

Dietary Supplementation of Mixed Yeast Culture Derived from *Saccharomyces cerevisiae* and *Kluyveromyces maxianus*: Effects on Growth Performance, Nutrient Digestibility, Meat Quality, Blood Parameters, and Gut Health in Broilers

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This study aimed to evaluate the effect of dietary supplementation of a mixed yeast culture (MYC; *Saccharomyces cerevisiae* YJM1592 and *Kluyveromyces maxianus* TB7258 in a 1:1 ratio) on growth performance, nutrient digestibility, meat quality, blood parameters, and gut health of broiler chickens. In total, 576 one-day-old male broilers (Ross 308) with an average initial body weight (BW) of 37 ± 0.51 g were used in a 35-day experiment with a completely randomized design. The broilers were randomly assigned to 3 dietary treatments: CON, basal diet; TRT1, CON + 0.1% MYC; and TRT 2, CON + 0.2% MYC. From days 8 to 21, the feed conversion rate (FCR) was significantly decreased in broilers fed MYC-supplemented diets. From days 22 to 35, BW gain (BWG) significantly increased with increasing MYC concentration. Throughout the experiment, BWG increased (linear effect, $P=0.002$) and FCR decreased with increasing MYC in the diet. MYC supplementation increased the digestibility of dry matter (DM) in broilers in a dose-dependent manner. Relative organ weight of the bursa of Fabricius linearly increased in broilers fed MYC-supplemented diets. The white blood cell count showed linear and quadratic increases in broilers fed increasing concentrations of MYC. The population of *Lactobacillus* in the excreta linearly increased $P=0.033$, whereas that of *Escherichia coli* tended to linearly decrease ($P=0.064$) in the MYC groups. This study provides a basis for future research on MYC as a growth promoter in broilers.

Key words: blood parameters, broiler growth performance, gut health, mixed yeast culture, nutrient digestibility

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Introduction

The use of antibiotics as growth promoters in the poultry industry has been under severe criticism. Therefore, it has become important to develop alternatives to antibiotics to provide more choices for poultry husbandry. Yeast has been used as a feed additive for several decades (Hatoum *et al.*, 2012). In recent years, different yeast products have been developed and used as growth promoters in livestock feed.

Yeast culture (YC) is an important widely used product type of yeast preparation and is defined as a product containing yeast and various metabolites of yeast fermentation (Shen *et al.*, 2009). *Saccharomyces cerevisiae* (SC) fermentation product is a widely used YC. SC is known as baker's yeast. It is one of the most widely commercialized yeast species, and it has been suggested to be the best source of

protein, vitamins, amino acids, and important trace minerals (Paryad and Mahmoudi 2008; Özsoy and Yalçın 2011; Adebisi *et al.*, 2012). SC has been demonstrated to possess antagonistic activity against various bacterial pathogens both *in vitro* and *in vivo* (Firman *et al.*, 2013). Gao *et al.* (2009) reported that SC affects various pathogens present in the intestinal tract of broilers. *Kluyveromyces maxianus* (KM), the species was transferred to the genus *Kluyveromyces* by van der Walt in 1956 (Fonseca *et al.*, 2008), has been recently approved by the Chinese Ministry of Health and European Union as a food-grade probiotic (Wang *et al.*, 2017). Commercial production of this species has gained increased attention because it has a higher growth rate, more active metabolic functions, and higher mannan content in the cell wall than SC (Lane and Morrissey, 2010; Lane *et al.*, 2011). However, there is limited information on the effects of KM supplementation in broiler diets.

Hooge *et al.* (2003) and Stanley *et al.* (2004) confirmed that YC has potential as an alternative to antibiotic-based drugs in broiler feed. Multiple other studies have reported the beneficial effects of feed supplementation with YC as a

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growth promoter that improves growth performance (Bai *et al.*, 2013), feed efficiency (Al-Mansour *et al.*, 2011), blood parameters (Xiao *et al.*, 2013), meat quality (Zhang *et al.*, 2005), immune function (Wang *et al.*, 2017), and intestinal microflora (Guo *et al.*, 2017) in broilers. However, Madriqal *et al.* (1993) failed to observe a positive effect of feeding YC on the body weight (BW) of broilers. Kanat and Calialar (1996) reported that active dry yeast effectively increases BW gain without affecting the feed/gain ratio in broiler chicks. However, these previous studies mostly focused on the effects of YC derived from a single species, whereas the effects of supplementation of mixed yeast culture (MYC) in broilers have not been systemically analyzed.

