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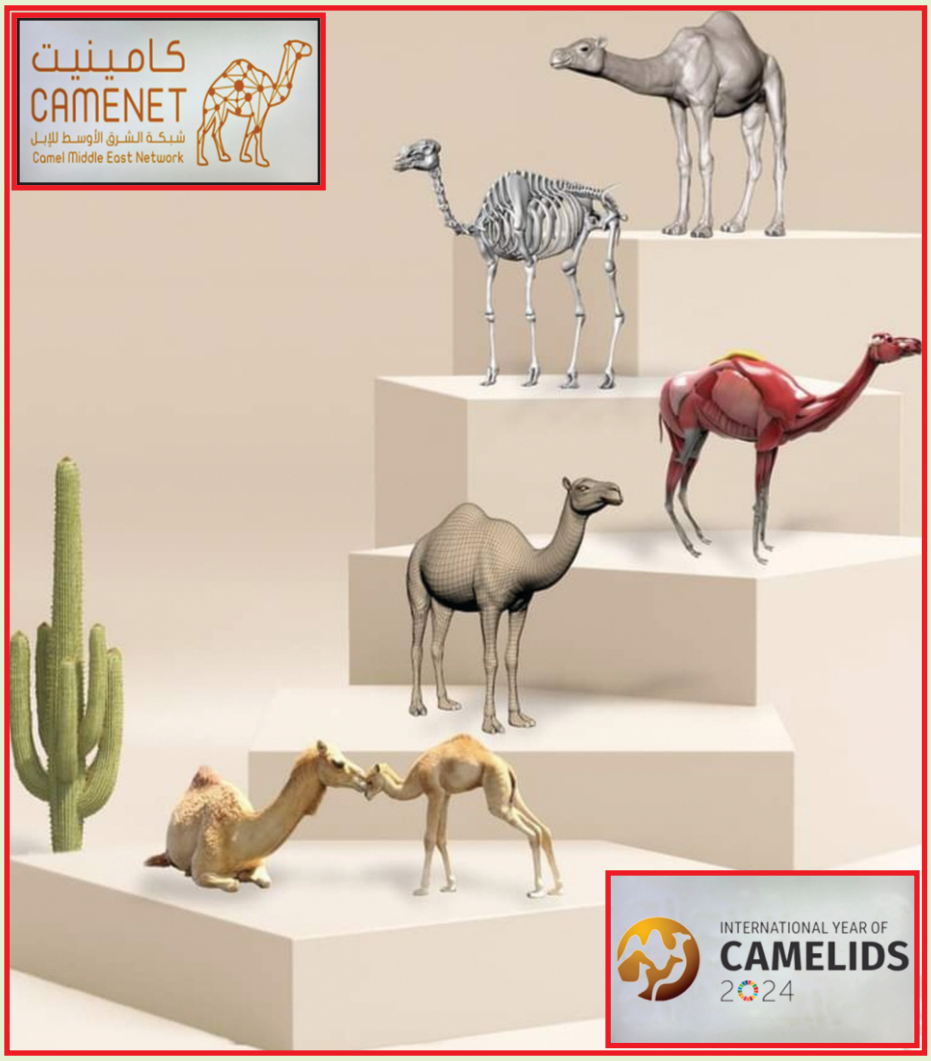

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Journal of Camel Practice and Research (JCPR) publishes only research and clinical manuscripts related to the Camelids (Old and New World camelids), hence published contents are consistent with the title and scope of the journal. Review articles on emerging research are invited and published. JCPR also publishes the news related to the New or Old World Camelids, specially those related to new products, conferences, books, trainings or workshops etc.

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CAMENET AND INTERNATIONAL YEAR OF CAMELIDS

The camel activities in the Middle East region and elsewhere were earlier monitored partly through ISOCARD and ICO but now CAMENET has also become active. CAMENET is a network of 9 camel rearing countries, including UAE, Saudi Arabia, Oman, Bahrain, Qatar, Kuwait, Jordan, Iran and Yemen. It is dedicated to dromedary camel health and husbandry. It was officially launched during the WOAH annual assembly meeting of the regional committee in Paris in May 2016. CAMENET started as an initiative of the Biological Standards Commission of the WOAH, to exchange knowledge and assist member countries to better control camel diseases in the Middle East region and neighboring countries. The development of CAMENET represents a strategic step forward in the GF-TADs agenda addressing the specific challenges of the growing camel sector in the sub-region with potential global impact on the camel economy. CAMENET overall goal is to assist members to develop their camel sector, particularly through the prevention, early detection, and control of camel diseases. The activities of the network will be supervised and managed by a Steering Committee, supported by a Technical Committee composed of experts from several veterinary sub-specialties.

The United Nations declared 2024 the International Year of Camelids (IYC 2024). The Year will highlight how camelids are key to the livelihoods of millions of households in hostile environments across over 90 countries, particularly indigenous peoples and local communities. From alpacas to Bactrian camels, dromedaries, guanacos, llamas, and vicuñas, camelids contribute to food security, nutrition and economic growth as well as holding a strong cultural and social significance for communities across the world. Camelids play a key role in the culture, economy, food security and livelihoods of communities in Andean highlands and in the arid and semi-arid lands in Africa and Asia, including Indigenous Peoples. Even in extreme climatic conditions they continue to produce fibre and nutritious food. Indeed, the International Year of Camelids presents a unique opportunity to raise awareness of the role of camelids in building resilience to climate change – particularly in mountains and arid and semi-arid lands.

The International Year of Camelids 2024 aims to build awareness of the untapped potential of camelids and to call for increased investment in the camelid sector, advocating for greater research, capacity development and the use of innovative practices and technologies.

The Journal of Camel Practice and Research would complete 3 decades with the release of December 2023 issue which is Volume 30 and Number 3. I am really thankful to all the members of the editorial board and contributors who laid a perfect trust and support to this exclusive journal of camelids which grew over the time and proved the biggest platform of camelids literature resource. Current issue contains two review papers, i.e. Camel cloning: achievements and consequences and camel milk and it's applications in treatment of diabetes. It has an interesting article on a quarter individual milking machine –StimuLactor, being used in a camel farm in Switzerland. Another interesting manuscript is based upon genome wide association for milk nutrition traits in Gobi Red Bactrian camel. Molecular assessment of kappa casein gene by sequencing in Bikaneri dromedary camels, technological and probiotic properties of *Enterococcus faecium* strains isolated from Tunisian camel milk, Unique development of the heart, fermented camel milk beverage, mandibular fractures in camels using modified IDW (IDW and transfixation of pins with fibre cast) technique, dermatophilosis and mange and imaging studies of cadaver mandible are among other interesting manuscripts of this issue. I am sure that JCPR will bring more interesting research and news in the International Year of Camelids 2024.

Merry Christmas and Happy New Year 2024 to all those thinking for welfare of camelids.

(Dr. Tarun Kumar Gahlot)
Editor

Civil society statement on the

INTERNATIONAL YEAR OF CAMELIDS 2024

As representatives of camelid herding communities, of NGOs and other stakeholder groups supporting them, and as entrepreneurs promoting cruelty-free camelid products, and their consumers,

- **Concerned** about the **future direction** of camelid husbandry and of camelid welfare,
- **Affirming** the **cultural meaning** of camelids in the world views of associated herding societies,
- **Apprehensive** about **climate change, biodiversity loss** and the degradation of the environment, including desertification,
- **Recognizing** the important role camelids are increasingly playing in enhancing pastoralists' **resilience** and adaptability to climate change,
- **Concerned** about the continuous loss of camelid **grazing lands** that restricts the mobility of our camelids and the availability of adequate feed for them,
- **Anxious** about limited **livelihood opportunities** for our youth, but aware that this situation could be turned around by means of supportive interventions and policies,
- **Noting** that certain **remote grazing** areas are no longer utilized, due to the hardships associated with mobility,
- **Convinced** of the enormous **potential** of camelids in producing food and fibre in extremely marginal and degraded environments, without external inputs and use of fossil fuels, and their comparatively low carbon footprint,
- **Honouring** our indigenous ecological knowledge, countless generations of **experience** in managing camelids sustainably in harsh environments and our innovative capacities to respond to changes,
- **Aware** of colonial **disruption** of traditional camelid management systems,
- **Disturbed** by the low resource allocation by governments and development partners to develop the camelid **value chains**,

on the occasion of the **International Year of Camelids** in 2024, we call on governments, scientists, donor agencies, local and regional decision makers, experts and the private sector to support camelid development efforts that consider the special ecological and cultural aspects of camelids by:

- Enabling **mobility** and ensuring **secure access** to ancestral grazing and browsing areas for our camelid herds to thrive, for example by recognizing them as **Indigenous Community Conserved Areas** or **Territories of Life**,

- Investing in **decentralized infrastructure**, such as networks of mini-diaries and local processing facilities[1] to link camelid herders in remote areas to value chains, while also respecting and supporting our traditional ways of processing,
- Fostering camelid-herding **community organizations** and their agency,
- Respecting and building on our **traditional knowledge** and related local innovations,
- Strengthening provision of **camelid healthcare**, including research into emerging diseases,
- Supporting investment on people-centred and -controlled camelid **research and development**,
- Recognizing camels as co-creatures and establishing **camelid welfare standards** into policy and practice worldwide,
- Carving out an alternative, cruelty-free **development trajectory** for camelid herding that conforms to the worldview of traditional camelid communities and avoids industrialization.

[1] In many areas, this would include local slaughtering facilities; however, some camelid-keeping ethnic groups object to these.

Contact: info@pastoralpeoples.org September 2023

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CAMEL CLONING: ACHIEVEMENTS AND CONSEQUENCES

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ABSTRACT

The natural process in producing an offspring is through *in vivo* fertilisation of an oocyte by sperm. Animal cloning via somatic cell nuclear transfer (SCNT) provided the possibility of producing live offspring independent of gametes' interaction. The procedure involves the reconstruction of an enucleated oocyte with a somatic cell followed by nuclear reprogramming of the differentiated diploid nucleus to an undifferentiated totipotent embryonic state. This allows the reconstructed embryo to grow and produce offspring nearly similar to the original animal that dedicated the somatic cell. SCNT is a very expensive technique and due to the numerous unknown signals involved in nuclear reprogramming and epigenetics, it has extremely low efficiency and low survival rates of offspring. Extensive use of this technique is associated with the reduction in genetic diversity. Nevertheless, it provides an opportunity to preserve an endangered breed of camel or to resurrect the deceased elite camel. This review concentrates on the efficiency of SCNT in dromedary camels and the possibility of using this technique in routine practice.

Key words: Animal cloning, camel, reproductive technologies

Based on the current status of animal reproductive technologies, the production of camel calves could be achieved by two main approaches: sexual and asexual. Sexual approach which relies on sperm and oocyte interaction, is the natural way of producing an offspring. Accordingly, several generations of reproductive technologies were used to assist the natural process of offspring production. Semen technology is the first generation of animal reproductive technologies that remains in the infancy stage in camels due to problems in the viscosity of semen. It could counteract semen processing and preservation and could prevent the production of camel calf using frozen semen (Niasari-Naslaji, 2023). The second generation of animal reproductive technologies that rely on the fertilisation of oocytes by sperm is the technique of embryo production, either *in vivo* or *in vitro* (Khatir and Anouassi, 2006; Anouassi and Tibary, 2013; Niasari-Naslaji and Nikjou, 2023). The *in vivo* production of embryos is very well-defined and routinely used in camel (Anouassi and Tibary, 2013; Ararooti *et al,* 2018; Niasari-Naslaji and Nikjou, 2023). However, *in vitro* embryo production has not had great progress since its introduction (Khatir and Anouassi, 2006), possibly due to the nature of follicle extrusion from the ovarian stroma, which might result in severe bleeding and adhesion following repeated ultrasound-guided transvaginal ovum pick-up and the problem of viscous semen that is not easy to be used for *in vitro* fertilisation.

Animal cloning is an asexual approach to producing offspring (Segers *et al,* 2019). Identical twins could occur naturally or through embryo splitting (Rahbaran *et al,* 2021). The birth of the first clone in mammals from an adult somatic cell provided a novel asexual approach to producing offspring (Campbell *et al,* 1996). Dolly was the only viable infant of 277 attempts created with mammary epithelial cells that developed into 29 early *in vitro* embryos. They were transferred into 13 surrogate females resulting in the production of a viable lamb. This indicated that the nucleus of the adult somatic cell could have a developmental competence nearly similar to the nucleus of the germ cell lineage. It took 14 years since the birth of Dolly, for the first dromedary camel cloned calf, Injaz, to be born using a similar approach (Wani *et al,* 2010). In 2017, the first Bactrian camel cloned calf was also born by interspecies SCNT (Wani *et al,* 2017). Since then, several articles published to optimise SCNT in camel (Wani, 2021; Hossein *et al,* 2023; Mansour *et al,* 2023; Moulavi and Hosseini, 2023). It was also claimed that the production of expensive camel calves could be possible via SCNT at the commercial scale (Olsson *et al,* 2021). The purpose of this review is not to provide the details of the technique for camel cloning, as this

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objective has already been accomplished (Wani, 2021; Moulavi and Hosseini, 2023). This review tries to summarise all results regarding camel cloning aiming at camel calf production to highlight the final result that one could expect using SCNT. Accordingly, the result of published articles was harmonised and a similar pattern in the report of result was followed to provide the possibility for comparing the results.

The impact of camel cloning on genetic diversity and camel industry

The existing genetic diversity in camels is the result of traditional camel breeding practices among ethnic groups. This in turn resulted in the production of different breed of camel (89 Dromedary camel breeds and 14 Bactrian camel breeds) according to cultural preferences throughout the centuries (Köhler-Rollefson, 2022). Extensive use of embryo transfer technology and camel cloning, in particular, could narrow the camel gene pool (Köhler-Rollefson, 2022) and possibly make the population vulnerable to particular diseases (Spielman *et al,* 2004). Therefore, any attempts to use cloning in the camel industry have to be conducted with great caution not to disturb the genetic diversity and not to disseminate particular diseases and abnormalities in camel. The genetic diversity allows us to select the racing camel with better performance, even better than those that died before. The expression of any particular traits such as meat and milk production, beauty and racing capabilities depend on the nature and the nurture of individuals. It might be possible to produce a camel calf very similar to the original donor camel genetically. However, it does not necessarily mean that such a cloned camel calf could perform as well as the original donor camel. Therefore, it is possible to produce a camel calf by the very expensive technique of SCNT which is as bad as any other camel calf in a group! According, trusting to the speculation that camel cloning could be used to produce animals with the highest potential for milk production or champions in racing and beauty contests and reintroduction of males of high genetic merit could be questionable. USA Food and Drug Administration addressed the safety of consumption of meat derived from cloned specimens (Animal Cloning, 2021). However, because the cost of obtaining such animals is extremely expensive, using cloned camel for meat consumption is not logical at the present time.

Camel cloning achievements and consequences

Very nice review articles on the scientific aspects and consequences of SCNT are available (Niemann, 2016; Malin *et al,* 2022; Mrowiec *et al,* 2022; French and Trounson, 2023). Production of an offspring through reproductive cloning is performed by *in vitro* or *in vivo* oocyte maturation, enucleation of the oocyte, reconstruction of the oocyte with somatic cell nuclear transfer, a fusion of somatic cell's nucleus (karyoplast) and oocyte (ooplast) followed by activation, *in vitro* embryo culture and transfer of the reconstructed embryo to a recipient animal. The first dromedary nuclear-transferred embryos derived from either adult fibroblasts or cumulus cells were successfully produced in 2008 (Khatir and Anouassi, 2008). Unfortunately, two detected pregnancies were lost by Day 60 following transfer and the success of producing the first cloned camel calf was postponed. Later on in 2010, the first camel calf, Injaz, was produced by reconstructing embryos following SCNT using cumulus cells of dromedary camel (Wani *et al,* 2010).

There are three main criteria for assessment of cloned animals including blastocyst development rate, birth rates and survival rate of newborns. In Table 1, blastocyst rates and birth rates in different studies were summarised. Out of more than 3000 reconstructed oocytes, 695 reached the blastocyte stage (23.1%). Of 915 recipients that received 1.67 blastocysts, on average, 105 were diagnosed pregnant by Day 60 of pregnancy (11.5%). The final achievement from more than 3000 reconstructed oocytes was 62 cloned camel calves from 2010 till 2023 (6.8%; Table 1). There is no information regarding the survivability and further performance of produced cloned camel calves. It was found that Injaz, the first cloned camel calf born on the 8th of April 2009, died several years ago. Early and late embryonic losses varied from 75 to 100%, were the main causes of failure in camel cloning.

Animal cloning remains inefficient compared with other assisted reproductive technologies, such as conventional embryo transfer, *in vitro* fertilisation, or artificial insemination. In general, the overall success rate from the creation of a viable and healthy camel calf remains at a similar and low level in literature similar to the achievements in other domestic animal species (Tsunoda and Kato, 2002; Oback, 2008; Czernik *et al,* 2019; Gouveia *et al,* 2020; French and Trounson, 2023). Despite the high cost and extreme difficulties, SCNT is often seen as a hope to restore extinct species or help preserve the endangered ones. Accordingly, the first cloned camel calf was produced from a decade-old vitrified tissue collected from a deceased champion show camel (Hossein *et al,* 2021).

Table 1. The result of camel cloning by SCNT technique from 2008 till 2023.

Authors	Year	Karyoplast	Oocyte maturation	NT oocyte	Fusion (%)*	Cleaved (%)*	Blastocyst (%)*	Recipients	Mean no embryos transferred	Pregnant ≥Day 60 (%)	Offspring (%)
Khatir and Anouassi	2008	Adult skin fibroblast	*In vitro*	369	247 (67)	217 (59)	52 (14)	5	---	1 (20)	0
		Cumulus cells	*In vitro*	363	225 (62)	162 (45)	55 (15)	7	---	1 (14)	0
Wani *et al*	2010	Cumulus cells	*In vivo*	75	60 (79.8)	---	26 (34.7)	26	2	4 (15)	1(3.8)
		Adult skin fibroblast-1	*In vivo*	98	87 (88.6)	---	29 (29.6)	29	1.7	2 (6.9)	0
		Adult skin fibroblast-2	*In vivo*	70	64 (92.1)	---	25 (35.7)	45	1.8	4 (8.9)	0
		Fetal fibroblast-1	*In vivo*	101	89 (88)	---	24 (23.8)	24	1.6	2 (8.3)	0
		Fetal fibroblast-2	*In vivo*	75	70 (92.8)	---	20 (26.7)	15	1.5	0	0
Wani and Hong	2018a	Adult skin fibroblast	*In vivo*	78	66 (85.2)	---	35 (44.9)	19	1.8	2 (10.5)	0
		Cumulus cells	*In vivo*	72	68 (94.2)	---	23 (31.9)	16	1.4	4 (25)	3 (18.7)
		Adult skin fibroblast-1	*In vivo*	86	75 (87.5)	---	21 (24.4)	17	1.2	4 (23)	3 (17.6)
		Adult skin fibroblast-2	*In vivo*	73	60 (82.6)	---	30 (41.1)	19	1.6	2 (10.5)	0
		Adult skin fibroblast-3	*In vivo*	92	86 (94)	---	25 (27.1)	22	1.1	2 (9.1)	1 (4.5)
		Adult skin fibroblast-4	*In vivo*	78	2 (79.3)	---	28 (35.9)	19	1.5	1 (5.3)	1 (5.3)
Wani and Hong	2018b	Adult skin fibroblast-1	*In vivo*	68	55 (80.5)	---	18 (26.5)	11	1.6	1 (9)	1 (9)
		Adult skin fibroblast-2	*In vivo*	79	67 (84.8)	---	25 (31.6)	19	1.3	2 (10)	2 (10)
		Adult skin fibroblast-3	*In vitro*	43	39 (91.1)	---	10 (23.2)	8	1.2	1 (12.5)	1 (12.5)
		Adult skin fibroblast-4	*In vitro*	32	24 (75.8)	---	24 (75.8)	3	1.7	0	0
Moulavi *et al*	2020	Adult skin fibroblast	HMC/ *In vitro*	---	---	---	---	47	2.6	5 (10.6)	4 (8.5)
		Adult skin fibroblast	HMC/ *In vitro*	---	---	---	---	15	2.4	1 (6.6)	1 (6.6)
		Adult skin fibroblast	Conventional/ *In vitro*	---	---	---	---	40	2.7	3 (7.5)	1 (2.5)
		Adult skin fibroblast	Conventional/ *In vitro*	---	---	---	---	5	2.4	1 (20)	0
Hossein *et al*	2021	Vitrified adult skin	*In vitro*	---	---	---	---	31	1.1	5 (16.1)	2 (6.4)
		Vitrified adult skin	*In vivo*	---	---	---	---	54	1.1	13 (24.1)	9 (16.1)
Olsson *et al*	2021	Adult skin fibroblast	---	---	---	---	---	286	---	31 (10.8)	19 (6.6)
Son *et al*	2022a	Adult skin fibroblast	*In vitro*	517	362 (70.0)	217 (41.9)	73 (14.1)	45	1.6	1 (2.2)	1 (2.2)
		Adult skin fibroblast	*In vivo*	309	223 (72.2)	183 (59.2)	101 (32.7)	62	1.5	10 (16.1)	10 (16.1)
Son *et al*	2022b	Adult skin fibroblast	*In vitro*	326	239 (73.3)	168 (51.5)	51 (15.6)	26	1.81	2 (7.7)	2 (7.7)
Summary				3004	2208	---	695 (23.1)	915	1.67	105 (11.5)	62 (6.8)

*All percentages were calculated based on NT oocyte.

Reconstructed embryos produced following SCNT have numerous biological problems with several undesired consequences following transfer to recipients and questionable survivability after birth. The main cause of these problems and consequences could be due to the failure or incomplete nuclear reprogramming (Bourc'his *et al,* 2001; Yang *et al,* 2007) and epigenetic modifications (Reik *et al,* 2001; Couldrey and Wells, 2013; Alsalim *et al,* 2018; Gao *et al,* 2018) resulting in the great differences in gene expressions (Li *et al,* 2005; Vassena *et al,* 2007). As a result, besides failure in maternal recognition of pregnancy (Arnold *et al,* 2006), high early and late embryonic death and abortion rates (Hill *et al,* 2000; Chavatte-Palmer *et al,* 2012), problems associated with the implantation, placenta development and function and also abnormal offspring syndrome (Niemann, 2016). Later obesity, immunodeficiency, respiratory defects and early death (Campbell *et al,* 2007; Loi *et al,* 2016) and the low birth rate (Gouveia *et al,* 2020; Yang *et al,* 2007) could be expected. The survival of offspring is also questionable. Using a neonatal intensive care unit (NICU) could be an essential part of maintaining SCNT calves due to several predicated and unpredictable problems. Information on cattle has estimated that nearly one in three cloned calves dies within the first 6 months of life (Chavatte-Palmer *et al,* 2004). Large/abnormal offspring syndrome, respiratory failure, abnormal kidney development, and cardiovascular and liver pathologies are often reported (Chavatte-Palmer *et al,* 2004; Watanabe and Nagai, 2009). SCNT technique could produce an offspring suffering from several health hazard problems that prevent the newborn from continuing a normal life. This could be a subject for those scientists who are dealing with animal ethics to consider some regulations to stop the commercial application of camel cloning until it is considered as a safe technique to produce offspring. Unraveling the molecular mechanism underlying SCNT-mediated nuclear reprogramming is needed to enhance the development of cloned embryos (Wang *et al,* 2020).

Conclusions

Several research groups using nearly similar and/or different approaches tried to clone camel. However, due to intrinsic problems associated with SCNT mainly because of epigenetic aspects of animal cloning involved in reprogramming the nucleus of an adult somatic cell, the final result of this technique is still extremely low and costly. With the small number of offspring produced by camel cloning during the last 13 years (not more than 70 cloned camels according to the published articles) without having any report on the health and survivability of cloned camel calves, any claims regarding the use of camel cloning to propagate elite racing, beauty and milking camel may not be valid and many years of innovative research with great investments are required for camel cloning to be recommended as a routine procedure in camel industry.

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CAMEL MILK AND IT'S APPLICATIONS IN TREATMENT OF DIABETES: SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Camel (*Camelus dromedarius*) milk has emerged as a plausible alternative treatment for diabetes. This systematic review and meta-analysis is aimed to report on the treatment applications of camel milk in diabetic patients. A comprehensive literature search was performed on PubMed.gov, Google Scholar and Cochrane Central Register of Controlled Trials (CENTRAL) databases to identify all published randomised clinical trials, clinical trials and experimental studies published within the past twenty years. The systematic review was conducted using a PROSPERO protocol prepared following the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). Nine studies were included in the systematic review. In a fixed-effects model, we conducted measurements and obtained the following results: the mean difference (MD) for haemoglobin A1c% (HbA1c%) was -0.85% with a 95% confidence interval (CI) of [-1.51, -0.18]; the MD for blood glucose in humans was -5.73 mg/dl with a 95% CI of [-14.92, 3.47]; the standard MD for blood glucose in rats was -5.73 with a 95% CI of [-14.92, 3.47]; the MD for plasma insulin was -5.73 with a 95% CI of [-14.92, 3.47]; and the MD for BMI was -0.22 with a 95% CI of [-1.09, 0.65]. Despite the high level of heterogeneity in the included studies, the findings had overarching evidence with high significance indicating that camel milk improves HbA1c% among patients with diabetes. We recommend that health sectors should properly position foods like camel milk between mainstream treatments and conventional foodstuffs.

Key words: Camel milk, diabetes, meta-analysis, review

In humans, diabetes is one of the most common chronic diseases. The World Health Organisation (WHO) found that between 2000 and 2016, diabetes was a major factor in the increase in premature mortality rates (deaths before the age of 70). Premature deaths in high-income countries due to diabetes fell between 2000 and 2010 but began rising again between 2010 and 2016 (WHO, 2021). During both periods, diabetes-related premature mortality increased in low- and middle-income countries. About 8.5% of the global population aged ≥18 had diabetes in 2014. Nearly half (48%) of all people died from diabetes before the age of 70, according to a WHO report in 2019 (WHO, 2021). However, between 2000 to 2016, the risk of death from non-communicable diseases, including diabetes among people aged 30–70 was reduced by 18% (WHO, 2021).

Studying diabetes is crucial for the significance of therapy allocation and informing community health services regarding the disease's dos and don'ts. Besides, reducing the prevalence of diabetes has been the main focus of most healthcare organisations. As reported by WHO, the prevalence of diabetes is affected low- and middle-income regions. These regions make up more than 80% of the global population living with diabetes (Mendenhall *et al*, 2014).

Type 1 and type 2 diabetes are the most dominant forms of diabetes. Type 1 diabetes is an immune-mediated, specifically autoimmune or idiopathic disease that attacks and damages insulin-producing beta cells of the islets Langerhans in the pancreas. As a result of damaged insulin-producing beta cells, the patient begins to experience a shortage of insulin (Turner and La Gruta, 2022). Type 2 diabetes, also called insulin-independent diabetes, is caused by defects in insulin receptors, diminish insulin release, or both.

Today, there are several treatment options in the form of oral (e.g., metformin, sitagliptin and

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sotagliflozin) and injectable (e.g. insulin, semaglutide and tirzepatide) medications to manage diabetes. Lifestyle and diet adjustments are also widely applied for patients with diabetes.

Before insulin and other advanced medical interventions came by, diabetes was treated using naturally-derived medicines (Boateng and Catanzano, 2015). Fujita *et al* (2003) researched the effects of touch-iextract on type-2 diabetic patients and whether it helped reduce their blood glucose. Touchi-extract is an α-glucosidase inhibitor that indicates a reduced postprandial rise in blood glucose levels (Fujita *et al,* 2003). The study reported that the fasting blood glucose and glycated haemoglobin levels and the triglyceride levels of the touchi-extract groups reduced significantly over four months. In a separate study, Lee *et al* (2003) investigated the usefulness of rice germ oil supplements on diabetes by researching their effect on serum and hepatic lipid levels. Patients who were not dependent on insulin were reported to have responded positively (reduced hyperglycaemic events and relieved symptoms) to sulfonylurea and biguanide preparations.

The primary treatment of type 1 diabetes remains insulin administration through parenteral routes. Camel (*Camelus dromedarius*) milk has emerged as a plausible alternative treatment for diabetes. This interest has come up due to camel milk showing exceptional broad therapeutic qualities on other serious human diseases. Zagorski *et al* (1998) investigated the popularly held belief that camel milk has large insulin concentrations. They studied the effects of camel milk on the blood glucose levels of laboratory rats. Evidence of insulin concentration in camel milk is undisputable and for this reason, camel milk reduces blood glucose levels in diabetic lab rats (Zagorski *et al,* 1998).

In an earlier study, (Agrawal *et al,* 2005a) conducted research aiming to observe the hypoglycaemic activity of camel milk. Diabetes was induced in 32 male albino rats using one intraperitoneal injection of streptozotocin 50mg/kg body weight (Agrawal *et al,* 2005a). Four treatment options based on camel milk were offered to four equal randomised groups. The groups were treated using either raw camel milk, pasteurised camel milk, raw camel milk + lactoferrin, or cattle milk and controlled for four weeks while conducting weekly blood estimations. This experimental study came to three sensible conclusions. Firstly, when diabetic rats were given raw camel milk, their mean blood glucose level dropped significantly. Secondly, adding lactoferrin to raw camel milk had no additional benefits. Finally, camel milk's hypoglycaemic activity reduces after pasteurisation. These findings confirmed camel milk's therapeutic potential, which can be used as an adjunctive treatment option in humans suffering from diabetes.

Looking at the current body of literature, there is much evidence surrounding the use of camel milk as a treatment option for diabetes. The successful application of camel milk in treating diabetes has also been reported by several investigations reviewed thus far. However, a gap still exists in the consolidation of evidence to inform further applications of camel milk in the treatment process. This systematic review and meta-analysis were carried out to provide credence to the current findings reported by various primary studies. The importance of natural substances in being preventive and therapeutic to major diseases has been the reason for focusing on camel milk. This systematic review and meta-analysis presented findings will give a lot of weight to current and developing applications of camel milk in treating diabetes. This systematic review and meta-analysis have been carried out to report on the treatment applications of camel milk in diabetes patients.

Methods

Protocol

Following the recommendations of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and the Cochrane Handbook for Systematic Reviews of Interventions, we conducted this systematic review and meta-analysis using a PROSPERO protocol. Cumpston *et al* (2019) detailed the PRISMA extension used to compile this review in Chapter 4 of the Cochrane Handbook for Systematic Reviews and Interventions.

Literature Search

We looked through a variety of sources for publications on clinical trials, randomised controlled trials and other forms of experimental experiments. The search took into account research from 2000 to 2022. We searched PubMed.gov, Google Scholar and the Cochrane Central Register of Controlled Trials (CENTRAL) for pertinent papers. We also carefully went through reference lists to back up our findings. We utilised focused filters to narrow down the most recent usage of camel milk for diabetes management. We used a combination of keywords and MeSH headings to discover relevant articles. These MeSH phrases and keywords were used to construct search

queries, which were then extended with Boolean operators (AND, OR), truncation methods (Asterix) and field tags. For diabetes, camel milk and treatment, we also evaluated keyword variations and related search phrases.

Eligibility Criteria

Eligible articles were required to meet the following criteria: (1) They had to be randomised clinical trials, clinical trials, or experimental studies without any quality restrictions to the methodology; (2) The investigation was to either focus on human subjects (primarily adults) diagnosed with type 1 or type 2 diabetes or animal subjects (primarily mice) with streptozotocin-induced diabetes; (3) Randomisation of the included studies must be reporting on the compared clinical outcomes of camel milk when used in treating diabetes to those using placebo modalities; (4) The outcomes of interest reported by the studies must be indicating the therapeutic potentialities of camel milk and the treatment success of the same. Publication formats were not used as restrictions. However, only studies published in the English language would be included. Articles published in non-English languages but had English translations were also included. Assessment for study eligibility was carried out by two independent reviewers participating in the systematic review and meta-analysis.

Data Extraction

Two independent reviewers used an Excel pre-designed data extraction form to extract data. Extracted data included first authors and last names, country, publication year, relevant demographic data (camel milk preparations (raw or pasteurised)), additional medications in the interventions, treatment duration, diabetes type under therapy and outcome improvement). The opinion of a third reviewer resolved any disagreements between the findings of the two data extractors. Extracted data were presented for quality screening and then for statistical analysis.

Statistical Analysis

Statistical analysis was done on Review Manager 5.4 (RevMan 5.4). The results from this phase provided a fixed effects odds ratio for the treatment success of diabetes using camel milk. The meta-analysis tested the effects of treatment using a mean difference (MD) and standard mean of difference (SMD) at a 95% confidence interval for the reduction of diabetic parameters when patients are treated with camel milk. The results of this meta-analysis were reported on forest plots which represented outcome comparisons of camel milk versus control. Additionally, a funnel plot was generated to represent the publication bias of the included studies. We also used the I^2 statistic to report on the studies' heterogeneity.

Results

Inclusion and Exclusion of Studies

We identified 543 articles from the primary search. In the first step, 430 articles were eliminated by Covidence automation tools for various predefined eligibility reasons, including duplication. We looked at 118 articles that underwent title and abstract screening for the first screening process. Out of these, 93 were eliminated and 25 remained for full-text screening. Eliminated studies were dropped for reasons such as observing camel milk interact with other diseases, missing parameters in the reports, studies focusing only on the antidiabetic composition of camel milk, reviews, book chapters and many more. We selected nine studies (Agrawal *et al,* 2005a; Agrawal *et al,* 2005b; Ejtahed *et al,* 2015; Mohamad *et al,* 2009; El-Sayed *et al,* 2011; Shareha *et al,* 2017; Fallah *et al,* 2020; Korish, 2014; Khan *et al,* 2013) from the 25 submitted for full-text screening. Eliminated 16 studies were dropped for lack of a control study group, missing data, lack of numerical data, studies not reporting on the effects of camel milk on blood glucose and others. Fig 1 below shows a PRISMA 2020 flow diagram for updated systematic reviews, which only searches for databases. The characteristics of the included studies are provided in Table 1.

The effect of camel milk on diabetic humans and rats

In this systematic review and meta-analysis, we looked at nine studies published within the last two decades and found evidence that consuming camel milk improves diabetes and its underpinning factors. In the statistical analysis section, we analysed the effects of camel milk consumed by diabetics on key diabetic indicators such as HbA1c%, fasting blood glucose (in humans and rat subjects) and plasma insulin concentration and body mass index (BMI).

We measured the mean difference in a fixed-effects model and found the mean difference (MD) for HbA1C% was -0.85 [95% CI: -1.51, -0.18] with a significant p-value (p = 0.01) (Fig 2). Other outcome measures, including fasting blood glucose in humans (MD -5.73 [95% CI: -14.92, 3.47], p = 0.22, Fig 3), plasma insulin in humans (MD -5.73 [95% CI: -14.92, 3.47], p = 0.69, Fig 4), BMI in humans (MD -0.22 [95%

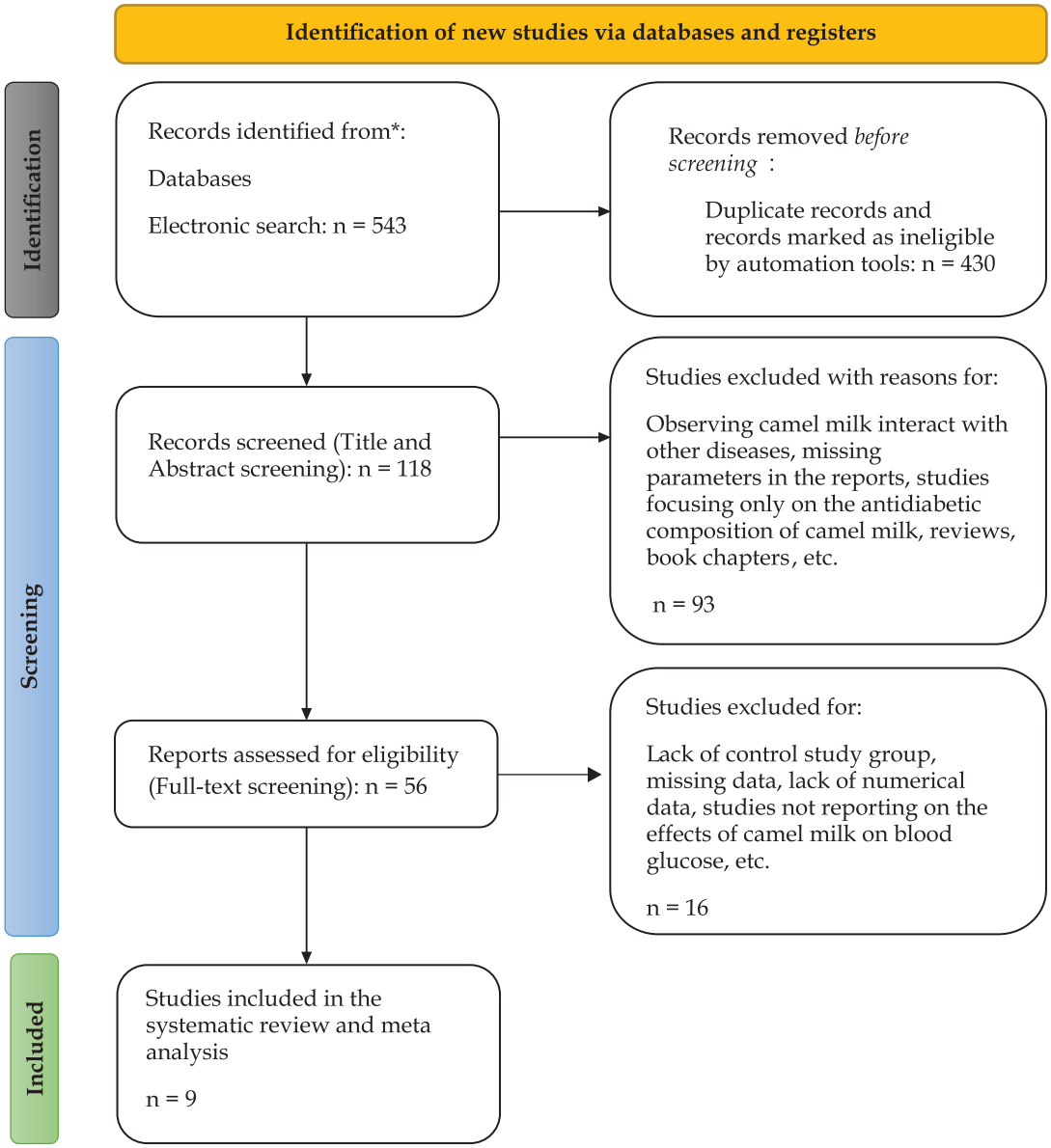

Fig 1. PRISMA flow diagram of studies in systematic review.

