Research Article

Efficacy of Yeast in Ameliorating Deleterious Effects of Coccidiosis in Sonali Chicken

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Abstract | This study aims at investigating the anticoccidial effect of yeast in sonali chicken based on possible ones on hematological parameters and histopathological changes. Sixty (60) sonali chickens of twenty-three days old were randomly divided into five groups and each group contained 12 chickens. All groups were supplied *E. tenella* orally except the T_0 group and after 3 days T_2 and T_3 group was treated with yeast 1g/kg and 1.5g/kg feed for 14 days and T_4 groups were treated with amprium-vet Powder (amprolium hydrochloride 20%) at a dose rate of 1g per 3 liters of drinking water for 7 days. Results showed that the body weight of T_0, T_1, T_2 T_3, and T_4 group at 30 days of post-infection were 627.96 ± 1.82, 585.52 ± 2.76, 595.66 ± 2.76, 743.17 ± 3.55, 734.88 ± 2.36 gm respectively which is statistically significant (P<0.01) and highest body weight gain was recorded from T_3 group treated with yeast 1.5g/kg feed. In the case of hematological parameters, the values of Hb and TLC were statistically highly significant (P>0.01) at 10 days and 30 days of post-infection. Total White Blood Cell (WBC) number was increased and Hb level was decreased in 10 days post-infection in all infected groups. Whereas the Total White Blood Cell number (WBC) was decreased and Hb level was increased in 30 days post-infection in all treated groups. Histopathological examinations were also performed which clearly indicated that T_2, T_3, and T_4 groups showed fewer abnormal lesions whereas T_3 showed significantly better lesions than others. Yeast showed good anticoccidial effects and can be used to explore novel therapy against coccidiosis.

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Introduction

The Sonali bird, brought to Northern Bangladesh between 1996 and 2000 via Smallholder and Participatory Livestock Development Project, is a crossbreed of Rhode Island Red (RIR) roosters and Fayoumi hens. It closely resembles the local chickens of Bangladesh in appearance (Uddin *et al*., 2015). In spite of many positive reasons behind the rearing of Sonali chickens, there are some causes that hamper in optimum production of Sonali chicken by smallholder households in Bangladesh (Biswas *et al*., 2006). Poultry diseases are one of the major constraints for developing the poultry industry in Bangladesh (Islam and Samad, 2004). However, very few studies have been reported on the prevalence of infectious diseases of chickens in the northern part of Bangladesh. Thus, this study was aimed at investigating the prevalence of various infectious diseases of Sonali chickens in the northern part of Bangladesh.

Avian Coccidiosis is one of the most important diseases of poultry in Bangladesh caused by single-celled protozoan parasites of the genus *Eimeria* (Lowery, 2023). Coccidiosis represents a severe economic loss in the poultry industry. Control of coccidiosis mostly depends upon chemoprophylaxis by using anticoccidial drugs, but, managerial skills are also important to get the maximum anticoccidial effect of these drugs (Tewari and Maharana, 2011). Chemical anticoccidial additives have been crucial for poultry industry growth, ensuring affordable and high-quality meat. However, their indiscriminate use has led to drug resistance issues and raised concerns about antibiotic presence, contributing to potential antibiotic resistance in bacteria, a worry for consumers (Mak *et al*., 2022). Due to consumer worries, poultry farmers seek antibiotic-free alternatives for growth. Ideal substitutes should be affordable, widely accessible, and health-conscious. Yeast, with its diverse therapeutic benefits like anti-inflammatory, antimicrobial, and anticoccidial properties, emerges as a promising solution. Yeast cell products are derived from yeast species such as *Saccharomyces cerevisiae* and *Pichia guillermondii* (De los Santos *et al*., 2007; Shanmugasundaram and Selvaraj, 2012). The yeast cell wall contains the polysaccharides mannan oligosaccharides and β-glucans (Thomas *et al*., 2022). These polysaccharides can have immunomodulatory effects in several species, including poultry (Yitbarek *et al*., 2012). Yeast products are crucial in poultry farming for their ability to combat coccidial issues, addressing concerns about drug resistance in pathogens (Bilal *et al*., 2022). Immunomodulation properties of the yeast derivatives have been shown to enhance cellular and humoral immunity in *Eimeria* challenge models which is critical for the effectiveness of coccidial vaccination. Moreover, yeast nucleotides have been shown to be beneficial in stimulating the healing of the intestinal mucosal surface. Among all the yeast species, *S. cerevisiae* has been used the most as a probiotic or prebiotic in poultry nutrition (Hooge, 2004; Dhama and Singh, 2010). Because it is readily available and easy to culture, the probiotic yeast *Saccharomyces cerevisiae* var boulardii is widely used as a low-cost and efficient adjuvant against gastrointestinal tract disorders such as inflammatory bowel disease and treatment of several types of diarrhea, both in humans and animals. Yeast cell surface has also been shown to be an effective oral *Eimeria* vaccine delivery vehicle. This study aims to compare the impact of amprolium and yeast on coccidiosis-infected Sonali chickens. The evaluation focuses on hematological parameters and histopathological changes.

