.70±0.06

Means carrying different letter are significantly different from each other, whereas means without letters are non-significant.

Fatty acids profile from MCP presented Linolenic acid (0.75±0.11g/100g) as the lowest while highest amount of Oleic acid (17.79±0.73g/100g) was found followed by Palmitic (10.42±0.45g/100g), Palmitoleic (6.13±0.09g/100g), lauric (5.86±0.09g/100g) and Stearic acid (5.83±0.51g/100g). Upper Punjab milk (MUP) samples exhibited mean values for Linolenic acid as (0.78±0.11g/100g) which was in lowest concentrations whereas, oleic acid (15.90±3.02g/100g) was found in highest concentration followed by Palmitic (10.34±0.19g/100g), palmitoleic (6.14±0.05g/100g), stearic (5.93±0.08g/100g) and Lauric acid (5.85±0.06g/100g).

Appreciable variations have been observed in lower Punjab milk samples. Oleic acid was found maximum (18.5±1.40g/100g) whereas, (17.79±0.73g/100g) and (15.90±3.02g/100g) of Oleic acid contents were quantified in central and upper Punjab regions, respectively. Linolenic was minimum among all fatty acids quantified in milk samples from all regions. Lowest amount of Linoleic acid (0.75 ±0.11g/100g) was found in milk from central Punjab samples and (0.78±0.11g/100g) was observed in upper Punjab, however, maximum Linoleic acid contents (1.06±0.06g/100g) was observed in the samples of lower Punjab region.

Variations in fatty acids composition were perceived with respect to camel milk from different locations. The reason can be attributed to regional difference, breed, feed, season and even lactation period of the animals. Konuspayeva *et al* (2007) explored fatty acids and cholesterol composition of camel milk in Kazakhstan and reported variation in fatty acids of camel milk during seasonal variations. In another study, Sheraz Ahmed *et al* (2013) agreed that variation in fatty acids was found in camel milk during fatty acid investigation through gas chromatography. Mohamed and Mustafa (2017) determined the fatty acid contents in camel milk and reported significant variation in milk samples in different rearing conditions, breeds, stage of lactation of camel in Sudan.

Insulin concentration in camel milk

The insulin concentration in camel milk samples were expressed in Table 5. Mean squares depicted that milk from lower Punjab region was found to be highly significant ($P<0.01$) while non-significant ($P>0.05$) results were observed in the milk from other two regions. Maximum concentration of insulin was (47.46 ±1.01IU) found in

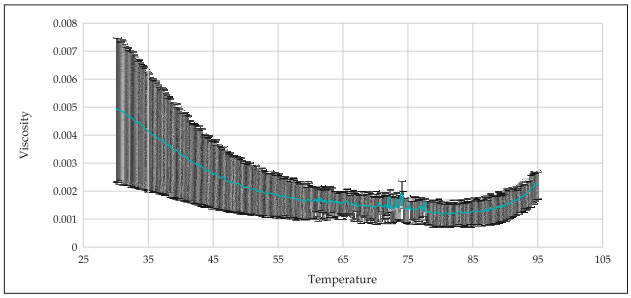

Fig 1. Viscosity 1-degree Temperature Ramp.

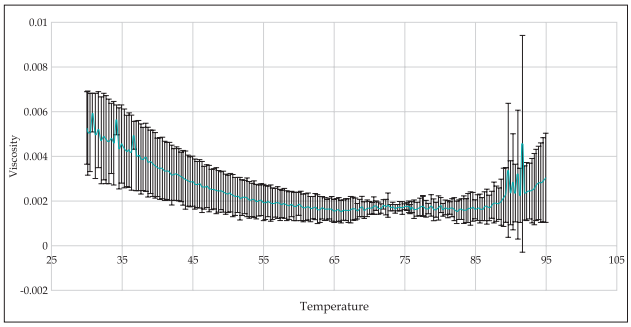

Fig 2. Viscosity 2-degree Temperature Ramp.

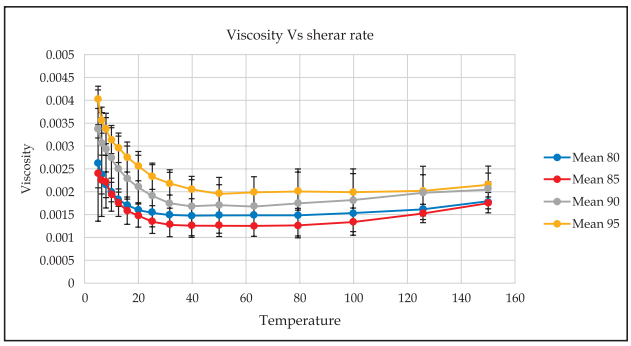

Fig 3. Viscosity Vs shear rate at four different temperatures (80, 85, 90 and 95°C).

