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The Siglec-5 Gene Expression and Histochemical Investigation of the Ileum of Camels (*Camelus dromedarius*) and Buffalo (*Bubalus bubalis*)

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Abstract | The research sought to examine the existence of sigalec-5 genes in the small intestine (ileum) of buffalo and camels. The RT-PCR amplification plots showed different Ct cycle numbers in the small intestine of buffalo and camels. The results were specific, and the amplification curves were consistent. The melting peaks siglec-5 genes were from 75 to 80°C and 65 to 80°C, respectively. Histologically, the ileum was delineated into three regions: proximal, middle, and distal. Four distinct layers were identified by microscopic examinations including the muscularis, serosa, submucosa, and mucosa. The small intestine mucosa in both animals consisted of simple columnar epithelium, containing superficial absorptive cells and goblet cells. Camels have a thick layer of muscle called the tunica muscularis this is composed of an exterior longitudinal layer and an interior circular layer. Buffaloes and camels' tunica serosa was noted to be a delicate layer of connective tissue. The ileal epithelium of both species is made up of three parts including proximal, middle, and distal. Each part has three components which are the tip of the villus, the space between the villus and the coelom, and the base of the coelom. The concentration of neutral and acidic mucopolysaccharides was elevated in the central region of both animals, with camels exhibiting higher amounts than buffaloes across all three areas. These finding highlight the importance of gene differentiation and diversity in histopathological characteristics in two important livestock species.

Keywords | Sigalec-5, Expression, Ileum, Camel, Buffalo

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INTRODUCTION

The (sialic acid-binding Ig-like lectins (siglec)) genes are crucial to the immune system and have been examined in multiple contexts, particularly regarding their association with the small intestine. The siglecs comprise a diverse array of cell surface proteins that identify sialic acids and play a role in immune control (Prenzler *et al.*, 2023). Some siglec genes have been identified as being expressed in multiple organs, including the small intestine. The expression of Siglec in the small intestine indicates its potential involvement in immune responses and the preservation of gut health. Moreover, siglec genes are recognized for their role in regulating inflammation and immune cell activation, both of which are essential activities in the gastrointestinal tract (Angata *et al.*, 2007).

Siglec genes, like SIGLEC12, can increase the chance of inflammation-related colorectal cancers (Cuello *et al*., 2024). The SIGLEC5 protein acts on immune regulation by inhibiting the activation of immune cells. It aids in maintaining immunological homeostasis and avoiding excessive inflammation. In addition, SIGLEC5 has been identified as communicating with pathogens such as Group B Streptococcus (GBS), enabling immune evasion by these bacteria (Pillai *et al*., 2012). SIGLEC-5 is an inhibitory immune checkpoint molecule that modulates immunological responses. It belongs to the SIGLEC family, including cell surface proteins that interact with sialic acids and play a role in immune modulation. SIGLEC-5 is predominantly expressed on the surface of immune cells, particularly monocytes, macrophages, and neutrophils (Vuchkovska *et al*., 2022; Ali *et al*., 2024; Al-Sailawi *et al*., 2024).

This study aims to identify the histological and histochemical characteristics of the ileum in buffalo and camels. It also aims to evaluate the gene expression of the SEGLC-5 gene in the different proximal, middle, and distal ileal regions in camels and buffalo.

MATERIALS AND METHODS

Histologically, the ileum of 20 healthy adult buffalo (*Bubalus bubalis*) and camels (*Camelus dromedarius*) of males and females was dissected. The ileum has three segments (proximal, middle, and distal) that were preserved by immersion in 10% neutral formal saline for 48 hours. The three segments of the ileum were dried, clarified, and placed in paraffin. Histochemical techniques were employed to identify mucin in goblet cells within the ileum of camels and buffalo, including periodic acid-Schiff (PAS) staining to glycogen and neutral muco-substances, and alcian blue at pH 2.5 for carboxyl groups of acidic muco-substances.

