Sudan Journal of Science and Technology (2016) **17**(1): 19-27

Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

A comparative Histometric Measurements and Carbohydrate Localizations on the Mandibular Gland of Camel, Ox, Sheep and Goat

Nada Juma Mursal[1*], Hassan Ahmed Ali[1], Zarroug Hassan Ibrahim[2]

1. College of Veterinary Medicine, Sudan University of Science and Technology, PO. Box: 204, Khartoum-North, Sudan.
2. College of Agriculture and Veterinary Medicine, Qassim University, KSA.
*Corresponding author: E- Mail: *nadajuma01@gmail.com*. Tel: +249 963 286 853.

ARTICLE INFO

ARTICLE HISTORY
Received: 4th January 2016
Accepted: 21 February 2016
Available online: June 2016

KEYWORDS:
Mandibular Gland,
Histology,
Histometry,
Histochemistry,
Camel, Ox, Sheep, Goat

ABSTRACT

The present study was conducted to describe the comparative histometry and histochemistry of mandibular glands in camel, ox, sheep and goat. Forty heads of apparently healthy adult animals (10 heads for each) were collected from Alssalam slaughter house, Omdurman, Sudan. The histometric measurements revealed three types of acini and four types of ducts according to diameter. The acini were either small and intermediate or large. The small acini revealed a big difference in diameter between camel and sheep and a similar diameter in goat and camel; ox and sheep. The intermediate acini showed a big difference between camel, ox and sheep. The large acini showed a big difference in diameter between camel, ox and sheep; ox, sheep and goat. The ducts were intercalated, striated, interlobular and interlobar ducts. The intercalated duct showed a slight difference in diameter among camel, ox, goat and sheep; a big significant difference between camel and sheep was observed. The striated duct showed a big significant difference between ox and goat and sheep and ox but the diameter was almost similar in camel, ox and sheep. The interlobular duct had the same diameter in camel, ox and sheep and a big difference was observed between goat and the rest of animals. The interlobar duct showed the greatest diameter in the goat, whereas in camel, ox and sheep the diameters were similar. The carbohydrate observations showed generally a difference in degree of PAS-positive, AB- positive and PAS/AB sequence reaction among the animals used and especially in the parenchyma of gland of camel.

INTRODUCTION

The salivary glands are envaginations from buccal epithelium into the lamina propria-submucosa (Banks, 1992) which develop at different sites. They have very different architectures and produce different types of saliva (Jaskoll *et al.*, 2002). Their secretion is mostly serous containing various enzymes, water, mucopolysaccharides and glycoproteins (Adnyane *et al*., 2010). The relative size of the salivary glands in different species of ruminants is closely associated with the composition of the diet (Kay 1987, 1989). This secretion has an important role in the moistening and swallowing of newly ingested food (Vissink, 2010) and maintenance of oral hygiene (Kay and Maloiy, 1989). In ruminants the salivary secretions regulate the digestion in the forestomach (Kay and Maloiy 1989). They have an important role in terrestrial animals by providing lubrication for eating and vocalization in addition to its aid in digestion and supply of saliva for pH buffering (YazdaniMoghaddam *et al.*, 2009). No data have been published on histometric measurements of acini of mandibular salivary glands of ruminants. The mucous and demilune cells contained an appreciable amount of acid and sulphated mucopolysaccharides in camel (Abdalla, 1979), ruminants (Shackleford and Wilborn, 1968), human (Munger, 1964) and spider monkey (Shackleford and Klapper, 1962). The mucosal units of mandibular gland of rodents reacted strongly with Alcian Blue (AB) and Periodic Acid Schiff (PAS) (Shackelford and Schneyer, 1964).

This study was conducted to:

1. Give information about the histometric measurements of the acini and ducts of the mandibular gland.
2. Perform some carbohydrate tests to evaluate the constituents of the parenchyma of the mandibular gland.

MATERIAL AND METHODS

Forty heads of apparently healthy adult camel, ox, sheep and goat (ten heads of each) were randomly collected from Alssalam slaughter house, Omdurman, Sudan. Samples (3-5 mm) thick were fixed in 10% buffered formalin, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene or chloroform and impregnated and embedding with paraffin wax (Drury and Wallington, 1980). Sections (5-7 µm) thick were cut in a rotary microtome and mounted on glass slides then stained with H&E.

lens 40X was used for determining the measurements after calibrating the ocular scale of the microscope (Thienpont *et al.*, 1986). Then the data were analyzed by the Statistical Tool for Agricultural Research (STAR).

