

Biochemical, Histochemical and Physiological Characterization of The Stomach and Cecum in Rabbits and Rats

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ABSTRACT

Objective: To assess physiological variations from gastric and cecal activities, compare the biochemical makeup of secretions in rabbits and rats, and correlate structural variations with functional adaptations. **Method:** Ten samples from the stomach and cecum; five from each animal; were used. **Results:** Rats' omnivorous diet necessitates effective protein digestion, which is reflected in their higher acid as well as enzyme output. Rabbits have more mucus production and lower enzymatic activity because they rely more on fermentation by bacteria in the hindgut. Pyloric, fundic, and cardiac glands are present in rabbits, whereas the stomach of rats is devoid of cardiac glands. Fibrous plant material is stored and first processed in a conspicuous non-glandular area found in rabbits, while rats have a more active glandular region ideal for quick digestion. The number of goblet cells in the rabbit's cecum was generally higher. The rabbit cecum is specialized to high-efficiency, long-lasting fiber fermentation along with nutrient recycling via cecotrophy. Significant histochemical, biochemical, and physiological variations were observed in the stomach and cecum of rats and rabbits due to differences in food patterns and digestion processes. **Novelty:** The study highlights comparative histochemical, biochemical, and physiological adaptations of the stomach and cecum in rabbits and rats, emphasizing the relationship between dietary patterns, glandular structure, goblet cell density, mucin composition, and digestive functional specialization.

INTRODUCTION

Numerous medical applications, especially experimental surgical treatments, depend on an understanding of rodent gut morphology [1]. Rabbits are distinguished by their distinct stomach anatomy, which includes a large, simple stomach [2]. Due to their meat and fur, their usage as pets, their importance in scientific and medical research, and the fact that rabbit meat is low in fat and high in protein, rabbits are regarded as economically valuable creatures [3]. Rats and rabbits are widely utilized in labs as traditional experimental animal models for a variety of studies, including nutritional research [4]. The gastrointestinal tract is the only one that provides the body with the energy it needs through the absorption of nutrients. By breaking down different kinds of food. Small herbivore species are limited by their nutritional apparatus systems because they require more energy and protein per unit mass than big herbivores [5], [6]. Therefore, in order to overcome the limitations of their small body mass in compared to larger animals, young herbivores need unique digestive strategies [7]. Despite having larger cecums, tiny herbivorous mammals differ in the flow and mixing patterns of digestion in the large intestine [8], [9]. Morphometric research on gastric mucosa are crucial in the identification of gastric ulcers. The digestive system is made up of the organs involved in the ingestion and digestion of food, its passage through the body, the

explosion of the remaining absorbed portion, and its primary storage of the digested food [10], [11]. Additionally, a variety of conditions, such as gastric reflux, dyspepsia, a gastroparesis, a pyloric a narrowing of and rapid gastric emptying, can be diagnosed using quantitative assessments of stomach muscle and its configuration [12], [13]. The stomach performs a variety of vital tasks, including direct digestion through the secretion of acids and enzymes, food storage, food milling and mixing with gastric secretion, controlling the amount of food that reaches the intestine, and reducing the number of microbes that enter the body through the mouth due to the strongly acidic characteristics of gastric juice, which also contains an intrinsic factor that facilitates the absorption of vitamins B12 [14]. The stomach's microscopic characteristics offer important insights into gut physiology and diet adaptation [15]. Large volumes of food are consumed by rabbits' digestive tracts, which also segregate the food particles that are fertile and digestible. Additionally, it quickly gets rid of fibrous waste materials that ferment slowly. The intestine has a substantial absorbable surface area. forty percent of intestinal contents are produced by the rabbit cecum, which is bigger than the stomach [16]. Research on the stomach and cecum of rats and rabbits is essential to understanding gastric malignancies caused by acidity.

RESEARCH METHOD

Ethical approval: Regulations pertaining to animal welfare were followed in every procedure. From January to March 2026, the Department of Dialysis Technology at Al-Furat Al-Awsat University-Al-Samawah Technical Institute used the animals' organs for study after they were killed in compliance with ethical and animal care standards.

