

Physiological changes in the gut of goats offered ration at different periods of twenty-four hours

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Abstract

Reports has shown that in mice and cattle feeding time has the capacity to impact nutrient use and assimilation by the gut. However, there is a little or no report of the impact of such on gut morphology of any ruminant species. Thus, this study aims to assess the effect of time of feeding on histology and histomorphometry of WAD bucks. The bucks were fed at either 06:00h, 12:00h or 18:00h for 115 days under natural light-dark cycles. Histomorphometry assessment was carried out on the rumen, duodenum, jejunum, ileum, and caecum. Mucosal thickness and villous width of the rumen, duodenum, jejunum, ileum, and caecum were measured. The villous height, villous width, of the duodenum, jejunum and ileum were measured. Duodenal mucous thickness was higher ($P=0.002$) in morning fed buck than afternoon and evening fed bucks. Bucks fed in the afternoon had higher ($P=0.032$) duodenal epithelial height compared to other treatments. Jejunal villous height ($P<0.001$) in evening fed buck and jejunal villous width ($P=0.006$) in afternoon fed bucks were higher compared to other treatments. Jejunal crypt depth ($P=0.015$) and jejunal mucous thickness ($P=0.001$) was higher in evening fed bucks compared to morning and afternoon fed bucks. Ileum villous height ($P<0.001$), crypt depth ($P=0.038$) and mucous thickness ($P=0.005$) in morning and afternoon fed bucks were similar, but higher compared to evening fed bucks. Caecum mucous thickness ($P<0.001$) and epithelial height ($P=0.003$) in morning fed buck was lower than bucks in afternoon and evening feeding regime. Rumen muscular wall thickness was higher ($P<0.001$) in evening fed bucks than in morning and afternoon fed buck which were similar. Morning and afternoon feeding in bucks caused duodenal villi stunting and increased glandular density. Again, morning and afternoon feedings were observed to increase the prominence of Peyer's patches and expansion of submucosa. Thus, due to the relatively low compromise of gut health in the evening-fed bucks compared to other regimes, feeding bucks in the evening is recommended for gut health.

Keywords: Chrono nutrition, duodenum, histomorphometry, histopathology

Les changements physiologiques dans l'intestin de chèvre offraient des rations à différentes périodes de vingt-quatre heures



Résumé

Les rapports ont montré que chez les souris et le temps d'alimentation du bétail ont la capacité d'impact de l'utilisation des nutriments et de l'assimilation par l'intestin. Toutefois, il existe un peu ou pas de rapport de l'impact de telle sur la morphologie des intestins de toute espèce de ruminant. Ainsi, cette étude vise à évaluer l'effet du temps d'alimentation sur l'histologie et l'histomorphométrie des daims de NAO. Les daims ont été nourris à 06h00, de 12h00 ou de 18h à 18h00 pendant 115 jours sous des cycles naturels-sombres lumineux. L'évaluation de l'histomorphométrie a été réalisée sur le rumen, le duodénum, le Jéjunum,

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l'iléon et le caecum. L'épaisseur des muqueuses et la largeur de la villeuse du rumen, duodénum, de Jéjunum, d'iléon et de caecum ont été mesurées. La taille de la villeuse, la largeur de la villeuse, du duodénum, de Jéjunum et d'iléon ont été mesurées. L'épaisseur muqueuse duodénale était plus élevée ($p = 0,002$) au mélange alimenté par l'argent que l'après-midi et la soirée fondue. Les daims nourris dans l'après-midi avaient une hauteur épithéliale duodénale ($p = 0,032$) par rapport à d'autres traitements. Jéjunal Hauteur vestimentaire ($p < 0,001$) en soirée nourris à l'argent et jéjunal largeur villeuse ($p = 0,006$) dans l'après-midi, des daims alimentés étaient plus élevés par rapport à d'autres traitements. La profondeur de crypte Jéjunal ($p = 0,015$) et une épaisseur muqueuse jéjunale ($p = 0,001$) était plus élevée dans les mâles de soirée alimentées par le soir par rapport aux daims nourris à la matinée et à l'après-midi. Hauteur vestimentaire d'Ileum ($p < 0,001$), la profondeur de crypte ($p = 0,038$) et l'épaisseur muqueuse ($p = 0,005$) au matin et à l'après-midi, les daims nourris étaient similaires, mais plus élevés par rapport aux daims nourris au soir. L'épaisseur muqueuse Caecum ($p < 0,001$) et une hauteur épithéliale ($p = 0,003$) au mélange nourri à la matinée était inférieure à celle de l'après-midi et du régime d'alimentation du soir. L'épaisseur de la paroi musculaire rumen était plus élevée ($P < 0,001$) dans des mâles alimentés en soirée que dans l'après-midi et l'après-midi, il était similaire. Le matin et l'après-midi se nourrissent de daims causés par des villons duodénaux et une densité glandulaire accrue. Encore une fois, les repas du matin et de l'après-midi ont été observés pour accroître la proéminence des patchs de Peyer et de l'expansion de la sous-muqueuse. Ainsi, en raison du compromis relativement faible de la santé intestinale dans les daims alimentés en soirée par rapport à d'autres régimes, il est recommandé d'alimenter des daims dans la soirée.

Mots-clés: nutrition chrono, duodénum, histomorphométrie, histopathologie

Introduction

Circadian rhythms are biological rhythms generated by endogenous clocks to allow animal to adapt to daily changes with environmental cues (Crnkoet *al.*, 2019). The time of nutrient availability is a potent zeitgeber (any external or environmental cues that entrains or synchronises an organism's biological rhythm) to body clocks that can cause a desynchronization between the rhythm of the master clock and peripheral circadian clocks such as present in the gut (Damiolaet *al.*, 2000). Time of eating is the zeitgeber for intestinal rhythm and gut bacteria rhythm (Voigt *et al.*, 2016). Feed intake can entrain peripheral circadian oscillators resulting in inversion of the pattern of gene expression in metabolic important organs such as enterocytes, caecum and jejunal mucosa (Hussain and Pan, 2009,) which may lead to intestinal and

systemic pathologies (Mukherjiet *al.*, 2013). There has also been changes in rumen epithelium and mucosal depending on the feeding regime practiced (Rompala and Hoagland, 1987). Furthermore, mice in the misalignment group experienced intestinal hyperpermeability and disrupted circadian gene expression in intestinal cells (Kaczmareket *al.*, 2017). Thus, changes in feeding pattern can alter the growth of mucosal component, surface area including the size and density of the villi, gut proliferation and vascularization, epithelial cell proliferation, motility, colonic motility, gastric secretion and enzymatic activities, maintenance, and repair of protective mucosal barriers (Hussain and Pan, 2009; Duarte *et al.*, 2013). The alternation in time of nutrient absorption also has the potential to affect the structure of the gut and other metabolically important organs in the body

(Tahara and Shibata, 2014). Therefore, understanding the impact of time of feeding on bucks productive is important. To the best of our understanding, this is the first study to evaluate the impact of time of feeding on the gut morphology of any ruminant livestock specie. Thus, this study is meant to evaluate the gut morphology of WAD goats under different feeding regime.

M a t e r i a l a n d m e t h o d s
The experiment was carried out at the goat unit, Teaching and Research farm of the Federal University of Technology, Akure, Nigeria. Goats were managed in accordance with the guide for the care and

use of Agricultural animals in Agricultural research and teaching (National institute of Animal Science) Nigeria.