RNA extraction and complementary (cDNA) synthesis

Total mRNA from the small intestine of cows was obtained using the Accuzol® reagent kit (Bioneer, Korea). A total of 200 mg of proximal, intermediate, and distal ileum have been placed in distinct 1.5 mL Eppendorf tubes (Jiangxi, China). Each tube was spun and incubated on ice for five minutes after 200 µL of chloroform was added. Centrifuging tissues at 14,000× g (at 4˚C) for 15 minutes produced the supernatant. 500 µL of isopropanol was added, mixed, and incubated for 10 minutes at 4˚C. The samples underwent another centrifugation for ten minutes at 4˚C and 14,000× g. Following supernatant removal, one milliliter of 80% ethanol was added, vortexed, and centrifuged at 4˚C for 10 minutes. The particle was air-dried in Eppendorf tubes under supernatant disposal after extraction. Pelletized RNA should be chilled at -20˚C after adding 50 µL of diethyl pyrocarbonate water. Each sample's RNA concentration was quantified with a Thermo Scientific, USA nanodrop spectrophotometer. Using manufacturer guidelines, samples were processed using a DNase I enzyme kit (Promega, USA). Using the manufacturer's instructions and the thermocycler's parameters, the DiaStarTM OneStep recombination PCR (RT-PCR) kit (China) converted total RNA into cDNA (Abed *et al*., 2024). Prior to usage, the cDNA concentrations were standardized and kept at -20˚C.

Real-time RT-qPCR

The RT-qPCR method was employed to determine the level of expression for the sigalic 5 gene utilizing the BioRad, USA, real-time PCR equipment. In this investigation, the following primers were used: Dehydrogenase of glyceraldehyde-3-phosphate in camels and buffalo (GAPDH) (accession number: XM_064489878.1) as the housekeeping gene, with the forward primer ATGCTTCGCTCAGCTTTCAC and the reverse primer ATCTCTGAGTACTCGGTGTTGC; and camels sigalic-like (LOC107317569) mRNA (XM_032448780.1 gene code). Forward primer: TGTGCTTGTGCCTCCTTTTC; reverse primer: ATGCCCCCATGTTTGTGATG. Buffalo sigalic, cell surface-associated (sigalic), mRNA, code: XM_045163275.1 Forward primer: ATGCCCCCATGTTTGTGATG; reverse primer: ACGATGCCAAAGTGGTCATG. The expression levels of GAPDH and sigalic genes were quantified using the SYBER Green dye qPCR master mix (Promega, USA) following the kit instructions (AccuPower™ 2× green Star qPCR master mix kit, Bioneer). The thermocycler protocol commenced with an initial denaturation at 50 ˚C for one hour, succeeded by cycles of denaturation at 95˚C for 20 seconds, 30 seconds of annealing or extension at 60˚C, followed by a final melting temperature step that alternates between 60˚C and 95˚C for 0.5 seconds, repeated repeatedly.

Our method was validated by the amplification and melting peaks, which showed a consistent curve free of any non-specific products or amplification and with melting peaks between 80˚C and 88˚C. The ileum's sigalic genes' RT-qPCR amplification plots were precisely detected, with threshold cycle (CT) values of expression ranging from CT 21.99 to CT 26.45.

Statistical analysis

RT-qPCR data were analyzed using $2^{-\Delta\Delta}$CT method and were used to measure the siglec-5 gene expression levels, and significance was assessed at a p-value ≤ 0.05 using one-way analysis of variance in the Statistica Package for the Social Sciences version 23.0 (IBM Corp., NY, USA).

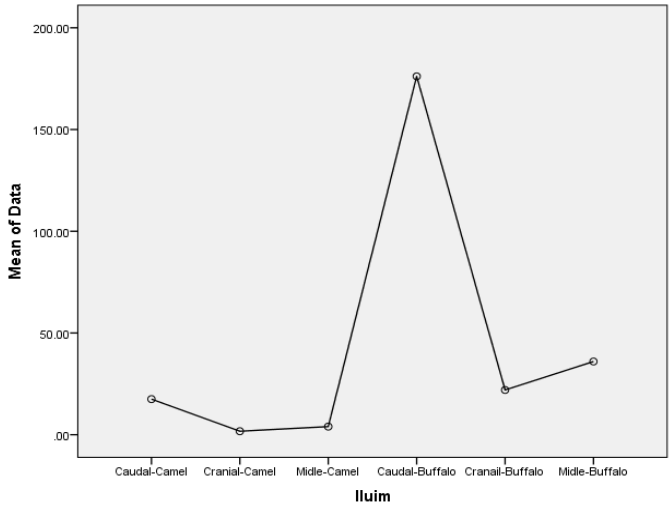

Figure 1: The amplification and melting peaks exited aflat curve devoid of any non-specific product or amplification with melting peak spanning from 180 to 188.