For histochemical investigation samples (3-5 mm) thick were fixed in 10% buffered formalin solution for neutral and acid polysaccharides. They were then processed for paraffin sections, cut at thickness of (5-7µm) on a rotary microtome and they were stained by Periodic Acid Schiff (PAS) technique, for neutral mucopolysaccharides. Control sections for glycogen were treated with 0.1 % amylase for 30 minutes at 37 Cº. For neutral and acid mucopolysaccharides sections were stained with PAS/AB sequence (Carleton, 1967). The slides were examined and photomicrographs were taken with a camera (Mottican U.K) attached to the Olympus microscope.

RESULTS

Histometric measurements: According to luminal diameters, there were three types of acini. The small acini had a small lumen, the intermediate acini had a

medium lumen and the large acini had a large lumen. The average number of lining cells was 5, 8 and 11 cells in the small, intermediate and large acini respectively (Figure 1. a. b, c and d). The diameters of the small, intermediate and large acini in camel, ox, sheep and goat were shown in (Table 1).

A comparison between the diameter of small, intermediate and large acini of the gland was shown in (Table 2). The small acini showed no significant difference (P>0.05) in the diameter in camel and goat; ox and sheep; goat and sheep. A significant difference (P>0.1<0.05) appeared between camel and ox and a high significant difference (P>0.01) was recorded between camel and sheep.

In the intermediate acini, there was no significant difference (P>0.05) between camel and goat; ox and goat; sheep and goat, whereas there was a significant difference (P>0.1<0.05) between camel and ox and high significant difference (P>0.01) between camel and sheep and ox and sheep.

In the large acini, there was no significant difference (P>0.05) between camel and ox, camel and sheep and camel and goat but there was a high significant difference (P>0.01) between camel ox and sheep; ox and goat; sheep and goat.

