Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) on performance, carcass and gut characteristics, blood profile, and antibody production to sheep red blood cells in broilers

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Primary Audience: Nutritionists, Researchers, Feed Formulators

SUMMARY

This study was conducted to determine the effects of dietary yeast autolysate on performance, carcass and gut traits, blood parameters, and humoral immune response of broilers. A total of 175 day-old Ross 308 male broiler chicks were allocated into one control group and 4 treatment groups each containing 5 replicate groups of 7 chicks. A basal diet was supplemented with 0, 1, 2, 3, and 4 g/kg of yeast autolysate (*Saccharomyces cerevisiae*, InteWall) to generate dietary treatments. The experimental period lasted 42 d. Supplemental yeast autolysate improved live weight gain ($P < 0.01$) and feed conversion ($P < 0.001$) during the starter period (1 to 21 d). Cumulative FCR was decreased ($P < 0.05$) during the overall period (1 to 42 d) with 2 and 3 g/kg of yeast autolysate supplementation when compared with the control diet. There were no dietary effects on final live weight, feed intake, excreta pH, excreta moisture, carcass yield, and the relative weight of gizzard, liver, heart, spleen, bursa of Fabricius, and the intestinal weights. Yeast autolysate supplementation decreased relative weight of abdominal fat ($P < 0.001$) and *Escherichia coli* count of the digesta ($P < 0.01$) and increased antibody titers to SRBC ($P < 0.001$). The pH of jejunal and ileal digesta was decreased at the 2, 3, and 4 g/kg of yeast autolysate supplementation compared with that of birds fed the control diet ($P < 0.001$). Dietary treatments did not significantly affect blood serum levels of cholesterol, triglyceride, protein, uric acid, aspartate amino transferase, and alanine amino transferase. It was concluded that the dietary supplementation at the level of 2 and 3 g/kg of yeast autolysate was an effective feed additive in broiler feeding because of the increased growth performance, increased immunocompetence, and the reduction of *E. coli* colonization in the intestine.

Key words: broiler, performance, gut trait, humoral immune response, yeast autolysate

2013 J. Appl. Poult. Res. 22:55–61
http://dx.doi.org/10.3382/japr.2012-00577

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DESCRIPTION OF PROBLEM

Yeast is a natural ingredient used daily in human diets in bread or fermented beverages, and therefore, consumer acceptability of yeast products is high [1]. Recently yeast and yeast products have also received considerable attention as effective growth enhancers in poultry nutrition. As yeast products, yeast autolysates consist of ruptured or lysed cells and contain both intracellular and cell-wall fractions. Yeast autolysis is a degradation process carried out by activating the yeast's own degradative enzymes to solubilize cell components within the cell. The cell wall is degraded by breaking its glucan and chitin fibers. The hydrolytic enzymes such as proteases and nucleases, which are located in the general matrix of the cell, are responsible for the degradation of yeast proteins and nucleic acids. Proteases break down yeast proteins into peptides and amino acid derivatives, whereas nucleases split nucleic acids, DNA, and RNA into nucleotides [2, 3].

Yeast and yeast products affect nutrient digestibility [4], increase humoral immune response [5–8], and reduce serum cholesterol [8] and egg-yolk cholesterol [8, 9] in poultry. Dietary yeast autolysate supplementation at the levels of 2, 3, and 4 g/kg had beneficial effects on performance, egg cholesterol content, and humoral response [8]. However, as far as we know there is no published report about the dietary yeast autolysate in broilers. Therefore, the aim of this study was to examine the effects of the dietary yeast autolysate (*Saccharomyces cerevisiae*) on performance, carcass and gut traits, blood parameters, and humoral immune response of broilers.

MATERIALS AND METHODS

Birds and Diets

All animal-use protocols were in accordance with the Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010, on the protection of animals used for scientific purposes [10]. A total of 175 day-old Ross 308 male broiler chicks were randomly assigned to one control group and 4 treatment groups each containing 5 replicate groups of 7 chicks. Chicks of each replicate group were placed in separate floor pens measured as 170 × 94 × 90 cm, width × length × height, respectively. Each pen had wood-shavings litter, 2 nipples, and one hanging suspended feeder. Feed and water were provided for ad libitum consumption, and the diets were presented in mash form. The lighting period was continuous lighting during the whole experiment. Average room temperature was 32 ± 2°C on the first week and then gradually lowered to average 24 to 26°C, and this temperature was maintained up to slaughter age. The experimental period lasted 42 d. Broilers were fed with starter diets during 1 to 21 d and fed with grower diets during 22 to 42 d. Basal diets were supplemented with the yeast autolysate (InteWall, *S. cerevisiae*, NCYC R 625, Integro Food and Feed Manufacturing Company, İstanbul, Turkey) at the level of 0, 1, 2, 3, and 4 g/kg. The ingredients and chemical composition of the basal diets are presented in Table 1.