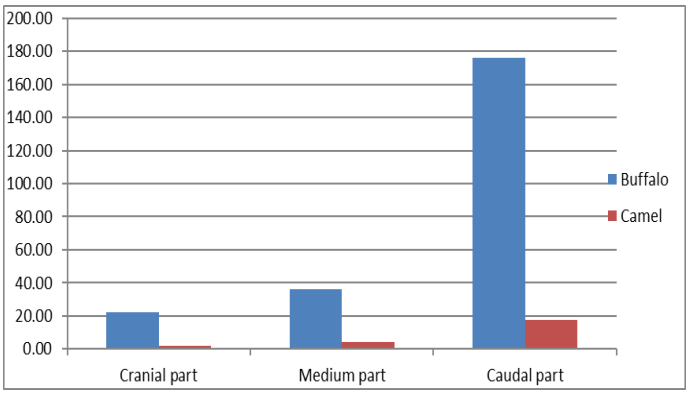

Figure 2: The expression level of sigalic-5 was comparatively elevated in the Distal region of buffalo comparing to that in camel the expression level of sigalic-5 was progressively elevated in the three regions of each animal.

RESULTS

Our work primarily investigated the presence and abundance of sigalic-5 genes. The RT-PCR amplification plots of the sigalic-5 genes in the proximal middle, and distal regions of the ileum in buffaloes exhibit distinct Ct cycle numbers, ranging 20 -25, while in camels, the range is from 30 to 35 (Figures 3 and 4). The siglec-5 and housekeeping (GAPDH) genes' RT-qPCR study showed consistent curve amplifications and great specificity, with buffalo showing clear melting peaks between 25 and 30.and in camels from 30 to 33 (Figures 5, 6). The melting peaks for siglec-5 in buffalo and camel were observed in a range of 75-80 °C and 65-80 °C (Figure 7 and 8). The melting peaks for the housekeeping gene, siglec-5, in buffalo and camel were observed in the ranges of 75-80°C for both species (Figures 9, 10). The RT-qPCR amplifications of siglec-5 genes demonstrated that mRNA from buffalo and camel expressed in the the proximal middle, and distal regions (Figures 1, 3), as shown in Tables 1 and 2. The concentration of siglec-5 was higher in the caudal regions compared to the proximal and middle regions; however, this difference did not reach statistical significance (Figure 1, 2 and Tables 1, 2). Siglec-5 in buffalo demonstrated a greater level of expression in the caudal region of the ileum compared to camels.

Table 1: Values of gene expression of siglec-5 and housekeeping gene of buffalo, analyzed using the $2^{\Delta\Delta CT}$ method.

Buffalo	Sag5	GAPDH	ΔCT test	Fold change	Mean
Caudal part	24.92	32.43	7.51	182.28	
Caudal part	24.15	31.55	7.40	168.90	176.156
Caudal part	23.97	31.44	7.47	177.29	
Cranial part	29.95	33.96	4.01	16.11	
Cranial part	26.91	31.29	4.38	20.82	21.991
Cranial part	28.62	33.48	4.86	29.04	
Medium part	27.42	32.28	4.86	29.04	
Medium part	27.83	33.62	5.79	55.33	35.986
Medium part	29.96	34.52	4.56	23.59	

Table 2: Values of gene expression 5 and housekeeping gene of siglec-5 of camel, analyzed using the 2^ΔΔCT method.

