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RESEARCH ARTICLE

HISTOMORPHOMETRIC CHANGES IN SHEEP GUT FOLLOWING NUTRITIONAL SUPPLEMENTATION WITH SACCHAROMYCES CEREVISIAE FERMENTATION PRODUCT (DIAMOND V XPC™)

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ABSTRACT: Saccharomyces cerevisiae fermentation product (Diamond V XPC™) was evaluated for effects on abomasal and small intestinal histomorphometry in Dorper lambs using qualitative and quantitative methods. Ninety lambs were weighed from birth at two-week intervals to six weeks, stratified by body weight, and assigned to two groups: control group (C1) and a treatment group (E2) receiving 2 g/head/day of the fermentation product through one-month post-weaning. Growth was monitored and at experiment end five lambs per group were randomly selected. They were euthanized, abomasa and small intestines excised, washed, fixed, and processed for stereological and histological analysis with Stepanizer software. Histomorphometric results indicated improved mucosal organization and more prominent intestinal villi in sheep supplemented with Saccharomyces cerevisiae fermentation product compared with the control group. Mean abomasal volumes were 116.01 ± 15.40 cm³ (C1) and 148 ± 87.71 cm³ (E1) intestinal volumes were 610.15 ± 99.9 cm³ and 564 ± 81.4 cm³, respectively. Villous length increased significantly with supplementation (503 ± 5.20 µm in C1 vs. 537.67 ± 37.98 µm in E1). Intestinal mucosal surface area rose from 4,641.63 ± 261.89 cm² to 7,038.59 ± 1,972.56 cm² with treatment; abomasal mucosal surface area increased from 200 ± 14.4 cm² to 380.67 ± 98.14 cm². Overall, S. cerevisiae supplementation produced statistically significant mucosal adaptations indicative of enhanced digestive and absorptive capacity in the gut. The study provides detailed histological evidence addressing an existing knowledge gap and supports the physiological relevance of S. cerevisiae fermentation products for improving gut health and informing feeding strategies in sheep.

Keywords: *Saccharomyces cerevisiae* fermentation product, sheep gut, stereology, histomorphometry

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Nutritional supplementation with *Saccharomyces cerevisiae* has proven beneficial in domestic animals, especially in as far as gut health is concerned. In broilers, supplementation can stimulate the growth by regulating the intestinal immunity and barrier function, and improving the intestinal morphology [1]. Previous studies found increase in the total thickness of the mucosa, the height of the villi and depth of the crypts and depth of the glands of the duodenum of rabbits supplemented with increased yeast doses [2]. Similarly, longer villi were reported in Awassi lambs supplemented with *Saccharomyces cerevisiae*[3].

Dorper sheep are recognized as a fast-growing breed with excellent body conformation and performance. A study investigating the relationship between body weight and morphological traits including heart girth, rump height, body length, withers height, and sternum height reported that these morphometric traits are strong predictors of body weight in Dorper sheep [4].

Numerous studies conducted in sheep have investigated histomorphological adaptations of the gastrointestinal tract (GIT) in response to nutritional management and physiological status. These studies have demonstrated that dietary composition and feeding strategies can markedly influence the structural organization of the GIT mucosa. For instance, variations in diet and lactation length have been shown to modify rumen tissue architecture in lambs. [5].

Over recent years, design-based stereology has become the state-of-the-art methodology in quantitative histologic analyses. The application of design-based stereological methods to the analysis of biological tissues has considerably contributed to our understanding of their functional and pathological morphology. It has been used to establish appropriate quantification strategies for obtaining unbiased estimates of liver cells that constituted a new solid quantitative background in normal conditions [6].

In sheep**,** the abomasum plays a crucial role in the chemica**l** digestion of ingested feed, especially after it has undergone microbial fermentation in the preceding forestomachs (The abomasum also prepares nutrients for absorption by liquefying the digesta and beginning the breakdown of complex proteins. [7]. Microscopic structure of the abomasa shows four layers of the wall, the uppermost being tunica mucosa, then tunica submucosa separated by muscularis mucosae, tunic muscularis and tunica serosa.

Nutritional manipulation can alter the epithelial morphological response in sheep and cow. This includes reduced acid and microbial load that protect abomasal mucosa from inflammation and lesions, enzyme activity and small-intestinal mucosal integrity and function [8].The small intestine of ruminants on the other hand is anatomically and functionally similar to non-ruminants and ranges in length between approximately 12 and 30 times the body length of the animal [9]. Structurally, besides being of considerable length, the small intestine has several folds and contains villi, approximately 0.5-1 mm in length, and microvilli which allow for increased surface area for enhanced final digestion and absorption of nutrients (Trahair & Sangild, 2010; Sun et al., 2022). The mucosal adaptations of this organ; including villus height, epithelial thickness, and glandular structures, play vital role in optimizing digestive and absorptive functions (Trahair & Sangild, 2010; Sun et al., 2022). Increased villus height is associated with greater absorptive surface area, while optimal crypt depth reflects balanced epithelial cell proliferation and turnover. Conversely, reduced villus height and excessive crypt depth are indicative of compromised gut integrity and reduced digestive efficiency[2].

In sheep, recent studies have demonstrated that dietary supplementation with *S. cerevisiae* improves both growth performance and intestinal

histomorphometry[12]. reported increased villus height and improved villus height-to-crypt depth ratio in the jejunum of lambs supplemented with yeast, indicating enhanced absorptive efficiency[2]. These structural improvements were accompanied by increased feed intake and weight gain, suggesting a direct link between gut morphology and animal performance.

Saccharomyces cerevisiae and its active products, such as beta-glucans, mannan oligosaccharides and nucleotides, are potential alternatives to antibiotics and play an important role in stimulating growth and immunity in small ruminants[13].Previous studies have shown that functional metabolites of *Saccharomyces cerevisiae* can help balance the immune response and stress hormone levels in production poultry increased [14]. *Saccharomyces cerevisiae* has also been shown to have a positive impact on weight gain, feed conversion and mortality in broilers, even in broilers that were challenged with ingestion of used litter during a heat-stress period [14] . In ruminants, bacterial and yeast probiotics are used as feed additive to enhance rumen fermentation as well as to promote immune function and general health [8].