Animals: Used ten samples from stomach and cecum; five from each of rabbit *Oryctolagus cuniculus* and rat *Rattus norvegicus*. Digesta is extracted from animals using a cannula. Phosphate-buffered saline is used to separate and cleanse the fundus, body, and pyloric sections of the stomach. A pH probe is used to measure the acidity of the stomach contents as soon as they are collected. A metallic spatula is used to remove mucus from the mucosa so that its thickness and gel characteristics may be examined. **Pepsin measurement:** In order to measure the pepsin enzyme, the animals are routinely slaughtered, their stomachs removed, and they are starved for 24 hours prior to sampling in order to standardize gastric secretion. The contents of the stomach are gathered, the gastric juice is placed in a highly acidic buffer, and the debris is centrifuged. Pepsin is an enzyme found in supernatant. In an acidic environment, combine the sample with hemoglobin substrate. At 37°C, incubate. Measure the absorbance of accessible peptides at 280 nm and stop the process with trichloroacetic acid. Short-chain fatty acids, propionic, acetic, and butyric acids, which are markers of fermentation activity, are analyzed using gas chromatography (GC); cecal contents are promptly kept at -20°C or -80°C to prevent degradation; add distilled water and centrifuge to remove particles. For GC analysis, gather the supernatant. Ketamine and xylazine intramuscular injections were used to deeply anesthetize the animals before they were killed for histochemical analysis. Five specimens from each animal's stomach (cardiac, fundic, and pyloric) and

cecum (base, body, and base) were collected and prepared for special staining. The results were interpreted using a variety of special stains, including Periodic Acid Schiff stain (PAS) for carbohydrates and Periodic Acid Schiff stain alongside Alcian blue (PAS-ABpH2.5) to neutral carbohydrates [17]. The staining intensity of the mucin of the mucous neck cells in the stomach and goblet cells in the cecum ranged from - (a weak staining) to +++ (strong staining) in all parts of the stomach and stomach. Analysis of variance, also known as ANOVA, was used to aggregate and statistically evaluate the data collected at the end of the experiment. The mean spacing was determined using Duncan's new multi-range testing (DMRT) when needed [4].

RESULT AND DISCUSSION

Compare the biochemical makeup of gastric secretions in rats and rabbits to assess physiological differences in stomach function and link structural variances with functional adaptations. Rats produce more acid and enzymes due to their omnivorous diet, which need effective protein digestion. Rabbits, on the other hand, rely more on fermentation by bacteria in the hindgut than on gastric digestion, which explains their increased mucus formation and decreased enzymatic activity. Rabbits have a noticeable non-glandular area that acts as a storage and preliminary processing compartment to fibrous plant material. Rats, on the other hand, have a glandular region that is more functionally active and ideal for quick digestion. shows notable biomechanical and histochemical variations consistent with their individual digestion processes (Histogram 1,2) (Fig. 1A-D), this similar to results of [18], [19], The rat's stomach exhibits a clear anatomical division which reflects a more varied diet and increased dependence on stomach-based digestion, whereas the rabbit's stomach is more homogeneous and supports a system centered on hindgut fermentation. Rats have a separate, non-glandular forestomach from a glandular corpus, whereas rabbits have a bigger, typically glandular stomach that has a noticeable forestomach. The mucosa layer in both rats and rabbits exhibits a significant PAS-positive response (Table 1) (Fig. 1A-D), suggesting a dense distribution of neutral polysaccharides, especially in surface cells with pyloric glands. The surface epithelial cells of the gastric pits and the entire stomach mucosa in rabbits exhibit strong PAS reactivity (Fig. 1A-C), indicating increased production of mucin for defense. Peptic and parietal cells are seen in the fundic glands of rats and rabbits (Fig. 1A-D). Rabbits have a high voluntary the consumption of feed, which influences gastric turnover and causes differences in gastric pH with acid output.