Feeding and experimental design

The experiment lasted for 115 days using 15 bucks randomly allocated into three treatments, in a completely randomized design. The bucks were housed individually in pen and had free access to food and were offered experimental diet of grass (*Panicum maximum*) and concentrate 50:50 dry matter (Tables 1 and 2). The bucks were fed once daily in the morning, noon, or evening at 06:00h, 12:00h or 18:00h respectively under the natural light-dark cycle.

Table 1: Chemical composition of concentrate and grass (g/100g)

| Parameters | Concentrate | Grass |
|-------------------------|-------------|--------|
| Dry matter | 86.96 | 27.91* |
| Crude protein | 10.70 | 7.81 |
| Crude fibre | 15.73 | 32.55 |
| Ether extract | 3.46 | 3.39 |
| Ash | 13.82 | 12.50 |
| Acid detergent lignin | 16.12 | 19.99 |
| Acid detergent fibre | 30.07 | 26.55 |
| Neutral detergent fibre | 72.03 | 63.94 |
| Hemicellulose | 41.96 | 37.39 |
| Cellulose | 13.95 | 6.55 |
| Nitrogen free extract | 56.30 | 43.75 |

* Dry matter of fresh grass

Table 2: Gross composition (g/100g) of the concentrate

| Ingredient | Diet |
|---------------------|-------|
| Cassavapeel | 40.00 |
| Palm kernel cake | 26.00 |
| Wheatoffals | 14.00 |
| Brewerdried grains | 15.00 |
| Bonemeal | 2.00 |
| Urea | 1.00 |
| Premix ^a | 1.00 |
| Salt | 1.00 |

^a Vitamin A 8,000,000 IU, vitamin D3 17,000,000 IU, vitamin E 5000 mg, vitamin K3 1500 mg, folic acid 200mg, niacin 1500mg, vitamin B2 3000 mg, vitamin B12 5 mg, vitamin B1 1000mg, vitamin B6 1000mg, iron 25,000 mg, manganese 45,000 mg, copper 3000 mg, zinc 35,000 mg, choline chloride 100,000 mg.

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Gut physiological evaluation

On the last day of the experiment, three bucks were randomly selected and slaughtered, and the gut segment of each buck harvested at the midpoint of the intestinal part by veterinarians to avoid error. Nine samples each of rumen, duodenum, jejunum, ileum, and caecum samples were harvested from the bucks. The gut segment tissues were harvested after taking the weight and length where appropriate. The tissues were washed in Normal saline and preserved in 10% formal saline solution. The samples were stained with staining with Haematoxylin and Eosin. Afterwards, Histopathology evaluation was conducted on all harvested tissues. Furthermore, histomorphometry assessment was carried out on the rumen, duodenum, jejunum, ileum, and caecum. The microscopic method (Snipes, 1981) using the light microscope with magnification x 100 was used to take the picture of tissues for histopathological and histomorphometry analysis.

Chemical analysis

Crude Protein (CP), Ether extract (EE) (method ID 920.39) according to. The Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent

lignin (ADL) were analyzed according to (Van Soest *et al.*, 1991). Hemicelluloses was calculated as NDF-ADF while cellulose was calculated as ADF-ADL.

Statistical analysis

Data collected from the bucks and does were analyzed as a completely randomized design. All data collected were subjected to analysis of variance using SPSS version 23.0 (SPSS 2015). The differences between treatments means were examined by Duncan's multiple range test of the same package.

Results

Feed intake and gut measurement

Feeding regimes did not affect ($P>0.05$) the feed intake of the WAD bucks fed at different times of the day (Table 3). Intestinal weight and length were not significantly ($P>0.05$) affected by time of feeding, but it showed that evening-fed bucks and afternoon-fed bucks had the highest small intestinal and large intestinal weight and length respectively. The afternoon-fed bucks and evening-fed bucks had the shortest small and large intestinal length respectively. It was observed that the feeding time with the highest small or large intestine weight had the longest intestinal length.

Table 3: Feed intake and gut measurement segment of WAD buck under different feeding regime

| Parameters | 06:00h | 12:00h | 18:00h | SEM | P-Value |
|----------------------------------|---------|-----------|---------|--------|---------|
| | Morning | Afternoon | Evening | | |
| <i>Feed intake</i> | | | | | |
| Initial weight (Kg) | 7.40 | 7.50 | 7.50 | 0.351 | 0.993 |
| Final weight (Kg) | 10.69 | 10.02 | 10.93 | 0.877 | 0.929 |
| Weight gain (kg) | 3.40 | 4.26 | 4.46 | 0.36 | 0.463 |
| Forage intake (g DM/day) | 103.19 | 109.45 | 95.40 | 6.69 | 0.723 |
| Concentrate intake (g DM/day) | 197.46 | 210.7 | 226.15 | 14.24 | 0.743 |
| <i>Gut measurement</i> | | | | | |
| Live Weight (Kg) | 10.69 | 10.02 | 10.93 | 0.877 | 0.929 |
| Small intestine weight (g/kg BW) | 18.33 | 17.80 | 18.41 | 1.012 | 0.973 |
| Large intestine weight (g/kg BW) | 16.59 | 18.91 | 16.87 | 1.481 | 0.819 |
| Small intestine length (cm) | 1237.00 | 1153.00 | 1358.67 | 67.136 | 0.516 |
| Large intestine length (cm) | 353.33 | 407.33 | 333.33 | 25.835 | 0.545 |

Histomorphometry

The histomorphometry of WAD bucks are shown in (Table 4). Duodenal mucous thickness was higher (P=0.002) in morning fed buck than afternoon and evening fed bucks that were similar. WAD bucks fed in the afternoon had higher (P=0.032) duodenal epithelial height compared to other treatments. Jejunal villous height (P<0.001) in evening fed buck and jejunal villous width (P=0.006) in afternoon fed bucks were higher compared to other treatments. Jejunal crypt depth (P=0.015) and jejunal mucous thickness (P=0.001) was higher in evening fed bucks compared to morning afternoon fed bucks. Ileum villous height (P<0.001), crypt depth

(P=0.038) and mucous thickness (P=0.005) in morning and afternoon fed bucks were similar, but higher compared to those found in evening fed bucks. Caecum mucous thickness (P<0.001) and epithelial height (P=0.003) in morning fed buck was lower than bucks in afternoon and evening feeding times. Rumen muscular wall thickness was higher (P<0.001) in evening fed bucks than in morning and afternoon fed buck which were similar. However, the different feeding regime had no impact (P>0.05) on the duodenum villous height, duodenum villous width, duodenum crypt depth, jejunum epithelial height, ileum villous width, ileum epithelial height, caecum crypt depth, rumen epithelial thickness and rumen papillae length of the WAD buck.