Previous literature has reported that supplementation with *Saccharomyces cerevisiae* can positively influence growth performance in animals [15]. They include increasing the nutrient utilization and villus height, bacterial modulation (increasing *Lactobacillu*s, decreasing *E. coli*), anti-toxic and anti-inflammatory properties, and in immune response as well as the provision of metabolites as nutrients. Functional metabolites derived from *Saccharomyces cerevisiae*, including products developed by Diamond V (Cedar Rapids, Iowa, USA), have been shown to help balance immune responses and regulate stress hormone levels in production poultry, supporting improved health and stress tolerance [16]. A study by WHO [15], showed increased villous height and superior ileal mucosa development at 21 days in chickens supplemented with a yeast cell wall product prepared from *Saccharomyces cerevisiae.*

Histomorphometric evaluation provides critical insight into tissue-level changes in response to dietary interventions.

Like other ruminant species, morphometric and biometric features of the gastrointestinal tract (GIT) have been studied in goat and camel[16]. Despite the importance of the histomorphometry of the GIT in education, researches, and disease diagnosis, the literature is insufficient to study the adaptations on the GIT of sheep describe only the histology of rumenal wall [17]. A study was conducted to compare the histomorphology of the GIT i.e. colon in sheep and goat which showed that, only some parts of the GIT i.e. rumen and colon are very briefly studied in sheep [18]. However, the detailed histomorphometry characteristics of the GIT and especially the small intestines and abomasum in dorper sheep have never been studied. Better understanding of the structure of sheep small intestines is crucial for enhancing production and general growth performance through proper care and feeding management.

Nutritional manipulation can alter the gut epithelial morphological responses in sheep through supplementation with probiotic[8].

There is a gap regarding the structural responses of the abomasum and small intestines to yeast supplementation, particularly in small ruminants such as sheep.

Therefore, the present study was carried out to describe the histomorphometry of the small intestines and abomasum in dorper sheep and contribute to a deeper understanding of how probiotic yeast influences gut morphology

MATERIALS AND METHODOLOGY:

Experimental Animals and Study Design

The study was carried out in Empakai farm in Kajiado County-Kenya. The study involved a total of 90 lambs used to evaluate growth performance under different feeding treatments. Body weights of the lambs were recorded at birth and subsequently at two-week intervals until six weeks of age in order to monitor growth performance throughout the experimental period. Prior to allocation, all lambs were screened for gastrointestinal (GIT) helminths and protozoa using fecal examination techniques[19]. Only lambs that tested positive for GIT parasites were included in the experiment.

Following screening, the lambs were weighed and stratified according to body weight to ensure balanced distribution between experimental groups. Lambs were ranked from heaviest to lightest and divided into blocks of three. Within each block, animals were allocated to two experimental groups (Control and supplemented) using an alternating assignment procedure to maintain comparable mean body weights between groups. In the first block, the heaviest lamb was assigned to the Control group while the remaining two lambs were assigned to the supplemented group in descending order of body weight. In the next block, assignments were made in ascending order (lightest to heaviest). This alternating descending–ascending allocation procedure was repeated until all lambs were assigned to their respective groups.

The lambs were assigned to two treatment groups: a control group (C1) and a treatment group (E2). Lambs in the control group were maintained on the standard feeding regimen without supplementation. Lambs in the treatment group received 2 g per head per day of the fermentation product as shown in **Table 1**.

Table 1: Animal groupings and treatment received

Groups	Treatment received	Management practices	No of animals
Group 1 (Control)	anthelmintic and anticoccidial treatment	Grazing; routine farm husbandry	30
Group 2 (Supplemented)	Diamond V XPC™ — 2 g per lamb/day+ anthelmintic and anticoccidial treatment	Grazing; routine farm husbandry	60

lambs were maintained under similar grazing and housing conditions throughout the study, with clean drinking water provided. Routine anthelmintic and anticoccidial treatments were administered uniformly to all lambs at the start of the trial and thereafter according to the farm's established parasite control schedule, to minimize variation in parasite exposure between treatment groups. The experimental period continued until one-month post-weaning, at which point all experimental procedures were concluded.

At the end of the experiment, five (5) lambs were randomly selected from the control group (Group 1), while ten (10) lambs were selected from the supplemented groups (Groups 2). The selected lambs were sacrificed following approved animal welfare procedures. Immediately after slaughter, the entire gut was removed and was then rinsed in clean water. The combined forestomaches were placed on a dissecting table with the parietal side facing up. The abomasa and the small intestines were separated from the rest of the forestomach compartments for morphometric assessment.

Following excision, the small intestine was carefully straightened without applying tension and the total intestinal length recorded. To ensure systematic uniform sampling along the longitudinal axis, the intestine was partitioned into five approximately equal segments as shown in **Figure1**. These segments constituted systematically distributed sampling units for macroscopic stereological assessment.

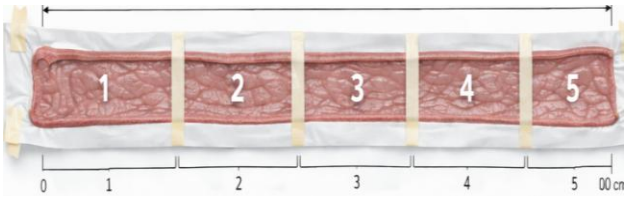

Figure 1. Representative photograph of sheep small intestines showing segments of the small intestines.

The intestine was severed at the pyloric junction and the ileo-cecocolic junction, opened along the mesenteric border and divided into five segments of approximately equal length.

Volume estimation

The volumes of the abomasa and small intestines were estimated separately through Scherle method of volume estimation [20]. The organs were fixed by total immersion in 10% Neutral buffered formalin in individually labelled containers for easier identification for at least five days before further processing.