Camel	Sag5	GAPDH	ΔCT test	Fold change	Mean
Caudal part	27.35	31.47	4.12	17.39	
Caudal part	26.16	30.44	4.28	19.43	17.495
Caudal part	25.32	29.29	3.97	15.67	
Cranial part	28.58	30.37	1.79	3.46	
Cranial part	30.49	30.98	0.49	1.40	1.719
Cranial part	31.26	29.51	-1.75	0.30	
Medium part	28.06	30.51	2.45	5.46	
Medium part	29.73	31.95	2.22	4.66	4.005
Medium part	29.64	30.56	0.92	1.89	

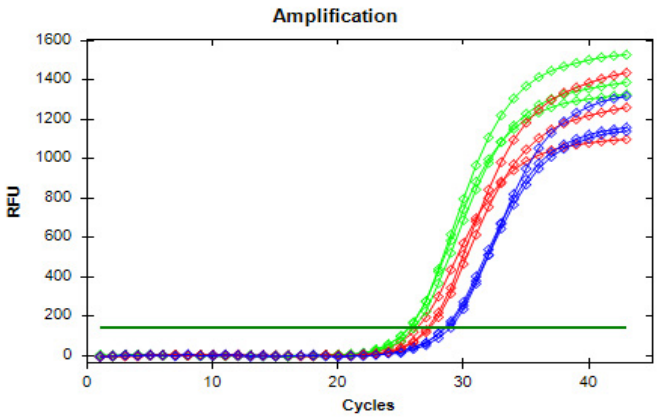

Figure 3: qPCR plots of SAG5 gene in buffalo tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

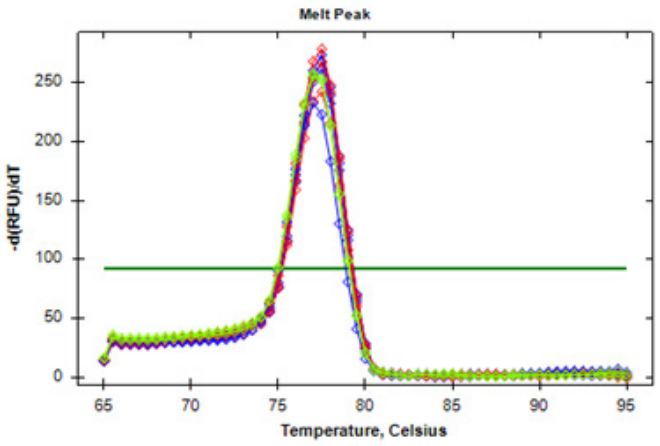

Figure 4: qPCR melt peak of *SAG5* gene in buffalo tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

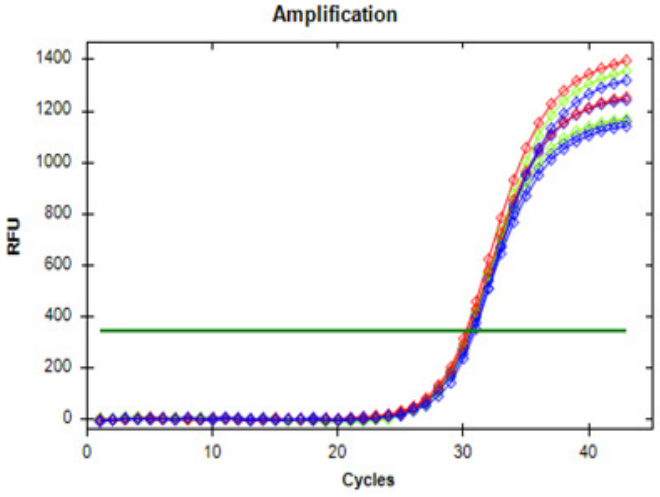

Figure 5: qPCR plots of GAPDH gene in buffalo tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

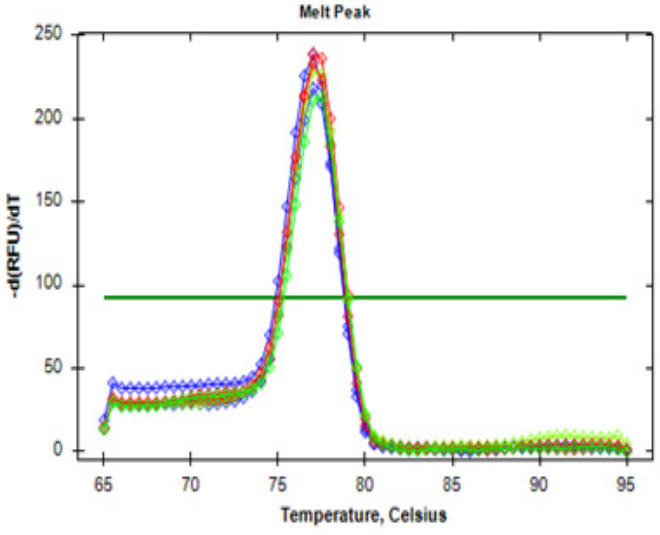

Figure 6: qPCR melt peak of GAPDH gene in buffalo tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

