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Histochemical and Immunohistochemical Examination of Cranial and Caudal Gland Alveolar Secretion of the Same Lactating Mammary Gland Quarter of One Humped She Camel (*Camelus dromedarius*)

Ola RH[1], Yasmine H. Ahmed[1], El-Saba AA[1], Khalifa EF[2] and El-Bargeesy GA[1]

[1]Department of Cytology and Histology, The Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Department of Anatomy and Embryology, The Faculty of Veterinary Medicine, Cairo University, Egypt
***Corresponding author:** olaraouf89@gmail.com

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ABSTRACT

The differences in milk composition between different parts of the same mammary gland in camel milk have been a point of interest in previous studies. Therefore, the aim of this study is to demonstrate the differences in the distribution of carbohydrates, mucins and caseins between the cranial and caudal gland of the same lactating mammary gland quarter through testing the histochemical differences by Periodic Acid Schiff (PAS), Alcian Blue (AB), and immunohistochemical differences by α and β-casein. The examined mammary glands samples were collected from 6 healthy mature one-humped she-camels (Camelus dromedaries) on mid of lactation just after slaughtering. Sections were fixed in 10% neutral buffer formalin then subjected to the histochemical and immunohistochemical examination. The intensity of the PAS reaction differed throughout the mammary gland; the cranial gland alveolar secretion showed more intense magenta color when compared to the caudal gland. The same lactating mammary gland quarter showed darker blue alveolar secretion in cranial gland when stained with AB in comparison with the caudal one which showed significant faint colored secretion. The alveolar secretion of the cranial gland showed stronger reaction for α- and β-casein than the caudal gland alveolar secretion. The positive staining of β-casein in cranial gland was localized on the alveolar secretion as well as in most of the cells surrounding the lumen of the alveoli. In contrast, week positive staining appeared in the lumen and was not observed in alveolar cells for α-casein in cranial gland and for α and β -casein in caudal gland.

Key words: She-Camel Mammary PAS, Alcian Blue, α-casein, β-casein.

INTRODUCTION

Camels have special anatomical and physiological features help them to survive, reproduce and produce meat and milk in areas where other species couldn't survive (Eisa 2006). In times of drought, the lactating camel loses water in its milk, a natural adaptation to provide the dehydrated calf with nutrients and the necessary fluid (Sisay and Awoke 2015). Camels can produce a sufficient amount of milk in drought areas where other domestic animals produce very little milk (Sisay and Awoke 2015).

Compared to dairy cows, camel milk has been neglected in milk production, and there is little literature on differences in milk yield and composition of the various udder parts. Recently, camel milk was given much attention in researches, due to its potential therapeutic properties (Agrawal et al. 2007; Quan et al. 2008; Ranjan et al. 2022). In addition, camel milk contains all the important nutrients that are also contained in cow's milk (Alhaj et al. 2010; Alhafiz et al. 2022) with recommendation for consumption by persons allergic to cow's milk (Elsayed et al. 2009). That leads to restudying the structure of the camel mammary gland to understand the variations in the anatomy of the mammary gland and the composition of its milk.

Rizk et al. (2017) reported that each teat of the she camel mammary gland is opened dorsally by cranial and caudal orifices, and each orifice leading to a separate distinct gland. They found that the cranial gland was more active than the caudal gland. On other hand, Rizk et al. (2017) mentioned some differences in the fluid produced from each mammary gland. This point is what will be discussed in the recent study from the histological point of view.

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Al-Bazii et al. (2019) and Hue-Beauvais et al. (2021) pointed to the fact that the milk secretion within the alveolar lumen and ducts of lactating mammary gland contains three main components of the milk (carbohydrates, proteins and lipids).

For demonstration of carbohydrates, Swapna et al. (2020) studied the usefulness of PAS staining in identification of carbohydrates in mammary gland by using the colour intensity of the PAS reaction. They concluded that the PAS technique is perhaps the most versatile and widely used technique for the detection of carbohydrates, mucins and glycoproteins. On the other hand, Sulochana and Singh (1995) and Shedge et al. (2018) used PAS and Alcian blue (AB) methods for carbohydrates detection in mammary gland parenchyma.

