Investigation into the Sensory-Behavioural Interactions Between a Dairy Camel and a Calf During Milking

By

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Bachelor of Science

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Summary

Feral Dromedary camels are increasingly being utilised in Australia and around the world to provide milk for human consumption. There are significant contradictions in the literature concerning the requirement of the presence of a calf for successful milking of the Dromedary camel. The first hypothesis tested in the current study was that presence of her own calf for the dairy camel is more successful than no calf or a non-kin calf, without any contact, for allowing milk let-down prior to machine milking. The second hypothesis was that full physical contact between the dairy camel and her calf is more successful than no calf or a non-kin calf (with contact) for allowing milk let-down prior to machine milking. An additional aim was to investigate the sensory behaviours associated with successful milking of the dairy camel.

A total of 9 camels and their respective year-old calves were used in the study. A total of twelve experimental sessions were conducted, six kin sessions and six non-kin sessions. On the kin day, after the cow was situated in the race and the udder was washed, let-down was attempted by manual stimulation firstly without a calf. If let-down was successful, the cow was milked and moved into the release yard with the calf. If let-down was unsuccessful, a transparent plastic barrier was moved into place between the cow race and calf race to block physical contact. The kin calf was let into the calf race and the milker continued to use manual stimulation to elicit milk let-down with the calf present. If let-down was successful, the cow was milked, then both cow and calf were let into the release yard. If let-down was unsuccessful, the barrier was removed, and the calf given full physical contact access to the cow including suckling. This procedure was repeated for the non-kin day. Success of let-down, time taken to let-down and cow and calf behaviours were recorded.

There was an overall effect of treatment (χ^2=37.2; P<0.0001), with the presence of the kin calf stimulating milk let-down by the cow on 73% (n=64) of attempts, compared to 20% (n=64) for the presence of the non-kin calf and 42% (n=108) when no calf was present. There was also a significant effect of the barrier (χ^2=24.8; P<0.0001), for when the barrier placed between the cow and calf, the kin calf elicited milk let-down on 50% of attempts, while the non-kin calf was unable to initiate let-down on any occasion. When the barrier was removed the kin calf successfully initiated let-down on 94% of attempts, while the non-kin calf was only successful on 40% of all attempts. The dominant behaviours associated with let-down were cow and calf vocalisations, vigilance of the cow looking at the calf, and udder nudges.

The findings of the current study partially agrees with the majority of literature that stated that the presence of the kin calf was "essential" for achieving milk let-down in Dromedary camels. However, it is clear from this study that it is still possible to achieve milk let-down using no calf or a non-kin calf. This research may act as a platform to launch future study into the management and understanding of Dromedary camels and may be used to improve industry practises within the camel dairy industry.

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Chapter 1. Literature review

The camel in Australia

The Dromedary camel (*Camelus dromedarius*) (NRMMC, 2010), although an introduced species, is quickly becoming an important livestock animal in Australia. Dromedaries are large, herbivorous pseudo-ruminants with a single hump and can weigh on average between 450 – 650 kg, (DPIRD, 2018) stand up to 2 metres tall and have an average lifespan of 40 - 50 years (DESWPC, 2010).

The Dromedary is one of two camel species; the other being the two-humped camel *Camelus bactrianus* (DESWPC, 2010). The Dromedary evolved in the Middle East and Northern Africa to survive in arid and inhospitable environments (Gauthier-Pilters & Dagg, 1981) While the Bactrian camel evolved in the cold deserts of Mongolia and China (DESWPC, 2010). For the remainder of this paper, the term "camel" will refer exclusively to the Dromedary camel.

Camels were only introduced to Australia in the 1840s for use in transport, exploration and building infrastructure such as railway lines (Short *et al.*, 1988; DPIRD, 2018). An estimated 20,000 camels were imported until 1907 but by the 1930s, camels had become obsolete and either escaped or were released into the desert after being replaced by motor vehicles (NRMMC, 2010). With the number of feral camels predicted to double every 8-10 years (Edwards *et al.*, 2004) and the current population being estimated at over a million (DESWPC, 2010; NRMMC, 2010); the wild Dromedary population in Australia is the largest in the world (NRMMC, 2010).

The Feral camels compete with livestock over food, shelter and water while also damaging fences and water troughs (DPIRD, 2018). Dromedaries destroy long lines of fences by leaning on them until they collapse; sometimes also leading to the escape and loss of livestock (NRMMC, 2010). Another agricultural concern is that dromedaries may act as potential reservoirs for serious exotic livestock diseases such as Bovine Tuberculosis and Brucellosis (Brown, 2004).

For these reasons, there have been previous culling programs (NRMMC, 2010) to decrease the population. Current non-commercial culling methods in use are aerial or ground culling and exclusion fencing. Commercial culling involves the capture of feral camels either for slaughter or live export (NRMMC, 2010). The control and management of feral camels costs approximately $2.35 million per year (DESWPC, 2010).

Currently the most common uses of the Australian feral camels are live export and the pet meat trade (Clarke, 2014). However, camels are increasingly valued for: tourism, racing, co-grazing, meat, wool and milk (Dörges & Heucke, 2003; Kaskous & Abdelaziz, 2014). Camels survive on sparse vegetation but maintain good body condition and successfully raise healthy calves even in harsh environments (DPIRD, 2018). To accomplish this, Dromedaries produce high quality milk. Therefore, the Australian camel dairy industry is seen as a practical approach to utilising this feral resource. (NRMMC, 2010).

Camel Milk Properties

Due to the reported health benefits discussed below, camel milk is being used in a variety of ways namely; consumption (milk, cheese, yoghurt, etc), for medicinal purposes and in cosmetics (Nelson *et al*., 2015; Secchi, 2008; Choi *et al*., 2014) Several factors impact the value of each milk constituent including the animals' age, breed, lactation stage and nutrition (Musaad *et al*., 2013). A camel may produce on average anywhere between 3.5L in desert conditions, to 20L per day under intensive management (Khan & Iqbal, 2001; Edwards *et al*., 2008). Although slightly saltier in taste, camel milk has been declared as palatable as cows' milk and is highly nutritious (Edwards *et al*., 2008).

Protein

Although milk contains a small percentage of immunoproteins; milk proteins can be classed into two main groups; whey proteins and casein complexes (Kula & Tegegne, 2016). Casein complexes are higher in proportion compared to whey proteins. The average value of total protein in camel milk is 3.1g per 100g of milk (Table 1).

Lipids

Milk fats consist of spherical globules of triglycerides surrounded by a membrane of complex lipids (Park *et al*., 2017). Camel milk is characterised by a high proportion of long-chain polyunsaturated fatty acids (Shabo *et al*., 2005).

Carbohydrates

Lactose is the principle form of carbohydrate in milk (Kula & Tegegne, 2016). Most milks contain *glucose* and *galactose* which are the biosynthetic precursors to *lactose* (Jensen, 1995). *Oligosaccharides* are constructed from 3 to 10 units of *monosaccharides* and exhibit probiotic, anti-inflammatory and anti-pathogenic properties (Albrecht *et al*., 2013). The concentrations of *oligosaccharides* vary between species (Jensen, 1995) but thus far only 12 *oligosaccharide* structures have been identified in camel milk compared to over 200 identified in human milk (Albrecht *et al*., 2013).

Water

Water is the main component of all milks, with an average range of 68% in reindeer milk to 91% in Donkey milk (FAO, 2013). The Dromedary camel average water content per 100g of milk is 89g (Table 1).

Table 1. Proximate Energy, Fat, Protein, Lactose and Water contents in Dromedary camel milk compared with human, cow, goat, sheep and buffalo milk. Values are average per 100 g of milk.

Species	Energy (KJ)	Total Fat (g)	Total Protein (g)	Lactose (g)	Water (g)
Camel	234	3.2	3.1	4.3	89.0
Human	291	4.4	1.0	6.9	87.5
Cow	262	3.3	3.3	4.7	86.1
Goat	270	3.9	3.4	4.4	87.7
Sheep	420	6.4	5.6	5.1	82.1
Buffalo	412	7.5	4.0	4.4	83.2

(FAO, 2013, p. 73-77)

Vitamins and Minerals

Vitamin C is a well-known antioxidant and the average concentration of vitamin C in camel milk is three times higher than compared with cow and goat milk (FAO, 2013; Edwards *et al*., 2008) (Table 2). The Vitamin C in camel milk is significant for the health of pastoralists in arid areas where fruit and vegetables are scarce (Yagil, 1982). The acidity from the Vitamin C in camel milk maintains a lower pH to allow the milk to be kept for longer without forming a cream layer (Kula & Tegegne, 2016). The high concentration of vitamin C also contributes to the antioxidant properties of the milk by reducing the concentration of damaging free radicals in the tissues (Masaki, 2010; Ganceviciene, 2012).

