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DIETARY SACCHAROMYCES CEREVISIAE PRODUCT ALTERS INTESTINAL HISTOMORPHOLOGY AND MUCIN HISTOCHEMISTRY IN BROILER CHICKENS

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ABSTRACT

The increasing withdrawal of in-feed antibiotics from poultry production has intensified the search for safe and effective alternatives capable of supporting growth and maintaining intestinal health. The present study evaluated the effects of a Saccharomyces cerevisiae fermented product on the gross morphology, histomorphometry and histochemical characteristics of the intestine in broiler chickens. 50-day-old Vencobb-430 broilers were randomly divided into control and treatment groups, with the treatment group receiving a maize–soybean basal diet supplemented with an S. cerevisiae fermented product. Six birds from each group were sacrificed on days 21 and 42 for assessment of intestinal morphology and histological changes. Live and dressed weights, intestinal weight and length were recorded. Tissue samples from the duodenum, jejunum, ileum and caecum were examined using routine histological and special staining techniques, including haematoxylin and eosin, Van Gieson, Verhoeff, Mallory's triple stain, periodic acid–Schiff and Alcian blue. Villus height, epithelial cell height, tunic thickness and caecal lymphoid-nodule diameter were quantified by image-based micrometry. Supplementation with the S. cerevisiae fermented product resulted in higher live and dressed weights, increased intestinal length and weight, and marked improvements in intestinal histomorphometry. Villus height was significantly greater in the supplemented birds across the small-intestinal segments, with the most pronounced increase observed in the duodenum. The mucosal and muscular layers were also thicker, accompanied by increased numbers of goblet cells and larger caecal lymphoid nodules. More intense PAS and Alcian blue reactions indicated increased glycogen and acid mucopolysaccharide content in the intestinal epithelium, goblet cells and glands. Overall, dietary supplementation with an S. cerevisiae fermented product enhanced intestinal structural development, mucosal protection and gut-associated lymphoid tissue in broilers. These findings suggest that S. cerevisiae fermented products may serve as promising antibiotic-free feed additives for supporting intestinal health and productive performance in broiler chickens.

KEYWORDS: Intestinal Histomorphology; *Saccharomyces cerevisiae*; Villus Height, Lymphoid Tissue.

1. INTRODUCTION

Poultry is among the fastest-expanding components of the global livestock economy and a principal source of affordable animal protein. India now ranks third worldwide in egg output, producing about 129 billion eggs and 10.36 million tonnes of meat in 2022 - 23, with poultry contributing close to one per cent of national gross domestic product (Agri. Stat,2023). Sustaining this growth depends heavily on the efficiency with which the bird converts feed into edible tissue, and for several decades that efficiency was underpinned by the routine inclusion of sub-therapeutic antibiotics in feed. Continued use of in-feed antibiotics has, however, been curtailed because of residue accumulation in meat and eggs and the emergence of antimicrobial resistance, both of which carry recognised risks for human health. Their restriction, and outright prohibition in many production systems, has created a clear need for growth-promoting agents that improve performance without leaving residues or selecting for resistant organisms (Dibner JJ and Richards JD,2005). Prebiotics and probiotics have emerged as the leading source because they favor a healthy gut ecology while remaining safe for the consumer (Dunkley C, 2008).

Prebiotics are non-digestible feed fractions that escape digestion in the upper gut and selectively promote beneficial microbes, chiefly in the caeca; the oligosaccharides used most often in poultry are fructo-, galacto- and mannan-oligosaccharides. Probiotics, a term introduced by (Lilly and Stillwell, 1965) and later refined by (Fuller, 1989) following the early substitution experiments of (Tortuero, 1973) are live micro-organisms that benefit the

host by rebalancing the intestinal flora. Colonizing bacteria adhere to the mucosa and form a physical barrier that excludes enteropathogens, so maintaining normal microflora, stimulating digestive-enzyme activity and reinforcing immunity. Among the products explored for this purpose, the yeast Saccharomyces cerevisiae has attracted particular attention. It supplies high-quality protein, vitamins and trace minerals, and its cell wall is rich in mannan-oligosaccharide and β-glucan, which decoy pathogens away from the enterocyte and modulate the immune response (Spring P et. al., 2000) Feeding S. cerevisiae has been reported to expand the absorptive surface of the gut by increasing villus height, crypt depth and goblet-cell numbers, to improve mineral retention and to lower the intestinal pH, ammonia and abdominal fat while raising short-chain fatty-acid production (Santin E, et. al., 2001).

