Lactobacillus (*L. plantarum* & *L. rhamnosus*) and *Saccharomyces* (*S. cerevisiae*): effects on performance, biochemical parameters, ammonium ion in manure, and digestibility of broiler chickens

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ABSTRACT Two strains of *Lactobacillus* combined with Baker's yeast (*Saccharomyces cerevisiae*) used as probiotics were evaluated to replace antibiotics in poultry flocks by reducing ammonia emissions in manure of broilers without comprising performance or health. One-day-old Cobb 500 broilers (600) were fed starter, grower, and finisher diets as control (**CON**); probiotic *S. cerevisiae*, inclusion rate at 4.26×10^6 *CFU/kg of feed* (**SCY**); probiotic *L. plantarum* and *L. rhamnosus*, inclusion rate at 4.35×10^8 *CFU/kg of feed* (**LPR**) for each; and a combination of *Lactobacillus plantarum* and *L. rhamnosus* at 4.35×10^8 CFU/kg of feed for each plus *Saccharomyces cerevisiae* and 4.26×10^6 CFU/kg of feed (**SWL**). The 4 treatments had 5 replicates (pens), each with 30 broilers. Performance was measured weekly as feed consumption, weight gain, BW, and feed conversion ratio (**FCR**) over a 6-wk grow-out period. Accompanying biochemical analyses included lipase activity of the pancreas, liver weight, and uric acid (**UA**) concentration in liver. Albumin, total protein, UA, ammonia, and blood urea nitrogen (**BUN**) were measured in serum. Ammonium (NH_4^+) in manure and apparent ileal digestibility from digesta were also measured. Significance was determined at $P \leq 0.05$. Results showed that biochemical analyses had no significant treatment effect; however, there were significant temporal changes in performance measures for individual treatments. Feed consumption increased over time for all treatments ($P = 2.00 \times 10^{-16}$). CON had lower weight gain in wk 2 ($P = 0.013$) compared to all treatment and the lowest BW in wk 5 ($P = 0.0008$) and wk 6 ($P = 0.0124$) compared to SWL. Specific probiotic strains, with well-defined inclusion rates, and surrounding environmental analyses of present microbes are needed to ascertain effects of probiotics. Other important areas for investigation include 1) confirmation of probiotics present in the digesta/ceca and how they alter the microbiota within the gastrointestinal (**GI**) tract and 2) the serum heterophil:lymphocyte ratio to further examine potential immune responses to the probiotics.

Key words: broiler, *Lactobacilli* & *Saccharomyces*, production, ammonia, biochemical analysis of tissues

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INTRODUCTION

World meat production increased 44%, reaching 337 million tons in 2019, with chicken being the most produced type, having the highest growth reaching 35% of the meat supply (FAO, 2021). In turn, farm-gate greenhouse gas and ammonia emissions in 2019 also increased 11%, with 55% of the increase coming from total agriculture (crops and animals) production (FAO, 2021). Ammonia has many adverse environmental effects including acid rain and soil acidification. High ammonia levels cause negative effects on the health of birds and staff (Naseem and King, 2018). Several investigators noted exposure to high levels of atmospheric ammonia negatively affected birds' performance, increased disease susceptibility, and induced changes in the gut microbiota (Charles and Payne, 1966; Al-Mashhadani and Beck, 1985; Beker et al., 2004; Han et al., 2021).

Reduction of Ammonia

Several techniques (nipple drinkers, litter type, and diet manipulation) have been developed to help reduce the levels of ammonia inside the aviary (Naseem and King, 2018). In combination with probiotics, discussed below, these techniques decreased levels of ammonia in aviaries with minimal to no negative side effects on production of white leghorn and broilers (Endo and Nakano, 1999; Liu et al., 2007; Ahmed et al., 2014;

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Jeong and Kim, 2014; Chen et al., 2017; Chen et al., 2018; Upadhaya et al., 2019; Such et al., 2021).

Primary Cause of Ammonia in Aviaries: Nitrogen Cycle and Excess Protein

Protein is an essential nutritional requirement for animals and how it is utilized depends on the amount, composition, and digestibility of amino acids (**AA**). Dietary proteins unlike carbohydrates or lipids have no dedicated storage mechanism within the body; thus, they are broken down into AA (Vilela et al., 2020). AA are further catabolized into uric acid (**UA**) and ammonia primarily by the liver (Campbell, 1991). Some UA is synthesized within the kidney and subsequently expelled (Chin and Quebbemann, 1978). However, the UA produced by the liver will travel through the bloodstream. Because there is no urinary bladder in avian species, UA will be expelled directly into the GI tract, finally passing through the large intestine and cloaca. This movement allows for microbes to further break down UA into ammonia, which could be reabsorbed by the host for making nonessential AA (Vispo and Karasov, 1997). However, not all excess ammonia from high protein diets is reabsorbed, resulting in the primary source of ammonia in poultry manure from the microbial breakdown of UA (Vilela et al., 2020; Swelum et al., 2021).

A delicate balance is needed to provide adequate levels of AA that are important to ensure proper protein deposition for growing tissue while avoiding excess consumption as it increases energy expenditure for excretion, thus reducing performance (Vilela et al., 2020; Swelum et al., 2021). Furthermore, in monogastric animals, UA has been associated with increased risk of disease (dysfunction in metabolism, renal disease, fatty liver, risk factor for gout, and cardiac disease) when at high levels within blood (de Oliveira and Burini, 2012; Kanbay et al., 2016).

Improvement in Production Performance

In a healthy gut microbiota, diverse microbes specialize in a variety of different functions. With the continuous growth of the poultry industry, manipulation of the microbiota is seen as a means to not only reduce ammonia emissions but also to improve production performance. This manipulation of the microbiome follows the practice for use of antibiotics in livestock that started in the 1940s to promote health rather than directly treating the illness. Early reports suggested that antibiotics, when fed, greatly reduced known pathogens within the gastrointestinal (**GI**) tract (Moore et al., 1946). However, prolonged usage of an antibiotic permanently alters the microbiota, potentially resulting in an imbalance (for instance reduction of symbiotic microbes within the GI tract) leading to disease and/or deficiencies in nutrient digestion (page number). Concerns over development of further antibiotic resistance, particularly for human pathogens, led to recommendations for banning subtherapeutic antibiotic use in animal feeds (Swann et al., 1969). With continuing concern from governments and consumers alike, antibiotic use declined over time in favor for usage of alternatives, such as probiotics (Sneeringer et al., 2015).

Lactobacillus and Yeast as Probiotics: Reduction of Ammonia, Improvement in Performance, and Reduction in Lipase Activity

Lactobacilli are species that produce lactic acid during glucose metabolism. *Lactobacilli* are separated into 7 family groups: *L. buchneri, L. casei, L. delbrueckii, L. plantarum, L. reuteri, L. sakei*, and *L. salivarius* (De Angelis and Gobbetti, 2011). *L. plantarum* and *L. rhamnosus* are both probiotics that can be added to animal feeds. Specifically, *L. plantarum* is thought to utilize a large variety of AAs via aminotransferases to form alpha-keto acids and specific AA (Leu, Ile, Val, Phe, Tyr, Trp, Cys, Met, and Asp) (De Angelis and Gobbetti, 2011). *L. plantarum* utilizes the arginine-deiminase pathway to produce citrulline, ornithine, and ammonia, altering the pH by acidifying the environment (Corsetti and Valmorri, 2011). However, Corsetti and Valmorri (2011) noted that some strains of *L. plantarum* were shown to have lower proteolytic activity, high levels of esterolytic activity, and high lipolytic activity. Additionally, other studies showed that *L. rhmanosus* and *L. plantarum* have a protective effect against pathogenic *Escherichia coli* and unspecified *E. coli* (Calasso and Gobbetti, 2011; Corsetti and Valmorri, 2011).

L. plantarum, L. paracasei, and *L. rhamnosus* were used individually and in varying combinations to determine effectiveness for reduction of ammonia levels in manure and improvement in performance of layers (Naseem and King, 2020b; Naseem et al., 2020). Results revealed that *L. plantarum* and *L. rhamnosus* numerically reduced ammonia emissions when both were present in the GI microbiota, but had no effect on performance. Naseem et al. (2021) suggested that *Saccharomyces cerevisiae's* ability to form synergistic relations with probiotics could improve the effect of *L. plantarum* and *L. rhamnosus* in reducing ammonia emissions in layer chicken manure. Possibly, yeast could affect performance through improved digestibility, specifically for proteins and minerals for the host and other microorganisms by providing growth factors, provitamins, and other bacterial growth stimulants (Candrawati et al., 2014; Gde Bidura et al., 2016). In turn, the survivability of probiotics within the GI tract could be improved in the presence of yeast (Candrawati et al., 2014; Gde Bidura et al., 2016). Adding the yeast strain, *S. cerevisiae*, also improved overall function of the probiotic leading to improved weight gain, BW, or FCR (Haldar et al., 2011; Bai et al., 2013; Hussein and Selim, 2018; Hoque et al., 2021).