Lactoferrin is a glycoprotein with reported anti-bacterial, anti-carcinogenic, anti-diabetic and anti-viral properties (Gizachew *et al*., 2014). It is also essential for iron transportation and storage (Al-Majali *et al.,* 2007) The lactoferrin content of camel milk is significantly higher than other milks (Gizachew *et al*., 2014).

Iron is essential for many important biochemical processes such as: the binding and transportation of oxygen, maintaining a healthy immune system, gene regulation and cell proliferation and differentiation (Beard, 2001). According to FAO, 2013, the Iron concentration in Dromedary camel milk is double that of cow and sheep milk (Table 2).

Table 2. Comparative Iron and Vitamin C content between camel, human, cow, goat, sheep and buffalo milk. Values are average mg per 100g of milk.

Species	Iron (mg)	Vitamin C (mg)
Camel	**0.2**	**3.8**
Human	**Tr**	**5.0**
Cow	**0.1**	**1.0**
Goat	**0.3**	**1.1**
Sheep	**0.1**	**4.6**
Buffalo	**0.2**	**2.5**

(FAO, 2013, p. 73-77) Tr: Traces

Medicinal Properties

Lactose intolerance, an impaired ability to digest lactose (Ibrahim & Gyawali, 2013), is becoming increasingly common, with approximately 65-70 per cent of the human population currently affected (Bayless *et al.,* 2017). Symptoms of lactose intolerance usually present as bloating, abdominal pain and diarrhoea (Swagerty *et al.*, 2002). Shabo, *et al*. (2005) claimed all eight participants with lactose intolerance in the study greatly improved with the regular consumption of camel milk.

Although more than one mechanism for milk allergy exists, the protein *β-lactoglobulin (β*-LG*)* and casein fractions have been identified as the main causative agents of milk allergies in humans (Gizachew *et al*., 2014; El-Agamy, 2006). *β*-LG is present in cow and sheep milk but absent in both human and camel milk (El-Agamy, 2006; Gizachew *et al.*, 2014). Common gastrointestinal milk allergy symptoms display as diarrhea, vomiting and nausea but in some extreme cases anaphylaxis (Shabo *et al*., 2005). To date, camel milk shows promise as a hypoallergenic alternative for people with milk allergies (El-Agamy, 2006; Ehlayel *et al.,* 2011; Shabo *et al*., 2005).

Although the mechanism behind it is not yet fully understood, camel milk also shows great promise as a potential treatment for diabetes (Agrawal *et al*., 2009). The current leading theories are: (1) camel milk contains insulin in a lipid capsule that can withstand proteolysis until the small intestine; (2) Some properties of the milk insulin make it easier to absorb than insulin from other sources or; (3) the milk contains insulin-like proteins that operate in the same way as insulin (Kula & Tegegne, 2016; Malik *et al.,* 2012; Agrawal *et al.,* 2003). It has been a long-held belief in the Middle East that camel milk is an effective treatment for diabetes (Malik *et al*., 2012). Some studies indicate that patients with diabetes that regularly drink camel milk may be able to decrease their dependence on insulin treatments (Agrawal *et al*., 2009).

Cosmetics

Camel milk contains α-hydroxy acids which are a group of organic acids that are recognised for their anti-inflammatory and anti-aging effects (Bhalla *et al.,* 2012). α-hydroxy acids, such as glycolic acid derived from milk sugars (Hong *et al*., 2001), thins the stratum corneum of the epidermis by promoting epidermolysis to reveal the new, fresh layer of cells beneath (Tung *et al*., 2012). α-hydroxy acids help to eliminate wrinkles, age spots and relieve dryness as they disperse basal layer melanin and increase collagen synthesis within the dermis (Tung *et al.*, 2012). Another example of an a-hydroxy acid in camel milk is lactic acid. Lactic acid is beneficial for dry skin as it exhibits moisturizing properties. (Bhalla *et al*., 2012).

Physiology and Behaviour

Desert Adaptations

Physiologically, Dromedaries developed several specialised adaptations for desert environments. They have slit-like nostrils that can close against blowing sands as well as long eyelashes and a third, clear eyelid to protect the eyes against sand and the sun (Gebreyohanes & Assen, 2017). They have long legs with broad, thick padded feet to prevent them sinking into the sand (DAF, 2016). Camels congregate in small herds of approximately 10 to 30 animals and can travel up to 70 kilometres a day (DESWPC, 2010).

Most importantly; camels have adapted to withstand significant water loss. Camels can lose up to 30% of their body mass in water, whereas in other mammals, excess of 15% is fatal (Gebreyohanes & Assen, 2017). The rumen contains a large volume of water which buffers against short term dehydration. This adaptation also prevents osmotic tissue shock during rehydration where a camel may drink up to a third of its body weight in a few minutes (Gebreyohanes & Assen, 2017). Like all camelids, the dromedary is a pseudo-ruminant and differs to true ruminants in that camels lack a distinct omasum chamber of the stomach. (Yagil, 1982).

Reproduction

For most of the year, wild camels will travel in small herds usually comprised of females and calves, bachelor groups and solitary older males (DAF, 2016). In Australia the camel breeding season is typically from May to October (DESWPC, 2010). Both males and females will come into heat concurrently for a few months in winter when food availability is most abundant (Khanvilkar *et al*., 2009). During the breeding season larger groups of sexually mature females will travel together and be escorted by a dominant bull. (DAF, 2016; DESWPC, 2010). Female camels reach puberty between 3 and 4 years old where males (bulls) mature at 5 to 7 years (Yagil, 1982).

Males will be in rut for 2-3 months and typically demonstrate restlessness, aggressive behaviour and excrete a pungent odour from the poll gland at the back of the head (Gauthier-Pilters & Dagg, 1981) Bulls are very dangerous during this time to the point they may not be able to be handled at all (El-Bahrawy *et al*. 2015; Khanvilkar *et al*., 2009). Bull camels display female defence polygamy (Venpé, 2005) and will compete for and vigorously defend a group of breeding females during the rutting period (NRMMC, 2010).

The female camel is seasonally polyoestrous with oestrous cycles lasting between 16-28 days with oestrus being displayed for 3-4 days (Khanvilkar *et al*., 2009; Yagil, 1982). The calving interval is considerably long in camels (2 to 2.5 years) due to the long gestation and lactation periods (Nagy & Juhasz, 2016). Camels typically produce a single calf (Figure 1) with the gestation period lasting approximately 13 months (Khanvilkar *et al*., 2009; Kadim *et al.,* 2008). Signs of parturition will involve restlessness, inappetence, frequent urination and vulvar swelling (Nelson *et al*., 2015). Camels give birth in a recumbent position with the calf forelimbs and head presenting first (Nelson *et al*., 2015).

Figure 1. A Photograph showing a Dromedary cow with her neonatal calf in Western Australia.

Lactation

Colostrum is the first milk produced post-partum and is essential for the health and survival of the calf (Wernery, 2006). Colostrum is produced for 7 to 10 days after calving. It contains high levels of maternal immunoglobulins and has a mild laxative effect (Wernery, 2006). There is a popular belief among camel herders and pastoralists in the Middle East and Africa; that colostrum causes extensive diarrhoea and is thought of as dangerous for the calves (Elmi, 1989). The colostrum is usually milked and wasted onto the ground (Farah *et al*. 2007; Elmi, 1989).

Camels lactate on average between 8 to 18 months depending on several factors including geographical location, the type of management system and nutrition (Nagy & Juhasz, 2016). However, unlike dairy cattle, pregnancy and lactation cannot overlap for an extended period in the camel (Nagy & Juhasz, 2016; Butler, 2003). If the camel becomes pregnant while lactating, milk production will cease at approximately 4 months after conception (Nagy & Juhasz, 2016).

Milk Let-Down

Understanding the morphological and physiological characteristics of the camel udder are essential in order to appreciate the mechanism behind milk let-down and the potential challenges of milking. The udder of the camel is situated in the inguinal area between the hind legs and has 4 glandular quarters connected to 4 individual, cone-shaped teats (Kaskous & Abdelaziz, 2014). Each teat has two narrow teat canals which lead into two milk cisterns per teat canal. (Wernery, 2006).

In many mammalian species, the hormone *oxytocin* is released from the posterior pituitary gland after pressure receptors in the teat are stimulated, which causes contraction of the myoepithelial cells surrounding the milk secreting cells (*alveoli*) of the mammary gland (Svennersten-sjaunja, 2004). Milk is then ejected (let-down) from the cistern into the teat canal of the udder to be made available to the offspring for passive removal (Nagy & Juhasz, 2016) (Figure 2).