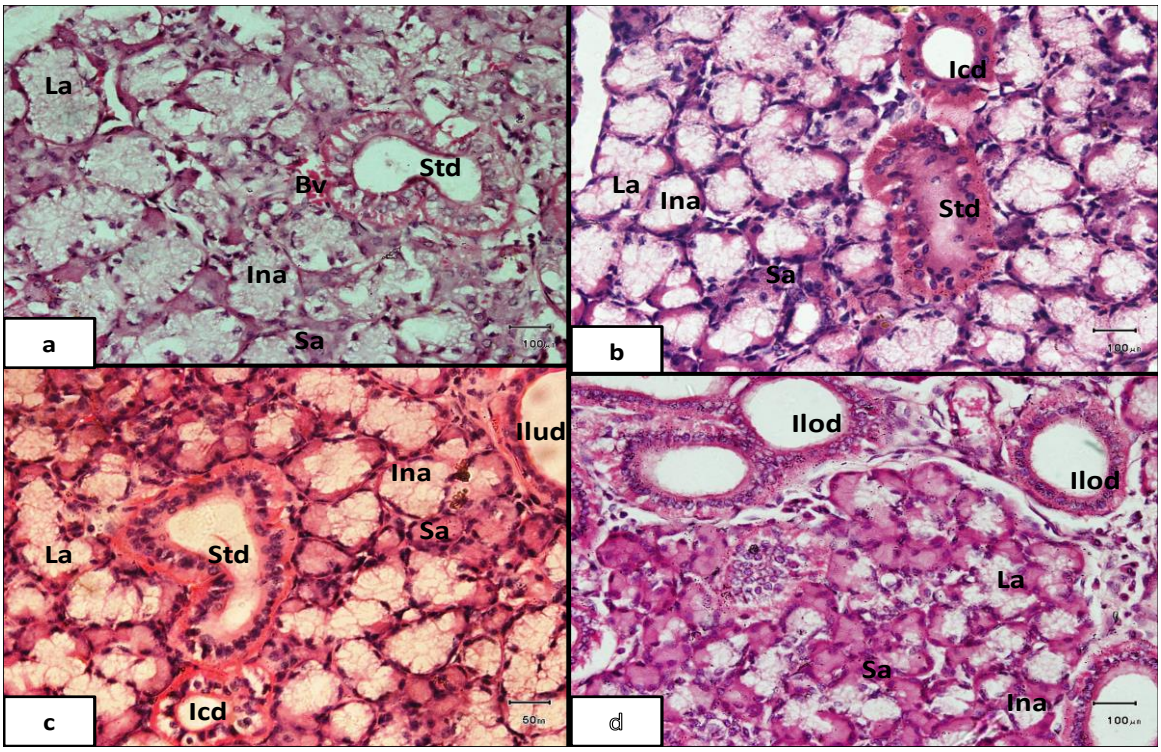

Figure 1: Micrograph of mandibular gland of camel (a), ox (b), sheep (c) and goat (d) showing small acini (Sa), intermediate acini (Ina), large acini (La),intercalated (Icd), striated duct (Std), interlobular duct (Ilud), interlobar duct (Ilod) and blood vessel (Bv). H&E stain.

Table 1: Diameter (μ) of mandibular acini of camel, ox, sheep and goat

Animal /parameters	Small acini	Intermediate acini	Large acini
Camel	18.57±0.42	26.48±0.60	38.52±0.57
Ox	20.38±0.25	28.28±0.25	39.70±0.43
Sheep	20.70±0.42	30.52±0.31	36.92±0.39
Goat	18.89±1.26	27.96±1.17	33.08±0.73

*Data were presented as M ±SE

Table 2: Comparison between mean diameter (μ) of mandibular acini of camel, ox, sheep and goat

Animals/parameters	Small acini	Intermediate acini	Large acini
Camel-ox	1.81±0.634*	1.79±0.528*	1.17±0.756 NS
Camel-sheep	2.13±0.427**	4.03±0.547**	1.600±0.764 NS
Camel-goat	0.3195±1.51 NS	1.47±1.51 NS	0.319±1.50 NS
Ox-sheep	0.319±0.557 NS	2.24±0.425**	2.77±0.553**
Ox-goat	1.49±1.23 NS	0.319±1.15 NS	6.62±0.801**
Sheep-goat	1.81±1.49 NS	2.56±1.42 NS	3.84+0.644**

M±SE
P< 0.01 highly significant (**)
P> 0.01 <0.05 Significant (*)
P> 0.05 $non-significant\ (NS)$.

The duct system composed of intercalated, striated, interlobular and interlobar ducts. The diameters of intercalated, striated, interlobular and interlobar ducts in camel, ox, sheep and goat were shown in (Table 3). Statistical analysis of the diameters of ducts in camel, ox, sheep and goat (Table 4) revealed that there was no significant difference (P>0.05) between ox and goat; sheep and goat in the diameter of the intercalated duct, whereas there was a significant difference (P>0.1<0.05) between camel and ox; camel and goat; ox and sheep and high significant difference (P>0.01) between camel and sheep. The striated duct showed no significant difference (P>0.05) between camel and ox; camel and sheep; ox and sheep. A high significant difference (P>0.01) between camel and goat was seen; there was a high significant difference (P>0.01) between ox and goat; sheep and goat. The interlobular duct showed no significant difference (P>0.05) between camel and ox; camel and sheep; camel and goat; ox and sheep; ox and goat; sheep and goat. Regarding the interlobar duct no significant difference (P>0.05) was recorded between camel and ox; camel and sheep; camel and goat; ox and sheep; ox and goat. A high significant difference (P>0.01) between sheep and goat was shown.