Additional uses of probiotics such as enhanced immune function are of interest (Alizadeh et al., 2021); however, the most appropriate quantity and duration for use are yet to be determined. Indeed, there is limited data showing how long broilers need to be given a probiotic (*Lactobacillus* spp.), a combination of probiotics, or *S. cerevisiae* combined with probiotics to determine if or when they become a part of the bird's gut microbiota.

Lipase is a class of enzymes responsible for the breakdown of fats and their subsequent transport, specifically triglycerides into free fatty acids and glycerol by catalyzing the hydrolysis of the ester bonds in triglycerides (Salah et al., 2006). It may take several weeks after hatching for lipase to increase in broilers; thus, lipid digestion and absorption are hampered in young broilers due to a deficiency in lipase production (Noy and Sklan, 1995; Al-Marzooqi and Leeson, 2000). Researchers have noted that *Lactobacillus* spp. lower lipase activity and reduce fat storage in mice (Aronsson et al., 2010). However, some studies with *Lactobacillus* spp. in chickens had no effect on lipase activity (Jin et al., 2000; Qing et al., 2017). Other investigators noted products from probiotics reduced lipase activity for corn, wheat and soy diets but improved that in feed contaminated with aflatoxins (Matur et al., 2010).

For this study, it was hypothesized that 1) *S. cerevisiae* in conjunction with *L. plantarum* and *L. rhamnosus* would improve measurements for performance as well as reduce ammonia (ammonium (NH_4^+)) in manure compared to either the *Lactobacilli* species or *S. cerevisiae* alone and 2) the results would determine the minimum length of time for which the combined probiotics should be provided. Thus, *L. plantarum* and *L. rhamnosus*, the *Lactobacilli* species plus *S. cerevisiae*, or *S. cerevisiae* alone were fed to broilers for 6 wk to determine performance, associated biochemical parameters, digestibility, and NH_4^+ in manure.

MATERIALS AND METHODS

Animals, Experimental Design, and Housing

All broiler husbandry and sample collection procedures were approved (protocol # 21596) by the Institutional Animal Care and Use Committee, University of California – Davis (Davis, CA). A total of 600 Cobb 500 broiler chicks were donated after hatch at Foster Farms Ellenwood Hatchery (Ellenwood, CA) and transported to a UC Davis grow-out facility. Control feed was provided while chicks were acclimating to the environment (32°C) for 12 h. Birds were weighed and randomly distributed into 4 dietary treatments (below) which contained 5 replications of 30 chicks over a 6-wk grow-out period.

Each replicate was floor-raised in 1.2 m × 1.8 m pens with a 16:8 h light/dark cycle. Pen temperatures were 32°C for the first 2 wk and were maintained at approximately 22°C for the remainder of the grow-out period. Average humidity was 40%. Monitoring of all birds occurred twice daily. Water and diets were given ad libitum, except on d 8, 15, 22, 29, 36, and 43 when all birds were fasted overnight prior to being weighed and randomly chosen for sample collection.

Diet Preparation and Inclusion of Probiotics

CFU for the *Lactobacillus* strains were provided by UAS Laboratory (Windsor, WI), while CFU of *S. cerevisiae* was determined by growth in potato dextrose agar (20 mL in a 100 mm × 15 mm plate) (Biological Media Services University of CA – Davis, Davis, CA).

To ensure no destruction of probiotics due to heat and pelleting, all treatments were prepared as a mash corn and soybean meal basal diet (Council, 1994; Table 1). The treatments were the basal diet (**CON**, Table 1); probiotic *S. cerevisiae*, inclusion rate of 4.26×10^6 CFU/kg of feed (**SCY**); probiotic *L. plantarum* and *L. rhamnosus*, inclusion rate at 4.35×10^8 CFU/kg of feed (**LPR**) for each; and a combination of *Lactobacillus plantarum* and *L. rhamnosus* at an inclusion rate of 4.35×10^8 CFU/kg of feed for each plus *Saccharomyces cerevisiae* and 4.26×10^6 CFU/kg of feed (**SWL**). Chemical and AA analyses of the basal diets were performed by Cumberland Valley Analytical Services (Waynesboro, PA; Table 2).

Feed Consumption, Weight Gain, BW, and FCR

Feed consumption was calculated from the difference in weight of bulk feeders (free of all adhering material) per replicate and the number of birds in each replicate (pen). Prior to obtaining live BW, all chicks were fasted overnight on d 8, 15, 22, 29, 36, and 43. Weekly weight gain was calculated. The weekly feed conversion ratio (**FCR**) was calculated as:

$$\text{FCR} = \frac{\text{Feed Consumed}}{\text{Weight Gained}}$$

Table 1. Composition of mash basal diets for starter, grower, and finisher.

Ingredient	Starter feed %	Grower feed %	Finisher feed %
Corn	47.78	54.97	63.14
Soybean meal	45.93	39.00	31.00
Soybean oil	1.80	1.80	1.80
Monocalcium phosphate	2.00	1.80	1.70
Limestone	1.40	1.40	1.40
Salt	0.45	0.45	0.45
DL-Methionine	0.27	0.21	0.14
Vitamin & mineral mix[1]	0.37	0.37	0.37

[1]Foster Farms vitamin and mineral premix, Foster Farms, Traver, CA. The mix was formulated to meet or exceed recommended quantities (Council, 1994) of the following: calcium, manganese, zinc, copper, vitamin E, niacin, iron, selenium, riboflavin, vitamin A, vitamin D3, menadione nicotinamide bisulfite, pyridoxine hydrochloride, thiamine mononitrate, folic acid, vitamin B12, biotin, and ethylenediamine.

Table 2. Nutrient analysis[1] of mash basal diet.

	Starter feed	Grower feed	Finisher feed
%			
Moisture	9.00	8.20	9.00
DM			
Dry matter (DM)	91.00	91.80	91.00
Crude protein (CP)	27.90	26.30	22.60
Soluble protein	6.30	6.50	5.10
Ash	6.59	8.09	7.18
Calcium	1.26	1.38	1.39
Phosphorus	0.83	0.78	0.75
Magnesium	0.20	0.33	0.30
Potassium	1.33	1.24	1.07
Sodium	0.21	0.30	0.32
CP			
ADF	3.50	4.30	10.40
aNDF	8.80	10.30	0.00
Soluble protein	22.70	24.80	22.60
NDF			
ADF	39.10	42.00	39.70
	——	PPM	——
Iron	238	304	310
Manganese	181	172	179
Zinc	178	227	231
Copper	265	237	335
Amino acids	——	mg/g diet	——
Essential			
Arginine	16.4	14.8	12.7
Cystine	4.3	3.0	3.1
Lysine	14.6	13.8	11.3
Methionine	6.5	4.7	4.9
Tryptophan	3.2	3.3	3.0
Glycine	11.2	10.8	9.1
Histidine	6.3	6.1	5.1
Leucine	22.6	21.8	19.0
Isoleucine	11.2	11.0	9.4
Phenylalanine	12.6	12.0	10.3
Threonine	9.8	9.5	8.2
Valine	13.4	12.6	11.1
Nonessential			
Alanine	13.5	13.1	11.3
Aspartic acid	28.5	27.2	23.1
Glutamic acid	51.8	50.4	42.5
Proline	14.9	14.5	12.6
Serine	13.7	13.4	11.1
Tyrosine	9.5	9.2	7.6

[1]Cumberland Valley Analytical Services (Waynesboro, PA).

Serum Collection and Analysis

Three birds were randomly selected from each replication per treatment and euthanized by CO_2 asphyxiation prior to sampling on d 15, 29, and 43. Blood was collected from the inferior vena cava at the heart immediately after euthanasia. It was then centrifuged at 841 × *g* for 10 min to separate the serum (International Equipment Co, IL). Serum was stored at −80°C. Prior to analyses, 30μL of serum was pooled by replication and analyzed for concentration of albumin, total protein, UA, ammonia, and blood urea nitrogen (**BUN**) at the Comparative Pathology Laboratory, University of CA − Davis (Davis, CA).