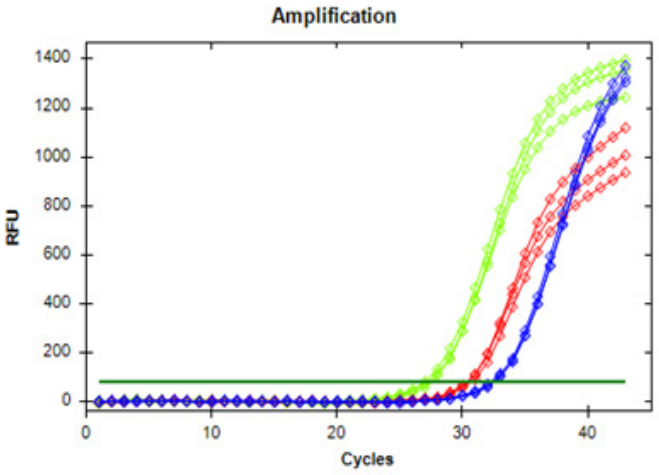

Figure 7: qPCR plots of SAG5 gene in camel tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

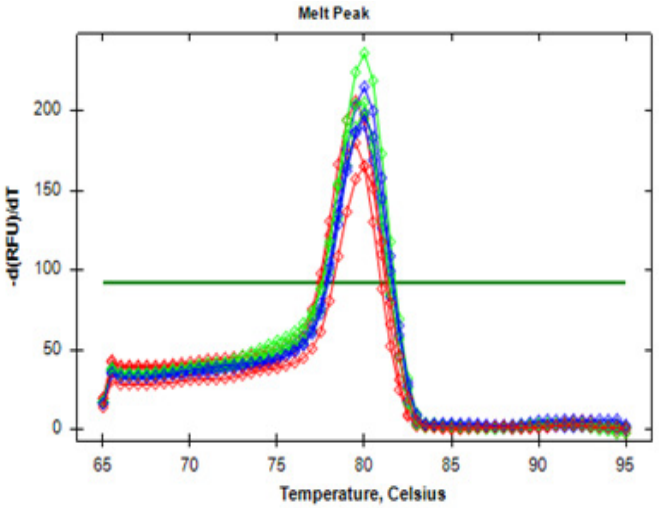

Figure 8: qPCR melt peak of SAG5 gene in camel tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

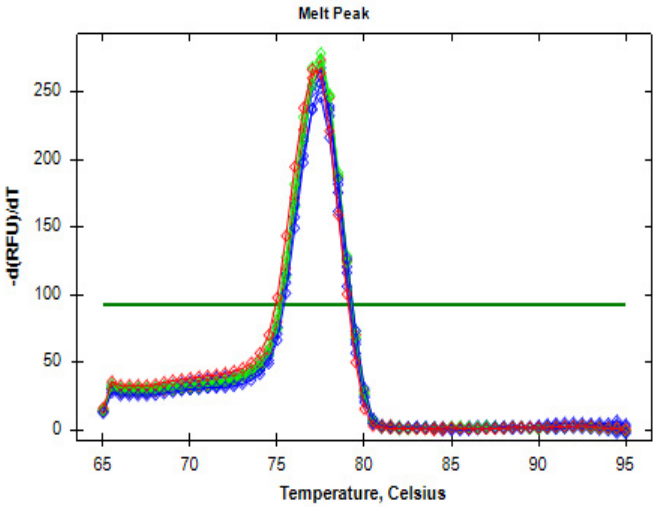

Figure 9: qPCR plots of GAPDH gene in camel tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

