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Effect of Autolyzed Yeast Supplementation on Productive Performance Meat Quality Visceral Organ and Cecum Bacteria of Broiler Chickens

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Abstract: We assessed the effects of dietary Autolyzed Yeast (AY; *Saccharomyces cerevisiae*) on the performance, meat quality, visceral organ weights, and cecum bacteria of broiler chickens. We utilized a total of 360 one-day-old male Ross308 chicks. We allocated the chicks to four different dietary treatments, each consisting of six duplicates. Each replica consisted of 15 chicks per pen. The study included four dietary treatments: Control (CON), CON diet supplemented with 0.125% AY, CON diet supplemented with 0.25% AY, and CON diet supplemented with 0.50% AY. These treatments were administered using a corn-soybean meal-based basal diet served as the basis for administering these treatments. The data on different parameters was analyzed using the analysis of variance technique with a completely randomized design. The treatment means were compared using Duncan's multiple range test. The research showed that between days 25 and 36, chicks that were given AY at concentrations of 0.125, 0.25, and 0.50% had lower feed intake and a better feed conversion ratio ($p<0.05$) than chicks in the control group. Supplementation of dietary AY did not have any impact on the meat quality and texture profile analysis of the broiler. However, the chicks supplemented with AY showed a slight tendency towards a decrease in abdominal fat. The inclusion of 0.25 and 0.50% of AY reduced the presence of *E. coli* in the cecal digesta when compared to the control diet. Chicks that received AY supplementation showed no significant differences in the levels of *Lactobacillus* spp. and *Salmonella* spp. *Saccharomyces cerevisiae* AY at a dosage of 0.125-0.25% resulted in improved feed efficiency and reduced fat deposition. As a result, the population of *E. coli* in the cecum decreased.

Keywords: Cecal Microbial, Growth Performance, Intestinal Organ, Sugarcane Byproduct

Introduction

Yeast cells and their derivatives have been incorporated into animal feeds since the 1980s. While research has primarily focused on ruminants, growing interest exists in their application for horses, pigs, poultry, and pets (Denev *et al*., 2007). Most commercially available yeast products originate from the abundant biomass waste generated by the distillery and yeast-based industries (Lyons *et al*., 1993). Among the various yeast species, *Saccharomyces cerevisiae*, commonly known as baker's yeast, stands out as the most widely used probiotic or prebiotic in poultry diets (Hooge, 2004; Dhama and Singh, 2010). Yeast offers valuable nutritional benefits due to its richness in protein, fiber, and minerals. Both living and non-living yeast cells provide essential B vitamins and inorganic acids for animals (Buzzini and Ann, 2006). However, the primary nutritional contribution of whole yeast cells comes from the intracellular components, such as proteins, peptides, vitamins, and minerals, which require cell wall disruption (lysis) for efficient digestion and absorption. Autolysis is a natural process occurring in non-viable *S. cerevisiae* cells at the end of their growth cycle. During autolysis, yeast hydrolase enzymes damage the cell wall, releasing nutrients and low-molecular-weight substances into the surrounding environment (Fornairon-Bonnefond *et al*., 2002). Autolyzed

Yeast (AY) offers a concentrated source of nutrients and facilitates the release of beneficial components from the cell wall, such as Mannan Oligosaccharides (MOS) and β-glucans (Song *et al*., 2014). Notably, β-glucans have been shown to promote broiler chicken growth and improve meat quality (Cho *et al*., 2013). Moon *et al*. (2016) suggested that dietary β-glucan can reduce oxidative stress in poultry, potentially leading to significant improvements in meat quality. Additionally, AY releases intracellular components like vitamins and nucleotides, which have been associated with enhanced liver function and growth performance in animals (Sauer *et al*., 2011).

In addition to its nutritional value, AY has the potential to provide health benefits, such as reducing populations of pathogenic microbes and stimulating the immune system. Research indicates that the active components, β-glucans and MOS, function as antibacterial agents (Tao *et al*., 2023). These components may regulate intestinal flora and stimulate immune function by preventing pathogen adhesion to the intestinal mucosa while stimulating the oxidative burst activity of heterophilic cells (Huff *et al*., 2010). Additionally, AY can increase cytokine production by macrophages, exerting an antipathogenic effect while promoting the growth of beneficial enzymes (Bortoluzzi *et al*., 2018). Furthermore, AY enhances the diversity of the gut microbiota in the cecum, which may result in enhanced animal growth and overall health (Lyons *et al*., 1993).