Materials and Methods

Animals and records of body mass

Sixty Sonali chickens, aged 23 days, were obtained from Bismillah Farm, Ramsagar, Dinajpur. Upon arrival, they were placed in the experimental shed with proper ventilation and lighting. The birds acclimated for 5 days before the 30-day experiment. Throughout, they experienced a 16-hour photoperiod (natural plus artificial light) in an open-sided house, with additional light from electrical bulbs at night. Weights were measured at the start, every 7 days, and the final weight was recorded on the 30th day.

Collection of feed and yeast

Polli mash commercial feed was collected from Griholokkhi Poultry Feed, Kalitola, Dinajpur from a reputed Sonali feed exporter. Mash and water were provided *ad-libitum* during the whole experimental period. Yeast (*Saccharomyces cerevisiae*), company name: Lihama was purchased from the local market of Dinajpur district. Birds were fed with commercial feed and drinking water *ad-libitum*. Glucose and vitamin C were supplied with drinking water for the first three days to overcome the transportation stress.

Experimental birds grouping

Sonali chickens were uniformly cared for in all groups during the study. Sixty Sonalis were divided into five groups to assess the impact of yeast (*Saccharomyces cerevisiae*) on *Eimeria tenella* infection, growth performance, blood parameters, and histopathology. The groups were as follows:

Group T_0 (Negative control): Received a normal diet, water ad-libitum, and periodic (10 days interval) weight recordings without *Eimeria tenella* infection. Blood parameters, and histopathological examination were measured at the times when that of other groups were measured.

Group T_1 (Positive control): Induced *Eimeria tenella* infection without antiprotozoal treatment.

Group T_2: Induced *Eimeria tenella* infection, started treatment with yeast at a dose of 1.5g/kg feed after 72 hours.

Group T_3: Sonali's were supplied with *Eimeria tenella* protozoa after acclimatization to induce *Eimeria tenella* infection as like as T_1 and T_2 group. Induced *Eimeria tenella* infection, started treatment with yeast at a dose of 1.5g/kg feed after 72 hours.

Group T_4: After acclimatization to induce *Eimeria tenella* infection, Sonalis of this group were supplied with *Eimeria tenella* protozoa as like as T_1, T_2 and T_3 group. This group left about 72 hours for the establishment of *Eimeria tenella* infection. After that, we started our treatment with Amprium-Vet (Amprolium hydrocloride 20%-Square Pharmaceuticals Ltd). It was preserved in a dry place at room temperature and was administered at a dose rate of 1g per 3 liters of drinking water for 3-7 days, to compare the antiprotozoal effect with yeast.

Examination of feces for protozoal oocyst investigation

Feces were examined by two different types of qualitative tests; namely direct smear and flotation techniques to identify the morphological features of eggs, cysts, and oocysts (Hendrin and Robinson, 2006; Soulsby, 1982). The direct smear technique was conducted by mixing a drop of water with a few feces using an applicator stick on a glass slide, covered with a coverslip, and examined under a microscope (Figure 1A).

