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Evaluation of yeast culture and direct-fed microbial on gut histology and serum components of broilers challenged with suboptimal diets under heat stress

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ABSTRACT. The use of antibiotics in feed for growth promotion has been restricted in many countries, thus it is of interest to investigate potential alternatives for enhancing growth performance in birds. An experiment was carried out to evaluate the concurrent use of prebiotic and probiotic on gut histology and some blood chemicals of broiler chickens during heat stress. A total of 144 day-old male Ross 308 broiler chicks were randomly divided into 3 treatments. The first treatment was a diluted diet including rice bran without probiotic or prebiotic. The other treatments were fed the diluted diets with a prebiotic (treatment 2) and concurrent use of that prebiotic along with a probiotic (treatment 3). Results showed that villus height was not influenced by feed additives. However, crypt depth significantly increased by feed additives in particular with combination of prebiotic and probiotic. The ratio of villus height to crypt depth significantly decreased in treatments fed prebiotic alone or prebiotic and probiotic together. This finding is indicating the fact that non-starch polysaccharides (NSPs) in high inclusion rate of rice bran caused to villus height erosion. It could be concluded that prebiotic and probiotic caused an increasing of enterocyte proliferation in the crypt of Liberkhun.

Keywords: blood constituents; chicken; prebiotic; probiotic; histomorphometry.

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Introduction

Heat stress is a major concern for poultry producers which has adverse impacts on various physiological aspects such as gastrointestinal tract (Akbarian et al., 2013). It has been well documented that exposing broiler chickens to continuously high ambient temperatures during the finisher phase leads to chronic heat stress, and could exert profound effects on health and performance of birds (Han et al., 2010; Hosseini, Afshar, Ahani, & Vakili Azghandi, 2015; Melesse, Maak, Schmidt, & Von Lengerken, 2011; Prieto & Campo, 2010; Quinteiro-Filho et al., 2010). The metabolic changes induced in chickens by high temperature decreases serum protein concentrations. Importantly, elevated ambient temperature results in impaired antioxidant status and caused oxidative stress in poultry (Mujahid, Akiba, & Toyomizu, 2007). Also high ambient temperature causes a disruption in the structure and function of gut epithelium including reduced regeneration and integrity of intestinal epithelium (Burkholder, Thompson, Einstein, Applegate, & Patterson, 2008; Soderholm et al., 2002).

Rice bran is an agricultural by-product which is used in some parts of the world as livestock and poultry feed ingredient. Albeit, it is cost effective as compared to the conventional feedstuffs. Meanwhile, it has limitations in poultry feeding due to some anti-nutritional factors and low nutritional value.

Feed additives research on different poultry species has been dramatically intensified in recent decade (Hajati, Gilani, & S., 2019; Seifi, Khoshbakht, Hajati, & Gilani, 2018). Interestingly, it has been shown that antibiotic replacers such as prebiotic and probiotic are more fruitful under suboptimal conditions such as stress (Fowler, Kakani, Haq, Byrd, & Bailey, 2015; Seifi, Khoshbakht, Sayrafi, & Gilani, 2018). Therefore, a trial was conducted to assess these additives under heat stressed broilers fed high inclusion rate of rice bran in diluted diets. On the other hand, the growth promoters were evaluated under multiple-stress circumstances.

Material and Methods

Birds, diets and experimental design

A total number of 144 day-old male chicks (Ross 308) were purchased form a local hatchery. All the chicks were weighed (Average weight = 46 g) and randomly divided into 3 dietary treatments. Each treatment consisted 4 replicates (floor pens) of 12 birds each. Pen dimensions were 120×120 cm, so that each chicken had 1200 cm^2 floor space. The initial house temperature was set at 32°C and gradually decreased to reach 24°C at 28d. For inducing heat stress, the birds were subjected to ambient temperature more than 30°C for at least 8 hours from day 20 afterward. Average relative humidity was kept at 60% during the experimental period. A lighting schedule of 24h illumination with approximately 20 lx was used for the entire period. Chicks were vaccinated for Infectious Bronchitis (IB) on day 4, and Avian Influenza (AI) + Newcastle Disease (ND) on day 14 of age.

The first treatment (control) fed a diluted diet including rice bran without probiotic or prebiotic (Table 1). All diets were formulated as 90% of nutrient requirements of the chickens as recommended by Ross 308 broiler management guide (Aviagen, 2009). The birds in treatment 2 received control diet which supplemented with 0.1% prebiotic A-Max Ultra® and treatment 3 fed the second diet plus 0.02% probiotic Multibehsil®.