As for proteins, casein is the main protein in camel milk and accounts for about 52-87% of the total proteins. Camel milk contains more β-casein than α-casein, 65 and 21% of total casein, respectively (Kumar et al. 2016). The β-casein is more digestible and less allergic to humans because it responds better to peptic hydrolysis in the intestine. The higher content of β-casein makes camel milk beneficial for human health (Kumar et al. 2016; Swelum et al. 2021).

Therefore, the aim of this study is to demonstrate the differences in the distribution of carbohydrates, mucins and casein as a main protein component in the cranial and caudal gland parenchyma of the same mammary gland quarter through testing the histochemical differences by PAS, Alcian blue, and immunohistochemical differences by α and β-casein.

MATERIALS AND METHODS

Sample Collection

The examined samples of mammary glands were collected from 6 healthy mature one-humped she-camels (Camelus dromedaries) on mid of lactation just after slaughtering. The study was conducted in accordance with the ethical principles of the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Cairo University, Egypt.

Small pieces (5mm in thickness) were taken from the glandular parenchyma of cranial and caudal glands of the same mammary gland quarter. These specimens were fixed in 10% neutral buffered formalin for 24 hours, then were embedded in paraffin in accord with standard procedures. The 4-5μm paraffin sections were prepared with a rotatory microtome and stained with Periodic Acid-Schiff (PAS) and Alcian Blue stain (AB) and examined by light microscopy (Bancroft and Gamble 2013).

Immunohistochemical Evaluation

The paraffin sections were prepared and processed according to the manufacturer's directions (Bioss Antibodies). Slides were put on a positively charged slide, baked at 60°C oven for 1 hour and then loaded onto the Benchmark GX. Slides were treated with the automated benchmark system through a series of deparaffinization and antigen retrieval steps. The antibodies were pre-diluted and incubated for 16 mins at 37ºC. The counterstain and post-counter stain comprise Hematoxylin for 4 mins and Post Counterstain by Bluing reagent for 4 mins.

Primary Antibodies Used

α-Casein polyclonal antibody (bs-8245R, 28kd, Rabbit, Bioss), β-Casein polyclonal antibody (bs-10032R, 24kd, Rabbit, Bioss).

Scanning and Photos Capturing

All histochemical and immunohistochemical stained-glass slides were scanned by using Philips digital pathology solution medical device, version 3.2, SN 62, REF 760001. The results were processed, stored and viewed on Image Management System 3.3 Application Server and Storage software and Image Management System 3.3 Viewer software.

RESULTS

PAS Staining Observations

Sections of lactating mammary gland stained with PAS, showed magenta color. The intensity of the PAS reaction differed throughout the mammary gland; the cranial gland alveolar secretion showed more intense magenta color (Fig. 1A) in comparison to the caudal gland (Fig. 1C). The alveolar epithelium in cranial gland (Fig. 1B) was comparatively darker than the alveolar epithelium of the caudal gland (Fig. 1D). In caudal gland, the alveolar epithelium was filled with negative stained cytoplasmic vacuoles (Fig. 1D).

Alcian Blue (AB) observations

The same lactating mammary gland quarter showed darker blue alveolar secretion in cranial gland (Fig. 2A) in comparison with the caudal one which showed significant faint colored secretion (Fig. 2B).

α and β casein investigations

In tissue sections from the same mammary gland quarters, positive staining for both α- and β-casein was found in the cranial glands (Fig. 3A, 3B; Fig. 4A, 4B). The alveolar secretion of the cranial gland showed stronger reaction for α- and β-casein than the caudal gland alveolar secretion (Fig. 3; Fig. 4).

