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Original Research Article

Study of Brunner's Glands Using Histology and Histochemistry in Various Age Groups of Cats, Dogs, and Goats

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Abstract: The purpose of this research is to clarify what Brunner's glands are and how they are distributed in the duodenum of three different dietaryly diverse animals: cats, dogs, and goats. Each species had a total of 20 individuals, which were then divided into four groups of five animals each. The animals were categorized according to their age, which spans from one day to sixty days. After collecting samples from several locations in the duodenum and preserving them with formalin, they were later analyzed histologically. The sections were stained with various dyes, including hematoxylin and eosin, Masson's trichrome, Periodic acid Schiff, Alcian blue PH 2.5, and a mixture of the two stains. All of the species included in the study exhibited an abundance of vacuolated columnar cells in their epithelium-lining villi at the one-day mark. Short and uneven, with intervals in between, were the villi. Except in canines, where the crypts were plainly apparent, the bases of the villi did not have crypts in the lamina propria, but they did contain clusters of immature cells. Also, the muscularis mucosa did not remain constant from one age group to the next. Furthermore, with the exception of goats, a Brunner's gland vanished from the submucosa in as little as one day. It wasn't until the animal was twenty days old, though, that this happened. The muscularis mucosa did not have a completely matured circular layer, but the mucosa contained thin cylindrical villi. During histogenesis, Brunner's glands and the crypts both grew. The villi changed into their mature form in forty days, the crypts were visibly expanding, and the muscularis mucosa formed a circular layer with more Brunner's glands in the submucosa. The mucosa developed mature villi resembling leaves after sixty days. An advanced circular muscular layer covered the muscularis mucosa, and the crypts were more along in their development. Goats have a mix of gland types, in contrast to canines and cats whose duodenal glands are mucous-based. Within the submucosa, the glands were composed of closely packed acini. Positive reactions were detected in dogs and cats only with Periodic acid Schiff, but in goats, they were seen with Alcian blue, Periodic acid Schiff, and a mix of the two. The villi's morphology, crypt count, and gland count were all greatly affected by the age and diet of the subject. Furthermore, this study used a number of morphometrical analyses to show how the lining changed with time.

Keywords: Development, Brunner's Gland, Cat, Dog, Goat.

INTRODUCTION

Digesting food, absorbing it, building immunity in the host, and excreting waste are all vital functions of the gut, an important organ. A tiny part of the small intestine called the duodenum is in charge of enzyme digestion of nutrients. Hormones released by the pancreas cause the pancreatic duct to open and release pancreatic juice and bile [1, 2]. The duodenal submucosal glands in the duodenum help all mammals absorb nutrients by secreting a mucus that digestive enzymes can use. The submucosa of the proximal duodenum is where the glands are located [3, 4]. Duodenal mucus secretion protects the duodenum from acidity, making it less sensitive to acidic chyme than other parts of the small intestine. So, before the chyme enters the jejunum, the duodenum balances its acidity, protecting the rest of the small intestine [5]. The secretory branches of Brunner's glands, which are tubuloalveolar glands, resemble mucous acini. The ducts that originate from these glands usually reach the deepest section of the Lieberkuhn crypts [6–8], and then pass through the muscularis mucosae to convey their secreted product into the duodenal interior. A mucus discharge is produced by glands located in the submucosa of the duodenum. All mammals possess these glands [9]. From infancy through maturity, the duodenal glands of animals grow and change in size and structure. As they continue to swell, the glands eventually form a compact cluster [10-13]. In Mole-rats, the submucosa of the duodenum forms coiled tubules after birth [14]. Multiple sugar molecules are found in mucins, which are also called glycosaminoglycans and mucopolysaccharides [15]. Both acid and neutral mucins are produced by epithelial cells. Sulfomucins include sulfate, while sialomucins contain sialic acid [4]. Acid mucins come in two different varieties. As a morphogenetic process, the creation of finger-like structures termed villi and crypts enhances the gut's ability to absorb nutrients, allowing it to satisfy the daily requirements [6-8]. Nutrient absorption and defense against commensal and harmful bacteria prevalent in the adult gut are both facilitated by the villi's unique cell types [10-14]. Analyze the evidence supporting the concept that the gut mucosa's overall differentiation is regulated by a complex network of interconnected signaling pathways. The intestinal epithelial layer frequently generates immature cells due to its fast and constant regeneration [15]. Rapid cell proliferation creates a transit amplification zone as these cells ascend the axis that links the crypt to the villus. This area is responsible for producing specialized epithelial cells such as paneth cells, enterocytes, goblet cells, and enteroendocrine cells [1-16].