Performance Analysis

Moisture, crude ash, CF, EE, and CP contents of basal diets were determined according to AOAC International [11]. The samples were ashed in a muffle furnace before the analysis of calcium [12] and total phosphorus [13]. Other minerals were determined with an Agilent 7500ce ICP-MS system (Yokogawa Analytical Systems, Yamanashi-Ken, Japan). Metabolizable energy levels of samples were estimated using the Carpenter and Clegg's equation [14].

Chicks were weighed individually at the beginning of the experimental period and weekly for calculating live weight gains. The birds were observed daily for evaluating mortality. Feed consumption was recorded weekly and expressed as grams per bird per week, and the FCR was calculated as grams of feed per gram of live weight gain.

Broilers in each replicate were put on a cleaned plastic sheet in a separate pen during 5 to 10 min to collect excreta. Then excreta samples of each replicate pen were collected and mixed. One gram of samples was diluted 1:3 (wt/wt) with deionized water in a beaker and vortex-mixed for 2 min, and then the pH values of the samples were measured with a Selecta pH meter (pH-2004, J.P. Selecta, Barcelona, Spain) [15]. The remaining amount of samples

Table 1. Ingredients and chemical composition of the basal diets (as-fed basis)

Item	Starter period, 1–21 d	Grower period, 22–42 d
Ingredients (g/kg)		
Corn	441	511
Wheat	30	30
Soybean meal	245	205
Full-fat soya	175	130
Meat and bone meal	40	40
Soybean oil	35	50
Limestone	15	15
Dicalcium phosphate	12	12
Salt	2.5	2.5
DL-Methionine	2.0	2.0
Vitamin mineral premix[1]	2.5	2.5
Chemical composition (analyzed)		
ME[2] (MJ/kg)	13.23	13.63
CP (g/kg)	229.0	202.0
Calcium (g/kg)	14.2	14.0
Total phosphorus (g/kg)	8.3	7.9

[1]Supplied the following per kilogram of diet: 12,000 IU of vitamin A, 2,400 IU of vitamin D_3, 30 mg of vitamin E, 2.5 mg of vitamin K_3, 2.5 mg of vitamin B_1, 6 mg of vitamin B_2, 4 mg of vitamin B_6, 20 µg of vitamin B_{12}, 25 mg of niacin, 8 mg of calcium-D-pantothenate, 1 mg of folic acid, 50 mg of vitamin C, 50 µg of D-biotin, 80 mg of Mn, 60 mg of Zn, 60 mg of Fe, 5 mg of Cu, 1 mg of I, 0.5 mg of Co, 0.15 mg of Se.

[2]Metabolizable energy content of diets was estimated using the equation of Carpenter and Clegg [14].

was dried in an air-forced oven at 60°C until reaching constant weight, and then the moisture of samples was determined according to AOAC International [11].

At the end of the experiment (on d 42) 20 broilers from each group (4 from each replicate) were weighed and slaughtered by exsanguination. Their gastrointestinal tracts were excised. Hot carcasses were weighed to determine the carcass yield. Absolute and proportional weights of abdominal fat, liver, heart, gizzard, spleen, and bursa of Fabricius were determined. Empty weight of duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to the cecal junction), and cecum parts were recorded. Weight of intestinal parts to slaughtering weight was calculated. Jejunal and ileal digesta contents were pooled and homogenized. The pH of jejunum and ileum contents was measured immediately by a pH meter (Selecta pH meter, pH-2004, J.P. Selecta). One gram of digesta and 9 mL of 0.9% sterile saline solution were mixed in a sterile tube. For each sample, 10-fold serial dilutions were made. Total aerobic bacteria and *Escherichia coli* were enumerated using the pour plate technique on Nutrient Agar (Merck 1.05450, Merck KgaA, Darmstadt, Germany) and on MacConkey Agar (Merck 1.05465), respectively. Plates were incubated at 37°C for 24 to 48 h. Colonies were enumerated and recorded. Results are presented as $\log_{10}$ colony forming units per gram of digesta [16, 17].