The cranial gland was less intensely stained for α-casein than for β-casein. The positive staining of β-casein in cranial gland was localized on the alveolar secretion in in cranial gland was localized on the alveolar secretion in the lumen as well as in most of the cells surrounding the lumen of the alveoli (Fig. 3A, 3B). In contrast, week positive staining appeared only in the lumen and was not observed in alveolar cells for α-casein in cranial gland or α and β-casein in caudal gland.

DISCUSSION

The gross examination of the camel mammary gland in previous studies revealed that it is composed of four teats. Each teat is ended by two separate orifices; cranial and caudal (Rizk et al. 2017; Ola et al. 2022). Each orifice leads to a separate streak canal and separate gland cistern (Zayeed et al. 1991; Kausar et al. 2001). On examination of the two glandular cisterns, it was noticed that the cranial glandular cistern was considerably wider than the caudal one (Rizk et al. 2017).

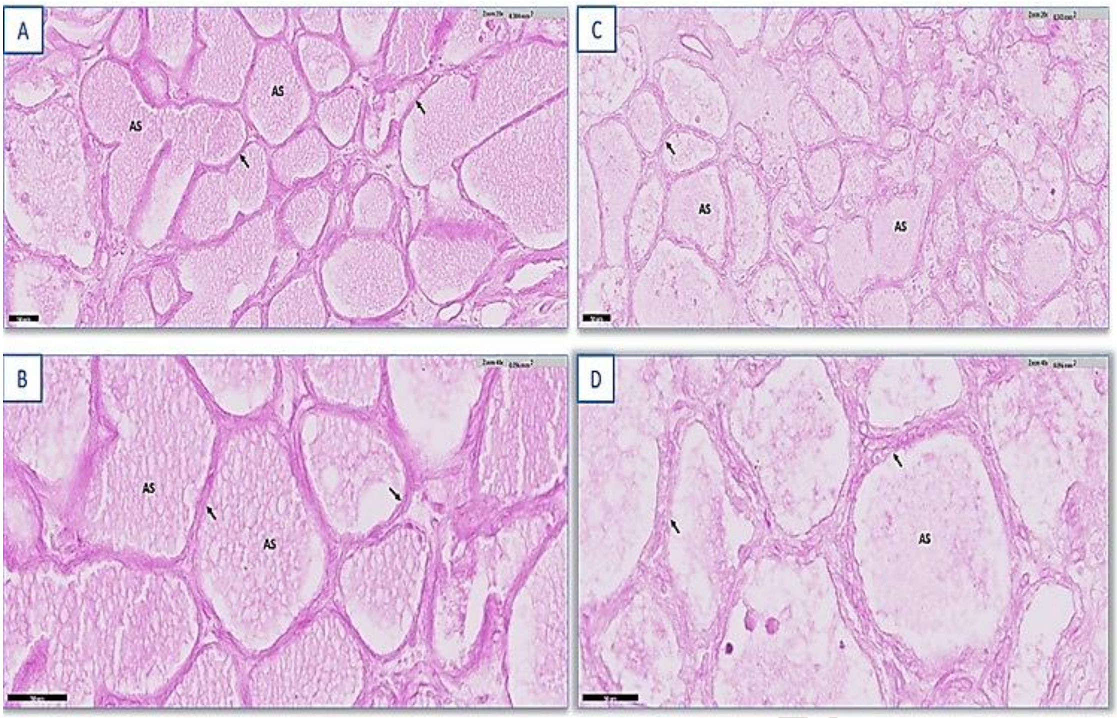

Fig. 1: Photomicrograph of the cranial and caudal gland parenchyma of the same quarter of mammary gland during lactation stained with PAS stain. A) Cranial gland contained dark magenta colored alveolar secretion (AS), lined by dark stained epithelium (arrow), B) Cranial gland contained dark magenta colored alveolar secretion (AS), lined by dark stained epithelium (arrow), C) Caudal gland contained faint magenta colored alveolar secretion (AS), lined by light-stained epithelial cells (arrow), and D) Caudal gland contained faint magenta colored alveolar secretion (AS), lined by light-stained epithelial cells filled with negative stained cytoplasmic vacuoles (arrow). PAS; A and C=X200; B and D=X400.