The purpose of the present study was to investigate the effects of MYC derived from SC and KM on growth performance, nutrient digestibility, meat quality, blood parameters, and gut health in broilers fed a corn-wheat-soybean diet to evaluate its usability as a growth promoter.

Materials and Methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University, South Korea.

Experimental Design, Animals, Diets, and Housing

In total, 576 one-day-old male Ross 308 broiler chickens with an average initial body weight of 37 ± 0.51 g were used in a 35-day growth assay. The experiment was conducted in three phases: phase 1 (days 1–7), phase 2 (days 8–21), and

phase 3 (days 22–35). Broilers were randomly assigned to one of three diets: CON, corn-wheat-soybean basal diet; TRT1, CON + 0.1% MYC; and TRT 2, CON + 0.2% MYC. All diets were formulated to meet or exceed the NRC (1994) requirement for broiler chickens (Table 1), and all diets were presented in mash form. The MYC was mixed properly using a mixer (DDK-801F; Daedong Tech, Korea) according to the manufacturer's protocol. There were 12 pens per treatment, with 16 broiler chickens per pen. The broiler chickens were housed in the animal building of Dankook University in all-in/all-out production system. The room was cleaned every week during the experiment and it was regularly disinfected. The temperature in the room was $33 \pm 1^\circ\text{C}$ for the first 3 days and was then gradually decreased by 3°C per week to 24°C , which was maintained until the end of the experiment. The humidity was kept around 60% throughout the experiment. Artificial light was provided 24 h per day by fluorescent lights. The broilers had free access to feed and water during the experiment. Each pen was equipped with two feeders on each side and two nipple drinkers.

MYC Production

The SC and KM culture strains were selected and procured from a local commercial brewhouse (Cheonan, Korea). For the production of yeast cultures, liquid medium was prepared by dissolving 20 g of glucose, 20 g of peptone, and 10 g of yeast extract in a liter of distilled water. The pH of the liquid medium was maintained at 6.5 ± 0.2 at 28°C . Sterilized liquid medium was inoculated aseptically with 10 ml of liquid

Table 1. Ingredient composition of experimental diets on as-fed basis

Ingredient, %	Phase 1 (days 1–7)	Phase 2 (days 8–21)	Phase 3 (days 22–35)
Corn	37.26	39.82	37.56
Wheat	15.00	15.00	20.00
Soybean meal	38.24	32.06	28.64
Corn gluten meal	2.04	3.86	3.24
Animal fat	2.85	5.07	6.61
Mono-di-calcium phosphate	1.22	1.08	0.90
Limestone	1.80	1.64	1.61
Salt	0.36	0.36	0.63
Choline	0.13	0.13	0.12
Lysine	0.37	0.37	0.36
Methionine	0.30	0.24	0.24
Threonine	0.11	0.07	0.07
Vitamin premix ¹	0.12	0.11	0.10
Mineral premix ²	0.10	0.10	0.10
Phytase 1000G	0.10	0.10	0.10
Calculated composition			
Metabolizable energy, kcal/kg	2,928	3,100	3,200
Crude protein, %	23.30	21.87	20.28
Lysine, %	1.42	1.27	1.17
Methionine, %	0.65	0.58	0.56
Calcium, %	1.00	0.90	0.85
Phosphorus, %	0.61	0.55	0.50

¹ Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D₃; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 µg vitamin B₁₂.

² Provided per kg of complete diet: 12 mg Cu (as CuSO₄·5H₂O); 85 mg Zn (as ZnSO₄); 8 mg Mn (as MnO₂); 0.28 mg I (as KI); 0.15 mg Se (as Na₂SeO₃·5H₂O).

culture and incubated at 32°C for 48 h under shaking at 60 rpm. The two strains were cultivated separately and dried to a moisture level of less than 10%. In the mixed culture, live yeast cultures of SC YJM1592 and KM TB7258 were mixed in a ratio of 1:1. The chemical composition of the MYC containing 5.2% of ash, 53.2% of crude protein, 1.8% of crude fat, 0.8% of crude fiber, and 6.8% of moisture. The digestive energy of MYC is 4240 kcal/kg.