Table 3: Diameters (µm) of mandibular ducts of camel, ox, sheep and goat

Animals/parameters	Intercalated duct	Striated duct	Interlobular duct	Interlobar duct
Camel	34.14±2.44	57.73±4.98	89.63±5.63	103.72±8.16
Ox	38.41±2.53	55.7±3.34	87.12±5.10	104.24±7.30
Sheep	45.38±2.09	54±3.11	89.63±2.85	99.66±4.92
Goat	45.24±2.24	59.98±4.65	93.53±4.36	102.50±5.48

M±SE

Table 4: Comparison between diameters (µm) of mandibular duct of camel, ox, sheep and goat

Animal	Intercalated duct	Striated duct	Interlobular duct	Interlobar duct
Camel-ox	4.270±1.53*	2.03±5.66NS	2.50±6.29NS	0.517±13.69NS
Camel-sheep	11.23±2.48**	3.73±6.32NS	0.0041±5.76NS	4.06±7.12NS
Camel-goat	11.10±4.35*	23±8.35*	3.91±5.40NS	17.78±10.55NS
Ox-sheep	6.83±2.64*	3.73±6.32NS	2.50±5.60NS	4.58±9.48NS
Ox-goat	6.83±4.54NS	25.28±5.63**	6.41±7.78NS	17.27±9.48NS
Sheep-goat	0.132±4.01NS	26.98±5.32**	3.9±4.94NS	21.85±5.18**

M±SE. P< 0.01 high significant (**), P> 0.01 <0.05 Significant (*) P> 0.05 $non-significant\ (NS)$

Carbohydrate localizations

Perodic Acid Schiff (PAS): The mandibular glands of camel, ox, sheep and goat showed strong PAS-positive reaction. The gland was non-resistant to diastase digestion in camel. However, the glands were resistant to diastase in ox, sheep and goat. The reactions were strong in the epithelial lining of acini and moderate in the epithelial lining of ducts and weak or negative in the connective tissue septa in all animals (Figure 2. a, b, c and d).

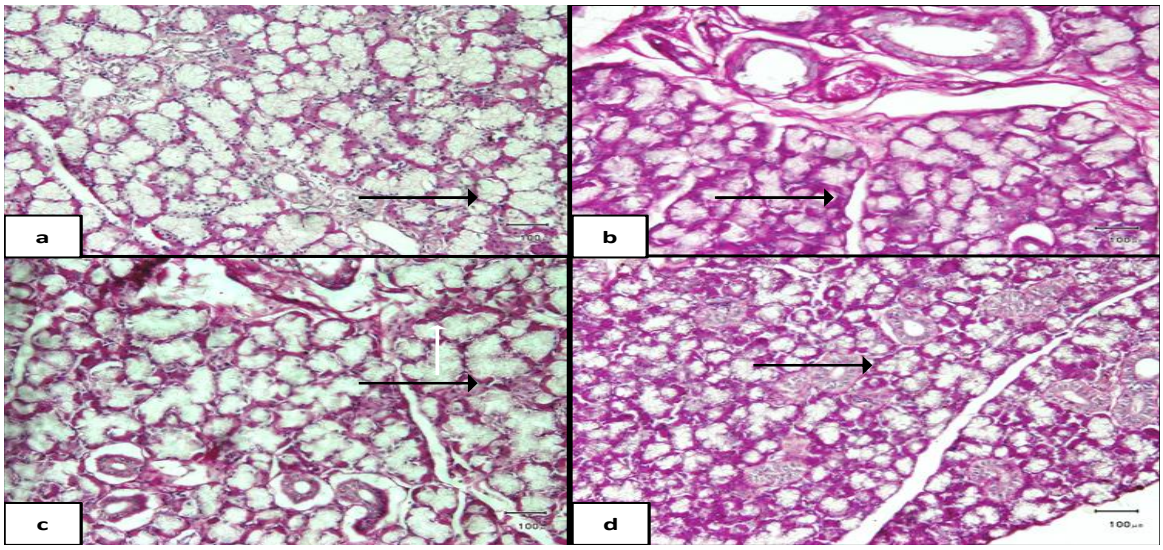

Figure 2: Micrograph of mandibular gland of camel (a), ox (b), sheep (c) and goat (d). Strong PAS-positive reaction in magenta colour on basal lining of serous, mucus and seromucus acini (arrows). The epithelial lining of ducts showed a weak reaction in (a), moderate in (c) and strong in (b and d). A negative reaction in the connective tissue septa is seen in (a), (b), (c) and (d). PAS stain without diastase.