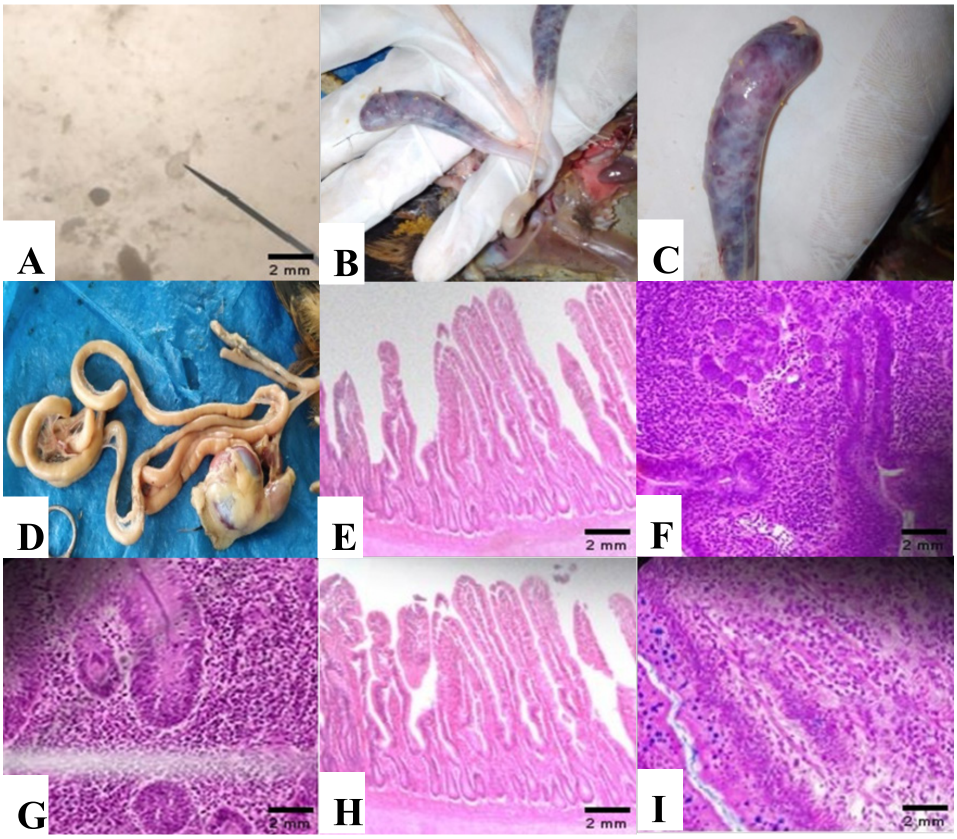

Figure 1: *(A) Microscopic examination of protozoal oocyst, (B) Pathological lessions in intestinal parts, (C) Haemorragic and distended intestine (ceca), (D) Nornal intestine and ceca after treatm, (E) Section of lower intestine fed with basal diet showing no haemorrhagic lesion & other changes (microscopic examination 100x, stained with hematoxylin & eosin: H & E), (F) section of lower intestine from infected bird without treatment showing thickening of intestinal lamina propia, presence of coccidial oocysts in lamina propia (microscopic examination 100x, stained with hematoxylin & eosin: H & E), (G) section of lower intestine from infected bird treated with yeast (1g/kg feed) showing mild congestion of sub-mucosal blood vessels, fibrosis & inflammatory infiltration (microscopic examination 100x, stained with hematoxylin & eosin: H & E), (H) section of lower intestine from infected bird treated with yeast (1.5g/kg feed) showing no haemorrhagic lesions, no inflammatory infiltration, sloughing off intestinal villi slightly & no other distinct changes in the intestinal epithelium (microscopic examination 100x, stained with hematoxylin & eosin: H & E), (I) Section of lower intestine from infected bird treated with amprolium showing inflammatory infiltration of intestinal mucosa & congestion of blood vessels (microscopic examination 100x, stained with hematoxylin & eosin: H & E).*

Parasite flotation was performed using a specific gravity solution (1.20) to exploit the density of parasite eggs. Five grams of feces were mixed with 5ml of saline, filtered, and centrifuged at 1500 rpm for five minutes. The sediment was mixed with 5ml of saturated sodium chloride, centrifuged at 2500 rpm for one minute, and then suspended with more saturated sodium chloride. After ten minutes without shaking, a coverslip was applied, transferred to a slide, and examined under a microscope.