Table 4: Histomorphometry of gut segments (µm) in WAD Buck under different feeding regime

| Parameters | 06:00h | 12:00h | 18:00h | SEM | P-Value |
|-------------------------------|---------------------|---------------------|---------------------|-------|---------|
| | Morning | Afternoon | Evening | | |
| Rumen epithelial thickness | 49.15 | 55.15 | 63.23 | 2.97 | 0.153 |
| Rumen papillae length | 608.86 | 488.32 | 636.47 | 40.44 | 0.288 |
| Rumen muscular wall thickness | 572.79 ^b | 651.18 ^b | 876.53 ^a | 33.82 | <0.001 |
| Duodenal villous height | 503.59 | 507.26 | 435.01 | 20.29 | 0.267 |
| Duodenal villous width | 100.13 | 101.50 | 88.18 | 3.745 | 0.286 |
| Duodenal crypt depth | 123.26 | 90.80 | 77.01 | 13.32 | 0.355 |
| Duodenal mucosal thickness | 356.74 ^a | 197.07 ^b | 207.23 ^b | 21.34 | 0.002 |
| Duodenal epithelial height | 25.02 ^b | 30.11 ^a | 24.79 ^b | 0.95 | 0.032 |
| Jejunavillous height | 383.14 ^b | 435.01 ^b | 602.24 ^a | 21.52 | <0.001 |
| Jejunavillous width | 102.03 ^b | 144.41 ^a | 104.75 ^b | 6.31 | 0.006 |
| Jejunacrypt depth | 92.61 ^b | 83.20 ^b | 113.97 ^a | 4.57 | 0.015 |
| Jejunalmucosal thickness | 197.32 ^b | 207.48 ^b | 305.04 ^a | 14.18 | 0.001 |
| Jejunalepithelial height | 28.55 | 24.38 | 25.34 | 0.94 | 0.168 |
| Ileavillous height | 533.42 ^a | 509.98 ^a | 344.11 ^b | 22.19 | <0.001 |
| Ileavillous width | 97.40 | 91.68 | 86.77 | 5.11 | 0.706 |
| Ileacrypt depth | 86.34 ^{ab} | 108.70 ^a | 80.17 ^b | 4.86 | 0.038 |
| Ileamucosal thickness | 252.19 ^a | 269.52 ^a | 181.48 ^b | 12.08 | 0.005 |
| Ileal epithelial height | 25.00 | 28.44 | 28.18 | 0.91 | 0.235 |
| Caecal crypt depth | 98.33 | 121.51 | 126.64 | 7.75 | 0.289 |
| Caecal mucosal thickness | 189.03 ^b | 337.19 ^a | 366.84 ^a | 16.53 | <0.001 |
| Caecalepithelial height | 17.51 ^b | 21.85 ^{ab} | 25.46 ^a | 0.99 | 0.003 |

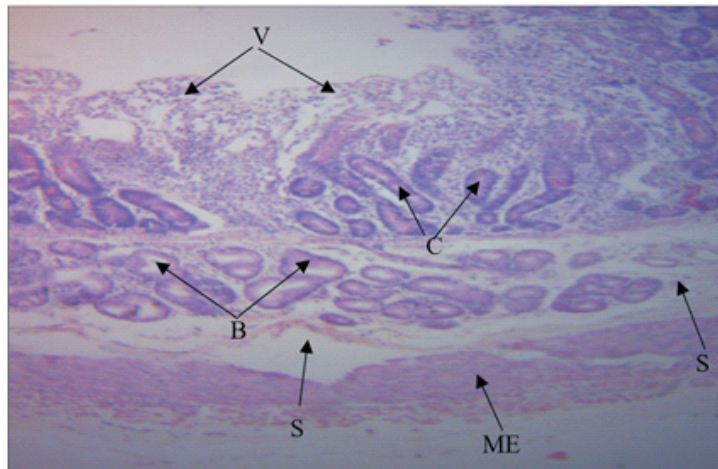
a, ab, b = Means in the same row but with different superscripts are statistically (P<0.05) significant; SEM: Standard Error Mean.

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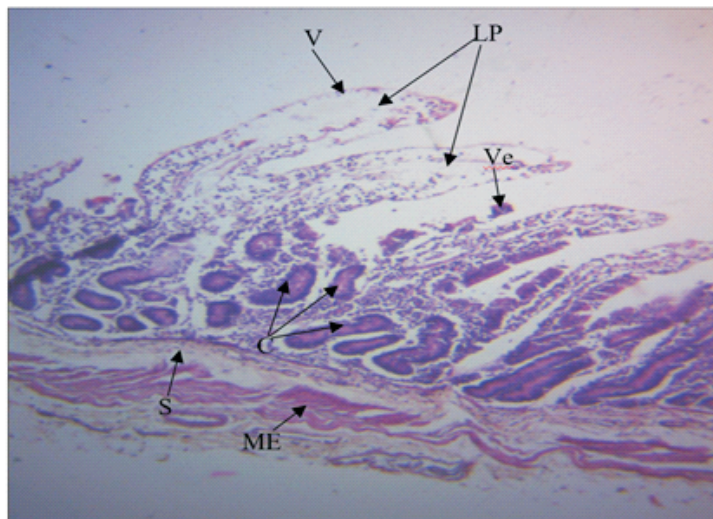
Histopathology of the duodenum of bucks under different feeding regimes

Figure 1 shows the photomicrograph of the duodenum of bucks fed in the morning.

Figure 2 shows the photomicrograph of the duodenum of afternoon-fed bucks. Figure 3 shows the histology of the duodenum of evening-fed bucks.

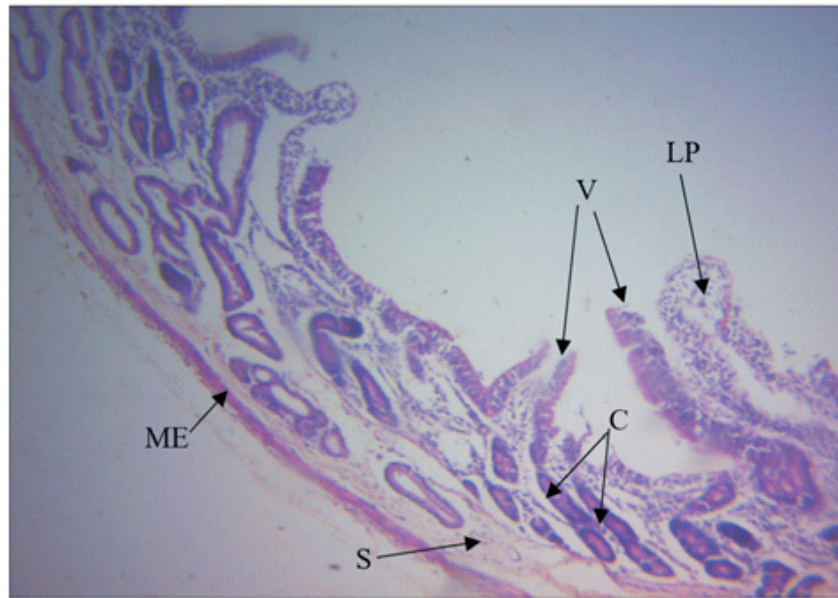


A

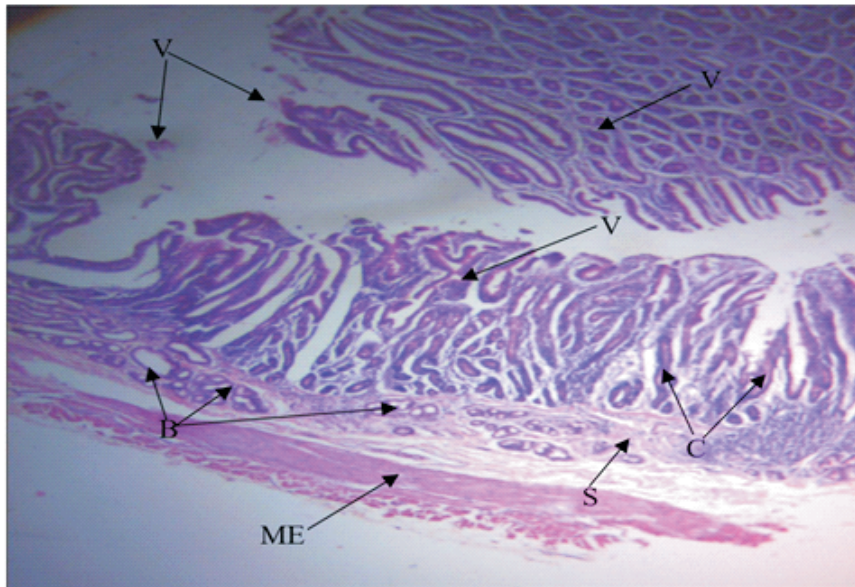


B

Figure 1: Photomicrograph sections of the duodenum of morning-fed bucks (A-B x 100 Magnification [H&E]). Plate A showed a moderate to severe stunting and erosion of the duodenal villi (V). The Brunner glands (B) appeared mildly distorted. The crypts (C), muscularis externa (ME) and submucosa (S) are seen. Plate B showed that there is severe erosion of the jejunal villi (V), villi epithelium (Ve) and lamina propria (LP). The Brunner glands are not seen. The Crypts (C) are shown and the submucosa area (S) is greatly reduced.