CI: -1.09, 0.65], p = 0.62, Fig 5) and blood glucose in rats (standard MD -5.73 [95% CI: -14.92, 3.47, p = 63, Fig 6]. The level of heterogeneity was considerable for three measures (HbA1C%, fasting blood glucose in humans and blood glucose in rats), while very low heterogeneity was seen for studies evaluating the effect of camel milk on plasma insulin and BMI in humans.

Discussion

In 2004, (Agrawal *et al,* 2004) published an observational study highlighting the prevalence of diabetes in camel milk-consuming Raica rural community of northwest Rajasthan, India. The community of Raica consumed a lot of camel milk and after observing them, the study found a very low prevalence of impaired glucose tolerance and diabetes (Agrawal *et al,* 2004).

It is widely accepted that camel milk had positive impact on diabetic patients (Sumaira *et al,* 2020). A systematic review had shown that camel milk had beneficial effects on diabetes mellitus by lowering blood sugar levels, reducing insulin resistance and improving lipid profiles (Mirmiran *et al,* 2017). In addition, the extended use of camel milk among patients with diabetes had the potential to serve as a beneficial supplementary therapy when combined with conventional medications. Particularly, it may aid in reducing the necessary insulin dosage and improving HbA1c levels (AlKurd *et al,* 2022).

Table 1. The study characteristics.

Author	N	Subject	Randomization	Setting	Intervention		Control	Outcomes measured	Results
					Dose	Duration			
Agrawal (2005a)	40	Male albino rats	Group I: (n = 8) on raw camel milk, Group II: (n = 8) on pasteurised milk, Group III: (n = 8) on a standard diet and raw camel milk, Group IV: (n = 6) on a standard diet and cow milk, Group V: (n = 8) on a standard diet	India	Group I - IV: 25 ml of camel milk daily, Group II: 25ml	4 weeks	Group V: Tap water	Blood glucose	Day 28= Group I: 81.54±11.43, Group II: 113.08±29.09, Group III: 93.24±11.57, Group IV: 203.79±40.66 vs Group V: 77.29±7.42
Agrawal (2005b)	24	Humans	Group 1: (n = 12) control group, Group 2: (n = 12) camel milk group	India	of pasteurised camel milk daily, Group III: 1mg of	1 year	Group 1: usual care	BMI (kg/m2), Blood sugar, Plasma insulin, HbA1c (%)	Group 1= BMI: 18.2 ± 3.8, Blood sugar: 105.25 ± 14.50, Plasma Insulin: 19.54 ± .43, HbA1c: 7.63 ± 1.03 Group 2= BMI: 19.7 ± 2.97, Blood sugar: 95.42 ± 15.70, Plasma insulin: 18.17 ± 7.12, HbA1c: 6 ± 0.96
Ejtahed (2015)	20	Humans	Intervention group (n = 11), Control group (n = 9)	Iran	lactoferrin, Group IV: 25ml of cattle milk	2 months	Control group: daily 500 mL of cow milk (250 mL morning and evening)	Blood sugar, Plasma insulin	Intervention= Blood sugar: 169.92 ± 45.9, Plasma insulin: 14.01 ± 13.31. Control= Blood sugar: 160.92 ± 57.96, Plasma insulin: 11.69 ± 6.25
Mohamad (2009)	64	Humans	Group 1 patients (n = 27), Group 2 patients (n = 27), Control (n = 10)	Egypt	Group 2: 500 ml camel milk in addition to usual care	16 weeks	Healthy individuals	BMI, fasting blood sugar, HbA1c (%)	Group 1= BMI: 18.43 ± 3.59, Blood sugar: 227.2 ± 17.7, HbA1c: 09.59 ± 2.05 Group 2= BMI: 24.3 ± 2.95, Blood sugar: 98.9 ± 16.2, HbA1c: 7.16 ± 1.84. Control= BMI: 25.3 ± 7.93, Blood sugar: 81.7 ± 9.16, HbA1c: 6.8 ± 1.08
El-Sayed (2011)	50	Humans	Group A (n = 15), Group B (n = 15), Group C (n = 15)	Yemen	Intervention group: daily 500 mL of camel milk (250 mL morning and evening)	12 weeks	Group A: usual care (diet, exercise and insulin injection)	BMI, fasting blood sugar, HbA1c (%)	Group A= BMI: 17.79±0.27, Blood sugar: 173.4±1.66, HbA1c: 9.27±0.36. Group B= BMI: 17.38±0.18, Blood sugar: 155.13±3.5, HbA1c: 7.28±0.23. Group C= BMI: 20.79± 0.28, Blood sugar: 147.26±1.89, HbA1c: 5.62±0.21
Shareha (2017)	43	Humans	Group 1 (n = 22), Group 2 (n = 21)	Libya	Group 1: Usual management for diabetes like diet, exercise and insulin mixtard. Group 2: daily 500 mL of camel milk in addition to the usual management for diabetes.	3 months	Group 1: Usual care i.e., diet, exercise and insulin dose	HbA1c (%), Fasting blood sugar	Group 1= HbA1c: 7.72 ± (0.10), Blood sugar: 198.86 ± (3.32). Group 2= HbA1c: 7.03 ± (0.06), Blood sugar: 187.05 ± (5.29)

Fallah *et al,* (2020)	40	Humans	Camel milk group (n = 19), Cow milk group (n = 17)	Iran	Group B: usual care (diet, exercise and insulin injection) + 500 mL/day of camel's milk, Group C: usual care (diet, exercise and insulin injection) + insulin mixed with 500 ml/ day of camel's milk	3 months	Cow milk group: 500 mL of raw cow milk daily	HbA1c (%), Fasting blood sugar	Camel milk group= HbA1c: 9.4±0.3, Blood sugar: 148.4±59.5 Cow milk group= HbA1c: 9.5±0.3, Blood sugar: 152±51.4
Korish (2014)	80	Wistar rats	Group C (n=20), Group C-CMK (n=20), Group D (n=15), Group D-CMK (n=20)	Saudi Arabia	Group 2: Usual care + 500 ml of fresh camel milk daily	8 weeks	Group C: Control normal rats receiving no treatment. Group C-CMK: Control normal rats treated with camel milk. (Approximately 35 ml/rat/day)	Fasting blood sugar, Plasma insulin	Group C= Blood sugar: 76.00 ± 3.88, Plasma insulin: 0.44 ± 0.03. Group C-CMK= Blood sugar: 76.20 ± 6.74, Plasma insulin: 0.43 ± 0.03. Group D= Blood sugar: 459.40 ± 100.04, Plasma insulin: 0.25 ± 0.03. Group D-CMK= Blood sugar: 198.70 ± 135.31, Plasma insulin: 0.38 ± 0.09
Khan *et al,* (2013)	40	Albino wistar rats	Group 1 (n=8), group 2 (n=8), Group 4 (n=8), Group 4 (n=8), Group 5 (n=8)	Saudi Arabia	Camel milk group: 500 mL of raw camel milk daily	30 days	Group 1: Normal control rats, Group 3: Diabetic control group injected with streptozotocin (STZ)-induced diabetes (55 mg kg-1 b.wt.).	Blood sugar	Group 1: 115.64±5.60, Group 2: 121.76±4.30, Group 3: 520.46±8.90, Group 4: 235.61±7.10, Group 5: 135.32±5.20
					Group D: diabetic rats receiving no treatment. Group D-CMK: Diabetic rats treated with camel milk (Approximately 35 ml/rat/ day)				
					Group 2: Normal rats fed with camel milk, Group 4: Diabetic rats fed with camel milk, Group 5: Diabetic rats treated with insulin (6 units kg-1 b.wt./day)				

There was a long-held notion in the Middle East that drinking camel milk on a regular basis would help prevent and manage diabetes. According to recent investigations, camel milk had characteristics that may substantiate these assertions. Camel milk insulin was thought to have special properties that improved absorption into the bloodstream when compared to insulin from other sources. This could be because insulin was coated in nanoparticles, allowing it to pass through the stomach and into circulation. Furthermore, several components found in camel milk were known to have anti-diabetic properties. The structure of camel insulin and its expected digestion pattern showed no significant alterations that

Human Subjects

Haemoglobin A1c% (HbA1c%)

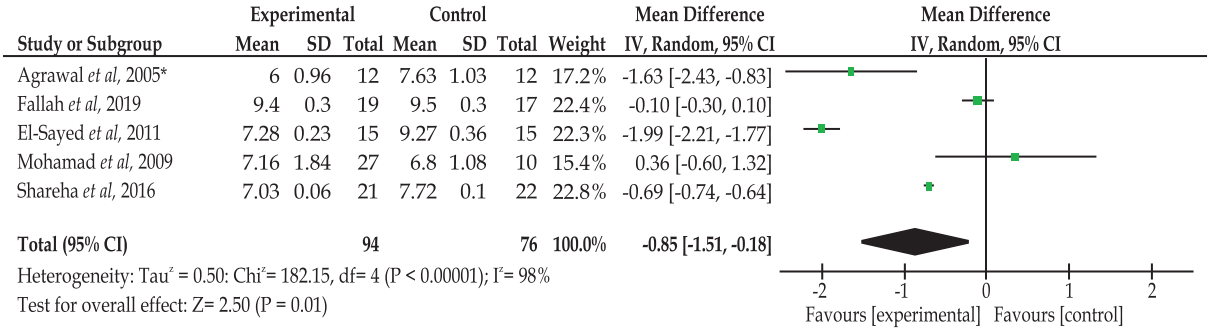

Fig 2. A forest plot of HbA1c in human subjects.

Fasting Blood Glucose

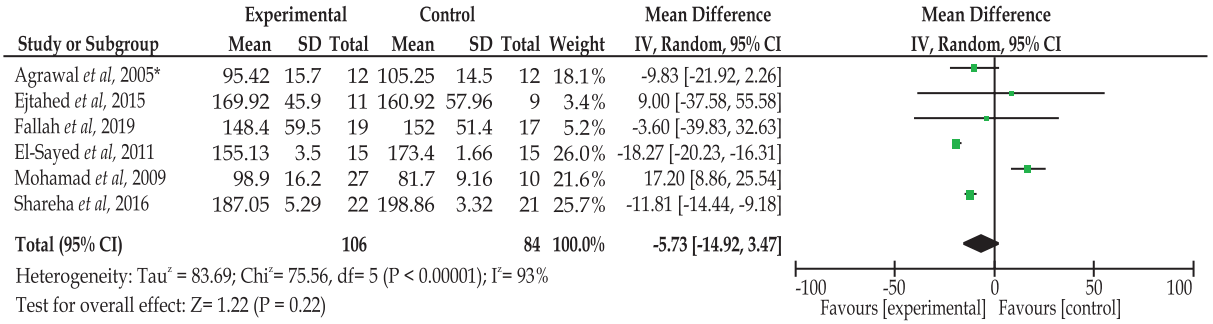

Fig 3. A forest plot of blood glucose in human subjects.

Plasma insulin

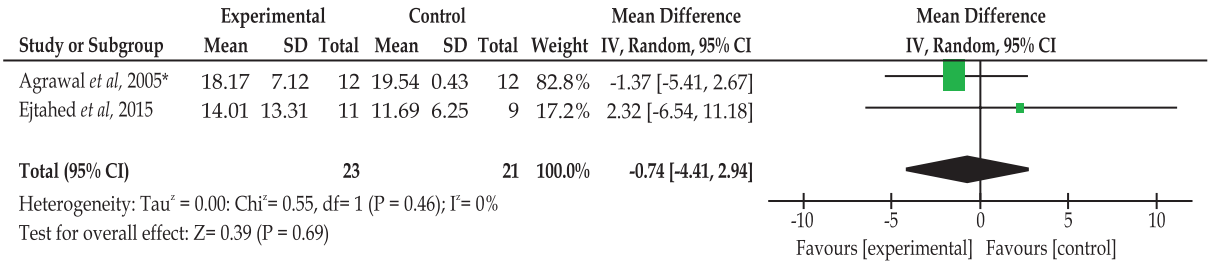

Fig 4. A forest plot of plasma insulin.

would allow it to overcome the digestive system's limitations before (Malik *et al,* 2012).

In a clinical trial, researchers separated 60 patients with Type 2 Diabetes who were already taking oral diabetes medications into two groups. The first group, known as group 1, was given 500 mL of raw camel milk twice a day (in the morning and at night) along with their prescription diabetes medications for three months. The second group, referred to as group 2, only got oral anti-diabetic drugs and did not consume camel milk. The study's findings revealed considerable improvements in a number of metrics. In both groups, fasting blood glucose and postprandial (after-meal) glucose levels decreased significantly. Furthermore, there was a significant decrease in HbA1c levels, which was a marker used to monitor long-term blood sugar levels. Furthermore, group 1 had a considerable drop in total cholesterol and triglyceride (TG) levels in addition to their prescribed drugs. Throughout the trial, however, there were no statistical differences in urea and creatinine levels, which were indications of kidney function (Sboui *et al,* 2022).

In this comprehensive examination and synthesis of existing research, we examined a collection of nine studies published over the past twenty years. Our investigation revealed compelling evidence suggesting that the consumption of camel milk offers benefits in managing diabetes by reducing HbA1c%, however, there were no significant benefits

BMI

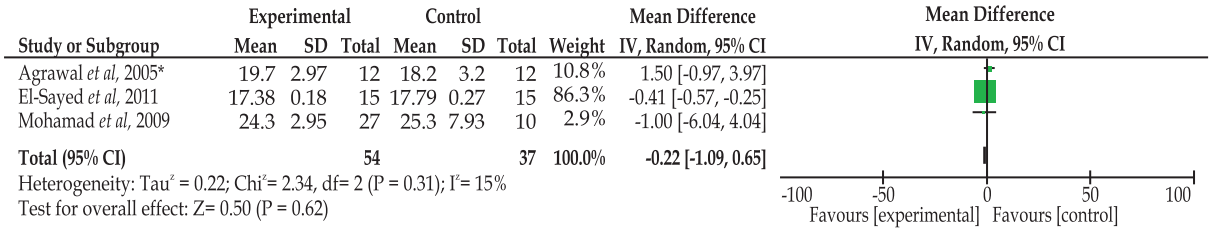

Fig 5. A forest plot of BMI.

Rat Subjects

Blood glucose

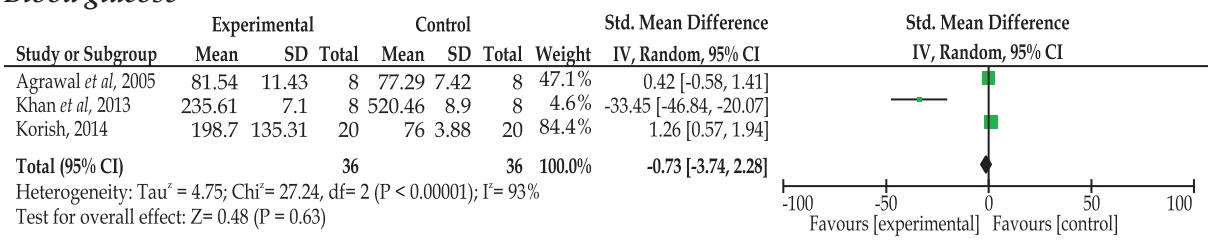

Fig 6. A forest plot of blood glucose in male albino rats.

regarding blood glucose level, plasma insulin level and BMI. These results indicated that camel milk might require consistent use for several months to show antidiabetic efficacy.

Agrawal *et al* (2005a), Korish (2014) and Khan *et al* (2013) present useful results obtained by inducing diabetes into rats and treating them with camel milk. Agrawal *et al* (2005a) pointed to the oxidative potentialities of camel milk due to its high vitamin-C concentration and mineral content (sodium, potassium, iron, zinc, copper and magnesium). As a result of these antioxidants, camel milk insulin receptors in the milk respond favourably to the presence of insulin (Agrawal *et al,* 2005a); (Korish, 2014); (Khan *et al,* 2013). In human subjects, Agrawal *et al* (2005b), Ejtahed *et al* (2015), Mohamad *et al* (2009), El-Sayed *et al* (2011), Shareha *et al* (2017), Fallah *et al,* (2020), Korish (2014) and Khan *et al* (2013) provided more reliable findings. In the study by (Ejtahed *et al,* 2015), the plasma profile contents rose while fasting blood glucose and blood pressure changed. The study demonstrated the success of treatment using camel milk. However, in both interventional and control groups demonstrated positive changes in diabetes risk factors (El-Sayed *et al,* 2011). In another instance, findings of Mohamad *et al* (2009) seemed to contradict those of Agrawal *et al* (2005b) in terms of C-peptide levels. More recent studies (Shareha *et al,* 2017 and Fallah *et al,* 2020) seem to come to a consensus on the reduction of the major diabetic parameter (blood glucose, HbA1c%, plasma insulin, BMI). As a result, the insulin doses used by diabetic patients are reduced (Agrawal *et al,* 2005b and Fallah *et al,* 2020).

In conclusion, despite the high level of heterogeneity in the included studies, the findings have overarching evidence with high significance indicating that camel milk improves HbA1c% among patients with diabetes. The antidiabetic effects of camel milk can be attributed to the existence of high insulin that has been encapsulated in nanoparticles. The insulin found in camel milk could be absorbed in the intestine as it is not coagulated by stomach acid and may have a role in reducing HbA1c%. (Korish 2014) also reports on insulin-like proteins, polyunsaturated fatty acids, minerals (sodium, potassium, iron, copper, zinc and magnesium), Vitamins C and B3 and immunoglobulins, with low fat and sugar contents. In the present systematic review and meta-analysis, camel milk only qualifies to be used as an adjuvant to other medications for diabetes. We recommend that health sectors properly position foods like camel milk between mainstream treatments and conventional foodstuffs. That way, camel milk will be used much more for therapeutic purposes.

Limitations

The included studies were not specific to one form of diabetes, which may affect the outcomes regarding blood glucose levels in human and animal subjects.

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Disclosure statement

The authors declare no conflict of interest.

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SELECTED RESEARCH ON
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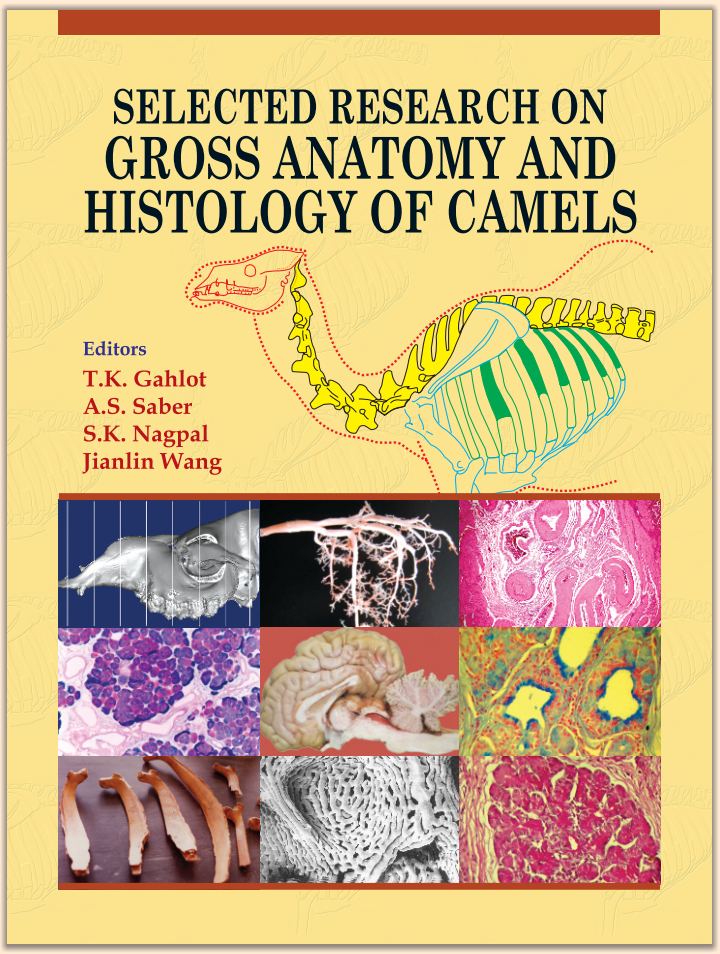
Selected Research on Gross Anatomy and Histology of Camels is a unique reference book on anatomy of dromedary and bactrian camels. This book contains a first ever wide spectrum of histological description of various organs of camels which is depicted by special stains and scanning electronmicroscopy in addition to the gross anatomy, histochemical and immunohistochemical studies. The book has 92 manuscripts in 9 sections, e.g. radiographic anatomy, anatomy of various systems (skeletal, digestive, respiratory, circulatory, urogenital and nervous), common integument and miscellaneous. These manuscripts were published by 158 authors working in 37 laboratories or colleges or institutions from 14 countries in the Journal of Camel Practice and Research between June 1994 to June 2010. Bactrian camel anatomy research was exclusively contributed by the researchers of China. The countries involved in camel anatomy research were China, Egypt, India, Iran, Saudi Arabia, Iraq, Jordan, Japan, Pakistan, Sweden, United Arab Emirates, United States of America, France and Germany. Camel Publishing House has taken a step forward to compile this knowledge in form of a book and this herculian task was accomplished by its dedicated editors, viz. T.K. Gahlot (India), S.K. Nagpal (India), A.S. Saber (Egypt) and Jianlin Wang (China). This classic reference book will serve as a one stop resource for scientific information on gross anatomy and histology of camels.



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A QUARTER INDIVIDUAL MILKING MACHINE "STIMULACTOR®" IN A CAMEL FARM IN SWITZERLAND: ACCORDING TO FIELD STUDY

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ABSTRACT

The aim of the field study was to test the performance of the quarter-individual milking machine "StimuLactor" (ST-C) in a camel farm in Switzerland. Eight one-humped lactating dromedaries were used for this purpose. The camels were milked twice a day with a unit milking machine-StimuLactor for camels. The setting of the milking machine was as follows: vacuum level 36 kPa, pulsation rate 90 cycles/min and, pulsation ratio 65/35. In addition, the milking machine was equipped with sequential pulsation (25% offset quarter to quarter) and the teat cups are equipped with round silicone liners and an air inlet valve (Bio-Milker). Daily milk yield was recorded over a period of one year and milk samples were taken for qualitative analysis. The results have shown that after using the new milking machine no pathogenic bacteria were detected in the milk produced during the trial period. The examined milk parameters fat, protein, lactose, somatic cell count (SCC) and non-pathogenic bacteria (NPB) were in the physiological range and the concentration were 3.33%, 2,39%, 4.09%, 79000 cells/ml and 6150 b/ml, respectively. Finally, milking with StimuLactor has shown very good results, since this milking machine has been adapted to the anatomical, morphological and physiological characteristics of the camel's udder.

Key words: Camel, fat, lactose, milking machine, protein, SCC, StimuLactor

Camel milk is still the most important nutritional source for pastoralists in rural areas in Asia and Africa. However, in the last 2 decades, camel milk has been in great demand in Europe and north America (Dijk, 2021), because camel milk has several minor components that have special bioactive properties. These are present at significant concentrations and are extremely important and beneficial for human diet and health (Kaskous, 2016; Kaskous and pfaffl, 2017; Sumaira Shah *et al,* 2020; Swelum *et al,* 2021; Ismail *et al,* 2022; Behrouz *et al,* 2022). Therefore, the amount of camel milk needs to be increased to meet the demand. To increase the milk yield for each camel and to improve the quality as well as the safety of raw camel milk, machine milking must be used instead of hand milking (Hammadi *et al,* 2010; Nagy and Juhasz, 2016; Kaskous, 2018). Nowadays, the development of milking technology is making great strides. A special modern milking machine for camels "StimuLactor" (ST-C) was developed in 2018 by Siliconform, Germany and has been used in practice since then (Kaskous, 2019a; 2021a; 2023). This new milking technique has not only improved milk yield and quality, but also the working conditions for the milkers and the welfare of the camels (Kaskous, 2023). Fast milking and above all complete milking is a matter of course in the StimuLactor milking system. In addition, this new milking technique was adapted to the morphological, anatomical and physiological requirements of camels (Kaskous, 2019a). The aim of this field study was therefore to demonstrate the efficiency of the StimuLactor milking machine on a commercial camel farm.

Materials and Methods

The experiments of this study were conducted in compliance with the requirements of the Swiss animal protection and welfare law.

Animals and Housing

Eight one-humped lactating camels from a commercial camel farm in Switzerland were used. The camels varied in parity numbers and stages of lactation. The camels were kept outdoor most of the time. However, at night and in the cold winter they were kept in a loose housing system. Camels were fed primarily on pasture grass and were also provided

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with grass hay and supplements of vitamins and minerals. Drinking water was administered *ad libitum*.

Milking Equipment and Properties

The camels were milked twice a day with a unit milking machine - StimuLactor for camels (Siliconform, Germany) (Fig 1). The working vacuum level was 36 kPa and sequential pulsation (25% offset quarter to quarter) was adopted. The pulsation rate was 90 cycles per minute and the pulsation ratio was 65/35 during the milking time. The main characteristics of the milking machine used were as follows:

- It was an easily handled and animal- as well as person-friendly semi-automatic milking system that differed technically and impressively from conventional milking machines.
- It was based on a quarter-individual milking system. This meant that teat cups worked completely independently of each other (without a claw).
- The teat cups were equipped with round silicone liners and an air inlet valve (called Bio-Milker).
- The teat cups could be easily attached to the teat with one hand. In this way, accidents, or injuries to the milker during milking were avoided.
- The milking machine milks as the calf suckles, so the calf did not have to be present during milking.
- In addition, the system included a very special pre-stimulation program and an excellent cleaning and sanitary process.

The milking routine

The milking routine was performed according to the usual routine of the farm. This included pre-milking preparations, in which the teats were cleaned with a wet udder tissue and afterwards dried with another tissue. Then, each teat cup was individually or in pairs manually attached to the teats. After this step, the system was started on the control display and stimulation began. The system was programmed to intensively stimulate through a standard pulse rate (90 cycles/min) and a reduced milking phase (bphase)of 10 % over a period of 90 s. Simultaneously, additional stimulation was reached through intensive movement of the teat cups by an actuator. This mechanical arm supports the four milk tubes. During the stimulation phase and the milking phases the arm moved up and down. This movement was transferred to the teat cups and made the teats erect. With this method, the liners apply a vibratory massage to the udder and teats, like the calf would do during suckling. After this stimulation phase the main milk phase began and the milk flow was observed on the display. When the milk flow decreased to a certain level, the milking process was automatically stopped by detaching the milking unit. After all animals had been milked, the milking system was cleaned.

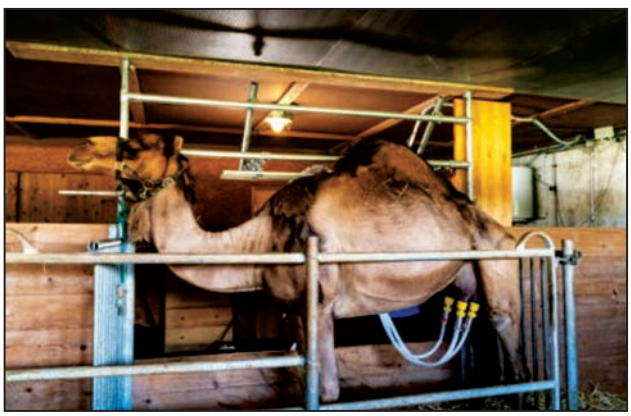

Fig 1. A StimuLactor milking machine during milking in a camel farm in Switzerland.

Milk sampling and milk analysis

Daily milk yields were recorded and milk samples were taken for qualitative analysis for a period of one year after the introduction of the new milking machine. The milk samples were examined by the animal health service and the Milchprüfring Bavaria e. V.

Statistical Analysis

For statistical analysis, the SAS program (version 9.2. SAS Institute Inc.) was used. The results were presented as arithmetic means and standard errors.

Results and Discussion

- Findings of bacteria in the examined milk samples.

The milk analysis has shown that after installing the new milking machine no pathogenic bacteria were to be found in the milk produced during the trial period. These results clearly showed that the StimuLactor milking machine is adapted to the camel's udder. Thus, milking machine design and function are critical for rapid and efficient removal of milk without damaging the teat and without transmitting pathogenic microorganisms that might cause mastitis. Similar results were shown by Kaskous (2019a). The milk remained clean and free from pathogenic bacteria. Furthermore, it has been shown that the use of machine milking is preferable to hand milking, since the contamination with pathogenic bacteria was very high in hand milking compared to

machine milking (Saleh and Faye, 2011). It has also been found that improper use of the milking machine, especially improper use of liners, can damage the camel's udder and lead to oedema and promote the colonisation of *Staphylococcus aureus* during the time of machine milking (Juhasz and Nagy, 2008). New results from Tunisia showed that the use of machine milking in the field was associated with increased milk yields but that in also caused an increased microbial load compared to hand milking (Atigui *et al,* 2023). These results emphasise that improper use of the milking machine has a negative impact on teat health. Apparently, the good results of this study are due to the use of a lower vacuum (36 kPa) in the milking machine. The use of high vacuum for camels could lead to udder health problems, which are reflected by high SCC in the milk produced and a negative impact on the health status of the teat (Kaskous, 2018).

Camel milk parameters

As shown in Table (1), the mean of fat concentration in the camel milk was 3.33±0.07%. This value was within the normal range for camel milk and meets other study results (Siboukeur, 2007; Chethouna, 2011; Nagy *et al,* 2013; Benyagoub *et al,* 2013; Alwan *et al,* 2014; Kaskous, 2019a). However, fat content of camel milk varies greatly between 2% and 5% in the literature depending on many factors such as parity, stage of lactation, breeds, weather conditions, feed, presence of water, country, weaning time and milking methods (Hassan *et al,* 2007; Haddadin *et al,* 2008; Bekele *et al,* 2011; Mustafa *et al,* 2020; Bakry *et al,* 2021; El-Hanafy *et al,* 2023).

The mean content of camel milk protein was 2.39±0.03% (Tab. 1) and it ranged between 2.17±0.05% and 2.57±0.07%. This protein concentration correlates with various studies (Ellouze and Kamoun, 1989; Raghvendar et al, 2004; Bakheit et al, 2008), while it appears quite low, compared to other author's results (Mal *et al,* 2006, 2007; Yadav *et al,* 2015). Indeed, this protein content in camel milk can be normal under Swiss conditions, since water is free and green fodder or hay is available all year round. Under these conditions, the protein synthesis in the udder were in the physiological range.

The average lactose content in camel milk was 4.09±0.03% and ranged from 3.91±0.04% to 4.16±0.04% (Tab. 1). Similar results were reported by Hassan *et al* (1987); Elamin and Wilcox (1992); Wangoh *et al* (1998); Raghvendar *et al* (2004); Kouniba *et al* (2005); Haddadin *et al* (2008) and Smits *et al* (2011). However, many researchers reported that lactose concentration of camel milk varied between 2.4% and 5.8% (Khan and Iqbal, 2001; Konuspayeva *et al,* 2009; Karaman *et al,* 2022). Faraz (2020) reported lactose contents from 4.8-5.8%, which are slightly higher than those of cow's milk.

The mean SCC in raw camel milk was 79000±23000 cells/ml and it ranged between 66000 and 118000 cells/ml (Tab. 1). Under Swiss conditions, this SCC concentration was normal and within the physiological range. Kaskous (2021b) reported that a SCC 150000 cells/ml in camel milk was a threshold value for healthy camels and that it is within physiological values. SCC in camel milk could be the main indicator of milk hygiene, milk quality and udder health (Hadef *et al,* 2016). Results from literature studies have shown that SCC were higher in camel milk compared to these results. The investigation by Hamed *et al* (2012) found that the arithmetic means of SCC in camel milk were 100000 cells/ml. Another study found that the mean SCC in raw camel milk from healthy udders under German conditions was 126430±7210 cells/ ml (Kaskous, 2019a). The concentration of SCC in Saudi Arabia has been obtained by Saleh and Faye (2011) and the mean value of SCC was 125000 cells/ml. The results from Golestan province in Iran have shown that out of 243000 cells/ml in camel milk samples from individual quarters (95 milking camels), 18.1% were subclinical mastitis and that SCC values beyond 306000 cells/ml could be considered as subclinical mastitis in the camel (Niasari-Naslaji *et al,* 2016). Abbood (2016) suggested that an SCC value of 250000 was specified as the limit value for a healthy camel.

The mean value of NPB in raw camel milk was 6150±230 b/ml and it ranged between 5780 and 6330 b/ml. This non-pathogenic germ count in the raw camel milk was normal during the experiment and the udders remained healthy. Kaskous (2019b) reported that raw milk from a healthy udder contained a very low concentration of microorganisms, typically less than 1000 colony-forming units of total bacteria per ml (cfu/ml). It is important to remember that the milk from a healthy udder was virtually sterile (Johnson *et al,* 2015), since the camels udder was protected by a variety of defense mechanisms such as innate or specific immunity and physiological peculiarities and that it was only contaminated with germs when passing through the teat canal (Zangerl, 2007). However, the germs that got into the milk cause from the surface of the udder and teats, the stall, the feed, the milker, the air, the water and the milking machine (Kaskous,

2019b). Anyway, the number of NPB was low in this study, because the farm condition is ideal.

Table 1. Average of camel milk parameters during the experimental period.

Parameter	N	Concentration
Fat %	208	3.33±0.07
Protein %	208	2.39±0.03
Lactose %	208	4.09±0.03
SCC Cells/ml	208	79000±23000
Non-Pathogenic Bacteria /ml	208	6150±230

Conclusion

- The results of this study clearly showed that the quarter-individual camel milking machine "StimuLactor" is adapted to the anatomical, morphological and physiological needs of camel udders.
- The milking machine ST-C is easy for the milker to use and requires less effort compared to conventional milking machine.
- The calves do not need to be present during milking as this milking machine mimics the way the calf suckles.

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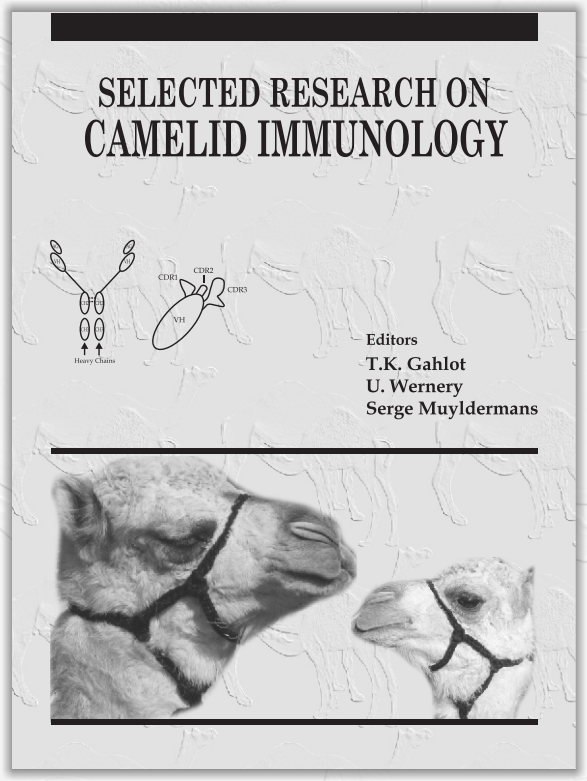
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

(Hard Bound, 392 pages, few figs coloured, Edition 2016)

In 1989 a group of biologists led by Raymond Hamers at the Free University Brussels investigated the immune system of dromedaries. This discovery was published in Nature in 1993. Based on their structure, these peculiar camelid antibodies have been named Heavy Chain Antibodies (HCAb), as they are composed of heavy chains only and are devoid of light chains. Sera of camelids contain both conventional heterotetrameric antibodies and unique functional heavy (H)-chain antibodies (HCAbs). The smaller size and monomeric single domain nature make these antibodies easier to transform into bacterial cells for bulk production, making them ideal for research purposes. Camelid scientists world over were greatly fascinated by a new field of research called "Camelid Immunology". Significant research has been done on camelid immunology in recent decade. In order to benefit future camelid immunology researchers, this book was planned in the series of "Selected Topics" by Camel Publishing House with a title- "Selected Research on Camelid Immunology" edited by T.K. Gahlot, U. Wernery and Serge Muyldermans. This book is a unique compilation of research papers based on "Camelid Immunology" and published in Journal of Camel Practice and Research between 1994-2015. Research on this subject was done in 93 laboratories or institutions of 30 countries involving about 248 scientists. In terms of number of published papers in JCPR on the immunology the following countries remain in order of merit (in parenthesis), i.e. Iran (1), India and UAE (2), China and Saudi Arabia (3), Sudan (4), Kenya and Belgium (5), USA (6), Germany (7) and so on. The book contains 11 sections and is spread in 384 pages. The diverse sections are named as overview of camel immune system; determinates of innate immunity, cells, organs and tissues of immune system; antibodies; immunomodulation; histocompatibility; seroprevalence, diagnosis and immunity against bacteria, viruses, parasites and combination of other infections; application of camel immunoglobulins and applications of immune mechanisms in physiological processes. The camelid immunology has to go a long way in its future research, therefore, this reference book may prove quite useful for those interested in this subject. Book can be seen on www.camelsandcamelids.com.



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GENOME WIDE ASSOCIATION STUDIES FOR MILK NUTRITION TRAITS IN GOBI RED BACTRIAN CAMEL

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ABSTRACT

Through our research, we explored a suite of novel single nucleotide polymorphisms (SNPs) associated with milk nutrition traits, and thus form a solid basis for eventually unraveling the causal mutations for milk production traits in the Bactrian camel. A genome-wide association study was conducted for four general nutritional indicators (milk lactose, dry matter, milk protein, and milk fat) and the composition of 17 amino acids in 158 Gobi Red Bactrian camels (*Camelus bactrianus*). Genome-wide association study was performed on the 548745 SNPs identified by genotyping-by-sequencing. The phenotypes of the milk nutrient traits were determined and the association effects of single nucleotide polymorphisms SNPs on each phenotype were evaluated using a mixed effect linear model. One or more than one of the milk traits were significantly associated with 46 single nucleotide polymorphisms. All the polymorphic sites were located in intergenic regions or non-coding regions of genes in the genome. Two single nucleotide polymorphisms were significantly associated with camel milk lactose and protein, and were located in the OSBPL8, MRPL37, SSBP3, and LOC102516351 genes. The results of the present study will be used to reveal the genetic basis of milk nutrition traits in domestic Bactrian camels.

