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Effect of Yeast Culture (*Saccharomyces cerevisiae*) Supplementation on Growth Performance, Excreta Microbes, Noxious Gas, Nutrient Utilization, and Meat Quality of Broiler Chicken

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The study was conducted to test the effects of using yeast culture (*Saccharomyces cerevisiae*) as feed additive on the growth performance, noxious gas emission, utilization of nutrients, excreta microbial count, and meat quality of broilers. In total, 360 one-day-old Ross 308 broilers with average body weight (BW) of 42.90±1.43 g were randomly selected and allotted to two groups; they were fed either a basal diet (control) or a basal diet supplemented with 1% yeast culture (YC). Each treatment group had 10 replication pens and each replication contained 18 birds. The experiment was divided into 3 phases (1 to 7, 8 to 21, and 22 to 35 days) for growth performance observation. In the 1st phase (1 to 7 days), only the body weight gain (BWG) significantly increased ($P<0.05$) in birds with the YC diet compared to the control diet. Significant effects on BWG ($P<0.05$) and feed conversion ratio (FCR) ($P<0.05$) were seen in birds receiving the YC-supplemented diet in the 3rd phase (22 to 35 days) as compared to the control diet. In addition, during the overall period (1−35 d), BWG was significantly higher ($P<0.05$) and FCR was reduced ($P<0.05$). Throughout this experiment, the meat quality, nutrient utilization, noxious gas emission, and bacterial count in the excreta did not vary significantly between the groups. This study proved that a higher dose of YC (*Saccharomyces cerevisiae*) supplementation could maintain the consistent positive effect on broiler growth but eliminated the speculated outcomes on digestibility, bacterial count, or excreta gas emission.

Key words: broiler, excreta microbes, growth performance, *Saccharomyces cerevisiae*

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Introduction

There have been several improvements in the strategies of livestock rearing. Antibiotics and probiotics have shown a great impact on animal growth. However, in the recent decade, the usage of antibiotics has increased the consumer's concerns about bacterial resistance, antibiotic residues in food, and adverse effects on human health. The use of antibiotics as growth promoters is prohibited in European countries (Eckert *et al*., 2010). In the USA, the application of antibiotics or antimicrobials to enhance livestock or poultry growth is also prohibited (FDA, 2015). As a result, probiotics, and prebiotics have garnered attention as substitutes for antibiotic growth promoters. They have reduced customer concerns about bacterial resistance and other safety issues. *Saccharomyces cerevisiae* (yeast) products have proved efficient in feed utilization, pathogen reduction (Haldar *et al*., 2011), and reducing negative environmental effects (Cheng *et al*., 2014). yeast is used in animal feed in the form of live yeast, dried form, fermented products, and yeast cell wall components. In broilers, yeast supplements have influenced performance, pathogen reduction, modification of microflora, immunomodulation, intestinal changes, and meat quality (Islam *et al*., 2004; Khaksefidi and Ghoorchi, 2006). yeast contains α-D-mannan, chitin, β-D-glucan with calcium, magnesium, and zinc. It also contains digestible proteins, Vit. B6, thiamin, biotin, riboflavin, nicotinic acid, and pantothenic acid (Elghandour *et al*., 2020). Yeast supplementation improves the immune system, inhibits toxins, supports nutrient utilization with microflora, reduces pathogenic microorganisms, lowers the cholesterol level, and increases the number of anaerobic bacteria that reduce noxious gases (Elghandour *et al*., 2020). Different forms and combinations of yeast supplementation display different and opposing results. Positive effects were observed on

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animal growth, digestion, and animal health by some researches (Medina *et al*., 2002; Van der Peet-Schwering *et al*., 2007; Kowalik *et al*., 2012), whereas Kornegay *et al*. (1995) and Elnager (2013) reported no influence.

The inconsistencies in the findings of different studies related to yeast indicate that further research is required to elucidate the effect of yeast culture (YC) on the performance and production of animals. Thus, the objective of the current study was to evaluate the effect of YC on growth performance, microbial count in excreta, noxious gas emission, nutrient utilization, and meat quality of broilers.