Measurement of the macroscopic mucosal surface areas

The macroscopic surface areas were then estimated using the point-associated area method. (Howard and Reed, 2005) whereby the surface of the abomasum was placed on a transparent counting grid with test points printed on it. The grid has a value representative of the area associated with a test point (a/p). The grid was placed on the mucosal surface of each abomasa surface and as described by [20]. The same was done on the mucosal surface of the intestinal surface. The number of test points hitting the surface of the abomasal and or the small intestinal area were counted. The total mucosal surface area of each test sample at gross level was then estimated by multiplying the total number of test points counted on each surface of the tissues area by the area associated with the test point on the counting grid.

The surface area for each abomasum and small intestines was obtained as $A = a * p$

Where, A is the surface area of the abomasa,

P is the total number of test points counted; a = is the area associated with a test point.

Point associated area is a(p);
Total points counted on segment 1 (Sgt1) = Pt;
Therefore, surface area of a segment 1 is
S(Sgt1) = Pt x a(p)
Total macroscopic surface area of the intestine
S(Si) = S(sgt1) + S(Sgt2) +…. + S(Sgt5).

Tissue sampling

Segments of the small intestines including the duodenum, jejunum and ileum were sampled in a systematic uniform manner. The same sampling method was used for abomasum. The samples were placed with their serosal surface on a flat surface dissection board while the mucosal surface faced up. It was serially cut transversely to obtain slices of tissue at intervals of approximately 3 cm apart as shown in Figure.2. The transverse slices were serially cut in a longitudinal direction (i.e. perpendicular to the first cut) at intervals of 3 cm to ultimately obtain smaller slices of the tissue. From the total number of the blocks of tissue obtained, a sub-sample was selected through systematic random sampling. The first block of tissue to be selected among the first five blocks was determined by randomly picking a number between 1 and 5. Thus the number picked determined the starting position for selecting the first block and subsequently every fifth additional tissue block was selected from the remaining lot. Selecting every fifth block of tissue was done serially through each row in alternate left and right directions for the successive rows. Thus if the first row was sampled from left to the right, the second row was sampled from right to the left; and this was continued alternately for all successive rows until the sampling of the entire Abomasum and small intestines tissues was accomplished. The five blocks of tissue from each intestinal segment and

abomasum were placed as a group in individually labelled containers with 10% NBF solution and stored until processing time.

Figure. 2. Representative photograph of a sheep abomasum showing tissue blocks for sampling

Histology

Selected tissue blocks from the abomasa and small intestines were dehydrated through ascending concentrations of ethanol starting with 50% to 100%. The tissues were then cleared in methyl benzoate which is miscible with paraffin wax to allow for infiltration process. The tissues were infiltrated and embedded in molten paraffin wax. The tissues were then taken through the embedding process with the preferred orientation to obtain vertical sections. Subsequently, sections were obtained by the use of microtome at a nominal thickness of 5 µm. They were then mounted on glass slides and then stained with hematoxylin and eosin (H/E) for observation under the light microscope. Digital images were obtained with a digital Light Microscope fitted with image j software. (Reed et al., 2010).

Villous lengths

Formalin-Fixed small intestines samples were divided into five to six segments of approximately equal lengths. Each segment was then virtually divided into ten equal sub segments by placing a paper tape with a ruler next to the segment. Finally, one segment was picked randomly and processed for histology. Histological sections were obtained by the use of microtome at a nominal thickness of 5 µm. They were mounted on glass slides and stained with H/E and examined under the light microscope. Digital images were obtained with a digital light microscope fitted with image J software. Vertical sections showing the entire thickness of the intestinal wall from serosa to tips of villi were selected for villous morphometry. The lengths of villi were taken from randomly selected villi and in which both the base and the tips were clearly identifiable (Figure.3). Measurement of the villous length was from the base to the tip and as demonstrated in [22] .

For each intestinal segment, at least ten villi were measured and the means and standard deviations calculated.

Digital images were obtained with a Digital Light Microscope (Leica DM750 digital light microscope) fitted with image J software.

After the light microscopy, the lengths of the villi were obtained using image J software (National Institutes of Health, Bethesda, MD, USA).

The images were obtained using a calibrated scale of 100µm from each segment of the small intestines. The intestines from each animal was divided into at least five segments. From each segment, at least four mucosal lengths were obtained.

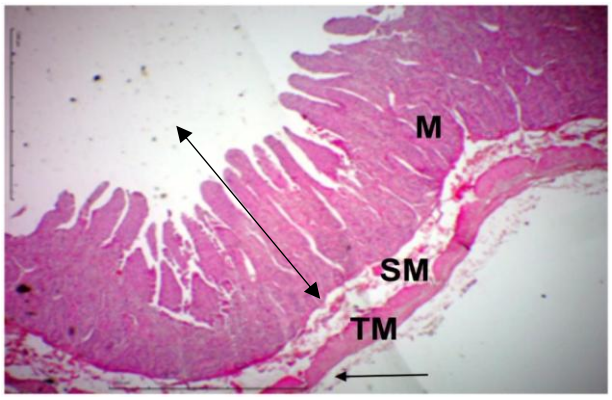

Figure 3: Histological section of the sheep intestine (duodenum) showing the landmarks for measurement of the villous lengths. Notice one tip of the arrow points to the villous base and the other one to the tip of the villus. The tissue layers denoted are the mucosa (M), the submucosa (SM), the tunica muscularis (TM) and the arrow showing serosa. Magnification X40

Microscopic morphometry

Quantitative data was analyzed and estimated at light microscopy levels for both the intestinal and abomasa layers. The parameters estimated included:

Volume densities

The volume densities of mucosa, sub mucosa, muscularis and serosa were estimated by the use of stepanizer software. This was done by using a point counting stereological grid as shown in Figure 5 and counting the number of points falling on particular layer in the abomasa wall and dividing it by the total no of points falling on the entire abomasum. For the intestinal layers, the volume density of each layer was calculated by counting the number of points on a particular layer divided by the number of points falling on the entire intestinal area.