Lipase Activity

Pancreases were collected from broilers and immediately frozen in liquid nitrogen, then stored at −80°C until further analysis. Tissue samples were homogenized prior to dilution at 10 × following manufacturer's protocols for an assay kit to measure lipase activity (MAK046, Sigma-Aldrich, St Louis, MO). Briefly, tissue from each pancreas was homogenized within 4 volumes of provided lipase assay buffer then centrifuged at 13,000 × *g* for 10 min to remove any insoluble material (Micro-centrifugette 4214, Astel Enterprises, Inc., Waltham, MA). Sample and buffer solution were added to the reaction mixture provided and mixed via pipetting. Plates were incubated at 37°C in the dark for 2 min prior to measuring absorbance at 570 nm using a BioTek Synergy HT running Gen 5 version 2.03.1 software (Agilent Technologies, Santa Clara, CA). Continuous incubation at 37°C in the spectrophotometer allowed for measurements to be taken every 10 min until the value of the most active sample was greater than the value of the highest standard. Final absorbance was the measurement prior to the most active sample exceeding the highest standard. Lipase activity was reported as nmole/min/mL = milliunit/mL. Lipase activity was calculated using the following equation:

$$\text{Lipase Activity} = \frac{B \times \text{Sample dilution Factor}}{(\text{Reaction Time}) \times V}$$

where B is the amount (nmole) of glycerol generated between T_{initial} and T_{final}, reaction time is $T_{\text{final}} - T_{\text{initial}}$ (minutes), and V is the sample volume (mL) added to the well.

Liver Weight and Uric Acid in Liver

Livers were collected from the same 3 broilers per replication euthanized for the determination of lipase activity. Whole livers were lightly dried with a clean paper towel, weighed, immediately frozen in liquid nitrogen, and stored at −80°C until analyzed. Tissue samples from the livers were homogenized within 4 volumes of UA assay buffer (provided) to dilute 10× following manufacturer's protocols to measure UA content (K608, Biovision Inc., Milpitas, CA). Samples were centrifuged (Micro-centrifugette 4214, Astel Enterprises, Inc., Waltham, MA) at 13,000 × *g* for 10 min to remove any insoluble material. Sample and buffer solutions were added to the preprepared reaction mixture (provided) and mixed via pipetting. Plates were incubated at 37°C in the dark for 30 min prior to measuring absorbance at 570 nm using a BioTek Synergy HT running Gen 5 version 2.03.1 software (Agilent Technologies, Santa Clara, CA). UA was reported as UA mg/g of liver. UA concentration was calculated using the following equation:

$$\text{Uric Acid Concentration} = \frac{A}{V} \times 1000$$

where A is the UA amount from the sample well in nmol, V is the sample volume added into the sample well in microliters, and molecular weight of UA is considered to be 168 g/mol.

NH_4^+ Concentration in Manure

Paper was placed in each pen on d 44 for a minimum of 2 h to collect manure. Fresh fecal droppings from each pen were pooled. Care was taken not to collect cecal droppings. Samples were weighed, dried for 24 h at 55°C, and stored at −20°C until analysis (Naseem et al., 2020). Concentration of NH_4^+ was measured by using a commercially available enzymatic colorimetric assay kit (K370-100, Biovision Inc., Milpitas, CA). Dried manure was homogenized within 4 volumes of provided assay buffer then centrifuged at 13,000 × *g* for 10 min to remove any insoluble material (Micro-centrifugette 4214, Astel Enterprises, Inc., Waltham, MA). Sample and buffer solutions were added to the reaction mixture provided and mixed via pipetting. Plates were incubated at 37°C in the dark for 60 min prior to absorbance reading at 570 nm using a BioTek Synergy HT running Gen 5 version 2.03.1 software (Agilent Technologies, Santa Clara, CA). Reported NH_4^+ was expressed in mM and was calculated using the following equation:

$$NH_4^+ \text{ Concentration} = \frac{S_a}{S_v}$$

where S_a is the sample amount in nmol from standard curve, S_v is the sample volume (μL) added into the wells, and the molecular weight of NH_4^+ is considered to be 18.04 g/mol.

Digestibility of AA

As noted above, AA analysis of feed was conducted (Table 2; Cumberland Valley Analytical Services; Waynesboro, PA). On d 8, 15, 22, 29, 36, and 43, all birds were fasted overnight. To determine digestibility, 3 randomly selected birds from each replicate were fed for 2 h with their corresponding treatment plus 0.3% titanium dioxide, as an inert marker. Digesta from the ileum, defined as the area from Meckel's diverticulum to 40 mm above the ileo-cecal junction, was collected by squeezing the contents into 1.5 mL microtubes. The digesta samples were stored at −20°C until analysis.

To determine titanium oxide concentration, 1 g of the digesta from each of the 3 birds per replication was pooled and dried for 15 h at 72°C in a vacuum oven (Precision Scientific Group, Chicago, IL). Dried samples were weighed in aluminum crucibles, then transferred to a muffle furnace (Thermolyne, Thermo Scientific, Headquarters, Waltham, MA). Replicate samples were heated to 575°C and maintained for 12 h then cooled to 200°C for 12 h. Samples were stored in a desiccator prior to determination of TiO_2 concentration using the procedures outlined by Short et al. (1996).

To determine the AA concentration of the digesta, 1 g of the digesta from each of the 3 birds per replication was pooled and provided to the Molecular Structure Facility Proteomics Core, University of CA − Davis (Davis, CA). Samples were dried in a hydrolysis tube to determine the dry mass prior to liquid phase hydrolysis using 6N HCl and 1% phenol at 110°C for 24 h in vacuo. Once cooled, the sample was dissolved in sample solution buffer then measured using the ion-exchange column of Hitachi 8800-A (Hitachi, Ltd., Tokyo, Japan).

Apparent ileal digestibility was determined using the following equation (Adeola et al., 2016):

$$\text{Apparent ileal digestibility} = 100 - \left(\frac{I_{feed}}{I_{digesta}} \times \frac{AA_{digesta}}{AA_{feed}}\right) \times 100$$

where AA is amino acid of interest and I is the inert marker (TiO_2).

Statistical Analysis

Analysis of data was performed in R software with version 4.0.3 (RCore Team, 2020). For all data, diet and time were considered fixed effects in the model. Time represented each of the grow-out periods (starter, grower, and finisher). Each pen was considered a replicate and an experimental unit for biochemical measurements and ammonia emission. In the absence of a replication effect, individual birds were the experimental unit for BW and liver weights. All data and residuals of models were tested for normality using the Shapiro-Wilk's test and visualized by residual plots using qqnorm. NLME, emmeans, and multicomp were used to conduct a single-step multiple comparison procedure, ANOVA, using the linear model (**lm**) function. The Tukey test was used to determine significance of pairwise differences of means using the LSMEANS and compact letter display (**cld**). Significance was determined as $P \leq 0.05$.

RESULTS AND DISCUSSION

Performance Measures

Feed Consumption. This parameter increased significantly over time ($P = 2.00 \times 10^{-16}$). However, feed consumption among treatments was not significantly different within the same week (Table 3). Total feed consumption across all weeks was not significantly different among treatments (Figure 1).

Weight Gain. Total weight gain was not significantly different among treatments; however, there were temporal differences. SWL was numerically higher compared to all treatments and during wk 2, SWL was significantly higher than CON ($P = 0.013$; Table 3). This finding could indicate that birds fed SWL started to gain more weight in wk 2 although the significant effect for BW was not apparent until wk 5. Results for the present study are consistent with those of other studies reporting a significant increase in growth for broilers fed diets containing *S. cerevisiae* (Haldar et al., 2011; Bai et al., 2013; Kaushal et al., 2019). Contrarily, another study with 7-day-old Arbor Acres broiler chicks fed *S. cerevisiae* did not have a different growth rate compared to the control (Hussein and Selim, 2018). The finding for Hussein and Selim (2018) along with that

Table 3. Performance parameters of broilers.