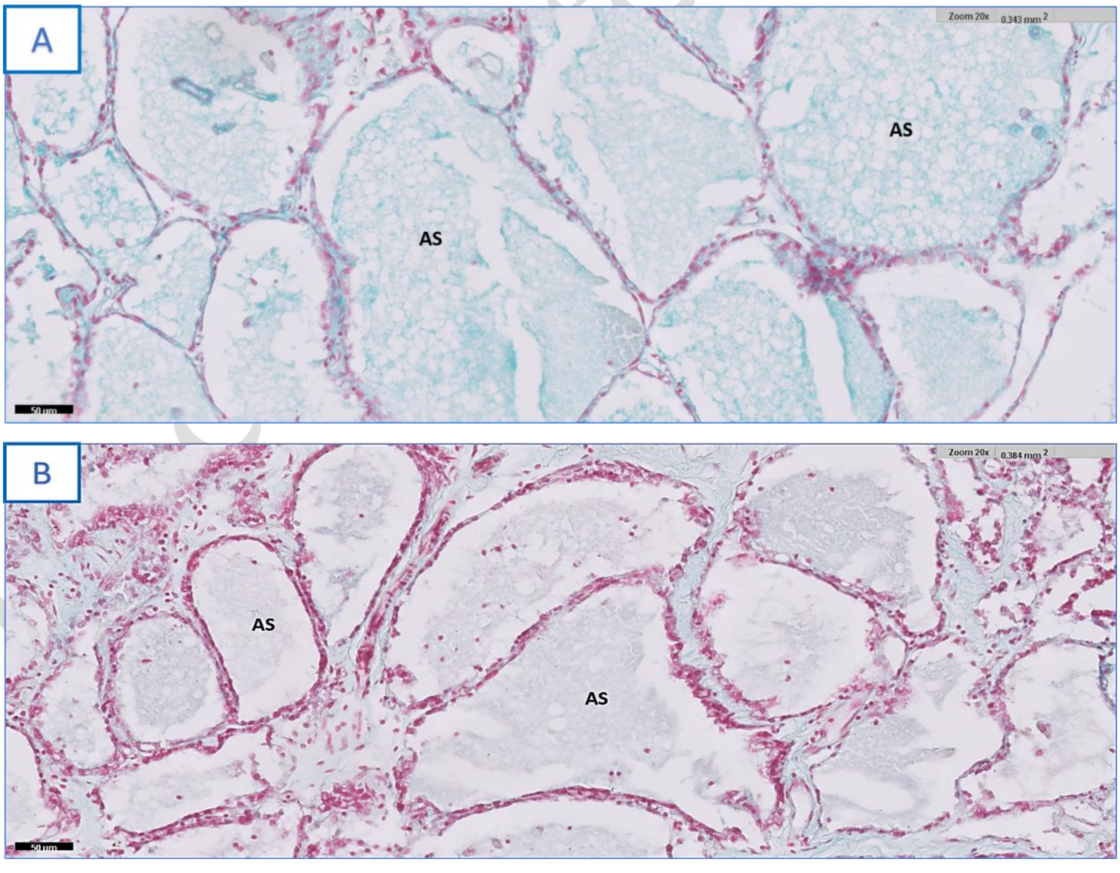

Fig. 2: Photomicrograph of the cranial and caudal gland parenchyma of the same quarter of mammary gland during lactation stained with Alcian Blue stain. A) Cranial gland contained dark blue stained alveolar secretion (AS), and B) Caudal gland of lactating mammary gland contained light-stained alveolar secretion (AS). Alcian Blue; X200.