Key words: Bactrian camel milk, correlation analysis, single nucleotide polymorphism

Bactrian camels are usually found in desert and semi desert areas of Asia, and were originally domesticated in eastern Iran or southern Turkmenistan in the 3rd millennium BCE (Peters and Driesch, 1997; Liang *et al,* 2020). They are used for working, and milk, meat, and wool production (Jirimutu *et al,* 2009). According to the statistical data, China and Mongolia are the main distribution area of Bactrian camel and there are five breeds in China: Alxa, Sonid, Qinghai, Tarim, and Junggar (Liang *et al,* 2022; Jirimutu *et al,* 2022); and three breeds in Mongolia: Galbiin Gobiin Ulaan, Haniin Hetsiin Huren, and Tokhom Tongalag (Chuluunbat *et al,* 2014).

The continued development of sequencing technologies means that milk traits might be improved via genetic selection based on high-throughput sequencing data. Genome wide association studies (GWASs) are considered a powerful method to identify the genomic regions that control prominent animal husbandry traits within collections of non-structured germplasm (Huang *et al,* 2012; Kang *et al,* 2015; Li *et al,* 2020; Peng *et al,* 2022). Genotyping-by-sequencing (GBS), a high throughput method of genotyping, is capable of identifying sufficient markers to support GWAS, even in species lacking genomic information or the tools to determine it. Currently, GWAS is being used to identify causative mutations affecting variation in milk production traits in dairy cattle (Bouwman *et al,* 2011; Eveline *et al,* 2016). However, associations of genome polymorphisms with milk production or nutrition traits have not been reported in domestic Bactrian camels. The present study was aimed to identify single nucleotide polymorphisms (SNPs) in Gobi Red Bactrian camel samples from China to reveal candidate genes or SNPs associated with milk nutrition traits.

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Materials and Methods

Ethical considerations

The animal use procedures and protocols used in the present study were performed according to the national codes of practice for the care and handling of farm animals (http://www.nfacc.ca/codes-of-practice) and the animal care committee of Inner Mongolia Agricultural University approved the study.

Camels and milk sampling

The Bactrian camel blood and milk samples from 158 Gobi Red Bactrian camels were collected from Bayanur city, Inner Mongolia province of China. During morning milking, whole blood (30mL) and milk (50mL) were collected from each animal. The collected samples were immediately transported in an ice bath to be stored at -80°C until analysis. The summary of the sequencing data has been presented in Table S1.

Milk composition analysis

The contents of milk fat (FP), milk lactose (LP), milk protein (PP), and dry matter (DM) were determined according to the method of GB5009.6-2016 (the Röse-Gottlieb method), GB5413.5-2010 (the Rhein-Enron method), GB5009.5-2016 (the Kjeldahl method), and GB5413.39-2010, respectively.

Amino acid profile analysis

Thawed milk samples were thoroughly mixed and hydrolysed using 6mol/L HCl, in an octanol stock solution, using superior grade concentrated hydrochloric acid in a sealed glass ampoule for 22-24h at 110°C (Bai *et al,* 2009). The hydrolysate was centrifuged and the supernatant was used for analysis after filtering through a 0.22µm syringe filter. The amino acids composition was determined using an automatic amino acid analyser (L-8900, Hitachi, Tokyo, Japan). SPSS (16.0) software (IBM Corp., Armonk, NY, USA) was used for data analysis.

DNA Extraction

A TIANamp Blood DNA Kit (Tiangen Biotech Co., Beijing, China) was used to extract genomic DNA from whole blood samples, following the manufacturer's protocol. A NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) and 1% agarose gel electrophoresis were used to determine the DNA quantity and quality.

GBS and SNP calling

The methods detailed by Elshire *et al*. were used to prepare and analyse the GBS libraries Elshire *et al* (2011). The libraries were sequencing using 150bp paired end 150bp sequencing (Illumina HiSeq 2000 system; Illumina Inc., San Diego, CA, USA). For analysis, low quality reads were removed from the raw data to obtain clean data; reads with adapter sequence were trimmed; reads with an N content in a single-ended sequence that exceeded 10% of the read length were removed, and the reads with low-quality (≥5) bases contained in the single-ended sequencing read that exceeded 50% of the read length were also removed. Sequences were mapped to the wild Bactrian camel reference genome (Jirimutu et al, 2012) by using BWA (Li and Durbin, 2009). The data were summarised filtered using Samtools v0.1.19 (http://samtools.sourceforge.net/) (Li *et al,* 2009), and the variants after filtering were annotated by ANNOVAR (Kai *et al,* 2010).

Deducing the population structure

The GCTA software (http://cnsgenomics.com/software/gcta/pca.html) was used to investigate the population structure, together with principal components analyses (PCA). The distance matrix was calculated using TreeBest (http://treesoft.sourceforge.net/treebest.shtml) by constructing a neighbor-joining tree (1000 bootstrap values).

Genome wide association analysis

A genome-wide genotype-phenotype association analysis was carried out to reveal associations between milk nutrition traits and SNPs. In the GWAS analysis, a mixed effect linear model (MLM) was used, as implemented in the GEMMA software (http://www.xzlab.org/software.html). The MLM is described as follows:

$$y = X\alpha + Z\beta + W\mu + e,$$

where y is the phenotype vector for each milk production traits measured in all the Bactrian camel samples; X represents the genotype, Z is the structure matrix and W is the relative kinship matrix. Fixed effects are represented by Xα and Zβ; e is the vector of residual errors with $e \sim (0, \delta e^2)$.

In this study, the Bonferroni method was used to avoid the high rate of false positives resulting from multiple testing. The corrected significant threshold is calculated as follows:

$$P_{adj=\alpha/n},$$

where α is the significance threshold, α = 0.05; n is the SNP number; the corrected threshold is

compared with the p value of each site using a hypothesis test. If the p value is less than the corrected threshold, it can be determined that there is a significant correlation between the SNP and the target trait. The corrected genome-wide level significant threshold was $P_{adj} = 9.11E^{-08}$ (0.05/548745).

Results

Sequencing results, identified SNPs, and classification

DNA samples from 158 Gobi Red Bactrian camels were sequenced using the GBS method on the Illumina HiSeq 2000 system (data available with authors). A total of 236.80Gb of raw data were generated and 236.52Gb of clean data were retained after quality checking. The clean reads were aligned to our previous reference genome (Jirimutu *et al*, 2012) assembly of the Bactrian camel for variant calling. After stringent filtering (dp2, Miss 0.5, Maf 0.01), 54,8745 SNPs were identified in the genome. Annotation of the variants showed that most of the identified SNPs were in intergenic regions (70.12%) or in the introns of genes (28.27%) (Table 1). Among the SNPs, only 2876 were located in exonic regions, of which 1380 were synonymous and 1496 were non-synonymous. Among the SNPs, 60.63% were transition and 39.37% were transversion, giving a ratio of transition to transversion of 1.54.

Genome wide associations among milk traits and SNPs

The significant associations of milk lactose (LP), milk protein (PP), cys aminoacid and dry matter (DM) with the SNPs were determined (Table S1,2,3,4). Significant GWAS results (-lg P > -lg (Padj) = 7.04) were recorded.

Two SNPs were significantly associated with milk lactose, and were located on scaffolds gi|558498741|ref|NW_006211618.1| and gi|558499717|ref|NW_006210642.1| (Table S1). One was located within the OSBPL8 gene (encoding oxysterol binding protein like 8), another one within MRPL37 gene (encoding mitochondrial ribosomal protein L37) and downstream of SSBP3 (single stranded DNA binding protein 3) and LOC102516351 genes

Table 1. The statistics of SNP classification.

Category	Number of SNPs
Upstream	2671
Stop gain	61
Stop loss	5
Exonic Synonymous	1380
Non-synonymous	1496
Intronic	155104
Splicing	37
Downstream	3099
upstream/downstream	28
Intergenic	384757
Ts[1]	332710
Tv[2]	216035
Ts/tv[3]	1.540
Total SNPs	548745

Note: [1]Ts, Transition. [2]Ts: Transversion. Ts/tv[3]: Transition/Transversion

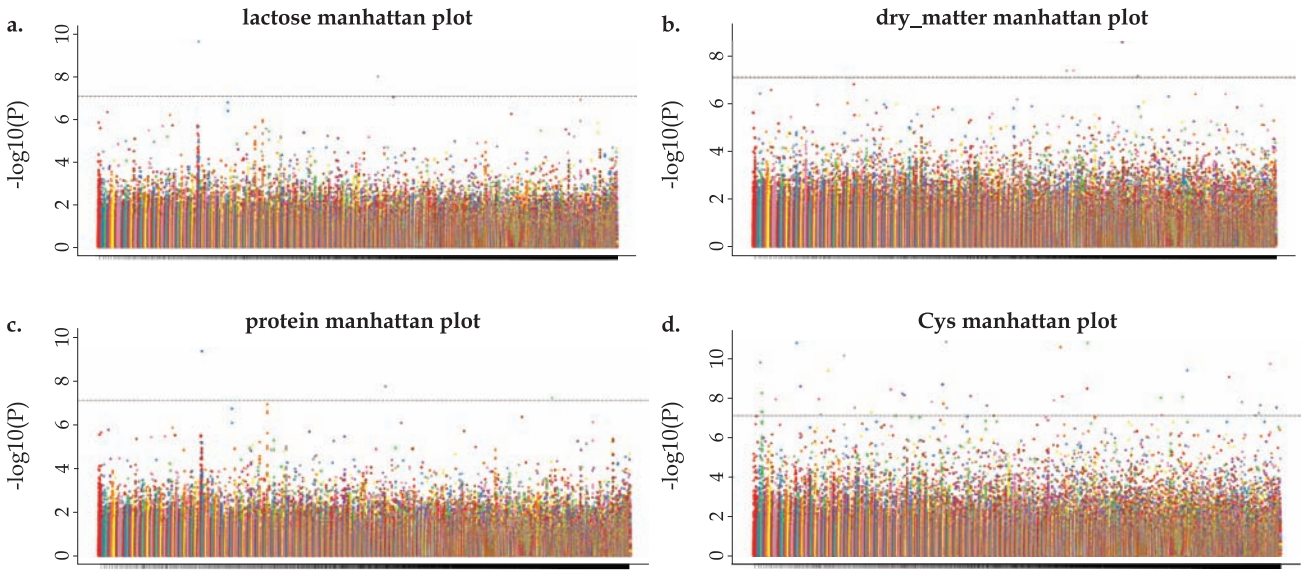

Fig 1. Manhattan plots of four milk traits in a genome-wide association study. a, b, c and d refer to plots for milk lactose, milk protein, dry matter and cys amino acid, respectively. Genome wide significance is indicated for values above -log10 (Observed value) > 7.04 (black horizontal).

(Fig 1a, Fig S1). Four SNPs were significantly associated with dry matter, and were distributed in scaffolds gi|558499724|ref|NW_006210635.1|, gi|558499606|ref|NW_006210753.1|, gi|558496357|ref|NW_006214002.1|, and gi|558499765|ref|NW_006210594.1| (Table S2). One site (g.1053778A>C) was located within the TAF13 gene (encoding TATA-Box binding protein associated factor 13), downstream of TMEM167B (transmembrane protein 167B); one site (g.859455T>C) was located within the TTN gene (titin), downstream of RDH8 (retinol dehydrogenase 8) and upstream of the LOC102516684 gene; and the last site was located downstream of COL5A3 (collagen type V alpha 3 chain) (Fig 1b, Fig S1, Table S2). Three SNPs were significantly associated milk protein, and were distributed in scaffolds gi|558498741|ref|NW_006211618.1|, gi|558498226|ref|NW_006212133.1|, and gi|558499717|ref|NW_006210642.1| (Table S3). Two mutation sites were located within the OSBPL8 and FRMPD4 (FERM and PDZ domain containing 4) genes, respectively. The last site was located within the MRPL37 gene, and upstream of SSBP3 gene and downstream of LOC102516351 gene (Fig 1c, Fig S1, Table S3).

Significant genome wide associations between amino acids and SNPs

Amino acid composition of Gobi Red Bactrian camel milk were shown in Table S4. Thirty-seven SNPs showed significant associations with cysteine (Cys) (Fig 1d, Fig S2, Table 3, Table S5), distributed

Table 2. Functional annotation for significant SNPs associated with camel milk lactose, protein, and dry matter in the GWAS analysis.

Trait	SNP	Scaffold	Gene/nearby gene			Minor Allele Frequency
			Gene name	Annotation	Distance/bp[1]	
Lactose	g.428152T>C	gi|558498741|ref|NW_006211618.1|	OSBPL8	oxysterol binding protein-like 8	Intron	0.0137
	g.859455T>C	gi|558499717|ref|NW_006210642.1|	MRPL37	mitochondrial ribosomal protein L37	Intron	0.0165
	g.859455T>C	gi|558499717|ref|NW_006210642.1|	SSBP3	single stranded DNA binding protein 3	-2391	0.0165
	g.859455T>C	gi|558499717|ref|NW_006210642.1|	LOC102516351	NADH-cytochrome b5 reductase-like	-2363	0.0165
Protein	g.428152T>C	gi|558498741|ref|NW_006211618.1|	OSBPL8	oxysterol binding protein-like 8	Intron	0.0137
	g.859455T>C	gi|558499717|ref|NW_006210642.1|	MRPL37	mitochondrial ribosomal protein L37	Intron	0.0165
	g.859455T>C	gi|558499717|ref|NW_006210642.1|	SSBP3	single stranded DNA binding protein 3	19892	0.0165
	g.859455T>C	gi|558499717|ref|NW_006210642.1|	LOC102516351	NADH-cytochrome b5 reductase-like	-2391	0.0165
	g.284381A>T	gi|558498226|ref|NW_006212133.1|	FRMPD4	FERM and PDZ domain containing 4	Intron	0.0950
Dry matter	g.1053778A>C	gi|558499724|ref|NW_006210635.1|	TAF13	TAF13 RNA polymerase II TATA box binding protein (TBP)-associated factor	Intron	0.0150
	g.1053778A>C	gi|558499724|ref|NW_006210635.1|	TMEM167B	transmembrane protein 167B	-12512	0.0150
	g.316487G>T	gi|558496357|ref|NW_006214002.1|	TTN	titin	Intron	0.0140
	g.353395A>T	gi|558499765|ref|NW_006210594.1|	RDH8	retinol dehydrogenase 8	-2509	0.0328
	g.353395A>T	gi|558499765|ref|NW_006210594.1|	LOC102516684	complement C3	6591	0.0328
	g.353395A>T	gi|558499765|ref|NW_006210594.1|	COL5A3	collagen type V alpha 3 chain	-14018	0.0328

Distance/bp[1]: the distance value is positive for a gene located downstream of the SNP, and the distance value is negative for a gene located upstream of the SNP.

on scaffolds gi|558499724|ref|NW_006210635.1|, gi|558499606|ref|NW_006210753.1|, gi|558496357|ref|NW_006214002.1|, and gi|558499765|ref|NW_006210594.1|. Among them, g.4896657C>T was located in the intron region of the LOC102511343 gene, g.8002660G>T was located in the a *FARP1* gene

Table 3. Functional annotation for significant SNPs associated with camel milk Cysteine amino acids in the GWAS analysis.

Significant SNP	Scaffold	Gene/nearby gene			Minor Allele Frequency
		Gene name	Annotation	Distance/bp[1]	
g.8002660G>T	gi|558491038|ref|NW_006218037.1|	FARP1	FERM RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	intron	0.0109
g.1116521C>A	gi|558499089|ref|NW_006211270.1|	FANCL	Fanconi anemia complementation group L	intron	0.0104
g.3630299G>T	gi|558499268|ref|NW_006211091.1|	DLG2	discs large MAGUK protein 2	intron	0.0115
g.460204G>T	gi|558499098|ref|NW_006211261.1|	PLEKHM2	pleckstrin homology domain containing family M (with RUN domain) member 2	473792	0.0106
g.1864716G>A	gi|558499340|ref|NW_006211019.1|	PRKCE	protein kinase epsilon	1384379	0.0123
g.1864716G>A	gi|558499040|ref|NW_006211319.1|	PCSK5	proprotein convertase subtilisin/kexin type 5	intron	0.0119
g.4152920C>A	gi|558499444|ref|NW_006210915.1|	MFSD6	major facilitator superfamily domain containing 6	-18375	0.0162
g.2404559T>A	gi|558499561|ref|NW_006210798.1|	SERTM1	serine-rich and transmembrane domain containing 1	768	0.0126
g.1077348G>T	gi|558499516|ref|NW_006210843.1|	BAG4	BCL2-associated athanogene 4	-3503	0.0139
g.1077348G>T	gi|558499516|ref|NW_006210843.1|	DDHD2	DDHD domain containing 2	10815	0.0139
g.894464G>A	gi|558499631|ref|NW_006210728.1|	LOC102507327	chromosome unknown open reading frame human C17 or f67	2013	0.0119
g.894464G>A	gi|558499631|ref|NW_006210728.1|	DGKE	diacylglycerol kinase epsilon	-9402	0.0119
g.1645465T>G	gi|558499491|ref|NW_006210868.1|	FAM89A	family with sequence similarity 89 member A	-16548	0.0122
g.730799C>G	gi|558499706|ref|NW_006210653.1|	PDE4D	phosphodiesterase 4D cAMP-specific	intron	0.0146
g.370107G>A	gi|558499585|ref|NW_006210774.1|	MYO1E	myosin IE	intron	0.0152
g.2048513C>A	gi|558499280|ref|NW_006211079.1|	CCDC82	coiled-coil domain containing 82	intron	0.0116
g.688272T>G	gi|558499584|ref|NW_006210775.1|	ANK3	ankyrin 3 node of Ranvier (ankyrin G)	intron	0.0107
g.1346840C>T	gi|558498060|ref|NW_006212299.1|	LOC113107804	lipopolysaccharide-responsive and beige-like anchor protein	intron	0.0105
g.178075T>G	gi|558499248|ref|NW_006211111.1|	ARID5B	AT rich interactive domain 5B (MRF1-like)	intron	0.0108
g.239530C>A	gi|558498708|ref|NW_006211651.1|	KLF12	Kruppel-like factor 12	intron	0.0357
g.225920C>A	gi|558500160|ref|NW_006210199.1|	LOC102515356	cysteine-rich protein 2-binding protein	intron	0.0841
g.225920C>A	gi|558500160|ref|NW_006210199.1|	PET117	PET117 homolog	8928	0.0841

Distance/bp[1]: the distance value is positive for a gene located downstream of the SNP, and the distance value is negative for a gene located upstream of the SNP.

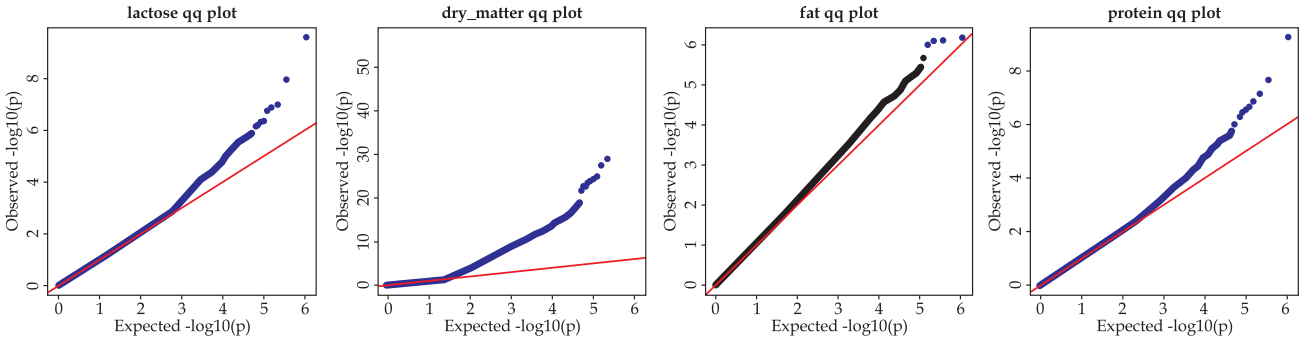

Fig S1. Quantile-quantile (Q-Q) plots for milk composition traits obtained by standard mixed linear model.

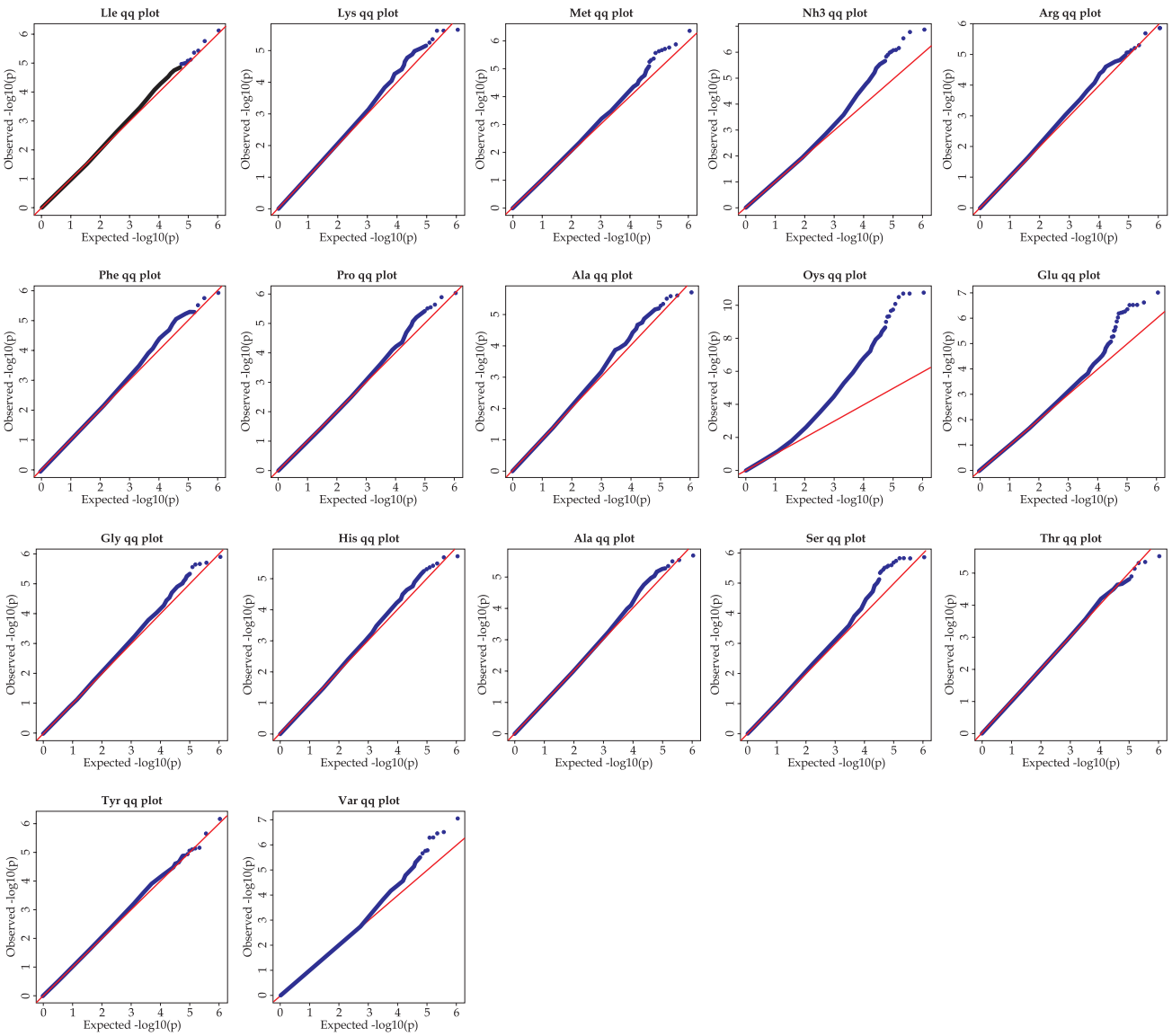

Fig S2. Quantile-quantile (Q-Q) plots for amino acid traits obtained by standard mixed linear model.

intron (FERM, ARH/RhoGEF and pleckstrin domain protein 1), g.1116521C>A was located in the intron region of the *FANCL* gene (FA complementation group L), g.3630299G>T was located in an intron of the *DLG2* gene (discs large MAGUK scaffold protein 2), g.460204G>T was located 13588 bp downstream of the *PLEKHM2* gene (pleckstrin homology and RUN domain containing M2), g.1864716G>A was located 12249bp upstream of *PRKCE* gene (protein kinase C epsilon), g.3761726C>A is located in an

Table S1. SNPs significantly associated with camel milk lactose at the genome level.

No.	SNP	Scaffold	Position(bp)	P-value
1	g.428152T>C	gi|558498741|ref|NW_006211618.1|	428152	9.5871
2	g.859455T>C	gi|558499717|ref|NW_006210642.1|	859455	7.9622

Table S2. SNPs significantly associated with camel dry matter at the genome level.

No.	SNP	Scaffold	Position(bp)	P-value
1	g.1053778A>C	gi|558499724|ref|NW_006210635.1|	1053778	7.3158
2	g.733072G>T	gi|558499606|ref|NW_006210753.1|	733072	7.3244
3	g.316487G>T	gi|558496357|ref|NW_006214002.1|	316487	8.5146
4	g.353395A>T	gi|558499765|ref|NW_006210594.1|	353395	7.0952

Table S3. SNPs significantly associated with camel milk protein at the genome level.

No.	SNP	Scaffold	Position(bp)	P-value
1	g.428152T>C	gi|558498741|ref|NW_006211618.1|	428152	9.2691
2	g.859455T>C	gi|558499717|ref|NW_006210642.1|	859455	7.6676
3	g.284381A>T	gi|558498226|ref|NW_006212133.1|	284381	7.1482

Table S4. Amino acid composition of Gobi Red Bactrian camel milk.

	Amino Acid	Mean ± SD/%
EAA (Essential Amino Acid)	Lysine (Lys)	0.306±0.04
	Leucine (Leu)	0.414±0.06
	Isoleucine (Ile)	0.223±0.03
	Valine (Val)	0.254±0.04
	Threonine (Thr)	0.161±0.02
	Histidine (His)	0.106±0.01
	Phenylalanine (Phe)	0.179±0.02
	Methionine (Met)	0.125±0.02
NEAA (Non-Essential Amino Acid)	Cysteine (Cys)	0.051±0.0042
	Aspartate (Asp)	0.257±0.03
	Glutamate (Glu)	0.839±0.11
	Serine (Ser)	0.187±0.02
	Proline (Pro)	0.452±0.07
	Glycine (Gly)	0.050±0.01
	Alanine (Ala)	0.093±0.01
	Tyrosine (Tyr)	0.161±0.02
	Arginine (Arg)	0.156±0.02
TAA (Total Amino Acid)		4.014±0.5342
EAA (Essential Amino Acid)		1.768
EAA/TAA (%)		44.05%
EAA/NEAA (%)		78.72%

Table S5. SNPs significantly associated with Cys amino acid at the genome level.

No.	SNP	Scaffold	Position(bp)	P-value
1	g.9758155T>A	gi|558499440|ref|NW_006210919.1|	9758155	9.7322
2	g.4896657C>T	gi|558498651|ref|NW_006211708.1|	4896657	8.1925
3	g.4957179T>C	gi|558498651|ref|NW_006211708.1|	4957179	7.2127
4	g.8002660G>T	gi|558491038|ref|NW_006218037.1|	8002660	7.8634

5	g.1116521C>A	gi|558499089|ref|NW_006211270.1|	1116521	10.7007
6	g.3630299G>T	gi|558499268|ref|NW_006211091.1|	3630299	8.5222
7	g.460204G>T	gi|558499098|ref|NW_006211261.1|	460204	7.8717
8	g.1864716G>A	gi|558499340|ref|NW_006211019.1|	1864716	7.12126
9	g.601785G>T	gi|558499693|ref|NW_006210666.1|	601785	9.3034
10	g.3761726C>A	gi|558499040|ref|NW_006211319.1|	3761726	10.0702
11	g.4152920C>A	gi|558499444|ref|NW_006210915.1|	4152920	7.4482
12	g.2404559T>A	gi|558499561|ref|NW_006210798.1|	2404559	7.1907
13	g.409215A>G	gi|558499330|ref|NW_006211029.1|	409215	8.3621
14	g.518889G>T	gi|558499708|ref|NW_006210651.1|	518889	8.1581
15	g.1077348G>T	gi|558499516|ref|NW_006210843.1|	1077348	8.0829
16	g.894464G>A	gi|558499631|ref|NW_006210728.1|	894464	7.7284
17	g.1645465T>G	gi|558499491|ref|NW_006210868.1|	1645465	8.6183
18	g.2093903C>A	gi|558499938|ref|NW_006210421.1|	2093903	7.6495
19	g.730799C>G	gi|558499706|ref|NW_006210653.1|	730799	10.7651
20	g.2615573G>A	gi|558499706|ref|NW_006210653.1|	2615573	7.4337
21	g.370107G>A	gi|558499585|ref|NW_006210774.1|	370107	8.0310
22	g.2048513C>A	gi|558499280|ref|NW_006211079.1|	2048513	7.5533
23	g.1574963C>A	gi|558499355|ref|NW_006211004.1|	1574963	7.0426
24	g.688272T>G	gi|558499584|ref|NW_006210775.1|	688272	7.8206
25	g.1346840C>T	gi|558498060|ref|NW_006212299.1|	1346840	10.4980
26	g.112029C>A	gi|558498825|ref|NW_006211534.1|	112029	8.0172
27	g.899410C>A	gi|558499185|ref|NW_006211174.1|	899410	8.41203
28	g.23055C>T	gi|558498804|ref|NW_006211555.1|	23055	10.6974
29	g.11511G>T	gi|558498763|ref|NW_006211596.1|	11511	7.9452
30	g.178075T>G	gi|558499248|ref|NW_006211111.1|	178075	7.0760
31	g.652656A>C	gi|558499080|ref|NW_006211279.1|	652656	7.9760
32	g.239530C>A	gi|558498708|ref|NW_006211651.1|	239530	9.3308
33	g.166087A>T	gi|558498770|ref|NW_006211589.1|	166087	8.9924
34	g.225920C>A	gi|558500160|ref|NW_006210199.1|	225920	7.7213
35	g.48114A>T	gi|558488424|ref|NW_006220651.1|	48114	7.0590
36	g.10008C>G	gi|558498711|ref|NW_006211648.1|	10008	9.6531
37	g.334G>T	gi|558488164|ref|NW_006220911.1|	334	7.4554

intron of the PCSK5 gene (proprotein convertase subtilisin/kexin type 5), g.4152920C>A was located 18375 bp downstream of the *MFSD6* gene (major facilitator superfamily domain containing 6), g.48114A>T was located in an intron of the CR2 gene (complement C3d receptor 2), g.225920C>A was located 8928bp upstream of the *PET117* gene (PET117 cytochrome C oxidase chaperone), g.2404559T>A was located 768bp upstream of the *SERTM1* gene (serine rich and transmembrane domain containing 1), g.1077348G>T was located 10815bp upstream of the *DDHD2* gene (DDHD domain containing 2) and 3503bp downstream of the *BAG4* gene (BCL2 associated athanogene 4), g.894464G>A was located 2013bp upstream of the LOC102507327 gene and 9402bp downstream of the *DGKE* gene (diacylglycerol kinase epsilon), g.1645465T>G was located 16548 bp downstream of the FAM89A gene (family with sequence similarity 89 Member A), g.2615573G>A was located in an intron of the *PDE4D* gene (phosphodiesterase 4D), g.370107G>A was located in the MYO1E gene (myosin IE), g.2048513C>A was located in an intron *CCDC82* (coiled-coil domain containing 82), g.688272T>G was located in an intron of ANK3 (ankyrin 3), g.1346840C>T was located in an intron of the LOC102505495 gene, g.178075T>G was located in an intron of the *ARID5B* gene (AT-rich interaction domain 5B), the g.239530C>A was located in an intron of the KLF12 gene (Kruppel like factor 12), and g.225920C>A was located in an intron

of the LOC102515356 gene. SNPs g.9758155T>A, g.4957179T>C, g.601785G>T, g.409215A>G, g.518889G>T, g.2093903C>A, g.730799C>G, g.1574963C>A, g.112029C>A, g.899410C>A, g.23055C>T, g.11511G>T, g.652656A>C, g.166087A>T, g.10008C>G, and g.334G>T were not annotated as being close to or in any genes.

Discussion

The present study involved a GWAS for camel milk nutrition traits using 158 Gobi Red Bactrian camels. To the best of our knowledge, this was the first GWAS to use GBS sequencing to investigate camel milk nutrition traits.

In GWAS, population stratification results in an increase in the false positive rate in the results. In a large population, subpopulations from different genetic backgrounds lead to pseudo-association of SNP markers with traits of interest, and although the results are correlated, the markers are not related to the trait genes (Cardon and Palmer, 2003). Therefore, group stratification is also considered as one of the most important factors affecting GWAS results (Jody and Machado, 2003; Madsen *et al*, 2006). Subpopulations with different allele frequencies in a population, which either have a common ancestor or are in a relatively consistent environment, can cause population stratification. Population stratification and SNP effects lead to false positives in the association analysis, which ultimately leads to many correlation results that are difficult to replicate by other research groups (Thompson *et al*, 2018). In our research, the Q-QPlot test showed that there was no obvious group stratification phenomenon, which demonstrated that the GWAS results based on the mixed linear model were relatively reliable.

GBS permits a higher-level determination of genetic variation within a population compared with current genotyping arrays and is less expensive. Approximately 88% of the SNPs were high quality, and may be correlated with camel milk nutrition traits, which will expand our understanding of genomic variation in domestic Bactrian camels, leading to dairy industry improvement. As far as we can tell, the significant SNPs annotated in this study were mostly located in non-coding regions and intergenic regions of the genome, and none of these candidate genes directly interacted with or affected the camel milk nutrition traits.

We speculated that the effects of these genes on the traits were achieved through interactions between molecules, including up- and down regulation, signal transduction, and membrane transport, which will require further investigation. The results of the present study will form the basis for replication studies, and the identification of possible mutations underlying milk nutrition traits in domestic Bactrian camels.

Conclusion

A genome-wide association study was conducted for four general nutritional indicators (milk lactose, dry matter, milk protein, and milk fat) and the composition of 17 amino acids in 158 Gobi Red Bactrian camels (*Camelus bactrianus*) from China. Two single nucleotide polymorphisms were significantly associated with camel milk lactose and protein, and were located in the OSBPL8, MRPL37, SSBP3, and LOC102516351 genes.

Conflict of interest

None. The authors declare that there is no conflict of interest.

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MOLECULAR ASSESSMENT OF KAPPA CASEIN GENE BY SEQUENCING IN BIKANERI DROMEDARY CAMELS

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ABSTRACT

The aim of the present study was to investigate the genetic and phylogenetic analysis of kappa casein gene in the 70 Bikaneri breed of camels which were blood sampled for isolation of their DNA. The polymerase chain reaction (PCR) products (488bp) of kappa casein gene were sequenced using the Sanger dideoxy chain termination method. The sequence analysis of kappa casein gene revealed presence of within-species variation. Intraspecies variation within camel were observed in the form of four SNPs. Generated Sequences were submitted on NCBI GENBANK. Furthermore, a Neighborhood Joining Phylogenetic tree was constructed through MEGA7 bioinformatics software. The sequencing of the kappa casein gene in Bikaneri camel may be useful for future studies on genetic variation within and between camel populations or on associated traits due to the therapeutic importance of camel milk for human consumption.

Key words: Camel, kappa casein, milk, polymorphism, SNP

The casein fraction comprises of 52-89% in the camel milk (Ereifej*et al,* 2011) and can be distributed into four fractions involving 1- CN (22%), as 2- CN (65%), b- CN (9.5%), and k-CN (3.5%) (El Agamy, 2009). These four fractions of casein are encoded by four genes such as CSN1S1, CSN1S2, CSN2 and CSN3, respectively (Kappeler *et al,* 1998). Out of all the casein protein, k casein plays a crucial role in stabilising milk micelles and keeping calcium phosphate in solution and further allowing the transfer of calcium and phosphorus from milk to consumers (Ikonen *et al,* 2008).

Substantially more research has been conducted on genetic variability of the kappa casein gene in other dairy farm animals in comparison to the camel (Dioli, 2016; Hemati *et al,* 2017). The first characterisation of the major components of camel milk casein was reported by Farah and Farah-Riesen (1985). Subsequent to this, a quantitative analysis was carried out by Kappeler *et al* (2003) on camel milk protein, which found significantly lower amounts of camel k-casein compared to the homologous cow's casein. Hinz *et al* (2012) have reported five different isoforms of k-casein in the camel milk as a result of a strong glycosylation of casein protein. Yamini *et al* (2019) carried out RFLP analysis through restriction digestion of amplified product of kappa casein gene with *Alu*I generated two genotypic patterns, CT and TT. Further, the CT pattern was identified by the presence of four separate intact fragments of 203-bp, 146-bp, 127-bp and 12-bp and the TT pattern produced three fragments 203-bp, 158-bp and 127-bp. Furthermore, Jadhav *et al* (2020) also reported polymorphism in the kappa casein gene in Indian dromedary. Further, Pauciullo *et al* (2013) characterised, for the first time, the nucleotide sequence of the whole k-casein encoding gene CSN3 plus 1045 nucleotides at the 5′ flanking region in the *Camelus dromedarius*. The length of camel k-casein gene is 13000 bp and it contains 5 exons and 4 introns.

We hypothesised that there may be genetic variation of the kappa casein gene in Indian dromedary camels. The aim of the present study was therefore, to the investigate genetic diversity in Indian dromedary camels through sequencing of the kappa casein gene along with detection of SNPs.

Materials and Methods

Sample collection

Blood samples were collected from 70 Bikaneri camels via jugular venipuncture in sterile vacutainer

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tubes containing EDTA as an anticoagulant. For this procedure, the camels were led gently into a squeeze chute facility, and blood was sampled using minimal restraint. After collection, blood samples were immediately placed on ice, and serum was separated through centrifugation at 3000 RPM for 15 min. Blood serum samples were stored at -20°C until DNA isolation.