METHODOLOGY

From February 2022 to February 2023, a total of forty healthy animals were used in this investigation. The felids (domestic cats), canids (domestic dogs), and herbivores (goats) were all part of the research. The parents of each species were housed in cages at the College of Veterinary Medicine at Al-Muthanna University. The cages were lit artificially (dark/light8-16) and a feeding system was set up so that the animals could breed and procreate. Each species' offspring were divided into four groups of five animals each according to their chronological age: one day at birth, twenty days at sucking time, forty days at preweaning age, and sixty days after weaning, when the goats were fed a solid diet and greenish food, and the cats and dogs were fed flesh. Every day, except for one (direct sloughing), the animals were put down using an overdose of xylazine and ketamine. Each animal had its abdomen ventral wall removed, and the duodenum was separated from the pylorus by severing it just before it joins the jejunum. Histological analysis was conducted on specimens fixed in formalin and collected from various locations within the duodenum. Multiple staining techniques were employed on the specimens, including hematoxylin and eosin (H&E), Periodic acid Schiff (PAS), and Masson's trichrome, which highlight collagen and smooth muscles, respectively. To identify acid mucopolysaccharides, researchers utilized Alcian blue with a pH of 2.5; to detect neutral mucin, they employed Alcian blue in conjunction with Periodic acid Schiff [17]. The user is inquiring about the quantities of Brunner's gland alveoli or acini and the thickness of the tunicae (mucosa, submucosa, muscularis, and adventitia) in a 200x magnified permiroscopic area [18].

RESULTS

There were four separate layers to the duodenum wall in all the animals and age groups that were studied: the mucosa, submucosa, muscularis, and serosa (Fig.1,2,3). A thin layer of smooth muscle called muscularis mucosa, loose connective tissue called lamina propria, and columnar epithelium make up the mucosa. Columnar epithelium lines the Lieberkuhn crypts, and hematoxylin and eosin staining revealed pale cytoplasm in individual mucous cells (Fig.1,3), While periodic acid Schiff stained them magenta (Fig.2), Masson's Trichrome pale green (Fig.4), Alcian (Fig. 5-7), and periodic acid Schiff combined with Alcian blue (Fig.8), the tissue seemed blue overall. Figures 2, 3, 9, and 10 show its inside structure, which includes gland-filled submucosal connective tissue, the muscularis externa, two layers of smooth muscles, and a thin serosa layer made up of simple squamous cells.

The villi epithelium consisted of columnar cells containing vacuoles at one day of age in all species studied. The individual villi were slender, brief, and perfectly symmetrical, with intervals in between. Clusters of immature cells were found at the villi bases. The muscularis mucosa was not continuous, and there were no crypts in the lamina propria. Furthermore, the absence of a Brunner's gland in the submucosa of cats and dogs was noted (Fig.1, 3). However, compared to other animals, goats showed fewer Brunner's gland acini on the first day of life (Fig.2). At one day of age, the duodenum walls of dogs measured 172.2±4.1μm for mucosa, 74.1±1.2μm for submucosa, 101.2±0.2μm for muscularis, and 15.1±0.3μm for serosa. Cats had thicknesses of 137.6±3.1μm, 57.6±0.1μm, 87.6±0.4μm, and 13.2±1.1μm, respectively. The thicknesses in goats were around 289.2±1.1μm, 86.5±3.4μm, 136.2±2.1μm, and 16.2±1.1μm, respectively. Goats' tunicae were thicker on average than cats' and dogs'. The number of acini in the Brunner's gland of the goat duodenum was 6±1, according to Table 1.

At the 20-day mark, a circular smooth layer had developed imperfectly on the muscularis mucosa, and the mucosa had thin, tube-like projections known as veil. The Lieberkuhn crypts were also seen in every single animal (Fig.4, 7). Goats' duodenal glands mature within the first twenty days following birth. In the submucosa of the duodenum, a small number of serous acini and a small number of Brunner's gland acini were noted. On the other hand, canines and felines did not exhibit these acini. At this age, the canine dimensions of the mucosa, submucosa, muscularis, and serosa were 201.6±2.4μm, 81.2±0.1μm, 113.2±0.3μm, and 19.2±0.2μm, correspondingly. Cats had thicknesses of 168.3±3.1μm, 66.3±1.2μm, 92.6±1.1μm, and 16.1±0.5μm, respectively. Thicknesses of 311.2±1.5μm, 97.2±0.4μm, 153.2±0.3μm, and

19.2±0.1µm were recorded in goats. In goats of this age, the number of Brunner's gland alveoli in the duodenum was 20±2. According to the data in Table 1, the duodenum of goats was thicker than that of cats and dogs. Goats show off their crypts around 20 days after birth.