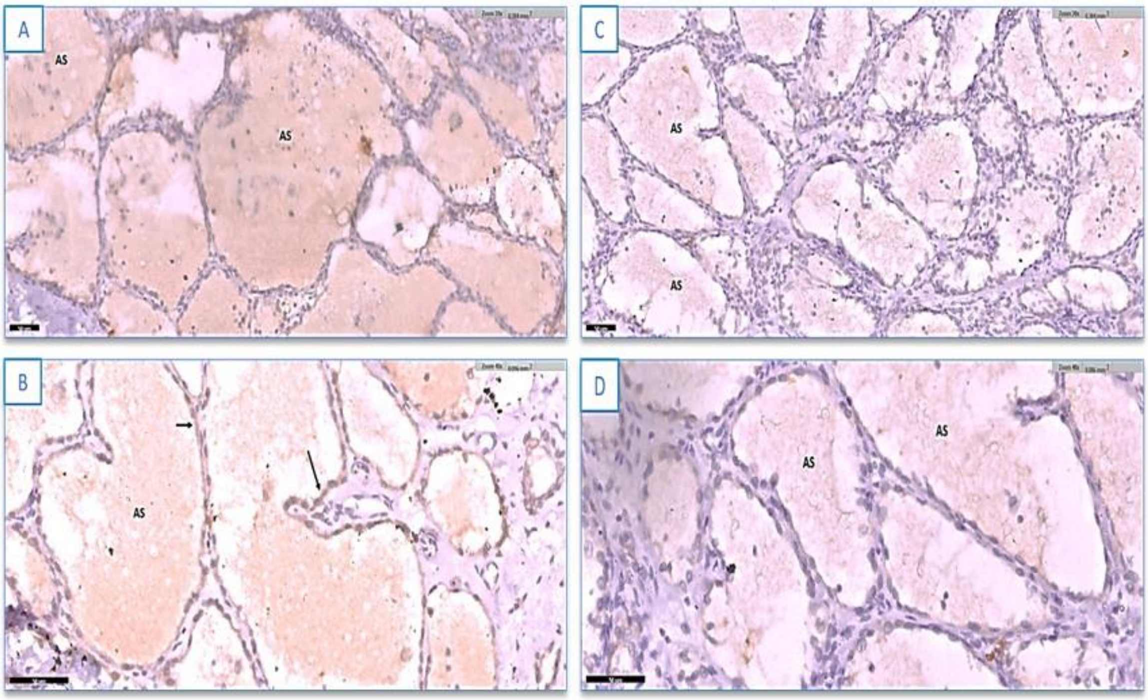

Fig. 3: Photomicrograph showing the difference in the expression of β-casein between cranial and caudal gland of the same lactating mammary gland quarter. A) Strong expression at the alveolar secretion (AS) and alveolar epithelium (arrow) of the cranial gland, B) Strong expression at the alveolar secretion (AS) and alveolar epithelium (arrow) of the cranial gland, C) Week expression at the alveolar secretion (AS) of the caudal gland, and D) Week expression at the alveolar secretion (AS) of the caudal gland. Fig. A and C=X200; B and D=X400.