Isolation of DNA

DNA was extracted from 200µL of blood by the spin column method following standard procedures as per the manufacture's protocol through a Blood Genomic DNA Purification Kit supplied by HIMEDIA Pvt. Ltd (Mumbai, Maharashtra). Horizontal agarose gel electrophoresis was carried out to check the quality of genomic DNA using 0.8% w/v agarose. The gel was visualised under UV trans-illuminator and documented by photography. DNA samples showing intact bands were used for further analysis.

PCR Conditions

PCR amplification of 488bp fragments were carried out using specific primers (Forward 5'- CAC AAA GAT GAC TCT GCT ATC G -3' and reverse 5'- GCC CTC CAC ATA TGT CTG -3') designed by Othman *et al* (2016). The PCR reaction was carried out using a final volume of 50 µl, containing GeneTaq PCR Master Mix 25 µl, Forward primer 0.5 µl, Reverse primer 0.5 µl, Template DNA 4 µl, and Nuclease free water 20µl. Thermal cycler conditions for PCR were initial denaturation at 95°C for 5-min, followed by 36 cycles of 94°C for 45s, 57.2°C for 1-min, 72°C for 1-min, a final extension at 72°C for 10-min, and then holding at 4°C for 5-min. PCR amplified DNA products were analysed by analytical agarose gel electrophoresis as per the procedure described by Sambrook *et al* (2001).

Restricted fragment length polymorphism

The PCR products for each tested gene were digested with *Alu*I restriction enzyme. A total 15µl PCR products were digested with1.5 µl *Alu*I overnight at the optimum temperature for maximum activity of each restricted enzyme. Molecular size of the digested fragments was measured by analyzing gel images with Gel Analyser software with 100 bp DNA ladder as the DNA size marker.

Sequence analysis and SNP's detection

The six representative samples of amplified products of each electrophoresed RFLP pattern were purified through the Exosap method and sequencing in both directions was carried out in a private laboratory (X Celris Genomics Pvt. Ltd, Ahmadabad, India) using an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) through the Sanger dideoxy chain termination method. Forward and reverse sequences of each gene fragment was assembled against the most closely related reference sequences of respective genes and similarity was checked into the non-redundant database of GenBank with BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST).The raw sequences were analysed by Codon Code Aligner for detection of sequence anomaly, if any, on the basis of the chromatogram generated. Multiple sequence alignment for each sequenced region of kappa casein gene was carried out by Clustal W software to detect the exact location of nucleotide where polymorphism was present. An total of 6 sequences were generated for kappa casein genes of different animals and were submitted to the National Centre for Biotechnology Information (NCBI) GenBank database, an international bank of gene and genomic sequences, through online gateway.

Phylogenetic analysis

Phylogenetic analysis was conducted using the Neighbor-Joining method (Tahmoorespur *et al,* 2016) with a bootstrap test of phylogeny in MEGA7 software to evaluate the evolutionary relationship within the studied indigenous camel breeds.

Results and Discussion

PCR Amplification and sequencing

The present study focused on the identification of genetic divergence and ev*Alu*ation of SNPs within the kappa casein gene of *Camelus dromedarius*. The genomic DNA of Bikaneri camel was successfully extracted from all of the blood samples and 488 bp specific fragments of exon4 of kappa casein (CSN3) gene were successfully amplified. Further, the nucleotide sequence information generated through Sanger sequencing was checked for accuracy through Codon Code Aligner software. The sequences were assembled with the help of reference sequence already available in the NCBI database (GenBank Accession No. KU055605). The assembled sequence of 488-bp, generated for camel were submitted to the NCBI GenBank database through the online Banklt gateway after proper annotation and accession numbers were obtained (Table 1).

The detection of sequence variation is the core of all genetic analysis for identifying areas across

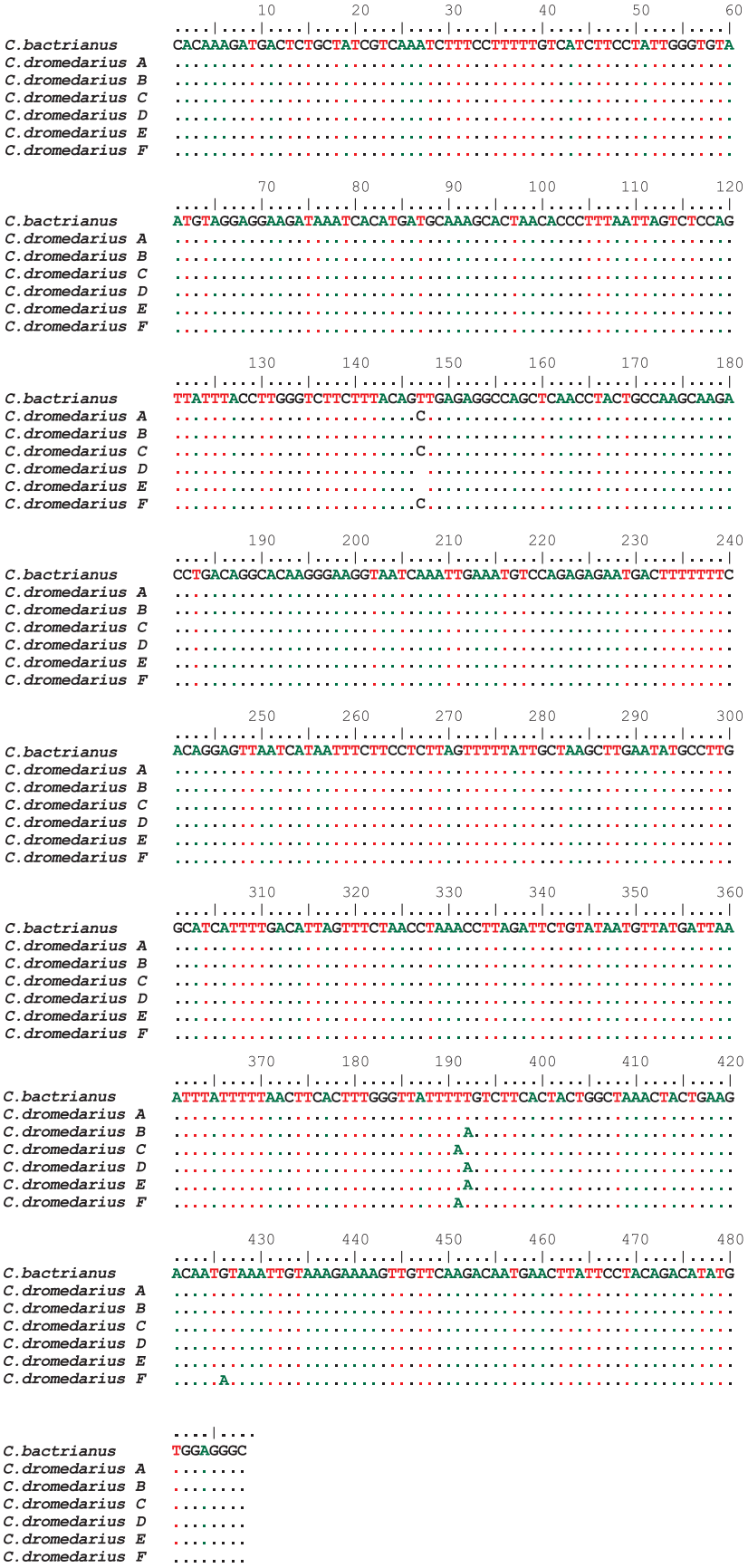

Fig 1. Multiple sequence analysis of the kappa casein gene in dromedary camel.

the candidate gene that may affect the trait of interest (Nickerson *et al*, 1997). Single nucleotide base changes including deletion, insertion and substitution, may play an important role in gene transcription and amino acid sequences of mature proteins. The RFLP banding patterns revealing different nucleotide sequences for different animals were sequenced to identify the position and nature of possible SNPs responsible for sequence variation. The multiple sequence alignment (MSA) of generated sequences (query sequences) was carried out with the help of Codon Code Aligner software to detect the position of SNPs in amplified fragments through comparison with the reference sequence for camel (Fig 1). Results reveal that polymorphism contained in different RFLP patterns of 488-bp fragments of the kappa casein gene in camel were the result of substitution of four bases at four different positions in the nucleotide sequences (Table 2). The four different SNPs were observed to be located at the 147th, 391st, 392nd and 426th position in the amplified fragment of 488-bp of the CSN3 gene. Two out of 4 detected SNPs were transition transitory in nature and the remaining two were transversion mutations (Table 2).

The nucleotide substitutions detected at the 391st position were found to be non-synonymous in nature, with the predicted protein sequence detecting the presence of tyrosine instead of leucine. The present study agrees with the study conducted by Othman *et al* (2016), which reported one SNP (C>T) at position 121 in the 488 bp amplified fragments which are responsible for destruction of site (AG/CT) in Maghrabi camels. Similarly, Pauciullo *et al* (2013) identified 17 SNPs in the kappa casein gene in *Camelus dromedarius* and thereof

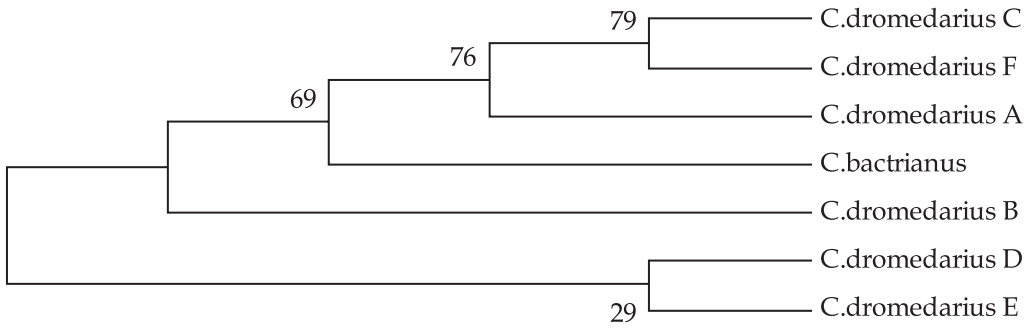

Fig 2. Phylogenetic analysis of the kappa casein gene by Neighbor-Joining method.

Table 1. List of accession numbers obtained for different RFLP patterns of the kappa casein gene.

RFLP Pattern	Product size	Accession number
kCN Variant A	488-bp	MG586889
kCN Variant B	488-bp	MG586890
kCN Variant C	488-bp	MG586891
kCN Variant D	488-bp	MG586892
kCN Variant E	488-bp	MG586893
kCN Variant F	488-bp	MG586894

Table 2. List of position and nature of SNPs observed in the kappa casein gene.

147	T>C	Transition	Synonymous	No change
391	T>A	Transversion	Non-Synonymous	(Leucine to Tyrosine)
392	T>A	Transversion	Synonymous	No change
426	G>A	Transition	Synonymous	No change

these SNPs were found in exon 4. Tahmoorespur *et al* (2016) using in SNP screening of the CSN3 gene in Iranian dromedarius and Bactrian camels, indicated that there were 3 and 1 single mutation found in 10 samples of dromedary and 5 samples of Bacterian camel, respectively. Consistent with our results, studies with other species such as buffalo, sheep, goat, and cattle, revealed many nucleotide changes in exon 4 of the kappa casein gene. Mukesh *et al* (2005) reported seven nucleotide changes: 14 (Asp–Glu), 19 (Asp/Ser–Asn), 96 (Ala–Thr), 126 (Ala–Val), 128 (Ala/Gly–Val), 156 (Ala/Pro–Val) and 168 (Ala/Glu–Val) and they were limited to exon 4 only. Rachagani *et al* (2008) revealed two allelic variants in exon 4 of bovine and these variants were differentiated by PCR-RFLP in indigenous Tharparkar and Sahiwal breeds. Our study concluded that increasing the number of camel populations in future studies may identify more SNPs in the CSN3 gene, which may be useful for determining effective milk camel breeding strategies.

Phylogenetic Analysis

The FASTA converted nucleotide sequences of the kappa casein gene of camel were tested for their evolutionary relationship with the variants using MEGA7 software. A dendrogram was considered an effective way to represent genetic distances through construction of tree-based diagrams. A phylogenetic tree was constructed through the algorithm-based Neighbor-Joining (NJ) method of Saitou and Nei (1987) to observe the evolutionary between the studied variants of the kappa casein gene (Fig 2). The Tamura 3-parameter model (Tamura, 1992) was selected from 24 different maximum likelihood substitution models for construction of the dendrogram as it had the lowest minimum BIC value (1481.512). Bootstrapping was carried out to obtain confidence intervals for the grouping of variants and to test the validity of the clusters obtained. A bootstrap consensus tree, inferred from 1000 replicates (Felsenstein, 1985), was taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates were collapsed. The NJ tree showed bootstrap values ranging from 29 to 79. The *Camelus dromedarius* C is identical to *Camelus dromedarius* F and they are forming one cluster and *Camelus dromedarius* A and *Camelus bactrianus* are separated and they are little bit distant and other forming two lineages with *C. dromedarius* D and E are more distant. While *Camelus dromedarius* D and E are in same cluster and they are identical.

In conclusion, the CSN3 gene of Bikaneri dromedary was investigated in order to detect molecular variation through sequencing methods and determine the divergent evolution of Bikaneri camel breed. The presence of within variation in Bikaneri camel suggests that it is non-conserved in nature and this may be due to dilution of the breed. Intra-specific variation suggests that increasing the number of camel populations included in future

studies could identify more mutations in the kappa casein gene which may be useful in determining camel breeding strategies.

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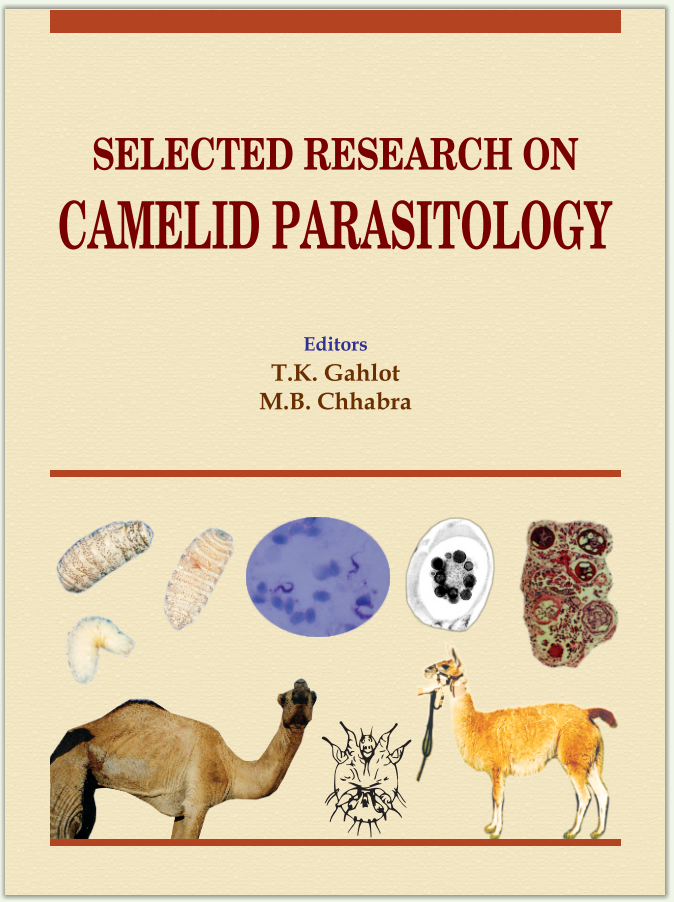

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TECHNOLOGICAL AND PROBIOTIC PROPERTIES OF *Enterococcus faecium* STRAINS ISOLATED FROM TUNISIAN CAMEL MILK

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ABSTRACT

Sixty-two lactic acid bacteria (LAB) strains isolated from Tunisian raw camel milk were studied by focusing on their technological and probiotic potential. Strains were tested for their acidification activity, proteolytic and lipolytic activity, ability to use citrate, biomass yield, growth rate, and Exopolysaccharide (EPS) production. Probiotic tests used were pH and bile tolerance, antimicrobial activity, antimicrobial susceptibility, adhesion, cell surface hydrophobicity and aggregation abilities. Twenty-six strains showed survival at pH 2; only 10 strains tolerated 0.3 % of bile salt and will therefore further assessed for their probiotic properties. These strains were identified by partial 16S rRNA gene sequencing and were represented by *Enterococcus faecium* as the only group. Eight strains present ΔpH ≥0.3 U and then were considered as rapid acidifier strains. All strains have a significant proteolytic power. All strains produced EPS.

Survival to the simulated *in vitro* digestion was strain-dependent. Strains were tested for cell surface acid-base properties and adhesion to gastric mucin and STC-1 cells. Isolates showed good adhesion rate to gastric mucin and only two strains (SCC1-33 and SLch6) were able to colonise STC-1 cells with an adhesion value of 7.8×10^3 and 4.2×10^3, respectively. Auto- and co-aggregation abilities were interesting and values were ranged from 33.10 to 63.10 %. Results from the cytotoxicity test showed a negative effect on STC-1cells of all the studied strains. *Enterococcus faecium* isolated from camel milk was characterised by their technological and probiotic properties.

Key words: Camel milk, *Enterococcus faecium*, probiotics, technological properties, Lactic Acid Bacteria

Camel milk, alone or in combination with bacterial strains having probiotic properties and/ or producing physiologically active metabolites, represents one of the technology options for manufacturing dairy functional beverages (Gomes *et al,* 1998). Probiotics are non-pathogenic microorganisms that, when ingested in adequate amounts, exert a positive influence on their host's health. The most studied probiotic strains are lactic acid bacteria (LAB) especially belong to the genera *Lactobacillus* and *Bifidobacterium*. However, other genera such as *Streptococcus, Lactococcus* and *Enterococcus*, are also considered as probiotics. *Enterococcus* identified as potential probiotics are available on the market known as *Enterococcus faecium* SF68® (NCIMB 10415, Cerbios-Pharma SA, Barbengo, Switzerland) and *Enterococcus faecalis* Symbioflor 1 (SymbioPharm, Herborn, Germany).

The nutritional health benefits (for consumers) attributed to probiotic bacteria include their role in enhancing the bio-availability of calcium, zinc, iron, manganese, copper and phosphorus, increasing the digestibility of protein and synthesis of vitamins (Sudha, 2014). Camel milk is also known as a source of LAB strains, mainly *Lactobacilli, Lactococci* and *Enterococci* (Abdou *et al,* 2018). *Lactobacillus* spp. was the major group which has been isolated from camel milk (Abushelaibi *et al,* 2017). According to Li *et al (*2020), lactococcus was used in the production and maturation of dairy fermented products. The *Lactobacillus* (Lb.) was added as fermenting agents for the manufacture of fermented milks and cheeses *Lb. plantarum, Lb. casei* and *Lb. acidophilus* involved in the development of the flavour and texture of cheese. Enterococci (*E. faecium* and *E. feacalis*) has an important role in the maturation of several varieties of cheese, probably because of their proteolytic activity, lipolytic, their capacity diacetyl and other volatile components contributing to flavouring, flavour and taste characteristic (Terzić-Vidojević *et al,* 2021). The isolation of the microflora in camel milk as a basis for possible development of suitable

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starter cultures for fermented camel milk products is therefore necessary.

The objective of this work was to isolate and characterise lactic acid bacteria from camel milk and to evaluate their technological and probiotic properties.

Materials and Methods

Bacterial strains and culture conditions

LAB strains were isolated from camel milk (*Camelus dromadarius*) belonging to the herd of Arid Lands Institute (IRA, Medenine, Tunisia). Milk samples were collected from camel flock from Arid Land Institute, Medenine, Tunisia. LAB were isolated on MRS agar (Pronadisa, Madrid, Spain) and incubated at 37°C for 24 to 48 h in order to apply the conventional tests for identification and screening of probiotics. The strains were tested for Gram staining, catalase and mobility. Gram-positive, catalase-negative and non-motile isolates were selected and stored in MRS broth (Pronadisa, Madrid, Spain) supplemented with 30% sterile glycerol and conserved at -80°C. At the time of analyses, the purified cultures were activated by sub-culturing twice in MRS broth before use.

16S rRNA gene identification

The genomic DNA of the strains was isolated using the DNA extraction and purification kit according to the manufacturer's instructions (Fermentas, Cambridge, UK). The PCR reaction mixture contained 0.5 µL of template DNA, 2.5 µL of reverse primer (10 mM), 2.5 µL of forward primer (10 mM), 2 µL of dNTP (25 mM), 4 µL of $MgCl_2$ (25 mM), 5 µL of PCR buffer (10X) and 1µL Taq polymerase, in a 50 µL final volume. The primers sequences used were S1 (5'AGAGTTTGATC (A,C) TGGCTCAG 3') and S2 (5' GG (A,C) TACCTTGTTACGA (T,C) TTC 3'). The cycling programme was 94°C for 3 min, 29 cycles at 94°C for 40 sec, 55°C for 50 sec and 72°C for 2 min. The PCR products were visualised on agarose gel electrophoresis and 1500 bp bands were purified. The resulted amplicons were cloned into the pGEM-T Easy Vector System (Promega Corp., Charbonnières-les-Bains, France), followed by plasmid extraction using Gene JET plasmid Mini prep (Thermo Fisher Scientific, Surrey, UK). Sequencing of the amplicons was performed by the sequencing facility offered by Eurofins (Ebersberg, Germany). The obtained nucleotide sequences, displayed by BioEdit software, were analysed using the blast tool of the NCBI site in order to find identity percentages with the sequences present in databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Probiotics screening tests

pH and bile tolerance

The tolerance of the isolated LAB to acidic pH was performed as described by Yu *et al* (2013) with some modifications. pH 2 was used as a representative gastric pH value. After 16 to 18 h of culture in aerobic conditions, cells were harvested by centrifugation for 10 min at 5000 rpm at 4°C. The pellets were washed once in phosphate buffered saline PBS (8 g/LNaCl, 0.2 g/LKCl, 1.44 g/L Na_2HPO_4, 0.24 g/L KH_2PO_4, at pH 7.2) then resuspended in PBS (pH 2) and incubated at 37°C. After 4 h of incubation, colonies were counted and the number of LAB was calculated according to the standard ISO 15214 (1998). The survival rate was calculated as the percentage of LAB colonies number grown on MRS agar (Oxoid CM0361, Thermo Fisher Diagnostics, Dardilly, France) after 4 h of incubation compared to the initial LAB colonies number. Strains showing resistance to low pH were tested for bile tolerance. The experiment was applied at this concentration of bile 0.3% (w/v) for 4 h. MRS medium containing 0.3% bile (Oxoid, Thermo Fisher Diagnostics, Dardilly, France) was inoculated with active cultures (incubated for 16-18 h). During the incubation for 4 h, viable cells were enumerated for every hour with pour plate technique and also growth was monitored at OD600. A non-probiotic strain: *Lactococcus lactis* was used as a negative control.

Adhesion tests

Adhesion to organic solvents

The bacterial adhesion to hydrocarbons test (BATH) was performed according to Sepova *et al (*2017) with some modifications.

Cells were washed once with phosphate-buffered saline PBS (pH 7.2) and resuspended in the same buffer to an optical density (OD_{600}) of about 0.25 ± 0.05 (OD_0) in order to standardise the number of bacteria (10^7-10^8 CFU/mL). Then an equal volume of solvent was added. Both phases system was mixed by vigorous mixing for 5 min. The aqueous phase was removed after 1 h of incubation at room temperature, and its optical density at 600 nm (OD_1) was measured. The percentage of bacterial adhesion to the solvent was calculated as follows: $[(OD_0-OD_1)/OD_0)]*100$

Two different solvents were tested in this study: chloroform, a monopolar and acidic solvent; and

ethyl acetate, a monopolar and basic solvent. The values obtained with the two solvents, chloroform and ethyl acetate, were considered as a measure of electron donor (basic) and electron acceptor (acidic) characteristics of bacteria, respectively.

Adhesion to gastric mucin

The isolated LAB strains were evaluated for adhesion to immobilised porcine stomach mucin (Type III, Sigma-Aldrich, St Louis, Missouri, USA), in 96-well polystyrene microplates (NuncMaxisorp, Thermo Fisher Scientific, Roskilde, Denmark) (Sanchez *et al,* 2010).

Each well was covered by 100 µL of porcine gastric mucin type III solution at 10 mg/mL in sterile PBS (pH 7) and microplates were incubated at 4°C, overnight. Wells were washed twice with 200 µL of sterile PBS to remove unbound mucin. A bovine serum albumin solution (200 µL at 2% (w/v) in PBS) was added and the microplates were incubated for 2 h at 37°C.

The wells were then washed twice with 200 µL of sterile PBS, before adding the bacterial cells. One mL of culture was collected at three different stages of growth (9, 12 and 24 h) by centrifugation at 3500 g for 5 minutes at 4°C. The obtained pellet was washed twice with 1 mL of Tris-HCl 0.1 M, pH 7.5 then centrifuged. Cells were resuspended in PBS and diluted to achieve an OD_{600} of 0.1±0.02 (which corresponds to about 3.10^7 CFU/mL). Each bacterial suspension (100 µL) was added per well and the microplate was incubated for 1 h at 37°C then the liquid was removed by pipetting. Each well was washed five times with 200 µL of PBS. The desorption of adherent cells was carried out by adding 200 µL of Triton X-100 0.5% solution (v/v) for 20 min at room temperature, with orbital stirring at 150 g and bacteria were counted on MRS plates. Adhesion to gastric mucin was performed in triplicate. Percentage adhesion was calculated from the viable counts adherent to the mucin with respect to the initial counts (%) = (CFU/mL recovered bacteria/CFU/mL initial bacteria) × 100.

Adhesion to STC-1 cells

Adhesion to mouse intestinal endocrine tumor cell line (STC-1) was investigated (Borah *et al,* 2019). Cells were cultured in DMEM medium (Dulbecco Modified Eagle's Minimal Essential Medium, high glucose, HEPES, GlutaMAX™, Gibco, Life Technologies, Paisley, United Kingdom) supplemented with 10% (v/v) calf serum (South America origin, Gibco), 100 µg/mL of streptomycin and 100 UI/mL of penicillin(Gibco) at 37°C in 95% air/5% CO_2. The medium was changed every 2 days and the cells were used after 15 days when they had a full confluence. Cells were inoculated at 5.10^4 cells per well, grown on 24 wells plates (Nunclon™ Surface, Nunc, Thermo Fisher Scientific, Roskilde, Denmark) at 37°C until a confluent monolayer was obtained. Prior to adhesion, wells were washed with a pre-warmed medium to discard antibiotics. Bacteria were grown in MRS medium for 18 h at 37°C then washed twice in 100 mM phosphate buffer (pH 7.0). Bacteria were then diluted in DMEM to obtain 1.10^7 CFU/mL and 1 mL was added in each well. After 2 h of incubation, free bacteria were removed by 1 mL of pre-warmed phosphate buffer. Then, 1 mL of 1% triton X100 was added and after 10 min, serial dilutions were plated on MRS medium and incubated 48h at 37°C. This test was done in triplicate.

Auto-aggregation assays

Auto-aggregation assays were performed according to (Holst *et al,* 2019) with certain modifications. LAB strains were grown on MRS broth for 18 h at 37°C. The cells were harvested by centrifugation at 5000 g for 15 min, washed twice and resuspended in phosphate buffered saline (PBS) to give viable counts of approximately 10^8 CFU/mL. Cell suspensions (4 mL) were mixed by vortexing for 10s. After incubation at 37°C for 4 h, 0.1 mL of the upper suspension was transferred to another tube with 3.9 mL of PBS and the optical density (OD) was measured at 600 nm. Aggregation was expressed as:

$$1 - (OD_{uppersuspension} / OD_{total\ bacterial\ suspension}) * 100$$

Co-aggregation assays with Saccharomyces cerevisiae

The co-aggregation test was performed as described by Ribeiro *et al* (2020) with some modifications. Briefly, bacterial suspensions were prepared as described for auto-aggregation analysis. Equal volumes of cells (100 mL) of the different *Enterococcus* strains and *Saccharomyces cerevisiae*; grown in Sabouraud Dextrose Broth (Sigma-Aldrich, St Louis, Missouri, USA) for 24h, were mixed and incubated at 20 and 37°C without agitation. Pure bacterial suspensions (200 mL each) were incubated under conditions similar to the mixtures in order to check self-flocculation. The optical density of the mixtures and the pure bacterial suspensions was measured at 600 nm after 4 h of incubation. The co-aggregation (%) was calculated according to the equation:

$[(OD_{Sac}+OD_{Bact}) - (OD_{Mix}) / (OD_{Sac}+OD_{Bact})] * 100$

Where: OD_{Sac} represent OD_{600} of *S. cerevisaeat* time T_0, OD_{Bact} represent OD_{600} of bacterial suspension at time T_0 and OD_{Mix} represents OD_{600} of the mixture after 4 h of incubation.

Survival to simulated in vitro digestion

Successive *in vitro* gastric and intestinal digestion was performed according to (Haghshenas *et al*, 2016). The strains were grown in skimmed milk for 8 h at 30°C. One gram of the resulting fermented milk was diluted to 1/10 in phosphate-buffered saline PBS. To simulate the gastric digestion, the sample was adjusted to pH 3.0 and Pepsin was added to a final concentration of 5% (w/v). The mixture was incubated at 37°C for 90 min with agitation at 110 rpm. To create intestinal digestion conditions samples were adjusted to pH 6.0 and solutions of pancreatin and bile salts at a final concentrations of 0.1% and 0.3% (w/v), respectively were added. Samples were incubated at 37°C for 150 min with agitation (110 rpm). After that, the number of cells was determined before and after both gastric and intestinal digestion. An aliquot was serially diluted and then plated on MRS agar in double. The plates were incubated under anaerobic condition for 48 h. The survival of LAB was expressed as log percentage of the final bacterial count (log_{10} CFU/mL) in comparison to their log of the initial count (log_{10} CFU/mL), which allowed the comparison of different isolates regardless of differences in initial counts.

Antagonistic Activity

The antibacterial activity of the selected isolates was determined by agar spot on-lawn test. The indicator bacteria used in this study were *Saccharomyces cerevisiaea, Listeria innocua, Micrococcus luteus,* and *Escherichia coli* (Lab collection).

One microlitre of each overnight culture of selected LAB was spotted on MRS plates (containing 0.2% glucose and 1.2% agar) and incubated under anaerobic conditions for 48 h to develop colonies. A portion of 0.25 mL of 1:10 dilution of an overnight culture of the indicator bacteria was inoculated in 9 mL of Brain Heart Infusion (Merck, Darmstadt, Germany) soft agar (0.7% agar). The medium was immediately poured over the MRS plate on which the tested *Enterococcus* was grown. The plates were incubated anaerobically at 37°C for 24 h. The antibacterial activity was related to the clear inhibition zone which calculated as the difference between the total of inhibition zone and the diameter of the growth spot of selected strains. Zones with diameters larger than 1 mm were determined to have antagonistic activity according to Nisin, used as a control, was prepared by dissolving the commercial preparation Nisaplin® (2.5% pure nisin, Sigma-Aldrich, St Louis, Missouri, USA) in 0.02 N HCl to assure complete solubilisation. Subsequently, sterile distilled water was added until a final concentration of 1mg/mL of nisin. The solution of nisin was prepared on the day of the experiment. The Antagonistic Activity was determined by replacing 1 µL of LAB strain culture by 1 µL of nisin solution.

Safety Evaluation of Enterococcus faecium strains

Antibiotic susceptibility

The antibiograms of the strains were determined using agar antibiotic diffusion discs. Strains were grown overnight in MRS broth at 37°C and 100 µL of the diluted culture (approximately 10^6 viable cells) were streaked on MRS agar. The antibiotics were used at the following concentrations: 30 µg tetracycline, 10 µg ampicillin, 1000 µg kanamycin, 15µg erythromycin, 30 µg rifampicin and 30 µg vancomycin. The plates were incubated at 37°C under anaerobic conditions for 18 h and the inhibition zones were measured. According to CLSI zone diameter interpretative standards, strains were considered resistant if the inhibition zone diameter was less than 17 mm for Vancomycin, Erythromycin and tetracycline; less than 16 mm for ampicillin and than 10 mm for Kanamycin CLSI (2017).

Cytotoxic assay on STC-1 cells

The cytotoxic assay was performed according to Espíndola *et al* (2022), where Caco-2 cell line was replaced by STC-1 cell line. Cells were seeded on a 96 well plate at 7000 cells per well in 150 µL of DMEM medium. A volume of 80 µL of minimum medium containing bacterial strains at 10^5 or 10^7 CFU/mL were added; sterile minimum medium was used as a control. Twenty microlitres of propidium iodide at 5 µg/mL were then added in each well. The emission was monitored during 60 minutes every 30 seconds using excitation/emission wavelengths of 575/615 nm and 5 nm slits for both on spectrofluorimeter (SAFAS, Monaco, France). Results where expressed in fluorescence emission "fold of control" observed with non-treated cells.

Technological characteristics of strains

Acidifying activity

Acidifying activity of strains was measured according to the International Dairy Federation (IDF)

standard 306 (1998), (Martínez-Cuesta *et al,* 2021) and (Ribeiro *et al,* 2021).

Acid production ability was assayed by inoculating 10% skim milk with 24 h old cultures at 1% level and incubation at 30°C. Acidity was determined during 24h of incubation.

Proteolytic activity

To determine the proteolytic activity of LAB, MRS agar supplemented with 10% skim milk was poured, solidified and then dried. Sterile Whatman paper discs were deposited on the surface of the agar. Each disk received a volume of 20µl of a young culture. After incubation at 37 ° C for 24 h, proteolysis is indicated by clear zones around discs (Artha *et al,* 2019).

Lipolytic activity

To determine the lipolytic activity, the strains were inoculated on agar spot in Tween 80 (1, 3, 5%). Incubation was carried out at 25°C for 72 hours. Strains with an opaque area due to the formation of esters with calcium liberated fatty acids were considered positive (Silva *et al,* 2019).

Biomass production

Strains were sub cultured on MRS broth; 100 ml of the medium were inoculated with 10% of the active culture. Bacterial growth was monitored by measuring the optical density at 600 nm (OD600) using a spectrophotometer (CECIL CE 2041/2000 Series) during 6 h. The difference between the initial OD and the OD at which cells were collected (ΔOD) was taken as an indication for the growth amount. The maximum growth rate was determined from the slope of the linear part of curve representing Log OD versus time. At the early stationary phase, 30 ml of culture were harvested by centrifugation (Sigma GmbH, Model 6K15, Gottingen, Germany) at 5000 g for 30 min at 4°C. The dry weight was determined after drying the pellet at 105°C for 24 h. The remaining 70 ml were used to study the separation of biomass by centrifugation and measurement of OD600 of supernatant (Berisvil *et al,* 2020).

Exopolysaccharides production

The cultures were streaked on modified MRS (m-MRS; glucose replaced with 100 g/l sucrose) (Prete *et al,* 2021) and incubated at the optimum growth temperature for 24 h, then tested for slime formation using the inoculated loop method (Mazlumi *et al,* 2022). Formed colonies were dragged up using a metal loop and the strains were considered positively slimy producer if the length of slime was above 1.5 mm (Berisvil *et al,* 2020).

Results and Discussion

Isolation of Lactic Acid Bacteria

A total of 62 strains were isolated from camel milk using MRS agar at 37°C under anaerobic conditions. All isolates were Gram positive, non-motile, cocci shaped and catalase negative as preliminary characteristics.

pH and bile resistance

The major selection criterion for probiotic strains is resistance to low pH because they have to pass through the stressful conditions of the stomach and reach the small intestine. In our case, only 26 isolates were tolerant to low pH, thereby tested for bile salt tolerance. At this stage, 10 strains were retained for further probiotic assessment and molecular identification. These strains showed a good resistance to bile salts: 8 strains (SSC1-2, SCC1-6, SCC1-8, SCC1-13, SCC1-15, SCC1-24, SCC1-33 and SLch14) were resistant after the incubation period with a survival rate higher than 0.5 unit of OD_{600} and only 2 strains (SCC1-7 and SLch6) had a survival rate greater than 1 unit.

Molecular identification of the selected isolate

At least a 1500 bp fragment of the 5′ region of the 16S rRNA gene was sequenced for the 10 retained strains. Comparison of sequences in the NCBI data base revealed that the 10 strains showed 99% of identity with *Enterococcus faecium* (Table 1). Sequences of the 16S rRNA genes have been deposited at the NCBI gene bank under the accession numbers: JN560903.1, KF149320.1, JX847611.1, JQ726533.1,

Table 1. Identification of LAB isolates from camel milk and their Genbank accession numbers.

Strains	Identification	% of similarity	NCBI accession number
SCC1-2,	*E. faecium*	99%	JN560903.1
SCC1-6	*E. faecium*	99%	KF149320.1
SCC1-7	*E. faecium*	99%	JX847611.1
SCC1-8	*E. faecium*	99%	JQ726533.1
SCC1-13	*E. faecium*	99%	EU878170.1
SCC1-15	*E. faecium*	99%	KC422716.1
SCC1-24	*E. faecium*	99%	JN560911.1
SCC1-33	*E. faecium*	99%	JN560898.1
SLch6	*E. faecium*	99%	HM162421.1
SLch14	*E. faecium*	99%	AY587799.1

EU878170.1, KC422716.1, JN560911.1, JN560898.1, HM162421.1 and AY587799.1 corresponding to the strains: SCC1-2, SCC1-6, SCC1-7, SCC1-8, SCC1-13, SCC1-15, SCC1-24, SCC1-33, SLch6 and SLch14 respectively. Ours results were according to previous reports finding that LAB isolates from Camel Milk were especially *Enterococcus* species (Abushelaibi *et al,* 2016).

Adhesion to organic solvents, gastric mucin and STC-1 cells

Adhesion to organic solvents

Bacterial adhesion to solvents is implicated in various interfacial phenomena such as microbial adhesion. Bacterial adhesion to chloroform and ethyl acetate was tested to assess the Lewis acid-base characteristics of the bacterial cell surfaces. Our findings showed that the strains have medium to low affinities to both solvents (Table 2). The greatest affinities with chloroform were observed in *E. faecium* SCC1-13 (30.4±7.58%), *E. faecium* SCC1-15 (28.6 ±5.05%) and *E. faecium* SLch6 (25.5±0.76%), while the least affinities were observed in *E. faecium* SCC1-2(12±5.66%). The bacterial affinities to ethyl acetate were relatively high when compared to chloroform and ranging from 0 to 34.56 ± 4.88%; the highest value was obtained for the strain *E. faecium* SCC1-8 and the lowest value was obtained for the strain *E. faecium* SCC1-13; indicating the acidic and electron acceptor property of most strains. However, the strain SCC1-13 showed higher affinity to chloroform than to the ethyl acetate (30.4% vs. 0.0%, respectively). As reported by [26] three strains of *Lactobacillus acidophilus* showed strong affinities to chloroform, which means they are strong electron donors. Unlike chloroform, the bacterial adhesion to ethyl acetate was low, ranging from 5.1 to 16.9%.