Significant histochemical and physiological differences can be seen in the stomachs of rats and rabbits, which are mainly due to their different eating patterns and digestive systems. Rabbits have a well-developed, dense, and lamina propria. in the cardiac region, with unique smooth muscle fibers inside the mucosa, whereas both species have an epithelium with a in their glandular regions. Rabbits have fundic, pyloric, and cardiac glands. cardiac glands are absent from the rat stomach. In the fundic region, both species possess chief and parietal cells; however, the parietal cells in rabbits are polymorphic that has a central nucleus, whereas those in rats are pyramidal (Fig. 1A-D),

the parietal cells are arranged differently, primarily in the upper portion of the gland [20]. The stomach has three layers of muscle: an exterior longitudinal layer with specific "sling" muscles, a middle circular layer, and an interior oblique layer. The muscularis in pyloric area is especially thick in rabbits [21]. Rabbits have high pyloric control and regional specialization (parietal cells), which is consistent with the requirement to carefully manage digesta prior to hindgut fermentation. Rats, on the other hand, have an increasingly mechanically adaptable stomach with sling fibers and layered musculature that enable active mixing and digestion of an omnivorous meal [22], the stomach of a rabbit functions as a continuous, acid-dependent sterilizing reservoir for a dense, fibrous diet, while the stomach of a rat is more functionally separated into non-glandular and glandular, corresponding with an omnivorous, a meal-based diet. Rabbits have high basal acid and pepsin secretion rates, frequently exceeding those of rats, and they rely heavily on vagal-cholinergic pathways. They are appropriate for various pharmacological and nutritional models due to these distinctions, especially the non-glandular part in rats and the unique glandular configuration in rabbits [23]. Research comparing gastric strips reveals species and location-dependent biomechanical characteristics. Studies on gastric strips demonstrate that the stomach's mechanical action is species-specific and regionally unique. These variations are closely related to eating habits, digestive strategies, and diet, guaranteeing that each animal breaks down food in the most effective manner possible for its biology [24], [25]. In general, glandular stomach strip are more rigid than non-glandular ones. In contrast to rabbit stomach cells, rat stomachs typically exhibit greater stiffness and stress levels, probably because to different ingested. The rat stomach has been developed with a unique non-glandular compartment for quick processing, whereas the rabbit stomach is designed to handle high-volume omnivorous intake through a huge, mostly glandular reservoir. Due to their lengthier retention of fibrous food, rabbits' stomachs are shielded from physical and chemical harm by their slower gastric motion and increased mucus secretion (Histogram 1). Rats' increased metabolic rate and diverse food are supported by their quicker digesting. Although the cecum in rats and rabbits is the main location for microbial fermentation for dietary fiber, its form and function change significantly depending on the digestive techniques of each species. Rats have a comparatively smaller cecum, but it is still essential to fermentation, whereas rabbits, being hindgut fermenters, have a large, specialized cecum for cecotrophy [26], the rat cecum is a typical, smaller hindgut fermentation chamber, the rabbit cecum is designed for long-term, high-efficiency fiber fermentation as well as nutrient recycling through cecotrophy. Studies comparing the cecum of rats and rabbits show notable histomorphological and histochemical variations in goblet cells (Fig. 2A-K), which are consistent with [27], [28], [29]. These differences are primarily caused by the rat's traditional digestive process [8], [30] and the rabbit's function as a cecotropher by nature (consuming fermented foods from the cecum).

Many goblet cells are found in the epithelium of cecal folds in rabbits. Goblet cells are especially common in the large intestine's crypts in both animals, but their density is typically higher in rabbits, which is consistent with the requirements of cecotrophy.

(Table 1). Goblet cells in rats and rabbits react favorably to Alcian blue (AB) to acidic mucins and Periodic Acid-Schiff to neutral mucins, appearing blue and magenta, respectively (Fig. 2A-K). On the surface of the epithelium and folds, neutral mucins are often predominant and are probably involved in the lubricating and movement of cecal contents [31], [32]. In order to protect the mucosa and help build cecotropes, the rabbit cecum, which is adapted for massive fermentation, produces a large amount of mucus. Rats frequently exhibit significant quantities of sulfated mucins in the cecum, while both species manufacture acidic and neutral mucins. The distribution and proportion of sulfomucins can differ between species. The rabbit cecum's high goblet cell density and unique mucin composition are adaptations to higher-intensity fermentation and the need to shield the mucosal surface from abrasive, quickly moving material during cecotrophy. By secreting the mucins which create the gastrointestinal tract's protective mucus barrier, goblet cells are essential for preserving intestinal health. These cells are critical for immunological defense, lubrication, and pathogen defense [8], [31].