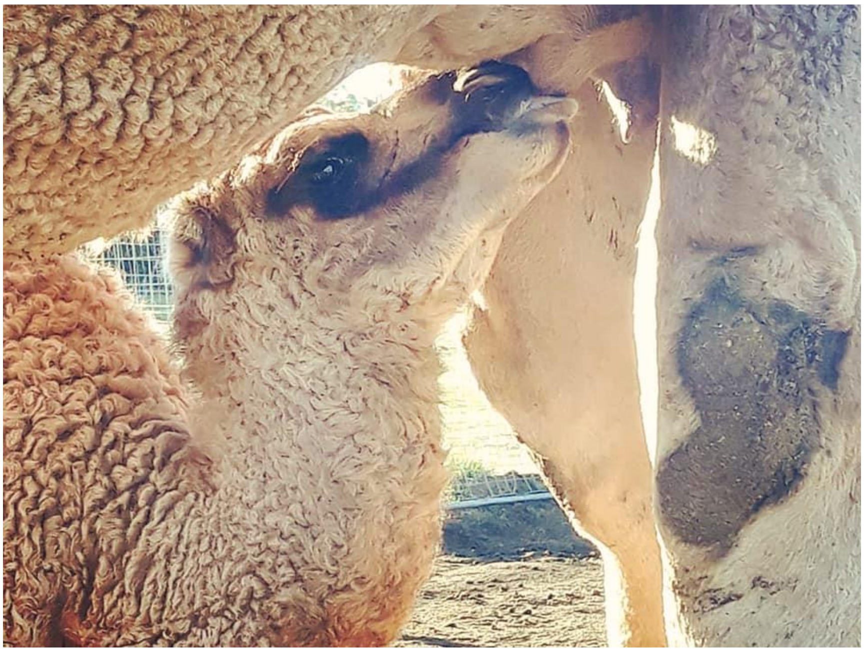

Figure 2. A photograph of a day-old dromedary calf suckling the dam's left fore-teat

Synthesising milk is a biologically expensive process therefore, an autoregulated negative feedback system alters milk production when milk is detected as being left for long periods in the milk cistern (Lollivier *et al*., 2002). This stimulus promotes the production of the glycoprotein Feedback Inhibitor of Lactation (FIL) which is thought to possibly reduce the sensitivity of mammary cells by reducing the number of prolactin receptors and inhibit the synthesis of lactose and caseins (Lollivier *et al*., 2002; Peaker & Wilde, 1996).

However, milk let-down can be inhibited at the site of release (central inhibition) or at the site of action (peripheral inhibition) (Bruckmaier, 2005). Central inhibition involves a lack of or an insufficient release of *oxytocin* from the posterior pituitary gland (Wellnitz & Bruckmaier, 2001). Central inhibition is usually caused by emotional stress which may be triggered by a new milking environment, loud noises during milking or aversive handling but may be overcome by administering exogenous *oxytocin* (Bruckmaier, 2005).

Posterior inhibition refers to when oxytocin is physiologically released but unable to act upon the mammary gland (Bruckmaier, 2005). This may be accomplished by administering an *oxytocin* receptor blocking agent or an α-adrenergic receptor antagonist (Bruckmaier, 2005; Wellnitz & Bruckmaier, 2001). The milk let-down response can become a conditioned response in modern dairy cattle and let-down can initiated by manual stimulation of the udder without the calf being present (Willis & Mein, 1983). It is believed that manual stimulation alone doesn't sufficiently stimulate milk let-down in the dairy camel without the calf being in full physical contact during milking (Eisa *et al*., 2010; Eisa & Mustafa, 2011; Kaskous & Abdelaziz, 2014). But there is also a report by Kaskous *et al*. (2006) of milk let-down with just the presence of the calf (i.e. no physical contact). This raises the question of the relative importance of the various cow-calf sensory mechanisms (sight, smell, sound, taste, touch) during the suckling process.

The presence of the calf is usually required to initiate milk let-down (ejection) in camels (Eisa & Mustafa, 2011; Kaskous & Abdelaziz, 2014). Although several studies indicate that camels may be tricked into fostering a non-filial calf or may let-down milk by using some form of dummy calf. Eisa & Mustafa (2011) stated that if a calf dies, the cow will dry up unless milking is continued by manual stimulation. They also claim a cow presented with a doll made with the skin of her deceased calf is enough to initiate milk let-down. In agreement with this, Elmi (1989) described in detail the following techniques used by Somali Ceeldheer camel herders to initiate milk let-down without a calf. He also described the techniques used to trick or punish a cow into accepting a foster calf.

Salaax: This term is used for the massage technique. According to herders, no calf is required, and the camel may potentially be milked in this way for up to 6 months after the calf has died (Elmi, 1989).

Magaar: Magaar is used to define two approaches that utilise the skin from a calf. These methods are commonly used if a calf has died or been culled. The skin of the calf is either placed over a foster calf and helped to suckle by pastoralists until the skin is no longer required; or may be presented by itself as a sort of glove. The herders claim this to be very successful in prompting milk let-down and successful fostering (Elmi, 1989).

Allosuckling

Allosuckling is the term used to describe when young suckle from a non-maternal female (Bradlová *et al.,* 2013). Allosuckling has been studied in several mammalian species including reindeer, giraffes, guanacos and Bactrian camels (Bradlová *et al.*, 2013; Gloneková *et al*., 2016; Engelhardt *et al*., 2014; Zapata *et al*., 2009). Research is ongoing to determine if this behaviour is a result of opportunistic milk theft by the non-filial calves, altruistic behaviour on the part of the female or misdirected parental care (Bradlová *et al.,* 2013*;* Zapata *et al*., 2009).

One such study by Bradlová *et al.* (2013) found allosuckling to be common in herds of captive Bactrian camels. The observations suggest that non-filial calves would adopt a perpendicular position when suckling and never the antiparallel position. This is thought to make identifying the calf as non-filial more difficult for the cow. In addition, the rate of allosuckling appeared to increase during weaning; and non-filial calves seem to prefer to suckle from a non-maternal dam while her own calf is also suckling. This observation accounted for over 80% of the recorded allosuckling incidences. The study concluded that the evidence supports the milk-theft hypothesis but cannot rule out altruistic behaviour from the dam.

Interestingly, allosuckling has yet to be properly researched in Dromedary camels. Packer *et al*., (1992) stated that Dromedaries do not tolerate allosuckling at all. However, this study is limited in that the research was based from previous research and a questionnaire survey. Contrary to Packer *et al*. (1992), Elmi, (1989) described attempts at allosuckling within Dromedary herds in Somalia.

Milking the camel

Pastoralists in Africa and the Middle East have been milking camels for centuries (Edwards *et al*. 2008) but until recently, no commercial dairies existed. The First ever large-scale, commercial camel dairy was opened in Dubai in 2006 (Nagy & Juhasz, 2016). Even in large-scale operations, milking a camel can potentially be very dangerous. Adults and immature camels can pose a serious safety risk. Besides being very large and heavy, the attack and defence behaviour of camels include kicking, striking out with the forelimbs and biting (Al-Hazami *et al*., 1993).

Management of a large-scale camel dairy may be more difficult with the required presence of the calves during milking compared to a cattle dairy where the calf is no longer needed for successful milking (Nagy & Juhasz, 2016; Willis & Mein,1983). Nagy & Juhasz (2016) described that in their study, calves had to be visible to the cows (partially separated) in the paddock to maintain milk production over time.

Wernery (2006) suggested that the goal for commercial camel dairies should be to remove the need for a calf from the milking process altogether. The intention behind removing the need for a calf is a reduction of labour and infrastructure expenses, an increase in milk production and milking efficiency (Singh *et al*., 2017).

Hypothesis

There are significant contradictions in the literature concerning the requirement of the presence of a calf for successful milking of the dairy camel. Wernery *et al.* (2004) stated that young camels could be removed from the dam without any negative effect on the milk yield. On the contrary, Eisa *et al*. (2010) observed that the presence of the calf is imperative for milk let-down in the camel.

There are no studies on the sensory behaviours between the camel cow and the calf that may be important for milk let-down. This project will lead to a deeper understanding of the importance of both the presence of a calf during milking, and the significance of sensory behavioural communication between cow and calf, with the specific hypothesis of:

i. The presence of her own calf for the dairy camel is more successful than no calf or a non-kin calf, without any contact, for allowing milk let-down prior to machine milking.

ii. Full physical contact between the dairy camel and her calf is more successful than no calf or a non-kin calf (with contact) for allowing milk let-down prior to machine milking.