Table 2. Adhesion to organic solvents (%), coaggregation (%) with S. cerevisae at 20°C and 37°C and auto-aggregation of the different *E. faecium* strains.

Strains	Adhesion to ethyl acetate	Adhesion to chloroform	Coaggregation with *S. cerevisae*		Auto-aggregation
			20°C	37°C	
SCC1-2	16± 5.66	12± 5.66	61.77± 11.05	46.78± 2.16	41.03
SCC1-6	26.0± 2.83	16.7± 2.62	58.10± 13.38	45.07± 2.23	62.76
SCC1-7	20.7± 9.75	14.4± 4.08	48.74± 1.99	43.31± 12.29	59.66
SCC1-8	31.8± 10.71	18.8± 2.95	48.43± 6.93	44.94± 18.76	34.48
SCC1-13	00± 00	30.4± 7.58	62.59± 0.68	43.41± 0.1	60.69
SCC1-15	25.0± 8.16	28.6± 5.05	55.28± 4.13	43.98± 0.29	33.10
SCC1-24	34.5± 4.88	14.8± 5.24	44.40± 7.2	43.46± 2.08	62.76
SCC1-33	18± 2.83	18.5± 00	48.64± 2.6	41.03± 19.46	40.34
SLch6	22± 2.83	25.5± 0.76	50.00± 1.57	42.15± 8.19	63.10
SLch14	15.4± 5.44	18.8± 4.42	55.15± 14.34	41.18± 10.72	60

Adhesion to gastric mucin and STC-1 cells

The capacity of adherence to mucus is a desirable property for potentially probiotic bacteria since it could allow the competitive exclusion of pathogens and also the interaction with epithelial and immune cells in the gut (Dell'Anno *et al,* 2021). Strains were tested for their adhesion to gastric mucin at different stages of growth. The results are shown in Fig 1. All *E. faecium* strains showed good adhesion rate to gastric mucin (higher than 60% at all growth stages) which decreased at stationary phase due to a possible reduction in nutrients and an increase in metabolic. Strains able to adhere to mucins, could allow them to interfere with pathogen binding and also interact with the mucosal immune (Han *et al,* 2021). Furthermore, the strains were also examined for their ability to adhere to STC-1 cells *in vitro*. Our results showed that *E. faecium* strains had low adhesion to STC-1 cells and only two strains: SCC1-33 and SLch6 were able to colonise epithelial cells with an adhesion value of 7.8 10^3 and 4.2 10^3 CFU/mL respectively.

Auto-aggregation and co-aggregation

Auto-aggregation and co-aggregation are essential properties of probiotic organisms as they prevent their elimination from gastro-intestinal tract environment. Co-aggregation with *S. cerevisae* and auto-aggregation of the different probiotic strains are presented in Table 2. Results showed that among the ten *E. faecium* tested, six strains showed a high auto-aggregation rate (higher than 60%). All tested strains were well aggregated with *S. cerevisae* and co-aggregation was higher at 20°C. The process of adhesion appears to be multifactorial because adhesion cannot be attributed to one component and includes electrostatic interactions, hydrophobic interactions, and specific bacterial structures (Elbourne *et al,* 2019).

Survival in simulated in vitro digestion

The ability to survive in the GIT is one of the main crucial properties required for probiotic strains. Since, the therapeutic effect of probiotic bacteria is correlated with their concentration in the intestine lumen. Thus, the selected strains were tested for their viability in simulated stressful conditions of gastric and intestinal digestion. Results in Fig 2 indicated that two strains (SCC1-2 and SCC1-7) were able to multiply in simulated intestinal fluid. Two strains (SCC1-5 and SLCch6) had a good survival rate higher than 50%. Three strains had low survival rate (between 11 and 37%) whereas, two strains did not survive simulated gastric nor intestinal transit (less than 5% survival rate). Similar results were found by (Coimbra-Gomes *et al,* 2022) for the strains *E. faecium* SJRP20 and *E. faecium* SJRP65.

Antagonistic effect

Antagonistic activity to inhibit the pathogens in the GIT is considered an important probiotic trait. The ten selected strains were tested for their antagonistic effect (Fig 3). All the *E. faecium* strains showed high antagonistic effect towards all tested pathogen strains with different intensity between strains except for SCC1-13 strain that did not inhibit the growth of *M. luteus*. Several studies reported the antibacterial effect of the *Enterococcus* genus.

E. faecium LCW 44, isolated from camel milk exhibited a large antibacterial spectrum with inhibitory activity against several Gram-positive strains belonging to the genera *Clostridium, Listeria, Staphylococcus,* and *Lactobacillus* (Choeisoongnern *et al,* 2021).

Safety evaluation of E. faecium strains

The susceptibility of *E. faecium* strains to several antibiotics was determined (Table 3). All the strains were susceptible to Tetracycline, Vancomycin, Erythromycin, Ampicillin and Kanamycin and resistant to Rifampicin. The antibiotic susceptibility profile of *E. faecium* SLCch6 is in agreement with previous reports concerning *E. faecium* strains that are commonly found in foods and have safety criteria (Golob *et al,* 2019). Results from the cytotoxicity test showed a negative effect on STC1- cells.

Technological properties

Study of technological properties of LAB strains isolated from camel milk is an important criterion for selection of starter cultures to be used in the standardised production of dairy products.

Table 3. Antibiotic resistance and susceptibility of the studied *E. faecium* strains.

Strains	T30	R30	V30	E15	A10	K1000
SCC1-2	S	R	S	R	S	S
SCC 1-6	S	R	S	S	S	S
SCC 1-7	S	R	S	S	S	S
SCC 1-8	S	R	S	S	S	S
SCC 1-13	S	R	S	S	R	S
SCC 1-15	S	R	S	S	S	S
SCC 1-24	S	R	R	S	S	S
SCC 1-33	S	R	S	S	S	S
SLC ch6	S	R	S	S	S	S
SLC ch14	S	R	S	S	S	S

T30: Tetracycline; R30: Rifampicin; V30: Vancomycin; E15: Erythromycin; A10: Ampicillin; K1000: Kanamycin
(R): resistant, (S): susceptible

Acidifying activity

The strains were characterised on the basis of acid production ability. The acidity increased during the fermentation time and there was variability in acidification rate between the different strains used to inoculate milk (Fig 3). The strain is considered fast, medium and slow when ΔpH reached 0.4 U for 3, 3 to 5 and > 5 h respectively (Ayad *et al,* 2004). This is applicable using cow's milk as a substrate. In our case, only strains with ΔpH ≥ 0.3 U after 6 h were kept for the next steps considering the antimicrobial activity of camel milk. Thus, the strains selected are: SCC1-33, SCC1-8, SCC1-7, SCC1-15, SCC1-6, SCC1-24 and SLCch14.

A rapid decrease in pH during the initial step of cheese preparation is crucial importance in cheese manufacture, since it is essential for coagulation and prevention or reduction of the growth of adventitious microflora. The fast acidifying strains are good candidate in the dairy fermentation process as primary starter organisms, whereas, the poor acidifiers strains can be used as adjunct cultures depending on their other important properties, e. g., proteolytic and autolytic activity.

The difference observed from one lactic acid bacteria species to another were suitably explained (Abozead *et al,* 2022). In fact, the acidifying activity of each strain is related to its specific capacity to break down the substances in the medium and render the capability of assimilation. On occasion, differences are also due to the presence or absence of nutrient transport systems (Mercha *et al,* 2020).

Proteolytic activity

The results obtained during the implementation of this test are summarised in Table 4. The table

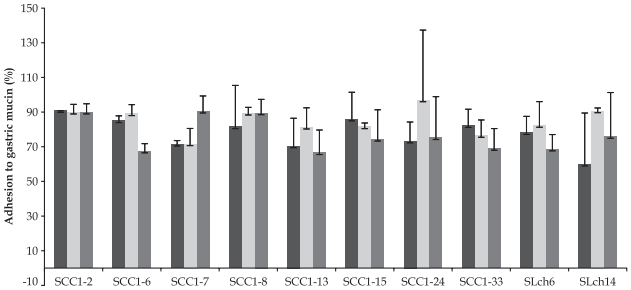

Fig 1. Adhesion to gastric mucin at three different stages of bacterial growth: exponential phase (■), early stationary phase (■) and stationary phase (■).

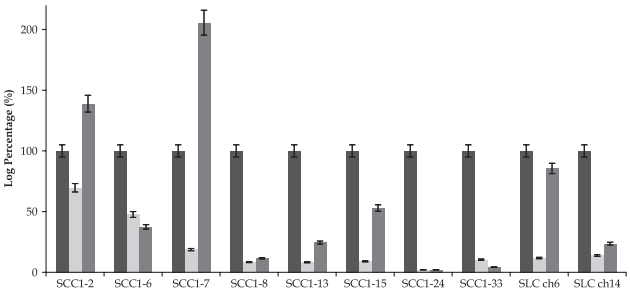

Fig 2. Resistance of *Enterococcus faecalis* strains to simulated *in vitro* digestion: viability at the beginning of the assay (■), viability after simulation of gastric conditions (■) and viability after intestinal digestion (■).

shows that all strains studied show growth with proteolytic activity resulted in the emergence of a clear halo around the discs. According to (Kieliszek *et al*, 2021), the strain is called proteolytic if it has a zone of lysis of diameter between 15 and 21 mm.

Table 4. Proteolytic activity of lactic isolates.

Strains	Diameter of inhibition zone en mm
SCC1-2	15±1.4
SCC1-6	15±0.0
SCC1-7	18±1.41
SCC1-8	16±0.0
SCC1-13	21±0.0
SCC1-15	16.5±3.53
SCC1-24	16.5±3.53
SCC1-33	16.5±3.53
SLch6	19±0.0
SLch14	18±0.0

The proteolytic activity of dairy lactic acid bacteria is essential for the bacterial growth in milk and involved in the development of organoleptic properties of different fermented milk products (Razzaq *et al*, 2019; Worsztynowicz *et al*, 2019). The production of high quality fermented dairy products is dependent on proteolytic systems of starter bacteria, since peptidase and amino acids formed have a direct impact on flavour or serve as flavour

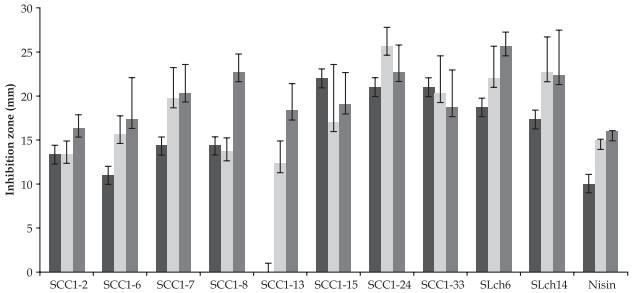

Fig 3. Antagonistic effect of *Enterococcus faecalis* strains against *Micrococcus luteus* (■), *Listeria innocua* (■) and *Escherichia coli* (■).

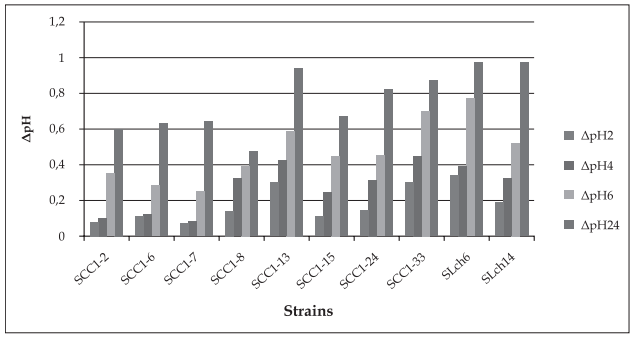

Fig 4. Evolution of ΔpH during the fermentation of camel milk after 2H , 4H , 6H and 24H inoculated with different lactic strains and incubated at 30°C.

precursors in these products. Several peptidases with different specificities have been identified in lactic acid bacteria; all peptidases have been found to be intracellular and liberated in fermented milk products after cell lysis (Sebastián-Nicolas *et al*, 2021; Razzaq *et al*, 2019).

Acidification and proteolytic activity are difficult to dissociate and the differences of acidifying power between the different strains were certainly related to a difference in the initiation of proteolytic activity (Raveschot *et al*, 2020).

Lipolytic activity

The results of the lipolytic activity of lactic strains are shown in Table 5. Lactic acid bacteria are considered weakly lipolytic (García-Cano *et al*, 2019) in comparison with other bacterial species such as *Pseudomonas*, *Acinetobacter* and *Flavobacterium* (Brennan *et al*, 2002).

Tavakoli *et al* (2019) stated that the addition of autochthonous LAB on dairy products contributes to the production of free fatty acids and linoleic acid from milk fat lipolysis, providing a hipolipidemic effect in the host. These bacteria are found in large amounts on lactic foods due to their adaptation capacity in this substrate rich in proteins, lipids and fatty acids. The wide distribution of these bacteria is attributed to their lipolytic and proteolytic properties,

capacity to ferment/assimilate lactose and to use fatty acids. The Free Fatty Acid contributes to the aroma and flavour of some foods, especially cheese (Gomes *et al,* 1998).

Table 5. Lipolytic activity of lactic acid bacteria.

Strains	Lipolytic diameter zone		
	1%tween80	3%tween80	5%tween 80
SCC1-2	11,375	9	9
SCC1-6	8,5	9	9,125
SCC1-7	9,5	10	9,875
SCC1-8	9,25	9	9,125
SCC1-13	13,5	9,75	9,5
SCC1-15	9,625	10,5	11,5
SCC1-24	8,875	9,5	10,25
SCC1-33	9,5	8,75	9,5
SLch6	8,625	9,5	11
SLch14	11,125	9	9,5

Biomass production and growth rate

The fermentation broth was centrifuged and the pellet was dried in order to determine biomass. The difference between the initial optical density (OD600) and the OD600 at which cells were collected (ΔOD600) as well as the dry weight of strains were used to reflect the growth amount (Table 6). Based on the biomass, cultures were divided into 3 groups: major yields when biomass ≥ 1.30 mg/L, an average yield when the formed biomass ranged from 0.6 to 1.29 mg/L, poor performance when the biomass was <0.6 mg/L (Berisvil *et al,* 2020). Strains SCC1-6, SCC1-15, SCC1-33 and SLCch14 were characterised by a high value of ΔDO600 and an important growth rate. The strains SCC1-24, SCC1-2, SCC1-13 and SCC1-13 presented a weak biomass and growth rate.

Table 6. Characteristics of starters growth.

Strains	ΔOD600*	Biomass (g/l)	µmax (h-1)	EPS	OD600 Supernatant
SCC 1-2	0.448	0.53	0.052	-	0.003
SCC1-6	1.136	0.81	0.132	-	0.008
SCC1-7	0.67	0.69	0.071	-	0.019
SCC1-8	0.864	0.20	0.080	+	0.106
SCC1-13	0.737	0.06	0.073	+	0.115
SCC1-15	1.322	0.79	0.123	+	0.024
SCC1-24	0,737	0,06	0,073	+	0.049
SCC1-33	1 ,322	0,79	0,123	+	0.056
SLch6	0.689	0.887	0.146	+	0.068
SLCch14	1.935	0.98	0.131	+	0.02

* ΔOD 600, difference between the initial optical density and optical density after 6 h of culture; +, EPS producing strains; -, non EPS producing strains.

Indeed, the production of small quantities of biomass could be an inconvenient for the industrial use of these strains. However, this low yield could be explained by the loss of biomass during centrifugation and this was due to the production of exopolysaccharides that prevent the separation of bacterial cells and culture medium. This was visualised in the OD values of supernatant (Table 6). According to Ren *et al* (2022), a good separation of biomass was represented by an OD600 ranging between 0 and 0.1. The majority of strains had an OD600 <0.1 reflecting a good separation of biomass. Only two strains SCCl-8 and SCCl-13 had values greater than 0.1. As mentioned earlier, this was due to the production of EPS which prevent separation during centrifugation.

Exopolysaccharide production

Many strains of LAB produce EPS that can be a capsule, closely attached to the bacterial cell, or loosely attached or excreted as slime (Angelin and Kavitha, 2020; Sørensen *et al,* 2022). The strains that showed poor and fair pellet separation after centrifugation (see above) were screened for EPS production.

Lactic acid bacteria have the ability to synthesise and excrete during their growth, extracellular sugar polymers called polysaccharides or exopolysaccharide (EPS), which can improve the texture and viscosity of the final product (Korcz and Varga, 2021). In general, the presence of polysaccharides in fermented products such as yogurt can increase the homogeneity of the product and make its presentation more enjoyable (Wa *et al,* 2021). The texture of fermented milk depends also on the interactions between bacteria and the different proteins (spatial conformation, interaction, pH, ionic strength) (Prete *et al,* 2021). Our results showed that seven strains (SCC1-8, SCC1-13, SCC1-15, SCC1-24, SCC1-33, SLch6 and SLch14) were able to produce EPS (Table 6).

A relatively high number of *Enterococci* strains were evaluated and the results indicated good production and technological performance of many strains of *E. faecium*. However, enterococci comprise a major part of the fresh cheese curd microflora and in some cases, they are the predominant microorganisms in the ripened cheese in other countries (Berisvil *et al,* 2020). The role of these strains in the manufacture of cheese has been investigated in the last few years (Dapkevicius *et al,* 2021; Rhoades *et al,* 2021; Sarkar *et al,* 2020).

Conclusion

Several LAB isolated from Tunisian camel milk and identified as *Enterococcus faecium* showing potentially important properties are valuable for practical application as starter and a potential probiotic. *E. faecium* SLCch6 strain had great potential to be used as an effective probiotic in both food industry or therapy purposes. This strain presented high tolerance to the passage through the GIT environment, high co-aggregation and auto-aggregation ability, high adhesion to gastric mucin and acceptable adhesion to epithelial cells suggesting high adhesiveness to the host tissues which favour the colonisation and survival in the GIT. Moreover, this strain showed strong antagonistic activity against pathogens. Furthermore, the susceptibility of *E. faecium* SLCch6 toward antibiotics suggested the absence of transferable antibiotic genes. However, careful safety investigations must be greatly emphasised before the application of *E. faecium* SLCch6 as probiotic.

Author Contributions

Imen Fguiri designed and performed the experiments. Imen Fguiri and Manel Ziadi analysed the data and wrote the manuscript. Samira Arroum contributed in the experimental analysis. Touhami Khorchani revised the paper

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Competing Interests

The authors declare that there is no Competing Interests regarding the publication of this paper.

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UNIQUE DEVELOPMENT OF THE HEART OF THE DROMEDARY CAMEL: A COMPARATIVE REVIEW

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ABSTRACT

The cardiovascular system was one of the first body systems to appear within the embryo. The heart was active by the beginning of the fourth week. The aim of this review is to describe the prenatal developmental changes that occur in dromedary camel foetus and compared the structures of the foetal camel heart to that of other mammals.

Key words: Camel, development, foetus, heart

The heart is the first organ to develop during embryogenesis, and the researchers places a great deal of importance on the heart's early circulation function. Therefore, both classical and molecular embryologists have given a great deal of attention to the developmental mechanisms that coordinate the formation and morphogenesis of this organ. Several vertebrate and invertebrate embryos have been studied in depth because of the evolutionary conservation of many of these processes (Zaffran and Frasch, 2002; Tan and Lewandowski, 2020; McGeady *et al*, 2017). In humans, the heart beats spontaneously by the fourth week of development. Its development begins with the formation of two endocardial tubes, which merge to form the tubular heart (primitive heart tube). It loops and become separated into four chambers and paired arterial trunks, (Moorman *et al*, 2003). According to Moorman and Christoffels (2003) and van den Hoff *et al* (2004) the heart development started when the first mesodermal cells migrated anterolateral and formed the bilateral heart- forming primordia during gastrulation. The main walls of the heart were formed between day 27 and day 37 of the development of the embryo. Growth began with two tissue masses, which were actively growing towards each other until they finally merged and split into two separate tubes (Fernández, 2002). Only one research in the available literature was conducting during pregnancy in the dromedary camel, the beats of the heart was detected on day 26 - day 28 (Alhaider, 2019).

Histological development of the heart:

Balogh and Sótonyi (2003) studied the early cardiac development in rabbit. They have stated that on the 9th day of gestation the embryonic disc appeared, on the 10th day the single cardiac tube was formed, on the 11th day the bulboventricular loop was formed and the heart consisted of three chambers. On the 12th day the partitioning of atria and ventricles was close to its end. On the 13th day the heart consisted of four chambers and on the 14th day the developmental stage of the heart was very similar to that seen in the newborn. In rabbits the most intensive development of the heart took place in the period between the 10th and the 13th day.

The smooth zones of the interventricular septum and the pulmonary and aortic roots, compared to the trabeculated parts of the right and left ventricles, were recognisable. The transition zone between compact and trabeculated tissues was called the spongy layer and it was recognised as a network of fine trabeculations, the different layers of the ventricular myocardium (Savolainen *et al*, 2009). Important studies were conducted in the development of dromedary camel heart (Babiker, 2016). At the stage of 2cm CVRL (71 days of gestation), the pericardium of the camel heart was associated with the diaphragm, liver, and thoracic vertebrae. The atrial outlines were irregularly showing many undulations, whereas the ventricular outlines were relatively regular. The epicardium appeared as a thin layer. The ventricular endocardium showed many trabeculae (Fig 1). At

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the stage of 17cm CVRL (112 days of gestation), the atria were formed of a thin layer of mesenchymal connective tissue covering the atrial myocardium. The endocardium was formed of an endothelium supported by a subendothelial layer of mesenchymal connective tissue. The atrial myocardium presented scattered and thick cardiac muscle bundles (Fig 2) (Babiker, 2016).

Ventricular myocardium was in the form of compact layers of cardiac muscle fibres rich on mesenchymal connective tissue and blood vessels with decreased trabeculation and wide luminae. The right ventricle at this stage showed mainly cardiac muscle fibres and mesenchymal connective tissues. The left ventricle had a thick layer of myocardium and a considerable amount of mesenchymal connective tissue. Many cardiac muscle fibres were closely surrounding the endocardium and some others were transformed from myofibroblasts to myocytes (Fig 3) (Babiker, 2016).

As foetal age increased, a gradual increase was also observed in the myocardial thickness of the ventricular wall and atrial pectinate muscles by an increase of cell layers. Large amount of adipose tissue was observed in the dromedary camel foetus in the epicardium in which the interventricular branches of coronary arteries and their branches were embedded (Fig 4) (Babiker, 2016).

Cardiac Conduction System

The components and structure of the specific conduction system in humans are like those found in commonly used laboratory animals. The conduction system was composed of specialised myocytes. Its atrial components, sinus node and atrioventricular node, are in contact with atrial myocardium. The bundle of His penetrates the right fibrous trigone, then divides into two specialised ventricular branches (right and left). It is surrounded by a fibrous sheath that separates the specialised myocytes from the ordinary myocardium. The fibrous sheaths disappear at the distal ramifications of the bundle branches, allowing continuity with the ventricular myocardium (Sánchez-Quintana and Yen Ho, 2003).

Miquerol *et al* (2010) stated that the propagation of electrical activity through the heart is regulated by the ventricular conduction system to synchronise cardiac contraction.

Sinoatrial Node (SAN)

The sinus node of yak contained an extensive framework of collagen and two main type cells: pacemaker cells (P cells) and transitional cells (T cells). The P cells had a perinuclear clear zone, contained less myofibrils. The T cells were longer and slender than P cells. At the periphery of sinus node, there were many nerve fibres and ganglions (Duan *et al,* 2012). Ghazi *et al* (1998) also found that, the SAN in the domestic cat contained normally a dense collagenous framework.

The sinus node in adult camel was located 0.5 mm beneath the epicardium, near the junction between the cranial vena cava and the right atrium at the sulcus terminalis. It's shape was elongated, and oblong; 28.25 mm in length, 5.75 mm in width and 5.38 mm in thickness. The adult camel's SAN histology described by Ghazi and Tadjalli (1996) was comparable to that of the yak described by Duan *et al* (2012).

The histological structure of the SAN in the dromedary camel foetus was investigated using routine histological techniques and some special stains (Marwa-Babiker *et al,* 2016b). The SAN in the first trimester camel foetus was found in subendocardial region cranial to the opening of cranial vena cava at the junction between the cranial vena cava and right atrium. Two types of cells were observed; the first type had dark cytoplasm and large spherical lightly stained nucleus. The second type were small and spindle in shape with dark small nuclei. The SAN in camel foetuses in the second and third trimesters; it had the same location as in the adult camel also had two types of cells as in other mammalian species like yak (Marwa-Babiker *et al,* 2016b).

Atrioventricular Node (AVN) and Atrioventricular Bundle (AVB)

The anatomy and histology of AVN and AVB were studied in the heart of the dromedary camel (Ghazi and Tadjalli, 1993). They stated that the trunk of the atrioventricular bundle (Bundle of His) was a direct continuation AVN with no sharp line of demarcation between the node and the bundle. The AVB ran through the fibrous trigone and entered the lower part of the interventricular membranous septum beneath the right endocardium. They lay then over or slightly to the side of the centre of the muscular interventricular crest. The AVB of camels measured 4.12±1.00 mm in length, 3.66±1.13 mm in width and 1.13±1.85 mm in thickness, it's maximum sectional area being 12.68±6.13 mm^2 (Ghazi and Tadjalli, 1993 and 1996).

Histologically, the AVB in the heart of camels comprised multiple strands of Purkinje cells separated

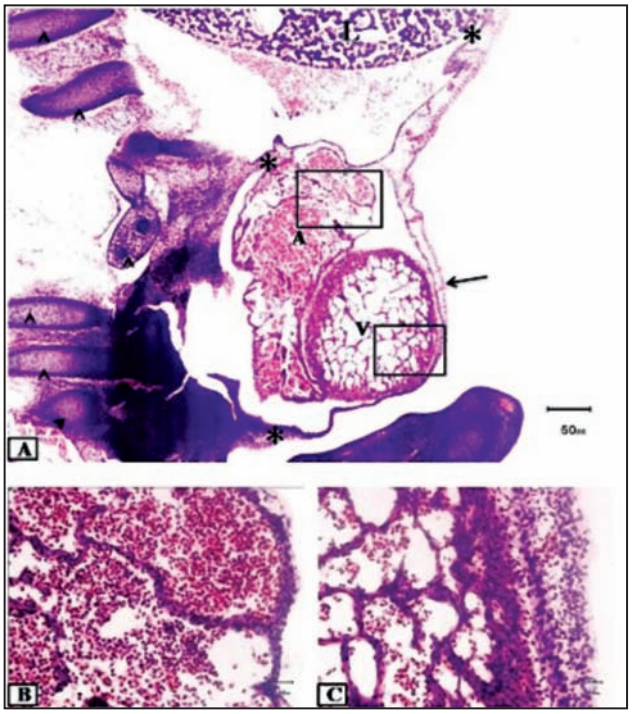

Fig 1. **A:** Photomicrograph showing the heart of 2 cm CVRL (71 days) camel foetus: right atrium A, pericardium (arrow), vertebrae (arrowheads), pericardial attachments (asterisk), right ventricle V, liver L, H and E (X4). **B:** Magnification of the upper rectangle in A: irregular outlines (X40). **C:** magnification of the lower rectangle in A: regular outlines (X40).

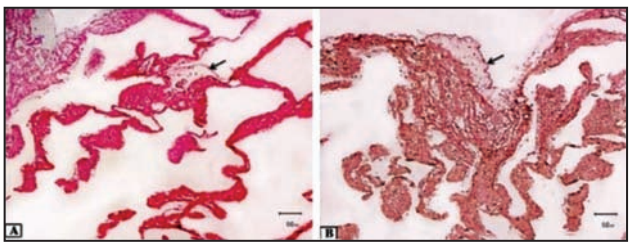

Fig 2. **A** and **B:** Photomicrographs showing the atrium of 17 cm CVRL camel foetus; epicardium (arrow) consisting of large amount of mesenchymal connective tissue and lined by simple squamous epithelium. A: H&E (X10). B: Verhoff's (X10).

by collagen fibres and surrounded by connective tissue. It resembled that in humans and dogs except that, in camels, intercalated discs were present at the intercellular connections in the AVB (Ghazi and Tadjalli, 1996). The development of the AVB and ventricular Purkinje system and their innervation has been studied in sheep foetuses from 27 to 140 days of gestation (term is 147 days) (Canale *et al*, 1987). The AVB initially consisted of a primordium, which lacked innervation and was composed of small, relatively undifferentiated myocytes. Differentiation of Purkinje-like cells within the AVB began near

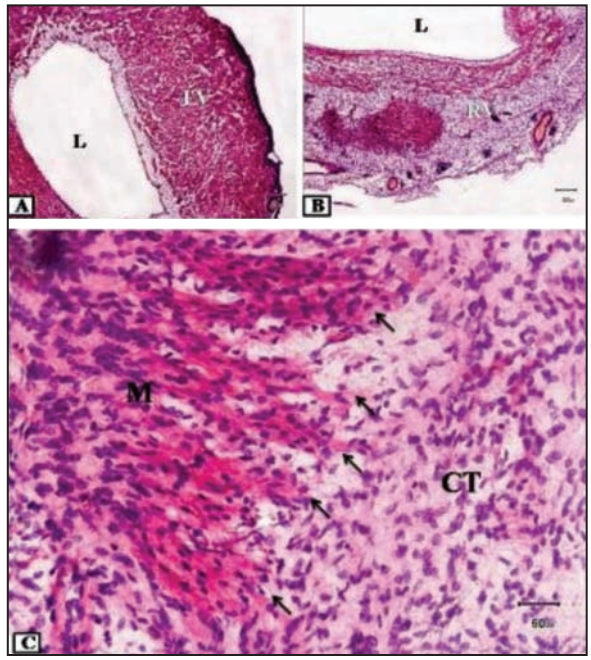

Fig 3. **A:** Photomicrograph showing histogenesis from connective tissue to muscular tissue of LV, left ventricle of 17.5 cm CVRL camel foetus. L, lumen of the left ventricle. H&E, (X4). **B:** Photomicrograph showing the right ventricle of the same heart the histogenesis was comparatively less than that in the left ventricle. L, lumen of the right ventricle. H&E, (X4). **C:** Photomicrograph showing higher magnification of the same section in B, histogenesis is very clear in the right ventricle (arrows). CT, connective tissue, M, myocardium. H&E, (X40).

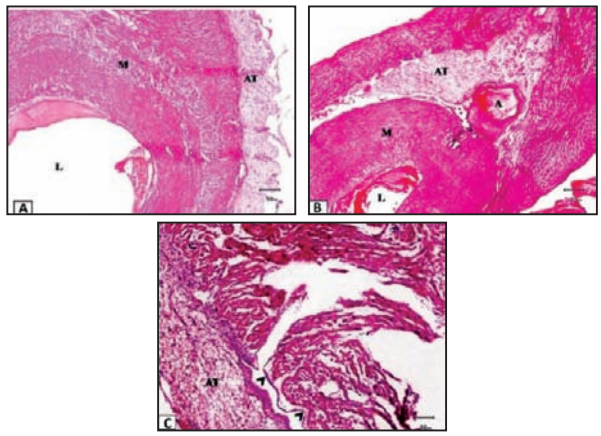

Fig 4. **A:** Photomicrograph showing the right ventricle of the heart of 30 cm CVRL camel foetus. Epicardium lined by simple squamous epithelium and has large amount of adipose tissue (AT) attached to the myocardium (M). L, lumen of the right ventricle. H&E (X4). **B:** Photomicrograph showing the left ventricle of the heart of 30 cm CVRL camel foetus. Epicardium lined by simple squamous epithelium. Large amount of adipose tissue (AT) in the myocardium (M) and around the aorta (A). L, lumen of the left ventricle. H&E (X4). **C:** Photomicrograph showing the atria of 68 cm CVRL of camel foetus containing large amount of adipose tissue (AT) covering epicardium (arrowheads). H&E (X4).

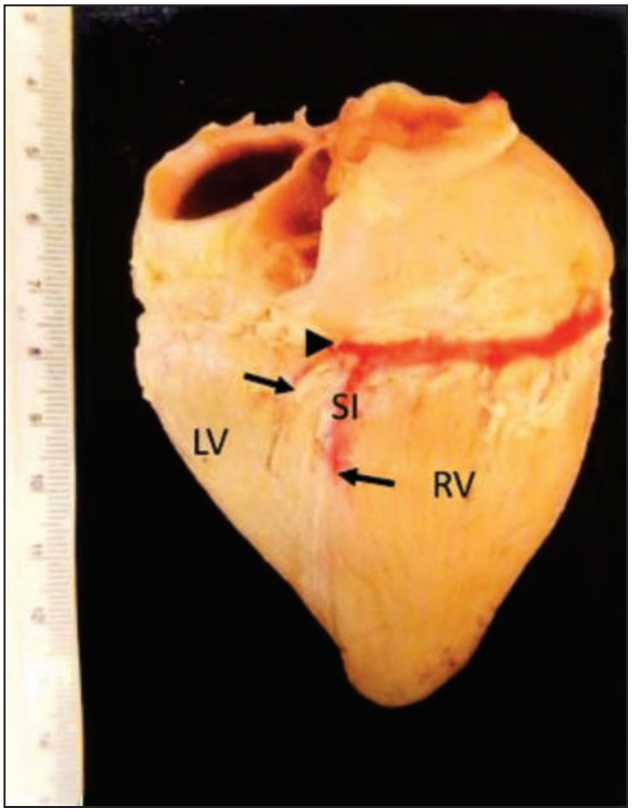

Fig 5. Photograph of a dissection of right aspect of the heart of a foetus of the dromedary camel showing that the subsinuosal interventricular branch (SI), is covered by type II myocardial bridge in the middle part of the subsinuosal groove. The arrow points to the site where the artery dips in the myocardium. Arrowhead the caudal branch of the right coronary artery, LV; left ventricle, RV; right ventricle.

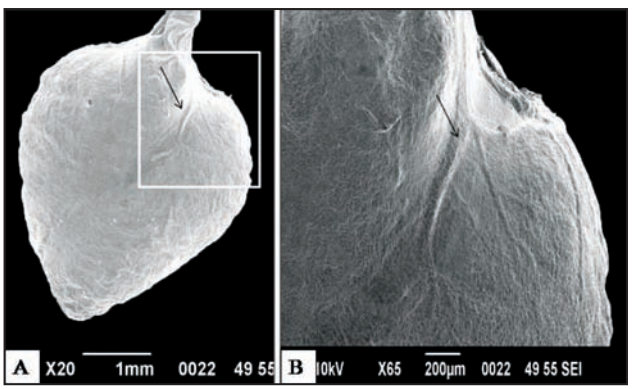

Fig 6. **A:** Scanning electron micrographs of the camel heart foetus of 6.8 cm CVRL showing two ventricles as semi-triangular in shape and a shallow left longitudinal groove (arrow). **B:** Magnification of A showing interventricular branches of coronary artery (arrow) in the upper part of the groove.

its distal end and extended towards the AVN. Differentiation of the ventricular Purkinje system extended distally from the region of bifurcation of the AVB from cells that were indistinguishable from

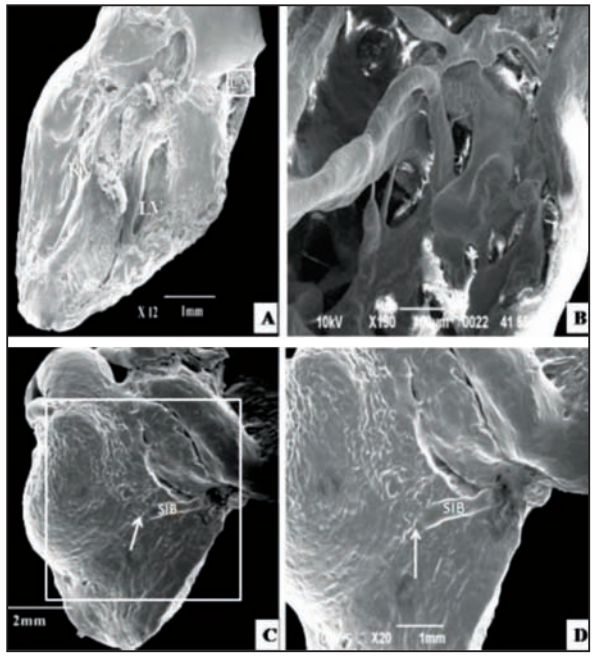

Fig 7. **A:** Scanning electron micrograph of the camel heart foetus of 23 cm CVRL. **B:** A higher magnification of the square in A showing atrial pectinate muscles in the form of cords; **C:** showing type II myocardial bridge over the subsinuosal interventricular branch of right coronary artery (SIB) dipping in the myocardium (arrow) without reappearing. **D:** Magnification of the square in C.

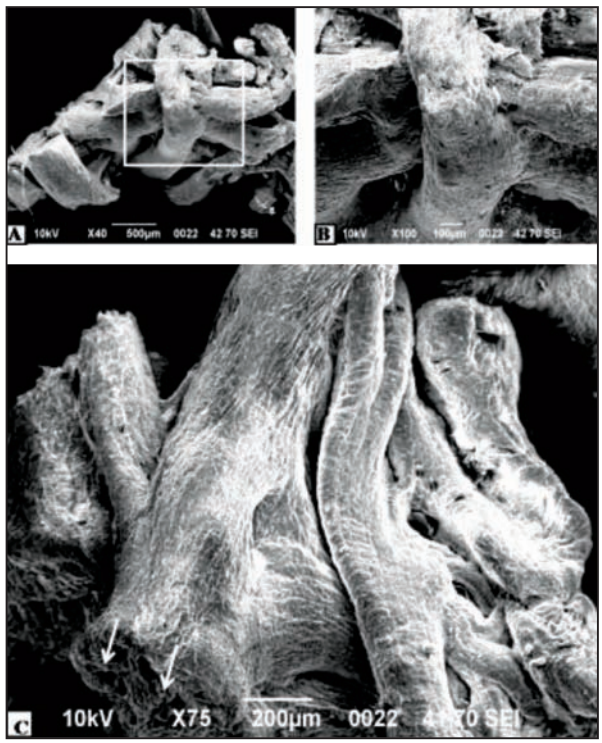

Fig 8. **A, B** and **C:** Scanning electron micrographs showing atrial pectinate muscles of 60 cm CVRL camel foetus as a plexus. B: Magnification of the square in A. C: Higher magnification of the same age showing some branches of pectinate muscles in cross section (arrows).

the working myocardium and continuous with the AVB primordium. Differentiation of Purkinje-like AVB cells was complete by 46 days gestation but Purkinje fibres were still differentiating within the ventricular wall at 60 days of gestation. The main morphological changes included a large increase in cell size and organisation into strands, development of characteristic glycogen-filled regions containing many intermediate filaments and early development of myofibrillar M lines compared to the working myocardium. The differentiation of AVB cells and the ventricular Purkinje system preceded their innervation (Canale *et al,* 1987).