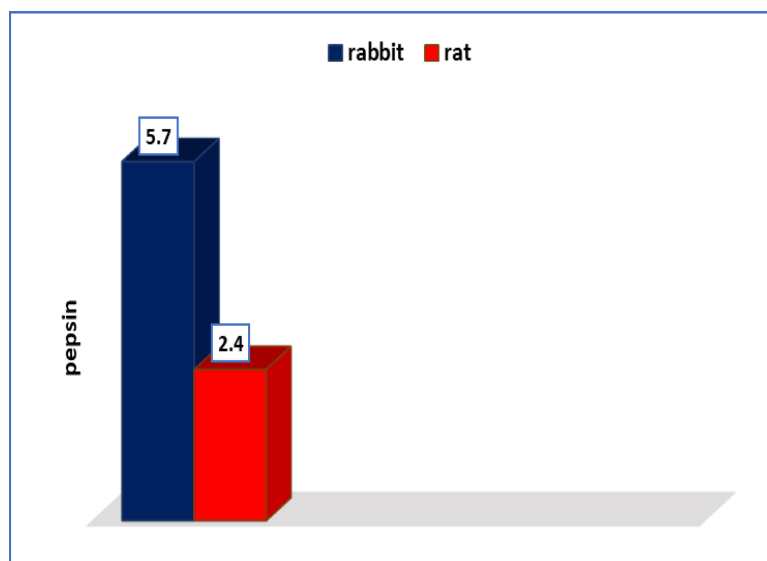
Gastric acid secretion was explained by biochemical characterization (Histogram 1,2); rats secreted less gastric acid than rabbits. Due to their herbivorous diet, rabbits showed comparatively increased acidity. a greater potential for gastric acid production to speed up the digestion of an omnivorous diet, according to comparisons of digestive physiology. On the other hand, despite having high pepsin activity, rabbits had lower basal secretion rates. Compared to rabbits' fermentation-heavy digestive systems, rats' shorter gut and greater metabolism cause them to create a greater volatile fatty acids within the cecum and need higher acid concentrations to break down different types of food. Enzymatic Activity: Rats had greater pepsin activity, which aided in the digestion of proteins. Rabbits' dependence on hindgut fermentation resulted in limited enzymatic activity. Mucus Production: Rabbits produced more mucus, which shields stomach lining from fermentation and extended food retention. Rats' omnivorous diet necessitates effective protein digestion, which is why they produce more acid and enzymes. Rabbits, on the other hand, rely further microbial fermentation within the hindgut than on gastric digestion, which accounts for their increased mucus formation and decreased enzymatic activity.

According to histochemical data, the goblet cell content varied among the study animals in the stomach and cecum sections (Histogram 1), with high mucous secretion and an increase in goblet cells from the cecum's base to its apex (Fig. 2A-K). The goblet cells' mucin staining intensity varied from weak, moderate, and strong via periodic acid Schiff, Alcian blue, and companied Alcian blue with periodic acid Schiff. Rabbits' goblet cells primarily showed moderate to high PAS reactivity, suggesting a higher neutral mucin concentration. Rats, on the other hand, displayed higher Alcian blue staining, indicating an abundance of acidic mucins; combination AB-PAS staining showed mixed mucins across both species, with an acidic bias in rats and a neutral leaning in rabbits. Neutral mucins, which are linked to lubrication and digestion, are more common in rabbits. Rats have larger levels of acidic mucins, that are more defensive (for example, against bacteria and irritation) [9], [10].

