



Prenatal Histochemical Study of Stomach in Kosali Cattle

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ABSTRACT

Background: Energy is the important part of any life, the potential energy present in the tissue in the form of chemical energy. Presence of glycogen as glucose is source of energy for cell division, differentiation, delamination and maturation process as essential process in histogenesis of foetal organ. Glycosaminoglycans, also known as mucopolysaccharides, are long chains of sugar occurs in different proportion in different tissue and their distribution in compartment of ruminant stomach varied in foetus of different CRL. Compartment of ruminant stomach abomasum, omasum, reticulum and rumen was contributed grossly in varied proportion from initial stage to full term of prenatal stage. In initial stage rumen is largest compartment with advancement of development omasum is largest compartment followed by reticulum and abomasum at full term. As per morphology and histology of varied compartment there could be probability of varied intensity of histochemical reaction in different layer of tissue as per their embryogenesis. Kosali cattle is registered 36th breed of India. Kosali the native breed of Chhattisgarh, known for its compact body, disease tolerance and well maintained in agricultural waste. More than total 60% Kosali cattle are concentrated in the central plains of the C.G. state. There is paucity of Cited literature, literally no scientific documentation regarding presence and distribution of glycogen and mucopolysaccharides in rumen, reticulum, omasum and abomasum of prenatal Kosali cattle. In view importance of Kosali cattle concern with livelihood of farmers in Chhattisgarh, varied degree of development of compartment of stomach and paucity of literature regarding intensity of histochemical reaction from initial to final term of prenatal stage present research was undertaken.

Methods: In light of above background in twenty four Kosali foeti were collected and on the basis of CR length categorized in four groups with six foeti in each group i.e. Group I (below 20 cm CRL), Group II (Above 20 up to 40 cm CRL), Group III (Above 40 up to 60 cm CRL) and group IV (Above 60 cm CRL), tissue samples of rumen, reticulum, omasum and abomasum were collected and processed for study of glycogen and mucopolysaccharide by PAS and AB-PAS method respectively.

Result: Apparently in group I PAS activity was either not observed as in reticulum or very mild in rumen, omasum and abomasum and was greater in abomasum. In epithelium, propria submucosa and serosa PAS activity increased slowly mostly from mild to moderate while in glands reaction was intense. However in tunica muscularis of rumen reaction decreased from group III to IV, in omasal tunica muscularis and omasal laminae from group II to IV. In group I PAS activity was higher in pyloric region than cardiac stomach. AB-PAS activity for neutral mucopolysaccharide was observed in epithelium and tunica muscularis of rumen, reticulum, omasum and abomasum in all groups, mixed mild activity for both neutral and acid mucopolysaccharide in propria submucosa in group I and II. In fundic region in group III and IV in glands intense acid mucopolysaccharide reaction was seen in patches in forerunner parietal cells.

Key words: Abomasum, AB-PAS activity, Kosali, PAS activity, Prenatal, Rumen, Tunica.

INTRODUCTION

The Kosali breed was registered as the 36th breed of cattle in 2012 (Accession No.: INDIA_CATTLE_2600_KOSALI_03036) and it is the first breed from Chhattisgarh. The breeding tract lies between 19.8° to 22.7° north latitude and 80.3° to 83.6° east longitude. The Kosali breed is mainly concentrated in the Central Plain region of Chhattisgarh state. The population of the Kosali breed is 31.32 lakh, spread over a geographical area of 68.49 lakh hectares. More than 60% of the cattle are concentrated in the central plains of Chhattisgarh state (Jain *et al.*, 2017). The average body weight for males is 260 kg, while for females it is 160 kg and the birth weight of Kosali calves varies between 11 and 18 kg (Jain *et al.*, 2018). The average milk yield per lactation is 210 kg, with an average milk fat percentage of 3.5%. Cattle are ruminants and have a unique digestive system that comprises a four-chambered stomach (rumen, reticulum, omasum and abomasum), which allows them to better utilize energy from fibrous plant

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material. The rumen serves as a fermentative chamber (Habel, 1975). The shape and size of the stomach vary with development, age and size of the animal Gupta *et al.* (2017).