Table 1: The thickness of the wall layers and the number of acini in Brunner's gland in the duodenum of rabbits and mice were measured in micrometers (X- ± S.E).

Tunica / Part	Mucosa	Submucosa	Muscularis Externa	Serosa	Number of acini
In 1 day age					
dog	172.2±4.1	74.1±1.2	101.2±0.2	15.1±0.3	-
cat	137.6±3.1	57.6±0.1	87.6±0.4	13.2±0.4	-
goat	289.2±1.1	86.5±3.4	136.2±2.1	16.2±1.1	6±1
In 20 day					
age dog	201.6±2.4	81.2±0.1	113.2±0.3	19.2±0.2	-
Cat	168.3±3.1	66.3±1.2	92.6±1.1	16.1± 0.5	-
Goat	311.2±1.5	97.2±0.4	153.2±0.3	19.2±0.1	20±2
In40 day					
age dog	241.4±3.5	106.4±2.1	196.4±2.3	18.4±0.2	18±1
Cat	203.9±4.1	93.2±3.2	171.2±3.4	17.3±0.3	24±3
Goat	343.2±0.3	143.2±0.3	221.1±2.1	21.4±0.1	29±3
In 60 day					
age dog	311.4±2.1	157.2±1.2	204.3±1.5	23.3±0.1	34±2
Cat	290.1±3.4	123.1±2.3	173.1±1.3	22.4±0.2	42±1
Goat	383.2±0.2	172.2±0.3	223.2±1.4	24.2±1.4	72±3

Cell vacuolation had decreased, villi had taken on their adult form, and crypts were obviously expanding after 40 days. There was an increase in the number of Brunner's gland acini and the duodenum submucosa, and the muscularis mucosa had produced a circular smooth layer (Figure 8-10). In the duodenum of preweaned dogs at forty days of age, the thickness of the mucosa, submucosa, muscularis, and serosa was measured to be 241.4±3.5µm, 106.4±2.1µm, 196.4±2.3µm, 18.4±0.2µm, and 18±1µm, respectively. It should be noted that Brunner's gland was only seen in the proximal part of the duodenum during this time (as shown in Figure 9,10).

The similar values in cats were 203.9±4.1µm, 93.2±3.2µm, 171.2±3.4µm, 17.3±0.3µm, and 24±3, in that order. Measurements of 243.2±0.3µm, 143.2±0.3µm, 221.1±2.1µm, 21.4±0.1µm, and 29±3 micrometers were recorded in goats, respectively. Goats also have thicker tunicae in their duodenums compared to cats and dogs (Table 1).

At 60 days of age, mature villi with a leaf form made up the duodenal mucosa. Cell vacuolation was not observed in the duodenal epithelium. Columnar epithelium lined the crypts, which had grown in depth and number throughout the years. The circular muscle layer, known as muscularis mucosa, was quite developed. Figure 11 shows the distribution of duodenal glands over the length of a goat's duodenum, which extends towards the jejunum, Dogs and cats have mucous glands in their duodenums, but goats had a mix of serous and mucous glands. At sixty days of age, the duodenal mucosa, submucosa, muscularis, and serosa thicknesses were measured. The thicknesses in dogs were 311.4±2.1µm, 157.2±1.2µm, 204.3±1.5µm, and 23.3±0.1µm, respectively. The thicknesses in cats were 24.40±0.2εm, 173.1±1.3µm, 123.1±2.3µm, and 290.1±3.4µm, respectively. The relevant thicknesses in goats were 383.2±0.2µm, 172.2±0.3µm, 223.2±1.4µm, and 24.2±1.4µm, respectively. When compared to dogs and cats, goats had thicker tunicae. At sixty days of life, we also counted the alveoli in the duodenal Brunner's gland. Goats had 72±3 alveoli, cats had 42±1 alveoli, and dogs had 34±2 alveoli. Goats had the highest concentration of Brunner's glands of any animal studied.