Given the potential benefits of yeast compounds in animal feed, research is crucial to optimize the use of autolyzed yeast for maximizing animal health and performance. This study investigates the effects of supplementing broiler chicken feed with autolyzed yeast derived from sugarcane fermentation at various inclusion levels (0.125, 0.25, and 0.50%) on their productive performance and pathogenic bacterial populations.

Materials and Methods

Ethical Approval

The animal care committee of the faculty of agriculture, Kasetsart University, Thailand accepted this study under ethics clearance number ACKU64-AGT-001. The experiment was conducted at the poultry research center farm of Kasetsart University. All experimental procedures adhered to the principles of Good Agricultural Practices for broiler farms (GAP). The authors prioritize animal welfare, food safety, and environmental safety throughout the study, following the standards established by the Guidance on the Application of Thai Agricultural Standard (TAS 6901(G)-2017) policies.

Animals, Diets, and Experimental Design

Three hundred sixty-day-old Ross male broiler chicks were randomly divided into four groups (6 replicates per treatment and 15 birds per replicate) under a completely randomized design. Four experimental diets were formulated at the starter (1-10 days), grower (11-24 days) and finisher phases (25-36 days) to include control diet (basal diet without AY), AY 0.125% (basal diet with 0.125% of AY), AY 0.25% (basal diet with 0.25% of AY) and AY 0.50% diet (basal diet with 0.50% of AY supplementation). The ingredients and chemical composition of the basal diets are presented in Tables 1-2. AY (*S. cerevisiae*) obtained from the fermentation of sugarcane was incorporated into the diet by adding a commercial feed additive (Mitr Phol Biofuel Co., Ltd., Thailand). The process of AY begins with the death of the cell and degradation of cellular constituents which is a proteolytic enzyme. The degradation of the cell wall by enzymes glucanase and proteinase. Birds were reared intensively in a house equipped with an evaporative cooling system, artificial programmable lighting, automated electric heating, and tunnel ventilation. The initial brooding temperature was set at 34°C and gradually decreased to 28°C over the first three weeks of the experiment. The lighting regimen consisted of 18 h of light followed by 6 h of darkness, repeated every 24 h for the duration of the trial. Ad libitum of feed and water were provided and vaccinations were administered according to regular commercial procedures.

Table 1: The ingredient of the control diet (Kg as fed-basis)

Ingredients (Kg)	Starter	Grower	Finisher
Corn	49.11	51.92	56.57
SBM 48%	40.73	37.30	32.27
Rice bran oil	4.95	5.99	6.71
MCP-22	1.54	1.36	1.22
Limestone	1.43	1.30	1.19
Salt	0.58	0.48	0.29
Sodium bicarbonate	-	0.15	0.30
DL-Methionine	0.34	0.28	0.26
L-Lysine	0.19	0.12	0.12
L-Threonine	0.10	0.07	0.04
Vitamin and mineral premix	0.24	0.24	0.24
Choline chloride 60%	0.08	0.08	0.08
Antioxidant and toxin binder	0.16	0.16	0.16
Anticoccidial	0.05	0.05	0.05
Autolysis yeast or corn cob	0.50	0.50	0.50
Total	100.00	100.00	100.00

Table 2: The chemical composition of the control diet

Chemical composition	Starter	Grower	Finisher
Metabolizable energy, Kcal/Kg	3000	3100	3200
Crude protein %	23.00	21.50	19.50
Fiber %	3.57	3.43	3.24
Fat %	7.32	8.40	9.21
Methionine %	0.68	0.61	0.56
Lysine %	1.44	1.29	1.16
Methionine + Cysteine %	1.08	0.99	0.91
Threonine %	0.97	0.88	0.78
Valine %	1.11	1.04	0.95
Calcium %	0.96	0.87	0.79
Total P %	0.72	0.67	0.62
Available P %	0.48	0.44	0.39
Na %	0.23	0.23	0.20
DEB, mEq/Kg	252	255	247

Productive Performance

Individual weights were taken of each broiler chicken on day one, as well as at the conclusion of each dietary phase on days 10, 24, and 36. The amount of feed consumed by each pen was measured at the end of each growth period. Body Weight (BW), Body Weight Gain (BWG), Average Daily Gain (ADG), Feed Intake (FI), and Feed Conversion Ratio (FCR) were measured during four different periods: 1-10 d (starter phase), 11-24 d (grower phase), 25-36 d (finisher phase) and 1-36 d (overall) after accounting for any mortality.