MATERIALS AND METHODS

Present study was carried out in twenty four Kosali foeti. Foeti were collected immediately after death of apparently healthy Kosali cow in nearby districts (Rajnandgaon, Balod and Durg). Crown rump length of foeti (Joubert, 1956) were measured immediately after collection. As per the CRL, foeti were categorized in to four groups with six foeti in each group *i.e.* Group I (below 20 cm CRL), Group II (Above 20 up to 40 cm CRL), Group III (Above 40 up to 60 cm CRL) and group IV (Above 60 cm CRL). After midventral abdominal, paracostal and flank incision, abdominal cavity were exposed, tissue sample of rumen, reticulum, omasum and abomasum were collected and processed and stains for presence and distribution of glycogen as PAS method and form mucopolysaccharide AB-PAS method as counterstain aniline blue was used at Ph 2.5 as per method of Bancroft and Cook (1994).

RESULTS AND DISCUSSION

PAS activity (Fig 1 to 4)

PAS reaction was very mild in basal epithelium, propriasubmucosa, tunica muscularis and serosa in Rumen in group I. PAS reaction was increased from group I to II from very mild to mild. In group III reaction was slightly increased in epithelium and propria submucosa, decreased in tunica muscularis and same in tunica serosa. In group IV intensity of reaction was same as in group III with intense reaction in keratin. Similarly Panchamukhi and Srivastava, (1979) at 10 days buffalo foetus reported glycogen and Patra, (2019) in sheep at 32 days of sheep foetus. As increased reaction in epithelium from group II to IV was contradict the finding of Arias *et al.* (1980) observed that in bovine fetal rumen, up to six months of pregnancy, the glycogen level of both basal and superficial cells increases in fetuses; after that, it declines in older fetuses and Franco *et al.* (1992). As finding of Patra, (2019) in sheep, feeble reaction in the muscular and serosal tunics present finding of decreased activity in tunica muscularis from group III to IV was corroborated.

In group I PAS activity was not observed in reticulum. Present finding of increased activity in epithelium, propriasubmucosa, tunica muscularis and serosa of reticulum from group II to IV was in agreement with observation of Patra, (2019) in sheep as weak PAS activity at day 32 which progressively increased to a moderate degree by 150 days of gestation, in contrast Ramkrishna and Tiwari, (1979) reported minimum glycogen concentration in middle age group and Franco *et al.* (1993b) in deer observed decreased PAS activity 46 days onwards.

In omasum increased PAS activity in epithelium, from group I to IV as mild to intense, from group I to III in propriasubmucosa and serosa. In submucosa reaction

progressively increased from mild to light intense and constant from group III to IV and decreased activity in tunica muscularis and core of laminae from group II to IV was in agreement with Ramkrishna and Tiwari, (1979) they

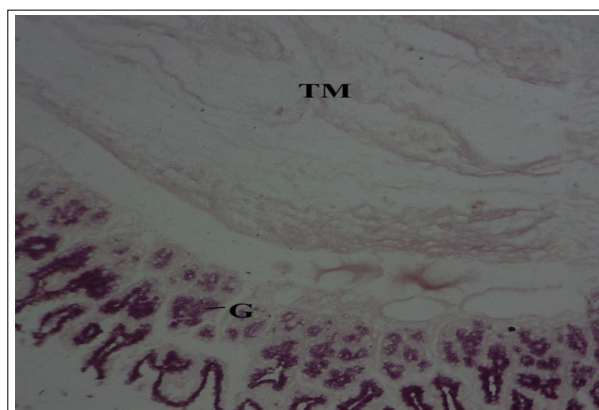


Fig 1: Photomicrograph of cardiac abomasum showing PAS activity in glands (G) and tunica muscularis (TM) in group III PAS 100 x.

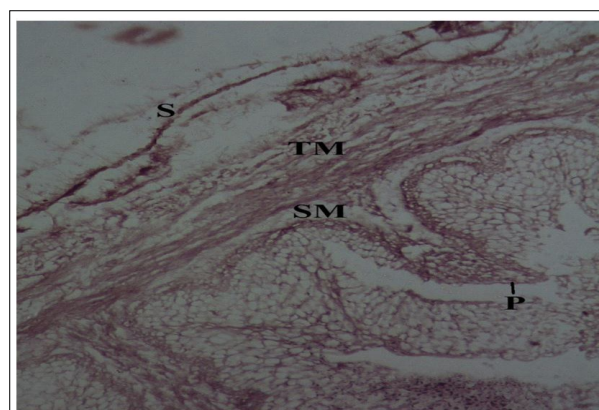


Fig 2: Photomicrograph of omasum showing PAS activity in papillae (P), submucosa (SM), tunica muscularis (TM) and serosa (S) in group II PAS 100 x.