Sampling and Measurements

Growth Performance and Nutrition Digestibility

The pen was used as the experimental unit in the growth performance experiment. On days 0, 7, 21, and 35, chickens and feeders were weighed to calculate BW gain (BWG), feed intake (FI), and feed conversion rate (FCR). From days 28 to 35, chromic oxide (0.2%) as an indigestible marker was added to the diets to determine the nutrient digestibility of dry matter (DM) and nitrogen (N). Fresh excreta samples were collected from each pen on days 33, 34, and 35 and were stored at -20°C until analyzed. Prior to chemical analysis, the excreta samples were thawed and dried at 70°C for 72 h, ground to a fine powder, and passed through a 1-mm screen. All feed and excreta samples were analyzed following procedures outlined by the Association of Official Analytical Chemists (2000). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201; Shimadzu, Kyoto, Japan). The apparent total tract digestibility of nutrients was calculated using the following formula: digestibility (%) = $\{1 - [(Nf \times Cd) / (Nd \times Cf)]\} \times 100$, where Nf = nutrient concentration in feces (% DM), Cd = chromium concentration in diet (% DM), Nd = nutrient concentration in diet (% DM), and Cf = chromium concentration in feces (% DM).

Blood Parameters, Meat Quality, and Organ Weight

At the end of the study (day 35), one chicken per pen (12 chickens in total per treatment) was randomly selected for blood collection from the wing vein into K₃EDTA vacuum tubes (BC Vacutainer System; Becton Dickinson, Franklin Lakes, NJ). The samples were centrifuged at 3,000 × g for 15 min to recover the plasma. White blood cells (WBCs), red blood cells (RBCs), lymphocytes, and glucose concentration were determined using an automatic blood analyzer (ADVIA 120; Bayer, New York). The chickens used for blood samples were weighed and slaughtered. Breast meat, abdominal fat, gizzard, liver, spleen, and bursa of Fabricius were removed by trained personnel. All samples were pat-dried to remove excess moisture and then weighed. Hunter lightness (L*), redness (a*), and yellowness (b*) of the breast meat were measured using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Drip loss percentage was determined on days 1, 3, 5, and 7 by the procedure described by Honikel (1998).

Excreta Microbial Analysis, Vent Score, and Excreta Gas Emission

At the end of the experiment, for all birds, four personnel members evaluated the vent and assigned a score of 1 to 5 according to the dirtiness of the vent following the method described by Lee (2014). At the same time, excreta samples

were collected from each pen and mixed. The samples were stored in 2.6-L plastic boxes, in duplicate. Each box had a small hole in the middle of one side-wall that was sealed with adhesive tape. The samples were allowed to ferment for 5 days at 25°C. After the fermentation period, a GV-100 gas sampling pump (Gastec Corp., Kanagawa, Japan) was used for the detection of ammonia (NH₃), hydrogen sulfide (H₂S), and total mercaptans (R.SH) using different detection tubes (No. 3L, No. 4LT, and No. 70L; Gastec). To this end, the seal was punctured, and 100 mL of headspace air was sampled approximately 2 cm above the excreta. After air sampling, each box was sealed again covered with adhesive tape. Head-space measurements were repeated after 58 h. The gas contents were averaged from two measurements.

One chicken per pen (12 chickens in total per treatment) was randomly selected and, by massaging the abdominal area, excreta was collected from the cloacae into a microtube. One gram of excreta from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and homogenized. Counts of viable bacteria in excreta samples were determined by plating 10-fold serial dilutions (in 1% peptone broth solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium agar plates (Medium 638; DSMZ, Braunschweig, Germany) to isolate *E. coli* and *Lactobacillus*, respectively. The *Lactobacilli* medium agar plates were incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator as described by Lee (2014).

Statistical Analysis

Data from the completely randomized design experiment were analyzed using a general linear model (SAS Institute Inc., Cary, NC, USA 1996). The pen was used as the experimental unit. For microbial counts, data were log-transformed prior to statistical analysis. Orthogonal polynomials were used to assess the linear and quadratic effects of increasing concentrations of supplemental MYC. Duncan's multiple range test was adopted to compare means of the treatments. Variability in the data was expressed as the pooled SE. $P < 0.05$ was considered statistically significant and $P < 0.1$ was considered a trend.

Results

Growth Performance

As shown in Table 2, the results of growth performance analysis indicated that from days 1 to 7, BWG, FI, and FCR were not affected by the treatments. From days 8 to 21, FCR linearly decreased ($P = 0.017$). From days 22 to 35, BWG linearly increased ($P = 0.019$) with increasing MYC concentration in the diet. Throughout the experiment, BWG linearly increased ($P = 0.002$) and FCR linearly decreased ($P = 0.002$) with increasing MYC concentration in the diet. FI was not affected throughout the experimental period.