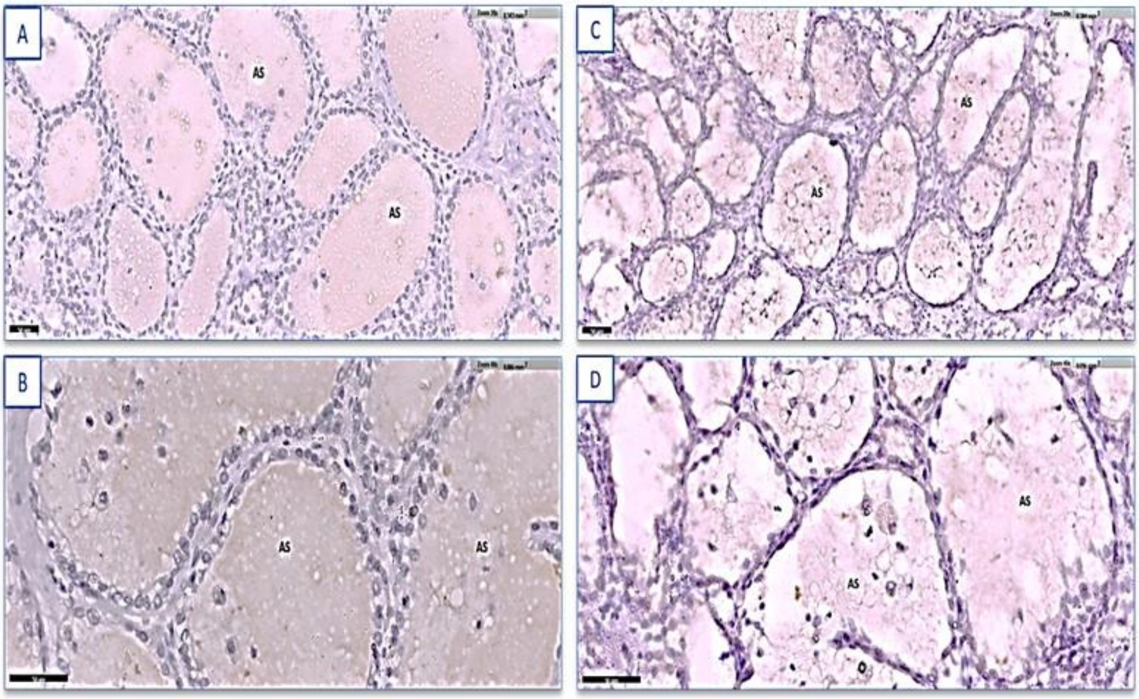

Fig. 4: Photomicrograph showing the expression of α- casein between cranial and caudal gland of the same lactating mammary gland quarter. A) Week expression at the alveolar secretion (AS) at the cranial gland, B) Week expression at the alveolar secretion (AS) of the cranial gland, C) scarce expression at the alveolar secretion (AS) at the caudal gland, and D) Scarce expression at the alveolar secretion (AS) at the caudal gland. Fig. A and C=X200; B and D=X400.

Rizk et al. (2017) found that the fluids produced from the two milk systems showed differences in consistency and color. The fluid from the caudal system was watery, whereas the cranial system was milky. The amount of fluid produced was greater in the cranial gland.

To identify the carbohydrates in the secretion of the lactating mammary gland, Ellen et al. (2017) and Swapna et al. (2020) used PAS stain during different stages of milk production. The results of these studies revealed that the intensity of the PAS reaction differed throughout the milk production process in a direct correlation to the level of carbohydrates and mucins in the secretion.

In the recent study, the intensity of the PAS and AB reaction differed throughout the same mammary gland. The cranial gland of the lactating mammary gland quarter showed more intense PAS and AB positive stained color when compared to the caudal gland. The alveolar epithelium in cranial gland was comparatively darker. That indicates the alveolar secretion of the cranial gland is richer in PAS and AB positive substances like carbohydrate, neutral and acid mucins than caudal one.

Leal et al. (2017) and Shedge et al. (2018) mentioned that mucins are high molecular weight glycoproteins known by their ability to form gels; therefore, mucins are an important component of most gel-like secretions. Therefore, the high level of mucins detected in the cranial gland secretion in the current study, may illustrate the findings of Rizk et al. (2017) that the produced fluids from the caudal lactating camel mammary gland was watery, while the cranial gland was milky.

In the present study, both cranial and caudal glands showed stronger reaction to β-casein than the α-casein. These results agreed with Kumar et al. (2016), Ryskaliyeva et al. (2018) and Swelum et al. (2021), that camel milk contains more β-casein than α-casein (65 and 21% of total casein, respectively). On the other hand, the alveolar secretion of the cranial gland showed slight stronger reaction for α- and β-casein than the caudal gland alveolar secretion.

This different distribution of casein and the dominance of β-casein in camel milk may be important in explaining some of the special properties of camel milk, especially in the processing of camel milk into yogurt and cheese (Huda et al. 2020; Kamal-Eldin et al. 2020).