Surface densities

Surface density of the abomasum was estimated by using cycloid test lines as shown in Figure 4. This surface density was estimated by counting the number of intersections between the test lines and the mucosal layer and dividing it by the length of the test system [20].

The surface density of the intestinal mucosa was also estimated by the use of cycloid test lines method. This was done by counting the number of intersections between the test lines and the mucosal layer and dividing by the length of the test system. [20]. Surface density of each tissue component was calculated as follows;

$$SV = 2 * \frac{\sum i}{\frac{l}{p} * \sum P}$$

Where, Sv is the surface density of the abomasal/small intestinal tissue;

$\sum i$ is the total number of intersections between the cycloid test lines and the mucosal surface for the small intestinal/abomasal;

l/P is the test line length per point.

$\sum P$ is the total number of points hitting the abomasal/intestinal mucosal surface.

Surface area of the mucosa

Surface area of the mucosae was estimated by multiplying the surface density with reference volume [20].

Absolute surface area of each abomasum and small intestine was calculated as follows,

$$Sa_{ab} = Sv * Vref$$

where,

SA_{ab} is the absolute surface area of the abomasum;

Sv is the surface density of the abomasal mucosa layer;

Vref is the reference volume of the abomasum/small intestine.

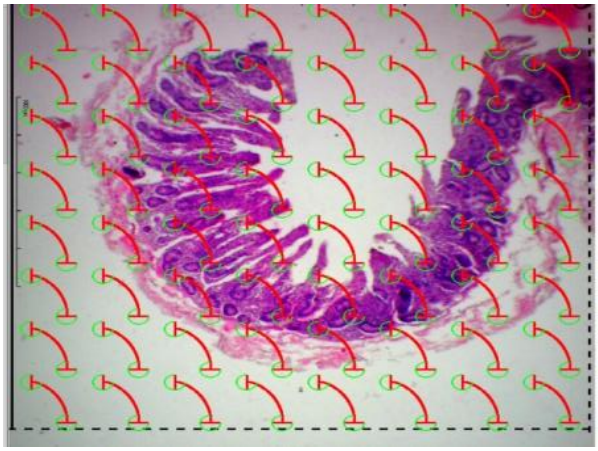

Figure 4: Histological section of the sheep intestine with an electronically superimposed grid showing the interaction between the arcs and the mucosal surface. Magnification X40

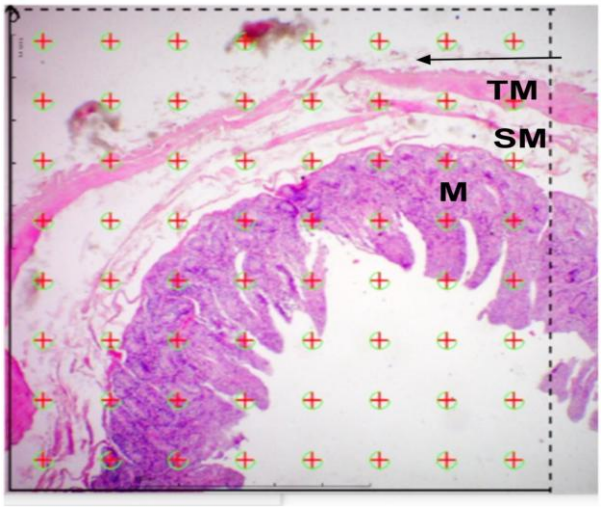

Figure 5: Histological section of the sheep small intestine showing the interaction between the electronically superimposed points and he various tissue layers for the measurement of volume densities. The tissue layers denoted are the mucosa (M), the submucosa (SM), the tunica muscularis (TM) and the serosa (Arrow) Magnification X40

RESULTS :

Small intestinal and abomasal macroscopic surface areas

The present study evaluated and compared the macroscopic surface areas of both the small intestine and the abomasum across two groups. The data obtained revealed consistent patterns in terms of increased surface area and variability in Group 2 relative to Group 1. For the small intestine, Group 2 demonstrated a higher mean macroscopic surface area (2150.38 cm^2) compared to Group 1 (1817.97 cm^2). Similarly, in the abomasum, Group 2 also showed a higher mean surface area (101.15 cm^2) than Group 1 (85.54 cm^2). These findings collectively suggest that Group 2 animals exhibited a generally larger gastrointestinal macroscopic surface area across both group.

Villous lengths

The data obtained in this study have established intestinal villi length using FIJI Image J software. This was achieved by getting the images of vertical sections of the small intestines microscopically.

The mean villous length of the sheep entire intestine showed moderate variation among individuals. In the first dataset, the overall mean villous length was **503.79 ± 5.20 µm**, with values closely clustered around 500 µm, indicating low inter-individual variability. In the second dataset, villous lengths ranged from approximately **501 µm to 589 µm**, with an overall mean of **537.67 ± 37.98 µm**, demonstrating greater variability and higher villous lengths in some animals. Lambs supplemented with *saccharomyces cerevisiae* fermentation product tended to have longer villous height than the control lambs as shown in **Figure. 6 and figure 7**.

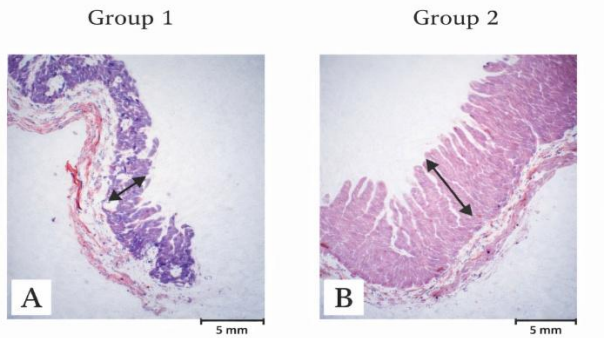

Figure 6: Histological section of duodenal intestinal mucosa illustrating higher villous length (double headed arrow) among the supplemented group (B), compared to control (A). Magnification X40.