Week	Broiler performance								*P* value
	CON		LPR		SCY		SWL		
	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	
Feed consumption									0.976
1	0.109^{a}	0.041	0.138^{a}	0.041	0.121^{a}	0.041	0.133^{a}	0.041	
2	0.412^{b}	0.041	0.425^{b}	0.041	0.433^{b}	0.041	0.415^{b}	0.041	
3	0.561^{b}	0.041	0.560^{b}	0.041	0.543^{b}	0.041	0.544^{b}	0.041	
4	0.782^{c}	0.041	0.813^{cd}	0.041	0.822^{cde}	0.041	0.837^{cde}	0.041	
5	1.028^{def}	0.041	1.088^{fg}	0.041	1.049^{ef}	0.041	1.133^{fg}	0.041	
6	1.231^{fgh}	0.041	1.297^{gh}	0.041	1.286^{gh}	0.041	1.367^{h}	0.041	
FCR									0.018
1	1.274^{a}	0.076	1.518^{abcde}	0.076	1.478^{abc}	0.077	1.435^{ab}	0.075	
2	1.843^{cdefgh}	0.081	$1.761^{bcdefgh}$	0.079	1.856^{cdefgh}	0.081	$1.665^{abcdefg}$	0.079	
3	1.590^{abcdef}	0.085	1.554^{abcde}	0.085	1.541^{abcde}	0.088	1.485^{abcd}	0.084	
4	$1.610^{abcdefg}$	0.092	$1.680^{abcdefg}$	0.093	$1.639^{abcdefg}$	0.096	1.636^{abcdef}	0.091	
5	1.930^{cdefgh}	0.100	1.967^{efgh}	0.102	$1.837^{bcdefgh}$	0.118	1.946^{defgh}	0.099	
6	2.091^{fgh}	0.111	2.120^{gh}	0.114	2.780^{i}	0.118	2.232^{hi}	0.110	
Weight gain									0.302
1	89.295^{a}	8.115	97.131^{a}	8.115	88.333^{a}	8.299	95.950^{a}	7.999	
2	230.713^{b}	8.671	247.403^{bc}	8.530	240.506^{bc}	8.744	279.403^{c}	8.462	
3	359.849^{d}	9.140	369.628^{d}	9.098	363.469^{d}	9.452	372.196^{d}	8.976	
4	497.301^{e}	9.849	504.689^{e}	10.013	513.845^{ef}	10.364	523.674^{ef}	9.746	
5	545.821^{efg}	10.755	565.230^{fgh}	10.968	591.943^{ghi}	11.353	598.313^{ghi}	10.620	
6	605.540^{hi}	11.967	624.083^{hi}	12.263	629.571^{i}	12.693	643.431^{i}	11.782	
Body weight									3.651×10^{-5}
0	45.581^{a}	11.293	45.554^{a}	11.255	45.395^{a}	11.293	45.416^{a}	11.255	
1	134.971^{b}	11.738	142.724^{b}	11.653	133.648^{b}	11.912	141.439^{b}	11.529	
2	366.451^{c}	12.542	389.605^{c}	12.338	373.784^{c}	12.648	421.624^{c}	12.234	
3	725.611^{d}	13.220	757.844^{d}	13.159	737.671^{d}	13.673	763.813^{d}	12.982	
4	1218.774^{e}	14.347	1263.308^{e}	14.402	1247.798^{e}	14.990	1291.432^{e}	14.096	
5	1764.488^{f}	15.361	1843.240^{fg}	15.864	1848.870^{g}[1]	16.540	1889.063^{g}	15.361	
6	2383.349^{h}	17.309	2480.323^{i}	17.448	2471.929^{hi}	18.359	2534.462^{i}	17.041	

Treatments included no alternations to the mash basal diet (CON); probiotic *Saccharomyces cerevisiae* inclusion rate at 4.26×10^{6} CFU/kg (SCY); probiotic *Lactobacillus plantarum* and *L. rhamnosus* inclusion rate at 4.35×10^{8} CFU/kg (LPR); and a combination of probiotics *L. plantarum, L. rhamnosus, Saccharomyces cerevisiae* inclusion rate of 4.35×10^{8} CFU/kg and 4.26×10^{6} CFU/kg, respectively (SWL).

Means with different superscript letters (a, b, c, d, e, f, g, h, i) differ significantly ($P \leq 0.05$).

[1]Actual *P* value is 0.0601 thus not considered significant.

from the present study could suggest that *S. cerevisiae* alone does not improve sustained BW gain.

BW. During the first 4 wk, all treatments maintained a similar BW ($P > 0.05$). As noted above at wk 5, CON had a significantly lower BW than SWL ($P = 0.0008$) and trends for lower BW than LPR ($P = 0.0799$) and SCY ($P = 0.0601$) (Table 3). However, during wk 6, CON was significantly lower than SWL ($P = 0.0124$) and LPR ($P = 0.0223$), with a trend for lower BW for SCY ($P = 0.0971$). While SCY had significantly higher BW than other treatments in wk 5, it was not significantly different from that of other treatments in wk 6, though it remained numerically higher than CON. CON had lower weight gain in wk 2 ($P = 0.013$) compared to all treatment and the lowest BW in wk 5 ($P = 0.0008$) and wk 6 ($P = 0.0124$) compared to SWL. A higher BW is consistent with previous findings when feeding *S. cerevisiae* to broilers and layers (Haldar et al., 2011; Bai et al., 2013; Sun et al., 2014; Hussein and Selim, 2018; Hoque et al., 2021). Cobb 400 broilers fed yeast (unspecified CFU) had higher BW on d 21 and d 35 of the grow-out period (Haldar et al., 2011). All male 1-day-old broilers supplemented with a combination of probiotics at 2×10^{6} CFU/g of *S. cerevisae* and 1×10^{7} CFU/g of *Lactobacillus fermentum* had higher BW at d 21 but not at d 42 (Bai et al., 2013). Arbor Acres broiler chicks given 8×10^{9} CFU/g of *S. cerevisae* in combination with 1×10^{9} CFU/g of *Lactobacillus acidophilus* had greater BW on d 35 compared to the control, but not when either probiotics was fed alone. Findings for BW in the present study were inconsistent with those of others in wk 6 (Mansour et al., 2011; Adebiyi et al., 2012).

Gao et al. (2008) suggested that lower concentrations of yeast culture provided significant increase in body weight; however, like many other studies, the CFU/kg of the yeast or yeast culture was not reported (Hassanein and Soliman, 2010; Haldar et al., 2011; Mansour et al., 2011; Adebiyi et al., 2012; AL-Zuhairi et al., 2014; Sun et al., 2014; Gde Bidura et al., 2016; Hussein and Selim, 2018; Azizi et al., 2021; Hoque et al., 2021). Thus, these results without CFU/kg are not highly useful in determining quantity and effective time frames for administration of probiotics. Additionally, it is important for researchers to clearly differentiate between yeast culture, metabolites, and yeast strains, possibly reducing contradictory information. Further evaluations of the CFU/kg inclusion rate and its effect on BW will help to determine the optimal concentration of yeast probiotic to include in the feed.

Treatments that included *Lactobacillus* species at 4.35×10^{8} CFU/kg in the present study numerically improved BW at 6 wk of age. The results were consistent with other studies evaluating a variety of *Lactobacillus* strains in diets that improved BW (Peng et al., 2016;