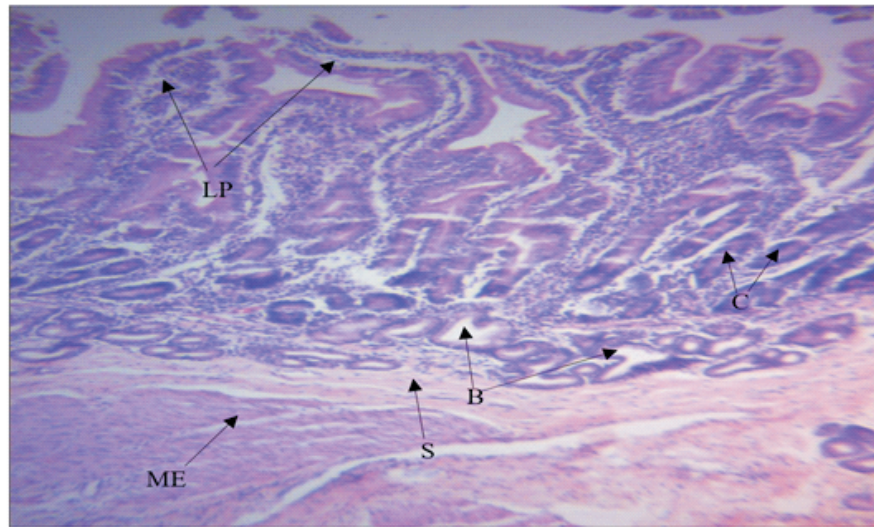
A



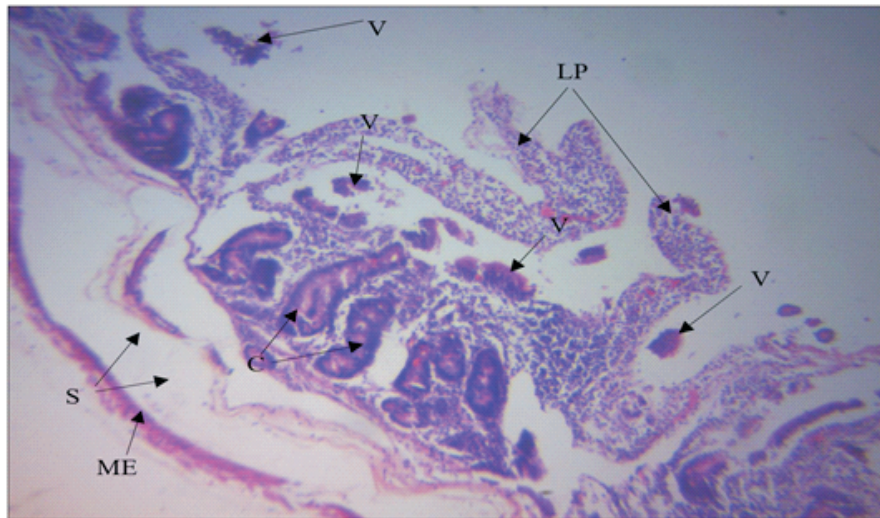
B

Figure 2: Photomicrograph sections of the duodenum of afternoon-fed bucks (A-B x 100 Magnification [H&E]). Plate A shows the erosion of the wear-off/erosion of the duodenal villi (V) and lamina propria (LP). The crypts (C), submucosa (S) and muscularis Externa (ME) are intact. Plate B shows the erosion of the duodenal villi epithelium (V). The Brunner's gland (B), crypts (C), submucosa (S) and muscularis externa (ME) are normal.

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A

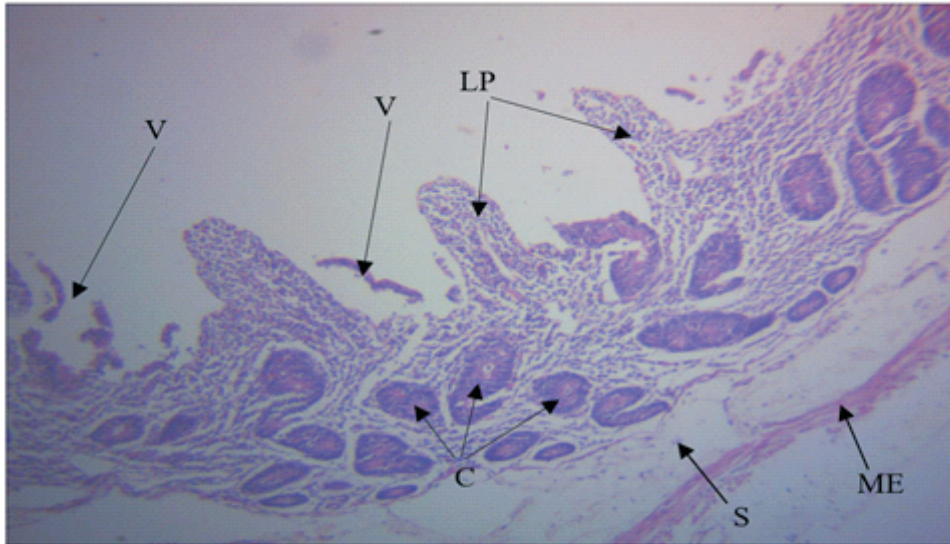


B

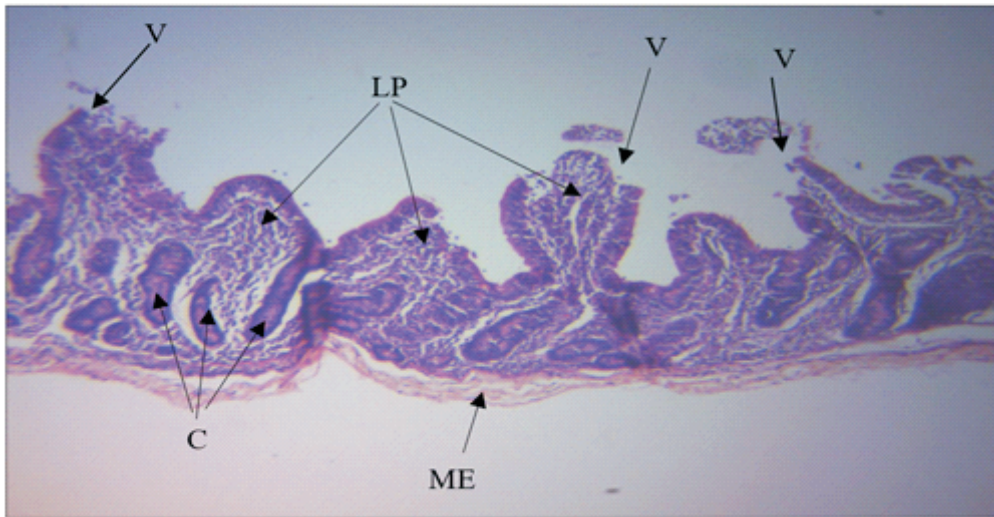
Figure 3: Photomicrograph sections of the duodenum of evening-fed bucks (A-B x 100 Magnification [H&E]). Plate A showed normal duodenal villi, with mild villi epithelium erosion (V). Distortion of the Brunner's gland (B) is seen. The lamina propria (LP), submucosa (S), crypt (C) and muscularis Externa (ME) are seen and normal. Plate B showed there are severe erosion of the duodenal villi (V) and Lamina propria (LP). The crypts (C), submucosa (S) and muscularis externa (ME) are seen.