Canale *et al* (1987) stated that in sheep the AVB became innervated earlier than ventricular Purkinje fibres. Intimate contacts between proximal AVB cells and nerve axons were present at 60 days of gestation. Nerve fibres were observed in the septomarginal band at this time. Although the AVB and ventricular Purkinje system of adult sheep composed morphologically of similar cells, they differed in origin and their mode of differentiation as well as timing and intimacy of innervation. Innervation was not part of the developmental mechanism leading to the differentiation of Purkinje fibres (Canale *et al*, 1987).

The AVN and AVB development in the camel heart were studied during the 1st, 2nd and 3rd trimesters of gestation using histological techniques (Marwa-Babiker *et al,* 2019b). AVN was found close to the atrioventricular opening in the first trimester and close to the opening of the coronary sinus in the second and third trimesters. It generally appeared as a group of large-sized and lightly stained cardiac muscle cells. AVB was embedded either in myocardium in the second trimester as a bundle of lightly stained fibres as strands located between the endocardium and myocardium or within the myocardium. At the early stages of the third trimester, they appeared as strands of fibres, which were covered by connective tissue between the endocardium and myocardium (Marwa-Babiker *et al,* 2019b).

Purkinje fibres (PF)

Canale *et al* (1987) in sheep and Miquerol *et al* (2010), in mammals, studied the development of Purkinje fibres. It is well known that Purkinje fibres are found in the subendocardium. They are larger than cardiac muscle cells, but have light glycogen content, fewer myofibrils and no T-tubules (Eliška, 2006). They are specialised conducting fibres, which extend from the interventricular septum, to the papillary muscles and up the lateral walls of the ventricles.

When compared to regular cardiac muscle, bovine Purkinje fibres were investigated by Forsgren and Thornell (1981). Gradually, it became clear that the two cell types differed in terms of the intercalated disc, myofibril content, mitochondrial organisation, glycogen content, and T-tubule development. The development of the PF was studied in dromedary camel foetuses during the three gestational periods (Marwa-Babiker *et al,* 2017). In the 1st trimester PF were embedded in cardiac muscle fibres close to the ventricular endocardium. Also, either between the endocardium or in the connective tissue. Some of Purkinje fibres were bi-nucleated. The striation was clear in peripheral parts of some fibres. At the 2nd trimester of gestation most of fibres were bi-nucleated and the cytoplasm around the nuclei was light. At the 3rd trimester PF appeared as bundles of fibres parallel to cardiomyocytes. Additionally, they were present as individual fibres or strands implanted in the endocardial mesenchymal connective tissue. Until the last stages of pregnancy, most Purkinje fibres in camel foetuses were not discovered in their typical place (Marwa-Babiker *et al,* 2017).

Myocardial Bridges (MBs)

MBs are defined as a congenital coronary abnormality in which a branch of a coronary artery passes intramurally through the myocardium (Kosinski and Grzybiak, 2001; Chen *et al,* 2004; Singh *et al,* 2005; Alegria *et al,* 2005; Demirsoy *et al,* 2006). They were also considered as structures consisting of heart muscle which passed above the coronary arteries or their branches (Chen and Lin, 2003; Kosinski *et al,* 2004; Aytan *et al,* 2006). The coronary arteries might dip into the myocardium for varying lengths and reappear on the heart's surface; this muscle overlying the segment of the epicardial coronary artery was termed a myocardial bridge (Loukas *et al,* 2006; Bharambe and Arole, 2008).

Iuchi *et al* (2013) stated that in human, the anatomical properties of MBs, especially of its length and thickness, played decisive roles as regulators of atherosclerosis in the left anterior descending coronary artery regardless of the amount of adipose tissue around it.

In bovines, the histological appearance of the pre-myocardial bridge segments differed from the other segments with or without a myocardial bridge

in that the intimal layer was well-developed (Shinjo *et al,* 2004).

Myocardial bridges in adult dromedary camel were studied previously by Taha and Abdel-Magied (1996). These were also found in 90% of dissected adult hearts of the dromedary camel (Marwa-Babiker and Taha, 2013). According to Marwa-Babiker and Taha (2013) and Marwa-Babiker *et al* (2015b) myocardial bridges in dromedary camel were classified into two types according to their relationship with the overbridged artery. In Type I, the descending interventricular subsinuosal or paraconal branches were bridged by one or two bands of cardiac muscle, in Type II, the descending interventricular subsinuosal or paraconal branches were noticed to dip in the myocardium without reappearing. Eight out of eleven hearts of foetuses had myocardial bridges (72.7%); seven hearts had myocardial bridges of Type II category in the subsinuosal interventricular branch (Fig 5), (87.5%); only one heart showed myocardial bridges on both sides (12.5%). In this heart, Type I category was observed over the subsinuosal interventricular branch whereas Type II category was confined to the paraconal interventricular branch. Type II myocardial bridges were observed in the intermediate stages of the first trimester (Marwa-Babiker *et al,* 2015b; Marwa-Babiker *et al,* 2016a).

Ultrastructure of the heart:

Chacko (1976) studied the ultrastructural differentiation of myocardium of Sprague-Dawley rat's embryos. Both thick (myosin) and thin (actin) filaments became identifiable for the first time in the tenth-day myocardium when the heart was pulsating, but circulation was not established. The appearance of the myofilaments and synthesis of Z lines was concomitant. There was a rapid proliferation and differentiation of most of the organelles by the eleventh day of gestation and during the subsequent days. The myofilaments became organised into fully formed striated fibrils. Intercalated discs appeared as small wavy lines on the eleventh day and became plicated in later stages and served as cell boundaries and points of attachment for myofilaments and fibrils. There was a tangible change in the number and morphology of mitochondria from the tenth to eleventh day and during later stages of development when the heart became functional. Similarly, there was a rapid proliferation and differentiation of granular endoplasmic reticulum and Golgi complexes. Large quantities of free ribosomes were dispersed in the cytoplasm of tenth-days myocardium. However, in later stages there was a progressive reduction in the distribution of these organelles. An intimate assembly of ribosomes and polysomes with the developing myofibrils was discernible.

The T-system and sarcoplasmic reticulum began to appear in myocytes at eleventh day. The embryonic myocardium displayed intense mitotic activity throughout its development. A unique feature of embryonic myocardial cells was the simultaneous occurrence of myofilament synthesis and mitotic activity within the same cells (Chacko, 1976).

The embryonic development of the heart of rat was greatly dependent on the myocyte's proliferation, which continued postnatally. The myocardium regeneration during postnatal period varied directly with the potential of cell proliferation. The electron microscopy showed myocytes well fixed with well-developed sarcomers disposed in an irregular form in the myocyte cytoplasm. The cardiac interstitium showed fibroblasts with characteristics of a great proteic synthesis. It suggested many binucleate cardiomyocytes during foetal period in the rat (Xavier-Vidal *et al,* 1997).

The internal cellular structures of the ventricular myocardium had been comparatively studied by transmission and scanning electron microscopy in sheep. The scanning electron microscopy demonstrated the relationships between organelles like mitochondria, sarcoplasmic reticulum, and nuclear envelope better than can be obtained by other methods (Sybers and Ashraf, 1975).

Myklebust *et al* (1975) investigated the sheep cardiac muscle cells using scanning and transmission electron microscopy. Later, Sheldon *et al* (1976) studied scanning electron microscopy in foetal and postnatal period in sheep between 90 days gestational age and 36 days postnatal age. Development of the transverse tubular system was visible as early as 90 days gestational age. Myofibrils in the 90-days foetus showed elongated mitochondria with constrictions and the mature myofibrils in later stages became oval and assumed their adult position in the perinuclear and interfibrillar regions. Myofibrillar development was sparse at 90 days and was most apparent in the subsarcolemmal region. Gradually the lateral addition of fibrils resulted in central displacement of the older myofibrils causing the sarcolemma to be drawn inward at its point of attachment at the Z-lines to form the T-tubules. At birth however, they resembled the adult configuration.

Kim *et al* (1992) studied the ultrastructure development of human foetal heart. Myofibril formation occurred by attachment of thin filaments into amorphous Z materials which were present in sarcolemmal plaques, sarcoplasmic condensations, desmosomes and in Z lines. Myofibrils radiated from these Z centers in many directions, branched, and anastomosed with further development. The myofibrillar growth pattern persisted throughout the entire foetal period. Mitochondria were well developed.

A transverse tubule system was clear in later foetal development in human. It occurred by invagination of sarcolemma into myocardial cells and by the formation of developed microvessels were found throughout the whole foetal period. Binucleated myocytes appeared by 32 weeks gestation, and this suggested that myocyte proliferation might cease before birth in humans. Development of the myocyte was an ongoing process (Kim *et al,* 1992).

Few studies were taken in the first, second and third trimesters of gestation of the camel foetus heart using scanning and transmission electron microscopy (Marwa-Babiker *et al,* 2015a; Marwa-Babiker *et al,* 2019a). Scanning electron microscopy (SEM) showed that during the early stages of the first trimester the heart of camel foetus was semi-triangular in shape (Fig 6) (Marwa-Babiker *et al*, 2015b). Both the coronary and longitudinal grooves were not clear. In addition, the atria were not clear at 84 days of gestation (6.8 cm CVRL) (Fig 6). The pectinate muscles were also not well developed, and the heart was only in the form of two ventricles separated by longitudinal grooves during that stage. During the intermediate stages of the first trimester, the longitudinal grooves and branches of the coronary arteries were clearly observed. The coronary groove was not observed until the end of the first trimester. Type II MBs were observed in this stage (Fig 7). Transmission electron microscopy (TEM) at (101 – 115 days) showed cardiomyofibres striations as irregular Z lines. Cardiomyofibres showed numerous mitochondria of different sizes and shapes around the nucleus and between fibrils. Rough endoplasmic reticulum was observed in the cytoplasm of cardiomyofibres in the form of few cisternae (Marwa-Babiker *et al,* 2015).

A comparative ultrastructure of the dromedary camel heart between second and third trimesters of gestation (131-426 days) has been studied (Marwa-Babiker *et al,* 2019a). At the second and third trimesters, the atrial pectinate muscles showed gradual development and appeared as large branching and anastomosing plexiform cords. Pectinate muscles were thicker in the second trimester than in the third trimester (Fig 8); (Marwa-Babiker *et al,* 2019a). Whereas Z lines with irregular striations were present in the second trimester, intercalated discs were not observed in this stage; they latter first appeared in the third trimester. Mitochondria were numerous around the myocyte's nuclei and between the fibrils. The sarcomere in the third trimester was thicker than in the second trimester. The length and width of mitochondria in the second and third trimester were constant. The organelles development started clearly in the second trimester and continued in the third trimester. Moreover, the transverse tubular system of the myocardium of the atria and ventricles showed obvious developmental changes during the second and third trimesters, (Marwa-Babiker *et al,* 2019a).

Ultrastructural measurements of the prenatal development in the ventricular myocardium and myocardial bridges of the dromedary camel

Measurements were done using the electron microscope processing software that included the dimensions of myofibrils nuclei, mitochondria of myofibrils at (X10000). Sarcomeres were measured at (X3000). The nuclei being oval, or semi oval were measured at the longest line (length) and the vertical line at the narrowest region (width), (Babiker, 2016).

Measurements at the 1st trimester was included the length of myofibril nuclei was 6.69±2.30 µm and the width was 3.19±1.60 µm. The length of mitochondria of myofibril was 1.13±0.29 µm and the width 0.55±0.12 µm. The sarcomere was measuring 1.19±0.23 µm (Babiker, 2016).

Measurements at the 2nd trimester included the length of myofibril nuclei of the ventricular myocardium was 6.29±1.29 µm and the width was 3.11±0.37 µm. The mitochondrial length was 0.85±0.23 µm and width was 0.75±0.02 µm. The sarcomere was measuring 1.21±0.16 µm (Babiker, 2016).

The length of the myocardial bridges myofibril nuclei was 6.71±1.52 µm while the width was 3.68±1.06 µm. The length of mitochondria of myofibril was 0.98±0.28 µm and its width was 0.83±0.23 µm. The sarcomere was measuring 1.08±0.17 µm.

Myofibril nuclei measurements of the ventricular myocardium of the 3rd trimester were 6.64±2.46 µm in length and 2.45±1.17 µm in width. Mitochondria of myofibril were measuring 0.45±0.25

µm for length and 0.40±0.08 µm in width. The sarcomere was measuring 1.8±0.04 µm (Babiker, 2016).

Nuclei of the myocardial bridges in the 3rd trimester were measuring; 8.81±1.45 µm and 2.73±0.10 µm in length and width, respectively. Mitochondria were measuring 1.05±0.16 µm, and 0.77±0.14 µm in length and width, respectively. The sarcomere was measuring 1.86±0.88 µm (Babiker, 2016).

Conclusion

During development of foetal camel hearts at the first, second, and third trimesters of gestation, many adipocytes were observed. The AVN was found close to the atrioventricular opening in the first trimester and close to the opening of the coronary sinus in the second and third trimesters. The AVB was embedded in the myocardium in the second trimester either between the endocardium and myocardium or within the myocardium in the third trimester. The PF were embedded in the myocardium in the first trimester and either between the endocardium and myocardium or within the myocardium in the second and third trimesters. The MBs were observed only histologically in the second and third trimesters. Type II MBs were observed in the late stages of the first trimester with SEM. MBs had a less developing transverse tubular system than the myocardium in the same stages. Pectinate muscles were thicker in the second trimester than in the third trimester. Mitochondria in the first trimester was longer than that in the second and third trimesters. Connective tissue nuclei in the first trimester were longer than those of the second and third trimesters. Connective tissue cell nuclei of MBs were longer in the third trimester than those of myocardium. It is concluded that the development of the camel heart had unique features during the three gestational stages.

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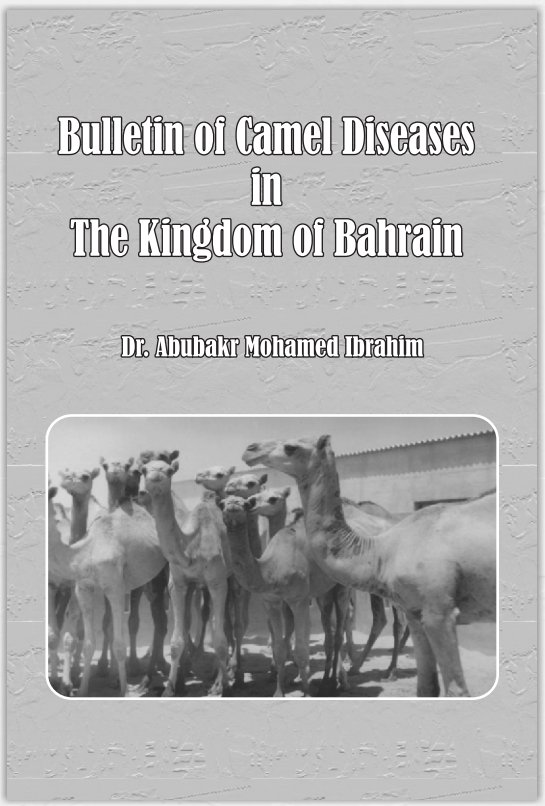
Bulletin of Camel Diseases in The Kingdom of Bahrain

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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DEVELOPMENT AND FUNCTIONAL APPRAISAL OF FERMENTED CAMEL MILK BEVERAGE

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ABSTRACT

In spite of the potential health benefits the camel milk possess, there is a scarcity of fermented camel milk products in India. This research explored fermentation as a means to develop a fermented camel milk beverage with acceptable sensory and enhanced health benefits. Lactic strains used in the study included *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Lactiplantibacillus plantarum* M11, *Lactobacillus helveticus* MTCC 5463 (V3) and *Lacticaseibacillus casei* NK9. Each lactic strains were studied for their growth behaviour in camel milk which was found insignificant among strains. The strains in different combinations (A=MD2+I4, B=MD2+M11, C=MD2+V3 and D=MD2+I4) were used to form starter cultures A, B, C and D for beverage preparation. The sensory acceptability and lactic count of beverage B was significantly ($p<0.05$) high, hence it was selected. To enhance the flavour of the beverage, cumin and black salt were added in different combinations on w/w basis (B1=0.25 cumin+0.3 black salt, B2= 0.5 cumin+0.4 black salt, B3=0.75 cumin+0.5 black salt, B4=0.3 cumin+0.4 black salt, B5= 0.5 cumin+0.4 black salt) in which combination B2 showed significantly higher ($p<0.05$) overall acceptability. Shelf life of fermented camel milk beverage was evaluated at 7±1°C. pH of the beverage decreased significantly ($p<0.05$) from 4.70 to 4.12 with significant ($p<0.05$) decrease in scores of flavour (8.25 to 5.57), body and texture (8.50 to 8.14) and overall acceptability (8.34 to 5.23) throughout the storage period. Overall acceptability score decreased to <6.0 on 18th day of storage. Beverage had a shelf life of 15 days. The percentage values of biofunctional attributes viz., ACE inhibition, α-amylase inhibition, α-glucosidase inhibition, antioxidant activity and proteolytic activity (mg/mL of histidine) of the fresh beverage was 49.86, 55.90, 35.96, 21.87 and 7.80, respectively which increased significantly ($p<0.05$) to 58.69, 58.23, 38.88, 28.50 and 8.27, respectively at the end of shelf life.

Key words: Biofunctional attributes, camel milk, fermented beverage, growth and acidification profile, shelf life, *streptococcus thermophilus* MTCC 5460

Camel milk is considered as a medicinal food owing to its strong immune-modulatory, antioxidative (Habib *et al,* 2013), antibacterial (Mojtahedi *et al,* 2018), antiviral, antifungal, anti-hepatitis, hypoglycemic and anti-cancerous activities (Gizachew *et al,* 2014; Kaskous, 2016; Jilo and Tegegne, 2016). But camel milk possesses a typical sensory characteristic contributed by its components and salty flavour which makes it less desirable for direct consumption by the consumer. Fermentation of camel milk may lead to a product with acceptable sensory attributes and enhanced nutritional and biofunctional activities.

Fermented camel milk products namely Gariss, Suusac and Shubat are traditionally consumed in countries such as Sudan, Kenya and Central Asia-particularly in Kazakhstan, Uzbekistan and Turkmenistan, respectively (Farah *et al,* 1990; Abdelgadir *et al,* 1998). Very scanty literature is available regarding fermentation of camel milk by lactic cultures. Additionally, in contrast to the milk from other dairy species, the viscosity of the product made from camel milk remains same during the fermentation process owing to the protein composition (Jumah *et al,* 2001) as well as the naturally occurring antimicrobial compounds present in camel milk (Attia *et al,* 2001). Fermented beverage type products therefore seems to have great promise.

In India, fermented camel milk products are not available in the market. This research explored fermentation as a means to develop a fermented camel milk beverage with improved sensory and functional attributes.

Materials and Methods

Lactic strains

Lactic Acid Bacteria (LAB) strains used in the study *viz. Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Lactiplantibacillus plantarum* M11 (M11), *Lactobacillus helveticus* MTCC 5463 (V3) and *Lacticaseibacillus casei* NK9 (NK9) were obtained from Dairy Microbiology

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Department, SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat, India. The strains were activated in sterilised reconstituted skim milk (12% T.S.) at 37±1°C and stored at 5±2°C. The strains were given three successive transfers prior to their use in reconstituted skim milk to ensure its activity during the course of study.

Camel milk

Camel milk was procured from Gujarat Cooperative Milk Marketing Federation Ltd., Anand, Gujarat, India. Cumin and black salt were purchased from local market, Anand, Gujarat, India. The roasted cumin powder was strained through wire mesh to obtain fine powder.

Evaluation of growth and acidification profile of Lactic Acid Bacteria (LAB) strains in camel milk

Each lactic strains were inoculated in the heated (90°C / 10 min), cooled (37°C) milk @ 2% and incubated at 37±1°C. During incubation, samples were drawn at interval of 0, 6, 12, 18, 24, 30, 36, 42 and 48 h and analysed for pH, titratable acidity and lactic count.

Estimation of Titratable acidity and pH

Titratable acidity (TA) was determined by method mentioned in FSSAI (2015). The product pH was determined using a pH meter (Chemi Line, Ahmedabad, India, Benchtop Meter with probe and stand). The time required to reach pH 4.7 was considered the fermentation time and expressed as hours.

Microbiological analysis

Microbiological analysis was performed as described in Chaudhary and Sreeja (2020). Briefly, serial dilutions of the samples were prepared in phosphate buffer and from selected dilution, 1 mL was transferred to petri dishes in duplicates. Then, 15-20 mL of sterile agar (de Man, Rogosa and Sharpe Agar for Lactobacilli and M17 for Streptococcal count) was poured in petri plates and mixed properly. Once the agar got solidified, second layer of respective agar was poured and allowed to solidify. Incubation was carried out at 37±1°C for 48-72 h. The typical colonies were counted and the count was expressed as log CFU/mL (IS: 1479-3, 1977).

Selection of starter culture for preparation of fermented camel milk beverage

Lactic strains were used in four combinations (A: MD2+I4, B: MD2+M11, C: MD2+V3 and D: MD2+NK9) in order to prepare the starter cultures. Fermented camel milk beverage was prepared according to the flow chart shown in Fig 1. The best starter culture was selected on the basis of sensory attributes and lactic count of the fermented beverage. This selected starter culture was used in the further study.

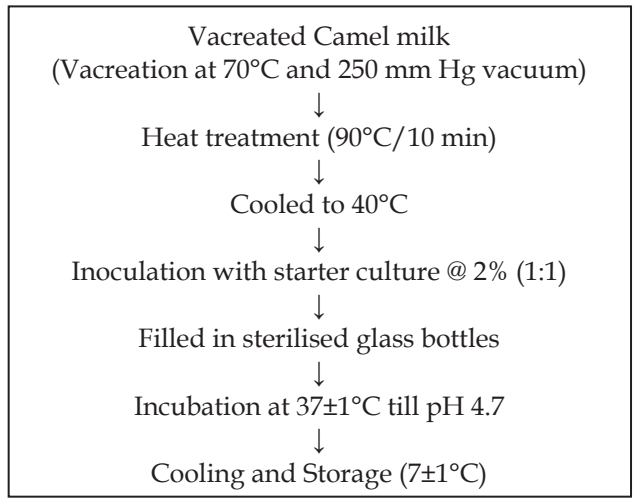

Fig 1. Method for preparation of fermented camel milk beverage.

Sensory evaluation

Sensory evaluation of the beverages were carried out by expert judges (n=10) using 9-point Hedonic scale.

Viscosity

Viscosity of the beverage was measured at 25°C using Brookfield viscometer (model LVDV-E Viscometer, Brookfield) with a constant shear rate using spindle No. 62 at 12 RPM. Viscosity was measured in terms of centipoise (cP).

Optimisation of level of cumin and black salt for the beverage

Fermented camel milk was incorporated with different levels (w/w) of cumin and black salt. Different combinations included B1=0.25 cumin and 0.3 black salt, B2= 0.5 cumin and 0.4 black salt, B3=0.75 cumin and 0.5 black salt, B4=0.3 cumin and 0.4 black salt and B5= 0.5 cumin and 0.4 black salt on w/w basis. The level of cumin and black salt was optimised based on the sensory score obtained on 9-point Hedonic scale. Highest scoring sample was taken as the optimised product.

Compositional Analysis

Camel milk and fermented camel milk beverage were analysed for its Total solids, fat, protein, lactose,

salt, vitamin C and ash). Total solid, fat and lactose content were estimated by Gravimetric, Gerber and Lane Eynon methods, respectively as described in the BIS Handbook (IS: SP-18, Part-XI, 1981). Protein content was estimated by following macro-Kjeldahl method as described in AOAC (2010). Ash content of the sample was estimated using the procedure in BIS handbook (IS: SP 18: Part XI, 1981). Mohr's method was used to estimate salt content (FSSAI, 2015). Vitamin C content was evaluated using 2,6-dichlorophenol indophenol method (IS: 5838, 1970).

Evaluation of shelf stability of fermented camel milk beverage

To determine the shelf life of fermented camel milk beverage, the beverage was packaged in sterilised glass bottles and stored at refrigerated condition (7±1°C) and was analysed for sensory evaluation attributes, titratable acidity, pH, lactic count at interval of 3 days till the end of its shelf life (sensory score <6 on 9-point hedonic scale).

Evaluation of Biofunctional attributes of fermented camel milk beverage

Fermented camel milk beverage was evaluated *in vitro* for its ACE inhibitory activity, antidiabetic activity, antimicrobial activity, antioxidant activity and proteolytic activity. Unfermented milk was used as control.

ACE-inhibitory activity, antidiabetic activity (measured as α-Amylase inhibitory activity and α-Glucosidase inhibitory activity), antimicrobial activity and antioxidant activity of samples was determined as described in Chaudhary and Sreeja (2020). Antimicrobial activity was evaluated against *Bacillus cereus* MTCC 1272, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* MTCC 1687, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* MTCC 737. Antioxidant activity was evaluated by 2, 2'-Azino-bis 3- ethylbenzothaizoline-6-sulfonic acid (ABTS) Assay. Proteolytic activity was evaluated using o-phthalaldehyde (OPA) method (Thakkar *et al*, 2018).

Statistical analysis

The data related to composition of milk and beverage were analysed using two sample t-test. The data related to Optimisation of starter culture, flavour, shelf life, biofunctional attributes, growth behavior and antimicrobial activity were analysed using Completely Randomised Design (CRD) and Factorial CRD.

Results and Discussion

Growth and acidification profile of LAB strains in camel milk

The comparative changes in pH and titratable acidity of camel milk fermented by different strains are shown in Fig 2 and 3, respectively. Decrease in pH of camel milk was found to be similar in all the cultures (Fig 2). Titratable acidity of the camel milk increased significantly ($p<0.05$) throughout the incubation of 48 hours. All cultures had shown similar increase in acidity up to 18 hours of incubation. After that, M11 and NK9 showed slow increase in acidity while MD2, V3 and I4 showed fast increase in acidity and then reached maximum at 48th hour of incubation (Fig 3).

Different cultures exhibited almost similar growth behaviour in camel milk (Fig 4). From 0 to 6 hours, all strains exhibited relatively slower growth. After that, the strains showed log phase up to 24 hours of incubation. V3 showed significantly ($p<0.05$) low microbial count than all other cultures. Stationary growth of cultures was observed from 24 to 36 hours, and after that the count decreased till the end of incubation.

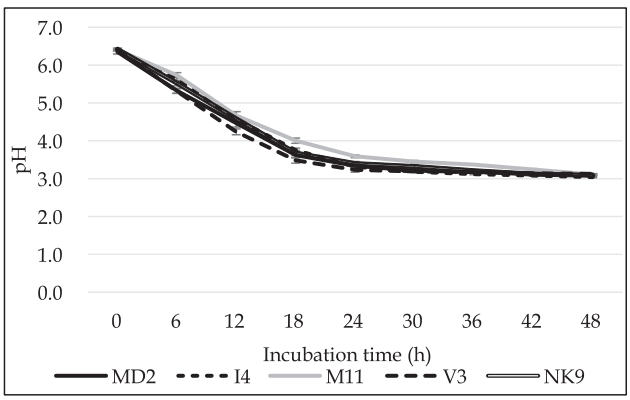

Fig 2. Comparative changes in pH of camel milk inoculated with different cultures.

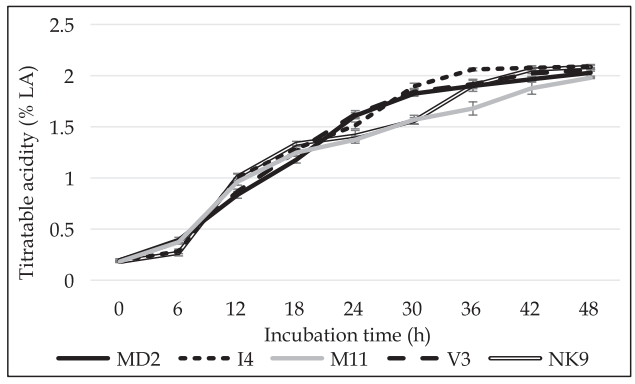

Fig 3. Comparative changes in titratable acidity of camel milk inoculated with different cultures.

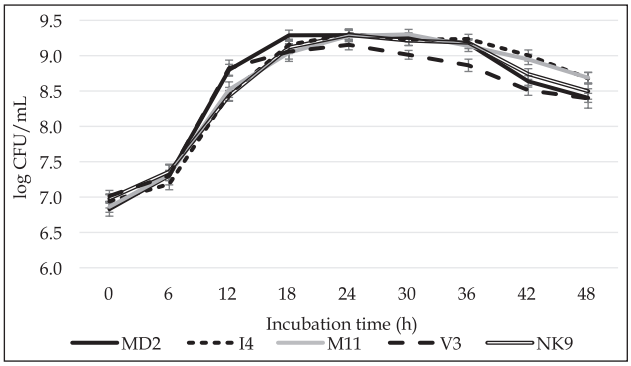

Fig 4. Comparative changes in lactic count of camel milk inoculated with different cultures.

Abu-Taraboush *et al* (1998) studied the growth characteristics of *Bifidobacterium breve* NCFB 2258, *Bifidobacterium bifidum* NCFB 2715, *Bifidobacterium longum* ATCC 15707 and *Bifidobacterium angulatum* ATCC 27535 in camel milk and bovine milk. They observed rapid growth of Bifidobacteria in both the milks. They also reported that decrease in pH during first 10 hours of incubation was minimum, but after that pH decreased significantly up to 36 hours of incubation. We have also observed that starter strains initially grew slowly but after 6 hours of incubation, they grew rapidly in camel milk.

Screening of starter culture for preparation of fermented camel milk beverage

Effect of starter cultures on the fermentation time

The fermentation time taken by different starter cultures to reach pH 4.7 was observed to be significantly ($p<0.05$) different (Table 1). Starter culture B took minimum time (9.54 h) to reach pH 4.7, which was followed by C (9.86 h) and A (10.10 h), respectively. D took the maximum time (10.73 h) to reach pH 4.7. Fermentation ability of the starter cultures was reported to be slower in camel milk in comparison to that of bovine, goat as well as sheep milk (El Zubeir *et al,* 2012; Berhe *et al,* 2018). Camel milk was reported to inhibit the growth of microorganisms owing to the presence of antibacterial and antiviral protective enzymes and proteins like lactoferrin, lactoperoxidase, lysozymes, immunoglobulins (Ig) and peptidoglycan recognition protein (El Sayed *et al,* 1992; Mojtahedi *et al,* 2018). This characteristic of camel milk has been proved a constraint in the manufacture of fermented milk products like yoghurt.

El Zubeir *et al* (2012) studied the processing properties of yoghurt made from nonbovine milk such as camel, goat and sheep milks and found that camel milk took maximum incubation time among all. Berhe *et al* (2018) compared the acidification activities of commercial starter culture in bovine and camel milk. Higher acidification activity was observed in bovine milk as compared to camel milk at their corresponding incubation temperature. Time taken by starter culture to reach pH 4.6 in case of camel milk was found to be significantly higher (1:15 to 4:10 h) than that in bovine milk.

Effect of starter cultures on the lactic count

Lactic count (Lactobacilli and Streptococcal count) of camel milk beverage fermented by different starter cultures is depicted in Table 1. Lactobacilli counts were highest in beverage B (9.89) and D (9.87) and were found to be at par with each other, which is followed by beverage A (9.57) and C (9.32), respectively. Similarly, Streptococcal counts (log CFU/mL) were highest in beverage B (9.85) and D (9.71) and were found to be at par with each other, which is followed by beverage A (9.58) and C (9.29), respectively. Beverages B and D had the highest Lactobacilli as well as Streptococcal counts and were found to be at par with each other.

Rahman *et al* (2009) studied the viable starter culture counts of camel milk fermented by selected bacterial starter cultures. After the fermentation of 6 hours, viable starter culture count of camel milk inoculated with St. *thermophilus* 37, Lb. *delbrueckii* sp. *bulgaricus* CH2, Lc. *lactis*, Lb. *acidophilus* and mixed yoghurt culture in log10CFU/mL were 7.61, 8.03, 6.71, 7.52 and 8.2, respectively. Varga *et al* (2014) evaluated the viability of cultures in honey

Table 1. Effect of different starter cultures on fermentation time, lactic count and viscosity of fermented camel milk beverages.

Beverages	Average fermentation time (h) to reach pH 4.7 at 37°C	Lactobacilli count (log CFU/mL)	Streptococcal count (log CFU/mL)	Viscosity (cP) at 25°C
A	10.10^{b}±0.12	9.57^{b}±0.11	9.58^{b}±0.13	15.13^{b}±1.78
B	9.54^{d}±0.09	9.89^{a}±0.16	9.85^{a}±0.14	34.38^{a}±2.07
C	9.86^{c}±0.07	9.32^{c}±0.12	9.29^{c}±0.10	17.63^{b}±1.95
D	10.73^{a}±0.09	9.87^{a}±0.12	$9.71a^{b}$±0.14	32.50^{a}±1.77

A = MD2+I4, B = MD2+M11, C= MD2+V3, D= MD2+NK9. Each observation is mean of four replications. Values with different superscripts in the column differ significantly ($p<0.05$).

enriched fermented camel milk. Starter culture used were consists of Lb. *acidophilus* LA-5, *Bifidobacterium animalis* spp. *lactis* BB-12 and *Streptococcus thermophilus* CHCC 742/2130. After the fermentation of milk till pH 4.7, Lb. acidophilus LA-5 counts were 7.11 and 7.58 $\log^{10}$CFU/mL, *Bifidobacterium animalis* spp. *lactis* BB-12 counts were 7.38 and 7.36 $\log^{10}$CFU/mL and *Streptococcus thermophilus* CHCC 742/2130 counts were 9.03 and 9.01 log10CFU/mL in control and honey enriched fermented camel milk, respectively.

Effect of starter cultures on the viscosity

Beverages B (34.38 cP) and D (32.50 cP) were reported to have significantly ($p<0.05$) higher viscosity as compared to that of beverages A (15.13 cP) and C (17.63 cP) as shown in Table 1. The effect of starter cultures on viscosity of a camel milk beverage has been least reported. However, the studies depicting the effect of incorporation of additives on the viscosity of fermented camel milk has been reported. Ibrahim and Khalifa (2015a) reported that fortification of fermented camel milk with dietary fibre had viscosity of 16.54 mPas and this viscosity was found to be increasing with increase in rate of addition of dietary fibre. EI-Deeb *et al* (2017) prepared the flavoured fermented camel milk with the addition of cinnamon and doum palm water extract. Cinnamon extract was added at the rate of 1%, 2% and 3% while doum palm extract was added at the rate of 5%, 7 % and 9 %. Viscosity of fermented camel milk was found to be 21 mPas and it was found to increase with the addition of cinnamon extract up to 2% while addition of cinnamon extract at the rate of 3% and addition of doum palm were found to decrease the viscosity. Shahein *et al* (2022) evaluated the effect of addition of date syrup on physicochemical properties of fermented camel milk. Viscosity of fermented camel milk was found to be 30 ± 4.84 cP and it was found a increased significantly with the increase in rate of addition of date syrup.

Effect of starter cultures on the sensory attributes

The beverages prepared using all starter cultures were found to be significantly ($p<0.05$) different from each other in terms of flavour, body and texture and overall acceptability (Table 2). Beverage B was found to be superior in flavour (7.77), body and texture (8.29) and overall acceptability (8.26). However, all the beverages were found to be at par with each other in terms of acidity and colour and appearance.

Rahman *et al* (2009) evaluated the sensory attributes of camel milk fermented by selected bacterial starter cultures. Starter cultures used in the study were *Streptococcus thermophilus* 37, *Lactobacillus delbrueckii* sp. *bulgaricus* CH2, *Lactococcus lactis*, *Lactobacillus acidophilus* and mixed yoghurt culture (*St. thermophilus* and *Lb. bulgaricus* in 1:1 ratio). Sensory scores indicated that camel milk fermented by mixed yoghurt culture was the most acceptable in all

Table 2. Effect of starter cultures on the sensory attributes of fermented camel milk beverages.

Beverages	Sensory scores (9-point Hedonic scale)			
	Flavour	Body and Texture	Colour and appearance	Overall acceptability
A	6.89^{d}±0.04	7.75^{b}±0.11	7.78±0.15	7.13^{c}±0.15
B	7.77^{a}±0.13	8.29^{a}±0.08	7.89±0.15	8.26^{a}±0.11
C	7.25^{c}±0.06	7.76^{b}±0.11	7.83±0.09	7.31^{c}±0.16
D	7.47^{b}±0.06	8.07^{a}±0.17	7.86±0.13	7.68^{b}±0.10

A = MD2+I4, B = MD2+M11, C= MD2+V3, D= MD2+NK9. Each observation is mean of four replications. Values with different superscripts in the column differ significantly ($p<0.05$).

Table 3. Effect of cumin and black salt addition on sensory attributes of fermented camel milk beverages.