Alcian Blue (AB)/ Periodic Acid Schiff (PAS): Three regions were identified in the mandibular gland of camel; red, blue and gray. The red region was PAS-positive, the blue was AB-positive and the gray was AB/PAS sequence positive (Figure 3. a). In the red region, the strong PAS-reaction was clearly seen in the epithelial lining and lumen of serous acini. The mucus acini showed a positive reaction in the epithelial lining and weak or negative in the lumen. In the blue region, the moderate AB-reaction was seen in the epithelial lining and lumen of the mucus acini and strong PAS reaction in the serous acini. In the gray region, the AB/PAS sequence was positive in the mucus acini and a positive PAS- reaction in the serous acini. In ox, sheep and goat the reaction varied markedly in its two different colours: red was PAS-positive and gray was AB/PAS positive (Figure 3.b, c and l). Intercalated, striated, interlobular and interlobar ducts demonstrated strong PAS-positive reaction in the epithelial lining in camel, ox, sheep and goat. A weak or negative PAS, AB and AB/PAS sequence reaction was seen in the connective tissue septa. The blood vessels were positive for PAS and negative for AB and AB/PAS reactions in all animals.

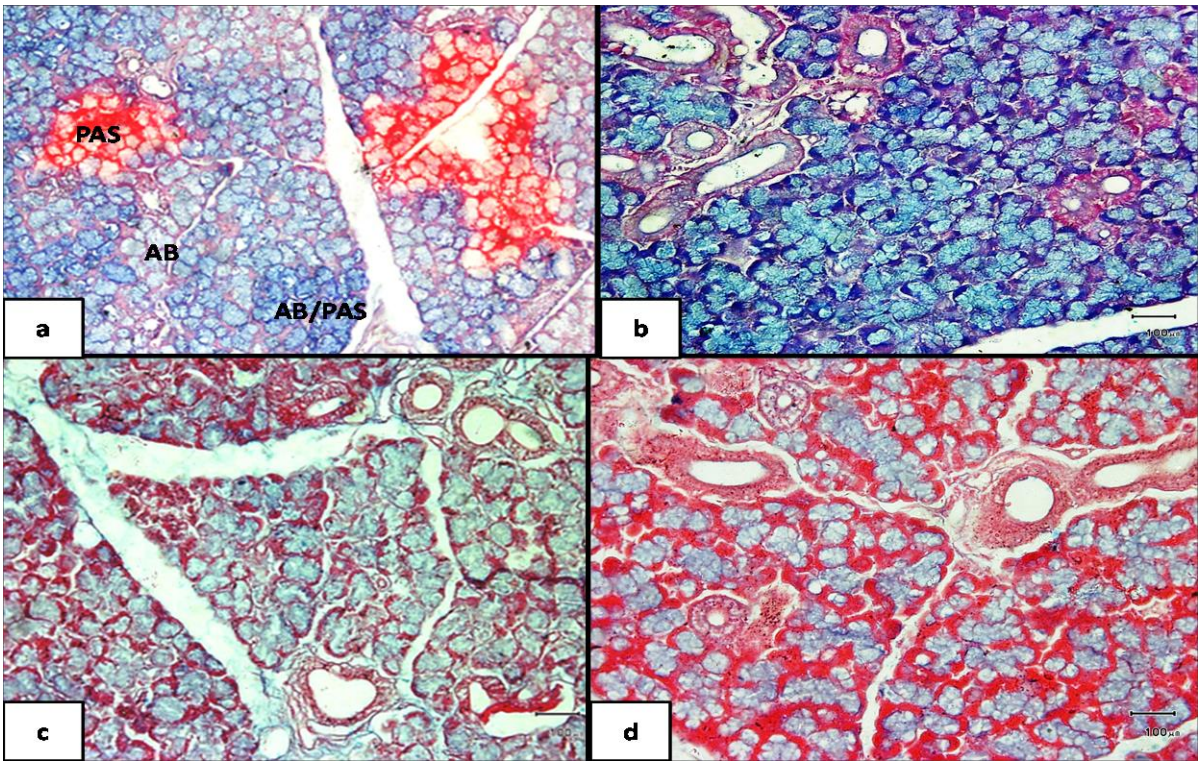

Figure 3: Micrograph of mandibular gland of camel (a), ox (b), sheep (c) and goat (d). AB/PAS sequence was shown in the epithelial lining of acini and ducts (arrows). Three regions in camel gland were identified, the strong PAS-positive reaction in the red region (PAS), strong AB-positive reaction in the blue region (AB) and AB/PAS sequence in the purple region (AB/PAS). Indicated PAS-positive reaction area in the epithelial lining of serous acini and ducts in red colour, AB-positive in the epithelial lining of mucus and seromucus acini in blue colour, AB/PAS reaction in mucus acini with gray colour and negative or week reaction in the connective tissue septa (arrows). AB/PAS sequence stain.