Meat pH and Drip Loss

The breast's pH was determined using a Hanna Instruments pH meter. The incision was made on the major pectoral muscle's cranial left side. After 45 min and 24 h, the average of three pH measurements for each sample had passed. The central portions of the left pectoralis major muscles from the slaughtered chicks were also collected, weighed, and then cut into a cube sized 25×25×15 mm. The samples were placed inside a plastic bag, sealed securely to prevent evaporation, and maintained in a refrigerated chamber at a temperature of 4°C. After 24 h, the meat was removed and its weight was measured after the drying process using filter paper. The drip loss was determined by calculating the percentage of breast meat yield (in grams).

Shear Force and Texture Profile Analysis

The tenderness was conducted using a texture analysis method outlined by Schilling *et al*. (2010). Shear force (N) was determined from breast samples. Two contiguous 25×25×15 mm cubes have been taken from the chilled breast. The Warner-Bratzler shear attachment on an Instron Tinius Olsen Testing Machine (model H5KS, Tinius Olsen Co., Horsham, PA) was to shear each cube once in a direction perpendicular to the muscle fibers. The machine was provided with a maximum 50-N load cell and a crosshead speed of 200 mm/min. The average was computed for each breast meat. Stable Micro Systems Ltd., located in Godalming, UK, manufactures the TA-HD Texture Analyzer, which we use to conduct the Texture Profile Analysis (TPA) of breast muscle.

The determination of TPA was done by scoring the cooked muscles aligned in the longitudinal direction using a handheld corer's assist measuring 1.5 cm in height and 2.5 cm in diameter. A cylinder piston with a diameter of 75 mm was used to compress the sample twice, compressing it by 80% of its original value. A 5-second interval separated the compression cycles. Force-time curves of deformation were obtained from the conditions laid down in the tetrameter. The velocities employed were 2.0, 5.0, and 5.0 millimeters per second throughout the pre-test, test, and post-test, respectively. The properties of hardness, chewiness, springiness, and cohesiveness were assessed based on the definitions suggested by Novaković and Tomašević (2017).

Visceral Organ

The feed was removed for 6 h at 36 days old before being processed. Twenty-four broilers from each group were killed for 1.5-2.0 min using CO_2 asphyxiation in an atmosphere with less than 2% oxygen (air displaced by CO_2). The major visceral organs (abdominal fat, liver, pancreas, spleen, bursa, gizzard, and proventricular) were collected, weighed, and documented. The relative weight of each organ was determined as a proportion of the live body weight.

Microbial in Cecum

After the end of the trial, the cecal samples were rapidly obtained when the chicks were killed. A quantity of approximately 0.5 g of digesta was utilized to extract DNA with the QIAamp DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany). 200 µL of ethanol was added to precipitate bacterial DNA, which was subsequently collected on a spin column. Consequentially, AW1 and AW2 washing buffers were added one at a time to the spin column. Then, elution was done in 50 µL of TE buffer. After the end of DNA extraction, the concentration of the DNA sample was determined using a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnology GmbH, Erlangen, Germany).

The DNA eluted was employed as a template for real-time PCR amplification using specific bacterial primers (Table 3). The total reaction volume was 20 µL and included 4 µL of 5× HOT FIREPol EvaGreen qPCR mix plus (Solid Biodye, Inc., Estonia), 1 µL of primer (Bio Basic, Markham ON, Cannada), 1 µL of template DNA (concentration 600 ng/µL) and 16 µL of nuclease-free water. The bio-rad CF ×96 real-time PCR detection system with CFX manager software (bio-rad, Hercules, CA) was used to perform the real-time PCR amplification. The activation step involved 5× HOT FIREPol EvaGreen qPCR Mix Plus and it was carried out for 15 min at 94°C.