At d 31, 20 broilers from each diet group (4 from each replicate) were randomly selected from each pen and injected with 0.1 mL of 0.25% suspension of sheep erythrocytes (SRBC) in phosphate buffer saline. Circulating anti-SRBC antibody titers were determined by the microhemagglutination technique from samples taken at 5 d after the immunization. All titers were expressed as the $\log_2$ of the reciprocal of the serum dilution [18].

At d 41, 10 broilers from each group (2 from each replicate) were randomly selected and bled from the brachial vein. Blood samples were taken in the tubes containing no anticoagulant and centrifuged at 3.220 × *g* for 8 min at 4°C. Serum was collected and stored at −20°C for determination of total protein, uric acid, triglyceride, cholesterol, and levels of aspartate amino transferase and alanine amino transferase by a Vitros 350 autoanalyzer (New York, NY; product code 680–2153) using their accompanying commercial kits (Vitros Chemistry Products, Ortho-Clinical Diagnostics, Johnson-Johnson Company, New York, NY).

Statistical Analysis

The replicate pens were the experimental unit for performance and excreta data. Statistical analyses were done using the SPSS program (SPSS Inc., Chicago, IL). The normality of data distribution was checked using the Kolmogorov-Smirnov test. One-way ANOVA was performed to examine differences among groups. The significance of mean differences between groups was tested by Duncan [19]. Values were given

Table 2. The effects of dietary supplementation of yeast autolysate on performance, abdominal fat, and anti-SRBC titer in broilers[1]

	Yeast autolysate (g/kg)					
Parameter	0	1	2	3	4	*P*-value
Final live weight (g)	2,555 ± 81	2,655 ± 41	2,687 ± 32	2,765 ± 60	2,601 ± 50	0.116
Live weight gain (g)						
1–21 d	626 ± 15^{b}	712 ± 21^{a}	713 ± 15^{a}	730 ± 12^{a}	682 ± 26^{a}	0.004
1–42 d	2,508 ± 61	2,609 ± 41	2,641 ± 43	2,719 ± 60	2,554 ± 50	0.114
Feed intake (g)						
1–21 d	1,036 ± 14	1,032 ± 14	1,025 ± 15	1,022 ± 11	1,012 ± 14	0.688
1–42 d	4,378 ± 35	4,353 ± 29	4,326 ± 26	4,272 ± 42	4,271 ± 25	0.099
FCR (g/g)						
1–21 d	1.66 ± 0.01^{a}	1.46 ± 0.06^{b}	1.44 ± 0.03^{b}	1.40 ± 0.02^{b}	1.49 ± 0.05^{b}	<0.001
1–42 d	1.75 ± 0.05^{a}	1.67 ± 0.02^{ab}	1.64 ± 0.02^{b}	1.57 ± 0.03^{b}	1.67 ± 0.03^{ab}	0.025
Abdominal fat (%)	1.83 ± 0.10^{a}	1.59 ± 0.08^{b}	1.53 ± 0.07^{bc}	1.45 ± 0.08^{bc}	1.29 ± 0.09^{c}	<0.001
Anti-SRBC titer (log_2)	7.10 ± 0.28^{c}	7.90 ± 0.19^{b}	8.85 ± 0.11^{a}	8.75 ± 0.18^{a}	8.65 ± 0.17^{a}	<0.001

$^{a–c}$Means in a row with different superscripts are significantly different ($P \leq 0.05$).

[1]The data are presented as mean ± standard error. n = 5.

as mean ± standard error. Level of significance was taken as $P < 0.05$.