MLP2 whereas, minimum concentration was (31.19 ±1.21 IU) recorded in MCP4. The results showed variation in insulin concentration in camel milk samples which could be due to feed, environment and health status of animal. In desert region, variety of natural shrubs and halophilic thorn plants are the main source that causes higher amount of mineral (Zinc) present in animals and ultimately bio-concentrated in animal tissues. Zinc triggers the insulin production in body that may be related to significant effect of lower Punjab habitat on insulin production. Results of insulin concentration in camel milk from different regions in current study were in agreement with many other studies which characterised insulin concentration in camel milk using different analytical techniques (Pourhosseini *et al,* 2007; Royatvand *et al,* 2013).

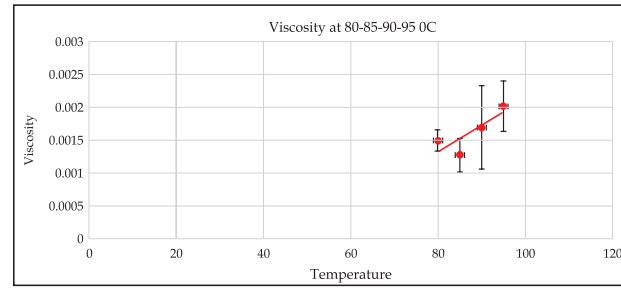

Fig 4. Viscosity trend at 80, 85, 90 and 95°C keeping shear rate (5-150 1/s).

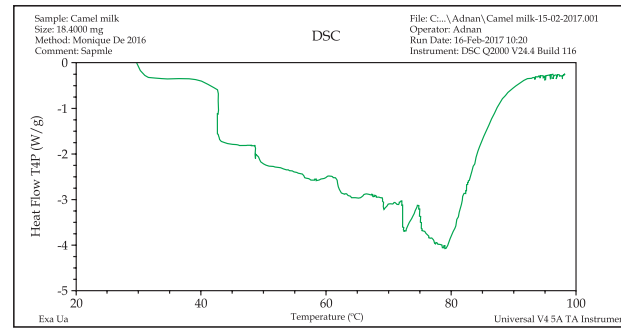

Fig 5. DSC Thermogram of camel milk Endothermic peak at 80°C (T_m of camel milk).

Table 5. Mean squares of insulin concentration in raw camel milk from different regions of Punjab.

Region	Insulin IU± S.D.
MLP1	45.38±1.77a
MLP2	47.46±1.01a
MLP3	45.48±2.12a
MLP4	43.44±1.08a
MLP5	44.47±1.09a
MCP1	34.25±1.06a
MCP2	36.25±1.11b
MCP3	31.22±1.18b
MCP4	31.19±1.21b
MCP5	31.23±1.06b
MUP1	33.25±1.08b
MUP2	34.18±1.16b
MUP3	31.26±1.05b
MUP4	36.29±1.11b
MUP5	36.28±1.17b

Means carrying different letter are significantly different from each other, whereas means without letters are non-significant.

3.6 Rheological behaviour of camel milk

Viscosity of camel milk was assessed with temperature sweep of 30-95°C, 1-2 degree temperature ramp, explained graphically in Fig 1 and 2. Camel milk viscosity was tending to decreasing behaviour from 30 to 80°C with 1-2

degree temperature ramp while after reaching at 80°C viscosity showed increasing behaviour that showed the denaturation in camel milk occurred on reaching 80°C as explained in Fig 1 and 2. Furthermore, viscosity of milk was assessed at combination of 80, 85, 90 and 95°C temperatures keeping the shear rate (1-150 1/s) for comprehensive rheological profiling of camel milk explained in Fig 3 and 4. It was clearly drawn from the Fig 3 and 4 that viscosity of camel milk increases after reaching at 80°C that confirms denaturation in camel milk occurred at this temperature as earlier epitomised in 1-2 degree temperature ramp.

Thermal characterisation of camel milk

Milk is a heat labile entity and certain thermal treatments for improving its keeping quality. Therefore, it is very important to understand the changes occurred in and functional properties of milk during the thermal treatment like industrial pasteurisation. Limited studies were carried out on thermal processing of camel milk globally. Camel milk was subjected to thermal analysis using differential scanning calorimetry (DSC) (TA Discovery series) that measures temperature and heat flows associated with thermal transition in food matrix. The DSC thermogram of camel (Fig 5) showed endothermic peak at 80°C that depicted thermal transition temperature of camel milk. The DSC theromogram of camel milk is given in Fig 5 that authenticated rheological results of camel milk.