Nutrient Digestibility

During the entire experiment, the apparent total tract digestibility of DM linearly increased ($P = 0.038$) with increas-

Table 2. Effect of dietary supplementation of MYC on growth performance in broilers

Parameter	CON	TRT1	TRT2	SE ²	P	
					Linear	Quadratic
days 1–7						
BWG ² , g	89	92	91	2	0.352	0.378
FI ² , g	105	103	105	1	0.705	0.247
FCR ²	1.187	1.128	1.153	0.026	0.370	0.203
days 8–21						
BWG, g	654	672	678	9	0.077	0.600
FI, g	1033	1022	1025	9	0.532	0.529
FCR	1.584 ^a	1.522 ^b	1.513 ^b	0.017	0.017	0.286
days 22–35						
BWG, g	983	1009	1035	16	0.019	0.568
FI, g	1778	1807	1802	23	0.446	0.524
FCR	1.809	1.794	1.743	0.024	0.077	0.990
Overall						
BWG, g	1726 ^b	1774 ^a	1805 ^a	17	0.002	0.372
FI, g	2917	2932	2932	27	0.673	0.794
FCR	1.690 ^a	1.655 ^b	1.625 ^b	0.012	0.002	0.472
Survival, %	96.02 ^b	98.44 ^a	98.44 ^a	0.79	0.057	0.253

Abbreviations: CON, basal diet; TRT1, CON + 0.1% MYC; TRT2, CON + 0.2% MYC; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; SE, standard error. Means in the same row with different superscripts differ ($P < 0.05$).

Table 3. Effect of dietary supplementation of MYC on nutrient digestibility in broilers

Items, %	CON	TRT1	TRT2	SE	P	
					Linear	Quadratic
Dry matter	67.79 ^b	69.24 ^{ab}	70.32 ^a	0.64	0.038	0.851
Nitrogen	63.67	63.72	63.66	1.92	0.998	0.977

Abbreviations: CON, basal diet; TRT1, CON + 0.1% MYC; TRT2, CON + 0.2% MYC; SE, standard error. Means in the same row with different superscripts differ ($P < 0.05$).

ing MYC concentration in broilers fed a MYC-supplemented diet (Table 3). However, no difference was observed in digestibility of N during the experiment.

Meat Quality and Relative Organ Weight

The relative organ weight of the bursa of Fabricius linearly increased ($P = 0.028$) with increasing MYC concentration in the diet (Table 4). We observed no effect of MYC supplementation on pH, breast muscle color, cooking loss, WHC, drip loss, and relative weights of breast muscle, liver, abdominal fat, spleen, and gizzard.

Blood Parameters

WBCs showed linear and quadratic increases ($P = 0.045$ and $P = 0.044$, respectively) with increasing MYC concentration in the broilers fed MYC-supplemented diets (Table 5). RBCs, lymphocytes, and glucose concentrations in the blood were not affected by the treatments.

Excreta Microbial, Vent Score, and Excreta Gas Emission

Excreta *Lactobacillus* linearly increased ($P = 0.033$), whereas *E. coli* tended to linearly decrease ($P = 0.064$, Table 6) with increasing concentration of MYC. No effects were detected on vent score as well as on the emission of NH₃, H₂S, and R.SH gases among the three treatments.

Discussion

The aim of the present study was to systematically evaluate the influence of dietary supplementation with MYC at two concentrations on the growth performance, nutrient digestibility, meat quality, blood parameters, and gut health in broilers. The results showed that the addition of MYC to the diet positively influenced BWG and FCR of broilers throughout the 35-day trial period compared to the control group, which is in agreement with previous findings (Santin *et al.*, 2001; Haldar *et al.*, 2011; Saied, *et al.*, 2011; Fathi *et al.*, 2012). However, in the present study, FI showed no significant response to MYC supplementation. There were no nutritional limitations in the diet of the control group as their diet met or exceeded NRC (1994) recommendations for nutrients and energy; thus, the improved growth performance upon MYC addition was not due to increased feed consumption, but due to improved efficiency of nutrient use by the birds. Similar results have been reported by Gao *et al.* (2009); addition of various amounts (2.5, 5.0, and 7.5 g/kg) of YC product did not affect feed consumption in broilers during a 42-day feeding trial. Reisinger *et al.* (2012) re-

Table 4. Effect of dietary supplementation of MYC on meat quality and organ weight in broilers