Prebiotic A-Max Ultra® yeast culture is *Saccharomyces cerevisiae* yeast grown on a media of sucrose and cane molasses, and dried with processed grain by-products (Arm & Hammer Animal Nutrition, the USA). Also, probiotic Multibehsil® was used in the current trial which is produced by Bahman Arad Company, Karaj, Iran. The number of microorganisms in this probiotic as CFU g^{-1} are as follow: 1×10^6 *Lactobacillus casei,* 1×10^5 *Streptococcus salvarious,* 1×10^{10} *Lactobacillus acidophilus,* 1×10^{10} *Bacillus subtillis,* 1×10^3 *Lactococcus lactis,* 1×10^3 *Lactobacillusramosus,* 1×10^8 *Bacillus coagulans,* 1×10^3 *Lactobacillus plantarum,* 1×10^5 *Bacillus lecinofermis,* 1×10^2 *Aspergillus oryzae,* 1×10^3 *Saccharomyses cerevisiae,* 1×10^8 *Bifidobacterium bifidium,* 1×10^3 *Lactobacillus delbrucci*, and 1×10^3 *Entroccucu faecium.*

Feed and water were offered ad-libitum throughout the trial. Vitamin C was also added in drinking water (0.1 g L^{-1}) of all groups during heat stress. The experimental protocol was approved by the Animal Care Committee of Amol University of Special Modern Technologies, Mazandaran, Iran.

Table 1. The composition of experimental diets.

Item	Starter (0-10d)	Grower (11-24d)	Finisher (25-47d)
Ingredients (%)			
Corn	43.92	53.99	55.27
Wheat	8.40	0	0
Rice bran	10	15	20
Soybean meal	33.83	27.89	21.66
Oyster shell	1.31	1.09	1.21
Dicalcium phosphate	0.74	0.32	0.16
Common salt	0.38	0.4	0.4
L-Lysine HCl	0.15	0.09	0.09
DL-Methionine	0.25	0.19	0.17
Vitamin and mineral premix[1]	0.5	0.5	0.5
Sodium bentonite	0.5	0.5	0.5
Enzyme (Multibehzyme®)	0.02	0.02	0.02
Calculated contents (%)			
ME (kcal/kg)	2850	2954	2960
Crude protein	21	19	17
Calcium	1.05	0.90	0.90
Available phosphorus	0.45	0.41	0.38
Sodium	0.17	0.18	0.18
Lysine	1.28	1.12	0.98
Methionine	0.42	0.54	0.49
Methionine+Cystine	0.96	0.85	0.77

[1]vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 9800 IU; vitamin E, 121 IU; B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 980 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

Blood sampling and determination of serum components

At the end of the experiment (47 d), 4 birds from each treatment were randomly selected and blood samples were collected from wing vein with a 25G needle. Serum was obtained by centrifugation of the coagulated blood (3000 rpm for 10 min). Glucose, aspartate amino transferase (AST), and alanine amino transferase (ALT) were analyzed by an automatic analyzer.

Histology

After slaughter, a 2-cm long segment was transected from the middle-length of jejunum; ingesta washed away using normal saline and fixated in 10% neutral buffered formalin. Following histological fixation, the tissues were processed through a standard alcohol dehydration-xylene sequence and embedded in paraffin. From each segment, 5 sections of 5-7 µ thickness were made and stained with haematoxylin and eosin (H & E). Morphometric analyses of digital photos of light microscopy were performed by means of an image analysis program (Image software). In each photo the villi height and crypts depth were determined by examining randomly 12 villi and 12 crypts. Then, an average of 60 values was obtained for each chick (Sayrafi, Shahrooz, Soltanalinejad, & Rahimi, 2011a).

Statistical analysis

All data were analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS, 2004). Duncan's multiple range test was used to compare the means. All statements of significance were based on probability of $p < 0.05$.

Results

Analyses of gut histology are presented in Figure 1 and Table 2. Villus height was not influenced by feed additives. Crypt depth was significantly increased by feed additives in particular with concurrent use of prebiotic and probiotic ($p < 0.05$). The ratio of villus height to crypt depth was significantly decreased in treatment containing prebiotic alone or both prebiotic and probiotic ($p < 0.05$).