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DETERMINATION OF THE BIOACTIVE POTENTIAL (ANTIOXIDANT ACTIVITY) OF CAMEL MILK DURING FERMENTATION PROCESS

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ABSTRACT

An experiment was conducted to explore the possibilities of utilisation of camel milk for production of bioactive peptides which have antioxidant potential by action of fermentation using 2 dairy cultures: *Lactococcus lactis* spp. *cremoris* and *Lactococcus lactis* spp. *lactis*. Pasteurised camel milk was incubated with these 2 cultures @ 1% at 37°C for a period of 12 hour fermentation. During this period change antioxidant potential was measured using ABTS and DPPH radical scavenging activity. According to ABTS and DPPH radical scavenging activity antioxidant activity of camel milk samples, the fermentative potential of *Lactococcus lactis* spp. *cremoris* was found significantly higher ($P<0.05$), when it was compared with *Lactococcus lactis* spp. *lactis*. Thus milk samples fermented with *Lactococcus lactis* spp. *cremoris* were used for production of fermented camel milk products at the time period of fermentation, where it showed highest antioxidant activity (both ABTS and DPPH) (*i.e.* 10 hours of fermentation for camel milk).

Key words: ABTS, camel milk, DPPH, fermentation

Camel milk is unique in terms of low fat (1.5-3%), low protein (2.5%) and longer shelf life, higher ratio of β-casein to k-casein, absence of Lysozyme-C and β-lactoglobulin and presence of Whey Acidic Protein (WHP) and Peptidoglycan Recognition Protein. There are reports on its antibacterial and other therapeutic properties. Camel milk differs from bovine milk in the composition and structure of protein content and thus has different functional and medicinal properties. Caseins (CNs) are the major proteins in camel milk and α, β and κ-CN constitutes about 65, 21 and 3.47%, respectively of total caseins present in milk (Kappeler *et al,* 2003).

Lactococcus lactis has 2 subspecies with few phenotype and genotype differences, *Lactococcus lactis* spp. *lactis* and *Lactococcus lactis* spp. *cremoris*, where the former is preferred for making soft cheese while later subspecies for hard cheese. *Lactococcus lactis* is a gram-positive bacterium and has crucial role in manufacturing dairy products, i.e. buttermilk and cheese. When *Lactococcus lactis* spp. *lactis* is added to milk, the bacterium uses enzymes to produce energy molecules (ATP) from lactose. Industrial research on *Lactococcus lactis* deals with the production of L-alanine, which is used as sweetener in dairy products.

The highest risk from oxidative metabolism by-products is the formation of free radicals. The damage to the organism caused by free radicals is immense and is a major threat for the welfare of the whole organism and is known to cause a variety of potentially fatal diseases. The objective of this research is to determine the antioxidative capacity of fermented camel milk with different types of microbiological strains.

Materials and Methods

A pre experimental trial was done by using different starter cultures of lactic acid bacteria procured from NCDC, NDRI karnal. On the basis of antioxidant activity, these 2 cultures *viz. Lactococcus lactis* spp. *cremoris* and *Lactococcus lactis* spp. *lactis* were choosen for the present investigation. About 2 litre of fresh camel milk was collected from camel dairy maintained at ICAR-NRC on Camel, at weekly interval for period of 2 months to perform the different experiments as mentioned below under the study.

Fresh camel milk was skimmed to bring the fat contents to below 0.5% using cream separator. The samples were heated to boil at least for 5 min

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to inactivate/kill the inherent microbial population present in milk. Then *Lactococcus lactis* spp. *cremoris* and *Lactococcus lactis* spp. *lactis* cultures were inoculated @ 1% and after proper mixing the samples were inoculated at 30°C and samples were drawn at 0, 2, 4, 6, 8, 10, 12 hours and were subjected to analysis for change in soluble protein concentration etc.

Bacterial cultures and their propagation:

Glass ampoules containing lyophilised powder of *Lactococcus lactis* spp. *cremoris* NCDC 81 and *Lactococcus lactis* spp. *lactis* NCDC 88 were obtained from the NCDC (National Collection of Dairy Cultures) Dairy Microbiology Division ICAR-National Dairy Research Institute, Karnal (INDIA). The organisms were stored at 4°C. The propagation for each strain was performed according to Donker *et al* (2007) with slight modification. Sterile 5 ml aliquots of reconstituted sterile skim milk (RSM) (Himedia Laboratories) were inoculated with each strain individually and incubated at 30°C for 24h in BOD incubator. After incubation, the pre-inoculated cultures were prepared by transferring loop full of activated culture to 10 ml aliquots of litmus milk (Himedia Laboratories) to determine the activation of culture activity by observing change in colour of litmus milk after 24 hour of inoculation. The skim milk and litmus milk were autoclaved by following the standard procedure (121°C for 15 min @15 lbs).