Table 3: The sequences of DNA primers used to investigate bacterial populations

Bacteria	Accession no.	DNA sequence (5′ 3′)	Annealing temperature	Source
Lactobacillus sp.	KACC 12419	F: AGCAGTAGGGAATCTTCCA R: ATTYCACCGCTACACATG	56°C	Vanhoutte *et al.* (2004)
Escherichia coli	ATCC 43894	F: TTGACCCACACTTTGCCGTAA R GCGAAAACTGTGGAATTGGG	60°C	Wang *et al*. (2007)
Salmonella spp.	ATCC 700220	F: GTGAAATTATCGCCACGTTCGGGCAA R: TCATCGCACCGTCAAAGGAACC	63°C	Malorny *et al.* (2004)

Note: F = Forward primer, R = Reverse primer

The first step involved denaturation, which was done for 2 min at 94°C. Then, there were 30 cycles of denaturation, annealing (Table 3), and extension at 72°C for 30 sec and a final extension step at 72°C for 10 min. The default setting was used to analyze the melting curve. The standard curve that was applied to each run served as the basis for the quantification. The number of bacteria was calculated based on their genomic size (Dumonceaux *et al*., 2006). The bacterial quantities are expressed in log Colony-Forming Units (CFU) per gram of digesta.

Statistical Analysis

The data were processed by statistical analysis using the general linear model procedure by SAS (SAS Institute, Inc., Cary, NC, USA, 1996) using a completely randomized design. The experimental unit was a pen. Before statistical examination, we converted the data on the bacterial population to log CFU/g. The means and standard error of the means were used to express the results. The differences between the groups were investigated using a one-way analysis of variance. Duncan's multiple range test (Steel and Torrie, 1981) was used to determine the significance of mean differences between groups. Significance was assigned to probability values below 0.05.

Results

Productive Performance

The effects of Autolyzed Yeast (AY) supplementation in broiler diets on productive performance are shown in Table 4. During the starter period (0-10 days) and the grower period (11-24 days), BW, BWG, ADG, FI, and FCR were not significantly affected by the inclusion of AY in the diets. The differences ($p<0.05$) in the FI were found among experimental treatments during the finisher period (25-36 days). The lower FI was observed in chicks receiving AY supplementation. The FCR was significantly ($p<0.05$) improved during the finisher period following the AY supplement groups compared to the control group. Furthermore, the FCR of the overall period (0-36 days) tended to be improved by supplementing AY in the diets ($p = 0.08$).

Meat Quality and Blood Profile

The broiler breast meat pH, drip loss, shear force, and Texture Profile Analysis (TPA) of the post-mortem are presented in Table 5. There was no significant difference in pH at 45 min and 24 h on the AY supplement breast meat sample. The AY supplementation had no significant effect on the broiler's breast meat drip loss and shear force. The AY supplementation did not affect the broiler breast meat TPA. Similar results for hardness, springiness, cohesiveness, gumminess, and chewiness were found in all treatment groups.

Visceral Organ

Visceral organs from broilers supplemented with AY are shown in Table 6. AY supplementation groups tended to decrease the abdominal fat percentage. The lower abdominal fat values were observed in chicks fed 0.50% of AY supplementation. However, 0.50% AY supplementation showed no difference in abdominal fat with control groups. The lower abdominal fat of 0.50%, followed by 0.25 and 0.125% AY supplement groups, tend to indicate that abdominal fat decreased with AY supplementation in broiler diets. In addition, there was no difference in the relative weights of the liver, gizzard with proventriculus, pancreas, and bursa gland.

Microbial in Caecum

The effects of AY supplementation in broiler diets on bacteria in the cecum are shown in Table 7. The chicks fed supplemented with AY 0.25 and 0.50% significantly decreased *E. coli* counts in the cecum ($p<0.05$). Nevertheless, dietary AY supplementation did not affect *Lactobacillus* sp. in cecal digesta, and *Salmonella* spp. was not detected in all treatment groups