Duodenal glands of the mucous type were found in the submucosa of preweaned cats and dogs. On the other hand, goats had the serous kind. Tightly packed acini made up these glands. Only when administered to goats with periodic acid Schiff did they exhibit a good reaction [2-14]. Figures 5, 6, 9, and 13 reveal that cats and dogs displayed a robust alcinophilia and a positive reaction to Alcian blue, suggesting the presence of substantial levels of weakly sulphated acid mucin. As people age, they enter the post-weaned era, As shown in Figures 8 and 10, the Brunner's glands were determined to be composed of mixed acini that contained neutral mucin, according to the PAS-AB reaction, suggesting the existence of a mixture of acids and bases. Weak reactions to PAS, increased concentrations of acid mucin and mild neutral mucin, and an increase in blue intensity were the causes (Fig. 2, 14). Due to differences in carbohydrate composition between the duodenum's crypts and Brunner's glands, the former were more likely to contain non-sulfated mucin, and the latter were more likely to contain neutral or sulfated acidic mucin in the vibroli. Glycosaminoglycan levels increased with age, especially in the postweaned years. An increasing number of secretory acini and simple branched tubule-alveolar formations were features of well-developed Brunner's glands (Fig. 10, 14). After 60 days of aging, Brunner's glands in all the animals that were studied show a combination of neutral mucin and mixed acini (Fig. 8, 10, 14). In contrast to mucus acini, which had a relatively broad lumen and pale-looking cells, serous acini had a smaller lumina and darker constituent cells with nuclei positioned centrally. The number of crypts and Brunner's glands, in addition to the villi's morphology, were all greatly affected by aging. In addition, detailed morphometrical studies were conducted in this work to show that the duodenum wall changes with age (Fig.14).

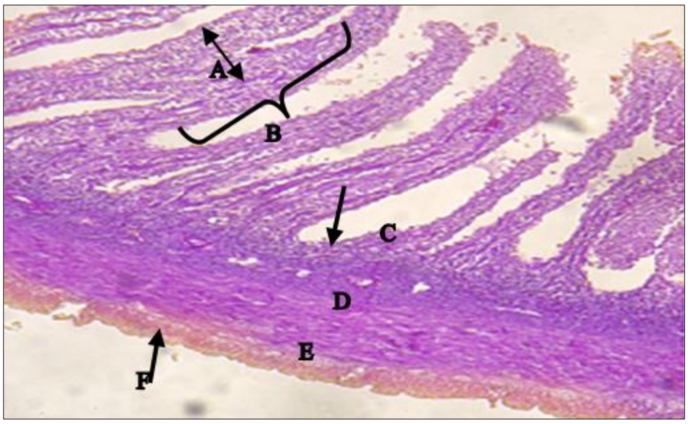

Fig. 1: Cross section of cat duodenum at 1 day age; mucosa (A), villi (B), intervillus space (C), submucosa (D), musclaris (E), Serosa (F), H&E 40X

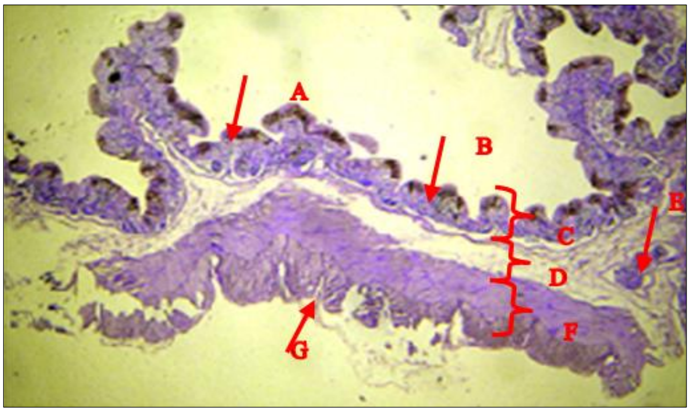

Fig. 2: Cross section of goat duodenum at 1 day age; mucosa (A), villi (B), intervillus space (C), submucosa (D), glands (E), muscularis (F), Serosa (G), PAS 100X