An additional aim was to investigate the sensory behaviours associated with successful milking of the dairy camel

Chapter 2. Scientific paper

2.1 Introduction

Feral Dromedary camels are increasingly being utilised in Australia and around the world to provide milk for human consumption. However, a significant management and production constraint is the need for the presence of the camel's calf at milking to allow milk let-down. There are significant contradictions in the literature concerning the requirement of the presence of a calf for successful milking of the dairy camel. Wernery *et al*. (2004) stated that young camels could be removed from the dam without any negative effect on the milk yield. On the contrary, Eisa *et al*. (2010) observed that the presence of the calf is imperative for milk let-down in the camel. Therefore, we hypothesised that the presence of her own calf for the dairy camel is more successful than no calf or a non-kin calf, without any contact, for allowing milk let-down prior to machine milking. Additionally, we hypothesised that full physical contact between the dairy camel and her calf is more successful than no calf or a non-kin calf (with contact) for allowing milk let-down prior to machine milking.

An additional aim was to investigate the sensory behaviours associated with successful milking of the dairy camel. There are no studies on the sensory behaviours between the camel cow and the calf that may be important for milk let-down. This project will lead to a deeper understanding of the importance of both the presence of a calf during milking, and the significance of sensory behavioural communication between cow and calf.

2.2 Materials and Methods

All procedures involving animals were approved by Murdoch University Animal Ethics Committee. The study was conducted at the Dromedairy Body + Skin dairy farm (Gidgegannup, WA) from April to August 2019.

2.2.1 Animals, dairy design and Location

2.2.1.1 Animals and Location

A total of 9 camels and their respective year-old calves were used in the study. The cows were collected from the wild in Western Australia along the trans access road near the border of South Australia and were trained for dairy use. The cows were pregnant at the time of capture and all calves were born on the DromeDairy farm in Gidgegannup Western Australia. Both groups were routinely milked daily at 6:30 am, with the normal milking routine carried out between experimental days.

2.2.1.2 Dairy Design

There were five separate holding yards and two undercover races that composed the layout of the dairy. Two of the yards (cow yards A and B) housed cows to be milked and likewise; two of the other holding yards (calf yards A and B) housed the calves. The final holding yard (the release yard) at the end of the races held both cows and calves together after milking for release into a paddock. The two races ran parallel to each other with cows entering from cow yard B and calves entering from calf yard B. The opposite ends of both races open into the release yard (Fig. 3).

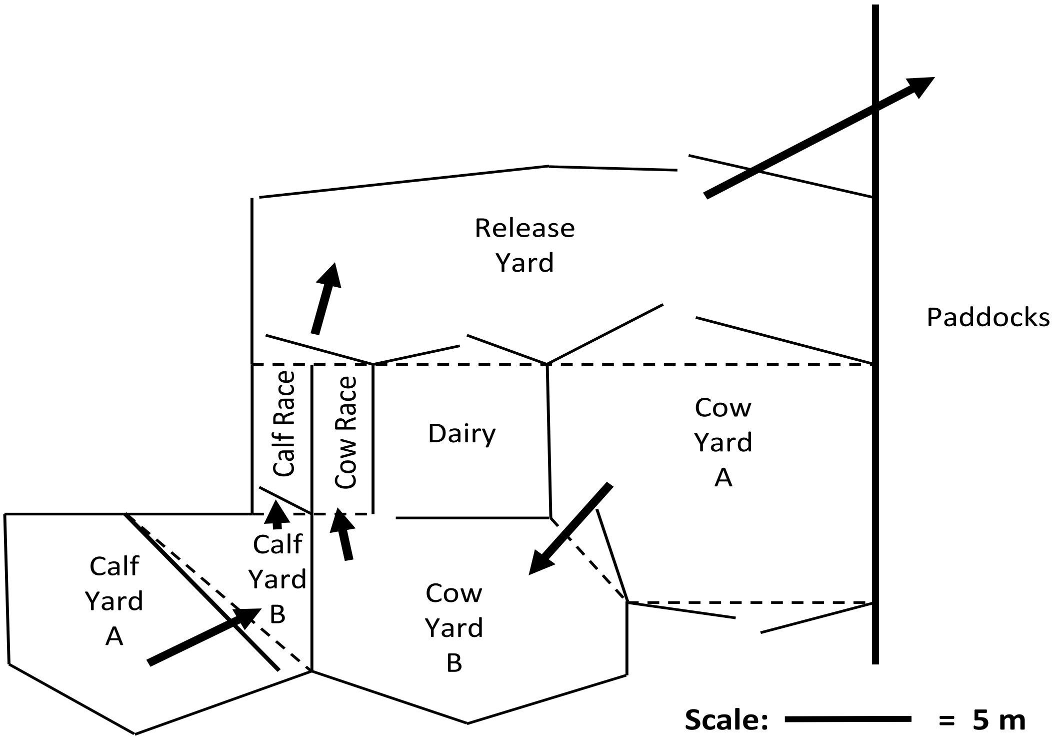

Figure 3. Schematic diagram of dairy layout and animal movement during the experiment

Figure 4. Photograph from camera angle one focused on the cow race, showing the anterior view of a camel being milked.

Figure 5. Photograph from camera angle two focused on the calf race, showing a calf with the barrier blocking physical contact.

Figure 6. Photograph from camera angle two focused on the calf race, showing a calf suckling from the maternal dam.

Figure 7. Photograph from camera angle three showing a lateral view of a camel in the race being suckled by the kin calf.

2.2.2 Equipment Used

- Polycarbonate plastic barrier – made from 3mm thick, 1.8m H x 1.22m W colourless polycarbonate plastic sheet with metal hooks and handles.

- 2 x GoPro Hero 3 (White Edition) cameras –

GoPro Pty Ltd (VIC, Australia)

1 x Panasonic Hybrid O.I.S camera –

Panasonic Pty Ltd (NSW, Australia)

DeLaval Milking Machine, Type DVP170/340. DeLaval Pty Ltd. (VIC, Australian)

2.2.3 Experimental Design

2.2.3.1 Baseline Sessions

The order in which the cows enter the race was determined by the animals themselves. On entry into the race the udder was washed with a cloth and warm water before the kin calf was presented and allowed to suckle to initiate let-down. The calf was allowed to continue suckling while two of the four teats were milked. After milking, both cow and calf were released into a release yard at the end of the races. Once both groups were milked, they were put out to pasture for the remainder of the day. At 7pm daily, the cows and calves were separated into adjacent holding yards over night. Baseline behavioural data and milk let-down time was gathered during three normal milking sessions in May before the commencement of the experiment and three more in August after the experiment.

2.2.3.2 Experimental Sessions

A total of twelve experimental sessions were conducted, six kin day sessions and six non-kin sessions. These were split across two days. Mondays focused on the presence or absence of the kin calf. Whereas Thursdays concentrated on the presence or absence of the non-kin calf. On Thursdays during the non-kin experiment, the ethical decision (based on possible stress to the cow if she wasn't suckled/milked) was made to introduce the kin calf if let-down was unsuccessful. The let-down and behaviour data for this kin calf was not taken.

Data was collected by direct observation on site and from subsequent video recordings.

Kin Day

On Monday (kin day), after the cow was situated in the race and the udder was washed, let-down was attempted by manual stimulation without a calf first. If let-down was successful, the cow was milked and moved into the release yard with the calf (Fig. 8).

If let-down was unsuccessful, the barrier was moved into place to block physical contact between the cow and calf. The kin calf was let into the calf race and the milker continued to use manual stimulation to elicit milk let-down with the calf present. If let-down was successful, the cow was milked, then both cow and calf were let into the release yard (Fig. 3).

If let-down was unsuccessful, the barrier was removed, and the calf given full physical contact access to the cow including suckling. When the calf had access to physical contact, the milker would not manually manipulate the udder but would only touch the teats to confirm if let-down was successful after being suckled by the calf. If let-down was successful, the cow was milked before being moved into the release yard with the calf.

On rare occurrences where let-down was no initiated by the kin calf with no barrier, the decision was made to release the cow and calf back into the paddock to hopefully allow natural suckling rather than increasing stress by keeping them in the race.