Histopathology of the jejunum of bucks fed under different feeding regimes

Figure 4 shows the photomicrographs of the jejunum of morning-fed bucks. Figure 5 shows the photomicrograph of the jejunum of afternoon-fed bucks. Figure 6 shows the Photomicrograph sections of the jejunum of evening-fed bucks.



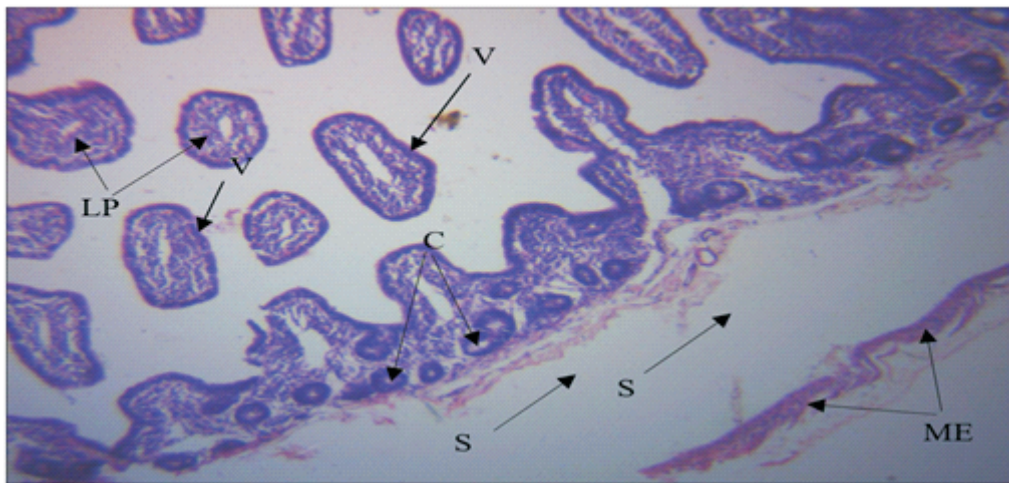
A



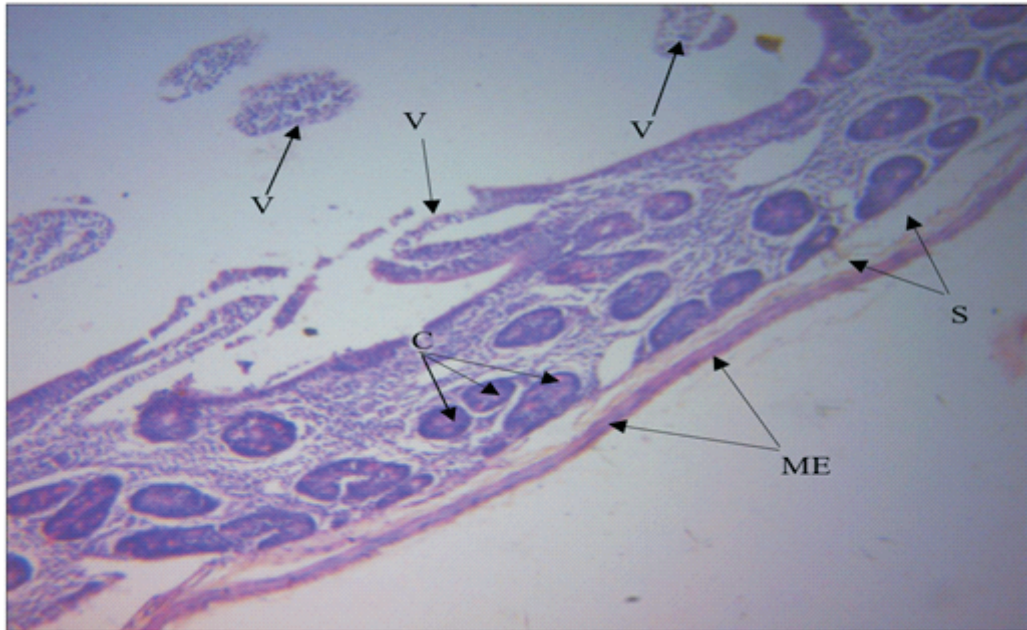
B

Figure 4: Photomicrograph sections of the jejunum morning-fed bucks (A-B x 100 Magnification [H&E]). Plate A showed there is erosion of the villi epithelium (V). The lamina propria (LP), crypt (C), submucosa (S) and muscularis Externa (ME) are seen and appear intact. Plate B shows there is a mild erosion of the villi epithelium (V). The lamina propria (LP), crypts (C) and muscularis externa (ME) are seen and they appear normal.

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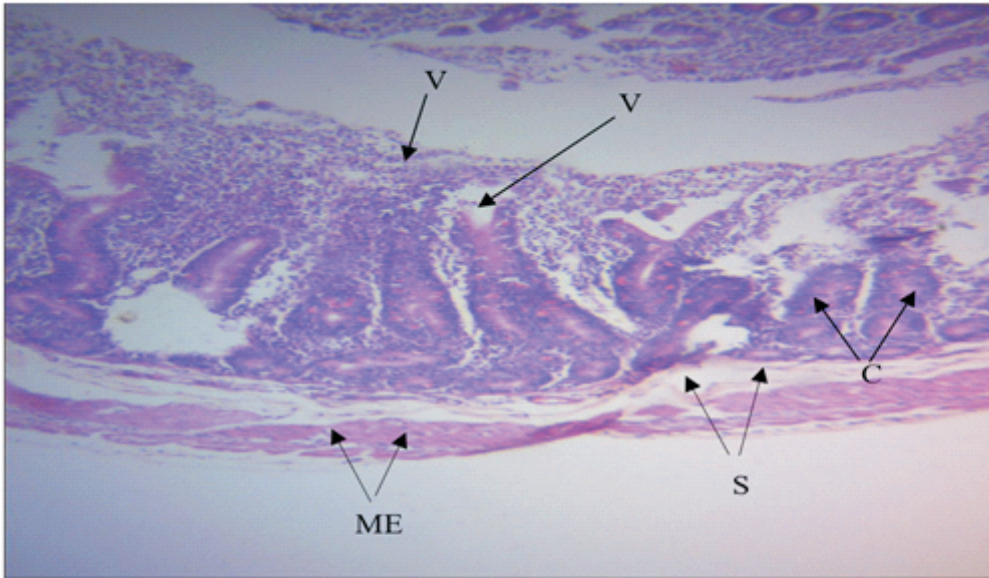