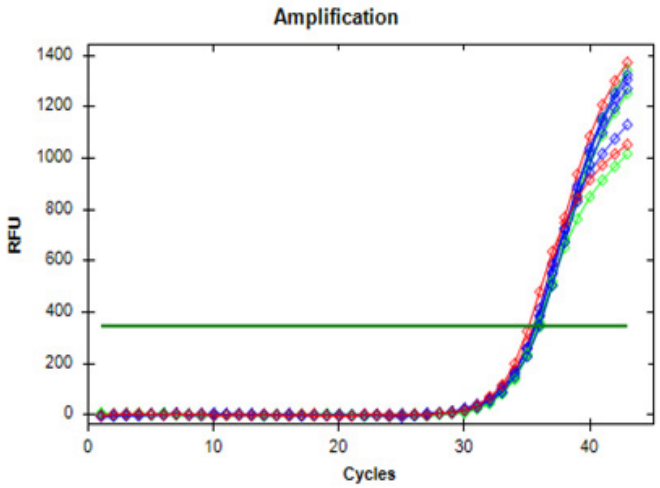

Figure 10: qPCR melt peak of GAPDH gene in camel tissue sample. The blue, red, green plots for cranial, medium, caudal part, respectively.

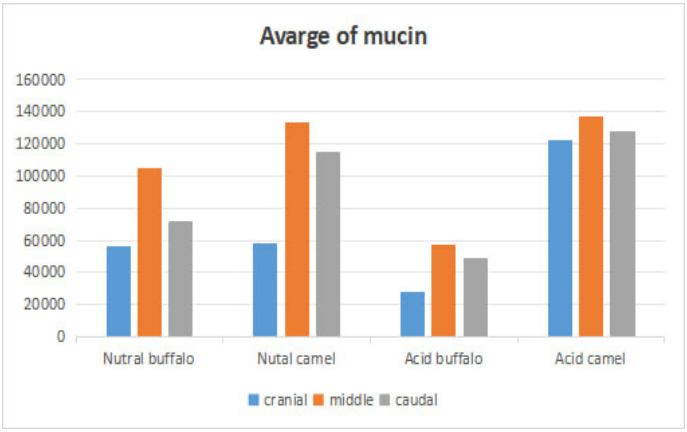

Figure 11: The semi-quantification of alcian blue (acidic mucin) is displayed in the bar graphs. The staining area of buffalo and camels is known as PAS, or periodic acid-schiff (neutral mucin). The data are displayed as the mean±SD of the percentage of the histological section's dependent histological section for each tissue type that expresses alcian blue staining.

Histologically, the ileum of buffalo and camel is classified into three regions: proximal, middle, and distal. Although the histological similarity of these sites is evident, some minor differences facilitate their identification. The luminal surface of the three parts of the ileum has been altered to enhance its surface area. Microscopic examinations revealed the presence of four layers: mucosa, submucosa, muscularis, and serosa. Microscopic and macroscopic folds (villi) were observed throughout the ileal mucosa. The mucosa of both animals' ileum was lined with simple columnar epithelium composed of surface absorptive cells and goblet cells (Figure 12). A diminished quantity of goblet cells was noted at the apex of the villi as one approached the terminus. A thick tunica muscularis was observed in camels, as indicated in the table. An inner circular layer and an outer longitudinal layer were present. The inner circular layer of the tunica muscularis exhibited a greater thickness in the Peyer's patches of camels compared to that of buffaloes. The tunica serosa of the camel and buffalo was noted to be a thin layer of connective tissue (Figure 13).