Items	CON	TRT1	TRT2	SE	P	
					Linear	Quadratic
pH	5.79	5.76	5.79	0.17	0.990	0.889
Breast muscle color						
Lightness (L*)	52.22	53.72	55.23	1.48	0.190	0.997
Redness (a*)	10.46	10.34	10.45	0.64	0.991	0.905
Yellowness (b*)	9.42	9.51	9.79	0.66	0.668	0.894
Cooking loss, %	33.49	33.84	31.41	1.34	0.567	0.642
WHC, %	54.83	52.48	51.07	2.71	0.404	0.897
Drip loss, %						
day 1	2.59	2.64	2.60	0.20	0.951	0.829
day 3	5.55	5.45	5.36	0.11	0.190	0.939
day 5	8.57	8.57	8.44	0.25	0.653	0.769
day 7	10.34	10.22	10.13	0.26	0.542	0.953
Relative organ weight, %						
Breast muscle	18.30	18.42	18.57	0.16	0.536	0.982
Liver	2.95	2.90	2.95	0.15	0.998	0.857
Bursa of Fabricius	0.09 ^b	0.12 ^a	0.13 ^a	0.01	0.028	0.357
Abdominal fat	3.16	3.56	3.67	0.25	0.195	0.640
Spleen	0.10	0.13	0.14	0.02	0.234	0.657
Gizzard	1.12	1.18	1.14	0.07	0.924	0.612

Abbreviations: CON, basal diet; TRT1, CON + 0.1% MYC; TRT2, CON + 0.2% MYC; SE, standard error; WHC, water-holding capacity

Means in the same row with different superscripts differ ($P < 0.05$).

Table 5. Effect of dietary supplementation of MYC on blood parameters in broilers

Parameter	CON	TRT1	TRT2	SE	P	
					Linear	Quadratic
WBC, $10^3/\mu\text{L}$	21.77 ^{ab}	24.175 ^a	14.42 ^b	2.64	0.045	0.044
RBC, $10^3/\text{MI}$	2.73	2.78	2.88	0.27	0.608	0.964
Lymphocytes ¹ , %	53.30	54.30	56.20	1.19	0.159	0.778
Glucose, mg/dL	225.00	225.00	228.75	9.58	0.784	0.868

Abbreviations: CON, basal diet; TRT1, CON + 0.1% MYC; TRT2, CON + 0.2% MYC; RBC, red blood cell; SE, standard error; WBC, white blood cell

¹ Values are presented as a percentage of the total WBC count.

Means in the same row with different superscript letters differ ($P < 0.05$).

Table 6. Effect of dietary supplementation of MYC on excreta microbial count, vent score, and excreta gas emission in broilers

Items	CON	TRT1	TRT2	SE	P	
					Linear	Quadratic
<i>Lactobacillus</i> , \log_{10} cfu/g	7.10 ^b	7.33 ^{ab}	7.41 ^a	0.07	0.033	0.468
<i>E. coli</i> , \log_{10} cfu/g	6.62	6.42	6.35	0.06	0.064	0.513
Vent score ¹	1.40	1.29	1.32	0.05	0.306	0.277
NH ₃ , mg/kg	28.6	27.9	25.9	1.2	0.101	0.640
H ₂ S, mg/kg	2.4	2.3	2.2	0.2	0.655	0.907
R.SH, mg/kg	1.3	1.3	1.1	0.1	0.548	0.713

Abbreviations: CON, basal diet; TRT1, CON + 0.1% MYC; NH₃, ammonia; H₂S, hydrogen sulfide; R.SH, total mercaptans; SE, standard error

¹ Vent score was assessed by four different personnel members and was scored from 1 = very clean to 5 = very dirty.

Means in the same row with different superscripts differ ($P < 0.05$).

ported that 0.1% of yeast derivative containing yeast cell-wall fragments from SC improved BW and BWG in broilers. MYC contains yeast cells as well as metabolites, peptides, organic acids, oligosaccharides, amino acids, flavor and aroma substances, and possibly some unidentified growth factors, which have been proposed to benefit performance in poultry. Furthermore, Al-Homidan and Fahmy (2007) reported that SC fermentation products have a positive impact on intestinal morphology. In this study, the increase in growth performance also may have been owing to benefits to intestinal health. Mortality was higher in the control group (96.02%) than in the treatment groups (98.44%) in the present study. This mortality might have been caused by natural bacterial challenge in the farm, although we observed no clinical signs. Jensen *et al.* (2008) reported that YC provides anti-oxidant, anti-inflammatory, and immunomodulatory activities *in vitro*. This might explain the tendency toward higher survival with the MYC-supplemented diets.