The small intestine is the principal site of digestion and nutrient uptake and therefore a key determinant of growth and immune competence in the bird. Its functional capacity is set largely by the surface area of the villi, which begin to mature in the late embryo and continue to develop rapidly after hatch (Uni Z, et. al., 2003). Because the mucosa is also the first barrier against the luminal environment, any structural injury to it is quickly reflected in impaired digestion and reduced performance (Sklan D. 2001). A feed additive that enlarges and protects this surface is thus doubly valuable.

Although the performance and blood responses to S. cerevisiae have been documented repeatedly, integrated descriptions that combine gross morphometry, quantitative histomorphology and mucin histochemistry across all intestinal segments and two ages remain scarce. The present study was undertaken to fill that gap. Its specific objectives were (i) to describe the histomorphological changes produced by an S. cerevisiae fermented product in the duodenum, jejunum, ileum and caecum of broilers; (ii) to quantify these changes by micrometry; and (iii) to localize glycogen and mucopolysaccharides in the mucosa by histochemistry, in each case comparing supplemented birds with control group at 21 and 42 days of age.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Ethics Committee, Nagpur Veterinary College, Nagpur, Maharashtra, India (approval no. GO/ReBi//S/CPCSEA/2000). Fifty day-old straight-run Vencobb-430 broiler chicks (*Gallus gallus domesticus*) from a central breeding farm were reared in a controlled-environment, deep-litter house on a routine vaccination schedule and divided into two equal groups of 25: controls received a maize-soybean basal diet, and the treatment group received the same diet supplemented with an S. cerevisiae fermentation product (Diamond V Original XPC) at 1.25 g/kg feed. Feed and water were offered ad libitum. Six randomly selected birds per group were humanely killed on day 21 and day 42 for gross and microscopic examination.

Gross morphometry, histology and histochemistry

Live and dressed weight, and intestinal weight and length, were recorded at slaughter. Samples of duodenum, jejunum, ileum and caecum were fixed in 10% neutral buffered formalin, processed through graded alcohols, embedded in paraffin and cut at 4-5 µm (Luna, 1968), then stained with hematoxylin and eosin for general structure and Van Gieson's, Verhoeff's and Mallory's triple stains for connective-tissue elements (Singh and Sulochana, 1996; Luna, 1968). Glycogen was localized by PAS (Bancroft and Stevens, 1982) and acid mucopolysaccharides by Alcian blue (Drury and Wallington, 1980), scored as low, moderate or intense. Villus height, columnar epithelial height (villus and crypt), tunic thickness and caecal lymphoid-nodule diameter were measured by image-based micrometry (Nikon 80i).

Statistical analysis

Data (mean ± SD, n = 6 per group per age) were analysed as a 2 x 2 factorial (treatment x age) by two-way ANOVA (Snedecor and Cochran, 1980) using IBM SPSS Statistics v27, with treatment means compared within age where the interaction was significant. Significance was set at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

RESULTS AND DISCUSSION

Gross morphometry: The duodenum, jejunum, ileum and caecum showed the expected gross topography. The duodenum originated from the distal end of the gizzard and formed a U-shaped loop enclosing the pancreas. The jejunum continued from the distal limb of the duodenal loop and formed several intestinal coils, while the ileum extended caudally towards the ileocecal junction, with the Meckel’s diverticulum serving as an approximate landmark between the jejunum and ileum. The paired caeca arose bilaterally at the ileocecal junction and extended as blind-ended tubular structures (Hodges, 1974; Turk, 1982) (Fig. 1).