Materials and Methods

All animals were raised, treated, and experimental processes were performed following the guidelines of the Animal Care Committee, Dankook University, Korea (Approval number DK-1-1901).

Animals and Diets

In total, 360 one-day-old Ross 308 broiler chickens of average body weight of 42.90±1.43 g were used in a 35-day experiment. For growth performance observations, the experiment was divided into three phases: phase 1 (days 1−7), phase 2 (days 8−21), and phase 3 (days 22−35). Broilers were randomly assigned to two treatment groups that consisted of one group fed the basal diet (CON) and the other group fed the basal diet supplemented with 1.0% yeast culture (TRT). Each group consisted of 10 replications (pen) and 18 birds were allotted in each pen. All feed was formulated to meet or exceed the National Research Council (NRC, 1994) recommendation of the broiler chicken nutritional requirement; mash form (Table 1) was used to feed the chicken. This experiment was conducted on a broiler farm under the Department of Animal Resource Science of Dankook University. The feed additive product (yeast culture) evaluated in this trial was a commercial product (XPC, Diamond V Original XPC^{TM} Yeast culture, Cedar Rapids, IA, USA). The room in which broilers were housed was cleaned weekly and routinely disinfected. The temperature in the room was controlled at 33±1℃ for the initial 3 days and then gradually decreased by 3℃ per week till it reached 24℃; this was maintained during the rest of the experiment. The humidity was maintained at around 60%. Fluorescent light was set to provide 24 hours/day of artificial light. Free access to feed and water was ensured. Each pen was equipped with two feeders and two nipple drinkers.

Table 1. **Ingredient composition of experimental diets as fed basis**

Ingredient, %	Starter	Grower	Finisher
Corn	54.19	55.38	56.77
Soybean meal	33.80	26.1	18.23
Canola meal	5.00	10.0	15.0
Soybean oil	2.10	3.62	5.07
MDCP[1]	—	1.28	1.12
DCP[2]	1.70	—	—
Limestone	1.15	1.34	1.22
L-lysine	0.50	0.65	0.81
DL-Methionine	0.46	0.47	0.52
L-Threonine	0.20	0.25	0.32
L-Tryptophan	—	0.01	0.04
$NaHCO_3$	0.10	0.10	0.10
Salt	0.30	0.30	0.30
Vitamin premix[3]	0.20	0.20	0.20
Mineral premix[4]	0.20	0.20	0.20
Choline	0.10	0.10	0.10
ME, kcal/kg	3,000	3,100	3,200
CP, %	23.0	21.5	20.0
Lys, %	1.50	1.40	1.30
Met + Cys, %	1.08	0.99	0.94
AP, %	0.48	0.44	0.41
Ca, %	0.96	0.87	0.81

[1] Monodicalcium phosphate
[2] Dicalcium phosphate
[3] Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D_3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg D-pantothenic acid; 166 mg choline; 33 μg vitamin B_{12}
[4] Provided per kg of complete diet: 12 mg Cu (as $CuSO_4 \cdot 5H_2O$); 85 mg Zn (as $ZnSO_4$); 8 mg Mn (as MnO_2); 0.28 mg I (as KI); 0.15 mg Se as $Na_2SeO_3 \cdot 5H_2O$