Some studies have reported that YC supplementation does not affect DM digestibility in broilers (Wang, 2007; Yu, 2008). Contrary to these findings, broilers that ingested MYC in the present study had higher DM digestibility. Previous studies have reported that supplementation with YC improves the feed utilization of animals (Bradley and Savage, 1995; Gao *et al.*, 2009). Because of the short digestive tract of broilers and the feed ingredients, the residence time in the gut is short; therefore, the addition of factors improving nutrient absorption in the formulation is not preferred. YC reportedly has the capability to increase villus height and intestinal surface area, thus improving nutrients absorption and, as a consequence, digestibility in broilers (Adebiyi *et al.*, 2012). Accordingly, Wang *et al.* (2017) reported that KM supplementation improves the intestinal structure in broilers. A combination of these factors likely caused higher DM digestibility in MYC-fed broilers in the present study.

Dietary supplementation of MYC had no significant effects on meat quality and relative organ weight, except for the bursa of Fabricius, the weight of which increased with MYC supplementation. Meanwhile, chickens supplemented with 0.2% MYC had the lowest WBC count. These two indicators reflect the immunologic system status of broilers. The bursa of Fabricius is the primary site of chicken B-cell development (Ratcliffe, 2006). B cells are a type of WBCs that functions in the humoral immunity component of the adaptive immune system by secreting antibodies (Murphy, 2012). In accordance with our findings, Gheisari and Kholeghipour (2006) observed a significant increase in relative bursa weight in broilers fed ration containing powdery yeast compared to other treatments. Moreover, Wang *et al.* (2017) reported that KM has a positive effect in broiler immune organs. However, some studies have reported that supplementation of YC did not influence carcass characteristics of broilers (Özsoy and Yalçın, 2011; Fathi *et al.*, 2012). The different findings may be associated with differences in the YC strains used in the studies. Saied *et al.* (2011) reported that control broilers had higher WBC counts than

broilers supplemented with YC. Blood parameters serve as indicators of the physiological state of birds (Smith *et al.*, 2005). WBCs are immune cells that protect the body against infectious disease and foreign invaders. As such, the WBC count is an important indicator of disease (Vital and Health, 2005). In a healthy, adult human, WBCs account for approximately 1% of the total blood volume (Alberts *et al.*, 2002). However, only a few studies have analyzed the correlation between the WBC count and health in chickens. In agreement with our findings, Al-Mansour *et al.* (2011) reported that broilers on 1.5 g/kg of YC supplementation had a lower WBC than chickens on 1 g/kg YC supplementation and control animals, and the authors hypothesized that YC improved the immunity through a reduction in WBCs. Paryad and Mahmoudi (2008) reported that the addition of increasing concentrations (0.5, 1.5, and 2%) of SC significantly improved the WBC count in broilers. Similarly, KM supplementation benefits the immune function of broilers (Wang *et al.*, 2017). The present results support the notion that supplementation of MYC in broiler diet influences immune organs, resulting in an improved humoral immune response. However, further studies are needed to investigate the decrease in WBC induced by the 0.2% MYC diet and the synergism or antagonism of SC and KM in the immune response.

Excreta microbial composition, vent score, and excreta gas emission were considered as indicators of gut health in this study. In agreement with our results, Muthusamy *et al.* (2011) reported that yeast cell wall influences the bacterial counts in the small intestine. It has been proven that YC influences intestinal morphology (Gao *et al.*, 2009; Adebiyi *et al.*, 2012). In this study, the population of *Lactobacillus* in the excreta was significantly increased with 0.2% MYC. *Lactobacillus* are major lactic acid bacteria that benefit intestinal microbiota balance and promote digestion (Makarova *et al.*, 2006; Martín *et al.*, 2013). It also plays important roles in host nutritional, physiological, and protective functions (Power *et al.*, 2014). KM supplementation reportedly has a role in modifying the gut microbial composition (Maccaferri *et al.*, 2012). Therefore, we assumed that MYC supplementation modulated the gut environment and enhanced gut barrier function via the fortification of the beneficial members of the intestinal microbiota. The increase in *Lactobacillus* in the intestine may explain the improved digestibility and growth performance.

In conclusion, supplementation of MYC improved the growth performance, enhanced the apparent total tract digestibility of DM, increased the contents of WBC and *Lactobacillus*, and positively influenced bursa weight in broiler chickens. This study provided a basis for future research on MYC derived from SC and KM as a growth promoter in broilers fed corn-wheat-soybean diets.