Fig. 1. Gross photograph of the broiler digestive tract: (A) gizzard, (B) duodenum, (C) jejunum, (D) mesentery, (E) ileum, (F) caeca, (G) colorectum, (H) pancreas, (I) proventriculus, (J) crop.

The body weight was higher in supplemented birds at both ages: live weight rose from 0.852 ± 0.047 kg (control) to 0.983 ± 0.056 kg (treatment) on day 21, and from 2.684 ± 0.14 to 3.178 ± 0.5 kg on day 42, with corresponding gains in dressed weight and a heavier, and by day 42 longer, intestine (Table 1) - responses consistent with earlier reports of dietary additives increasing gut size in proportion to their trophic action (Peric et al., 2010; Markovic et al., 2009).

Table 1. Body weight and gross intestinal morphometry of broilers on day 21 and day 42 (mean ± SD, n = 6).

Parameter	Day 21 - Control	Day 21 - Treatment	Day 42 - Control	Day 42 - Treatment
Live weight (kg)	0.852 ± 0.047	0.983 ± 0.056	2.684 ± 0.140	3.178 ± 0.500
Dressed weight (kg)	0.486 ± 0.029	0.653 ± 0.032	1.741 ± 0.139	2.304 ± 0.272
Intestinal weight (kg)	0.095 ± 0.013	0.140 ± 0.028	0.134 ± 0.017	0.152 ± 0.018
Total intestinal length (in)	5.917 ± 0.52	6.017 ± 0.16	6.733 ± 0.47	7.817 ± 0.33

Histomorphology and micrometry: The duodenum, jejunum and ileum showed the tongue-shaped, leaf-shaped and spatula-shaped villi respectively that are typical of the fowl gut (Gabella, 1985; Kachave et al., 2009), with a zig-zag villus arrangement in the treatment-group jejunum thought to further enlarge the absorptive surface (Yamauchi, 2002) (Figs 2-4).

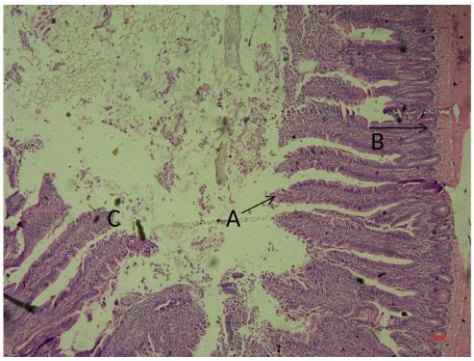

Fig. 2. Transverse section of duodenum (day 21): surface epithelium, lamina propria and tongue-shaped villi. H&E, x100.

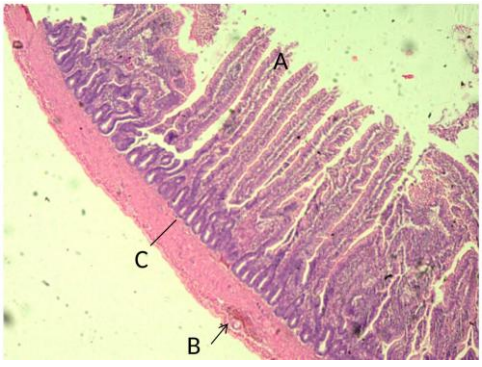

Fig. 3. Transverse section of jejunum (treatment group, day 21): leaf-shaped villi, artery, tunica muscularis. H&E, x40.

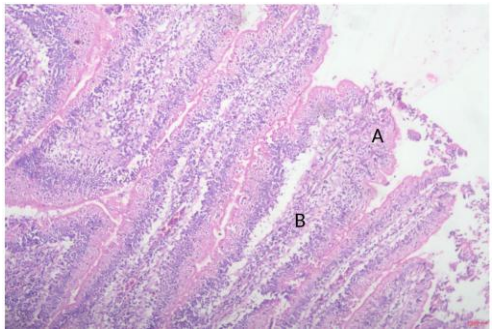

Fig. 4. Transverse section of ileum (day 42): spatula-shaped villi, core of villus. H&E, x200.