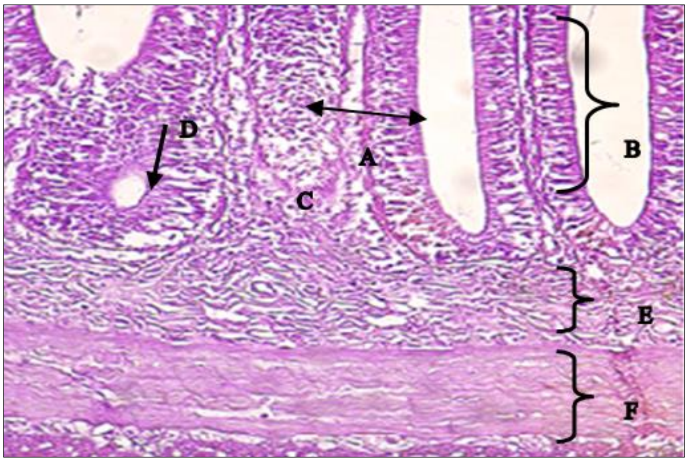

Fig. 3: Cross section of dog duodenum at 1 day age; mucosa with vacuolated cells (A), villi (B), lamina propria (C), crypt (D)

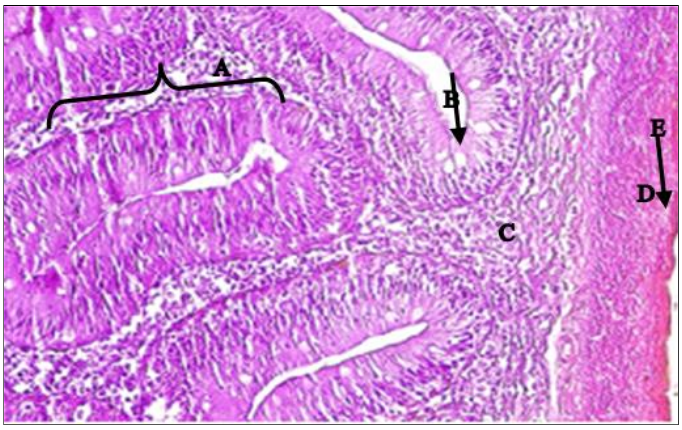

Fig. 4: Cross section of dog duodenum at 20 day age: crypt (A), goblet cells (B), submucosa (C), muscularis (D), serosa (E), masson trihrome 100X

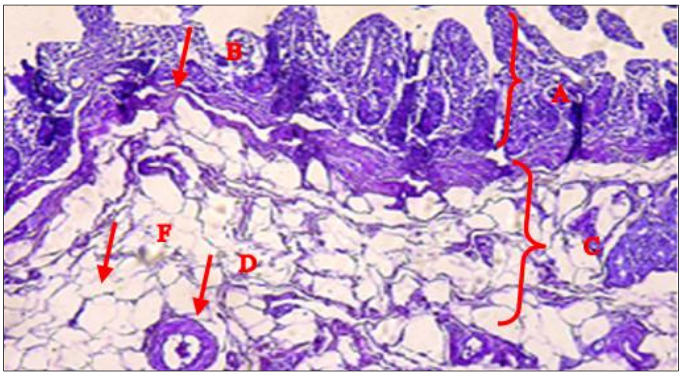

Fig. 5: Cross section of cat duodenum at 20 day age, mucosa (A), muscularis mucosa (B), Submucosa (C), blood vessel (D)

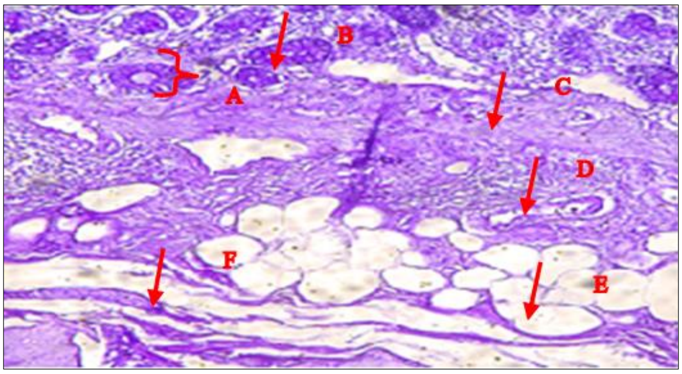

Fig. 6: Cross section of dog duodenum at 20 day age: crypts (A), goblet ceels (B), muscularis mucosa (C), blood vessel (D), adipose tissue (E), fibers (F), AB 400X