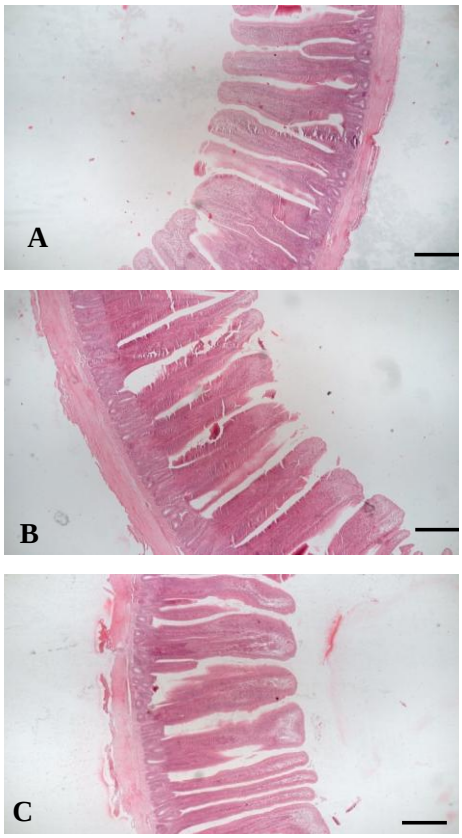

Figure 1. Histological sections of jejunal tissue. A: control group, B: prebiotic group, C: probiotic+prebiotic group. Hematoxylin and eosin staining (40X). Scale bars represent 400 µm.

Table 2. Evaluation of prebiotic and probiotic on intestinal histomorphometry of broiler chickens at 47 days of age.

Treatments	Villus height (µm)	Crypt depth (µm)	Villus height/crypt depth
Control	1135	138[b]	8.3[a]
Prebiotic	1158	196[ab]	5.9[b]
Prebiotic and probiotic	1184	224[a]	5.7[b]
SEM	66	20	0.54
P-value	0.87	0.03	0.01

[a, b] Means with different superscripts in each column are significantly different ($p < 0.05$).

Analyses of serum components are presented in Tables 3. Serum components were not significantly influenced by the additives. Interestingly, simultaneous use of prebiotic and prebiotic in the diets significantly improved overall FI (feed intake) and BWG (body weight gain) ($p < 0.05$). The FCR (feed conversion ration) did not dramatically change because both BWG and FI increased (Table 4).

Table 3. Evaluation of prebiotic and probiotic on serum components of broiler chickens at 47 days of age.

Treatments	Glucose (mg dL^{-1})	AST* (IU L^{-1})	ALT* (IU L^{-1})
Control	237	150	34
Prebiotic	190	203	33
prebiotic and probiotic	207	190	35
SEM	25	26	2
P-value	0.455	0.118	0.869

*AST: aspartate aminotransferase; ALT: alanine aminotransferase

Table 4. Evaluation of prebiotic and probiotic on overall feed intake, body weight gain and feed conversion ratio (FCR) of broilers.

Treatments	Feed intake (g)	Body weight gain (g)	FCR
Control	3365 [b]	1293 [b]	2.60
Prebiotic	3436 [b]	1391 [b]	2.47
prebiotic and probiotic	3924 [a]	1524 [a]	2.57
SEM	100	48	0.100
P-Value	0.004	0.001	0.121

[a, b] Means within each column with no common superscript are significantly different ($P < 0.05$).

Discussion

Antibiotic replacers such as prebiotic and probiotic have been shown to be a moderator in the inflammatory changes in gut structure and micro-ecology (Lambert, 2009). For example, the transport-stress drastically increased oxidative burst and this increment was significantly modulated in yeast extract fed poultry. Moreover, it has been shown that ileum villus height, surface area, crypt depth and goblet cell density were enhanced with a yeast product on d 7 and 21. Surface area and crypt depth were consistently higher for the yeast extract group compared with the control on d 7 and 21. Duodenum villus height, surface area, and goblet cell density were higher for a yeast extract fed group on d 7 (De Los Santos et al., 2007). Also, prebiotic addition increased the villus height and width in duodenum and ileum width compared with other treatments (Sayrafi et al., 2011a). In a recent study by Hutsko, Meizlisch, Wick, and Lilburn (2016) in young poultry, both of villus height and villus area increased with probiotic and mannan-oligosaccharide supplementation and there was a significant treatment interaction effect for crypt depth. They concluded that addition of probiotic and prebiotic to the diet may improve the intestinal microenvironment.