Culture performance during cultivation in milk

Formation of serial dilution of the culture was done and appropriate dilution was selected for enumeration by the pour plate technique. All samples were enumerated on MRS agar at 30°C for 24 hours. Plates containing 30–300 colonies were enumerated and the colony forming units (CFU) per gram of the product was calculated. These cultures were then used for fermentation of fresh pasteurised milk samples for 12 hour at 30°C corresponding to cell count 10^7–10^8 CFU/ml as suggested by Ramesh *et al* (2012). The supernatants were collected by centrifugation of camel milk during fermentation and then utilised for antioxidant assay (ABTS, DPPH, etc.).

DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The ability to scavenge DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical by adding antioxidants in samples was estimated by following the method of Brand-Williams *et al* (1995) with slight modification. About 2 ml of DPPH reagent (100 µM) was mixed with 0.50 ml of 0.1 M Tris-HCl buffer (pH 7.4) and 50 µ of hydrolysate sample in test tubes and the content was gently mixed and immediately absorbance was measured at 517 nm (nanometre) by using a spectrophotometer and then the sample tubes were incubated at room temperature under dark for 20 minutes and then again measured the absorbance. Ethanol was used as blank. The free radical-scavenging activity was calculated from the following equation:

DPPH radical Scavenging activity (% inhibition) =

$$100-[(At_{20}/At_0)x100]$$

Where At_{20}= absorbance at 20 minute

At_0= absorbance at zero minute

ABTS+ (2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity

The spectrophotometric analysis of ABTS+ radical-scavenging activity was determined according to method described by Salami *et al* (2011). ABTS radical cation (ABTS+) was produced by reacting ABTS+ stock solution with equal volume of 2.45mM potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 hours before use. For making working solution of ABTS, the stock solution of ABTS was diluted with distilled water to make its absorbance 0.70 and equilibrated at 30°C exactly 6 min after initial mixing. About 4 ml of ABTS+ working standard solution was mixed with 40µl of hydrolysate/standard and absorbance was measured after 20 minutes @ 734 nm by using spectrophotometer.

The ABTS+ activity was calculated by using the following formula

ABTS activity (% inhibition) =

$$[(0.70-At_{20})/\ 0.70]\ x\ 100$$

Where At_{20}= absorbance of mixture at 20 minute

0.70=absorbance of working solution of ABTS

Results and Discussion

ABTS activity (% inhibition) of camel milk during fermentation

The data related to ABTS activity (% inhibition) of camel milk has been shown in table 1. The ABTS radical-scavenging activity increased significantly ($P<0.01$) with the advancement fermentation time up to 10 hour, after that, decrease in activity was observed.

Milk inoculated with *Lactococcus lactis* spp. *cremoris* had the highest antioxidant capacity which increased from mean value of 1.57 ± 0.001% at zero hour (fresh milk) in camel milk to 18.46 ± 0.013% at 10 hour, after that it decreased significantly. Similar

trends were observed with *Lactococcus lactis* spp. *lactis* during the same incubation time and similar free radical scavenging activity at zero hour which reached to 13.64 ± 0.031% in camel milk samples, at 10 hour of fermentation. After that a significant fall in ABTS free radical scavenging activity takes place. Results were showing similar trend with Ramesh *et al*, (2012). The overall ABTS activity (% inhibition) for camel milk was observed 9.94 ± 0.897 and 8.19 ± 0.688, for *Lactococcus lactis* spp. *cremoris* and *Lactococcus lactis* spp. *lactis*, respectively.

Table 1. ABTS (Mean ± SE) activity (% inhibition) of camel milk during fermentation.

Treatment	*Lactococcus lactis* spp. *cremorsis*	*Lactococcus lactis* spp. *lactis*	Over all
Hour 0	1.57±0.001	1.57±0.001	1.57^{a}±0.001
Hour 2	4.74±0.054	3.24±0.015	3.99^{b}±0.227
Hour 4	5.31±0.070	5.85±0.016	5.58^{c}±0.089
Hour 6	10.82±0.022	8.73±0.020	9.78^{d}±0.316
Hour 8	14.80±0.022	12.74±0.018	13.77^{f}±0.311
Hour 10	18.46±0.013	13.64±0.031	16.05^{g}±0.726
Hour 12	13.84±0.010	11.53±0.019	12.69^{e}±0.349
Overall	**9.94^{b}±0.897**	**8.19^{a}±0.688**	**9.061±0.570**

Note – Means bearing different superscripts differ significantly.