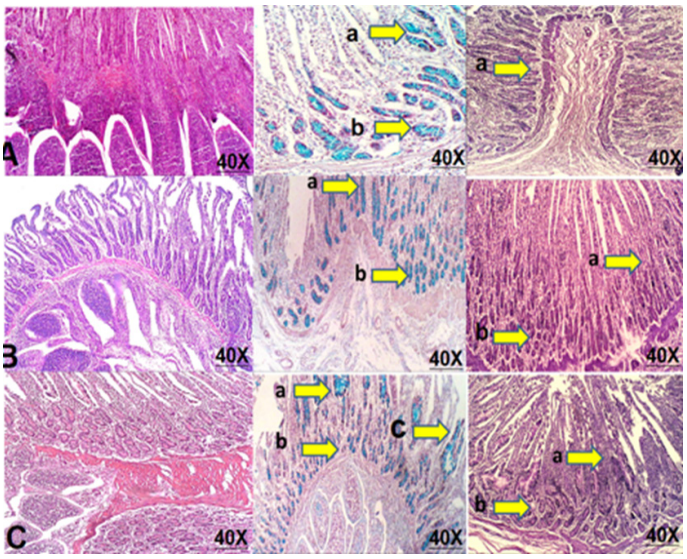

Figure 12: Histology of buffalo ileum. Representative histology sections of different parts of the cranial (A), middle (B) and caudal (C) ileum stained with H & E, and a alcian blue and PAS stains are shown. Photographs are labelled with (a) epithelial tissue, and (b) intestinal glands were positive stained with alcian blue, whereas (c) epithelial tissue was stained with PAS stain (40x).

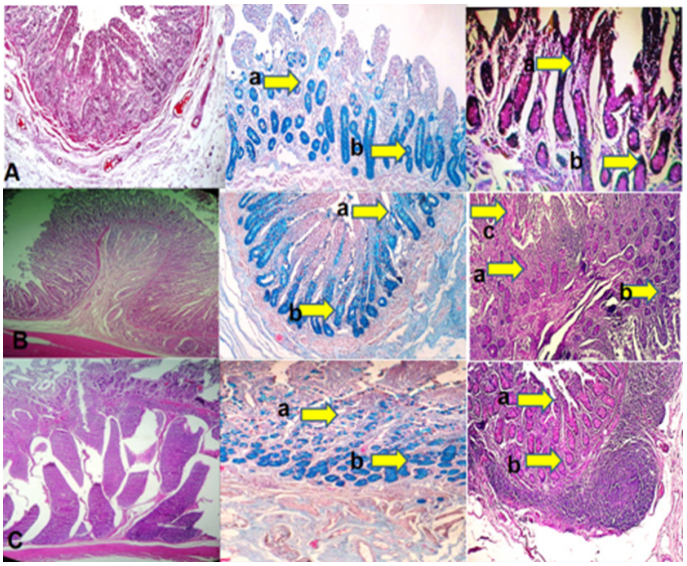

Figure 13: Camel ileum histology. Here are representative histology sections of various cranial (A), middle (B), and caudal (C) ileum sections stained with PAS, alcian blue, and H&E. Intestinal glands were positively stained with alcian blue in the photographs labeled (a) epithelial tissue, while (b) epithelial tissue was stained with PAS stain (40x).

Histochemically, the ileal epithelium of both species is separated into three sections: Proximal, middle, and distal, each comprising three components: the tip of the

intestinal villus, the villus-crypt space, and the crypt base. The histochemical characteristics of these components in the three regions were analyzed, and the data were appraised subjectively. In all parts of the camel's ileum, the goblet cells at the top of the intestinal villi, the space between the villi and crypts, and the bottom of the crypts showed much stronger staining with PAS and AB than those in the buffalo (Figure 11, 13). The epithelium of the three sections of the ileum did not contain any glycogen-containing cells. In camels, goblet cells had a lot of neutral and carboxylic acidic mucopolysaccharides but only a little bit of sulfated mucopolysaccharides. The concentration of neutral and acidic mucopolysaccharides in both animals was elevated in the middle area, with camels exhibiting higher levels of these mucopolysaccharides compared to buffalo throughout all three regions. The three parts of the ileum showed much higher amounts of neutral mucins and a large presence of acidic mucins (Figure 12, 13).