In another broiler research with low digestible diets has been shown that yeast prebiotic had resulted in more enzyme activity in duodenum. The presence of prebiotic reduced the stimulation of the enterocyte turnover rate caused by the presence of non-starch polysaccharides in diets with canola meal and dried distillers grain with soluble. The higher enzyme activity in duodenum and ileal nutrient digestibility imply higher digestibility with the inclusion of prebiotic (Gomez, 2012).

Intestinal epithelial cells are changed constantly and compensate villi cell loss through proliferation and maturation inside crypts and upward migration. Crypts depth is correlated with the intestinal cells turnover rate and increase in crypts depth indicates the need for enterocyte replacement and higher tissue turnover. Such a need could be due to increase in dimensions of villi or maintenance of the dimension as a result of increased destruction (Marković, Šefer, Krstić, & Petrujkić, 2009). In another study, increased depth of the duodenal crypts in the antibiotic treatment could be explained by increased height of intestinal villi and subsequent need for intestinal cells turnover (Sayrafi, Soltanalinejad, Shahrooz, & Rahimi, 2011b).

Meanwhile, complicated results have been reported due to various components of prebiotic. For instance, Zhang et al. (2005) compared three types of yeast products as whole yeast, cell wall, and yeast extract. They reported that villus height was greater in whole yeast and cell wall as compared with those in control and yeast extract. No differences were found in crypt depth among 4 treatments. The villus height to crypt depth ratios in whole yeast and cell wall was greater than those of the control and yeast extract. Both yeast extract and cell wall had oxidation-reducing effects (Zhang et al., 2005).

The current results about serum components are not in line with pronounced results of some previous studies. For example, serum levels of calcium, phosphorus, and triglycerides were decreased and uric acid level were increased by yeast extract supplementation (Huff et al., 2010). Also, Toghyani, Tohidi, Gheisari, Tabeidian, and Toghyani (2012) reported a significant reduction of blood triglycerides and abdominal fat pad in birds fed prebiotic A-MAX compared to control birds. Moreover, Jahanian and Ashnagar (2015) indicated that triglycerides and LDL concentrations in blood serum were significantly decreased in laying hens challenged with *E. coli* fed another MOS product. It is postulated that oligosaccharides particularly MOS are substrates for lactic acid producing bacteria like *Lactobacillus spp.* in the gut. Subsequently, these bacteria can effectively reduce the activity of acetyl coenzyme A carboxylase leading to decreased lipid synthesis, and consequently reduction of serum triglycerides (Van Loo, 2004) In overall, this biochemical process might cause to lower carcass fat especially in belly of the broilers (Toghyani et al., 2012).

Nevertheless, there are some reports indicating no significant impacts of these additives on gut and/or blood variables of poultry. Huff et al. (2015) indicated that prebiotics did not consistently prevent the effects of the cold stress or *Escherichia coli* challenge. Also, supplementation with a prebiotic had no considerable effect on performance, immunity, and stress indicators (blood glucose, cholesterol, and corticosterone) in a study (Houshmand, Azhar, Zulkifli, Bejo, & Kamyab, 2012). In another research, no significant changes in the gut morphology were mentioned between MOS treatment and negative or positive control at d 14, but birds in positive control group had significantly higher jejunal villi and mucosal alkaline phosphatase activities than MOS-supplemented birds at d 35 (Zhang et al., 2005). In another study, addition of MOS did not show a clear positive effect on performance or intestinal morphology and function (Yang, Iji, Kocher, Mikkelsen, & Choct, 2007).

All in all, specific mechanisms about the influences of prebiotic and probiotic on the host and digestive tract microbiota remains unknown yet. According to more detailed research conducted with nonconventional poultry, it is likely that structurally distinct prebiotics will influence not only the gastrointestinal tract microbiota differently, but potentially interact directly and/or indirectly with the bird host in distinguishable approaches, too (Ricke, 2015).

In conclusion, it can be concluded that prebiotic and probiotic cause to more enterocyte proliferation in the crypt of Liberkhun. In the current trial, crypt depth has been significantly increased, but villus height did not significantly change. Maybe, the villus erosion due to NSP of rice bran in particular under suboptimal conditions would be occurred during upward migration of cells to the top of villus. Therefore, further experiments are needed to determine whether prebiotic and/or probiotic supplementation affect gut and serum components of broilers according to suboptimal circumstances.

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