According to degree of reaction among the animals used in this study, the PAS-reaction was very strong in ox, strong in goat, moderate in sheep and weak in camel. AB/PAS sequence was very strong in ox, strong in camel and moderate in sheep and goat (Table 5).

Table 5: Summary of carbohydrate reactions in mandibular gland in camel, ox, sheep and goat

Animals	Periodic Acid Schiff (PAS)	Alcian Blue (AB)	AB/PAS Sequence
Camel	+	+++	+++
Ox	++++	++++	++
Sheep	++	++	++++
Goat	+++	+	+++

DISCUSSION

The present study has shown differences in diameters of acini in the gland of camel, ox, sheep and goat. The small acini revealed a slight difference in diameter between camel and sheep but a similar diameter in goat and camel, ox and sheep and goat and sheep. The intermediate acini showed a slight difference between camel and ox, ox and sheep and sheep and goat. The large acini showed clearly a small difference in diameter among these animals.

In this study the mandibular gland has four classes of ducts which include; intercalated, striated, interlobular and interlobar duct. However, Islam (1981) has reported that the mandibular gland of goat has intercalated, striated and excretory duct.

In present study the mandibular ducts are variable in diameters. This is confirmed by histometry. The diameters of the intercalated duct revealed a slight difference between camel and ox and almost identical in sheep and goat. The diameters of striated duct of camel revealed a big variation in diameter between the goat and other animals but the diameter is almost similar in camel, ox and sheep. The diameters of interlobular duct are similar in camel, ox and sheep and a big difference is shown between goat and the rest of animals. The diameters of interlobar duct reveal greatest diameter in the goat but are similar in camel, ox and sheep.

In the current investigation the gland of camel has shown a negative PAS reaction, non-resistant to diastase digestion while in the ox, sheep and goat a positive reaction resistant to diastase has been demonstrated. This is in agreement with Nawar and El-Khaligi (1977) in camel, Shackleford and Wilborn (1968) in ruminants and Gurusinghe (1983) in bovine who reported that the mandibular mucus cells contained neutral mucopolysccharides. But there is a weak reaction in the lining epithelium in the ducts of camel and strong reaction in the epithelial lining of intercalated, striated, interlobular and interlobar ducts in the gland of ox, sheep and goat. Van Lennep (1957) claim that glycogen is often found in the cells of the intercalated and striated ducts in camel.

In this study the PAS reaction is moderate in the lining epithelium of mucus acini and strong in the lining epithelium of serous acini and weak in the connective tissue in the gland of ox, sheep and goat. Acid myocopolysccharides as indicated by PAS/AB positive reaction are present in the glands of camel, ox, sheep and goat. The strong reaction is seen in the mucus acini and negative in the serous acini in all

animals. The intercalated, striated, interlobular and interlobar ducts show a week reaction in camel and sheep, but weak or negative reaction in ox and goat. The reaction is negative in the capsule and connective tissue in gland of camel, ox, sheep and goat. Abdalla, (1979) in camel, Shackleford and Wilborn, (1968) in ruminants, Munger, (1964) in human, Shackleford and Klapper, (1962) in spider monkey, Gurusinghe, (1983) in bovine and Al-Saffar (2014) in rabbits had detected the presence of acid myochopolysccharides in acini and demilunes of mandibular gland.

REFERENCES

Abdalla, A.B. (1979). Structure of the secretory cells of the salivary glands of the dromedary camel. *Sudan Journal of Veterinary Science & Animal Husbandry***. 20**: 65-76.

Adnyane, I.K.M, Zuki, A.B. Noordin, M.M and Agungpriyono, S. (2010). Histological study of the parotid and mandibular glands of Barking Deer (*Muntiacus muntjak*) with special reference to the distribution of carbohydrate content. *Anatomy Histology Embryology*. **39**: 516-520.