Hematological parameters

Blood samples were collected from the wing vein of chicken of all groups at the age of 38 days and 58 days. The following parameters were observed:

a. Total Leukocyte Count (TLC) [Thousand/mm^3]

b. Hemoglobin estimation (Hb) [mg/dl]

TLC and Hb were performed by Mindray BC-20 Auto Hematology Analyzer at Chatra Bazar, Frakkabad, Birol, Dinajpur.

Postmortem and Histopathological examination

Before and after treatment three chickens from each group were slaughtered to count the number of protozoa (*Eimeria tenella*) and to see if there were any pathological changes present (Figure 1B, C, D). Histopathological examinations were performed by Rotary Microtome (Tissue sectioning diameter 5 µm, Staining- Hematoxylin and Eosin (H and E), Microscopic examination 100x, 200x) at Veterinary Health Care, Satmatha, Rangpur.

Statistical analysis

Each experiment was repeated at least three times unless otherwise mentioned. The results were expressed as a ratio against each control as mean ± SEM. Single-factor analysis of variance (ANOVA) was used to analyze the statical differences where significances were considered at p<0.05. Furthermore, the Student-Newman-Keul's test was used to compare the two groups. Differences were considered significant at the level of p<0.05.

Results and Discussion

Body weight

In (Table 1) showed that after post infection body weight of birds in different dietary treatments was statically significant (p<0.01). The effect of dietary supplementation of yeast showed statically significant (p<0.01) all over the experimental period. Among the different treatments of combination maximum body weight (743.17±3.55^a gm) was observed under yeast at 1.5g/kg feed (T_3) whereas the minimum body weight (595.66±2.76^c) was observed under yeast at 1g/kg feed (T_2). Meanwhile, the second-highest body weight (734.88±2.36^a) was observed under amprolium (T_4) with the recommended dose. In addition, the lowest body weight (585.52±2.76^c) was found in the T_1 group compared to other groups whereas the body weight (627.96±1.82^b) of the T_0 group was heavier rather than T_1 and T_2 groups.

Table 1: *Effect of yeast at a different level and amprolium on body weight of Sonali chicken (10 days to 30 days post-infection).*

Treatment	38 day	48 day	58 day
T_0	422.40±1.02^a	520.48±1.10^a	627.96±1.82^b
T_1	402.70 ± 4.34^b	491.24 ± 3.45^b	585.52 ± 2.76^c
T_2	406.36 ± 3.82^{ab}	513.00 ± 1.55^a	595.66 ± 2.76c
T_3	417.99 ± 1.35^{ab}	525.61 ± 2.89^a	743.17 ± 3.55^a
T_4	415.47 ± 5.41^{ab}	515.11 ± 5.46^a	734.88 ± 2.36^a
P-value	0.016459*	0.000232***	0.000***

*All values indicate mean ± standard error of the mean. The mean value with different superscripts within the same column is statistically Significant at a 1% level of significance. ***Significant at the 0.01 level.*

Hematological parameter

Effect of yeast and amprolium on blood profile of sonali chicken infected with *Eimeria oocysts* given below:

Estimation of hemoglobin: All four experimentally infected groups revealed statistically highly significant decreases in hemoglobin (Hb) (p<0.01) when compared to the control group (10 days of post-infection, Table 2). Furthermore, chickens of T_1, T_2, T_3, and T_4 showed a highly significant reduction (p<0.01) in Hb values from 7.84 ± 0.37 to 9.51±0.30 in comparison to the control group (T_0) on 10 days of post-infection. On the other hand, chickens of T_0, T_1, T_2, T_3, and T_4 showed a statistically significant (p<0.05) increase in Hb values from 10.44±0.24 to 11.15±0.29 at 30 days of post-infection). Among all groups, the highest (11.15±0.29) Hb level was found in the T_3 group and the lowest (10.15±0.20) in the T_1 group.

Total leucocyte count (TLC)

All four experimentally infected groups revealed statistically highly significant ($p<0.01$) increases in total leucocyte count when compared to the control group (10 days of post-infection, Table 3). Furthermore, chickens of T_1, T_2, T_3, and T_4 showed a highly significant increase in TLC values from 10.64±0.33 to 12.86±0.46 in comparison to the control group (T_0) on 10 days of post-infection. On the other hand, chickens of T_0, T_1, T_2, T_3, and T_4 showed statistically significant ($p<0.05$) decreases in TLC values from 10.71±0.47 to 9.00±0.36 at 30 days of post-infection). Among all groups, the highest (10.71±0.47) TLC level was found in the T_1 group and the lowest (9.00±0.36) in the T_3 group.