Sampling and Measurement

Growth Performance and Nutrient Utilization

Each group consisted of 10 pens that were the experimental units for the growth performance experiment. Body weight and feed intake were recorded on the 0th, 7th, 21st, and 35th day to calculate the body weight gain (BWG) and the feed intake (FI). The feed conversion ratio (FCR) was calculated by dividing FI with BWG. The nutrient utilization assessment was performed in terms of dry matter utilization (DM), nitrogen utilization (N), and energy utilization (ME) by adding chromium oxide (Cr_2O_3), an indigestible marker, to the diets seven days before excreta sample collection. Fresh excreta samples were collected from each pen on the 33rd, 34th, and 35th day. Collected samples were stored at −20℃ until chemical analysis was performed. Before conducting chemical analysis, the excreta samples were thawed and dried at 70℃ for 72 h. They were then finely ground to pass through a 1-mm screen. All feed and excreta samples were analyzed following procedures of the Association of Official Analytical Chemists (2000). They were analyzed for dry matter (DM) using method 934.01 (AOAC, 2000). Nitrogen (N) was determined by the machine (Kjeltec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes Sweden) according to method 968.0 (AOAC, 2000) and crude protein (CP) was calculated as N×6.25. For energy measurement, an oxygen bomb calorimeter (Parr 6100 Instrument Co., Moline, IL, USA) was used. Chromic oxide concentration in the feed and excreta samples was determined by ashing and digestion of ground samples with phosphoric acid-manganese sulfate and potassium bromate (Williams *et al*., 1962). The washed digest was kept overnight in a calcium chloride solution and then filtered. The detection was performed by UV absorption spectrometry (Shimadzu UV-1201; Shimadzu, Kyoto, Japan). The gross total tract utilization of nutrients was estimated using the following formula: Nutrient utili-

zation (%)＝{1－[(Nf×Cd)/(Nd×Cf)]}×100, where Nf＝nutrient concentration in feces (% DM), Cd＝chromium concentration in the diet (% DM), Nd＝nutrient concentration in the diet (% DM), and Cf＝chromium concentration in feces (% DM).

Fecal Microbial Analysis and Excreta Gas Emission

At the end of the experiment, excreta samples from each pen were collected and mixed. The samples were stored in 2.6-L plastic boxes in pairs. Each box had a center hole on one side wall that was closed with adhesive tape. The samples were left at 25℃ for 5 days for fermentation. Then, a GV-100 gas sampling pump (Gastec Corp., Kanagawa, Japan) was used to measure ammonia (NH_3), hydrogen sulfide (H_2S), acetaldehyde, CO_2, acetic acid, and propionic acid within the range of 5.0 to 100.0 ppm (No. 3La, detector tube; Gastec Corp.) and 2.0 to 20.0 ppm (4LK, detector tube; Gastec Corp.). For measurement, the seal was penetrated. For each box, 100 mL of headspace air was sampled from around 2 cm above the excreta sample. After sampling the air, each box was re-sealed with an adhesive tape. Headspace measurements were repeated after 58 h. Average data were recorded from two measurements. On day 35, composite excreta samples were collected from each pen and placed on ice. The samples were then taken to the laboratory to perform immediate analysis. From each sample, one gram of excreta was mixed with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and homogenized. Counting of viable bacteria in the excreta samples was performed by plating 10-fold serial dilutions (in 1% peptone broth solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI), Lactobacilli medium agar plates (Medium 638; DSMZ, Braunschweig, Germany), and Salmonella Shigella (SS) agar plates (Becton, Dickinson and Co.) to isolate *E. coli*, *Lactobacillus,* and *Salmonella,* respectively. The plates were incubated for 48 h at 39℃ under an anaerobic condition for the *Lactobacillus* bacterial count. The MacConkey agar plates and Salmonella Shigella (SS) agar plates were incubated at 37℃ for 24 h. *E. coli*, *Lactobacillus,* and *Salmonella* colonies were counted soon after removal from the incubator by the procedure of Lee (2014).