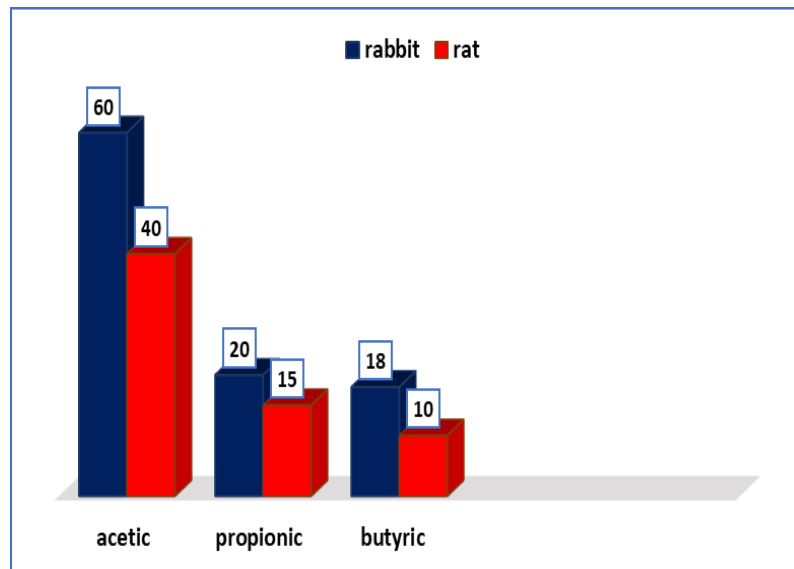
Table 1. Histochemical reaction of mucin of the mucous neck cell in (cardiac, fundic and pyloric of stomach) and the goblet cells of (base, body and apex of cecum) in rabbit and rat

Staining Part	PAS	AB	AB-PAS
Cardiac in rabbit	+++	++	+++
In rat	++	+	++
Fundic in rabbit	+++	+++	+++
In rat	++	++	++
Pyloric in rabbit	+++	+	+++
In rat	++	+	++
Base of cecum in rabbit	+++	+++	+++
In rat	++	++	+++
body of cecum in rabbit	+++	+++	+++
In rat	++	++	++
apex of cecum in rabbit	+++	+++	+++
in rat	++	++	++

Intensity level: weakly positive reaction (+), moderate positive reaction (++) and strong positive reaction (+++).



Histogram 1. Measurement of the pepsin in rabbit and rat, U/mL



Histogram 2. Measurement of the short-chain fatty acids in rabbit and rat, mmol/L