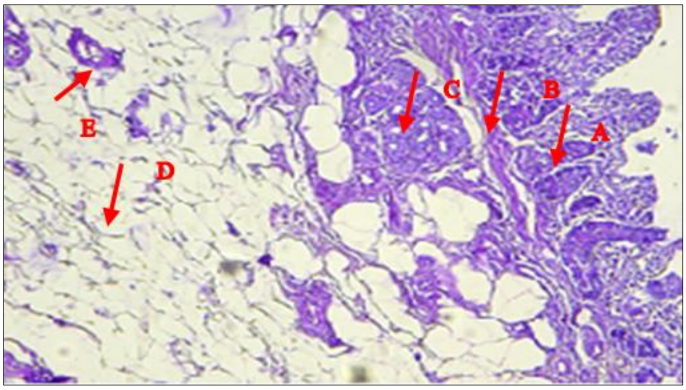

Fig. 7: Section of goat duodenum at 20 day age: goblet cell (A), muscularis mucosa (B), Brunner glands (C), adipose tissue (D), blood vessels (E), AB 200X

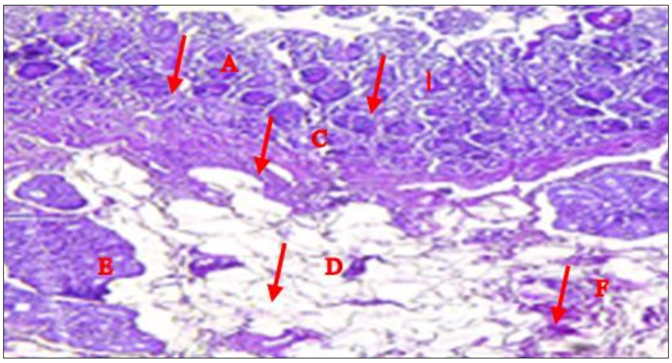

Fig. 8: Section of cat duodenum at forty day; goblet cell (A), crypt (B), muscularis mucosa (C), adipose tissue (D), Brunner gland (E), Fiber (F), PAS-AB 200X

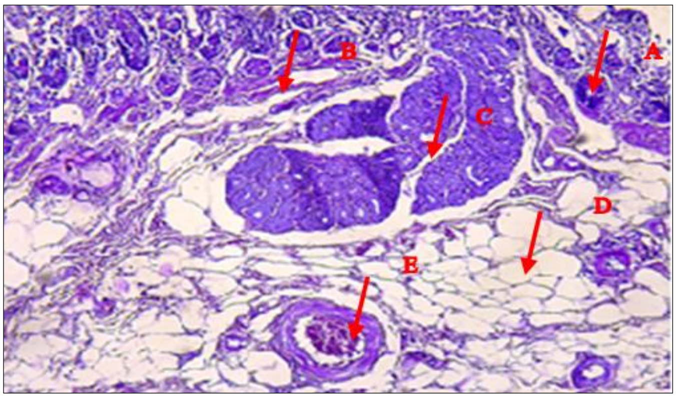

Fig. 9: Cross Section of cat duodenum at 40 day; goblet cell (A), musclaris mucosa (B), Brunner glands (C), adipose tissue (D), blood vessels (B), AB 200X

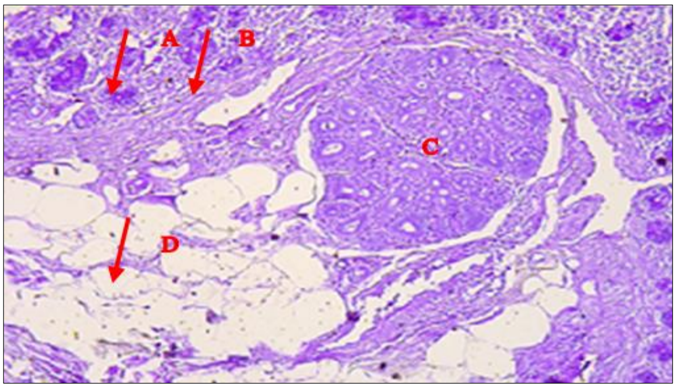

Fig. 10: Cross Section of dog duodenum at 40 day age; crypt (A), muscularis mucosa (B), Brunner glands (C), adipose tissue (D)