Table 2: Mean intestinal villous lengths (µm) for group 1

	Intestinal segment					
Animal Number	**1**	**2**	**3**	**4**	**5**	**Intestinal mean**
1	485.78	489.65	495.22	505.02	501.36	495.41 ±7.96
2	510.5	499.87	497	508.06	509.65	505.02 ±6.15
3	503.38	505.91	495.21	505.58	538.68	509.75 ±16.71
4	505.95	502.62	507.92	503.09	503.59	504.63 ±2.2
5	497	499.56	508.31	503.62	512.26	504.1 ±65.62
Mean ± SD	500.52 ±23.98	499.52 ±6.08	500.73 ±6.78	505.07 ±1.95	513.11 ±14.96	**503.79** ±5.20

Entire intestine refers to intestinal mean for the entire group

Table 3: Mean intestinal villous lengths (µm) for group 2

	Intestinal segment					
Animal Number	**1**	**2**	**3**	**4**	**5**	**Intestinal mean**
1	502.07	484.03	504.67	508.59	508.27	501.53 ± 10.14
2	502.81	503.86	492.35	503.07	505.07	501.43 ± 5.15
3	502.23	505.36	515.16	496.19	496.04	502.99 ± 7.89
4	513.1	502.29	505.41	495.19	508.42	504.88 ± 7.72
5	491.34	508.84	501.24	503.92	501.93	501.454 ± 6.39
6	629.21	553.44	552.09	633.79	515.26	576.76 ± 52.29
7	610.59	604.59	486.87	596.49	503.96	560.5 ± 59.29
8	570.12	632.17	595.69	604.49	542.52	588.99 ± 34.15
9	536.79	507.32	586.78	580.32	596.96	561.63 ± 38.07
10	632.01	528.05	556.13	630.32	536.37	576.58 ± 50.87
Mean± SD	549.03 ± 56.62	532.99 ± 48.99	529.64 ± 39.83	555.24 ± 58.87	521.48 ± 30.46	537.67 ± 37.98

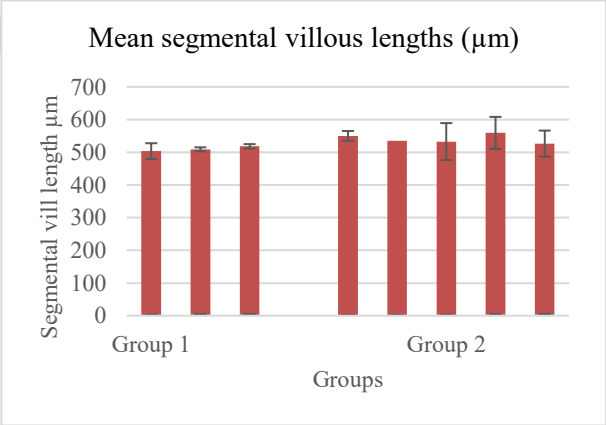

Figure 7: A bar graph showing comparison of sheep intestinal mean villous length between supplemented (Group 2) and control group (Group 1).

Small intestinal surface areas, surface densities and volume densities

The small intestinal mucosal surface areas (SA) and surface densities (Sv) varied between the two study groups. In Group 1, mucosal surface area ranged from **4397.3 to 5048.0 cm²**, with a mean of **4641.63 ± 261.89 cm²**, indicating relatively low inter-individual variability. Surface density values ranged from **155.8 to 220.0 cm⁻¹**, with a mean of **191.16 ± 24.30 cm⁻¹**.

In Group 2, mucosal surface area was markedly higher, ranging from **4708.2 to 9501.2 cm²**, with a mean of **7038.59 ± 1972.56 cm²**, demonstrating substantial inter-individual variation. In contrast, surface density values were comparatively uniform, ranging from **8.1 to 13.4 cm⁻¹**, with a mean of **11.21 ± 0.73 cm⁻¹**. The result shows that there were significant differences on small intestinal surface areas and Surface densities as shown tabulated in **Tables 4 and 5**.

Small intestinal volume (SV), measured using the Scherle method, showed variability within and between the two experimental groups.

In **Group 1**, animals had a mean body weight (BW) of **22.13 ± 5.43 kg** and a mean SV of **564.85 ± 81.11 cm³**. Individual SIV values ranged from approximately **491 to 699 cm³**, indicating moderate inter-animal variation.

In **Group 2**, the mean BW was slightly higher at **23.90 ± 5.53 kg**, with a correspondingly higher mean SIV of **610.15 ± 99.97 cm³**. The range of SIV values in this group was wider (**486 to 837 cm³**), reflecting greater variability compared to Group 1.

Overall, Group 2 exhibited a higher mean small intestinal volume and a larger standard deviation than Group 1, suggesting increased intestinal volumetric development.

ABOMASUM

Abomasal mucosal surface area (SA) and surface density (Sv) were assessed in two experimental groups. In **Group 1**, the mean abomasal mucosal surface area was **200.00 ± 14.14 cm²**, with individual values ranging from **180 to 220 cm²**. The corresponding mean mucosal surface density was **12.38 ± 1.54 cm⁻¹**, indicating relatively uniform mucosal organization among animals in this group.

In **Group 2**, abomasal mucosal surface area was markedly higher, with a mean value of **380.67 ± 98.02 cm²** and a wide range from **220 to 600.6 cm²**. Despite this substantial increase in total surface area, the mean mucosal surface density (**12.58 ± 1.38 cm⁻¹**) was comparable to that observed in Group 1. Variability in surface density within Group 2 remained moderate, despite pronounced differences in absolute surface

Overall, Group 2 exhibited a substantially larger abomasal mucosal surface area than Group 1 as shown **Figure.7** and tabulated in **tables 4 and 5**, while mucosal surface density remained relatively consistent between the two groups.

The volume density (Vv; %) of the abomasal wall components—mucosa, submucosa, muscularis, and serosa was quantified in the two experimental groups.

In **Group 1**, the mucosa had a mean volume density of **55.69 ± 7.84%**. In **Group 2**, the abomasal mucosal layer had a higher mean volume density of **61.45 ± 5.07%**. Compared to Group 1, Group 2 exhibited a higher relative mucosal proportion than group 1.