The free radical scavenging activity in all samples changed significantly (P< 0.01) from 0 to 12 hour. On the data basis shown in fig 1, the ABTS anti oxidant activity (% inhibition) of *Lactococcus lactis* spp. *cremoris* was significantly higher, when compared with *Lactococcus lactis* spp. *lactis* in camel milk samples during fermentation process.

According to Donkor *et al* (2007) the variations of biological activities may be attributed to the production of different bioactive peptides, which may or may not have antioxidant properties and it is likely to be strain dependent. These findings of proteolytic activity were in accordance with the findings of Salami *et al* (2011) and Jrad *et al* (2014) but the method of production of bioactive peptides from milk sample were different (digestive enzymes v/s LAB fermentation).

DPPH activity of camel milk during fermentation

The mean data related to DPPH activity of camel milk has been presented in table 2. The DPPH activity of fermented camel milk increased significantly (P<0.01) with the progress in fermentation time and a positive relationship between fermentation time and DPPH activity could be established. However, the higher DPPH-scavenging activity was decreased after 10 hour of fermentation. Data show in table 2 revealed that, at 10 hour of fermentation, the DPPH activity of camel milk samples were highest (9.29±0.005 and 6.22±0.004 respectively for *Lactococcus lactis* spp. *cremoris* and *Lactococcus lactis* spp. *lactis*). Results demonstrated a pattern of fermentative potential which was similar to those reported by Ramesh *et al* (2012).

Table 2. DPPH (Mean ± SE) activity (% inhibition) of camel milk during fermentation.

Treatment (Hour)	*Lactococcus lactis* spp. *cremoris*	*Lactococcus lactis* spp. *lactis*	Over all
0	0.67±0.001	0.68±0.005	0.67^{a}±0.003
2	1.95±0.004	1.76±0.007	1.85^{b}±0.028
4	3.78±0.004	2.32±0.005	3.05^{c}±0.220
6	4.84±0.047	3.07±0.006	3.95^{d}±0.267
8	6.86±0.012	5.87±0.004	6.36^{f}±0.151
10	9.29±0.005	6.22±0.004	7.75^{g}±0.464
12	5.25±0.062	4.56±0.004	4.90^{e}±0.109
Overall	**4.66±0.420**	**3.50±0.305**	**4.08±0.266**

Note – Means bearing different superscripts differ significantly.

The free radical scavenging activity in all samples changed significantly (P<0.01) from 0 to 12 hour. Milk inoculated with *Lactococcus lactis* spp. *cremoris* had the highest antioxidant capacity which increased from mean value of 0.67±0.001 at zero hour (fresh milk) in camel milk to 9.25±0.005 at 10 hour of fermentation after that it decreased significantly. Similar trends were observed with *Lactococcus lactis* spp. *lactis* during the same incubation time and similar free radical scavenging activity at 0 hour in camel milk samples, which reached to 6.22±0.004 at 10 hour of fermentation, respectively. Subsequently, a significant fall in DPPH radical scavenging activity took place.

The DPPH antioxidant activity (% inhibition) of *Lactococcus lactis* spp. *cremoris* was significantly higher, when compared with *Lactococcus lactis* spp. *lactis* during fermentation process.

Conclusion

The fermentative potential of *Lactococcus lactis* spp. *cremoris* was more compared with *Lactococcus lactis* spp. *lactis*, thus milk samples fermented with *Lactococcus lactis* spp. *cremoris* of camel milk can be used for production of fermented camel milk products at the time period of fermentation, where it show by highest antioxidant activity (both ABTS and DPPH basis) (i.e. 10 hours of fermentation for camel milk).