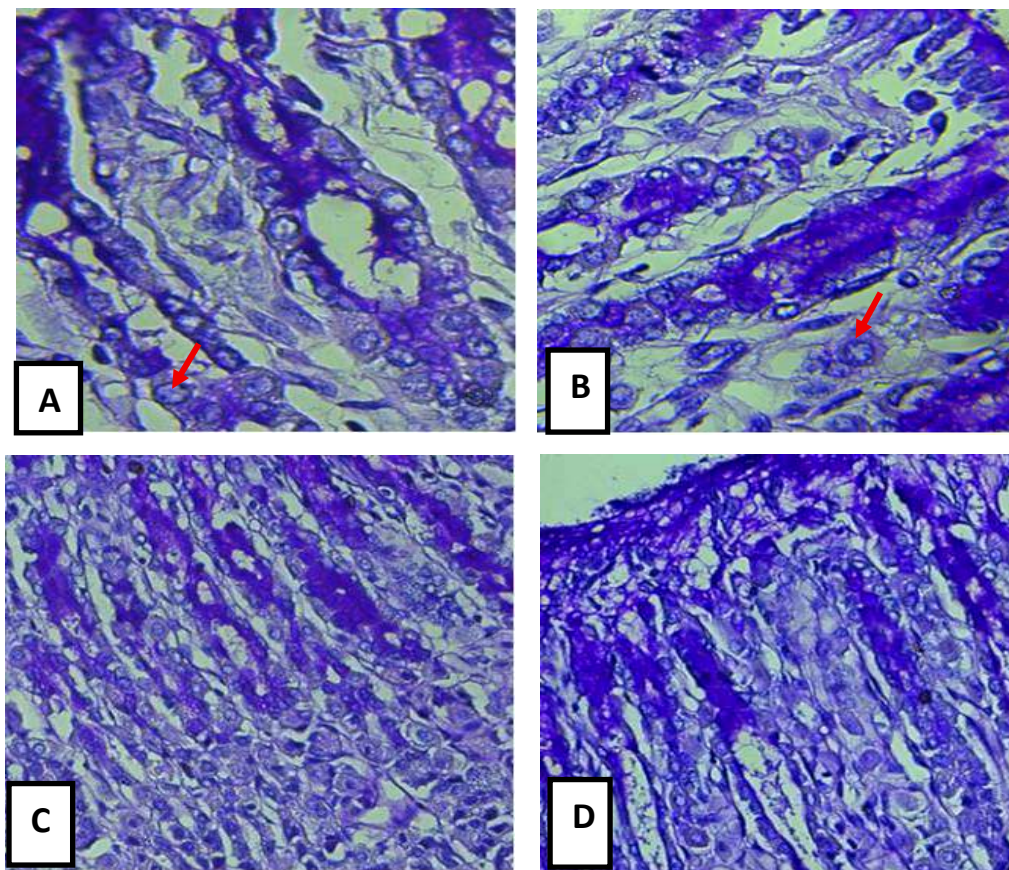
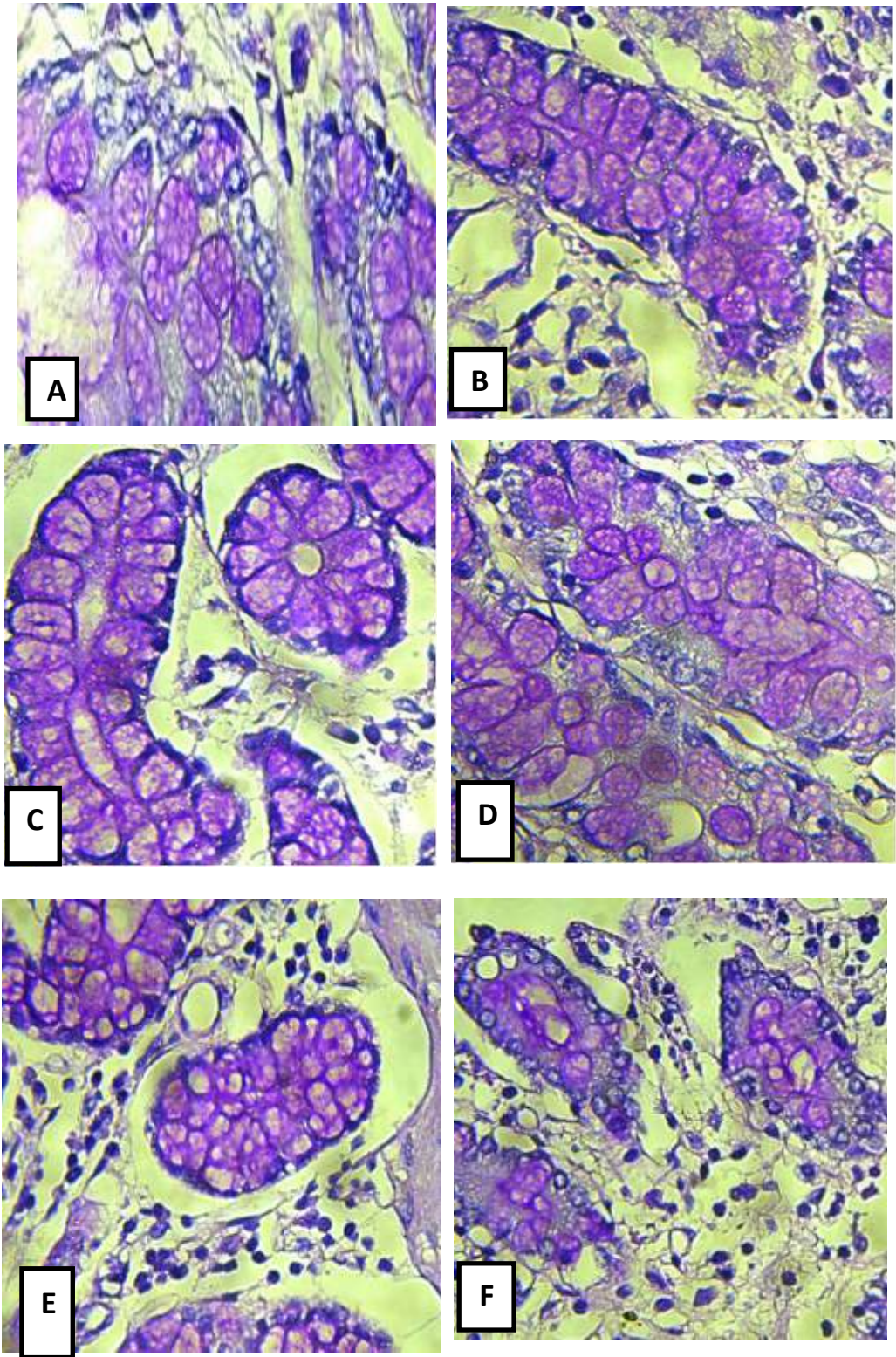