RESULTS AND DISCUSSION

The effects of dietary yeast autolysate on performance, abdominal fat, and anti-SRBC titer in broilers are given in Table 2. Dietary treatments did not significantly affect final live weight, total weight gain, and feed intake. Weight gains during the starter period (1–21 d) of broilers fed the diets containing 1, 2, 3, and 4 g/kg of yeast autolysate were significantly higher than those of the control group ($P < 0.01$). Feed conversion during the starter period was improved by yeast autolysate supplementation at the levels of 1, 2, 3, and 4 g/kg ($P < 0.001$). Cumulative feed conversion was improved ($P < 0.05$) by yeast autolysate supplementation at the levels of 2 and 3 g/kg. This improvement could be due to the yeast reducing the pathogenic bacterial load in the intestine as reported by Haldar et al. [5]. Zhang et al. [20] reported that the live weight gains by broilers fed whole yeast and cell walls were greater than those of the control broilers from 4 to 5 wk of age and from 0 to 5 wk of age. Haldar et al. [5] showed higher live weight gain during 1 to 21 d and 22 to 35 d and improved feed efficiency when the yeast and the yeast-protein-concentrate additives were supplemented to the broiler diets compared with the control group. The best results in performance of broilers fed yeast cell wall–supplemented diets might be due to the improvement of the intestinal lumen health, thereby increasing the absorption and utilization of the dietary nutrients [21, 22].

Live weight gain [1, 23], feed intake [4, 5, 20, 23], and feed conversion [1] were not affected by using yeast and yeast products in some studies. The differences in animal response may be related to differences in yeast products such as active dried yeast, live yeast culture, yeast cell wall, mannan oligosaccharide (**MOS**), β-glucan, fermented yeast culture, or yeast autolysate. No mortality was observed in the groups during the whole experimental period. Similarly some researchers [4, 5, 23, 24] reported that dietary supplementation of yeast or yeast products had no effect on mortality.

Microflora in the gut can be changed by dietary supplementation, and thus diet has an effect on excreta. Dietary yeast autolysate supplementation did not significantly affect excreta pH and excreta moisture (data not shown). Similarly Juskiewicz et al. [25] reported that excreta pH was not affected by dietary MOS in turkey.

No significant differences in the carcass yield and the relative weight of gizzard, liver, heart, spleen, and bursa of Fabricius were observed among groups (data not shown). By contrast, the relative abdominal fat weight was significantly lower ($P < 0.001$) in birds fed with diets containing yeast autolysate than in birds fed with the control diet (Table 2). This result shows a change in energy partitioning. It might be that the extra energy that was not being stored by the

birds fed yeast autolysate–supplemented diets was being used to upregulate the immune system and increase the titer response to SRBC. Intestinal weight proportions were not influenced by the addition of yeast autolysate to the diet (data not shown). In agreement with previous reports, different yeast products had no significant effect on gizzard weight [1], relative spleen weight [23], and relative weight of bursa of Fabricius [23, 26]. Corduk et al. [27] reported that MOS (BioMos) supplementation did not significantly affect carcass yield and the relative weights of abdominal fat and gizzard.

The effects of yeast autolysate on pH, total aerobic bacteria count, and *E. coli* count in jejunal and ileal digesta of broilers are presented in Table 3. The naturally established protective microflora is stable, but it can be influenced by dietary, disease, and environmental factors. Dietary factors such as composition, feed additives, processing, and digestibility may all disturb the balance in the gut ecosystem, especially in broilers [28]. In the present study pH values of jejunal and ileal digesta were decreased by yeast autolysate supplementation at the levels of 2, 3, and 4 g/kg. Low pH of the digesta given yeast autolysate could improve utilization of the diets as reported in the study of Afsharmanesh et al. [29]. In contrast to our findings, some researchers observed that MOS supplementation did not affect [30] or increased [31] the ileal pH. *Escherichia coli* count was decreased and total aerobic bacteria count was not affected by the yeast autolysate supplementation in jejunal and ileal digesta. Similarly, Yang et al. [31] showed that ileal populations of coliforms in broilers were reduced with dietary mannooligosaccharides supplementation. In vitro studies showed that MOS is very effective in binding *E. coli* [32]. Haldar et al. [5] and Ghosh et al. [24] reported that supplementation of yeast and yeast products reduced *E. coli* numbers in the digesta as compared with the control. Mourao et al. [33] reported that the total bacteria count reduction in ileal and cecal contents showed that MOS also influence other intestinal microflora. The dietary addition of MOS resulted in significantly higher total aerobic bacteria counts in quails [34]. However, some researchers reported that MOS supplementation had no significant difference on total aerobic bacteria count [27, 35] and *E. coli* count [35, 36] in the intestinal digesta in quails and broilers.