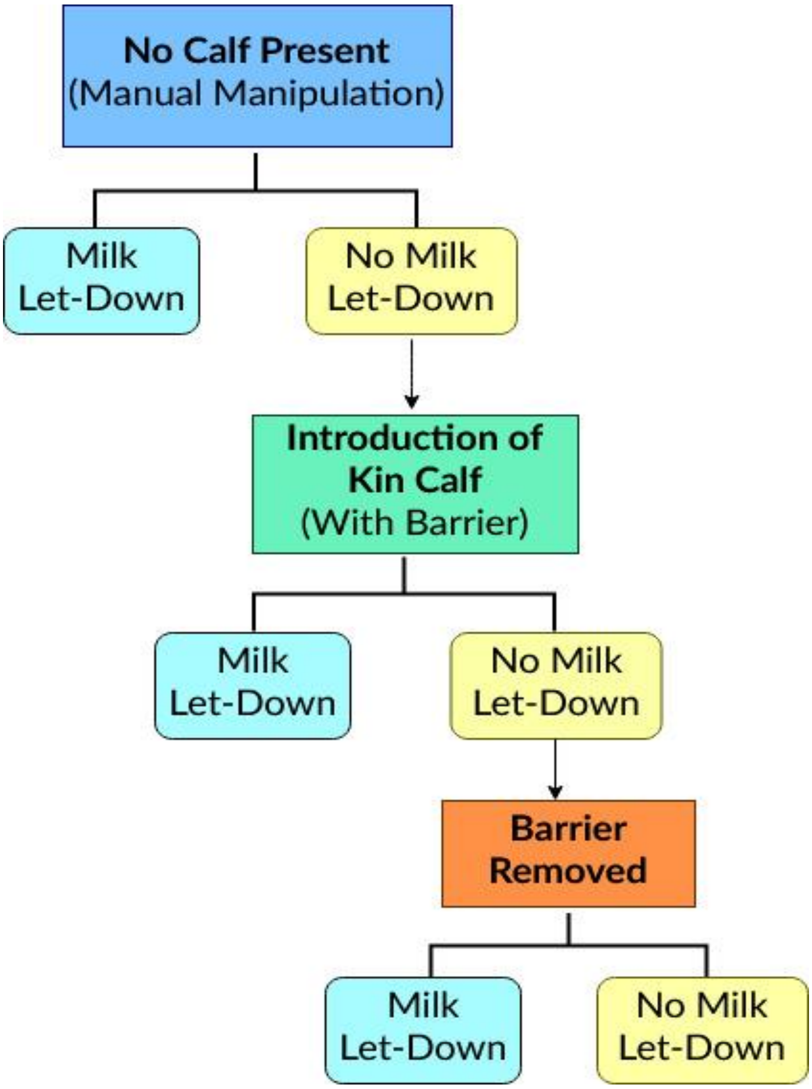

Figure 8. A flowchart illustrating the experimental procedure using kin calves

Non-Kin Day

On Thursday (non-kin day), after the cow was situated in the race and the udder was washed, let-down was attempted by manual stimulation without a calf first. If let-down was successful, the cow was milked and moved into the release yard with the calf (Fig. 9).

If let-down was unsuccessful, the barrier was moved into place to block physical contact between the cow and calf. A non-kin calf was let into the calf race and the milker continued to use manual stimulation to elicit milk let-down with the calf present. If let-down was successful, the cow was milked, then both cow and calf were let into the release yard (Fig. 3).

If let-down was unsuccessful, the barrier was removed, and the calf given full physical contact access to the cow including suckling. When the calf had access to physical contact, the milker would not manually manipulate the udder but would only touch the teats to confirm if let-down was successful after being suckled by the calf. If let-down was successful, the cow was milked, and the non-kin calf was returned to calf yard B. The kin calf of the cow just milked was then let out with the maternal cow into the release yard.

If let-down was unsuccessful, the non-kin calf was returned to calf yard B. The kin calf would then be released into the calf race and allowed to suckle. If let-down was successful, the cow would be milked, and the kin calf and cow were let into the release yard.

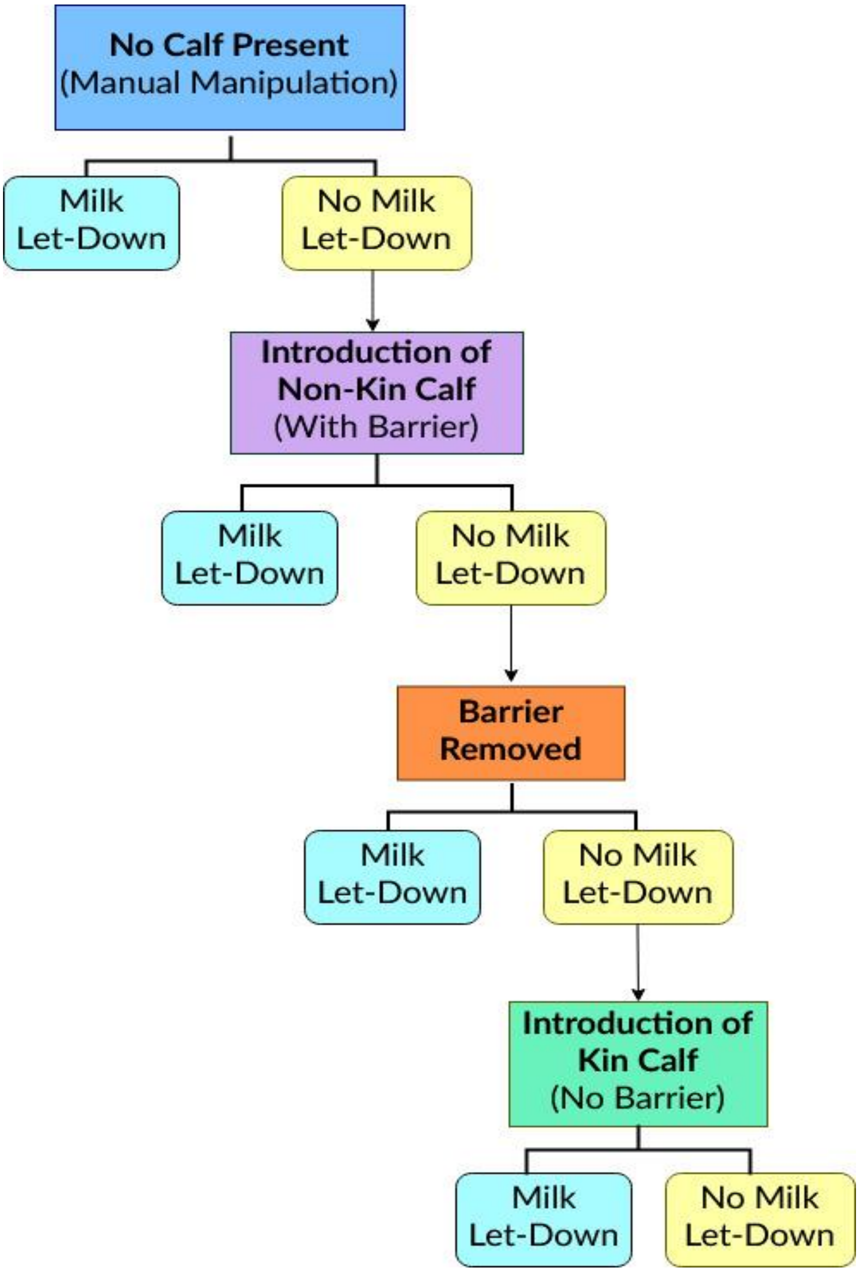

Figure 9. A flowchart illustrating the experimental procedure using non-kin calves.

The second last cow would be milked and moved into the release yard and her calf would remain or be returned to calf yard B to be potentially used as the non-kin calf for the last cow.

On rare occurrences where let-down was not initiated by the kin calf with no barrier, the decision was made to release the cow and calf into the paddock to hopefully allow natural suckling rather than increasing stress by keeping them in the race.

2.2.4 Treatment Conditions:

No calf – The milker uses manual stimulation to try and elicit milk let-down.

Kin calf with barrier – The kin calf is let into the calf race with the Perspex barrier placed on the fence to prevent physical contact between cow and calf.

Kin calf without barrier – The barrier is removed in order to allow physical contact and suckling by the kin calf.

Non-kin calf with barrier – The non-kin calf is let into the calf race with the Perspex barrier preventing physical contact between cow and calf.

Non-kin calf without barrier – The barrier is removed to allow physical contact and suckling by the non-kin calf.

2.2.5 Timing

The maximum time for each experimental condition was three minutes. Three minutes was decided as the maximum time allowed for each condition based on observations of six normal milking routines where the mean average let-down time was one minute and thirty seconds (90 seconds). After the three minutes finished, if no let-down had been achieved, the experiment would progress to the next treatment condition (Fig. 8 and Fig. 9).

Under the "No Calf" condition, timing was started from the point of udder contact by the milker. Timing started from the point of entry of a calf into the race under the conditions; Kin with barrier and Non-kin with barrier. For the conditions "Kin with no barrier and Non-kin with no barrier" timing started from the point of the milker exiting the calf race after removal of the barrier.