Villus height was significantly increased in the treatment group across all intestinal segments at both sampling ages, with the most pronounced increase observed in the duodenum (Table 2). Similarly, the heights of surface and crypt columnar epithelial cells, as well as the thickness of the mucosa and muscularis, were greater in supplemented birds, with the differences being particularly evident on day 21 (Table 3). In the caecum, lymphoid nodules appeared larger in the treatment group and exhibited increased lymphocytic infiltration, indicating enhanced development of the caecal lymphoid tissue (Fig. 5).

Table 2. Villus height (µm) in the small intestine (mean ± SD, n = 6).

Segment	Day 21 - Control	Day 21 - Treatment	Day 42 - Control	Day 42 - Treatment
Duodenum	270.68 ± 64.05	367.85 ± 39.23	1146.45 ± 233.60	1435.28 ± 142.29
Jejunum	420.38 ± 91.04	701.15 ± 143.72	678.17 ± 105.14	875.00 ± 235.65
Ileum	203.40 ± 62.84	463.70 ± 129.91	633.85 ± 51.62	697.57 ± 45.93

Table 3. Tunic thickness (µm) in the small intestine (mean ± SD, n = 6). Serosal thickness did not differ significantly between groups and is omitted here for space; full values are in the source data.

Segment / tunic	Day 21 - Control	Day 21 - Treatment	Day 42 - Control	Day 42 - Treatment
Duodenum - mucosa	316.16 ± 70.34	474.49 ± 52.68	1417.32 ± 282.42	1643.77 ± 112.00
Duodenum - muscularis	125.65 ± 24.56	162.30 ± 74.06	238.29 ± 63.08	354.91 ± 48.09
Jejunum - mucosa	598.43 ± 114.05	1074.49 ± 236.42	987.02 ± 153.14	1123.61 ± 249.87
Jejunum - muscularis	100.19 ± 28.71	160.14 ± 28.64	143.59 ± 36.91	237.58 ± 65.73
Ileum - mucosa	298.90 ± 66.56	671.47 ± 116.83	695.31 ± 55.20	884.31 ± 77.97
Ileum - muscularis	83.27 ± 29.78	215.72 ± 59.35	118.68 ± 26.34	320.62 ± 84.42

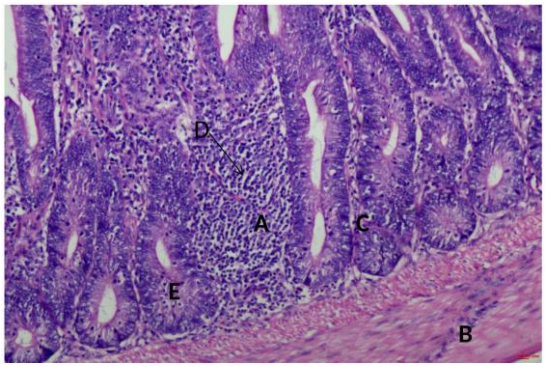

Fig. 5. Transverse section of caecum (treatment group, day 42): prominent lymphatic nodule, crypts of Lieberkuhn, dense lymphocyte population. H&E, x400 [see discrepancy note above].

Histochemistry: PAS staining showed comparatively stronger glycogen-associated reactivity, while Alcian Blue staining demonstrated increased reactivity for acidic mucopolysaccharides in the supplemented group, particularly within the striated border, goblet cells and glandular epithelium on day 21 (Figs. 6–7). At day 42, the ileum of supplemented birds continued to exhibit intense Alcian Blue reactivity, whereas the corresponding control

sections showed relatively weak staining. The enhanced PAS and Alcian Blue reactions suggest greater accumulation of glycogen and acidic mucous substances within the intestinal mucosa of yeast-supplemented birds, particularly in mucin-producing epithelial components. These findings are consistent with enhanced mucosal secretory activity and may contribute to the maintenance of the intestinal mucus barrier (Zhang et al., 2025).