Figure 1. (A) Histological section of the stomach of rabbit showed strong response to stain; parietal cell (arrow); PAS 400 \times . (B). section in stomach of rat; showed the moderate response to stain; parietal cell (arrow); PAS 400 \times .; (C). section of the stomach of rabbit showed strong response to stain; PAS-AB stain, 400 \times . (D) section in stomach of rat; showed the moderate response to stain; PAS-AB stain 400 \times .



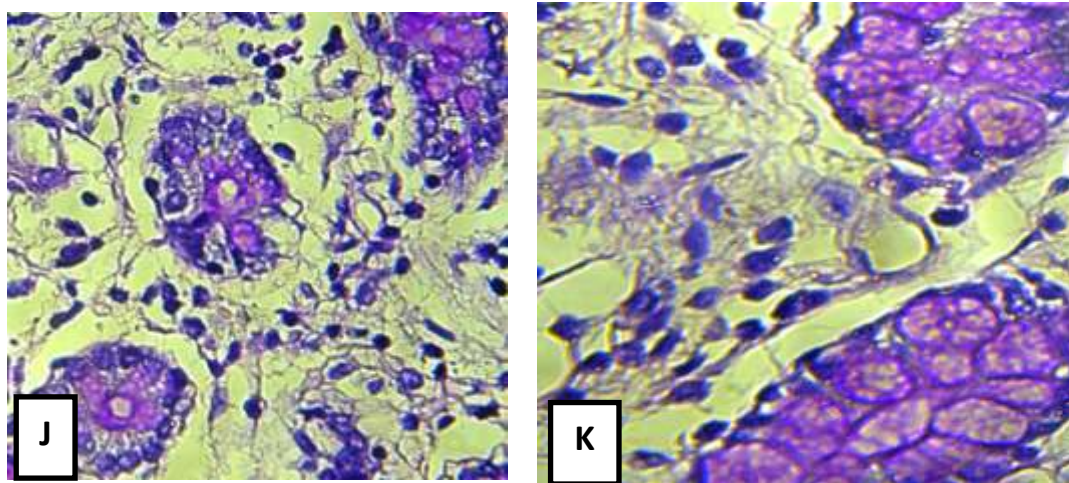


Figure 2. (A) Histological section of the cecum of rabbit showed moderate response of goblet cells to stain; PAS 400×. (B). section in cecum of rat; showed the strong response of goblet cells to stain; PAS 400×.; (C). section of the cecum of rabbit showed strong response of goblet cells to stain; AB stain, 400 ×. (D) section of the cecum of rabbit showed strong response of goblet cells to stain; AB stain, 400 ×. (E). section of the cecum of rabbit showed strong response of goblet cells to stain; PAS-AB stain, 400 ×. (F). section of cecum of rabbit showed moderate response of goblet cells to stain; PAS-AB stain, 400 ×. (J). section of the cecum of rabbit showed weakly response of goblet cells to stain; PAS-AB stain, 400 ×. (K). section of the cecum of rabbit showed strong response of goblet cells to stain; PAS-AB stain, 400 ×.

CONCLUSION

Fundamental Finding: This study shows that the stomachs of *Rattus norvegicus* and *Oryctolagus cuniculus* differ significantly in terms of biochemistry, histochemistry, and physiology. These differences are strongly linked to eating patterns and digestive techniques. **Implication:** A number of medical applications, including experimental surgical techniques, the detection of stomach problems, and the provision of information regarding diet adaption and gut physiology, depend on an understanding of rabbit and rat gastrointestinal morphology. This study advances our knowledge of how dietary practices affect the architecture and physiology of the stomach. **Limitation:** The study did not examine the molecular underpinnings behind these adaptations, developmental architecture, gut microbiota interactions, and enzymatic activity. **Future Research:** Future studies could examine the molecular underpinnings behind these adaptations, developmental architecture, gut microbiota interactions, and enzymatic activity.

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