All behaviours were recorded for each treatment condition only in the race during the three-minute timing intervals.

2.2.6 Behaviours Recorded:

- Number of vocalisations from cow and calf
- Number of urinations from both cow and calf
- Number of defaecations from cow and calf
- Number of stomps from the cow
- Number of Kicks from the cow
- Number of times the cow bit the milker
- Number of times cows bit the calf
- Number of times the cow looked towards the milker
- Number of times the cow looked at the calf

2.2.7 Statistical Analysis

Milk let-down data was analysed using Chi-square analysis of contingency tables using Statview (version 4.57, Abacus Concepts inc. NJ, USA). Behavioural data was analysed using the Mann Whitney non-parametric test for non-normally distributed variables, with Bonferroni correction of p-values for multiple comparisons, using Statview (version 4.57, Abacus Concepts inc. NJ, USA).

2.3 Results

2.3.1 Milk Let-Down

During the baseline sessions the normal milking protocol, i.e. presence of the kin calf with no barrier, elicited successful milk let-down on 100% of attempts. During the treatment sessions, there was an overall effect of treatment, regardless of the barrier being present or not, on successful milk let-down ($\chi 2$=37.2; d.f.=2; P<0.0001), with the presence of the kin calf stimulating milk let-down by the cow on 73% (n=64) of attempts, compared to 20% (n=64) for the presence of the non-kin calf and 42% (n=108) when no calf was present (Table 3). There was also a significant effect of the barrier ($\chi 2$=24.8; P<0.0001), for when the barrier placed between the cow and calf, the kin calf elicited milk let-down on 50% of attempts, while the non-kin calf was unable to initiate let-down on any occasion. When the barrier was removed the kin calf successfully initiated let-down on 94% of attempts, while the non-kin calf was only successful on 40% of all attempts.

Table 3. Milk Let-Down across Treatment Conditions

	No Calf	Kin Calf	Non-kin Calf
Overall*	**41.7% (45/108)**	**73.4% (47/64)**	**20.3% (13/64)**
Barrier	**NA**	**50.0% (15/30)**	**0% (0/32)**
No Barrier	**NA**	**94.1% (32/34)**	**40.6% (13/32)**

Values are percentage successful let-down (number of successful let-downs/total attempts).* Total for No Calf, total (No Barrier and Barrier) for Kin and Non-kin. Assumption: carry-over effects from previous treatment not taken into consideration.

2.3.2 Behaviour During Milking

For cow vocalisations there was an increase from baseline levels to levels when no calf was present ($U=1818$, $P<0.0005$) but a decrease to levels for a kin calf with no barrier ($U=808$, $P<0.0001$). There was an increase in cow vocalisations comparing baseline to non-kin calf with barrier ($U=50$, $P<0.0004$).

Table 4. Cow behaviours in relation to treatment condition regardless of successful let-down. Values are mean (min. - max. range).

Behaviours	Baseline	No Calf	Kin Calf +Barrier	Kin Calf + No Barrier	Non-Kin Calf + Barrier	Non-Kin Calf + No Barrier
Vocalisations	0.222[a] (0-2)	1.196[b] (0-9)	1.166[ab] (0-6)	0.101[c] (0-3)	0.627 (0-11)	0.518 (0-11)
Urination	0.166 (0-1)	0.314 (0-2)	0.055 (0-1)	0.027 (0-1)	0.059 (0-2)	0.065 (0-2)
Defaecation	0.574[a] (0-2)	0.435 (0-2)	0.333 (0-2)	0.185[b] (0-2)	0.242 (0-2)	0.250 (0-2)
Stomping	0.037 (0-1)	1.000 (0-16)	0.370 (0-7)	0.037[a] (0-2)	0.759[b] (0-6)	0.846 (0-16)
Kicking	0.000 (0-0)	0.192 (0-6)	0.037 (0-1)	0.009 (0-1)	0.085 (0-5)	0.288 (0-5)
Biting Milker	0.037 (0-2)	0.009 (0-1)	0.018 (0-1)	0.009 (0-1)	0.018 (0-1)	0.000 (0-0)
Biting Calf	0.037 (0-1)	NA	0.000 (0-0)	0.046 (0-4)	0.074 (0-3)	0.384 (0-8)
Looks at Milker	2.037[a] (0-5)	4.583[b] (0-13)	2.407[abef] (0-10)	0.444[d] (0-6)	3.648[acg] (0-12)	2.576[abe] (0-16)
Looks at Calf	2.000[a] (0-7)	NA	2.611[ac] (0-12)	0.657[bd] (0-8)	2.425[abe] (0-11)	2.423[abce] (0-11)

Superscripts that are different between treatments within rows indicate significance using the Bonferroni correction of P-value.

For cow defaecations there was a decrease from baseline to kin-calf with no barrier (U=1979, P<0.0009).

For cow stomping there was a decrease in levels comparing non-kin with barrier to kin no barrier (U=297, P<0.001).

For cow looks at milker, there was an increase from baseline levels to levels when no calf was present (U=1080, P<0.0001), when a kin calf was present without a barrier (U=720, P<0.0001). There was a decrease in levels comparing no calf with a kin calf with a barrier (U=544.4, P<0.0001). There was an increase in levels comparing a kin calf with barrier to a non-kin calf with barrier (U=256, P<0.0016). There was a decrease in levels comparing a kin calf with barrier to a kin calf without barrier (U=150.5, P<0.0001). There was an increase in levels comparing non-kin calf to a kin calf without a barrier (U=1071.5, P<0.0001) and comparing a non-kin calf without a barrier to a kin calf with a barrier (U=228, P<0.0001). However, there was a decrease in levels comparing a non-kin calf with a barrier to a non-kin calf without a barrier (U=284, 0.0036).

For cow looks at calf there was a decrease from baseline levels comparing a kin calf without a barrier (U=1630.5, P<0.0001). There was a decrease in levels comparing a kin calf with barrier to a kin calf without a barrier (U=241.5, P<0.0001), comparing a non-kin calf with a barrier to a kin calf without a barrier (U=323.5, P<500) and comparing a non-kin without barrier to a kin calf without a barrier (U=326.5, P<0.0055).

Table 5. Calf behaviours in relation to treatment condition. Values are mean (min - max range)

Behaviours	Baseline	No Calf	Kin Calf + Barrier	Kin Calf + No Barrier	Non-Kin Calf + Barrier	Non-Kin Calf + No Barrier
Vocalisations	0.092[ac] (0-3)	NA	2.888[b] (0-19)	0.120[c] (0-6)	4.740[bd] (0-19)	2.442[be] (0-15)
Urination	0.277 (0-2)	NA	0.333 (0-2)	0.148 (0-2)	0.296 (0-2)	0.173 (0-2)
Defaecation	0.018 (0-1)	NA	0.000 (0-0)	0.009 (0-1)	0.018 (0-1)	0.000 (0-0)
Udder Nudges	29.09[a] (10-82)	NA	NA	8.70[b] (0-84)	NA	6.37[c] (0-42)

Superscripts that are different between treatments within rows indicate significance using the Bonferroni correction of P-Value.

For calf vocalisations there was an increase in levels comparing baseline to a kin calf with barrier (U=808.5, P<0.0001). There was a decrease in levels comparing a kin calf with a barrier to a kin calf without a barrier (U=200, P<0.0001), comparing a non-kin calf with barrier to a kin calf without barrier (U=40.5, P<0.0001) and a non-kin calf without barrier (U=262.5, P<0.0013). There was a decrease in levels comparing a non-kin calf without a barrier to a kin calf without a barrier (U=244, P<0.0001).

For calf udder nudges there was a decrease in levels comparing baseline to a non-kin calf without a barrier (U=308, P<0.001), and a kin calf without a barrier (U=815.5, P<0.0001). However, there was an increase in levels comparing a non-kin calf with a barrier to a kin calf without a barrier (U=247, P<0.0001).

For milk let-down, there was a decrease in levels from no calf to kin calf without a barrier (U=257, P<0.0001) and non-kin calf without a barrier (U=123, P<0.0016).

For cow vocalisations, there was an increase from no calf levels to levels with a kin calf with a barrier (U=1226, P<0.0001) However, there was a decrease in cow vocalisations when comparing no calf to a kin calf without a barrier (U=3281, P<0.0001) and a non-kin calf without a barrier (1359, P<0.0004).

For calf vocalisations, there was a decrease in levels comparing a kin calf with a barrier to a non-kin calf with no barrier (U=232, P<0.0023) and a decrease in vocalisations comparing a non-kin calf without a barrier to a kin calf without a barrier (U=244, P<0.0001).

For cow urination, there was a decrease in urination events comparing no calf present to a kin calf present with no barrier (U=1617.5, P<0.0018).