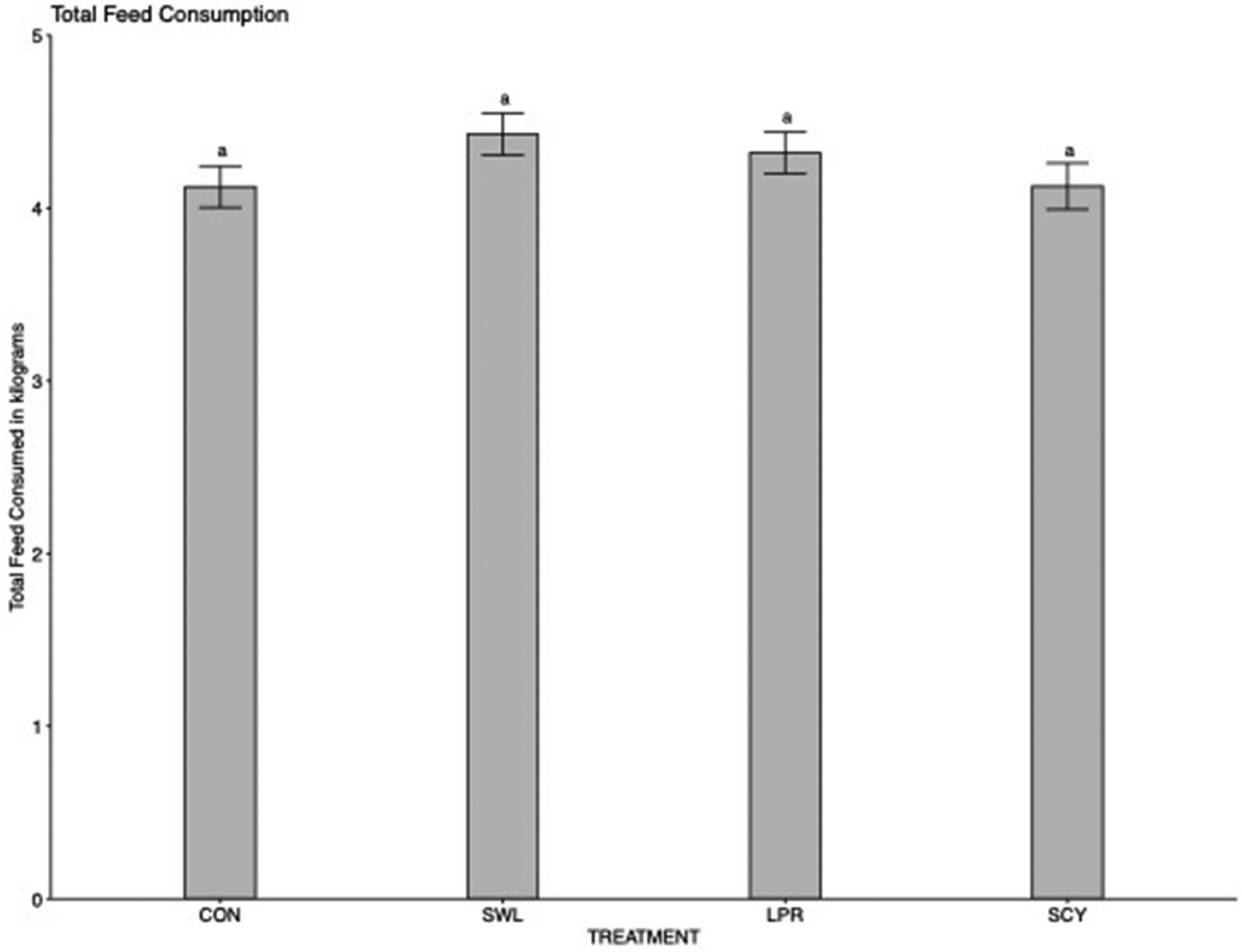

Figure 1. Total individual feed consumption of Cobb 500 broiler chickens during a 6-wk growth period. Treatments included the mash basal diet (CON); probiotic *Saccharomyces cerevisiae* inclusion rate at 4.26×10^6 CFU/kg (SCY); probiotic *Lactobacillus plantarum* and *L. rhamnosus* inclusion rate at 4.35×10^8 CFU/kg (LPR); and a combination of probiotics *L. plantarum, L. rhamnosus, Saccharomyces cerevisiae* inclusion rate of 4.35×10^8 CFU/kg and 4.26×10^6 CFU/kg, respectively (SWL). Means with different superscript letters (a, b) differ significantly ($P \leq 0.05$).

Fesseha et al., 2021). However, many researchers found no difference in BW when feeding *Lactobacillus* strains for the complete grow-out period (Awad et al., 2009; Foltz et al., 2017; Pender et al., 2017; Ding et al., 2019). For instance, Such et al. (2021) noted that feeding *Lactobacillus farciminis* at an inclusion rate of 5.0×10^6 CFU/kg did not improve production measures of broilers. Several studies with no improvement for BW did not report CFU/kg of feed or had lower CFU/kg ($\sim 3.35 \times 10^8$) than that in the present study, which may suggest that there is a minimum inclusion rate required to improve BW (Awad et al., 2009; Adebiyi et al., 2012; Foltz et al., 2017; Ding et al., 2019; Sugiharto et al., 2019; Fesseha et al., 2021; Hoque et al., 2021).

Overall, results in the present study suggested a possible cumulative effect on weight gain and BW for SCY and SWL. Effects for these treatments were significantly greater than CON by wk 5 but were not sustained in wk 6. These findings, along with those of other investigators, indicate that in a future study, levels of *S. cerevisiae* and *Lactobacillus* spp. should begin at a magnitude at 10^6 CFU/kg and extend 10^9 CFU/kg to determine the range that is adequate to sustain an increase in weight gain from 1 to 6 wk of production. In addition to indicating the quantity of microbes producing increased performance during the grow-out period, information learned from the proposed study may also show the week where improvement begins and is sustained throughout the 6-wk period.

FCR. This measurement slightly increased numerically for all treatments over time, though it was only significant when comparing wk 1 to wk 6 (Table 3). The overall FCR for wk 1 was 1.35 and 2.50 for wk 6. During wk 6, SCY was significantly higher than CON and LPR but not SWL ($P = 0.02$). This finding was inconsistent with other studies providing *Lactobacillus* spp. or *S. cerevisiae* as a feed additive for broilers (Awad et al., 2009; Hassanein and Soliman, 2010; Adebiyi et al., 2012; Bai et al., 2013; Peng et al., 2016; Foltz et al., 2017; Hussein and Selim, 2018; Fesseha et al., 2021; Hoque et al., 2021). Awad et al. (2009) provided 1-day-old Ross 306 broilers *Lactobacillus* spp. at 10^8 CFU/kg over 35 d and noted that birds produced a higher BW with a lower FCR. Seven-day-old Arbor Acres Plus

broilers chicks fed *L. plantarum* at 2×10^9 CFU/kg over 42 d had a lower FCR compared to the control (Peng et al., 2016).

Yeast culture (a mixture of unidentified strains) is sometimes preferred for use in broiler feed due to low cost (Phaff, 2022). Investigators who have provided yeast culture to 1-day old Ross 308 broiler chicks for 35 d reported no difference between the treatment and control; however, they did not provide the *critical* CFU for combined yeast species, making comparisons of results from other studies impossible (Hoque et al., 2021). Hussein and Selim (2018) who fed 7-day-old Arbor Acres Plus broilers yeast (*S. cerevisiae* containing 8×10^9 CFU/g) and a combination of other microorganisms (*L. acidophilus* at 1×10^9 CFU/g, *Bacillus subtilus* at 1×10^9 CFU/g, and *Aspergillus oryzae* at 2×10^7 CFU/g) for an additional 35 d reported a lower FCR, but not when the microorganisms were fed separately. As noted above, studies with inclusions rates from 10^6 CFU/kg to 10^9 CFU/kg feed for slow and fast growing broilers are needed to indicate how *Lactobacillus* spp. and/or *S. cerevisiae* improve performance measurements.

Biochemical Analyses

Serum. Albumin was not significantly different among treatments ($P = 0.90$), however, serum had significantly lowered albumin during wk 4 compared to wk 2 and 6 ($P = 0.04$) (Table 4). Other serum measurements were not different among treatments for total protein ($P = 0.87$), UA ($P = 0.73$), ammonia ($P = 0.62$) and BUN ($P = 0.40$) (Table 4). The insignificance in serum parameters from this study are consistent with those of others who provided either *Lactobacillus* spp. or *S. cerevisiae* to broilers, layers, and breeder hens (Matur et al., 2010; Chen et al., 2018; Khanian et al., 2019; Wang et al., 2019; Wu et al., 2019; Naseem and King, 2020a,b; Naseem et al., 2020; Osita et al., 2020; Azizi et al., 2021; Naseem et al., 2021). Contrary to these findings, Osita et al. (2020) noted that *S. cerevisiae* lowered serum albumin in broilers compared to the control. Azizi et al. (2021) noted that 1-day-old female broiler chicks provided with *Lactobacillus* spp. combined with *S. cerevisiae* lowered serum albumin and BUN; however, they did not note the CFU/kg for *S. cerevisiae* for comparison. Chen et al. (2018) found that *L. rhamnosus* at 10^{10} CFU/g fed to 7-day-old male White Leghorns reduced ammonia content in serum. The serum measurements from the current study suggested that the concentration of probiotics did not affect AA digestibility and synthesis within the bird's body.

UA in Liver. As ammonia increases, UA increases in the liver and excreta (Okumura and Tasaki, 1969). For the present study, UA in the liver increased for all groups over time ($P = 0.0005$) with LPR having the lowest rate of change from 4.64 mg to 10.26 mg; however, there were no significant differences among treatments (Table 5). To our knowledge, there are few or no reported results on the effect of *Lactobacillus* spp. and *Saccharomyces cerevisiae* on UA in broiler liver tissue; however, there are comparable results for *Lactobacillus* spp. in layers. In 52- to 62-wk-old White Leghorn laying hens, a combination of different *Lactobacillus* spp. did not alter UA concentration in the liver (Naseem and King, 2020a). Dietary treatments with 0, 1, 2, 3, and 4 g/kg of yeast autolysate and *S. cerevisiae* fed to 1-old-day Ross 308 broiler chicks until 42-days-old did not have a significant effect on serum levels and several blood parameters including UA (Yalcin et al., 2010).

Table 4. Biochemical analyses measurements.