Al-Saffar, F.J. (2014). Histomorphological and Histochemical study of the major salivary glands of adult local rabbits.*International Journal of Advanced Research*, **2**: 278-402.

Banks, W.J. (1992). *Applied Veterinary Histology*. 3rd *ed*. Mosby year book. United State of America.

Carleton, H.M. (1967). *Histological Technique*. 4th ed. New York London and Toronto. Oxford University Press.

Drury, R. A. B. and Wallington, E. A. (1980). *Carleton's Histological Technique*. *5th ed*. New York. Toronto. Oxford University Press.

Gurusinghe, C. J. (1983). *A histochemical study of bovine salivary gland secretory products and aninvestigation of intraepithelial granular duct cells of parotid gland*. MSc thesis, Massey University, Massey UK.

Islam, M. N. (1981). Gross anatomy of the salivary gland of the Black Bengal goat. *Bangladesh Veterinary Journal*. **15**: 17-21.

Jaskoll, T., Zhou, Y.M., Chai, Y., Makarenkova, H.P., Collinson, J.M., West, J.D. and Carvalho, A.D. (2002). Embryonic submandibular gland morphogenesis; stage-specific protein localization of FGFs, BMPs, Pax6 and Pax9 in normal mice and abnormal phenotypes in FgFR2-111 c (+/Delta), BMP7 (-/-) and Pax6 mice. *Cells Tissues Organs*, **170;** 83-90.

Kay, R.N.B. (1987). Weights of salivary glands in some ruminant animals. *Journal of Zoology London*. **211**: 431- 436.

Kay, R.N.B. (1989). Adaptation of ruminant digestive tract to diet. *Acta Veterinaria Scandinavica suppl*. **86:** 196-203. Kay, R.N.B., and Maloiy, G.M.O. (1989). *Digestive Secretions in Camels*. Options mèditerreane-series seminaries-n. 2º.

Munger, B.L. (1964). Histochemical studies on seromucous and mucous secreting cells of human salivary glands. *American Journal of Anatomy*, **115:**411-429.

Nawar, S.M., and El-Khaligi G.E. (1977). Morphological and Histochemical

Studies of the mandibular salivary glands of one-humped camel (*camelus dromedarius). Anatomischer Anzeiger*, **142:** 346-362.

R-STAR (2013). *Statistical Tools for Agriculture Research*. Version 1.1, international rice research institute (IRRI) Los banos, Philippines.

Shackleford, J.M. and Klapper, C. E. (1962). Structure and carbohydrate histochemistry of mammalian salivary glands. *American Journal of Anatomy*. **111:** 25-48.

Shackleford, J.M., and Schneyer, C.A. (1964). Structural and functional aspect of rodent salivary glands including two desert species. *American Journal of Anatomy*, **115:** 279-307.

Shackleford, J.M., and Wilborn, W.H. (1968). Structural and histochemical diversity in mammalian salivary glands. *Alabama Journal of Medical Sciences*, **5:** 180-203.

Thienpont, D., Rochette, F. and Vanparijs, O.F.J. (1986). *Diagnosing Helminthiasis by Coprological Examination*, 2nd ed. Janssen Research foundation. Beers, Belgium.

Van Lennep, E.W. (1957). The glands of the digestive system in the one humped camel, (*Camelus dromedarius*). 1. The salivary glands. *Acta Morphologica Neerlando Scandinavica*, **1**: 286-292.

Vissink, A., Mitchell, J.B., Baum, B.J., Limesand, K. H., Jensen, S.B., Fox, P.C., Elting, L.S., Langendijk, J.A., Coppes, R.P. and Reyland, M.E. (2010). Clinical management of salivary gland hypo-function and xerostomia in head and neck cancer patients: Successes and barriers. *International Journal Radiation Oncology Biology Physiology*, **78**: 983-991.

YazdaniMoghaddam, F., Darvish, J., Mahdavi, S.N., Abdulamir, A. S., Mousavi, M. and Daud, S. K. (2009). Comparative histological and histochemical inter-species investigation of mammalian submandibular salivary glands. *Research Journal Applied Science*, **4:** 50-56.