For cow defaecation, there was an increase in cow defaecation when comparing a kin calf with a barrier to a non-kin calf without a barrier (U=232, P<0.0023). However, there was a decrease in levels comparing a non-kin calf without barrier to a kin calf without a barrier (U=258, P<0.0021).

For cow stomping, there was an increase in levels comparing no calf present to a kin calf with a barrier (U=1475, P<0.0056).

For cow udder nudges, there was a decrease in levels from a kin calf with no barrier to a non-kin calf with no barrier (U=46.500, P<0.0001).

For cow looks at the milker, there was a decrease in levels comparing no calf present to a kin calf with a barrier (U=1453.5, P<0.0041) However, there was a further decrease in levels when comparing no calf to a kin calf without a barrier (U=1258, P<0.0001) and a non-kin calf without a barrier (U= 1379.5, P<0.0006).

For cow looks at the calf, there was a decrease in levels comparing a kin calf without a barrier to a non-kin calf without a barrier ($U = 151$, $P < 0.0001$)

Table 6. Time to Let-down (seconds) and behaviours where let-down was successful per treatment condition. Values are Mean ± SE

Behaviours	No Calf	Kin + Barrier	Kin + No Barrier	Non-Kin + Barrier	Non-kin + No Barrier
Milk Let-down Time	108.62 ± 4.65^{a} *(n=45)*	92.60 ± 4.53^{b} (n=15)	76.10 ± 4.17^{c} (n=31)	NA	84.15 ± 6.56^{bc} (n=13)
*** Cow Vocalisations**	0.42 ± 0.12^{ac}	2.25 ± 0.38^{bdf}	0.31 ± 0.11^{beg}	NA	0.15 ± 0.10^{e}
Calf Vocalisations	NA	5.57 ± 0.88^{ac}	0.37 ± 0.21^{df}	NA	0.61 ± 0.33^{be}
Cow Urination	0.11 ± 0.04^{a}	0.10 ± 0.10	0.09 ± 0.05^{b}	NA	0.08 ± 0.08
*** Calf Urination**	NA	0.64 ± 0.13	0.46 ± 0.10	NA	0.15 ± 0.10
Cow Defaecation	0.05 ± 0.10	0.64 ± 0.13^{a}	0.60 ± 0.10^{ad}	NA	0.85 ± 0.19^{bc}
*** Calf Defaecation**	NA	0	0.02 ± 0.02	NA	0
Cow Stomping	0.36 ± 0.17^{a}	0.71 ± 0.29^{b}	0.11 ± 0.07	NA	0
Cow Kicking	0.02 ± 0.02	0.07 ± 0.05	0.03 ± 0.03	NA	0
Cow Biting Milker	0	0.04 ± 0.04	0.03 ± 0.03	NA	0
**** Cow Biting Calf**	NA	NA	0.14 ± 0.11^{a}	NA	23.77 ± 2.91^{b}
**** Calf Udder Nudges**	NA	NA	26.86 ± 3.35^{a}	NA	23.76 ± 2.91^{b}
Cow Looks at Milker	3.64 ± 0.44^{a}	3.00 ± 0.41^{c}	1.37 ± 0.27^{d}	NA	2.00 ± 0.57^{b}
***Cow Looks at Calf**	1.76 ± 0.30	4.50 ± 0.56^{b}	2.03 ± 0.32	NA	1.77 ± 0.52^{a}

Bonferroni correction: No astrix P<0.008, *P<0.017, **P<0.05. (K+B, n=15). (K+NB, n=31). (NC, n=45). (NK+NB, n=13). (NK+B, n=0).

For cow vocalisations, there was a decrease in level when comparing no calf present to a kin calf without a barrier ($U=3281$, $P<0.0001$) and a non-kin calf with a barrier ($U=1203.5$, $P< 0.0009$). However, there was an increase in cow vocalisations when comparing no calf present to a kin calf with a barrier ($U=1226$, $P<0.001$) and a non-kin calf without a barrier ($U=1359$, $P<0.0004$).

For calf vocalisations, there was an increase in levels comparing a kin calf with a barrier to non-kin with a barrier ($U=197.5$, $P<0.0004$). However, when comparing kin calf with barrier to kin calf without barrier ($U=193$, $P<0.0010$) and non-kin calf without barrier ($U=195.5$, $P<0.0004$), there was a decrease in levels.

For cow urination there was a decrease in levels when comparing no calf to kin calf with no barrier ($U=1617.5$, $P<0.0018$) and non-kin calf with no barrier ($U=1518$, $P<0.0048$).

For cow defaecation, the levels for kin calf with a barrier were the same for non-kin calf with barrier ($U=232$, $P<0.0023$). There was a decrease in levels when comparing kin without barrier to non-kin without barrier ($U=258$, $P<0.0021$).

For cow stomping, there was a decrease in levels comparing no calf to kin calf with barrier ($U=1475$, $P<0.0056$), non-kin with barrier ($U=1309.5$, $P<0.005$). There was a decrease in levels comparing a non-kin calf with a barrier to a kin calf without a barrier ($U=318.5$, $P<0.0026$) and a non-kin calf without a barrier ($U=162$, $P<0.0070$). However, there was an increase in levels comparing no calf to non-kin without a barrier ($U=1513$, $P<0.0045$).

For calf udder nudges there was a decrease in levels from kin calf with a barrier to non-kin calf without a barrier ($U=46.5$, $P<0.001$).

For looks towards the milker, there was a decrease in levels comparing no calf to kin calf with a barrier (U=1453.5, P<0.006) and kin calf without a barrier (U=1258, P<0.001). However, there was an increase in levels comparing kin calf with barrier to a non-kin calf with a barrier (U=145, P<0.001) to non-kin without a barrier (U=145, P<0.0001).

For the cow looks towards the calf, there was an increase in levels comparing a kin calf without a barrier to a non-kin calf with a barrier (U=300.5, P<0.0012) and a non-kin calf without a barrier (U=151, P<0.0001).

Table 7. Time to Let-down (seconds) and behaviours where let-down was NOT successful per treatment condition. Values are Mean ± SE

Behaviours	No Calf	Kin + Barrier	Kin + No Barrier	Non-Kin + Barrier	Non-Kin + No Barrier
Milk Let-down Time	NA	NA	NA	NA	NA
Cow Vocalisations	1.69 ± 0.29[a]	3.57 ± 0.49[c]	1.00 ± 0.71[d]	1.61 ± 0.34[b]	2.00 ± 0.78[b]
* Calf Vocalisations	NA	7.00 ± 1.52[a]	1.50 ± 1.50 [d]	7.80 ± 0.88[b]	6.61 ± 1.11[c]
Cow Urination	0.48 ± 0.08[a]	0.14 ± 0.10	0.25 ± 0.25 [bc]	0.06 ± 0.04	0.39 ± 0.14[b]
* Calf Urination	NA	0.50 ± 0.20	0.50 ± 0.29	0.48 ± 0.11	0.39 ± 0.14
Cow Defaecation	0.41 ± 0.07	0.42 ± 0.14[a]	0.25 ±0.25[abd]	0.42 ± 0.09[b]	0.11 ± 0.08[c]
*Calf Defaecation	NA	0	0	0.03 ± 0.03	0
Cow Stomping	1.57 ± 0.39[a]	1.07 ± 0.49[bed]	0[acd]	1.32 ± 0.29[b]	2.44 ± 1.09[c]
Cow Kicking	0.31 ± 0.13	0.14 ± 0.10	0.25 ± 0.25	0.16 ± 0.08	0.83 ± 0.37
Cow Biting Milker	0.02 ± 0.02	0.07 ± 0.07	0.25 ± 0.25	0.03 ± 0.03	0
* Cow Biting Calf	NA	NA	1.00 ± 1.00	NA	1.11 ± 0.50
* Calf Udder Nudges	NA	NA	11.75 ± 2.56[a]	NA	1.22 ± 0.73[b]
Cow Looks at Milker	5.10 ± 0.29[ae]	5.07 ± 0.54[c]	2.50 ± 1.32[d]	6.03 ± 0.39[e]	6.00 ± 0.80[b]
* Cow Looks at Calf	NA	4.21 ± 0.76	3.50 ± 1.85[b]	4.19 ± 0.54[a]	5.72 ± 0.63[c]

Bonferroni Correction: No astrix P<0.005. With * Correction is P<0.008. (KB, n=14). (K+NB, n=4). (NC, n=61). (NK+NB, n=18). (NK+B, n=31).