Serum parameters									
Week	CON		LPR		SCY		SWL		*P* value
Serum	Mean	SE	5Mean	SE	Mean	SE	Mean	SE	
Albumin	—————		g/dL				—————		0.901
2	1.334	0.058	1.280	0.065	1.343	0.0645	1.350	0.074	
3	1.216	0.058	1.230	0.058	1.200	0.0576	1.206	0.058	
4	1.212	0.058	1.298	0.058	1.290	0.0576	1.312	0.058	
Total protein	—————		g/dL				—————		0.866
2	2.572	0.127	2.428	0.142	2.635	0.142	2.683	0.164	
4	2.424	0.127	2.576	0.127	2.470	0.127	2.482	0.127	
6	2.432	0.127	2.584	0.127	2.546	0.127	2.554	0.127	
Uric acid	—————		mg/dL				—————		0.727
2	15.790	1.125	12.566	1.258	14.903	1.258	14.373	1.453	
4	13.886	1.125	14.156	1.125	13.974	1.125	13.654	1.126	
6	11.704	1.125	12.274	1.125	11.576	1.125	11.012	1.125	
Ammonia	—————		μg/dL				—————		0.616
2	2459.000^{abc}	266.679	2566.750^{bc}	298.156	2895.125^{c}	298.156	2906.833^{c}	344.280	
4	1711.700^{abc}	266.679	1823.800^{abc}	266.679	1710.200^{abc}	266.679	1824.200^{abc}	266.679	
6	1346.100^{ab}	266.679	1164.900^{a}	266.679	2031.000^{abc}	266.679	1346.100^{ab}	266.679	
BUN	—————		mg/dL				—————		0.405
2	3.00^{bcd}	0.240	2.95^{bcd}	0.268	3.60^{cd}	0.268	4.00^{d}	0.309	
4	2.38^{abc}	0.240	2.24^{ab}	0.240	2.48^{abc}	0.240	2.22^{ab}	0.240	
6	1.44^{a}	0.240	1.40^{a}	0.240	1.68^{a}	0.240	1.58^{a}	0.240	

Treatments included no alternations to the mash basal diet (CON); probiotic *Saccharomyces cerevisiae* inclusion rate at 4.26×10^6 CFU/kg (SCY); probiotic *Lactobacillus plantarum* and *L. rhamnosus* inclusion rate at 4.35×10^8 CFU/kg (LPR); and a combination of probiotics *L. plantarum, L. rhamnosus, Saccharomyces cerevisiae* inclusion rate of 4.35×10^8 CFU/kg and 4.26×10^6 CFU/kg, respectively (SWL).

Means with different superscript letters (a, b, c, d) differ significantly ($P \leq 0.05$).

Table 5. Lipase activity and liver parameters.

Week	CON		LPR		SCY		SWL		*P* value
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Lipase activity	—————		milliunits/mL				—————		1.000
2	0.576	0.167	0.582	0.167	0.570	0.167	0.541	0.173	
4	0.567	0.167	0.546	0.167	0.570	0.167	0.578	0.167	
6	0.581	0.167	0.615	0.167	0.602	0.167	0.605	0.167	
Liver weight	—————		Grams				—————		0.298
2	14.220^{a}	1.183	14.753^{a}	1.183	12.988^{a}	1.145	14.279^{a}	1.224	
4	29.713^{b}	1.183	28.260^{b}	1.183	29.267^{b}	1.183	31.660^{b}	1.183	
6	47.820^{c}	1.183	50.433^{c}	1.183	51.033^{c}	1.183	50.240^{c}	1.183	
UA in liver	—————		Milligrams				—————		0.545
2	3.654^{a}	5.170	4.639^{a}	5.170	2.856^{a}	5.170	3.877^{a}	5.351	
4	7.591^{ab}	5.170	9.079^{ab}	5.170	14.525^{ab}	5.170	9.861^{ab}	5.170	
6	13.127^{ab}	5.170	10.277^{ab}	5.170	29.325^{b}	5.170	21.144^{ab}	5.170	

Treatments included no alternations to the mash basal diet (CON); probiotic *Saccharomyces cerevisiae* inclusion rate at 4.26×10^{6} CFU/kg (SCY); probiotic *Lactobacillus plantarum* and *L. rhamnosus* inclusion rate at 4.35×10^{8} CFU/kg (LPR); and a combination of probiotics *L. plantarum, L. rhamnosus, Saccharomyces cerevisiae* inclusion rate of 4.35×10^{8} CFU/kg and 4.26×10^{6} CFU/kg, respectively (SWL).

Means with different superscript letters (a, b, c) differ significantly ($P \leq 0.05$).

However, Sugiharto et al. (2019) reported that formic acid (2.0%), *S. cerevisiae* and formic acid (2.0%), and *S. cerevisiae* increased concentration of serum UA when compared with the control for Indonesian crossbred chicken.

UA is formed in the liver and excreted from the body by the kidneys when excess nitrogen is consumed. It was reported that UA can be further metabolized into ammonia by microbes in the GI tract in avian species to form nonessential AA once reabsorbed (Vispo and Karasov, 1997). This process mostly occurs in the cecum, where UA degrading bacteria are in high abundance. However, there is limited information supporting the recycling of nitrogen into AA for avian species (Singer, 2003).

Serum UA and apparent digestibility of AA were not significantly different among treatments under unstressed conditions. Similar results were reported by Okumura and Tasaki (1969) for 5-mo leghorn cockerels provided varying levels of protein. Probiotics supplements (*Lactobacillus* or *S. cerevisiae*) in the present study, did not influence UA synthesis within the liver. Birds fed high protein diets, as in the present study, can consume more nitrogen than required, leading to UA being formed in the liver from the excess nitrogen present (Swelum et al., 2021). However, the UA is deposited into the blood stream, where the kidneys of avian species will collect and expel it from the body via the intestines. In monogastric animals, such as humans, high levels of UA in the blood can lead to several diseases such as gout, inflammatory disease, kidney disease, cardiovascular disease, and hypertension (Kanbay et al., 2016; Wu et al., 2020).

While results suggested that the probiotics provided in the present study did not affect UA levels, more work needs to be done to determine if higher concentrations of probiotics could be important for lowering disease risks in flocks and increasing protein utilization within the ceca. In broiler production, healthy livers are included in a pack of giblets that are placed in the body cavity of the bird for retail. Thus, if probiotics could reduce UA in the liver, from a human health standpoint, this information could alter the recommendation that foods having high levels of purine, such as chicken liver, not be ingested in excess quantities (de Oliveira and Burini, 2012).

Lipase Activity. Lipid digestion and absorption is hampered in young broilers due to a deficiency in lipase production. It may take several weeks after hatching for lipase to increase in broilers (Noy and Sklan, 1995; Al-Marzooqi and Leeson, 2000). Therefore, lipase activity can be used to determine overall fat digestion during the sixth week production of broilers (Klasing, UC Davis, Davis, CA, personal communication). Lipase activity remained consistent over time with no significant difference among treatments (Table 5). These results are consistent with results of others who showed no difference in lipase activity induced by *Lactobacillus* spp. (Jin et al., 2000; Qing et al., 2017). Other researchers noted that *Lactobacillus* spp. induced more lipoprotein lipase inhibitor than that of *Bifidobacterium,* thus lowering the lipase activity and reducing fat storage in mice (Aronsson et al., 2010). This finding by Aronsson et al. (2010) also suggested that some *Lactobacillus* spp. may be more effective than others in influencing lipase activity.

Investigators noted that in breeding hens, *S. cerevisiae* extract (containing unknown strains) reduced lipase activity compared to the control but improved the lipase activity in feed contaminated with aflatoxins (Matur et al., 2010). This finding suggested that the *S. cerevisiae* probiotic does not influence lipase activity as well as an extract with unknown strains of *S. cerevisae.* Thus, care must be taken to clearly identify active ingredients in all extracts. Lipase activity may change with varying levels of fat in the diet; however, in the present work soybean oil was consistently 1.8% of all diets. Thus, no change by any combination of probiotics suggest that lipids were digested at the same rate over time regardless of treatment.

Liver Weights. Over time, all liver weights increased ($P = 2.0 \times 10^{-16}$) with no significant differences among

treatments (Table 5). Thus, the results were consistent with that of other studies indicating that neither *Lactobacillus* nor *S. cerevisiae* influenced liver weight (Matur et al., 2010; Brzóska et al., 2012; Khanian et al., 2019; Slizewska et al., 2019). Results for liver weights in the present study were inconsistent with that of Osita et al. (2020), who observed larger liver weights when chicks were provided *S. cerevisiae*. However, the concentration was recorded at g/kg (0.7, 1.2, and 1.7 g/kg of *S. cerevisiae* supplement) without providing a CFU/kg for comparison to the present study. This finding could suggest that lower CFU/kg, similar to the present study, does not impact the liver weight and that as noted above, greater magnitude of combined probiotics should be investigated.