A

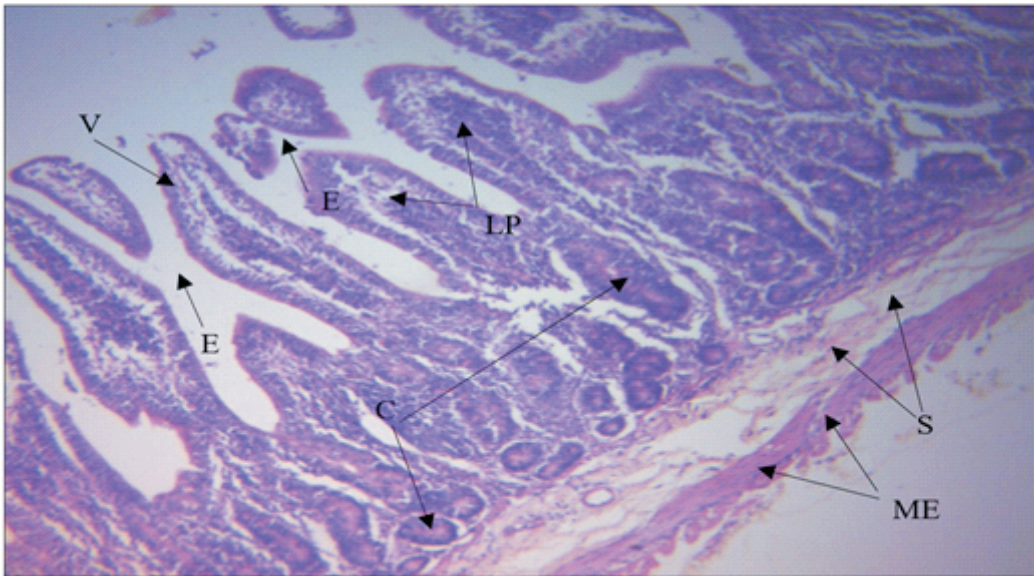


B

Figure 5: Photomicrograph sections of the jejunum of afternoon-fed bucks (x 100 Magnification [H&E]). Plate A showed severance or erosion of the villi (V). The submucosa area (S) is expanded. muscularis externa (ME), crypts (C) and lamina propria (LP) are seen. Plate B showed that there are foci (areas) of severe villi erosion (arrows). The submucosa (S) is normal. The Crypts (C) and muscularis externa (ME) are visible and normal.



A



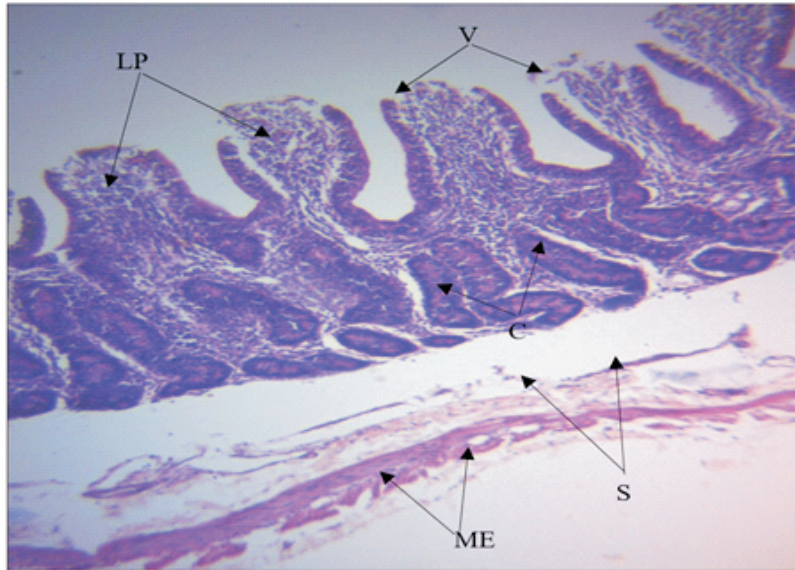
B

muscularis externa (ME), submucosa (S), and crypts (C) are normal. Plate B showed long villi (V), however, lesion occurred in some villi (E). The muscularis externa (ME), submucosa (S), and crypt (C) seen appear normal.

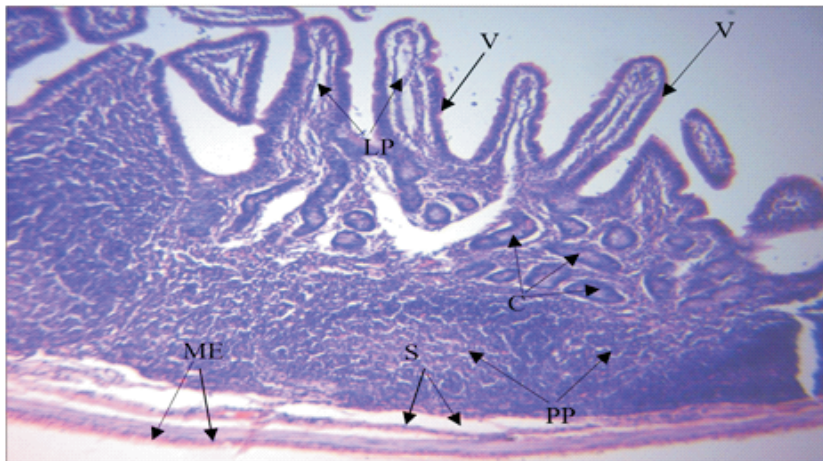
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Histopathology of the ileum of bucks under different feeding regimes

Figure 7 shows the photomicrograph sections of the Ileum of morning-fed bucks. Figure 8 shows the photomicrograph of the ileum of afternoon-fed bucks. Figure 9 shows the photomicrograph sections of the Ileum of evening-fed bucks.