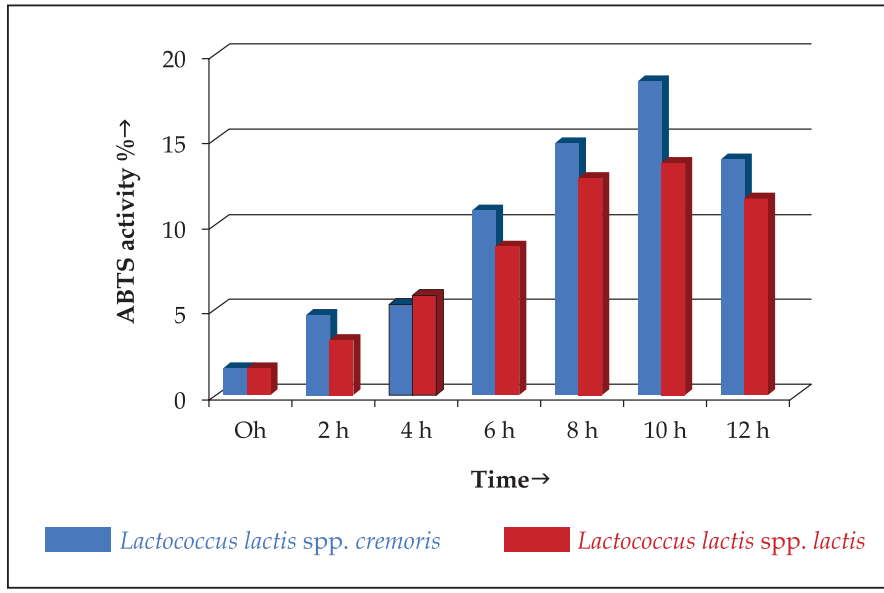

Fig 1. ABTS activity (% inhibition) of camel milk during fermentation.

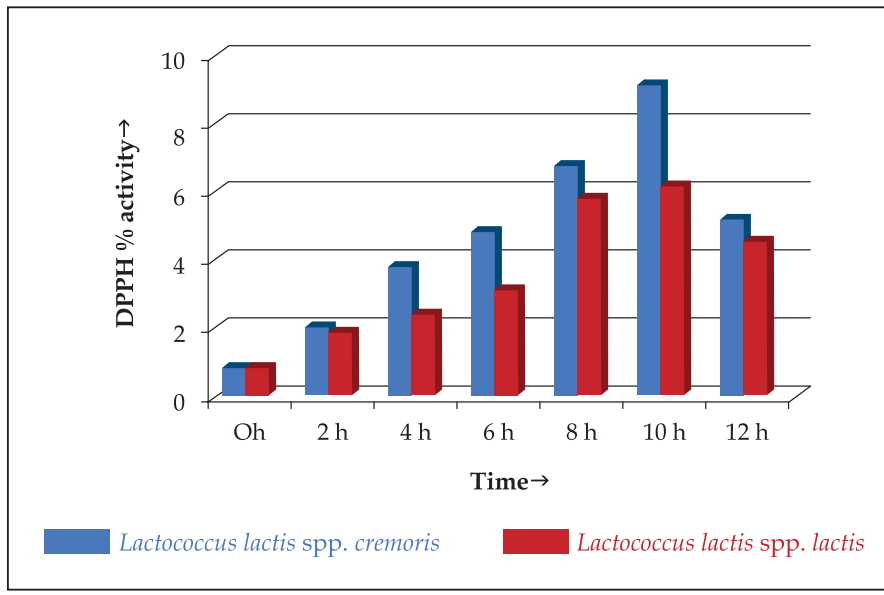

Fig 2. DPPH activity (% inhibition) of camel milk during fermentation.

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SERO-EPIDEMIOLOGY OF CAMEL (*Camelus dromedarius*) BRUCELLOSIS

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ABSTRACT

Brucellosis is an emerging zoonotic bacterial disease of ruminants and also reported in camels. Camels are one of the most important sources of livelihood for the poor nomadic population in Gujarat. The present study was aimed to determine the brucella specific antibodies in camel using RBPT and i-ELISA. On screening of 658 serum samples, 131(19.90%) and 78 (11.85%) samples found to be positive by RBPT and i-ELISA, respectively. Prevalence rate of brucellosis in different categories *viz.* herd size, physiological status, sex, district, region and breed were calculated. Susceptibility of brucellosis in different categories was also compared by using chi-square test.

Key words: Brucellosis, camels, i-ELISA, RBPT, seroprevalance

Brucellosis is caused by various species of the genus *brucella*, which is the 2nd most widely spread zoonosis worldwide (Dawood, 2008). brucella can affect almost all domestic species and cross transmission can occur between cattle, sheep, goat, camel and other species (Ghanem *et al,* 2009). Brucellosis is associated with abortion in females, orchitis and epididymitis in males (Radostits *et al,* 2007). In addition to that it also causes infertility, retention of placenta, chronic inflammation of joints, tendon sheath and synovial bursa especially at the carpus (Abbas and Agab, 2002). The disease is an important public health concern in many parts of the world including India (Pal, 2007; Hadush and Pal, 2013). Brucellosis is a burning problem in Gujarat, where cases of human brucellosis are reported along with high sero-prevalence in animals (Chauhan *et al,* 2017).