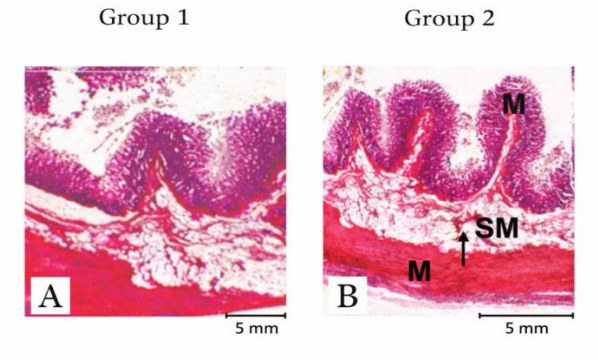

Figure 8: A pictorial presentation showing differences in sheep abomasal mucosal surface across treatment groups, with *Saccharomyces cerevisiae* supplementation increasing mucosal surface. The tissue layers denoted are the mucosa, the submucosa (SM), the tunica muscularis (TM) and the serosa (Arrow)

Table 4: Sheep small intestines and abomasum surface area and surface density data – Group 1

	Small intestines		Abomasum	
Animal Number	Mucosal surface area (SA)-(cm2)	Mucosal surface density (Sv)-cm-1	Mucosal surface area (SA)-(cm2)	mucosal Surface density (Sv)-(cm-1)
1	4397.3	155.8	180	15
2	4504.9	180	200	11.8
3	4752.44	200	220	11.6
4	5048	220	200	11.1
5	4505.5	200	200	12.375
Mean	4641.63	191.16	200	12.38
SD	261.89	24.3	14.14	1.54

Sv is the surface density of the tissue component
SA is the mucosal surface area
Mean refers to the mean of the abomasal and intestinal mucosal component

Table 5: Sheep small intestines and abomasum surface area and surface density Data-Group 2

	Small intestines		Abomasum	
Animal Number	Mucosal surface area (SA)-(cm2)	Mucosal surface density (Sv)- cm-1	Mucosal surface area (SA)-(cm2)	mucosal Surface density (Sv)-(cm-1)
1	4864	10	339	11.3
2	5483.2	9.7	339.3	11.7
3	6037.8	9	352.8	12.6
4	5294.6	9.3	316.4	11.3
5	4708.2	8.1	390.6	12.6
6	7336.4	13.3	408	12
7	8904.7	13	420	14.5
8	9003.1	13.2	220	11
9	9252.7	13.4	600.6	14.3
10	9501.2	13.1	420	14.5
Mean	7038.59	11.21	380.67	12.58
SD	1972.56	0.73	98.02	1.38

Sv is the surface density of the tissue component
SA is the mucosal surface area
Mean refers to the mean of the abomasal and intestinal mucosal component

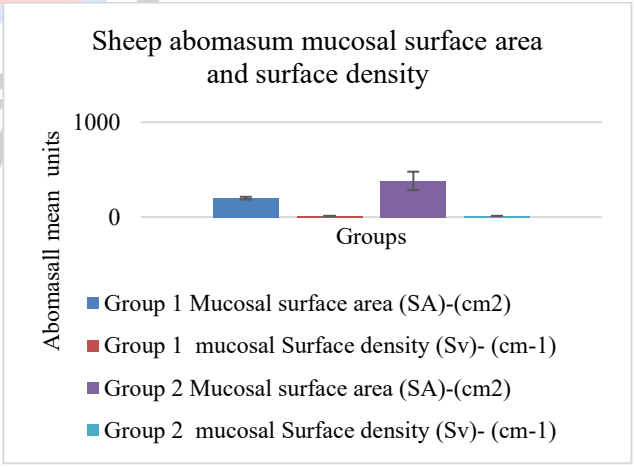

Figure 9: A bar graph showing comparison of sheep abomasal mucosal surface areas and surface densities between supplemented (Group 2) and control group (Group 1)

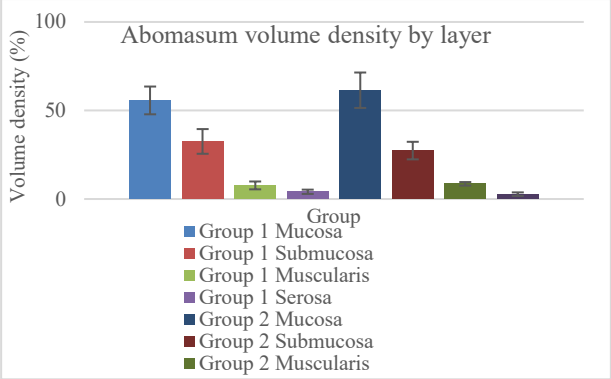

Figure 10: A graph showing the volume density of intestinal mucosa in sheep in control group

Table 6: Abomasal and small intestinal volume by Scherle Method-Group 1

Animal Number	Bwt (kg)	V(Ab) (cm^3)	V(Si), (cm^3)
1	23.68	111	491
2	28.9	135.47	699.3
3	17.6	100.4	555.23
4	24.82	107.3	512.97
5	15.64	95.87	565.73
Mean	**22.13**	**110.01**	**564.85**
SD	**5.43**	**15.4**	**81.11**

Bwt- Body Weight
V(Ab)- Volume of the entire Abomasum
V(Si)–Volume of the entire small intestines

Table 7: Abomasal and small intestinal volume by Scherle method- Group 2

Animal Number	Bwt (kg)	V(Ab) (cm3)	V(Si), (cm3)
1	17.18	64.6	486.3
2	20.4	122.3	644.57
3	31.38	113.33	517.53
4	24.92	110.5	644.8
5	29.24	176.37	659.7
6	24.3	163.47	637.07
7	18.88	126.3	584.77
8	25.28	126.03	551.9
9	30.96	382.2	836.57
10	16.44	101.37	538.27
Mean	**23.9**	**148.65**	**610.15**
SD	**5.53**	**87.71**	**99.974**

Bwt- Body Weight
V(Ab)- Volume of the entire Abomasum
V(Si)–Volume of the entire small intestines