Table 4: Effects of autolyzed yeast supplementation on productive performance

Items	Control	AY 0.125%	AY0.25%	AY 0.50%	SEM	P-value
Starter (1-10 days)						
BW (g/bird)	356.52	353.49	356.37	361.32	1.061	0.408
BWG (g)	310.51	307.50	310.38	315.30	1.062	0.417
ADG (g/day)	31.05	30.75	31.04	31.53	0.016	0.417
FI (g)	328.58	314.67	319.30	328.98	2.039	0.181
FCR	1.06	1.02	1.03	1.04	0.006	0.678
Grower (11-24 days)						
BW (g/bird)	1587.72	1564.36	1567.63	1568.52	5.780	0.497
BWG (g)	1231.20	1210.87	1211.26	1207.20	5.170	0.365
ADG (g/day)	87.94	86.49	86.15	86.23	0.370	0.365
FI (g)	1395.51	1400.94	1402.37	1392.44	8.510	0.923
FCR	1.13	1.16	1.16	1.15	0.010	0.197
Finisher (25-36 days)						
BW (g/bird)	3035.72	3001.69	3033.76	2956.04	15.500	0.233

Table 4: Cont.

BWG (g)	1448	1437.33	1466.13	1387.52	8.500	0.978
ADG (g/day)	120.67	119.77	122.18	115.63	1.080	0.169
FI (g)	2111.98[a]	2020.64[b]	2021.14[b]	1979.96[b]	16.210	0.019
FCR	1.46[a]	1.41[b]	1.38[b]	1.43[ab]	0.030	0.014
Overall (1-36 days)						
BW (g/bird)	3035.72	3001.69	3033.76	2956.04	15.500	0.232
BWG (g)	2989.71	2955.70	2987.77	2910.02	12.110	0.234
ADG (g/day)	83.04	82.10	82.99	80.83	0.340	0.234
FI (g)	3836.07	3736.25	3742.81	3701.38	14.820	0.198
FCR	1.28	1.26	1.25	1.27	0.004	0.080

Note: BW = Body Weight; BWG = Body Weight Gain; ADG = Average Daily Gain; FI = Feed Intake; FCR = Feed Conversion Ratio; EPEF = European Efficiency Factor, [a b]Mean values within a column with unlike superscript letters are significantly different ($p<0.05$)

Table 5: Effects of autolyzed yeast supplementation on meat quality and texture profile analysis

Items	Control	AY 0.125%	AY 0.25%	AY 0.50%	SEM	P-value
pH 45 min	7.18	7.34	7.25	7.27	0.003	0.44
pH 24 hr.	5.77	5.77	5.69	5.78	0.003	0.67
Drip loss (%)	2.92	2.92	3.06	3.06	0.019	0.94
Shear forced (N)	8.95	9.17	9.18	9.20	0.042	0.98
Texture Profile Analysis (TPA)						
Hardness (N)	8.74	10.50	10.27	10.62	0.035	0.31
Springiness (ratio)	0.59	0.61	0.62	0.62	0.001	0.11
Cohesiveness (ratio)	0.48	0.50	0.49	0.50	0.004	0.40
Gumminess (N)	4.24	5.12	5.04	5.38	0.020	0.26
Chewiness (N*mm)	2.50	3.13	3.17	3.40	0.138	0.16

Note: [a,b,c]Mean values within a column with unlike superscript letters are significantly different ($p<0.05$)

Table 6: Effects of autolyzed yeast supplementation on relative visceral organ

Items	Control	AY 0.125%	AY 0.25%	AY 0.50%	SEM	P-value
Abdominal fat (%)	1.47	1.37	1.34	1.32	0.002	0.006
Gizzard (%)	1.12	1.23	1.23	1.22	0.002	0.013
Liver (%)	1.82	1.84	1.81	1.91	0.003	0.060
Spleen (%)	0.12	0.12	0.10	0.12	0.003	0.044
Pancreases (%)	0.16	0.17	0.16	0.17	0.003	0.055
Bursal of Fabricius (%)	0.07	0.07	0.07	0.08	0.002	0.015

Note: [a,b]Mean values within a column with unlike superscript letters are significantly different ($p<0.05$)

Table 7: Effects of autolyzed yeast supplementation on microbial in caecum

Items	Control	AY 0.125%	AY 0.25%	AY 0.50%	SEM	P-value
Lactobacillus sp.	12.59	12.05	12.05	12.26	0.12	.049
Escherichia coli	12.17[a]	11.34[ab]	11.18[b]	10.91[b]	0.17	0.04
Salmonella spp.	ND	ND	ND	ND	-	-