Brucellosis was reported in camels as early as 1931 (Solonitsuin, 1949); since then the disease has been reported from all camel-keeping countries (Gwida *et al,* 2012). Camels are frequently infected with *brucella* organisms, especially when they are in contact with infected large and small ruminants (FAO/WHO, 1986; Radwan *et al,* 1992). Camels are not known to be the primary host for any of brucella organisms but they are susceptible to both *B. abortus* and *B. melitensis* (Musa and Shigidi, 2001). The relation between *brucella* infection and abortion in camels has been reported (Al-Majali *et al,* 2008; Higgins, 1986; Agab and Abbas, 1999). Serological evidence for Brucellosis in camels has been reported from Asia and Africa (Dawood, 2008). Prevalence may vary according to climatic conditions, geography, species, sex, age and diagnostic tests used (Gul and Khan, 2007).

The present study was planned for the detection of brucella antibody from camels using RBPT and i-ELISA because brucellosis has been reported from various species in different parts of Gujarat and limited work on camel brucellosis.

Materials and Methods

This study was conducted between October 2012 and September 2016 under DBT network project on Brucellosis.

Collection of samples

Approximately 10 ml of blood was collected from individual animal aseptically from jugular vein using serum activator vacutainer (BD vacutainer). The vacutainer tubes were kept in slanting position at room temperature for 2 hours and centrifuged at 3000 rpm for 10 minutes. The separated serum was collected in screw capped plastic vial and stored at –20°C temperature till further use.

A total of 658 sera were collected from camel of Gujarat state for detection of *brucella* antibodies by RBPT and i-ELISA.

Rose Bengal Plate Test (RBPT)

The RBPT antigen was procured from the Institute of Animal Health and Veterinary Biological

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(IAH and VB), Hebbal, Bangalore. One drop (0.03 ml) of serum was taken on a glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (0.03 ml) of the antigen was added. The antigen and serum were mixed thoroughly with sterile tooth picks and then the slide was rotated for 4 minutes and result was read immediately. Definite clumping/ agglutination was considered as positive reaction, whereas no clumping/agglutination was considered as negative.

Indirect-enzyme linked Immunosorbenly Assay (i-ELISA)

Indirect ELISA kit was procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bangalore and used as per manufacturer's protocols. The kit detects the antibodies against brucella lipopolysaccharide (LPS) in serum samples.

Results and Discussion

According to the Food and Agriculture Organisation (FAO), the World Health Organisation (WHO) and the World Organisation for Animal Health (OIE) brucellosis is one of the most important and widespread zoonoses in the world (Young, 1995). It has high public health significance and may poses threat to all human as diseases may transmit through consumption of raw and under cooked milk and milk products (Schelling *et al,* 2003). However, Dubey *et al* (2017), Chauhan *et al* (2017) and Patel *et al* (2017) reported 27.05%, 15.38% and 20.43% of seroprevalence of brucellosis in milk.

Overall seroprevalance

Total 658 serum samples were screened, 131 (19.90%) were found positive by RBPT (Table 1). In accordance to the present finding, Zewold and Haileselassie (2012), Dawood (2008), Patel *et al* (2017) and Chauhan *et al* (2017) reported 11.90%, 14.20%, 15.75% and 11.54% seroprevelence respectively. However, in-contrast to present findings, Ghanem *et al*(2009) and Shome *et al* (2013) reported 3.9% and 8.9% of seroprevalence in camel by RBPT, respectively.

In indirect ELISA, 78(11.85%) sera were found positive (Table 1). Similarly, Patel *et al* (2017) reported 13.47% seroprevelence using i-ELISA. In contrast to our findings, a lower rate of brucella antibodies were reported 3.10%, 3.50%, 4.90% and 4.54% by Ghanem *et al* (2009), Azwai *et al* (2001), Shome *et al* (2013) and Chauhan *et al* (2017), respectively. The difference in seroprevalence might be due to differences in sample size, management condition, herd size or due difference in seroprevalence in the two study areas. According to Radostits *et al* (2007), herd size and management condition determine rate of transmission of brucella infection in different study areas.

Sera were collected from three different herd size of camels. The highest seroprevalence was reported in medium size herd (22.07% and 16.55%) while in case of the small size herd (7.69% each), lowest both, by RBPT and i-ELISA, respectively. Univariate analysis showed that medium and large herd size was significantly associated with brucellosis but small herd size showed non-significant Chi-square value and also indicate that animals belonging to medium herd size are more susceptible than large herd size (Odd ratio= 2.37). This may be due to higher numbers of positive animals in medium herd size.