Fig 3: Photomicrograph of omasum showing PAS activity in epithelium (E), basal epithelium (BE) and submucosa (SM) in group III PAS 100 x.

observed maximum amount of glycogen in third trimester. In contrast to present finding, Lubis and O'Shea, (1978) in sheep foetus reported incredibly abundant glycogen in omasal epithelium, then rapidly decreased until it was nearly nonexistent in post-natal tissues, Franco *et al.* (1993c) in sheep, Patra, (2019) in sheep fetuses and Gupta *et al.* (2016) in goat foetus reported decreased amount of glycogen from second to third trimester.

In group I wall of abomasum shown relatively more PAS reaction than rumen, reticulum and omasum. PAS reaction was moderate in epithelium, mild in upper cellular zone of propria submucosa, very mild in lower zone, mild in tunica muscularis and very mild in serosa. Reaction was increased apparently from cardiac to pyloric region and specially in epithelium core of villi and tunica muscularis. In group I to II increased reaction was observed in gland. Intensity was increased from group II to III with notable increase in epithelium of villi with high intense PAS reaction and intense PAS reaction in gland. From group III to IV intensity was increased in gland and high intense PAS reaction was observed. In support of the result of present finding Ramkrishna and Tiwari, (1979) found maximum glycogen in fundic area during later stages of development, Franco *et al.* (1993a) in sheep at birth found enhanced secretion of neutral glycogen, Masotet *et al.* (2007) reported neutral mucopolysaccharides in the wall of abomasum of red deer from the age 97 days of gestation to term end, Ozbek *et al.* (2018) in sheep foetus observed the neutral in the superficial epithelial cells of the abomasal pyloric region and Patra, (2019) in sheep fetuses' abomasum reported PAS activity was mild at 32-48 days, moderate from 63-94 days and strong from 111-150 days and Poradowski and Chrószcz, (2022) reported increased pas activity in equine from 7 to 11 months of foetal period. In contrast to present finding Gupta, (2016) in goat reported surface epithelium of fundic part of abomasum showed intense PAS positive reaction in group I and this activity gradually decreased in group II and III. Present observation of higher PAS activity in pyloric region than fundic region in group I was in complete harmony with Gupta *et al.* (2020) they noticed in goat foeti that surface epithelial cells of pyloric region showed intense PAS activity than fundic part. The increased PAS activity could be attributed to supply of increased demand and supply of energy required for histological differentiation and maturation of tissue.

AB-PAS activity (Fig 5 to 7)

Absence of acid mucopolysaccharide activity in epithelium and tunica muscularis from group I to IV in rumen was in agreement with report of Franco *et al.* (1992) and Franco *et al.* (2004a) as absence of the alcianophilic activity in the rumen of sheep and red deer foeti respectively. In contrast Singh, (2002) reported weak AMPS reaction in the cells of basal and superficial layer of buffalo foetal rumen, Gupta, (2016) and Patra, (2019) in goat reported increase in reaction for acid mucopolysaccharide with advancement of foetal age. Absence of acid mucopolysaccharide activity in tunica muscularis throughout the study was in agreement

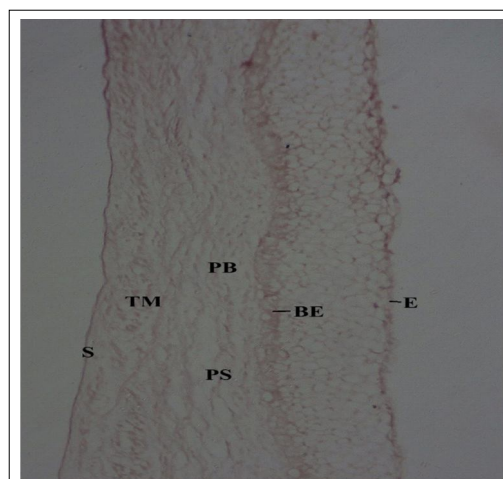


Fig 4: Photomicrograph of rumen showing PAS activity in superficial epithelium (SE), basal epithelium (BE), propria submucosa (SM), pluripotent blastemic cells (PB), tunica muscularis (TM) and serosa(S) in group I PAS 100 x.