The lower expression of PAS, AB, α- & β-casein in caudal gland than cranial gland can be explained with the results of Rizk et al. (2017) who found that the caudal gland secretion is less in amount than the cranial one. The causes of that variations are of a very complex nature. Eisa and Hassabo (2009) attributed this to either increased growth and number of secretory cells or increased secretory activity of the udder tissue, while Rizk et al. (2017) referred that to the adaptation to water deprivation as well as to physiological rest between the successive births.

Conclusion

The results obtained in this study suggest that the alveolar secretion of the cranial gland of the same lactating mammary gland quarter of the she camel contain higher amount of carbohydrates, mucins, α- and β-casein than caudal gland of the same quarter. While both alveolar secretion of cranial and caudal glands contain higher level of β-casein than α-casein.

Authors Contribution

El-Bargeesy GA and Khalifa EF set up the study design. Ola RH and Yasmine HA carried out the scientific study protocol. El-Saba AA and Khalifa EF were involved in the analysis of the data and scientific discussions. Yasmine HA and El-Bargeesy GA reviewed and edited the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Agrawal RP, Budania S, Sharma P, Gupta R and Kochar DK, 2007. Zero prevalence of diabetes in camel milk consuming Raica community of northwest Rajasthan. Diabetes Research and Clinical Practice 76(2): 290-296. https://doi.org/10.1016/j.diabres.2006.09.036

Al-Bazii SJ, Al-Masoudi FJ and Obeid AK, 2019. Histological effects of sesamum indicum seeds on mammary gland IOP Conference Series: Materials Science and Engineering. https://doi.org/10.1088/1757-899X/571/1/012057

Alhafiz GA, Alghatam FH, Almohammed H and Hussen J, 2022. Milk immune cell composition in dromedary camels with subclinical mastitis. Frontiers in Veterinary Science 14(9): 885523. https://doi.org/10.3389/fvets.2022.885523

Alhaj, Omar K and Hamad, 2010. Compositional, technological, and nutritional aspects of dromedary camel milk. International Dairy Journal 20: 811-821. https://doi.org/10.1016/j.idairyj.2010.04.003

Bancroft JD and Gamble M, 2013. Theories and practice of histological techniques. 7th Ed. Churchill Livingstone, London.

Eisa MO and Hassabo AA, 2009. Variations in milk yield and composition between fore and rear udder-halves in she-camel (*Camelus dromedarius*), Pakistan Journal of Nutrition 8(12): 1868–1872. https://doi.org/10.3923/pjn.2009.1868.1872

Eisa MO, 2006. Udder conformation and milk ability of she-camel (*Camelus dromedarius*) in EL- Showak. Ph.D thesis, University of Khartoum, Sudan.

Ellen CRL, Luiz RFJ, Silvana GPC and Sebastião RT, 2017. Histological and immunohistochemical characterization of the Mongolian gerbil's mammary gland during gestation, lactation, and Involution. Acta Histochemica 119: 273-283 http://dx.doi.org/10.1016/j.acthis.2017.02.003

Elsayed IE, Mohsen N, Sherif MS, Sameh A and George FWH, 2009. Are camel milk proteins convenient to the nutrition of cow milk allergic children?. Small Ruminant Research 82(1):1-6. https://doi.org/10.1016/j.smallrumres.2008.12.016

Huda M, Monika J, Åse L, Peter N and Afaf K, 2020. Caseins and α-lactalbumin content of camel milk (*Camelus dromedarius*) determined by capillary electrophoresis. Journal of Dairy Science 103 (12):11094-11099. https://doi.org/10.3168/jds.2020-19122

Hue-Beauvais C, Faulconnier Y, Charlier M and Leroux C, 2021. Nutritional regulation of mammary gland development and milk synthesis in animal models and dairy species. Genes 12(4): 523. https://doi.org/10.3390/genes12040523

Kamal-Eldin A, Alhammadi A, Gharsallaoui A, Hamed F and Ghnimi S, 2020. Physicochemical, rheological, and micro-structural properties of yogurts produced from mixtures of camel and bovine milks. NFS Journal 19: 26-33. https://doi.org/10.1016/j.nfs.2020.05.001

Kausar R, Sarwar A and Hayat CS, 2001. Gross and microscopic anatomy of mammary gland of dromedaries under different physiological conditions. Pakistan Veterinary Journal 21:189-193.