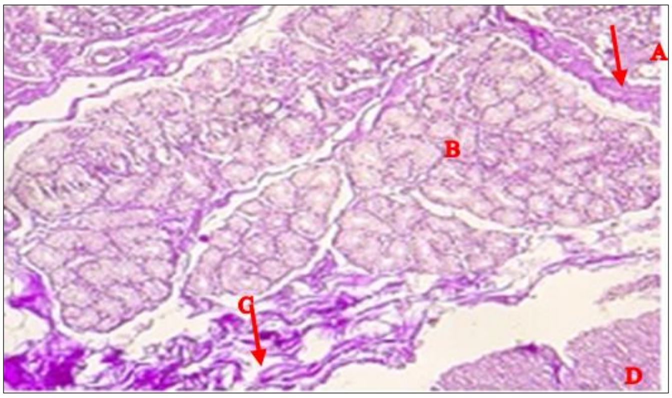

Fig. 11: Cross Section of goat duodenum at 60 day age; epithelium (A), Brunner glands (B), blood vessels (C), submucosa (D)

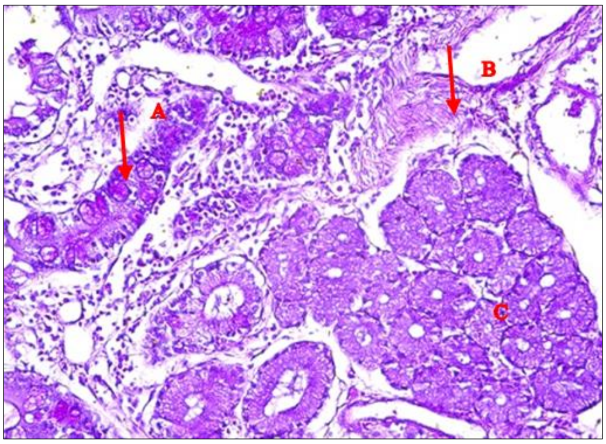

Fig. 12: Cross Section of dog duodenum at 60 day age; muscularis mucosa (A), Brunner glands (B), connective tissue (C), masson

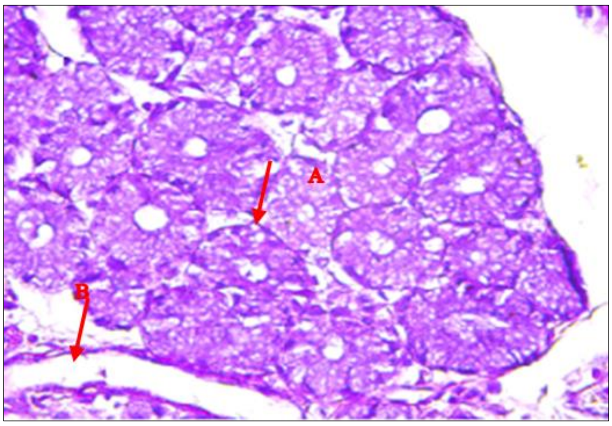

Fig. 13: Cross Section of cat duodenum at 60 day age; acini of the Brunner glands take the positive reaction to stain (A), connective tissue (B), AB 400X

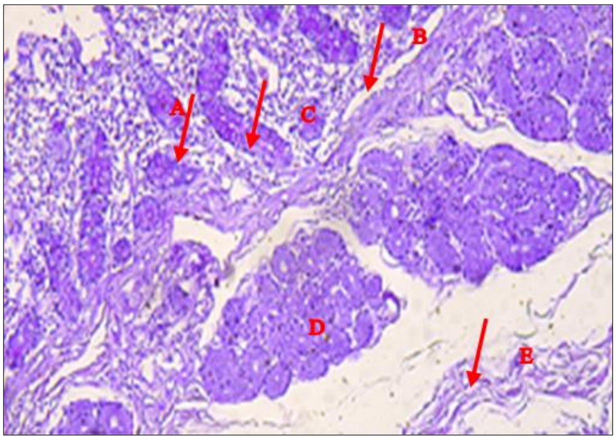

Fig. 14: Cross Section of goat duodenum at 60 day age; goblet cell (A), musclaris mucosa (B), crypt (C), Baunner glands (G)

DISCUSSION

Study animals' duodenal walls vary and the organ's histological structure changes with age; the intestinal mucosal barrier is not fully formed in newborns; the proportion of goblet cells and Brunner glands grows gradually with age, reaching its peak at sixty days; and so on. Since the majority of their distinctive histomorphological alterations happened within this sixty-day window, it is considered the essential period of development for these changes [19]. Mention The majority of food breakdown occurs in the duodenum, despite its modest size within the small intestine. While the size and shape of the intestinal villi might reveal something about the mucosa's functionality, some have argued that villi morphology isn't necessarily indicative of a person's health [20]. Some, however, contended that villi's compact and folded structure reduced the amount of surface area that came into direct touch with digestive tract contents, leading to ineffective absorption. Another possibility is that the villous morphology and layout are affected by the nutritional makeup. Genetic strains and species determine [1-19]. Dietary fibers, like cellulose and pectin, can sharpen and compress the villi, which in turn increase the small intestine's surface area and aid digestion and absorption [21].