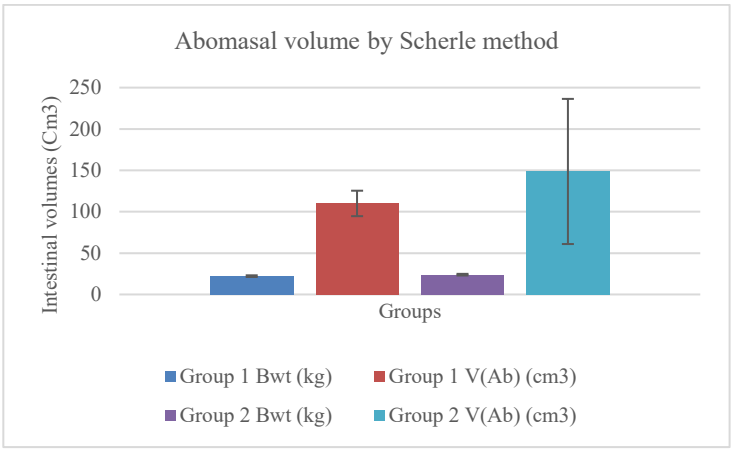

Figure 11: A graph showing sheep Abomasal volume by Scherle Method

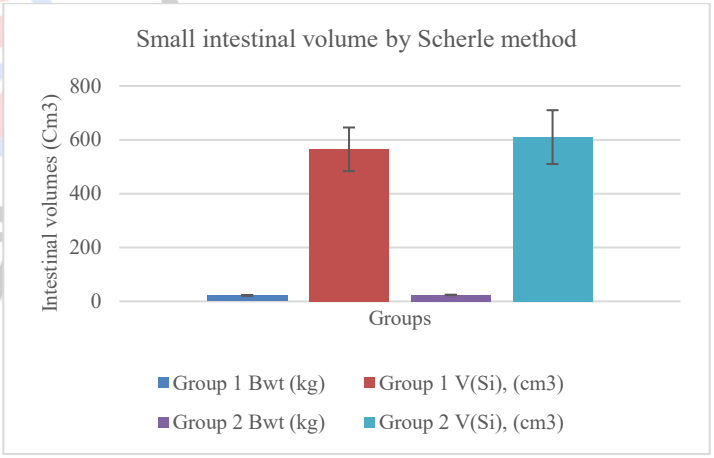

Figure 12: A graph showing sheep small intestinal volume by Scherle Method

STATISTICAL ANALYSIS

Morphometric data were analyzed using IBM SPSS Statistics[22]. Data distribution was assessed for normality using the Student T-test. Results are presented as mean ± standard error of the mean (SEM). Differences among tissue groups were tested by one-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test. Differences with $P < 0.05$ were considered statistically significant.

Statistical analysis for small intestine

Villous Length-One-way Anova

The f-ratio value is 12.21. The p-value is 0.00081. The result is significant at $p < .05$. Group two had a higher average intestinal segment value than Group one, showing that the *Saccharomyces cerevisiae* fermentation product significantly increased villous length.

Small intestines mucosal surface area -One way Anova

The f-ratio value is 7.05427. The p-value is .019785. The result is significant at $p < 0.05$.

It shows that there is a **highly significant difference** in mucosal surface area between the groups. This indicates that the *Saccharomyces Cerevisiae* supplemented had a **strong effect** on the intestinal mucosal surface area.

The results for mucosal surface area provide as follows:

Group 1 vs Group 2: Significant difference (**p = 0.019785**). Group 2 had a much larger mucosal surface area than Group 1.

Small intestines mucosal surface density-one way anova

The f-ratio value is 17.51745. The p-value is .001068. The result is significant at $p < .05$.

The anova results indicate that:

1. **Mucosa:** There is a highly significant difference among groups ($p < 0.05$). Group 2 have higher mucosal values than Group 1, indicating strong effect of the *Saccharomyces Cerevisiae* supplementation product on the mucosa.
2. **Surface Density (Sv):** There's significant difference among groups (p = 001068). This means the *Saccharomyces Cerevisiae* supplementation product strongly altered the surface density values.
   Group 1 vs Group 2: Large, highly significant difference; Group 2 mucosa much higher.

Anova results for abomasal mucosal surface area

The one-way ANOVA showed a **highly significant difference** in abomasal mucosal surface area between the treatment groups. The f-ratio value is 16.20641. The p-value is 0.001441. The result is significant at $p < .05$.

These findings suggest that Group 2 exhibits a significantly larger mucosal surface area compared to Group 1

This means that the *Saccharomyces Cerevisiae* supplementation product had a strong influence on the abomasal mucosal architecture, leading to measurable changes in absorptive surface area.

Tests of between- subjects' effects for abomasal volume density

Decision

- **F (1,13) = 3.02**
- Critical value at $\alpha = 0.05 \approx$ **4.67**
- Since **3.02 < 4.67**, **p > 0.05**

There is **no statistically significant difference** in mucosa volume density between **Group 1** and **Group 2**.

Although Group 2 shows a slightly higher mean, the difference is **not enough to be statistically meaningful**. **Group differences** indicate the *Saccharomyces Cerevisiae* supplemented group does not significantly influence volume density.

DISCUSSION:

The present study demonstrates that supplementation of Dorper sheep with *Saccharomyces cerevisiae* fermentation product is associated with coordinated structural adaptations of the gastrointestinal tract that are directly relevant to improved feed efficiency. By integrating morphometric indices of the abomasum and small intestine, including surface characteristics and villous architecture, the findings provide mechanistic evidence supporting the role of yeast supplementation in enhancing digestive and absorptive efficiency.

In supplemented animals, increases in small intestinal volume and surface area indicate an enhanced capacity for digesta handling and nutrient absorption. These gross anatomical changes likely facilitate prolonged contact between digesta and the intestinal mucosa, improving the extent of nutrient extraction from the diet. However, the functional significance of these changes is best understood when considered alongside surface density and villous mean length measurements. The villous length was measured from the tip of villus to the base of the villus as shown in **Figure 1**. The mean intestinal villous length for group 1 was 503.79 um and group 2 villous length was 537.67 um.