The seroprevalence data thus obtained were further analysed according to the clinical conditions of the animals at the time of testing the samples. Seroprevalence recorded in clinically healthy animals 42 (18.18%) and 17 (7.35%), animals with the history of abortion 36 (24.65%) and 26 (17.80%), hygroma 03 (14.28%) and 01 (4.76%), pregnant 8 (13.33%) and 05 (8.33%), non-pregnant 15 (19.48%) and 12 (15.58%), status unknown 19 (18.62%) and 12 (11.76%), still birth 00 (0.00%) and 00 (0.00%), orchitis 08 (50.00%) and 05 (31.25%), respectively by RBPT and i-ELISA. The camels found positive for orchitis showed highest seroprevalence both by RBPT and i-ELISA. Univariate analysis showed that animal having orchitis is 5 times more likely to be seropositive than clinically healthy (Odd ratio=5.72)

Sera were collected from both sex. The highest seroprevalence was reported in male (30.64% and 19.35%) while in case of female (18.79% and 11.07%), it was lowest both, by RBPT and i-ELISA. In contrast to the present findings, Chauhan *et al* (2017) and Adamu *et al* (2014) reported higher rate of seroprevalance in female than male. Univariate analysis showed that seroprevalence observed in male and female animal was significantly associated with Chi-square value and odds ratio indicates that male animal was more likely to be seropositive than female animal (Odd ratio=1.92)

In district wise analysis of results, it was found that the Banaskantha, Patan and Kuchchh have (19.59% and 22.36%), (16.24% and 5.40%) and (20.12% and 3.55%) seroprevalence using RBPT and i-ELISA, respectively. In accordance to the present findings, Chauhan *et al* (2017) reported higher prevelance

Table 1. Seroprevalence of brucella antibodies in camel.

Attributes	No. of sample tested	No. of sample found positive			
		RBPT +Ve	Percentage (%)	I-ELISA +Ve	Percentage (%)
HERD SIZE					
Small size (1-50 animals)	13	01	7.69	01	7.69
Medium size (upto 100 animals)	308	68	22.07	51	16.55
Large size (above 100 animals)	337	62	18.39	26	7.71
PHYSIOLOGICAL-STATUS					
Clinically Healthy	231	42	18.18	17	7.35
Aborted	146	36	24.65	26	17.80
Hygroma	21	03	14.28	01	4.76
Pragnant	60	08	13.33	05	8.33
Nonpragnant	77	15	19.48	12	15.58
Status unknown	102	19	18.62	12	11.76
Still birth	05	00	0.00	00	0.00
Orchitis	16	08	50.00	05	31.25
SEX					
Male	62	19	30.64	12	19.35
Female	596	112	18.79	66	11.07
DISTRICT					
Banaskantha	148	29	19.59	08	5.40
Patan	313	70	22.36	63	20.12
Kutchch	197	32	16.24	07	3.55
REGION					
North region	628	125	19.90	72	11.46
Kuchchh region	30	06	20.00	06	20.00
BREED					
Bikaneri	123	34	27.64	24	19.51
Kuchchhi	436	75	17.20	40	9.17
Jaisalmeri	99	22	22.22	14	14.14

in Banaskantha district followed by Patan and Kachchh using RBPT and i-ELISA. Univariate analysis showed seroprevalence observed in all 3 district was significantly associated with Chi-square value and Patan district is more likely to be seropositive than Kuchchh and Banaskantha (Odd ratio=6.84 and 4.41).

Concerning the two regions of Gujarat tested, the seroprevalence found was slightly different. In North region, overall seroprevalence found was 19.90% and 11.46% by RBPT and i-ELISA, respectively while in Kuchchh region, the seroprevalence found was 20% by both test Univariate analysis showed that Kuchchh region is more likely to be seropositive than North region (Odd ratio=1.93) and seroprevalence was significantly associated with Chi-square value.

Comparing the seroprevalence of the 3 different camel breeds tested, the results showed that Bikaneri breed showed a higher prevalence (27.64% and 19.51%) followed by Jaisalmeri 22.22% and 14.14%) and Kuchchhi (17.20% and 9.17%) breed by RBPT and i-ELISA tests, respectively. Univariate analysis showed that Bikaneri breed is more likely to be seropositive than Kuchchhi and Jaisalmeri (Odd ratio =2.40 and 1.47). This variation may be due to genetic structure of breed.

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