DISCUSSION

This study aims to examine the gene expression, histological, and histochemical properties of the siglec-5 gene in the proximal, middle, and distal regions of the ileum in buffalo and camel. RT-qPCR was used to assess gene expression. The results provide major new insights into the differences in histological composition and gene expression between the two species. Data from RT-qPCR show that in both buffalo and camels, the siglec-5 gene is expressed in all regions of the ileum (proximal, middle, and distal), although there are no previous studies of this gene in the digestive tract, especially the intestine. Still, however, the two species showed differences in expression levels. While the cycle threshold (Ct) values of the siglec-5 gene in camels ranged from 30 to 35, in buffalo they ranged from 20 to 25. The difference was due to the difference in the nature of the food and the nature of the environment in which both animals live, which is what Toivonen *et al.* (2016) explained, where it was explained that the effect of food and environment on the difference in the levels of the siglec gene in the intestines. Melting curves clearly showed a melting peak at 75-80 °C for buffalo and 65-80°C for camels, thus confirming the specificity of the amplification. This difference lacked statistical significance, although the distal part of the small intestine compared to the proximal and middle regions had a higher concentration of siglec-5. This is consistent with Kopat (2015) and Shahraz (2016). who explained the data associated with Siglec 11 that disorders and inflammatory reactions in the central nervous system are associated with Siglec receptors. The expression of the Siglec-5 gene in the distal small intestine of buffalo was found to be somewhat elevated compared to that in camels. These data may indicate a potential role of the Siglec-5 gene in immunological or metabolic functions that vary between the two species. The prebiotics that enhance gut microbiota development, gut maturation, pathogen resistance, immunomodulation, anti-inflammation, and neurodevelopment are Siglec bioactive compounds (Jahan *et al.*, 2021).

The ileum in both buffalo and camels is anatomically divided into three sections: Proximal, middle, and distal. Though there was general histological similarity among these areas, a few minor differences were observed that helped to distinguish them. Each species displayed the four main layers of the ileum: mucosa, submucosa, muscularis, and serosa. In the mucosa of the ileum, also seen were large folds (plicae) and microfolds (villi). Simple columnar epithelium made of surface absorptive cells and goblet cells made up buffalo and camels' mucous membrane. This is the same thing he reached (Jarrar and Faye, 2013) in camels (Watanabe *et al.*, 1983; Ahmed and Saleh, 2022) in buffalo. At the top of the villi, one observed a drop in goblet cell count as one neared the terminal section of the ileum. Particularly in areas of Peyer's patches, the inner circular muscle layer in camels was more robust than in buffaloes. These differences could point to functional modifications relevant to absorption or digestion in every category. The mature lymphocytes subsequently enter systemic circulation and migrate to various mucosa-associated lymphoid tissues throughout the body, ultimately homing to the intestine via high endothelial venules and gut-associated lymphoid tissue, delivering antigen-specific lymphocytes to locations likely to re-encounter the antigen (Heel *et al.*, 1997).

Signifying a higher amount of neutral and acidic polysaccharides in camels. Histochemical, the goblet cells in camels showed stronger staining with PAS and Alcian Blue (AB) than those in buffaloes agreeing with (Mohamedien, 2015; Al-Mahanna *et al.*, 2024) in camels and (Singh, 2015) in buffalo. In all categories, the middle area of the ileum showed higher levels of both neutral and acidic polysaccharides; the camel showed excellence in this respect. Still, none of the three sections of the ileum in either type revealed any cells with glycogen-containing capacity. The results show clear differences in gene expression and histological structure between the two species, maybe related to functional adaptations connected to their environment or feeding pattern. The increased expression of siglec-5 in buffalo relative to camels could point to a possible role of this gene in immune response or metabolic processes particular to buffalo. Furthermore, variations in histochemical characteristics, particularly with relation to polysaccharide concentration, may suggest differences in mucosal functions between the two species. Further studies are advised to investigate the functional analysis of siglec-5 and its participation in the

immune response in order to improve our knowledge of the function and the ileal tissue features in buffaloes and camels. determining how environment and diet affect tissue characteristics and gene expression. a thorough study of the histochemical characteristics of the two species applying modern methods like mass spectrometry. Future research will improve knowledge of the functional and molecular adaptations within ruminant species' digestive systems.

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NOVELTY STATEMENT

This study presents a novel comparative histological, histochemical, and gene expression analysis of the ileum in dromedary camels (*Camelus dromedarius*) and water buffaloes (*Bubalus bubalis*). To our knowledge, this is the first research to investigate the expression of Siglec-5 in the ileum of these two species. The findings provide new insights into species-specific adaptations at the tissue and molecular levels, contributing to the understanding of gastrointestinal immune regulation in large ruminants.

AUTHOR'S CONTRIBUTION

Jaafar Anwar Jaafar: Conceptualization, sample collection, laboratory work, data analysis, writing original draft. Prof. Dr. Sameer Ahmed Abid Al-Redah: Supervision, methodological guidance, critical revision of the manuscript.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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