References

- Adebiyi OA, Makanjuola BA, Bankole TO and Adeyori AS. Yeast culture (*Saccharomyces cerevisiae*) supplementation: Effect on the performance and gut morphology of broiler birds. *Global*

- Journal of Science Frontier Research Biological Sciences, 12: 6. 2012.
- Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P. Molecular Biology of the Cell, 4th ed. 2002.
- Al-Homidan and Fahmy MO. The effect of dried yeast (*Saccharomyces cerevisiae*) supplementation on growth, performance, carcass chemical analysis, immunity, ileum villi heights and bacterial counts of broiler chickens. Egypt. Poultry Science, 27: 613–623. 2007.
- Al-Mansour S, Al-Khalf A, Al-Homidan I and Fathi MM. Feed efficiency and blood hematology of broiler chicks given a diet supplemented with yeast culture. International Journal of Poultry Science, 108: 603–607. 2011.
- Bai SP, Wu AM, Ding XM, Lei Y, Bai J, Zhang KY and Chio JS. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry Science, 923: 663–670. 2013.
- Bradley GL and Savage TF. The effect of autoclaving a yeast culture of *Saccharomyces cerevisiae* on turkey poult performance and the retention of gross energy, and selected minerals. Animal Feed Science and Technology, 55: 1–7. 1995.
- Fathi MM, Al-Mansour S, Al-Homidan A, Al-Khalaf A and Al-Damegh M. Effect of yeast culture supplementation on carcass yield and humoral immune response of broiler chicks. Veterinary World, 5: 11. 2012.
- Firman JD, Moore D, Broomhead J and McIntyre D. Effects of dietary inclusion of a *Saccharomyces cerevisiae* fermentation product on performance and gut characteristics of male turkeys to market weight. International Journal of Poultry Science, 12: 141–143. 2013.
- Fonseca GG, Heinzle E, Wittmann C and Gombert AK. The yeast *Kluyveromyces marxianus* and its biotechnological potential. Applied microbiology and biotechnology, 79: 339–354. 2008.
- Gao J, Zhang HJ, Wu SG, Yu SH, Yoon I, Moore D, Gao YP, Yan HJ and Qi GH. Effect of *Saccharomyces cerevisiae* fermentation product on immune functions of broilers challenged with *Eimeria tenella*. Poultry Science, 88: 2141–2151. 2009.
- Gheisari A and Kholeghipour B. Effect of dietary inclusion of live yeast (*Saccharomyces cerevisiae*) on growth performance, immune responses and blood parameters of broiler chickens. XII European Poultry Conference, Verona, Italia, 6p. 2006.
- Guo XH, Liu M, Li WH and Yang FJ. Effect of yeast culture on growth performance, meat quality and intestinal microflora of broilers. China Animal Husbandry and Veterinary Medicine, 44: 1673–1679. 2017.
- Haldar S, Ghosh TK and Bedford MR. Effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with *Salmonella enteritidis*. Animal Feed Science and Technology, 1681: 61–71. 2011.
- Hatoum R, Labrie S and Fliss I. Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Frontiers in Microbiology, 3: 421. 2012.
- Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat Science, 49: 447–457. 1998.
- Hooge DM, Sims MD, Sefton AE, Connolly A and Spring P. Effect of dietary mannan oligosaccharide, with or without bacitracin or virginiamycin, on live performance of broiler chickens at relatively high stocking density on new litter. Journal of Applied Poultry Research, 124: 461–467. 2003.
- Jensen GS, Patterson KM and Yoon I. Yeast culture has anti-inflammatory effects and specifically activates NK cells. Comparative Immunology, Microbiology and Infectious Diseases, 31: 487–500. 2008.
- Kanat R and Calialar S. A research on the comparison effect on broiler chickens performance of active dried yeast and inactivated and stabilized probiotic yeast supplemented to the rations in different levels. Poultry Science, 75(Suppl 1): 123. 1996.
- Lane MM and Morrissey JP. *Kluyveromyces marxianus*: a yeast emerging from its sister's shadow. Fungal Biology Reviews, 241: 17–26. 2010.
- Lane MM, Burke N, Karreman R, Wolfe KH, O'Byrne CP and Morrissey JP. Physiological and metabolic diversity in the yeast *Kluyveromyces marxianus*. Antonie Van Leeuwenhoek, 1004: 507–519. 2011.
- Lee KW. Feed passage rate in broiler chickens fed on rye-based diet supplemented with essential oil components. International Journal of Poultry Science, 133: 156. 2014.
- Maccaferri S, Klinder A, Brigidi P, Cavina P and Costabile A. Potential probiotic *Kluyveromyces marxianus* B0399 modulates the immune response in Caco-2 cells and peripheral blood mononuclear cells and impacts the human gut microbiota in an in vitro colonic model system. Applied and environmental microbiology, 784: 956–964. 2012.
- Madrigal SA, Watkins SE, Skinner JT, Adams MH, Waldroup AL and Waldroup PW. Effect of an active yeast culture on performance of broilers. Poultry Science, 72(Suppl 1): 87. 1993.
- Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E and Shakhova V. Comparative genomics of the lactic acid bacteria. Proceedings of the National Academy of Sciences, 103: 15611–15616. 2006.
- Martín R, Miquel S, Ulmer J, Kechaou N, Langella P and Bermúdez-Humarán LG. Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. Microbial cell factories, 121: 71. 2013.
- Murphy KM. Comprar Janeway's Immunobiology 8th Ed. | Kenneth M. Murphy | 9780815342434 | Garland Science. 2012.
- Muthusamy N, Haldar S, Ghosh TK and Bedford MR. Effects of hydrolysed *Saccharomyces cerevisiae* yeast and yeast cell wall components on live performance, intestinal histo-morphology and humoral immune response of broilers. British poultry science, 526: 694–703. 2011.
- NRC Nutrient Requirements of Poultry, 9th rev. ed. National Academies Press, Washington, DC. 1994.
- Paryad A and Mahmoudi M. Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broiler chicks. African Journal of Agricultural Research, 312: 835–842. 2008.
- Power SE, O'Toole PW, Stanton C, Ross RP and Fitzgerald GF. Intestinal microbiota, diet and health. British Journal of Nutrition, 111: 387–402. 2014.
- Özsoy B and Yalçın S. The effects of dietary supplementation of yeast culture on performance, blood parameters and immune system in broiler turkeys. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 58: 117–122. 2011.
- Ratcliffe MJ. Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development. Developmental and Comparative Immunology, 301: 101–118. 2006.
- Reisinger N, Ganner A, Masching S, Schatzmayr G and Applegate TJ. Efficacy of a yeast derivative on broiler performance, intestinal morphology and blood profile. Livestock Science, 1432: 195–200. 2012.
- Santin E, Maiorka A, Macari M, Grecco M, Sanchez JC, Okada TM