Kumar D, Verma AK, Chatli MK, Singh R, Kumar P, Mehta N and Malav OP 2016. Camel milk: alternative milk for human consumption and its health benefits. Nutrition & Food Science 46 (2):217-227. https://doi.org/10.1108/NFS-07-2015-0085

Leal J, Smyth HDC and Ghosh D, 2017. Physicochemical properties of mucus and their impact on transmucosal drug delivery. International Journal of Pharmaceutics 532(1): 555–572. https://doi.org/10.1016/j.ijpharm.2017.09.018

Ola RH, Ahmed YH, El-Saba AA, Khalifa EF and El-Bargeesy GA, 2022. Comparative histological, histochemical and immunohistochemical examination of the cranial and caudal gland of each lactating mammary gland quarter of one humped she camel (Camelus Dromedarius). International Journal of Veterinary Science 11(3): 336-343. https://doi.org/10.47278/journal.ijvs/2021.120

Quan S, Tsuda H and Miyamoto T, 2008. Angiotensin I-converting enzyme inhibitory peptides in skim milk fermented with Lactobacillus helveticus 130B4 from camel milk in Inner Mongolia. Journal of the Science of Food and Agriculture 88: 2688-2692. https://doi.org/10.1002/jsfa.3394

Ranjan R, Narnaware S and Prakash V, 2022. Incidence, risk factors and economic impact of clinical mastitis in dromedary camel (Camelus dromedarius). Trop Anim Health and Production 54(31): https://doi.org/10.1007/s11250-021-03035-0

Rizk HM, Khalifa EF and Abdelgalil AL, 2017. Comparative morphometric overview between the two milk systems of the mammary gland of one humped camel Camelus dromedaries. Research journal of Pharmaceutical, Biological and Chemical Science 8(3): 2417-2425.

Ryskaliyeva A, Henry C, Miranda G, Faye B, Konuspayeva G and Martin P, 2018. Combining different proteomic approaches to resolve complexity of the milk protein fraction of dromedary, Bactrian camels and hybrids, from different regions of Kazakhstan. PLOS ONE 13(5): e0197026. https://doi.org/10.1371/journal.pone.0197026

Shedge SA, Priya PR, Megha AD, Pratibha P and Ajay JS, 2018. Histochemical Characteristics of Mucus substances in Normal Human Mammary Gland 7: 241-246 http://dx.doi.org/10.21088/ija.2320.0022.7318. 3

Sisay F and Awoke K, 2015. Review on production, quality and use of camel milk in Ethiopia. Journal of Fisheries and Livestock Production 3: 145-149. http://dx.doi.org/10.4172/2332-2608.1000145

Sulochana S and Singh Y, 1995. Histochemical studies of mammary gland parenchyma in pregnant Nali ewes. Small Ruminant Research 18: 189-192. https://doi.org/10. 1016/0921-4488(95)00693-F

Swapna SA, Priya R, Ajay S and Megha AD, 2020. Periodic Acid Schiff (PAS) Staining: A Useful Technique for Demonstration of Carbohydrates. Medico-legal Update 20(2): 353–357. https://doi.org/10.37506/mlu.v20i2.1129

Swelum AA, El-Saadony MT, Abdo M, Ombarak RA, Hussein E, Suliman G, Alhimaidi AR, Ammari AA, Ba-Awadh H, Taha AE, El-Tarabily KA and Abd El-Hack ME, 2021. Nutritional, antimicrobial, and medicinal properties of Camel's milk: A review. Saudi Journal of Biological Sciences, 28(5): 3126-3136. https://doi.org/10.1016/j. sjbs.2021.02.05

Zayeed AA, Magdub AB, Shareha AM, El-Sheikh A and Manzally M, 1991. Camels in the Arab World. 1st Ed. University of Omar El-Mukhtar, Libya.