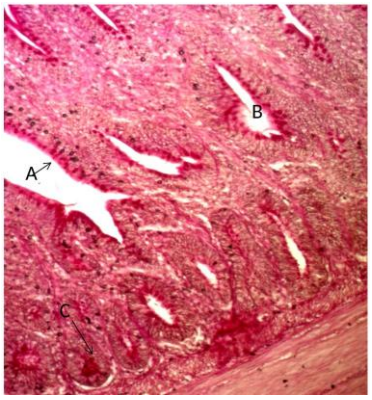

Fig. 6. Duodenum: PAS-positive striated border, luminal border of the intestinal gland, goblet cell. PAS, x100.

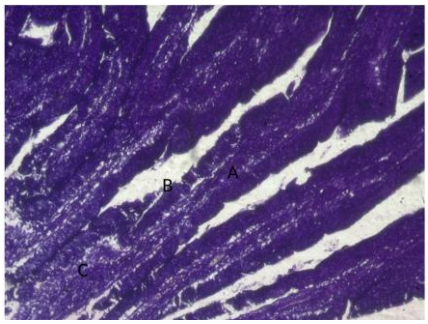

Fig. 7. Duodenum: Alcian blue-PAS reaction showing villi, striated border, core of villi. AB-PAS, x200.

Interpretation

The increase in villus height observed in supplemented birds indicates an expansion of the intestinal absorptive surface and have contributed to the improved growth performance observed in the treatment group. Similar increases in villus height and goblet-cell density following supplementation with *Saccharomyces cerevisiae* or its cell-wall derivatives have been reported previously (Teng et al., 2017; He et al., 2021; Padihari et al., 2014; Tarekar et al., 2023; Roy and Ray, 2023). The beneficial effects may be associated with the bioactive components of the yeast cell wall. Mannan-oligosaccharides can interfere with the adhesion of enteric pathogens by binding to type-1 fimbriae, thereby potentially reducing pathogen attachment and associated epithelial damage (Kyoung et al., 2023). β-glucans, in contrast, can interact with pattern-recognition receptors on innate immune cells and modulate mucosal immune responses, which may support epithelial integrity and tissue renewal (Bar-Dagan et al., 2022). Collectively, these mechanisms provide a plausible explanation for the enhanced intestinal architecture observed in the supplemented birds.

The effects of supplementation were more pronounced at day 21 and became less distinct by day 42, particularly in the jejunum. This temporal pattern may be related to the rapid post-hatch development and maturation of the intestinal mucosa, during which the intestine may exhibit greater responsiveness to nutritional and trophic stimuli (Uni et al., 1999; Uni et al., 2003; Ottinger, 2001). Nevertheless, the persistence of increased muscularis thickness at day 42 suggests that certain structural effects of supplementation may be maintained beyond the early developmental period. However, the present study evaluated a single supplementation level and therefore does not permit assessment of dose-dependent responses. In addition, feed intake, feed conversion ratio, microbiological characteristics and molecular markers of intestinal barrier function were not evaluated. Future studies incorporating graded supplementation levels together with growth-performance parameters, microbiome profiling and expression of intestinal barrier- and mucin-associated genes would help clarify the mechanisms underlying the observed morphological changes.

CONCLUSIONS

Dietary supplementation with a *Saccharomyces cerevisiae*-fermented product improved the gross, histomorphological and histochemical characteristics of the broiler intestine. Supplemented birds exhibited increased villus height, mucosal and muscularis thickness, goblet-cell abundance, PAS and Alcian Blue reactivity, and caecal lymphoid nodule development, accompanied by greater intestinal weight and length and higher body weight. These effects were most pronounced at 21 days of age. Collectively, the observed structural and histochemical changes indicate improved intestinal development and mucosal function in supplemented birds and

provide morphological evidence supporting the potential of *S. cerevisiae*-based supplementation as a nutritional alternative to antibiotic growth-promoting strategies in broiler production.

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DECLARATIONS
Ethics approval: approved by the Institutional Animal Ethics Committee, Nagpur Veterinary College, Nagpur, Maharashtra, India (approval no. GO/ReBi//S/CPCSEA/2000); birds were handled humanely and euthanized by approved humane methods.
Competing interests: the authors declare no competing interests.
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Availability of data and materials: the datasets generated and analysed are available from the corresponding author on reasonable request.

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