Meat Quality Analysis

At the end of the experiment, one chicken per pen (10 chickens per treatment) was weighed and slaughtered. Breast meat, abdominal fat, gizzard, liver, spleen, and bursa of Fabricius were removed by experienced personnel. All samples were pat-dried to eliminate excess moisture and then weighed. After 24 h of slaughter, the muscle color parameters, lightness (L*), redness (a*), and yellowness (b*) were recorded in triplicates using a CR-410 Chroma Meter (Konica Minolta Sensing Inc., Osaka, Japan) as described by Chen *et al*. (2009); the average value of each of these scores was recorded. The drip loss percentage was determined on days 1, 3, 5, and 7 by following the procedure described by Honikel (1998). The pH value of each sample was observed twice via the insertion of a glass electrode pH meter (Testo 205, Testo, Germany). For determination of the water-holding capacity (WHC), a 0.3-g sample was pressed at 3,000 psi for 3 min on a 125-mm-diameter filter paper. The area of the original sample and the expressed moisture area after pressing were outlined and determined by a digitizing area-line sensor (MT-10S, M.T. Precision Co. Ltd., Tokyo, Japan). The ratios of water area and meat area were calculated to give a measure of WHC, with a smaller ratio indicating higher WHC. Meat samples were cooked at 80℃ in a water bath to bring the core temperature of the fillet to 72℃. After cooking, the samples were weighed again and the cooking-loss percentage was calculated (Albrecht *et al*., 2019)

Statistical Analysis

The data were analyzed for two-sample t-test using the SAS software (SAS Institute, Inc, Cary, NC, USA) and significant differences were determined by $P<0.05$ level of significance.

Results

Growth Performance

The effects of YC on the growth performance of broilers are shown in Table 2. The body weight gain (BWG) during the 1st phase (days 1−7) was significantly higher ($P=0.013$) in the treatment group. In the 3rd phase, the BWG ($P=0.027$) and FCR ($P=0.026$) showed positive responses in the treatment group. In the end, the overall result indicated an improvement in the final body weight gain ($P=0.030$) and the final feed conversion ratio ($P=0.009$). Other factors were not affected by the treatment.

Nutrient Utilization

The effects of YC supplementation on nutrient utilization are presented in Table 3. There were no differences in the nutrient utilization between the control and treatment groups.

Microbial Study

The effects of YC supplementation are presented in Table 4. There was no effect of YC supplementation on the *Lacto-*

Table 2. **The effect of yeast culture supplementation on growth performance in broilers**

Items	CON	TRT	SEM	*P*-value
d 1 to 7				
BWG, g	133[b]	141[a]	2	0.013
FI, g	172	179	3	0.055
FCR	1.299	1.276	0.040	0.520
d 8 to 21				
BWG, g	589	597	11	0.496
FI, g	853	856	14	0.832
FCR	1.449	1.436	0.033	0.686
D 22 to 35				
BWG, g	1663[b]	1710[a]	13	0.027
FI, g	2810	2835	24	0.542
FCR	1.690[a]	1.658[b]	0.020	0.026

Abbreviation: CON, Basal diet; TRT, CON + 1% Diamond Co. yeast culture; SEM, Standard error of means; BWG, Body weight gain; FI, Feed intake; FCR, Feed conversion ratio; [a, b] Means in the same row with different superscripts differ ($P<0.05$). Values represent the means of 10 replication pens with 18 birds per pen.

Table 3. **The effect of yeast culture supplementation on nutrient utilization in broilers**

Items, %	CON	TRT	SEM	*P*-value
Finish				
Dry matter	72.55	74.20	0.91	0.492
Nitrogen	68.55	70.41	0.24	0.245
Energy	73.13	74.54	0.17	0.173

Abbreviation: CON, Basal diet; TRT, CON + 1% Diamond Co. yeast culture; SEM, Standard error of means; [a, b] Means in the same row with different superscripts differ ($P<0.05$). Values represent the means of 10 replication pens with 18 birds per pen.

Table 4. **The effect of yeast culture supplementation on the microbial count in broilers**

Items, lig_{10} cfu/g	CON	TRT	SEM	*P*-value
Lactobacillus	7.10	7.18	0.38	0.387
E. coli	6.47	6.44	0.68	0.680
Salmonella	2.98	2.97	0.92	0.924

Abbreviation: CON, Basal diet; TRT, CON + 1% Diamond Co. yeast culture; SEM, Standard error of means; [a, b] Means in the same row with different superscripts differ ($P<0.05$). Values represent the means of 10 replication pens with 18 birds per pen.