Beverages	Sensory scores (9-point Hedonic scale)			
	Flavour	Body and Texture	Colour and appearance	Overall acceptability
B1	7.38^{b}±0.17	7.59±0.11	7.47^{bc}±0.14	7.29^{b}±0.09
B2	8.11^{a}±0.11	7.86±0.08	8.06^{a}±0.14	8.06^{a}±0.15
B3	7.67^{b}±0.17	7.68±0.20	7.29^{c}±0.09	7.58^{b}±0.20
B4	7.46^{b}±0.23	7.80±0.12	7.61^{bc}±0.15	7.32^{b}±0.11
B5	7.41^{b}±0.15	7.52±0.18	7.73^{b}±0.13	7.38^{b}±0.17

B1 = 0.25 w/w cumin and 0.4 w/w black salt, B2 = 0.5 w/w cumin and 0.4 w/w black salt, B3 = 0.75 w/w cumin and 0.4 w/w black salt, B4 = 0.5 w/w cumin and 0.3 w/w black salt, B5 = 0.5 w/w cumin and 0.5 w/w black salt. Each observation is mean of three replications. Values with different superscripts in the columns differ significantly ($p<0.05$).

attributes, which is followed by camel milk fermented by *Lb. acidophilus, Lb. bulgaricus, St. thermophilus* and *Lc. lactis*, respectively. Ibrahim (2015) studied the effect of using EPS producing starter cultures on sensory attributes of fermented camel milk. He reported that yoghurt made with EPS producing starter culture were preferred for body and texture, colour and appearance and overall acceptability while yoghurt made with the use of non-EPS producing starter cultures was preferred for flavour. This was because yoghurt made with EPS producing starter cultures were reported to have significantly lower acetaldehyde content.

From the above observations, beverage B prepared using starter culture comprising of MD2 and M11 was found to be the most acceptable in terms of sensory attributes and lactic count.

Optimisation of level of cumin and black salt for the beverage

In order to improve the flavour of the beverage, we incorporated cumin and black salt into it and various rates on sensory score of fermented camel milk beverage is shown in Table 3. Combination B2 scored highest marks for flavour (8.11), colour and appearance (8.06) as well as overall acceptability (8.06). Body and texture as well as acidity were found to be similar in all combinations. Thus, combination B2 (0.5 w/w cumin and 0.4 w/w black salt) was selected. Studies depicting the use of cumin and black salt as flavouring for fermented camel beverage were not seen previously.

EI-Deeb *et al* (2017) studied the effect of addition of water extract of *Cinnamomum verum* (cinnamon) and *Hyphaene thebaica* (doum palm) in fermented camel milk. Sucrose solution @ 6% was added to milk before heating. Cinnamon extract was added at the rate of 1%, 2% and 3% while doum palm extract was added at the rate of 5%, 7% and 9%. Sensory evaluation has shown that addition of cinnamon extract at the rate of 1 and 2% and addition of doum palm extract at the rate of 5% significantly improved the total scores. Addition of cinnamon extract at the rate of 3 % and addition of doum palm extract at the rate of 7 and 9 % resulted in significantly fewer total scores in sensory evaluation.

Shahein *et al* (2022) investigated the effect of incorporation of date syrup on sensory attributes of fermented camel milk. Date syrup was added at the rate of 6% and 8% in camel milk before pasteurisation. They found that fermented camel milk added with 8% date syrup had significantly high scores for all sensory attributes, i.e. flavour, consistency, appearance and total score which is followed by fermented camel milk added with 6% date syrup.

Compositional Analysis of beverage

The composition of camel milk and fermented camel milk beverage is shown in Table 4. Total solids, ash and chloride content increased significantly ($p<0.05$) in fermented camel milk beverage than that in camel milk. This was due to addition of cumin and black salt in the beverage. Fat and protein content of camel milk and fermented camel milk were found to be similar. However, lactose and vitamin C content of camel milk were found to be higher in camel milk as compared to that in fermented camel milk. Lactose was utilised by starter culture during fermentation as an energy source which resulted in decrease in lactose in fermented camel milk beverage. However, vitamin C is heat sensitive and thus, it might get destroyed during the heat treatment given to milk which resulted in decrease in vitamin C content of fermented camel milk beverage.

Our results were in agreement with that of Magdi *et al* (2010) who found a significant decrease in the vitamin C content in the fermented camel milk in comparison to that in camel milk. They also found a significant reduction in lactose content in fermented camel milk than that in camel milk. Yoganandi *et al* (2014) evaluated composition of camel milk from Kutch region of Gujarat, India. Average total solids, fat, protein, lactose, ash and chloride content reported by them were 9.95, 2.90, 2.66, 3.77, 0.84 and 0.25%, respectively. These results were found to be similar to our results.

Determination of shelf stability of beverage

To evaluate the shelf life of fermented camel milk beverage, it was filled in sterilised glass bottles

Table 4. Composition of camel milk and fermented camel milk beverage.

Parameter	Total Solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Chloride content (%)	Vitamin C (mg/100mL)
Camel milk	9.34±0.03	2.83b±0.04	2.39b±0.02	3.51±0.01	0.749± 0.005	0.201±0.002	1.31±0.04
Fermented camel milk beverage	10.43±0.04	2.88a±0.04	2.42a±0.03	3.02±0.02	0.873±0.004	0.248±0.006	0.46±0.01

Each observation is mean of three replications.

and stored at refrigeration temperature (7±1°C). Beverage was evaluated for pH, titratable acidity, lactic count, sensory evaluation and biofunctional attributes.

Effect of storage period on pH and titratable acidity

The changes in pH and titratable acidity (% LA) of fermented camel milk beverage during storage are shown in Table 5. pH was found decreased significantly ($p<0.05$) due to slow and continuous lactic acid production by starter cultures used in beverage. Fresh beverage had an average pH of 4.70 which decreased to 4.12 on 18th day of storage. Titratable acidity of the fresh beverage was 0.71 % LA which increased significantly ($p<0.05$) throughout the storage period and reached 1.25 % LA on 18th day of storage. Ibrahim and Khalifa (2015a) studied the changes in pH and titratable acidity of fermented camel milk yoghurt fortified with fibre. They found significant reduction in pH and significant increase in titratable acidity of all yoghurts during the storage of 21 days. El-Deeb *et al* (2017) studied the changes in pH and titratable acidity of fermented camel milk flavoured with cinnamon and doum palm extracts. They found significant decrease in pH and simultaneously significant increase in titratable acidity of all fermented camel milks during the storage of 21 days. However, increase in acidity of flavoured fermented camel milk was less than that in control (fermented camel milk).

Effect of storage period on Lactobacilli and Streptococcal count (Table 5)

Both counts increased significantly ($p<0.05$) initially up to 6 days and then significant decrease was found ($p<0.05$) throughout the storage period. Hassan *et al* (2007) reported an increase in the lactobacilli counts of Gariss during first 6 days of storage and a subsequent significant decrease in the counts throughout the storage period of 10 days.

Ibrahim and Khalifa (2015a) studied the starter culture count of fermented camel milk yoghurt fortified with orange fibre and date fibre individually. Rate of addition of fibre were 1.5, 3 and 4.5%. *Streptococcus thermophilus* counts were found to be increased significantly up to 7 days of storage and later decrease a significantly up to 21 days of storage was seen. However, increase in *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* counts were observed up to 14 days of storage, and later a decrease significantly up to 21 days. All the counts were found to be increased with increase in

Table 5. Changes in pH, titratable acidity and lactic count of fermented camel milk beverage during storage.

Storage period (in Days)	pH	Titratable acidity (% Lactic Acid)	Lactobacilli count (log CFU/mL)	Streptococcal count (log CFU/mL)
0	4.70^{a}±0.01	0.71^{g}±0.01	9.67^{cd}±0.10	9.51^{d}±0.11
3	4.59^{b}±0.02	0.83^{f}±0.02	10.11^{ab}±0.17	9.99^{b}±0.13
6	4.54^{c}±0.02	0.94^{e}±0.01	10.25^{a}±0.14	10.29^{a}±0.09
9	4.43^{d}±0.01	0.99^{d}±0.01	10.14^{ab}±0.11	10.03^{b}±0.11
12	4.33^{e}±0.01	1.05^{c}±0.02	9.87^{bc}±0.09	9.76^{c}±0.10
15	4.25^{f}±0.02	1.11^{b}±0.01	9.53^{d}±0.14	9.57^{cd}±0.10
18	4.12^{g}±0.01	1.25^{a}±0.01	9.15^{e}±0.12	9.18^{e}±0.07

Each observation is mean of three replications. Values with different superscripts in the columns differ significantly ($p<0.05$).

Table 6. Changes in the sensory attributes of fermented camel milk beverage during storage.

Storage period (Days)	Sensory scores (9-point Hedonic scale)				
	Flavour	Body and Texture	Acidity	Colour and appearance	Overall acceptability
0	8.25^{a}±0.10	8.50^{a}±0.11	8.40^{a}±0.05	8.50±0.12	8.34^{a}±0.07
3	7.96^{b}±0.07	8.38^{ab}±0.08	7.99^{b}±0.07	8.50±0.10	7.84^{b}±0.11
6	7.59^{c}±0.11	8.35^{ab}±0.07	7.54^{c}±0.07	8.46±0.08	7.58^{c}±0.11
9	7.25^{d}±0.08	8.34^{ab}±0.05	7.29^{d}±0.10	8.44±0.10	7.31^{d}±0.09
12	6.80^{e}±0.18	8.27^{bc}±0.09	6.74^{e}±0.17	8.37±0.06	7.04^{e}±0.13
15	6.53^{f}±0.11	8.24^{bc}±0.08	6.52^{f}±0.13	8.33±0.06	6.76^{f}±0.15
18	5.57^{g}±0.14	8.14^{c}±0.11	6.20^{g}±0.06	8.30±0.05	5.23^{g}±0.16

Each observation is mean of three replications. Values with different superscripts in the columns differ significantly ($p<0.05$).

rate of addition of date fibre and these were further higher in orange fibre. Ibrahim (2015) prepared camel milk yoghurt made with EPS producing and non-EPS producing starter cultures. *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were found to be increased up to 14 days of storage and later, the counts decreased up to 21 days of storage. All these studies supported our findings that culture count were found to be increased in initial storage days and later, these were found to be decreased till the end of storage study.

Effect of storage period on sensory attributes (Table 6)

Sensory scores of fermented camel milk beverage during shelf life decreased significantly ($p<0.05$) to 5.57, 8.14, 6.20 and 5.23 on 18th day of storage. However, the change in the colour and appearance score was not significant. Judges reported a slight bitter off-flavour which may be due to proteolytic activity. On the basis of sensory score, fermented camel milk beverage was rejected on 18th day of storage. The camel milk beverage had a shelf life of 15 days. Ibtisam and Marowa (2009) reported that Gariss (fermented camel milk) prepared from pasteurised milk had a shelf life of 17 days at 8°C. Ibrahim and Khalifa (2015b) reported sensory scores of camel milk yoghurt added with stabilisers decreased significantly during the 21 days of storage. EI-Deeb *et al* (2017) studied the sensory attributes of fermented camel milk flavoured with cinnamon and doum palm extract. Flavour, body and texture, acidity, appearance and total score of all fermented milk were found to be decreased significantly throughout the storage of 21 days.

Evaluation of the biofunctional attributes of fermented camel milk beverage (Table 7)

ACE inhibitory activity of fresh fermented camel milk beverage was 49.86% which increased significantly ($p<0.05$) to 59.56% on 18th day of storage. This increase in ACE inhibitory activity might be due to proteolytic activity leading to peptides possessing such activity. High proline content in camel milk is another reason responsible for higher ACE inhibitory activity in fermented camel milk (El-Salam and El-Shibiny, 2013).

The α-amylase inhibition activity of fermented camel milk beverage increased throughout storage period. Fresh beverage had inhibitory activity of 55.90% which increased to 58.69% on 18th day of storage. However, the inhibition activity of beverage on days 3, 6 and 9 were found at par with each other. α-glucosidase inhibition activity of fresh beverage was 35.96% which increased significantly ($p<0.05$) to 39.15% on 18th day of storage. α-glucosidase inhibition activity was highest on 9th day of storage and after that, change in the activity was not significant.

Fresh beverage had antioxidant activity of 21.87% which increased significantly ($p<0.05$) to 29.52% on 18th day of storage. Higher proteolytic activity resulted in productions of functional peptides which are responsible for high antioxidant activity. Further, presence of several amino acids in peptides can improve antioxidant properties (Aluko, 2012).

Ayyash *et al* (2018a) evaluated the biofunctional attributes of fermented camel milk and compared it with fermented bovine milk. *Lactococcus lactis* KX881782, one of the probiotics isolated from camel milk, was compared with probiotic strain *Lb. acidophilus* DSM9126. Camel milk fermented by *Lactococcus lactis* KX881782 had significantly higher ACE inhibitory activity than that in bovine milk throughout the storage. Further, the ACE inhibitory activity increased significantly throughout the storage. α-amylase inhibition activity of fermented camel milk was found to be increased significantly throughout the storage of 21 days while that of change in fermented bovine milk was found to be

Table 7. Changes in the biofunctional attributes of fermented camel milk beverage during storage.

Storage period (Days)	ACE inhibitory activity (%)	α-amylase inhibition activity (%)	α-Glucosidase inhibition activity (%)	Antioxidant activity (%)	Proteolytic activity (mg/mL)
0	$49.86^{g}\pm0.45$	$55.90^{f}\pm0.35$	$35.96^{d}\pm0.37$	$21.87^{g}\pm0.23$	$7.80^{f}\pm0.03$
3	$52.68^{f}\pm0.34$	$56.51^{e}\pm0.15$	$37.21^{c}\pm0.33$	$23.72^{f}\pm0.51$	$7.88^{e}\pm0.03$
6	$54.28^{e}\pm0.48$	$56.94^{de}\pm0.20$	$38.70^{b}\pm0.25$	$25.57^{e}\pm0.31$	$7.97^{d}\pm0.04$
9	$56.55^{d}\pm0.40$	$57.12^{d}\pm0.15$	$39.28^{a}\pm0.21$	$26.39^{d}\pm0.19$	$8.08^{c}\pm0.03$
12	$57.86^{c}\pm0.24$	$57.63^{c}\pm0.21$	$39.11^{ab}\pm0.11$	$27.45^{c}\pm0.24$	$8.15^{c}\pm0.02$
15	$58.69^{b}\pm0.40$	$58.23^{b}\pm0.12$	$38.88^{ab}\pm0.13$	$28.50^{b}\pm0.28$	$8.27^{b}\pm0.03$
18	$59.56^{a}\pm0.21$	$58.69^{a}\pm0.14$	$39.15^{ab}\pm0.28$	$29.52^{a}\pm0.25$	$8.35^{a}\pm0.03$

Each observation is mean of three replications. Values with different superscripts in the column differ significantly ($p<0.05$).

nonsignificant. Camel milk fermented by *Lactococcus lactis* KX881782 had shown higher α-amylase inhibition activity than that in bovine milk. However, they could not observe any significant changes in the α-glucosidase inhibition activity during storage. Proteolytic activity was found to be increased significantly with the increase in storage period. Camel milk fermented by *Lactococcus lactis* KX881782 had higher antioxidant activity than that in camel milk fermented by *Lb. acidophilus* DSM9126. However, change in antioxidant activity throughout the storage was found to be nonsignificant except in bovine milk fermented by *Lb. acidophilus* DSM9126, in which it was found to be increased during storage. Ayyash *et al* (2018b) compared the biofunctinal attributes of camel milk and bovine milk both of which were fermented by probiotic strains. Probiotic strains used in the study were *Lb. plantarum* DSM2648, *Lb. reuteri* KX881777, Lb. *plantarum* KX881772 and *Lb. plantarum* KX881779. They observed significantly higher ACE inhibitory activity in fermented camel milk than that in fermented bovine milk except in case of *Lb. plantarum* DSM2648, in which fermented bovine milk possessed significantly higher ACE inhibitory activity than that in camel milk. They also observed that ACE inhibitory activity of all fermented milk increased significantly during the storage of 21 days. All fermented milks were reported to have α-amylase inhibition of more than 34%, except camel milk fermented by *Lb. plantarum* KX881772. Further, camel milk fermented by *Lb. plantarum* KX881772 was reported to have significantly lower α-amylase inhibition activity than that in bovine milk fermented by same strain. Except bovine milk fermented by *Lb. plantarum* KX881779, α-amylase inhibition activity of all were found to be increased significantly throughout the storage of 21 days. They observed that α-glucosidase inhibition activity and proteolytic activity was increased significantly during the storage of 21 days. Antioxidant activity of camel milk fermented by all strains were reported to be significantly higher than that in fermented bovine milk. Further, antioxidant activity was reported to increased significantly throughout the storage of 21 days. Camel milk fermented by *Lb. reuteri* KX881777 and *Lb. plantarum* KX881779 shown higher antioxidant activity than camel milk fermented by *Lb. plantarum* DSM2648 and *Lb. plantarum* KX881772. Lafta *et al* (2014) evaluated the antimicrobial activity of fermented camel milk. Starter cultures used in the study were *Lb. delbrueckii subsp. bulgaricus* and *St. thermophilus*, individually and mixed culture. Zone of inhibition (mm) of camel milk fermented by *Lb. delbrueckii subsp. bulgaricus* against *P. aeruginosa*, *E. coli* and *S. aureus* were 30.1, 29.8 and 23.5 mm, respectively while that of camel milk fermented by *St. thermophilus* against the same were 27.2, 25.4 and 22.1 mm, respectively. Zone of inhibition of camel milk fermented by mixed culture against *P. aeruginosa, E. coli* and *S. aureus* were 32.4, 30.2 and 25.5 mm, respectively.

Conclusion

Type of starter culture has a significant impact on the quality attributes of fermented camel milk beverage, hence, proper selection of starter culture is an important step. As per our results, *Streptococcus thermophilus* MTCC 5460 + *Lactiplantibacillus plantarum* M11 can be successfully employed for preparation of fermented camel milk beverage. Sensory attributes of fermented camel milk beverage can be further improved by addition of cumin and black salt. The developed beverage had a shelf life of 15 days at refrigeration temperature. The beverage had promising biofunctional attributes. The study concluded that camel milk can be successfully processed to fermented camel milk beverage having acceptable sensory attributes as well as enhanced biofunctional activities.

Conflict of interest

The authors declare no competing interests.

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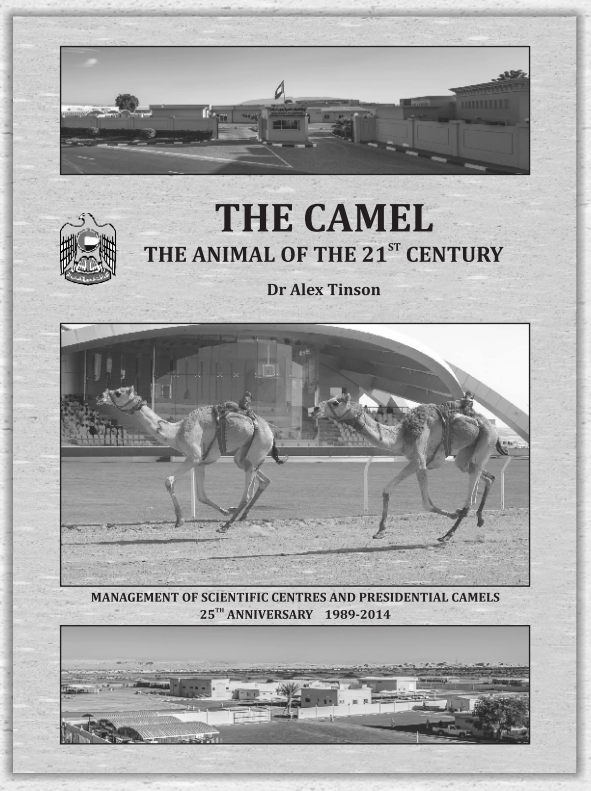
THE CAMEL

THE ANIMAL OF THE 21ST CENTURY

This book authored by Dr Alex Tinson is an acknowledgement to the support and inspiration that His Highness Sheikh Khalifa Bin Zayed Al Nahyan has provided to the centre and to research in general. The last 25 years has been an incredible adventure for us, the noble camel and the people of the U.A.E. Dr Tinson has been involved with many world first's since moving to Abu Dhabi 25 yrs ago. First there was the establishment of pioneering centres in exercise physiology and assisted reproduction. The establishment of the Hilli Embryo Transfer Centre led to five world firsts in reproduction. The world's first successful embryo transfer calf birth in 1990, followed by frozen embryo transfer births in 1994, twin split calves in 1999, pre-sexed embryo births in 2001 and world's first calf born from A.I. of frozen semen in 2013. The hard bound book is spread in 288 pages with 5 chapters. The first chapter involves early history of the centre, world's firsts, world press releases, history of domestication and distribution, evolution of camel racing in the U.A.E. and historical photos the early days. Second chapter comprises camel in health and disease and it involves cardiovascular, haemopoetic, digestive, musculoskeletal, reproductive, respiratory, urinary and nervous systems in addition to the description of special senses. This chapter describes infectious, parasitic and skin diseases in addition to the nutrition. The third chapter is based on Examination and Differential Diagnosis. The fourth chapter is based on special technologies bearing description of anaesthesia and pain management in camels, diagnostic ultrasound and X-Ray, assisted reproduction in camels, drug and DNA testing and surgery. The last chapter entailed future scope of current research.



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SURGICAL MANAGEMENT OF MANDIBULAR FRACTURES IN CAMELS USING MODIFIED IDW (IDW AND TRANSFIXATION OF PINS WITH FIBRE CAST) TECHNIQUE

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ABSTRACT

In the present study, the clinical and radiological assessment of mandibular fractures in 6 male camels (*Camelus dromedarius*) of 3-14 years age was carried out. The fractures observed were bilateral and compound (83.33%) and bilateral and closed (16.66%) in nature. However, the fracture site at horizontal ramus recorded was anterior to tushes (33.33%) and across the alveoli (66.66%). Mandibular fracture cases were treated by Modified Interdental Wiring (IDW and Transfixation of Pins with Fibre Cast) technique. The animals were sedated with xylazine @ 0.4 mg/kg BW. Clinical and radiographical fracture union was observed within 06.83±0.31 weeks in the animals. During fracture healing complications of pin migration (33.33%), fibre cast bite wound (33.33%), development of sub mandibular abscess (16.66%) and mucosal wire gall wound (100%) were observed. Osteomyelitis was not observed as a complication after surgical repair of mandibular fracture.

Key words: Camel, fibre cast, interdental wiring, mandibular fracture, transfixation of pins

Mandibular fractures are the most common type of fractures in camels which often occur during breeding (rut) season in males following fighting with each other (Ahmed, 2011; Rastabi *et al*, 2017; Singh *et al,* 2020), wherein the animals become active, vicious and tend to bite each other leading to abnormal stress on the mandible leading to fracture (Gahlot, 2000). Mandibular fracture occurs commonly in male camels because of powerful forces generated by the jaw muscles action on the weakest part of mandible. Long inter-dental space, presence of alveoli of first premolar or tushes and presence of mental canal make the lower jaw weak and fracture occurs at this point (Gahlot, 2010). The affected animals are unable to prehense due to ventral deviation of the mandible and lower lips owing to fracture (Rastabi *et al,* 2017). Different surgical techniques have been used for fixation of mandibular fractures in camels including interdental wiring, U-bar application, combination of cross pin fixation, and tension band wiring and bone plating (Rastabi *et al,* 2017). The success of fracture repair depends mainly on the use of suitable methods of immobilisation (Al-Sobayil *et al*, 2020). Interdental wiring technique has been reported by Gahlot *et al* (1989) and Bhabbhor *et al* (2020) to repair mandibular fractures in camels.

The present study is based upon evaluation of modified interdenatal wiring technique to repair mandibular fractures in dromedary camels.

Materials and Methods

Six adult male camels aged between 3-14 years having mandibular fracture were subjected to immobilisation at Surgery section of Veterinary Clinical Complex of College of Veterinary and Animal Science, RAJUVAS, Bikaner. These animals were not able to prehense food as both the lips were set apart due to the hanging of fractured rostral fragment of the mandible. The clinical examination was carried out by a careful per-oral examination of lower jaw. The separation of both the lips, drooling of saliva and gingiva were noted for its discontinuity and wounds. The location of fracture was ascertained in terms of anterior, posterior or across the alveoli of tushes. The nature of fracture was also noted in terms of whether, transverse, oblique or multiple. Lateral radiographs of horizontal ramus of camels were analysed for confirmation of type of fracture (Fig 2). Pre-operative

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antibiotics (Oxytetracycline @5mg/kg IV OD) and non-steroidal anti-inflammatory agent (NSAID) (Meloxicam @0.5mg/kg IV OD) were administered in all the animals. Animals were secured in sternal recumbency following sedation with Xylazine @ 0.4 mg/kg BW, intravenously. Oral cavity was irrigated with light solution of potassium permanganate and the fracture was immobilised by modified Interdental Wiring (IDW and Transfixation of Pins with Fibre Cast) technique (Fig 3). Interdental wiring was performed as per the technique (IDW) of Gahlot *et al* (1984). The copper wire (2 mm diameter) was passed bilaterally and both the ends were knotted separately. Excessive length of wires was trimmed with a wire cutter and knot ends were twisted up towards the incisor teeth. Two intramedullary pins (3.5mm) were inserted transversely into the horizontal ramus at caudal part latero-medially and two pins were inserted into rostral fractured part ventro-dorsally. Adequate cotton padding was applied between the all pins then a fibre cast bandage was applied. The fibre cast bandage (5inch x 3.5metre) was dipped into water for half a minute and then it was applied across the all four pins in a square fashion. Both the fractured ends of mandible were kept in perfect apposition at the time of fibre cast bandage application. The cast was allowed to dry for 2-3 minutes.

Post-operative Care

All animals were kept under clinical observation for at least 10 days and postoperatively, broad spectrum antibiotic (Oxytetracycline @5mg/Kg IV OD) and NSAID (Meloxicam @0.5mg/kg BW IV OD) and B-complex IM OD, intramuscularly were administered for 7 days, 5 days and 5 days, respectively The oral cavity was flushed daily with light potassium permanganate solution for 10- 15 days. All the animals started prehensing soon after application of IDW and transfixation of pins with fibre cast. Animals were offered soft leaves fodder, devoid of straws for two weeks and thereafter routine roughage was allowed. A submandibular abscess at the fracture site on ventral aspect developed in one case, 10-15 days after fracture. It was drained by incising at the depending part and was dressed routinely. The owner was advised to keep the animal confined alone in a separate enclosure for a period of at least six-eight weeks. On follow-up further, post-operatively radiographs were taken for evaluation of fracture healing at a different time interval, i.e. 20 or 30 days, wherever feasible (Fig 4). The wires and pins were removed following satisfactory clinical and/or radiological union.

Results and Discussion

In animals of present study this technique proved efficacious in transverse mandibular fractures. In cases of present study, tightening of wires at knots was not required. However, clinical and radiological union took place in 6-8 weeks in the present study (Fig 6). These points to the fact that transfixation of pins together with fibre cast provided most adequate immobilisation. The transverse and oblique fractures were managed more efficiently with the technique (IDW and Transfixation of pins with fibre cast). In a previous study IDW alone led to overriding and shortening of jaw (Gahlot *et al,* 1989).

Bilateral mandibular fractures were observed in all camels of present study. These findings confirmed with the observations reported by Gahlot (2000), Purohit *et al* (2019) and Singh *et al* (2020). In the present study, the site of mandibular fracture was either anterior to tushes or across the alveoli. Similar findings were also recorded by Gahlot *et al* (1989) who found factures either anterior to tushes or posterior to tushes or across the alveoli in 50 camels. Rastabi *et al* (2017) and Saharan *et al* (2018) also recorded the site of mandibular fracture which was posterior to tushes. In the present study, all the cases of mandibular fracture were repaired by modified IDW (Interdental Wiring and Transfixation of pins with fibre cast) technique. In previous studies Gahlot (2000), Saharan *et al* (2018) and Singh *et al* (2020) used IDW technique. The development of a sub-mandibular abscess in open fracture was a common sequel during the healing period (Gahlot *et al*, 1984) as noted in one case of present study. However, a space between fibre cast and sub-mandibular area allowed easy drainage of sub mandibular abscess. The gingival wounds and buccal mucosa wire gall wounds occurred due to bite of wires or knots of interdental wiring in animals of present study which usually healed within one week after removal of the wire due to rich blood supply of oral mucosa. However, occurrence of such wounds was also reported previously by Ram (1997); Ahmed (2011) and Rastabi *et al* (2017). External callus formation at the fracture ends at various time intervals was noted. In the present study, none of the cases recorded osteomyelitis during the healing period. Similarly Ramzan (2008) reported a good surgical reduction, complications were rare and osteomyelitis seemed not to be a common sequel. Ahmed (2011) and Ahmed and Al-Sobayil (2012) recorded osteomyelitis as a potential complication in their studies. The migration of pin in one animal of present study could have been a result of over drilling of intra medullary

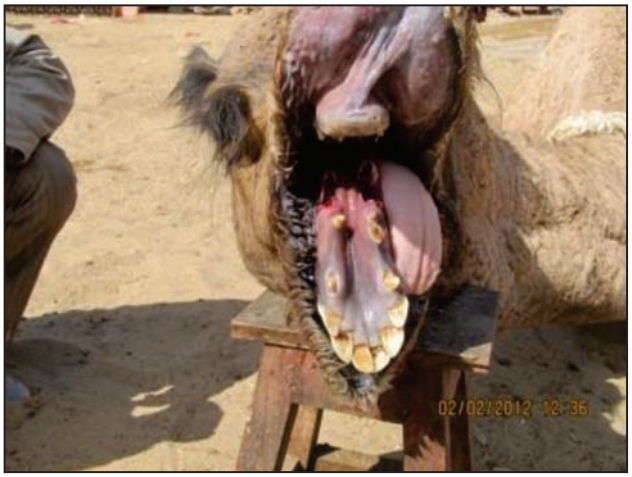

Fig 1. Mandible showing a bilateral compound transverse fracture of horizontal ramus.

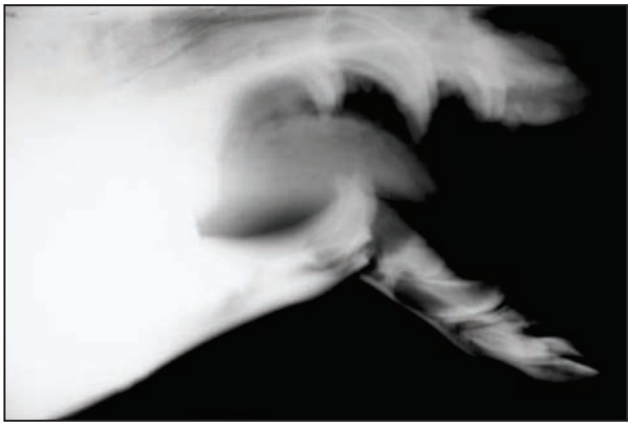

Fig 2. Lateral radiograph of mandible showing transverse fracture of horizontal ramus anterior to tushes.

Fig 3. Fracture was immobilised by modified interdental wiring (IDW and Transfixation of Pins with Fibre Cast) technique.

pin crossing both the cortexes of horizontal ramus while drilling. The loosening of pin as seen in two cases of present study was due to presence of local infection at the site of embedding of pins in rostral

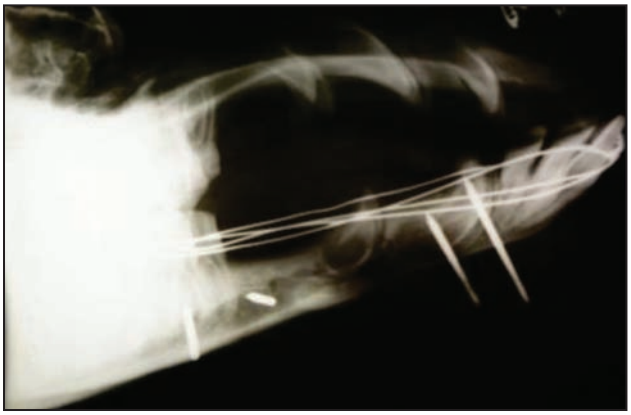

Fig 4. Lateral radiograph of mandible showing adequate reduction and immobilisation of fracture fragments repaired by IDW and transfixation of pins with fibre cast.

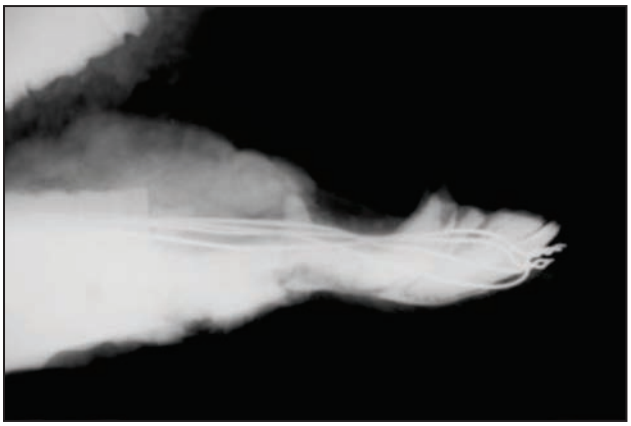

Fig 5. Lateral radiograph showing radiological union of mandibular fracture after removal of Transfixed pins with fibre cast at 7th week.

fractured fragments. However, the fibre cast remained in position. Eventhough, the fibre cast is applied after a careful padding with cotton wool, sometimes it is displaced and leads to bite wounds on the skin due to friction. However, such wounds do not cause a serious complication.

Acknowledgement

An authors are grateful to the Dean, College of Veterinary and Animal Sciences, Bikaner for providing adequate facilities for surgical treatment of camels.

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CONCURRENT INFECTION OF DERMATOPHILOSIS AND MANGE IN A CAMEL-A CASE REPORT

Tresamol P.V.[1], Suchithra S.[2], Mery Rincy K.[3], Prasanth S.[4] and Vinod Kumar K.[5]

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ABSTRACT

The present paper reports a case of mixed infection of sarcoptic mange and dermatophilosis in a camel reared in a farm. Animal had generalised skin lesions with alopecia and scab formation with matting of hairs especially on the rump region, legs and perineum. Microscopy of skin scrapings revealed presence of sarcoptic mites. Giemsa-stained scab smears revealed parallel rows of cocci arranged in typical tram track appearance suggestive of *Dermatophilus congolensis*. Cultural examination of skin scabs yielded haemolytic, greyish adherent colonies in sheep blood agar in presence of 10 per cent carbon dioxide, which was confirmed by morphological and biochemical characteristics. Molecular confirmation of the isolate was done using species specific PCR. The animal was treated successfully with two doses of long acting oxytetracycline @ 20mg/kg body weight at 3 days apart along with weekly injections of ivermectin @ 200µg/kg body weight and topical application of povidone iodine for four weeks.

Key words: Camels, *Dermatophilus congolensis*, sarcoptic mange, therapy

Dermatophilosis has a worldwide distribution and reported most frequently in tropical and subtropical countries with high ambient temperature and torrential rain patterns. First report of natural *D. congolensis* infection in camels was among camel calves reared on a commercial farm in a semi-arid area in Kenya (Gitao *et al*,1990). Khodakaram-Tafti *et al* (2012) reported 13.6 percent of prevalence of dermatophilosis among camels in Iran. Hughes and Anderson (2020) reported *D. congolensis* as a pathogen of zoonotic significance in camels. Bodinga and Shehu (2019) reported occurrence of dermatophilosis among 16 percent of camels slaughtered in an abattoir in Sokoto.

Gitao *et al* (1998a) reported an outbreak of dermatophilosis among camels from the Butana region of Eastern Sudan where camel calves were more likely to be infected (34%) than adults (8.9%), and lesions were more severe and involved most parts of the body. Mixed infection of *D. congolensis* and M. gypseum in camels reared on a dairy farm in Saudi Arabia was reported by Gitao *et al* (1998b). Gitao (1993) used enzyme linked immunosorbent assay (ELISA) to determine the epidemiological prevalence of *D. congolensis* infection in camels reared in a pastoral area of Kenya. Molecular diagnosis by using polymerase chain reaction (PCR) was described as a highly specific and sensitive test in comparison with the widely used conventional microbiological methods (Shaibu *et al*, 2010; Tresamol and Saseendranath, 2015).

Sarcoptic mange is a highly contagious zoonotic skin disease and in camels it is caused by *Sarcoptes scabiei* var *cameli* (Singh and Veer, 2005). The present paper reports a case of mixed infection of sarcoptic mange and dermatophilosis in a camel and it's successful therapeutic management.

Materials and Methods

A camel reared at a farm in Thrissur district was presented to University Veterinary Hospital, Mannuthy with generalised skin lesions which included alopecia and scab formation (Fig 1). It was previously treated for mange infestation using ivermectin and topical application of scabimide lotion without much improvement.

Skin scabs were collected aseptically from the lesions and were subjected to direct microscopy and bacterial and fungal culture. Smears were stained with Gram's and Giemsa stain. The scab materials were inoculated on sheep blood agar in the presence of 10 per cent carbon dioxide and incubated at 37°C in incubator. The isolates were further confirmed by the macroscopic and microscopic morphology of

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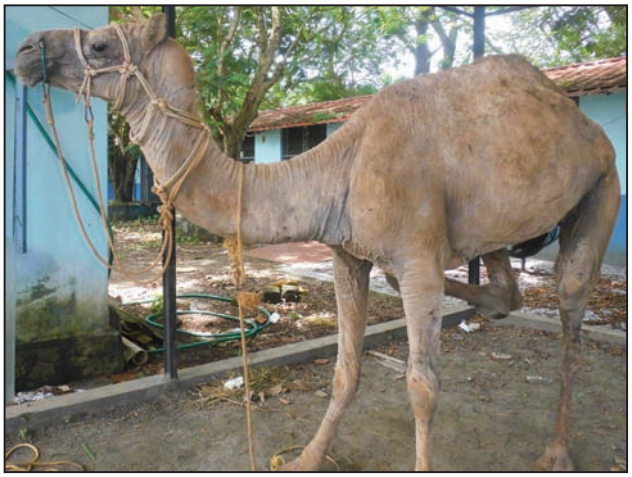

Fig 1. Camel with generalised skin lesions with alopecia and scabs.

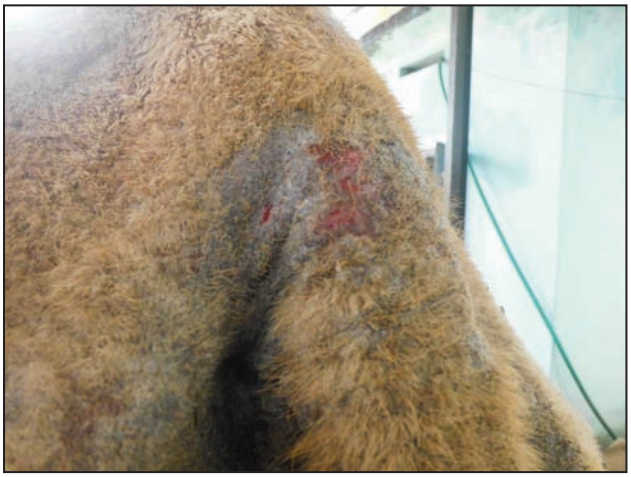

Fig 2. Thick scabs with matting of hairs on legs.