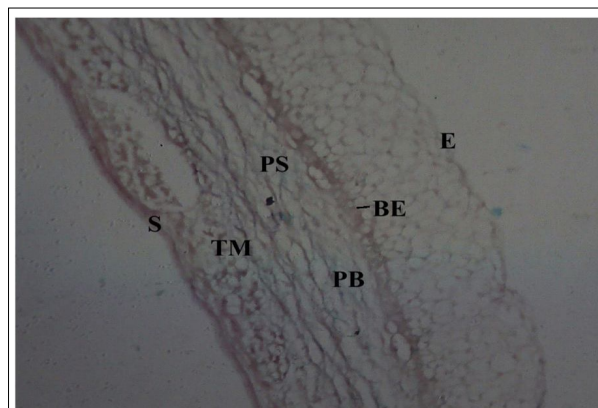


Fig 5: Photomicrograph of rumen showing ABPAS activity in superficial epithelium (SE), Basal epithelium (BE), Propria submucosa (PS), Pluripotent blastemic cells (PB), Tunica muscularis (TM) and serosa (S) in group I ABPAS 100 x.

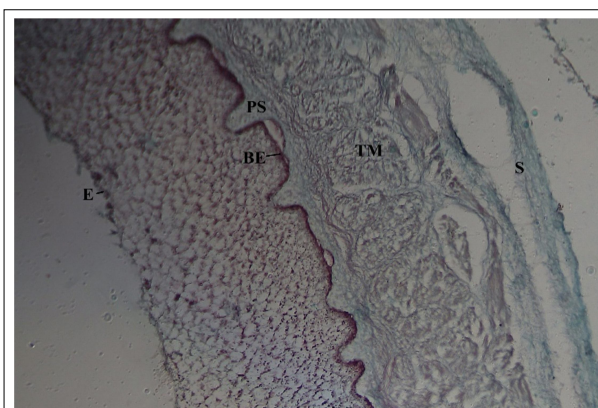


Fig 6: Photomicrograph of rumen showing ABPAS activity in superficial epithelium (SE), basal epithelium (BE), propria submucosa (SM), tunica muscularis (TM) and serosa(S) in group II ABPAS 100 x.

with Gupta, (2016) who reported negative reaction for AMPS in all stages. Presence of mixed mucopolysaccharide activity in submucosa in group I to IV and in serosa mixed to mild acid mucopolysaccharide observed from group I to IV was in complete harmony with Singh, (2002) observed moderate AMPS reaction in buffalo foetal rumen, Franco *et al.* (2011) in deer reported that the neutral mucopolysaccharides were first detected in epithelial cells at 46 days of fetal life, with their numbers gradually decreasing with advancement of foetal life, Gupta, (2016) and Patra, (2019) in goat as propria-submucosa and tunica serosa showed consistently weak to mild activity. The AMPS reaction found in propria submucosa and serosa could be required for the strengthening activity during development, differentiation and maturation of rumen.

In reticulum in group I and II epithelium, tunica muscularis and serosa shown absence of acid mucopolysaccharide was in agreement with presence finding Franco *et al.* (1993b) not observed acid mucopolysaccharides, mucins and mucoid compounds throughout the development. In contrast Franco *et al.* (2004b) noticed secretion of acid mucopolysaccharides by the reticular epithelium at 67 days of gestation in red deer and Patra, (2019) observed the basal zone shown strong activity from 32 to 63 days, moderate from 67 to 94 days and strong again from 111 to 150 days. Propri-submucosa shown very mild mixed mucopolysaccharide reaction in group I and II. In group III and IV reaction was same as in earlier group II with increased intensity. In harmony of present finding Patra, (2019) observed variable reaction in proprio-submucosa. As per discussion of Patra, (2019) these acidic mucins might be involved in the process of cellular differentiation as well as cell maturation in reticular wall.