Table 5. **The effect of yeast culture supplementation on gas emission in broilers**

Items, ppm	CON	TRT	SEM	*P*-value
Finish				
NH_3	11.56	11.54	0.61	0.971
H_2S	3.90	4.24	0.75	0.663
Acetaldehyde	2.52	2.58	0.62	0.920
CO_2	1960	1940	158.7	0.902
Acetic acid	1.06	0.92	0.31	0.664
Propionic acid	2.96	3.74	1.13	0.511

Abbreviation: CON, Basal diet; TRT, CON + 1% Diamond Co. yeast culture; SEM, Standard error of means; [a, b] Means in the same row with different superscripts differ ($P<0.05$). Values represent the means of 10 replication pens with 18 birds per pen.

bacillus, E. coli, or *Salmonella* count in the fecal samples of the broilers.

Noxious Gas

The results of YC supplementation on noxious gas analysis are shown in Table 5. No significant difference was found in the noxious gas emission between the control and treatment groups for hydrogen sulfide, ammonia, acetaldehyde, acetic acid, propionic acid, and carbon dioxide.

Meat Quality

The results of the YC supplementation on meat quality are shown in Table 6. The values for the pH, color parameters, relative organ weight, water holding capacity, and drip loss were not different between the two groups

Table 6. **The effect of yeast culture supplementation on meat quality in broilers**

Items	CON	TRT	SEM	*P*-value
pH value	7.47	7.60	0.07	0.07
Breast muscle color				
Lightness (L^*)	55.64	56.19	0.69	0.43
Redness (a^*)	12.47	12.56	0.58	0.86
Yellowness (b^*)	12.94	13.15	0.71	0.77
WHC, %	43.41	44.76	2.68	0.62
Cooking loss	18.69	18.69	0.42	1.00
Drip loss, %				
d 1	4.40	4.32	0.16	0.59
d 3	7.45	7.46	0.10	0.95
d 5	9.95	9.85	0.24	0.70
d 7	12.10	12.04	0.26	0.79
Relative organ weight, %				
Breast muscle	18.63	18.56	0.62	0.89
Liver	2.85	2.86	0.17	0.98
Bursa of fabricius	0.13	0.12	0.01	0.66
Abdominal fat	1.15	1.16	0.10	0.90
Spleen	0.14	0.13	0.10	0.90
Gizzard	1.01	1.02	0.03	0.73

Abbreviation: CON, Basal diet; TRT, CON + 1% Diamond Co. yeast culture; SEM, Standard error of means; [a, b] Means in the same row with different superscripts differ ($P<0.05$). Values represent the means of 10 replication pens with 18 birds per pen.

Discussion

Yeast and YC were recognized as animal feed components in 1980. During that time, the research was centered on their effects on ruminant animals alone; the other species were neglected. Research on SC or YC in mono-gastric animals and poultry began around the year 2000 (Auclair, 2001). Currently, some conflict exists regarding the more beneficial form of yeast for animal feeding. YC is quite different from live yeast or yeast extracts. In most cases, yeast products show beneficial results although their working mechanisms are still unclear. Different doses of a similar YC were implemented by Gao *et al*. (2008) at 0.25%, 0.5%, 0.75%; Al-Mansour *et al*. (2011) used 0.1%, 0.12%, 0.15%, and Özsoy and Yalçin (2011) used 0.1%, 0.2%, 0.3% of YC in broilers. However, they presented inconsistent results about the effectiveness of different doses. The current experiment was conducted to check the consistency of a higher dose and mechanism of YC.