Manure NH_4^+

In the current study, concentration of NH_4^+ in the manure was numerically higher for CON in wk 6 compared to the other treatments (Figure 2). While there were no significant differences ($P \leq 0.05$) observed among treatments, this finding indicated a possible beginning, but unsustainable influence, for all probiotic treatments. Results of the present study were consistent with results of other studies that provided only *Lactobacillus species (L. rhamnosus, L. paracasei*, and *L. plantarum)* to White Leghorns (32−40 and 65−74 wk). These studies found no significant difference between NH_4^+ for layers provided the probiotics and the control (Naseem and King, 2020b; Naseem et al., 2020, 2021). Again, our results as well as those of others suggest that feeding probiotics in excess of 10^6 CFU/kg is warranted.

In contrast to reporting NH_4^+, many researchers evaluated effects on ammonia emissions by several single species of probiotics including *Lactobacillus* spp. alone, *Bacillus* spp. and *Lactobacillus* spp. combined with other species. For instance, Ahmed et al. (2014) fed *Bacillus amyliquefacins* to 1-day-old male Ross 308 broilers for 35 d (no specified CFU) and revealed that ammonia emissions were lower than that of the control. Results of another study also reported lowered ammonia emissions compared to the control when feeding 1×10^9 CFU/g of *Bacillus subtilis* to 1-day-old male Ross 308 broilers for 35 d (Jeong and Kim, 2014). *Lactobacillus reuteri* at an inclusion rate of 10^9 CFU/g supplemented in diets of 1- to 37-day-old Arbor Acres broilers decreased ammonia emissions compared to the control (Liu et al., 2007). Additionally, in a study by Such et al. (2021) where *Lactobacillus farciminis* at 5×10^9 CFU/kg was provided for 40 d to Ross 308 broilers, ammonia concentrations from excreta were numerically lower than the control. The results from Such et al. (2021) and the present study were inconsistent with the results from Chen et al. (2018) who noted that

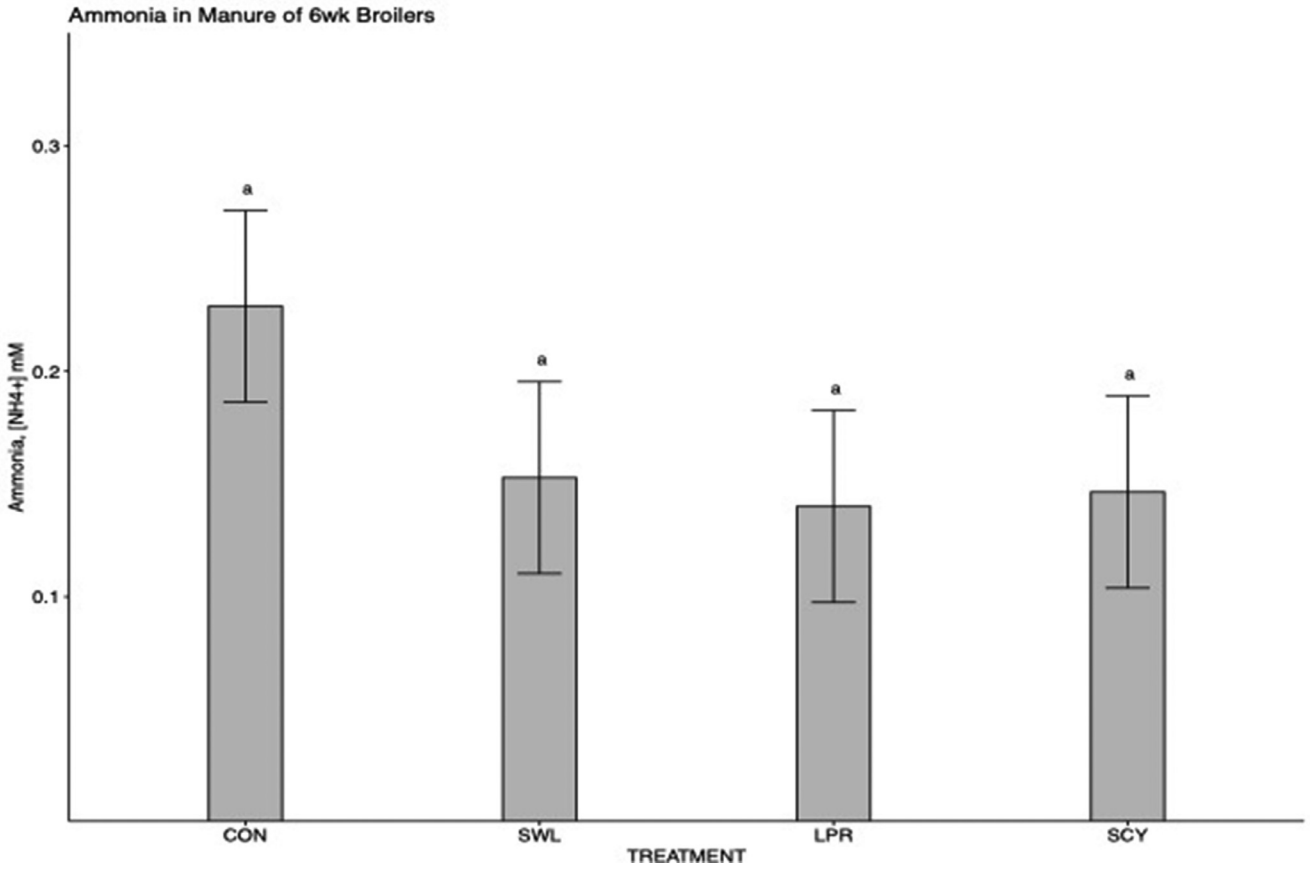

Figure 2. Manure ammonia levels Cobb 500 broiler chickens at the end of the finisher phase. Treatments included no alternations to the mash basal diet (CON); probiotic *Saccharomyces cerevisiae* inclusion rate at 4.26×10^6 CFU/kg (SCY); probiotic *Lactobacillus plantarum* and *L. rhamnosus* inclusion rate at 4.35×10^8 CFU/kg (LPR); and a combination of probiotics *L. plantarum, L. rhamnosus, Saccharomyces cerevisiae* inclusion rate of 4.35×10^8 CFU/kg and 4.26×10^6 CFU/kg, respectively (SWL). Treatments without superscripts do not differ significantly at $P \leq 0.05$).

providing *Lactobacillus rhamnosus* to 7-day-old male White Leghorns at 2×10^8 CFU/g for 24 d significantly lowered ammonia emissions compared to the control.

A study in which 5-wk-old White Leghorns were fed a mixture of *Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces*, and *Candida* spp. at 10^{7-8} CFU/g for 4 wk had lower numerical ammonia emissions compared to the control but was not significantly different from other treatments (Endo et al., 1999). In contrast, researchers fed *Lactobacillus* alone at 10^7 CFU/g and in combination with *S. cerevisiae* (unspecified CFU) and bacteriocin (1 mg/g *S. cerevisiae*) to Arbor Acres broilers for 35 d and reported significantly lowered ammonia emissions compared to the control (Chen et al., 2017). As well, Cobb broilers fed a mixture of *Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces*, and *Candida* spp. at 10^{7-8} CFU/g for 35 d also had lower emissions compared to the control (Endo and Nakano, 1999). Upadhaya et al. (2019) reported that 1-day-old Ross 308 broilers fed *Bacillus subtilis* at 8×10^5 CFU/kg for 6 wk had lower ammonia emission compared to the control.

In the findings above, investigators utilized different breeds of birds alone with various species or strains of probiotics, and ages of animals with little overlap between studies. The inconsistences within the literature could suggest that either the local environment, combination of probiotics, or breed of bird influences the effectiveness of the probiotics to lower concentration of NH_4^+ or ammonia emissions. Additionally, the methodology used could contribute to lack of differences as previous studies capturing gases from wet manure by various methods also reported significant differences (Endo and Nakano, 1999; Endo et al., 1999; Liu et al., 2007; Ahmed et al., 2014; Jeong and Kim, 2014; Chen et al., 2017; Upadhaya et al., 2019). Drying the manure may not be the most efficient procedure for measuring ammonia, due to significant losses of ammonia occurring during handling and storage (Chastain et al., 2022). Research focusing on specific probiotic strains, well-defined inclusions rates that include CFU/kg of feed preferably at higher quantities and surrounding environmental analyses (ammonia and microbes) are needed to ascertain the effectiveness of specific quantities of probiotics to lower ammonia emissions in chicken manure.