Table 2: *Estimation of hemoglobin (mg/dl) during the experimental period.*

Treatment	38 Day	58 Day
T_0	10.43 ± 0.34[a]	11.08 ± 0.28[a]
T_1	7.84 ± 0.37[b]	10.15 ± 0.20[a]
T_2	9.28 ± 0.23[a]	10.44 ± 0.24[a]
T_3	9.53 ± 0.25[a]	11.15 ± 0.29[a]
T_4	9.51 ± 0.30[a]	10.63 ± 0.29[a]
P-value	0.000***	0.049*

*All values indicate mean ± standard error of the mean. The mean value with different superscripts within the same column is statistically Significant at a 1% level of significance. ***Significant at the 0.01 level.*

Table 3: *TLC (Thousand/mm^3) during the experimental period.*

Treatment	38 Day	58 Day
T_0	8.70 ± 0.29[c]	9.35 ± 0.29[ab]
T_1	12.86 ± 0.46[a]	10.71 ± 0.47[a]
T_2	10.94 ± 0.34[b]	9.51 ± 0.33[ab]
T_3	10.72 ± 0.36[b]	9.00 ± 0.36[b]
T_4	10.64 ± 0.33[b]	9.35 ± 0.29[ab]
P-value	0.000***	0.014*

*All values indicate mean ± standard error of the mean. The mean value with different superscripts within the same column is statistically Significant at a 1% level of significance. ***Significant at the 0.01 level.*

Histopathology

In histopathological observation of lower intestine, Figure 1E: Intestinal sample obtained from birds based on basal diet (control negative group) showed absence of coccidial oocysts, no inflammatory infiltration and intestinal villi lined by simple columnar epithelium. Figure 1F: Intestinal sample obtained from infected non-treated birds (control positive group) showing thickening of intestinal lamina propia, presence of coccidial oocysts in lamina propia, degeneration, and loss of nuclei. Figure 1G: Intestine of infected birds treated with yeast (1g/kg) chickens, showing mild congestion of sub-mucosal blood vessels, fibrosis and degeneration of sub-mucosal glandular epithelium, and inflammatory infiltration. Figure 1H: Intestine of infected birds treated with yeast (1.5g/kg) showed no hemorrhagic lesions, no inflammatory infiltration, sloughing off intestinal villi slightly and no other distinct changes in the intestinal epithelium. Figure 1I: The infected birds treated with amprolium showed mild inflammatory infiltration of intestinal mucosa and congestion of blood vessels.

In our study, we aimed to highlight the anticoccidial effects of yeast compared to amprolium in inhibiting *Eimeria tenella* infection. We assessed efficacy based on mean body weight, blood analysis, and histopathological examination. The 35-day study indicates a decrease in body weight in group T_1. There was significantly higher at 30 days post-infection bodyweight of sonali chicken than the positive control group. A similar finding was also observed by (Lee *et al*., 2007; Priya and Babu, 2013; Hana *et al*., 2015) where significant differences ($p<0.05$) indicated that feeding yeast to chicks improves body weight gain and feed/gain ratio. The obtained results confirmed the previous findings of several researchers (Zhang *et al*., 2005; Paryad and Mahmoudi, 2008) that yeast supplementation in broiler ration had a significant effect on body weight gain and feed conversion ratio. Shankar *et al*. (2017) and Mulatu *et al*. (2019) also reported that *Saccharomyces cerevisiae* improved feed/gain ratio and body weight gain. The present study revealed that baker's yeast supplementation had a positive effect on the bodyweight of sonali chickens in T_2 and T_3 for the whole trial period which was similar to Islam (2022). Compared with the groups T_0, T_1, and T_4. Kabir (2009) stated that the yeast acts by (i) maintaining normal intestinal microflora by competitive exclusion and antagonism (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (iii) improving digestion, and (iv) stimulating the immune system.