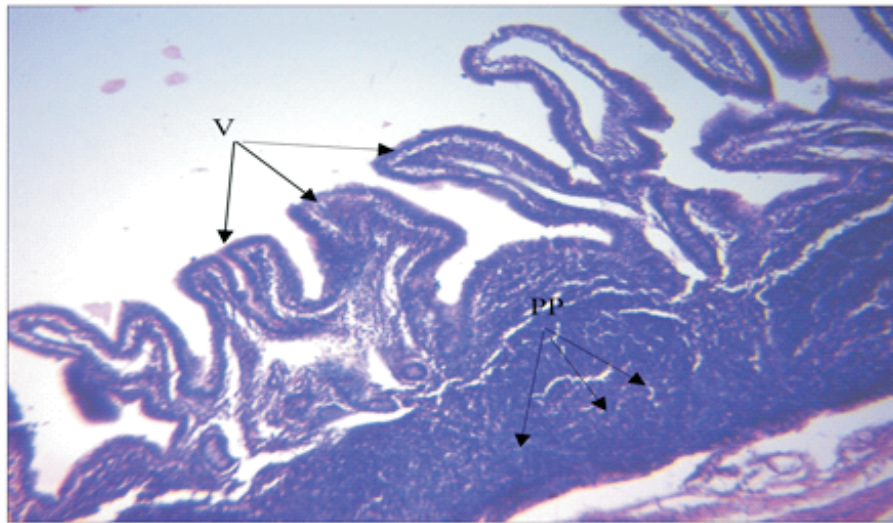


A

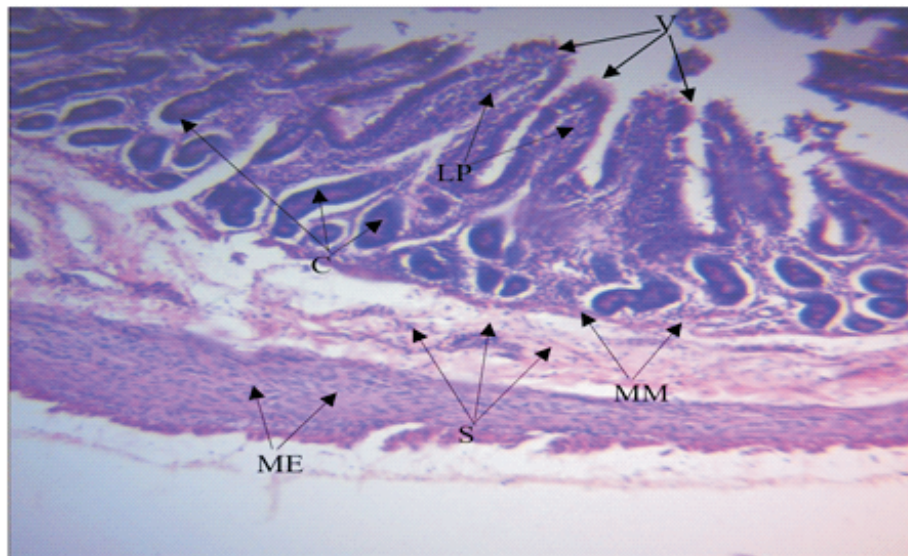


B

Figure 7: Photomicrograph sections of the ileum of morning-fed bucks (A-B x 100 Magnification [H&E]). Plate A showed there were erosions of the ileal villi (V). The submucosa layer (S) is expanded. The lamina propria (LP), and the muscularis Externa (ME) are normal. In Plate B, no lesions were seen on the villi (V) but the Peyer's Patches (PP) are very prominent in the submucosa. The submucosa (S), crypts (C) and muscularis externa (ME) appear normal.



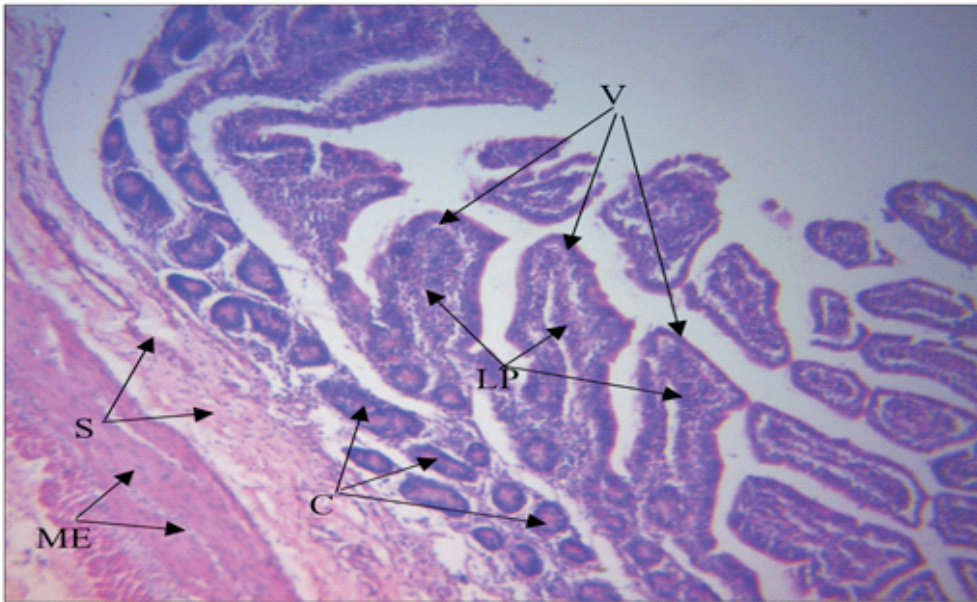
A



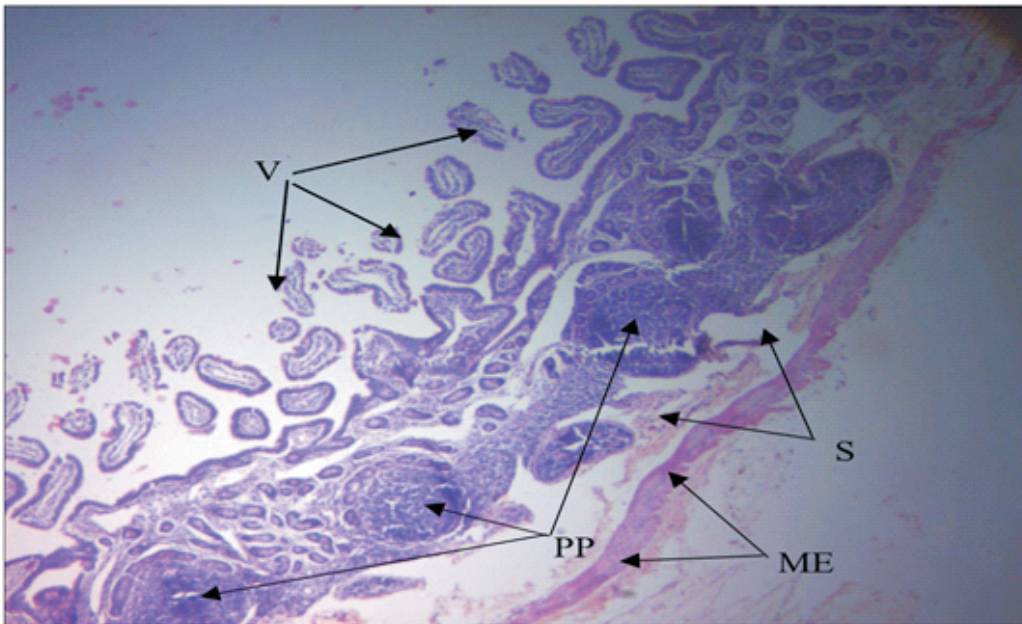
B

Figure 8: Photomicrograph sections of the ileum of Afternoon-fed bucks (A-B x 100 Magnification [H&E]). Plate A showed no lesions in the villi (V), but the Peyer's Patches (PP) are very prominent in the submucosa. **Plate B** showed expansion/edema in the submucosa area (S) and muscularis externa (ME). The villi (V), crypts (C), lamina propria (LP), muscularis mucosae (MM) appear normal.

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A



B

Figure 9: Photomicrograph sections of the ileum of evening-fed bucks (A-B x 100 Magnification [H&E]). Plate A showed no visible lesions in the villi (V). **The submucosa (S), muscularis Externa (ME), lamina propria (LP) and crypts (C)** seen are normal. Plate B showed the erosion and stunting of the ileal villi (V). The Peyer's patches (PP), submucosa (S) and muscularis externa (ME) are seen, and they appear normal.

Discussion

Histomorphometry of gut segments

Proliferation of intestinal epithelial and crypt cells and differentiation of villus enterocytes are under circadian control, and alteration in time of nutrient availability can affect many gut functions like epithelial cell proliferation and repair of protective mucosal barriers (Hussain and Pan, 2009), which may result in intestinal and systemic pathologies (Mukherji *et al.*, 2013). Changes in feeding pattern can alter the growth of mucosal component, surface area including the size and density of the villi, gut proliferation, and vascularization (Duarte *et al.*, 2013). The rumen is an important ecosystem where food is stored, mixed/churned regularly and microbial fermentation occurs. Gut motility and emptying are more effective in the morning than in the evening hours in diurnal animals (Serin and Tek, 2019). Gut motility and emptying slows down at night, when the evening bucks are eating, thus, the increase in rumen wall thickness might be an adaptive response to increase the strength required to mix the rumen content (Ulyatt *et al.*, 1984). Furthermore, rumen epithelial and wall thickness were found in efficient ruminant than less efficient counterpart (Lam *et al.*, 2017). This suggest that evening fed bucks had to adapt through thicker rumen muscular wall and epithelium, and papillae length to increase the surface area for nutrient absorption to counter the lower gut motility due to the evening time of feeding. The jejunum receives and absorb small nutrient particles that were enzymatically digested in the duodenum while the ileum is the third part of small intestine and contains villi like those in jejunum. In the intestine, the epithelium is constantly renewing, a balance gut epithelial cell proliferation is inseparable for its physical immunological barrier function which plays important role in the maintenance and regulation of