Measuring blood serum parameters could be more useful to provide important information about the diagnosis of diseases and dysfunctions. The mean values of serum triglycerides, cholesterol, protein, uric acid, and the activities of aspartate amino transferase and alanine amino transferase were not affected by dietary yeast autolysate supplementation (data not shown), and it can be emphasized that yeast autolysate caused no adverse effects on broilers. Yalçın et al. [8] reported that yeast autolysate supplementation in laying hens had no effect on serum total protein and uric acid but reduced the levels of serum cholesterol and triglyceride significantly. It was also reported that yeast culture supple-

Table 3. Effects of dietary supplementation of yeast autolysate on pH and *Escherichia coli* count (log_{10} cfu) in intestine in broilers[1]

	Yeast autolysate (g/kg)					
Parameter	0	1	2	3	4	*P*-value
pH						
Jejunum	6.35 ± 0.09^{a}	6.23 ± 0.08^{ab}	6.00 ± 0.09^{bc}	5.87 ± 0.08^{c}	5.85 ± 0.08^{c}	<0.001
Ileum	7.38 ± 0.10^{a}	7.21 ± 0.07^{ab}	7.02 ± 0.09^{bc}	6.86 ± 0.08^{c}	6.86 ± 0.07^{c}	<0.001
Total aerobic bacteria (log_{10} cfu)						
Jejunum	5.95 ± 0.03	5.92 ± 0.02	5.92 ± 0.03	5.93 ± 0.02	5.98 ± 0.02	0.243
Ileum	5.99 ± 0.03	5.99 ± 0.02	5.97 ± 0.03	5.97 ± 0.01	6.02 ± 0.02	0.374
Escherichia coli (log_{10} cfu)						
Jejunum	4.57 ± 0.05^{a}	4.28 ± 0.08^{b}	4.21 ± 0.09^{b}	4.17 ± 0.09^{b}	4.15 ± 0.09^{b}	0.004
Ileum	4.73 ± 0.04^{a}	4.59 ± 0.04^{b}	4.57 ± 0.04^{b}	4.53 ± 0.04^{b}	4.52 ± 0.04^{b}	0.002

$^{a–c}$Means in a row with different superscripts are significantly different ($P \leq 0.05$).

[1]The data are presented as mean ± standard error. n = 5.

mentation had no effect on the levels of serum cholesterol, triglyceride, total protein, and aspartate amino transferase but increased serum uric acid in laying hens [9]. Similar to the present study, Saoud and Daghir [37] observed that single-cell protein had no effect on serum uric acid in broilers.

Antibody responses have been used as measures of the humoral immune status of poultry [38]. The higher antibody titer in broilers supplemented with yeast autolysate in this present study (Table 2) might be due to the combined effect of the nucleotides and the glucans and the mannans present in the yeast autolysate on the immune system [4, 5, 7]. Oligosaccharides in prebiotics increase the protective antibody response and improve resistance to diseases. By direct action, it can be assumed that prebiotics would bind to macrophage reception sites by recognizing specific sugars found in glucoproteins of the epithelial surface, triggering a cascading reaction that would eventually activate macrophages and release cytokines, thereby activating the acquired immune response [39, 40]. In agreement with the present study, greater antibody production against SRBC in laying hens fed 2, 3, and 4 g/kg of yeast autolysate [8] and in laying hens fed 1 g/kg of yeast [6] in the diet were observed compared with the control group. Other researchers also observed higher antibody responses in broilers fed diets supplemented yeast and yeast protein concentrate [5] and in broiler breeders fed MOS [7].

CONCLUSIONS AND APPLICATIONS

1. Yeast autolysate improved growth performance, particularly during the starter period (1 to 21 d). Dietary yeast autolysate supplementation at the levels of 2 and 3 g/kg resulted in more consistent responses, possibly indicating a dose-dependent relationship.
2. Possible mechanisms for the increased growth response could be decrease in intestinal pH and reduced pathogenic load or increased immunocompetence (increased SRBC titer).
3. Yeast autolysate was effective in reducing *E. coli* colonization in the intestine of the broilers.
4. Further studies are needed to investigate the effect of dietary yeast autolysate under stressed conditions such as health, environmental, or nutritional challenges and to establish the optimal application of yeast autolysate including its optimal dosage level in the feed to obtain maximum effects.

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Acknowledgments

The authors thank Beypiliç for chicken material.