2.4 Discussion

In support of the first hypothesis, the most successful condition involved in achieving successful let-down was the presence of the kin calf. Regardless of the barrier, the overall success rate for milk let-down using a kin calf was 73% compared to 20% for the non-kin calf and 42% for no calf present. This agrees with the majority of literature that found the presence of the kin calf was “essential” or the most commonly practised method, for achieving milk let-down in dromedary camels (Eisa *et al*., 2010; Eisa & Mustafa, 2011; Eyassu, 2009). However, it is clear from this study that it is still possible to achieve milk let-down using no calf or a non-kin calf. These findings indicate that although the presence of the kin calf is better for initiating milk let-down, it is not always an absolute requirement. Therefore, this study adds weight to the claims within the minority of literature, such as Werney *et al.,* (2004) and Moufida *et al.,* (2014), that state the presence of the calf is unnecessary. Moreover, let-down was achieved without a calf on 42% of all attempts, and by a non-kin calf (without a barrier) on 20% of all attempts. While this result may lead to novel management strategies within the camel dairy industry, it also emphasizes the importance of sensory interactions provided by the non-kin calf including physical contact during milking.

In support of the second hypothesis, the most successful condition for inducing let-down with physical contact (i.e. no barrier) was the kin calf with a success rate of 94% compared to 41% for the non-kin calf and 42% with no calf. The addition of physical contact and suckling seems to be the most efficient method of eliciting milk let-down (Gjøstein *et al*., 2014; Combellas & Tesorero, 2003; Orihuela, 1990). As considered by Gjøstein *et al*. (2014), it is also possible that (regardless of kin or non-kin) having audio, visual and olfactory communication with a calf, the inability to come into physical contact may cause some level of emotional stress for the camel cows and may be why the presence of a calf alone was not more successful in initiation let-down. The physiology of milk let-down is a neurohormonal reflex arc that partially relies on stimulation of the afferent nerves in udder that transmit a neural signal to the brain which releases the hormone critical for milk let-down, *oxytocin* (Wellnitz & Bruckmeir, 2001; Bruckmier, 2005). Negrão (2014) reported that the Holstein Friesian cows, a very well-established dairy breed, produced the highest concentrations of *oxytocin* and *prolactin* when they were suckled by their own calves. Future work that may help to quantify and understand the impact that different treatments (no calf or non kin calf) has on the dromedary camel would be to measure *oxytocin* during the let down and milking period.

Dromedaries are a relatively naïve species to the dairy industry overall and in Australia many camels used for dairy are still captured from the wild. Considering this, it is not unreasonable to suggest that the maternal instincts of the camel are preventing reliable milking without a calf. This has also been speculated upon in reindeer by Gjøstein *et al*., (2014), who also discussed the possibility that the reindeer does were suppressing milk ejection to save milk for the calf. Alternatively, there may be other significant primitive sensory pathways that are not fully understood in naïve dairy species. However, it is evident from this study that it is possible to achieve let-down in a Dromedary camel without a calf present, or by using a non-kin calf. This raises the question of which behaviours between cow and calf are the most important in achieving let-down. To our knowledge, no studies have been undertaken to analyse which specific senses or behaviours between cow and calf may be the most influential in initiating let-down in the camel.

By comparing the baseline sessions to the experimental sessions, the dominant behaviours associated with let-down were cow and calf vocalisations, vigilance of the cow looking at the calf and udder nudges. Regardless of success of let-down, levels of vocalisations were increased across all experimental conditions compared to baseline levels. Looks towards the towards the calf were also increased during the experimental conditions compared to the baseline sessions, with the exception of a kin-calf without a barrier where levels were actually decreased compared to baseline. These behaviours may be possible signs of stress or agitation. Vocalisations and vigilance have been used as indicators of stress in studies experimenting with the presence of a calf in primitive cattle species (Rushen *et al*., 2001; Welp *et al.*, 2004). Moufida *et al*. (2014) discussed that camel cows in their study would sometimes display behaviours that were deemed stress indicators and yet still let-down. Therefore, it cannot be discounted that the cow may be just trying to visually locate or audibly communicate with her own calf. Atigui *et al.*, (2014) have shown that the milking routine and environmental perturbation will alter the time to let down and reduce milk yield, highlighting the importance of better understanding of behaviours associated with mild let down. To investigate the significance and to better interpret camel behaviours during the let-down period the collection of behavioural data could be coupled with blood metabolites associated with stress such as cortisol, for example.

The apparent significance of udder nudges is an understandable result considering the neurosensory pathway of milk let-down that requires physical stimulation of the nerves in the teats. However, it is interesting to note that under experimental conditions, the kin-calf nudged the udder more compared to the non-kin calf. Moreover, by far the highest values for udder nudging were observed by the kin calves in the baseline sessions.

Frequency of udder nudging may indicate perceived likelihood by the calf in achieving milk let-down, i.e. a non-kin calf may give up earlier if it realises the cow is not its own mother, or certain sensory or behavioural cues between the cow and calf are not indicative of let-down success.

A limitation within the experimental design of the current experiment included not being able to fully test visual, olfactory and auditory cues. Due to ethical and logistical limitations it was possible to only somewhat block physical contact using the polycarbonate barrier. Strategies from experiments or studies using other species could be adapted to investigate the importance of these specific senses in future camel research. Future research in this area would be sensory deprivation to explore which specific sense has the greatest impact on milk let-down and if any manipulation of the senses could lead to reliable let-down without a calf.

Recommendations for future study also include investigating the use of a dummy calf in stimulating let-down. Elmi 1989 and Eisa & Mustafa, 2011 agree that the use of a glove made from the skin of a deceased calf can initiate let-down in camels and Singh *et al.*, 2017 found success in initiating let-down in buffaloes using a model calf.

Although allosuckling was not specifically researched for in this experiment, it was observed. Allosuckling has been researched in several mammalian species including Bactrian camels but it is accepted as non-applicable in Dromedary camels (Bradlová *et al*., 2013; Packer *et al*., 1992). Given the results of this study and the lack of literature surrounding allosuckling in Dromedaries, it is evident that this area is severely unexplored. Knowledge in this area could greatly contribute to our understanding of

Dromedary camels behaviour and physiology and therefore, may lead to changes in management strategies.

The findings from this study that demonstrated a higher rate of successful let-down for a calf (kin or non-kin) without a barrier, indicate that physical contact is important. The industry implications for this could be to specialise the design of milking machine cups to fit the camel teat better or to consider more efficient pressure and pulsation rates. Pre-stimulation protocols of machines may also be changed in order to more closely mimic the natural udder nudging behaviour of a camel calf to improve let-down time.

The genetic improvement of Australian dairy camels should be emphasised in the near future. There is a lack of information and records of individual camels and their progeny as many are still being captured from the wild therefore, breeding and genetic records should be encouraged among Australian camel farmers to avoid inbreeding and improve desired characteristics. Camels have a long lifespan, a long gestation period and subsequently, a long generation interval (Hermes, 1998; Nagy & Juhasz, 2016; Khanvillkar *et al.*, 2009) all of which work against the process of selective genetic improvement. To overcome this, camels should be synchronised early in the breeding season, be given supplemental feed and calves should be weaned earlier (Hermes, 1998).

The use of breeding technologies such as artificial insemination (AI) and embryo transfer (ET) could prove advantageous in shortening the generation interval, increasing the selection intensity and improving the genetic pool available for use (Yagil *et al*., 1994; Hermes, 1998; Skidmore *et al*., 2011). Methods such as these have been thoroughly researched and implemented within the sheep and cattle industries (Rosendo-Ponce &

Becessil-Pérez, 2002; Barillet, 2007; Miller, 2010) so it may be concluded that similar breeding records and practises will yield beneficial results in the camel industries.

2.5 Conclusions

This experiment has shown that it is possible to stimulate milk let-down in the Dromedary camel without the calf suckling. This is an important finding as camel dairies throughout the world currently require the calf to be present at milking in the dairy.

This experiment has contributed to understanding of the behaviours associated with successful let-down and milking in the Dromedary camel. Identifying these behaviours will assist with developing better stimulatory milking procedures and ultimately improve the efficiency of milking in the dairy. Ultimately, if milk let-down can further be enhanced the presence of the calf in the dairy may be limited or unnecessary as it is in domestic dairy cattle.

The outcomes from this project have opened new lines of questioning that warrant further investigation. The collection of physiological data that may measure and quantify the hormones associated with milking (e.g. *oxytocin* and *prolactin*) would be beneficial to better evaluate let down techniques when compared to baseline data. Additionally the stress that various let down techniques elicit on the camels may be better linked to behaviours by collecting information on stress associated hormones (e.g. cortisol) Therefore, this experiment may act as a platform to launch future study into the management and understanding of Dromedary camels and may be used to improve industry practises within the camel dairy industry.

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