Note**:** ND = Not Detected, [a b]Mean values within a column with unlike superscript letters are significantly different ($p<0.05$)

Discussion

Autolyzed Yeasts (AY); yeast cells and their extracts have shown beneficial effects on the growth performance of broilers. The beneficial production responses in animals have been attributed to enzymes, vitamins, and other nutrients or growth factors contained in yeast products (Shen *et al*., 2009). The composition of AY has been summarized as: 3.5-3.9% nucleic acids, 11-22% β-glucan, 3-12% MOS, 30.0- 41.1% crude protein and 2.51-5.00% crude fat (Namted *et al*., 2022).

The present study did not find any significant difference in BW, BWG, and ADG during the whole experimental period. It might be associated with various factors, such as types of yeast products, inclusion levels of yeast, and management. Similarly, the experiment of Mohamed *et al*. (2015) reported no difference in growth performance rate supplementation AY during 0-3 weeks of broiler. In addition, the experiment of Bortoluzzi *et al*. (2018) with AY during the first 2 weeks showed no difference in productivity. However, the present study during the finisher period showed decreased FI and better FCR in AY supplementation groups and cumulative FCR tended to improve ($p = 0.08$) by AY supplementation at the levels of 0.25%. This improvement in broilers fed AY-supplemented diets could be due to increased

absorption and utilization of dietary nutrients. Ahiwe *et al.* (2020) reported that yeast could increase protein digestibility and show prebiotic effects on broiler weight gain and FCR. Zhang *et al*. (2005) found that yeast supplementation in broilers showed improved FCR compared to the control group. Moreover, Shen *et al*. (2009) reported higher nutrient digestibility in pigs fed with yeast supplementation in the diet. Gao *et al*. (2008) found that 2.5 g/kg yeast culture was the optimum dosage for the growth of broilers. The optimal inclusion of the yeast products would account for the requirement of a broiler. At the same time, a higher level of yeast inclusion (20 g/kg diet) decreased productive performance by increasing the FCR of broilers (Pappas *et al*., 2010; Chen *et al*., 2016; Cheng *et al*., 2016).

pH has a direct bearing on meat quality attributes such as tenderness, water-holding capacity, color, juiciness and shelf life The pH of broiler meat is the function of the amount of glycogen in the muscle before slaughter and the rate of glycogen conversion into lactic acid after slaughter. Identification of color is an easy way to determine the pH of meat. If the meat is very dark, it will have a high pH and if it is very light, it will have a low pH (Hinkelmann *et al*., 2011). Askri *et al*. (2022) stated that the beginning of the onset of rigor mortis was around 6 h post-mortem and yeast supplementation in broiler diets did not significantly affect ultimate pH. Similar to the current study, AY supplementation did not affect ($p>0.05$) the pH of breast meat. Opposite to our findings, Konca *et al*. (2009) showed that dietary mannan-oligosaccharides 1 g/kg diet (another main component of the yeast cell wall) nor live yeast affected meat pigmentation and the pH value of finishing turkeys. In addition, the result of the study showed that drip loss was not affected by AY supplementation. Zhang *et al*. (2005) reported that the shear force in raw breast meats showed no significant difference in whole yeast and yeast extract supplementation. In contrast, Cho *et al*. (2013) reported that dietary β-glucan (1 g/kg diet), one of the major components of the yeast cell wall, decreased the drip loss and cooking loss in the breast meat of broilers. Meat tenderness can be estimated by measuring the shear force; a lower shear force indicates tender meat. However, in our experimental conditions, the AY supplementation in the diet (0.125-0.50%) did not affect the shear force value. The muscle TPA is related to meat properties (Godschalk-Broers *et al*., 2022). Based on this study, similar TPA values were observed in all experiments. These results agreed with Grigore *et al*. (2023), who reported no significant effect on breast meats of broiler pH, hardness, springiness, cohesiveness, gumminess, and chewiness. The abdominal fat deposition in poultry is related to nutritional factors and is used as the parameter for judging total body fat content (Fouad and El-Senousey, 2014). In the present study, AY supplementation tends to decrease relative abdominal fat weight in broilers. Yalçın *et al*. (2013) reported that the relative abdominal fat weight was decreased in chicks fed with diets containing yeast autolysate. Similar results indicated that dietary supplementation of YC (*S. cerevisiae*) could decrease the abdominal fat percentage in broilers (Afsharmanesh *et al*. 2010). The reduction of abdominal fat deposition might be possible due to the components of yeast (MOS, β-glucans, and others), which reduces the absorption and accumulation of fat in the adipose tissue of yeast supplementation, and most of the feed energy was utilized efficiently in the growth of chicks (Roy and Ray, 2023). In line with this statement, previous studies have reported that supplementation of yeast (*S. cerevisiae)* in diets in the broiler's diet significantly reduced abdominal fat (Toghyani and Tabeidian, 2011). Furthermore, we observed that dietary AY supplementation did not affect the relative weight of the gizzard, liver, pancreas, spleen, and bursa gland among groups. The findings in the present study agree with Yalçın *et al*. (2013) as the supplementation of yeast autolysate (*S. cerevisiae*) did not affect broilers' intestinal weights.