In omasum in group I and II epithelium, tunica muscularis and serosa shown absence of acid mucopolysaccharide and presence of neutral mucopolysaccharide from group I to IV Similar to present findings of Lubis and O'Shea, (1978) and Franco *et al.* (1993c) in sheep omasum could not observed acid

mucopolysaccharide throughout development in sheep. Contradictory in buffalo foetus Singh, (2002) observed weak moderate reaction in tunica muscularis and in sheep foetus Patra, (2019) observed varied intensity of reaction in epithelium and tunica muscularis. Presence of mild mixed mucopolysaccharide reaction in propriasubmucosa and serosa from group I to III, in omasal lamina from group III and mixed mucopolysaccharide reaction to neutral in group IV was in line with finding of Singh, (2002) in buffalo foetus and Patra, (2019) in sheep foetus and contradict the earlier report of Gupta, (2021) as negative reaction in the proprio-submucosa in goat.

In abomasum in group I and II in villi and tunica muscularis reaction was mild for neutral mucopolysaccharide and mixed mild reaction was observed in propria submucosa and serosa in cardiac, fundic and pyloric region. In group II in fundic region reaction for neutral mucopolysaccharide was two fold increased in villi, tunica muscularis, blood vessel and serosa. In group II reaction was more in fundic stomach than cardiac stomach. In pyloric region mild increased activity was observed in serosa only. In cardiac region in group III and IV reaction was increased in villi, gland and tunica muscularis, lamina propria and submucosa shown mixed reaction as in group I and II. In fundic region in group III and IV in glands intense acid mucopolysaccharide reaction was seen in patches in forerunner parietal cells. In group IV in pyloric region similar intensity of acidic mucopolysaccharide was seen in gland but number of patches were one fourth less than fundic region. In group III and IV in submucosa with increased fibers intensity reaction for neutral mucopolysaccharide was remarkably increased. In epithelium presence of neutral mucopolysaccharide from group I and IV and appearance of acid mucopolysaccharide activity in glands from group III and intense activity in group IV was in harmony with earlier finding Ozbek *et al.* (2018) in sheep fetus, they reported neutral and mixed in the superficial epithelial cells of the abomasal pyloric region, acidic mucins were detected in the secretory ducts and corpus of the glands and contradict to Patra (2019) as the epithelium was intensely alcianophilic from 32-75 days, moderating afterward. In group I and II in propria submucosa and serosa mild affinity for acid mucopolysaccharide was observed as earlier report of Patra, (2019) and present finding of decreased activity from group III onwards in serosa and submucosa was similar and contradict respectively to the finding of Patra, (2019). Contradictory to finding of higher acid mucopolysaccharides activity in pyloric region by Gupta *et al.* (2020) present reported shown number of glands showing affinity for acid mucopolysaccharide was one fourth in pyloric region than fundic region.

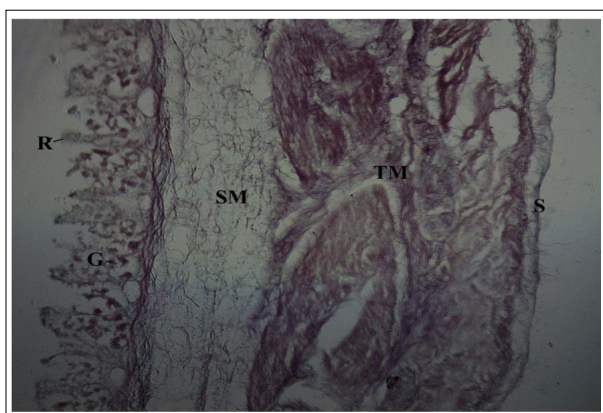


Fig 7: Photomicrograph of abomasum showing ABPAS activity in rugae (R), glands (G), submucosa (SM), tunica muscularis (TM) and serosa(S) in group III ABPAS 100 x.

CONCLUSION

In the present study PAS activity increase from group I to IV in all compartments of the stomach, maximal intensity was observed in the epithelium and keratinized layers. In reticulum and omasum PAS reactivity was more in

epithelium and submucosa, while activity decreased in the tunica muscularis. Intense PAS activity was observed in glands of abomasum. Intensity of reaction was progressively increased from cardiac to pyloric regions. AB-PAS staining was non-reactive for epithelium and tunica muscularis of all part of stomach, but mild to moderate mixed reactivity was observed in submucosa and serosa. In the abomasum glands showed presence of acid mucins from group III onwards.

Conflict of interest

All authors are agree for publication in this esteemed journal. Authors highly acknowledged the farmer dean Dr. S.K Tiwari for support, valuable suggestion and encouragement throughout research work.

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