The significantly greater villous mean length observed in supplemented Dorper sheep represents a critical microstructural adaptation with direct implications for feed efficiency. Elongated villi increase the epithelial surface area available for nutrient uptake without requiring substantial increases in intestinal length or tissue mass. This efficient expansion of absorptive surface enhances the uptake of amino acids, glucose, fatty acids, and minerals, thereby improving the conversion of feed into body tissues. Such microstructural optimization is particularly advantageous in production systems where maximizing nutrient utilization per unit of feed is essential. These results are similar to those obtained in sheep [22] The small intestine is the main site of nutrient absorption in animals and plays a major role in animal growth Studies have shown that the shortening of villi in the small intestine affects the number of absorbing cells per villus, reduces digestion and absorption capabilities, and reduces animal productivity[11].

Surface density results further support this interpretation by indicating more efficient packing of absorptive surface within a given intestinal volume. Higher surface density, in combination with increased villous height, suggests that *S. cerevisiae* supplementation promotes mucosal remodeling that favors functional efficiency rather than mere organ enlargement. This aligns with previous observations that yeast supplementation stabilizes gut microflora, reduces pathogenic load, and enhances epithelial turnover, thereby promoting a healthier and more absorptive intestinal lining.

Abomasal adaptations observed in supplemented sheep provide complementary support for improved feed efficiency. The marked increase in abomasal mucosal surface area and mucosal volume density suggests enhanced secretory capacity, including greater production of hydrochloric acid and proteolytic enzymes[23]. Improved abomasal digestion enhances the breakdown of microbial protein and dietary components, ensuring that substrates entering the small intestine are more readily absorbable. Importantly, the maintenance of relatively constant abomasal surface density indicates that this enhancement is achieved through expansion of functional tissue rather than structural inefficiency.

The combined effects of enhanced abomasal digestion and optimized small intestinal absorption create a synergistic improvement in feed utilization. *Saccharomyces cerevisiae* is known to improve rumen fermentation by stabilizing pH and stimulating beneficial microbial populations. The present morphometric findings extend these functional effects downstream, demonstrating that improved rumen and abomasal conditions are accompanied by structural adaptations in the small intestine that maximize nutrient capture.

From a production perspective, these gastrointestinal adaptations provide a morphological basis for improved feed conversion efficiency in Dorper sheep receiving yeast supplementation. Enhanced digestion, increased absorptive surface area, and efficient mucosal organization collectively reduce nutrient losses and increase the proportion of ingested feed converted into growth or production outputs.

In conclusion, supplementation of Dorper sheep with *Saccharomyces cerevisiae* is associated with functionally meaningful gastrointestinal remodeling that supports improved feed efficiency. The observed increases in villous length, intestinal surface area, and abomasal mucosal development provide mechanistic evidence linking yeast supplementation to superior nutrient utilization. These findings highlight the value of integrating gross anatomical, stereological, and histomorphometric analyses when evaluating nutritional interventions aimed at improving ruminant productivity.

Other studies were done to determine the effects of yeast metabolites (Diamond V XPC) and enzyme complex (Allzyme SSF) on the performance of broiler chickens. Broilers fed diets supplemented with yeast metabolites had longer jejunal villi heights [1]. In ruminants, approximately 10-15% of the animal's energy requirement is supplied through microbes in the caecum[24]. Like functional differences, morphology and physiology differences are nutritionally important in judging local sheep breeds, which is important not only for preserving genetic diversity, but also for sustainable rural development in developing countries[25].

Studies have also been done on the role of yeast supplementation in modifying the gut microbiome, which can impact intestinal morphology and functions, including the villous and microvilli architecture of the small intestine [26]

These findings are consistent with previous reports that dietary additives and functional feed supplements can positively modulate gut morphology, leading to enhanced nutrient utilization and growth performance[16]. Therefore, the present results highlight that use of a higher dose of *Saccharomyces Cerevisiae* fermentation product is a promising nutritional strategy to improve intestinal function and overall productivity in sheep.

The use of *Saccharomyces* (a genus of yeast, commonly ***S****accharomyces cerevisiae*) as a feed additive in ruminant diets has been studied primarily for its effects on rumen fermentation, gut health, and overall animal performance[27]. Saccharomyces has been shown to impact the abomasa and small intestine's surface area and surface densities indirectly by influencing microbial populations, fermentation products, and overall gut health as shown in **Tables 4, 5 and 6**.

The significant gastrointestinal morphometric changes observed in supplemented sheep may reflect cellular adaptations within the gut wall that enhance local resilience and immune competence. Under light microscopy, these effects are most plausibly associated with increased enterocyte proliferation and villous

elongation, which strengthen the epithelial barrier and absorptive surface.

CONCLUSION:

Based on the findings of the present study, it was concluded that:

Histomorphometric evaluation of the sheep gastrointestinal tract demonstrated that supplementation with *Saccharomyces cerevisiae* fermentation product led to measurable improvements in gut morphology in Dorper sheep. Specifically, supplementation increased the mucosal surface area of the abomasum, enhanced duodenal villous surface area, and increased villous density in the jejunum, indicating improved absorptive capacity of the gastrointestinal tract. So, there is a necessity to supplement dorper sheep with yeast metabolites.

However, Further studies are needed using different (higher) levels of yeast metabolites and to determine the optimal levels which promote gut health and overall growth performance of dorper sheep.

RECOMMEDATION

1. Incorporate histomorphometry as a standard method in ruminant nutrition research to objectively evaluate gut health and treatment efficacy.
2. Encourage the use of natural feed additives such as *Saccharomyces cerevisiae* fermentation products alternatives to synthetic growth promoters, to improve gut morphology and overall productivity.
3. Apply histomorphometric findings to optimize feeding strategies that support sustainable livestock production.
4. Promote farmer awareness on gut health as a key driver of feed efficiency, animal welfare, and reduced dependency on antibiotics.

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