- and Myasaka AM. Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *Journal of Applied Poultry Research*, 103: 236–244. 2001.
- Saied JM, Al-Jabary QH and Thalij KM. Effect of dietary supplement yeast culture on production performance and hematological parameters in broiler chicks. *International Journal of Poultry Science*, 105: 376–380. 2011.
- SAS. SAS User's Guide: Statistics. Version 7.0. SAS Institute, Cary, NC. 1996.
- Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I and Zhen YG. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. *Journal of Animal Science*, 87: 2614–2624. 2009.
- Smith TK, Boermans HJ and Woodward B. Effects of feed-borne *Fusarium* mycotoxins on hematology and immunology of laying hens. *Poultry Science*, 84: 1841–1850. 2005.
- Stanley VG, Gray C, Daley M, Krueger WF and Sefton AE. An alternative to antibiotic-based drugs in feed for enhancing performance of broilers grown on *Eimeria* spp.-infected litter. *Poultry Science*, 83: 39–44. 2004.
- Vital and Health Statistics Series 11, No. 247 (03/2005).
- Wang DM. Effect of yeast culture on modulating digestion and immunity in broilers. Doctoral dissertation. Jilin Agricultural University, 2007.
- Wang W, Li Z, Lv Z, Zhang B, Lv H and Guo Y. Effects of *Kluyveromyces marxianus* supplementation on immune responses, intestinal structure and microbiota in broiler chickens. *PLoS one*, 12: e0180884. 2017.
- Xiao M, Gao ZH, Li XH, Zhang SC, Chen XY, Zhang XH, Dong AH, Cao Z and Chen GX. Effects of Yeast Culture on Growth Performance, Nutrient Apparent Metabolic Rates and Serum Biochemical Indices of Broilers. *Journal of Henan Agricultural Science*, 8: 031. 2013.
- Yu SH. Effects of yeast culture on performance and its mechanism in broiler chicks. Doctoral dissertation. Northwest Agriculture and Forestry University, 2008.
- Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB and Lee CH. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. *Poultry Science*, 84: 1015–1021. 2005.