The submucosal glands that line the duodenum help absorb nutrients, produce an alkaline mucus, and assist the work of intestinal enzymes. The pancreatic duct enters the duodenum via the descending bile duct and pancreatic juice. Bile aids in the digestion and absorption of fats, whereas pancreatic juice contains enzymes that aid in these processes [22, 23]. Due to hormones secreted by the duodenum, the pancreatic duct is stimulated to release pancreatic juice and bile [24]. In addition, the duodenum is much less acid-sensitive than the remainder of the small intestine due to mucus secreted by submucosal glands [25]. Consequently, the duodenum protects the remaining small intestine from harm by eliminating the infection [26].

Sulphomucin and sialomucin are subtypes of the acidic mucins, which can be either neutral or slightly acidic. The acid mucin that is produced is a combination of sialomucin and sulphomucin, with sialomucin being the more predominant component. Weaning is characterized by a sharp decrease in sialomucin concentration [23]. Differences in goblet cell quantity have also been seen in other mammalian species; the amount of goblet cells present in the intestine may be affected by the type of diet ingested at different postnatal ages. One of the defenses that mucin uses against bacteria is goblet cells [1-3]. Intestinal goblet cell density may be affected by the maximum viscosity of luminal materials. The amount of gut crypt cells can actually be affected by one's diet [24].

Rather than being found on the first day of life, the glands were discovered in the third week. Concurring with the findings of [23-25]. Present evidence suggests that after weaning, animals start eating large quantities of solid feed. Goat duodenal glands were seromucous, in contrast to the mucous glands found in dogs and cats. The crucial role of Brunner's glands in shielding the mucosa from the acidic effects of gastric juice was associated with these anatomical changes. Mucus production, alkalinity, and maybe the ability of its level of bicarbonate to act as a buffer against digestive tract acidity [5-7]. Consequently, the duodenal glands in goats reach the jejunum, unlike in dogs and cats where they stop short, according to a recent study [23-26]. The number of Brunner glands is highest in herbivores and lowest in carnivores [23]. Along with producing urogastrone, an immunoglobulin demonstrator, and inhibiting production of gastric acid, these glands' secretions also lubricate and preserve the duodenal lining [27].

As an organ, the adult gut is intricate and multipurpose. After the stomach has digested and broken-down food, it interacts with the small intestine. The intestinal enterocytes are part of a complex circulatory network that facilitates the absorption of minerals, proteins, carbohydrates, and lipids [23-27]. The epithelial layer acts as a barrier between the intestinal contents and the outside environment, preventing the body from being infected by local microorganisms [28]. In addition to nutrient-rich mucus, the epithelium secretes mucus, a thick barrier that covers the intestines and serves to both prevent infection and trap germs. Another important role that epithelial cells play in immunity is the secretion of peptides that inhibit the growth of bacteria and fungi [29, 30]. The villi showed a wide range of shapes, and their heights varied widely. This could be because the very tall villi have such a large surface area, suggesting that absorption is highest in older animals (as seen in Angora rabbits, according to [24]. The results in cape hare were comparable to those in Lieberkuhn's crypts, which are surrounded by columnar epithelium [4]. These cavities help break down food by secreting digestive juices [23-33]. Gut paneth cell differentiation, which happens simultaneously with crypt emergence, generates antimicrobial defense proteins to ward off infection by pathogens. New research has shown that the maturation of the intestinal epithelium may depend on substantial changes in its metabolism throughout development [23].

CONCLUSION

The current study adds new knowledge and documents the structural differences in Brunner's gland growth in animals were unique in feed, age has a significant impact on villi shape and number of crypts and glands, and numerous morphometrical analyses showed how the duodenal wall altered with age and goats had thicker tunicae and more glands than dogs and cats.

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