Our study revealed that yeast had a significant impact on BWG and FCR in the later stage of production. Zhang *et al*. (2005); Gao *et al*. (2008); Paryad and Mahmoudi (2008); Koc *et al*. (2010), Özsoy and Yalçin (2011); and Sun and Kim (2019) found positive effects of different yeast products (SC) on the BWG and FCR of the broilers. In contrast, Brummer *et al*. (2010) studied yeast cell wall extracts for only fifteen days and Adebiyi *et al*. (2012) used a lower concentration of yeast for the broilers. Both experiments indicated no significant difference in the BWG and FCR. The feed intake remained unaffected in the current study;

this observation was in agreement with the findings of Gao *et al*. (2008); Ahiwe *et al*. (2019), and Sun and Kim (2019). We observed a significantly different feed conversion ratio. Therefore, it is common to find no effect on feed intake (FI).

Here, nutrient utilization was not affected by YC supplementation; this observation is supported by findings of Gao *et al*. (2008). Sun and Kim (2019) used mixed yeast (*Saccharomyces cerevisiae and Kluyveromyces marxianus*) cultures in broilers and found significant differences in dry matter digestibility and insignificant differences in nitrogen digestibility. Chen *et al*. (2009) also found gross energy digestibility significant, whereas, protein and dry matter digestibility were insignificant. Possibly, we can say that yeast alone is not responsible for nutrient utilization performance. Different forms and combinations with other additives may be responsible for the positive changes.

Compared to processed yeast, live yeast affects the gut microorganisms (Zhu *et al*., 2017). Therefore, our dried YC could not change the digestive tract bacterial population. Moreover, microbiological findings were not constant with yeast. Yan *et al*. (2011) mentioned that a higher nutrient digestibility would cause less noxious gas emission. As our experiment showed no variation in nutrient utilization, it was reasonable that there was no change in noxious gas emission as well. Similarly, Sun and Kim (2019) also found no significant change in noxious gas emission in the broilers.

Meat quality parameters were also not affected by the addition of YC; this result agreed with the findings of Sharif *et al*. (2018). Sun and Kim (2019) also did not find any difference in the meat quality parameters except in the bursa of Fabricius, which might be for immune response.

Previous literature (Auclair, 2001; Santin *et al*., 2001; Gao *et al*., 2008) suggested some possible roles of yeast in animal growth performance. They include increasing the nutrient utilization and villus height, bacterial modulation (increasing *Lactobacillus*, decreasing *E. coli*), anti-toxic and anti-inflammatory properties, and in immune response as well as the provision of metabolites as nutrients. However, in our study, nutrient utilization or villus height may not be the reason for better growth performance. We did not find any difference in nutrient utilization. Although we did not measure the villus height, an increment in the villus height would have increased the nutrient absorption and utilization and this was not observed. Therefore, villus height is also not responsible for better growth performance. Again, bacterial modulation was not supported by our results. It is possibly expected only in live yeast supplementation. Here, a simple explanation could be that the YC is the combination of dried yeast and culture media. Yeast fermentation occurs and it contains fermentation metabolites, peptides, organic acids, oligosaccharides, amino acids, and unknown growth factors that are beneficial to animal growth (Eltazi *et al*., 2014). YC did not work like a probiotic or antibiotic; it just made more nutrients available to the animal like a feed element. The possibility of anti-inflammatory and increased immune responses is not negligible. Song and Di Luzio (1979) mentioned glucan, a cell wall component of yeast cells, as an immune amplifier. It increases the anti-inflammatory function under stress conditions and stimulates the phagocytic function of the reticuloendothelial system. Auclair (2001) suggested the protective effects of yeast by being anti-toxic and reducing toxin amounts produced by pathogens. It does not reduce the pathogen population but it prevents the pathogen-produced toxins from binding to the epithelial cells.

Overall, YC at a 1% level of supplementation proved its beneficial effect on the broiler growth performance. Unexpectedly, the supplementation of 1% YC in the present study, which is comparatively higher than the doses used in the previous studies, did not show any beneficial effect on nutrient utilization, bacterial modulation, or excreta gas emission. To find a feed additive that influences all these parameters, yeast can be studied in different forms and combinations with other yeast and probiotic components.

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Conflicts of Interest

The authors declare no conflict of interest.

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