gastrointestinal health (Liu *et al.*, 2014). Furthermore, intestinal mucosa consists of epithelial cells, acts as an important barrier to toxic and harmful materials and protects an individual from pathogens and foreign particles (Johansson *et al.*, 2013). The increased duodenal epithelial height and mucous thickness in morning and afternoon fed bucks respectively compared to evening bucks might be due to increased influx of nutrients per time which necessitates the need for more nutrient absorption, and protection of such region from pathogen or foreign materials. The decrease in epithelial height and mucous thickness in evening fed bucks might be related to the abnormality associated with feeding at the "wrong" time of the day. The jejunum is an important site largely responsible for absorption, digestion and had the most osmotic pressure in the small intestine segment for absorption of all the major nutrients (Nkukwana *et al.*, 2015). Intestinal absorptive surface via elongation of intestine villi and microvilli are an adaptive mechanism or capacity of an animal to facilitate available nutrient uptake (Chevalier *et al.*, 2015). The increase in jejunal villous height and width, crypt depth and mucosal thickness in evening fed bucks may be due to the need to increase the absorptive capacity of jejunal region to counter the slowdown of the gut motility and gastric emptying. Increased jejunal villus development may be induced by prolonged retention of intestinal contents, including hyper-nutrients, because of greater need for digestive capacity (Yamauchi *et al.*, 2010; Svihus, 2014). This imply that with reduced rate of gut emptying, there might be increased accumulation of nutrients for a prolonged period at the jejunum, necessitating increased surface area (villous height and width) to facilitate nutrient absorption. This is further confirmed by the deeper crypt – a site to produce enterocyte that migrates to

the villus tip. The deeper jejunal crypt suggests demand for fast tissue turnover to permit renewal of the villus which indicates that the host intestinal response mechanism is trying to compensate for slower gut motility to aid nutrient absorption (Barasch, 2012). In addition, the deeper crypt also benefits the host, because as the crypt deepens, there is an increase in the proliferating stem cells which result in increased number of mucins producing goblet cells that are important constituent of host innate defense system (Loeffler, 2014). Variation in the jejunum between evening fed bucks and morning and afternoon fed bucks may be an adaptive response to the slower gut emptying in evening fed bucks rather than abnormality in jejunal segment of buck fed at other times. Overall, the improvement in jejunal absorptive surface area suggest greater nutrient absorption, improved feed efficiency than other treatments. In contrast, there was a decrease in the villous height and width, crypt depth and mucous thickness in evening fed bucks compared to morning and afternoon in the ileum. The ileum does not need an absorptive function but when it receives nutrient-rich contents, it might lead to increase villi growth to accommodate the inflow of nutrients (Yamauchi *et al.*, 2010). The lower ileum villi in evening fed buck compared to morning and afternoon might be because nutrient-rich ingested content have been absorbed in the jejunum causing nutrient poor intestinal content to reach the ileum. Therefore, due to lower nutrients reaching the ileum, there is no need for quick turnover of tissues for ileum villi in the evening fed bucks compared to morning and afternoon fed bucks. The caecum is an important fermentation sites and an absorptive area in the hindgut of ruminants and pseudo-ruminants; and it accounts for 12% of the total volatile fatty acid production in sheep. There is a possibility

for inflow of nutrient to the caecum and as such, osmotic pressure for nutrient inflow and absorption may be a problem for epithelial barrier and affect the permeability of caecal epithelia which may initiate inflammatory response (Liu *et al.*, 2014). The increase in mucosal thickness and epithelial height in the caecum suggest an improvement in the gut protective capacity in afternoon and evening-fed buck compared to morning fed buck. This study indicates that altering feeding time can affect gut histomorphometry because the animal intestinal segment will be forced to adapt to the new feeding system by adjusting their gut absorptive and protective capacity.

Histological changes in gut segments

Intestinal epithelium, digestion, intestinal absorption all function in a circadian rhythm and their rhythmic oscillation at molecular level can be affected by feeding rhythms (Pacha and Sumov, 2013). Villi erosion and stunting, submucosal reduction, and Brunner's gland distortion were observed in the duodenum of morning-fed bucks. Inadequate alkaline fluid secretion because of Brunner's gland distortion might have caused the erosion and stunting of the villi in the duodenal region due to acidic digesta coming from the stomach (Kurellaet *al.*, 2008). Feeding in the afternoon improved mucosal glands prominence and density but reduced villi in afternoon-fed bucks suggest that changes in feeding time aided proliferation of mucosal glands on the duodenal surfaces. These mucosal glands produce mucins that protect the intestinal surface areas and increase duodenal enzyme secretions. No visible intestinal anomaly was observed in the duodenum of evening-fed bucks. Stunting in the histology of jejunum villi observed in morning and afternoon relates to the histomorphometry measurement of the region. The increased jejunal villi erosion in morning and afternoon-fed bucks compared

to evening-fed bucks villi suggest that there was lower surface area for nutrient absorption which affected efficient nutrient absorption in morning and afternoon-fed bucks. This suggest that evening feeding improves the rate of nutrient absorption or removal from the jejunum. Peyer's patches are organized lymph nodes which functions as the immune surveillance system of the intestinal lumen and facilitate the generation of immune response within the mucosa. Although there was no lesion in the ileum, the prominence of peyer's patches in morning-fed bucks suggest increased immune responses to protect the intestine from infiltration of pathogens into the submucosal layer. This response may be attributed to erosion of the villi which permit foreign materials to infiltrate the gut segment. However, in afternoon-fed bucks, Peyer's patches were prominent and there was also expansion/edema of the submucosa. Feeding regimes can increase infiltration, epithelial changes and deterioration of mucosal architecture which suggest weakening of the epithelial barrier (Erbenet *al.*, 2014). This suggest that feeding in the afternoon might have increased the infiltration of foreign materials causing immunological response due to submucosal expansion and edema. Also, heat stress during afternoon feeding might have caused gut patho-morphologies, resulting in inflammation of the submucosal, which compromises gut integrity (Sammadet *al.*, 2020). In the morning and afternoon-fed bucks, the increase in villi suggest that more nutrients rich digesta were entering the ileum compared to evening-fed bucks. The histomorphometry of the ileum villi of morning and afternoon-fed bucks were higher than evening-fed bucks while their jejunum villi were lower than evening-fed bucks. This suggest that nutrients were not efficiently absorbed in the jejunum of

morning and afternoon-fed bucks which caused more nutrient-rich digesta to flow to the ileum, which might have triggered the prominence of the Peyer's patches in morning and afternoon-fed bucks in response to the higher-than-normal influx of nutrients into the ileum. This indicates that afternoon feeding compromised ileal integrity.

Conclusion

Evening-feeding increased jejunum height, crypt depth, and mucus thickness than other feeding regimes. Ileum villi height, crypt depth and mucous thickness in afternoon-fed bucks are higher than evening-fed bucks. Morning and afternoon feeding in bucks caused some histopathological changes in the duodenum such as stunting in villi, increased glandular density, which were not observed in evening-fed goat. Furthermore, morning and afternoon feeding were observed to increase the prominence of Peyer's patches and expansion of submucosa, and this was not observed in evening-fed bucks. Overall, the gut integrity of evening-fed bucks was observed to be less compromised compared to the two other feeding regimes. Thus, due to the relatively low compromise of gut health in evening-fed bucks compared to other regimes, feeding bucks in the evening is recommended for gut health.

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