Decreased mean hemoglobin (Hb) concentration had been recorded in infected groups T_1, T_2, T_3, and T_4 in comparison with the control group on 10 days post-infection. The research findings are in accordance with Ogbe *et al*. (2010) and Matthew *et al*. (2022)

who were reported a decrease in the Hb counts in *E. tennela* infected chickens. This could be attributed to the reduction of blood components due to hemorrhage caused by the disease. These findings are similar to Razzaq *et al.* (2003). A significant increase in the mean Hb values was observed in all treated groups when compared with the control group on 30 days post-infection. A similar finding was also observed by Mulatu *et al.* (2019) and Mares *et al.* (2023). This indicates that there was a dietary effect of yeast on Hb.

Increased mean total leucocyte count (TLC) concentration had been recorded in infected groups T_1, T_2, T_3, T_4 in comparison with the control group on 10 days post-infection. The study findings are in accordance with Hirani *et al.* (2018) who reported an increase in the TLC counts in *E. tennela* infected chickens. A significant increase in TLC has also been reported by Padmavathi and Muralidharan (1986), Panda *et al.* (1997), Kumar and Padmavathi (2000), Jaipurkar *et al.* (2004), and Ogbe *et al.* (2010). Adamu *et al.* (2013) and Shekhar *et al.* (2020) also reported similar findings of higher TLC with increased numbers of lymphocytes, eosinophils, and heterophils in *E. tenella* and *E. brunetti* infected broiler. In this study chickens (Sonali) of T_1, T_2, $T_{3,}$ and T_4 showed a highly significant increase in TLC values from 10.64 ± 0.33 to 12.86 ± 0.46 in comparison to the control group (T_0) on 10 days of post-infection. The increased total leucocytes count in coccidia-affected birds might be due to suppressed body immune system from the infection (Hirani *et al.*, 2018; Stephen, 1965). This increase was suggestive of increased leucopoiesis as the first step of defense mechanism to infection (Padmavathi and Muralidharan, 1986). A significant decrease in the mean TLC values was observed in all treated groups when compared with the control group on 30-day post-infection. These findings clearly indicated the ability of yeast to produce immunity against coccidiosis. Yitbarek *et al.* (2012), Shanmugasundaram and Selvaraj (2012) stated that the whole yeast cell is a good source of mannan oligosaccharides and β-glucans known for their immune-modulatory effects. Lee *et al.* (2007), also stated that Saccharomyces-based probiotic, when included in the diet may improve the resistance against *E. tenella* and *E. acervulina* by enhancing humoral immune response as well as reducing oocyst production in birds.

Conclusions and Recommendations

In conclusion, the current experimental findings strongly support the notion that yeasts exhibit a notable anticoccidial effect against *Eimeria tenella* in Sonali chickens, concurrently influencing the growth performance of the poultry. Given the observed drug resistance and adverse effects associated with amprolium, yeast emerges as a preferable alternative. The absence of adverse effects at dosage rates of 1g/kg and 1.5g/kg feed further underscores the safety profile of yeast during this study. This suggests that yeast could offer a promising therapeutic approach against *E. tenella*, considering its accessibility and efficacy. The potential of yeast as a viable agent, particularly in the face of multidrug-resistant species like *Eimeria tenella*, warrants further consideration and exploration in future research endeavors.

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Novelty Statement

This helps to combat against multidrug-resistant *Eimeria tenella*. Through this work we tried to focus on *Eimeria tenella* infection by using yeast and amprolium as a potential anticoccidial and evaluate their effeteness. Finally, this research helps to reduce coccidial treatment cost in sonali chicken as well as creating public awareness against coccidiosis.

Author's Contribution

Conceptualization: MMMP, RI, MS. Research work: MAA, MMMP. Draft writing: MAA, MMMP, MS, RI, MBR. Data arrangement, reviewing and final manuscript writing: MS. All authors reviewed the final version of the manuscript.

Ethical statement

This research work was approved based on University laws and National Law especially following the Bangladesh Veterinary Council (BVC) laws. In this study ethical approval for the use of Pigeon was granted by the Institute of Research and Training (IRT), and Department of Physiology and Pharmacology (Memo No: HSTU/VAS/PPH/1352), Faculty of Veterinary

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Conflict of interest
The authors have no potential conflicts of interest.

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