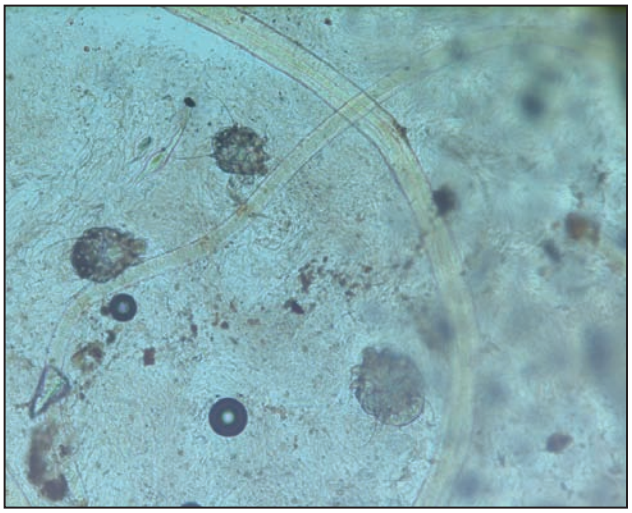

Fig 3. Sarcoptes mites in skin scrapings.

the colonies and biochemical tests.The PCR assay was carried out using the primers targeting the 16S rRNA gene of *D. congolensis* as described by Shaibu *et al* (2010). Skin scrapings (collected in 10% potassium hydroxide) were subjected to microscopical examination for detection of fungal spores or mites.

Results and Discussion

Clinical examination revealed thick scabs with matting of hairs especially on the rump region, legs and perineum (Fig 2). Osman (2014) reported lesions in the form of exudative dermatitis, thick greasy scabs and long hairs collected to form paint brush appearance in clinically affected camels. Removal of these hairs in the early stage of the disease revealed severe pain leaving bleeding area beneath it. Microscopy of skin scrapings revealed sarcoptes mites (Fig 3). Giemsa-stained and Gram's-stained scab smears revealed parallel rows of cocci arranged in typical tram track appearance

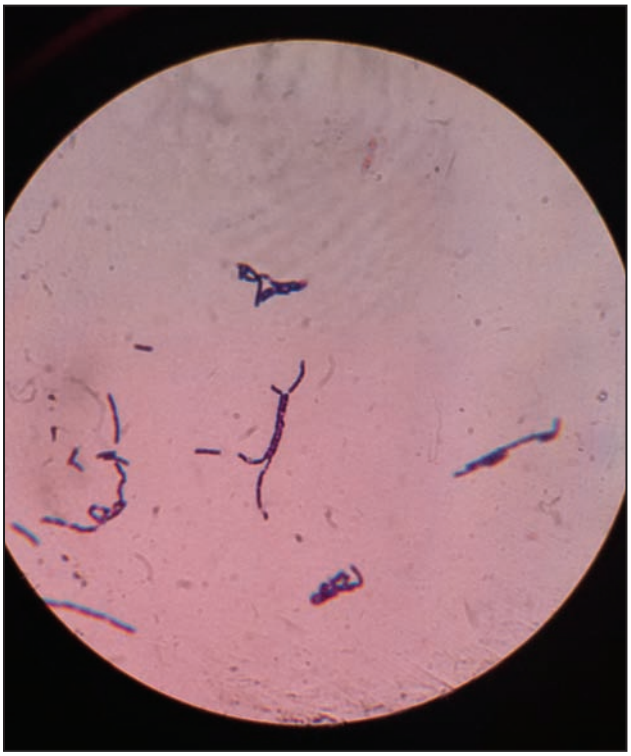

Fig 4. Gram's-stained scab smears with parallel rows of cocci suggestive of *D. congolensis*.

suggestive of *Dermatophilus congolensis*. Cultural examination of skin scabs yielded haemolytic, greyish adherent colonies in sheep blood agar in presence of 10 per cent carbon dioxide, which was confirmed by morphological and biochemical characteristics. Microscopical appearance of organisms in Gram-stained smears from colonies were also highly variable with Gram-positive branching filaments in different stages of segmentation, packets of coccoid forms, germinating spores or combinations of the above forms depending on the age of the culture and strain of the isolate (Tresamol *et al*, 2015a,b).

Fig 5. Haemolytic, greyish adherent colonies of *D. congolensis* in sheep blood agar.

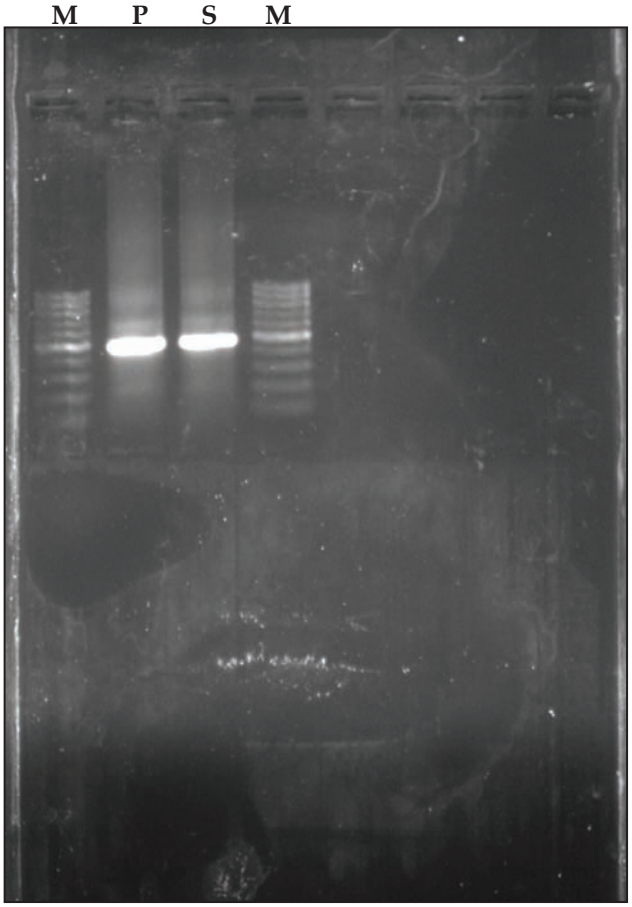

Fig 7. PCR amplification products of 500bp of *D. congolensis*.
M - Molecular marker 100 bp
P - Positive control - *D. congolensis* from cattle
S - Test sample from camel

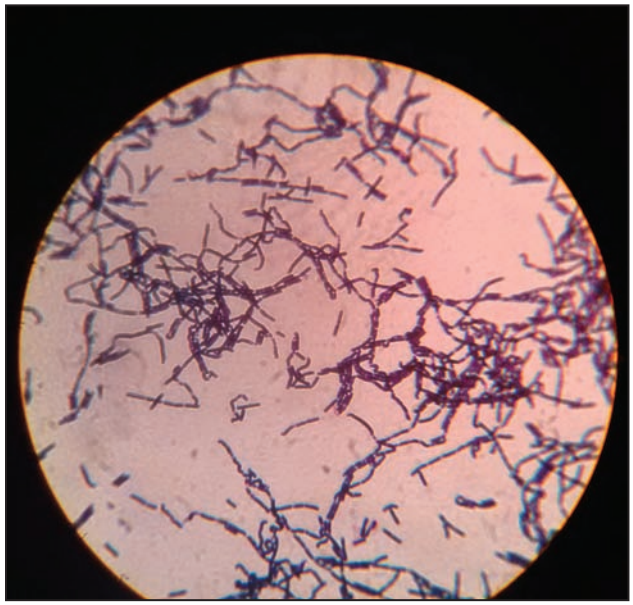

Fig 6. Gram-positive branching filaments of *D. congolensis* in different stages of segmentation agar.

Molecular confirmation of the isolate through PCR was done using primers targeting the 16S rRNA, and were found positive for *D. congolensis* amplifying a 500bp product (Fig 7). PCR has been adjudged as an effective tool for the definitive identification of *D. congolensis* in cattle, sheep and goats (Samon *et al,* 2010). The primers used in present study was found to be highly specific in detecting *D. congolensis* isolates from cattle, sheep, and goat (Tresamol and Saseendranath, 2015; Oladunni *et al,* 2016). Microscopical examination of skin scrapings revealed sarcoptes mites, however, fungal isolates could not be obtained by fungal culture. The concurrent infections might have resulted in severe lesions in the present case. Concurrent infections of dermatophilosis with other diseases such as camel pox (Abd, 2018), caseous lymphadenitis (Tarazi and Al Ani, 2016) and dermatophytes (Gitao *et al,* 1998b) were also reported previously in camels.

The camel was treated successfully with two doses of long acting oxytetracycline @ 20mg/kg BW at 3 days apart along with weekly injections of ivermectin @ 200mg/kg BW and topical application of povidone iodine for four weeks. Tarazi and Al-Ani (2016) reported successful treatment of affected camels using long-acting oxytetracycline injection in a dose rate of 10 mg/kg body weight every 48 hours for three successive treatments, and local antiseptic and antibiotic cutaneous spray treatment for five successive days. Osman (2014) also reported efficacy of long-acting tetracycline in treatment of dermatophilosis in camels. Branford *et al* (2021)

carried out the first detailed genomic study on *D. congolensis*, including observation of a tetracycline resistance-conferring gene tet (Z). Treatment with ivermectin was reported to be effective on sarcoptic mange infestation, and was found beneficial on clinical and body condition scores in camels (Feyera *et al*, 2015).

Prolonged wetting of the skin by daily bathing as reported by the owner might be one of the predisposing factor for dermatophilosis (Aliye *et al*, 2020). Vitamin deficiency and tick infestation were also reported to cause severe skin lesion of camel dermatophilosis (Osman, 2014; Gitao, 1993). Managemental factors such as avoidance of trauma and persistent moisture and control of ticks are important in preventing the recurrence of the condition.

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IMAGING STUDIES OF CADAVER MANDIBLE OF CAMEL AND ITS CORRELATION WITH MANDIBULAR FRACTURE

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ABSTRACT

The imaging study was conducted on 3 cadaver mandible of male camel. CT scan and computed radiography were performed for detailed morphological study of horizontal ramus of mandible in order to correlate with common fracture sites. The anatomical details of horizontal ramus of mandible viewed in both of the diagnostic imaging techniques correlated with findings of twelve mandibular fracture cases in camels and proved the weakest site was found in the vicinity of the alveolus of 1st premolar at horizontal ramus of mandible.

Key words: Cadaver mandible, camel, computed radiography, computed tomography, mandibular fracture

Mandibular fracture is the most common serious problem in camels which is higher in males than female camels (Al-Mujalli, 2012). It occurs commonly during rut season (Siddiqui *et al,* 2012; Parashar, 2013) and seen at cranial or caudal, or across the 1st premolar (Gahlot, 2000). Computed Tomography (CT) is an excellent diagnostic tool to study the detailed anatomy of horizontal ramus of mandible which is useful to evaluate the weakest point at mandible in camels (Parashar, 2013; Tucker and Farrell, 2001). Camel has a different anatomy of mandible hence imaging technique enables studying the surgical anatomy of mandible that would help identifying more susceptible region in mandible which predisposes lower jaw to fracture and help developing diverse immobilisation techniques for repair these fractures. It is imperative to augment the skill and knowledge for improvement of external fixation techniques with the help of advance diagnostic imaging to achieve rapid and effective outcome in mandibular fracture management. Present study was therefore done to evaluate the complex anatomy of the mandible by CT scan and Computed Radiographic imaging techniques in camels.

Materials and Methods

The computed radiography of three cadaver mandible of adult male dromedary camel was performed using factor 60kVp and 10mAs. A positive contrast radiograph using barium sulphate @ MICROBAR was taken by introducing the contrast material into the mandibular foramen to detail out the mandibular canal. The computed tomography of three cadaver heads was done using factor 100kVp and 120mAs (HITACHI) (Fig 1). Both imaging techniques were performed for detailed morphological study of horizontal ramus of mandible and to correlate with common fracture sites.

The common fracture sites were examined by clinical and radiological examination of 12 adult male camels (*Camelus dromedarius*) with mandibular fractures which were presented in Veterinary Clinical Complex of the College of Veterinary and Animal Science, Bikaner.

Results

Computed Radiography

Computed radiography (CR) of cadaver mandible was performed in both lateral and dorso-ventral views. In lateral view of mandible, the height of horizontal ramus was more at caudal ramus (10.43±0.21 cm) than rostral ramus (3.26±0.16 cm). The average length of diastema measured was 15.80±0.15 cm. However, it was further divided into two regions, i.e. rostral to the 1st premolar (rostral interdental space) (5.47±0.14 cm) and caudal to the 2nd premolar (caudal interdental space) (9.93±0.60 cm) (Fig 2). The mandible of camel was composed of two horizontal ramus which were fused rostrally

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Fig 1. Obtaining CT scan of camel head.

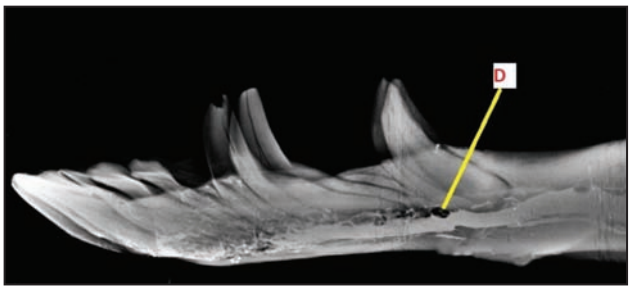

Fig 3. Lateral positive contrast radiograph showing opening of mandibular foramen into mental canal rostrally (D).

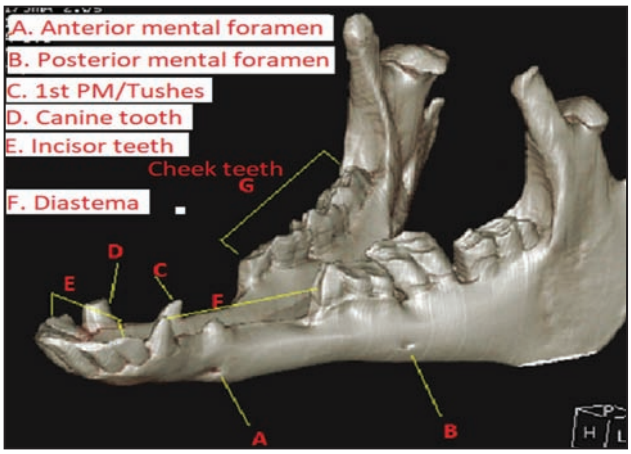

Fig 5. Three dimensional reconstructed CT image of cadaver mandible showing mental foramen, 1st premolar, canine tooth, incisor tooth and diastema.

(mandibular symphysis) midway between the roots of 1st premolar.

There was distinct presence of 1 pair of canines, 3 pair of incisors, i.e. central, intermediate and laterals

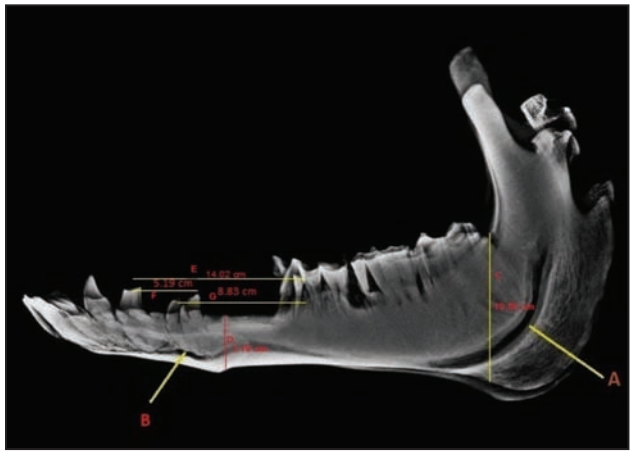

Fig 2. Lateral radiograph showing biometry of cadaver mandible, showing mandibular canal (A), mental canal (B), caudal horizontal ramus (C), rostral horizontal ramus (D), interdental space/ diastema (E), rostral interdental space (F) and caudal interdental space (G).

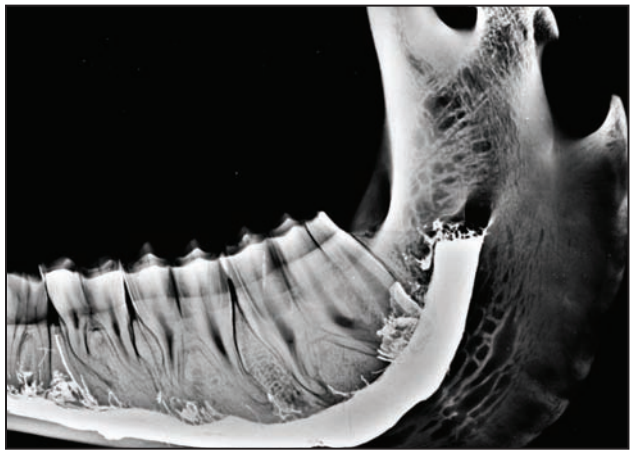

Fig 4. Positive contrast radiograph showing opening of mandibular foramen at vertical ramus of mandible.

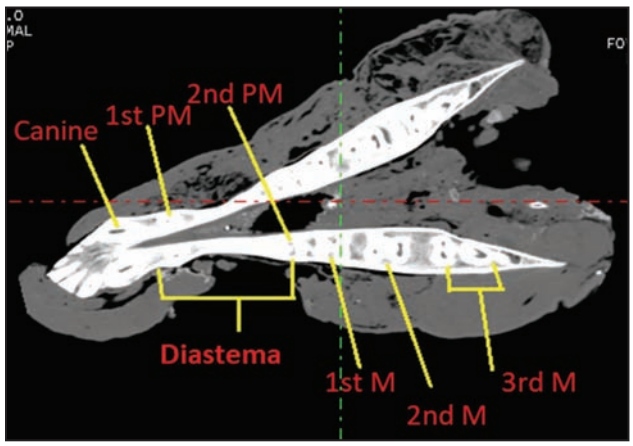

Fig 6. Multiplanar CT images in dorsal plane demonstrated arrangement of teeth in horizontal ramus with diastema.

and 1 pair of premolar at diastema. These are also known as tushes or wolf tooth. At the end of the diastema the horizontal ramus had 1 pair of 2nd

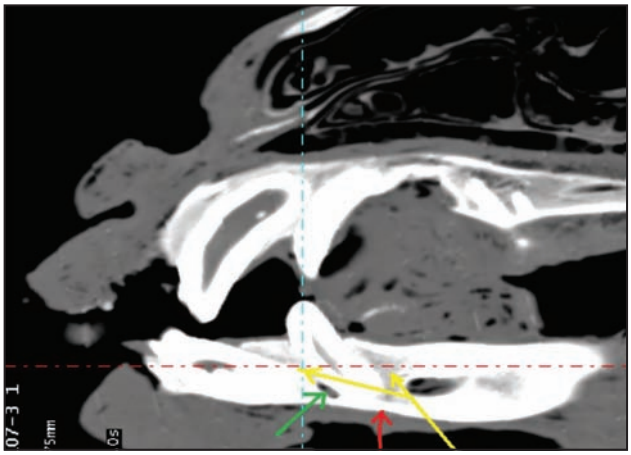

Fig 7. Sagittal plane of CT showing hollowness at either side of alveoli (yellow arrow), opening of anterior mental foramen (green arrow) and less osteogenic thickness (red arrow).

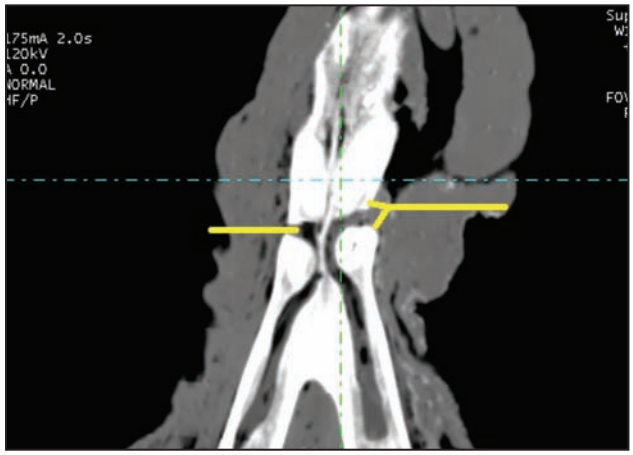

Fig 8. Dorsal plane of CT showing hollow passage of mandibular and mental canal communicating particularly at cranial to the alveolus of 1st premolar tooth.

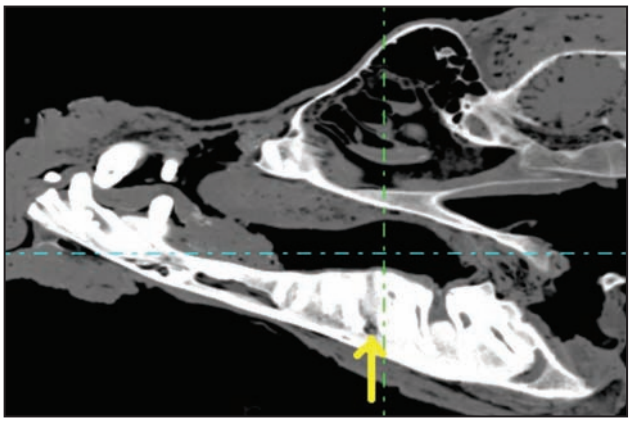

Fig 9. CT image of dorsal plane showing posterior mental foramen situated just caudal to the alveoli of 1st molar tooth.

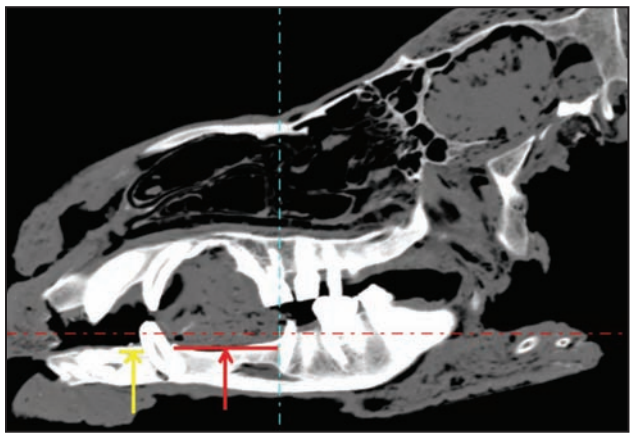

Fig 10. Sagittal plane of CT showing short cranial interdental space (yellow arrow) than caudal interdental space (red arrow).

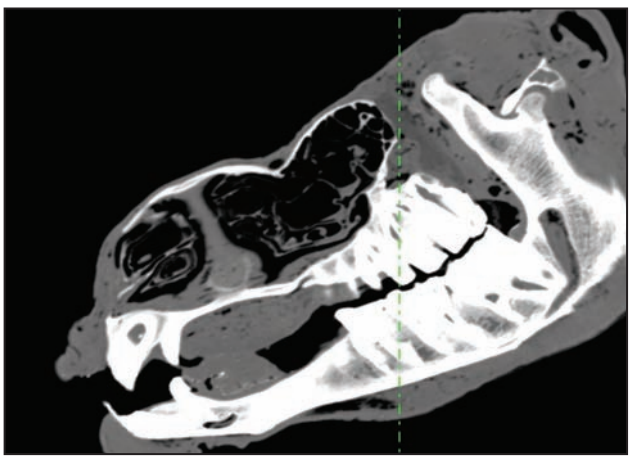

Fig 11. CT image of sagittal plane showing gradually increased width of horizontal ramus caudally merged into vertical ramus.

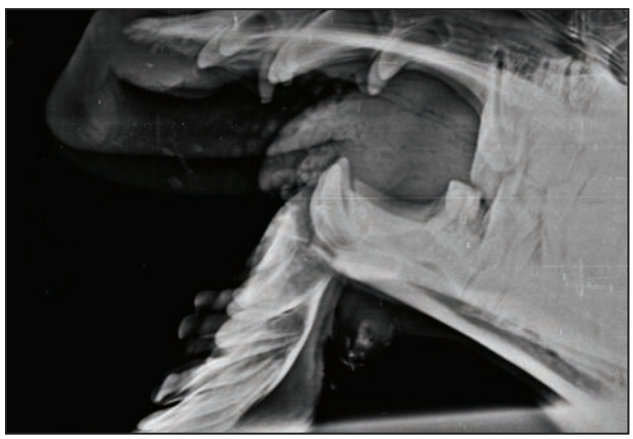

Fig 12. Lateral radiograph of mandible showing transverse fracture of horizontal ramus anterior to tushes

premolars and 3 pairs of molars. The posterior mental foramina was located between roots of 1st molar & 2nd molar and anterior mental foramina was located just posterior to the roots of 1st premolar.

The boundary of horizontal ramus remained up to roots of 3rd molar and thickness of ramus was more

Fig 13. Lateral radiograph showing oblique fracture of horizontal ramus of mandible.

till here. The vertical ramus began just posterior to the roots of last molar and it lost its thickness and became a flat bone here containing mandibular foramina on medial aspect and mandibular condyles on dorsal aspect. There was evidence of medullary canal in horizontal and vertical ramus. However, it appeared wider in the vertical canal and became narrower progressively in horizontal canal towards the incisors.

The contrast radiograph revealed a long mandibular canal travelling through vertical ramus to horizontal ramus and was in continuation to the mental canal. It became narrower progressively towards the incisors (Figs 3 & 4).

Computed Tomography

Three dimensional (3-D) reconstructed images of cadaver mandible showed 3 pairs of molar teeth, 2 pairs of premolar, 1 pair of canine and 3 pairs of incisor teeth (Fig 5). The mandible had long interdental space which was divided in two parts by the presence of alveoli of 1st premolar (Fig 6). A distinct hollowness was evident at the alveolus of 1st premolar. A distinct bulge of alveoli of 1st premolar was evident with presence of anterior mental foramina just below to it. The osteogenic thickness of horizontal ramus ventral to the alveolus of 1st premolar was comparatively less (Fig 7). A distinct hollow passage of mandibular and mental canal of both the horizontal rami communicated rostrally cranial to the alveolus of 1st premolar (Fig 8). The posterior mental foramen was distinct at horizontal ramus just caudal to the alveolar of the 1st molar tooth (Fig 9). The cranial interdental space was shorter than the caudal interdental space. The horizontal ramus evidenced thickness at the interdental space which gradually increased towards caudal interdental space (Fig 10). The horizontal ramus gradually increased in width until the alveolus of last molar tooth and then it got merged with vertical ramus of mandible which was almost like a flat bone having a mandibular foramen on medial aspect (Fig 11).

Study of Mandibular fracture

Clinical and radiographical examination of fracture sites of mandible was done in twelve cases. Among the cases of mandibular fracture, most common fracture site was anterior to 1st premolar/ tushes (n=6, 50%) (Fig 12) followed by between premolars (n=5, 41.67%) (Fig 13) and fracture site was different on both sides, i.e. between premolars on right side and between 2nd premolar and 1st molar tooth on left side (n=1, 8.33%). Out of 12 cases of mandibular fracture, the oblique fracture (open/close) was observed in 2 (16.67%) cases and transverse fracture (open/close) in 10 (83.33%). Eleven (91.67%) fractures were bilateral and one (8.33%) was unilateral.

Discussion

Computed tomography is considered the best valuable imaging options for the assessment of outlining the details of camel bone structures (Emam *et al,* 2020). The narrowest part of the horizontal ramus was at the interdental space and at the same site there was distinct presence of alveoli of 1st premolar, which predisposed the site to the fracture than other parts, which are comparatively wider. The presence of the mental canal and alveoli of the 1st premolar render this site of horizontal ramus quite weak and become prone to fracture (Siddiqui *et al,* 2012). In sagittal reconstructed images predicted by CT scan, the comparative bone thickness at rostral ramus was less than caudal ramus. Gahlot and Chouhan (1994) also reported that mandible of camel was anatomically weak at horizontal ramus due to presence of alveoli of 1st premolar and mental canal in the region.

CT scan and contrast radiography of mandible explained the intricacies of anatomy of mandible which helped in understanding the weakest site of mandible, i.e. close or across the alveoli of 1st premolar, at which the incidence of mandible fracture was highest in camel.

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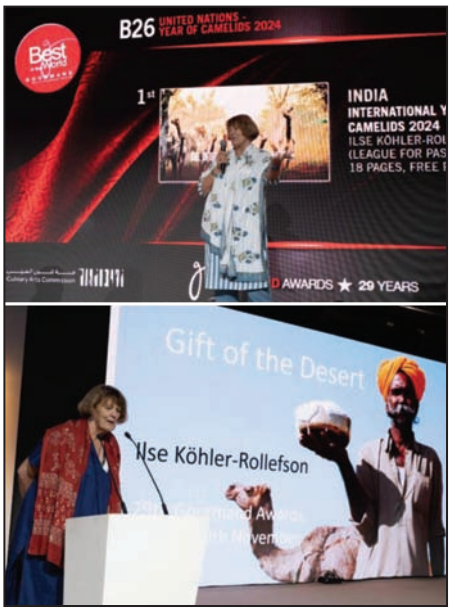
CAMENET Technical Committee Meeting held at Abu Dhabi, UAE

Fifth CAMENET technical Committee Meeting, was held on November 20-21, 2023 in Abu Dhabi, United Arab Emirates. This event was aimed to deliberate and strategise on matters integral to collective mission of CAMENET. The meeting was hosted by the Abu Dhabi Agriculture and Food Safety Authority. CAMENET achievements for the year 2023 and action plan for the year 2024 were discussed. By and large the discussion revolved around camel diseases and laboratory testing standards development and validation. Network stressed on standardised and accurately validated diagnostic methods according to WOAH guidelines, recommendation, and procedures. CAMENET focused on strategies and plan of action for scientific development and progress in camel health and welfare-related issues. Provisions of capacity-building and exchange of expertise and cooperation in controlling camel disease, through conferences and events to enhance knowledge sharing were also discussed. Facilitating R&D projects through collaboration between the national, regional, and international institutions and FAO/WOAH reference laboratories and collaborating centres and academia were agreed upon. Finally one health and public health, economy, and societal issues related to camelids were also discussed.

Ilse Köhler-Rollefson work on camelids is named "Best in the World" in Riyadh, Saudi Arabia



Ilse Köhler-Rollefson won the Gourmand World Cookbook Award "Best in the World" for her work with the League for Pastoral People preparing the UN International Year of Camelids 2024.

Founded in 1995 by Edouard Cointreau and with 205 participating countries, the Gourmand World Cookbook Awards are the only international competition for food culture content. Every year, they honour the best books, printed or digital, as well as television and social media. The competition is free and open to all languages.

This year, the Gourmand Awards Ceremony was hosted by the Saudi Feast Food Festival, the largest of its kind in the Middle East. Organised by the Saudi Culinary Arts Commission of the Ministry of Culture, the festival gathered hundreds of thousands of visitors, and food culture professionals from seventy countries coming from five continents.

AUTHOR INDEX

SUBJECT INDEX

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INSTRUCTIONS TO CONTRIBUTORS

(Effective from year 2023)

(Journal of Camel Practice and Research - triannual -April, August and December issues every year)

The Journal of Camel Practice and Research (JCPR) is a triannual journal (April, August and December issues) published in the English language by the Camel Publishing House, 67, Gandhi Nagar West, Near Lalgarh Palace, Bikaner, 334 001 (India). It is in offset print size of 20.5x27.5 cm in two columns with a print area of 17x22 cm. It will be known as **Journal of Camel Practice and Research** with **Volume number** on yearly basis and **Number** on issues per volume basis (in exceptional cases there can be more than three issues in a volume). The editorial policies of JCPR are established by the editor-in-chief and is detailed in this section. Views expressed in papers published in JCPR represent the opinions of the author(s) and do not necessarily reflect the official policy of the author's affiliated institution, or the editor-in-chief.

Nature of coverage: This journal is dedicated to disseminate scientific information about new and old world camelids in form of **Original research** articles in camel science, health, husbandry, pastoralism, sports, specific behaviour, history and socio-economics. **Reports** on unusual clinical case(s) or unreported management of clinical case(s) are also published. Review articles will be accepted on invitation only. **Book review** directly or indirectly related to camels will be reviewed by subject-matter specialists and included if sent to the journal for this purpose. The Journal of Camel Practice and Research will occasionally contain an **invited editorial** commenting on the current research and papers in the issue.

Submission of manuscript: Manuscripts should be submitted in word files to **Dr. Tarun Kumar Gahlot**, Editor, Journal of Camel Practice and Research at tkcamelvet@yahoo.com or editorjcpr@camelsandcamelids.com by online submission portals available at www.indianjournals.com or www.camelsandcamelids.com. The figures can be submitted preferably as a high pixel JPEG or other format. The manuscript should be accompanied by a covering note and author consent letter from the author responsible for correspondence. It should also contain a statement that manuscript has been seen and approved by all co-authors. Editor and members of the editorial board are not responsible for the opinions expressed by authors and reserves the right to reject any material or introduce editorial changes. Material will be accepted for publication on the understanding that it has not been published in any other form and is not being considered elsewhere. Any material once accepted for publication may not be republished in any form without prior permission of the author. **Single blind peer review policy** is used for the manuscripts submitted and the quality standards are maintained by the JCPR editorial board and by authors who submit manuscripts for publication. A preprint PDF is provided to the author in correspondence for verification of contents and corrections, if any.

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PROTEOMIC CHARACTERISATION OF SERUM DURING THE BREEDING CYCLE IN MALE BACTRIAN CAMELS

Le Hai[1], Rendalai Si[2], Fu-Cheng Guo[1], Jing HeI, Li Yi[1], Liang Ming[1], Jun-Wen Zhou[3], La Ba[3], Rigetu Zhao[3] and Rimutu Ji[1,2]

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Wilson R Trevor. The one-humped camel in Eritrea and Ethiopia: a critical review of the literature and a bibliography. Journal of Camel Practice and Research. 2020; 27(3):229-262.

For edited symposium/congress/proceedings: Abdalla HS. Camel trypanosomiasis in the Sudan. Proceedings of the First International Camel Conference, Dubai (UAE), February 2-6, 1992; pp 401-403.

Books (Personal authors): Faye B and Bengoumi M. Camel Clinical Biochemistry and Haematology: Springer International Publishing. 2018; pp 275-286.

Chapter from multiauthored books: Wernery U, Kinne J and Schuster RK. Unusual arboviruses and other minor viral infections. In: Camelid Infectious Disorders. OIE Book. 2014; pp 319-322.

Thesis: Rathod Avni. Therapeutic studies on sarcopticosis in camels (Camelus dromedarius). Unpublished Masters Thesis (MVSc), Rajasthan Agricultural University, Bikaner, Rajasthan, India. 2006.

Commercial booklets: Anonymous/Name. Conray-Contrast Media. IIIrd Edn., 1967; pp 12-15, May and Baker Ltd., Dagenham, Essex, England.

Magazine articles: Taylor D. The Constipated Camel. Reader's Digest. Indian Edn. RDI Print & Publishing (P) Ltd., Mehra House, 250-C, New Cross Road, Worli, Bombay, India. 1985; 126:60-64

News paper articles: Christina Adams. Camel milk: a miracle cure for children with autism?. Gulf News, Published: April 09. 2014.

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The International Year of Camelids

The International Year of Camelids (IYC) was inaugurated on 4th December at FAO headquarters in Romein the form of a 90 minute side-event during the FAO Council. The official slogan for the IYC is *Heroes of Deserts and Highlands: Nourishing People and Culture*. This has a nice ring, but the 'traditional' communities and indigenous peoples who in turn are nourishing camelids – whether it is alpacas and llamas in the Andes of South America, dromedaries in the arid zones of Africa, the Middle East and South Asia, or Bactrians in the steppes of Mongolia and China – are also true heroes. If we, as humanity, want to benefit from the special adaptations and potential of camelids in generating food in extremely marginal areas, then industrial scale stall-feeding systems as they have cropped up in oil rich countries are not the answer. These may look shiny and scientific and progressive compared with the mobile husbandry systems in which camelids have been raised until now, but they are basically unsustainable, depending on imported feed and fossil fuels, apart from not providing an environment in which camels thrive and are happy. Like people, camels love to wander around and browse ('shop') on different types of plants, composing menus according to their own individual tastes.

In short, putting camelids into stall-feeding systems with standard diets of alfalfa hay and grain defeats their ecological purpose. They are biologically designed to be kept in nomadic systems, and to do so requires the knowledge and dedication of traditional or modern pastoralists who are willing to undertake the hardship of managing and caring for camelids in marginal areas with harsh climates. And that is a challenge ever fewer young people want to engage with – and who can blame them, considering the hardships of living in remote areas. The average age of alpaca breeders in Peru is mid-sixties; in India it is probably similar for dromedary breeders.

All of us in the camelid world are grateful that the International Year of Camelids will shine the spotlight on our favourite animals. But we must make sure that the IYC's activities and outcome support their guardians: the communities who have nurtured and nourished them over thousands of years and for whom these animals are co-creatures and not meat and milk generating units crammed into feedlots or mere status symbols. A Civil Society statement that already has been signed onto by 23 organisations calls for carving out an alternative cruelty-free development trajectory that conforms to the worldview of traditional camelid keeping communities and avoids industrialization.

The organisations elaborate the kind of support and interventions they wish for:

- Enabling mobility and ensuring secure access to ancestral grazing and browsing areas for our camelid herds to thrive, for example by recognizing them as *Indigenous Community Conserved Areas or Territories of Life*
- Investing in decentralized infrastructure, such as networks of mini-diaries and local processing facilities to link camelid herders in remote areas to value chains, while also respecting and supporting our traditional ways of processing,
- Fostering camelid-herding community organisations and their agency,
- Respecting and building on our traditional knowledge and related local innovations,
- Strengthening provision of camelid healthcare, including research into emerging diseases,
- Supporting investment on people-centred and -controlled camelid research and development,
- Recognizing camels as co-creatures and establishing camelid welfare standards into policy and practice worldwide.

(Courtesy- Ilse Köhler-Rollefson)