Chicken gut microbiota plays a vital role in nutrient digestion, intestinal barrier, and gut health function of broilers, which promote productive performance (Pan and Yu, 2014). In cecum, digesta contains various gut microflora and could be used as a gut health index for broilers (Lin *et al*., 2023). Our results showed that broilers fed 0.25 and 0.50% of AY had lower *E. coli* counts in cecal than chicks fed without the AY supplementation group. Similar results reported that yeast supplementation reduces the *E. coli* loads compared to chicks fed without AY in diets (Bortoluzzi *et al*., 2018; Yalçın *et al*., 2013; Yang *et al*., 2020). We hypothesized that the component of AY is an active ingredient that could modulate gut microbiota, which may have a role in improving gut health in broilers. Besides, the MOS in yeast cell walls could alter the gastrointestinal microorganisms into beneficial organisms (Spring *et al*. 2000). Kim (2018) stated that MOS of yeast cell walls could be used by microorganisms in the intestine of chicks to produce organic acids, protecting the intestinal from pathogen invasion. Previous studies have demonstrated significant decreases of *E. coli*, *C. perfringens*, Coliforms, and *Salmonella* in the cecal of chicks fed MOS (Wexler, 2007; Yang *et al*., 2008a; Baurhoo *et al*., 2009). Additionally, Yang *et al*. (2018b) observed a decrease in ileal coliform populations with dietary mannooligosaccharide supplementation in broilers. In vitro studies further highlight the effectiveness of MOS in binding *E. coli* (Mourão *et al*., 2006). Interestingly, while Bonos *et al*. (2011) reported a reduction in total bacteria count in cecal content with MOS supplementation, they also found a significant increase in total aerobic bacteria count. This suggests a potential shift towards beneficial bacterial

populations. Furthermore, AY supplementation increased the abundance and prevalence of *Lactobacillus* sp. by decreasing pathogens to support mucin production and gut immunity (Chen *et al*., 2017; Kim, 2018). Thus, yeast cells prevent pathogen attachment and maintain gut microbial homeostasis. However, this study did not find the effect of AY supplementation on *Lactobacillus* sp. The absence of detectable *Salmonella* spp. in the present study could be attributed to the implementation of rigorous hygiene practices during the experiment.

Conclusion

Dietary AY supplementation in broilers significantly improved FCR during the finisher phase and, consequently, for the entire experiment. Supplementation with 0.25 and 0.50% AY reduced body fat accumulation without compromising meat quality, as evidenced by no significant changes in drip loss, shear force, and Texture Profile Analysis (TPA). Additionally, AY effectively decreased *E. coli* colonization in the broilers' intestines. Based on these findings, the recommended level of AY supplementation is 0.25%. These results provide a strong foundation for further development of dietary AY as a functional feed additive for broilers, with the potential to improve both performance and gut health.

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Author's Contributions

Sirinapa Taechanan: Data registration and writing of the manuscript.

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Choawit Rakangthong: Advised during the written manuscript and evaluated the final draft before submission.

Ethics

This article is original and contains previously unpublished material. It has been confirmed by the corresponding author that all of the other authors have read and approved the article and that there are no issues of ethics related to the paper.

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