Apparent Ileal Digestibility

Neither serum UA (above) nor apparent digestibility of AA (Table 6) were significantly different among treatments. For use of single probiotics, Upadhaya et al. (2019) reported that *Bacillus subtilis* at 8×10^5 CFU/kg diet fed to 1-day-old Ross 308 broilers for 6 wk did not affect crude protein digestibility. On the other hand, 1-day-old Ross 308 broilers fed *Bacillus amyloliquefaciens* at an inclusion rate of 1×10^6 CFU/g for 1 to 35 d had an increase in digestibility of AA (Gharib-Naseri et al., 2021). Poberezhets et al. (2021) noted that when 1-day-old Ross 308 broilers were supplemented with *Lactobacillus bulgaricus* and *Enterococcus faecium*, both at 2.0×10^{10} CFU/kg, for 42 d, digestibility improved for AA compared to the control. As well, researchers noted that when 1-day-old Ross 308 broilers were supplemented with *S. cerevisiae* at an inclusion rate of 2×10^9 CFU/g for 35 d, digestibility increased for Leu, Met, Phe, Ser, and decreased for His,

Table 6. Percent apparent ileal digestibility of amino acids.

	Apparent digestibility %												
	CON			LPR			SCY			SWL			
Week	2	4	6	2	4	6	2	4	6	2	4	6	*P* value
Essential													
Arginine	69.488	32.247	64.901	33.017	***	49.426	***	36.212	46.132	14.426	***	35.735	0.7384
Cystine	***	***	***	***	***	***	***	***	***	***	***	***	***
Lysine	43.316^{ab}	45.886^{ab}	69.039^{b}	52.896^{ab}	25.544^{a}	51.198^{ab}	42.466^{ab}	46.104^{ab}	45.609^{ab}	40.841^{ab}	32.725^{ab}	44.676^{ab}	0.2859
Methionine	***	***	***	***	***	***	***	***	***	***	***	***	***
Tryptophan	***	***	***	***	***	***	***	***	***	***	***	***	***
Glycine	56.833	49.526	64.185	57.124	30.895	49.301	56.629	62.661	45.744	50.675	36.288	40.137	0.2630
Histidine	61.370	50.106	72.979	53.317	40.872	60.835	58.888	63.630	59.460	53.303	45.560	55.941	0.7681
Leucine	45.115	53.000	69.807	45.333	33.656	56.606	45.493	56.104	49.728	40.286	35.035	51.928	0.5382
Isoleucine	53.778^{ab}	58.172^{ab}	73.372^{b}	54.669^{ab}	32.603^{a}	61.755^{ab}	54.965^{ab}	60.838^{ab}	57.923^{ab}	50.487^{ab}	33.533^{a}	56.451^{ab}	0.2634
Phenylalanine	47.944	57.470	72.287	48.352	32.917	61.364	48.817	59.443	55.086	44.967	40.471	56.070	0.4207
Threonine	61.981^{ab}	56.496^{ab}	76.883^{b}	62.069^{ab}	33.062^{a}	67.733^{b}	62.624^{ab}	68.230^{b}	65.986^{b}	55.254^{ab}	45.293^{ab}	61.682^{ab}	0.1383
Valine	52.235	46.548	72.132	52.998	40.343	60.734	54.369	58.151	56.239	47.040	34.044	54.437	0.6438
Nonessential													
Alanine	48.668	51.405	70.093	48.464	29.950	56.464	48.909	56.027	51.689	41.863	33.614	51.660	0.4164
Aspartic acid	45.847^{ab}	58.223^{ab}	72.538^{b}	49.249^{ab}	35.242^{a}	62.257^{ab}	47.343^{ab}	61.108^{ab}	57.188^{ab}	42.365^{ab}	42.799^{ab}	55.648^{ab}	0.3262
Glutamic acid	40.257	41.160	64.348	37.319	18.682	49.149	41.568	45.840	40.683	33.640	28.209	44.414	0.4999
Proline	45.216	44.532	70.755	44.744	34.134	60.701	44.354	59.416	56.043	34.502	31.110	55.107	0.5560
Serine	38.32^{ab}	55.113^{ab}	71.747^{b}	40.796^{ab}	36.257^{ab}	60.362^{ab}	43.499^{ab}	57.016^{ab}	56.268^{ab}	28.200^{a}	36.469^{ab}	54.312^{ab}	0.6376
Tyrosine	48.114^{ab}	47.756^{ab}	69.026^{b}	38.436^{ab}	25.351^{ab}	54.004^{ab}	42.276^{ab}	49.243^{ab}	47.470^{ab}	34.677^{ab}	25.874^{a}	47.775^{ab}	0.6520

Treatments included no alternations to the mash basal diet (CON); probiotic *Saccharomyces cerevisiae* inclusion rate at 4.26×10^6 CFU/kg (SCY); probiotic *Lactobacillus plantarum* and *L. rhamnosus* inclusion rate at 4.35×10^8 CFU/kg (LPR); and a combination of probiotics *L. plantarum, L. rhamnosus, Saccharomyces cerevisiae* inclusion rate of 4.35×10^8 CFU/kg and 4.26×10^6 CFU/kg, respectively (SWL).

Means with different superscript letters (a, b) differ significantly ($P \leq 0.05$).

Missing values (***) occurred when the amino acid did not survive the hydrolysis.

Ile, Lys, Thr, Val, Ala, Asp, Gly, Glu, Tyr, and Pro, though there were no significant differences for Arg, Cys, and Orn (Barbosa et al., 2018). Hy-line Brown laying hens (54−62-wk-old) supplemented with yeast culture (no CFU/kg reported; 2 g/kg diet) had no significant differences from the control for crude protein digestibility though there was numerical improvement compared to the control (Liu et al., 2021). In contrast to the present study, Barbosa et al. (2018) reported that sugarcane yeast (no specified CFU) fed to Cobb 500 broilers (1−38 d) had variability in AA digestibility dependent on the variety of yeast. Although not in broilers but in monogastric animals, results of another study for supplementing *S. cerevisiae* (unspecified CFU/kg) within the commercial product (Original XPC^{tm}, Diamond V North America, Cedar Rapids, IA) for domestic cats noted an increase in digestibility compared to the control (de Oliveira Matheus et al., 2021).

It is possible for AA to stimulate urate synthesis and, in turn, increase blood and liver synthesis of urate (Burns and Buttery, 1981). However, as discussed above, *Lactobacillus* or *S. cerevisiae* in the present study did not influence UA synthesis within the liver under unstressed conditions, indicating no likely significant difference. Thus, there being no significant differences among treatments for digestibility of AA in the present work was consistent with no significant differences between treatments for UA in the liver or serum. As noted above, there are limited studies that have investigated AA digestion within broilers when probiotics are provided. Investigations into specific probiotic strains and well-defined inclusions rates for CFU/kg diet are needed to fully ascertain the effect of probiotics on the digestibility of AA.

CONCLUSIONS AND PROPOSED FUTURE STUDIES

As the poultry industry continues to grow worldwide, excess greenhouse gases and ammonia are produced as genetic factors increase growth, body weight, and the need for protein. Researchers continue to search for ways to reduce various forms of ammonia, deleterious to birds, humans, and the environment. While many ways to reduce ammonia in grow-out facilities have been proposed, use of *L.* plantarum, L. *rhamnosus* and *S. cerevisiae* as combined probiotics in poultry diets has been proposed to 1) replace antibiotics that are restricted for use as growth promoters and 2) reduced production of ammonia in birds so that it is not released into the environment. Commercial *S. cerevisiae* (Baker's or Brewer's yeast) is a byproduct from breweries and is already commonly used in commercial poultry feed yet it is still unclear which variety and concentration is effective for improving performance measures or reducing NH_4^+.

Treatments were not significantly different for the biochemical analyses, digestibility, and NH_4^+ in the manure; however, there was significant temporal changes in performance measures for individual treatments. SWL had the highest BW starting in wk 5 compared to the CON, although there were no significant differences from LPR and SCY. While SWL had numerically highest weight gain for all weeks, it was only significantly different from CON in wk 2. This observation could suggest that probiotics initially influenced the birds' performance after wk 2; however, the quantity of probiotic was not enough to maintain higher growth throughout the grow-out period. Since there were numerically higher concentrations of NH_4^+ in the manure for CON compared to all other treatments, it could indicate that there were also initial effects on ammonia reduction for the probiotic combination. Thus, higher quantities of probiotics should be used for future studies.

Results of the present study did not fully support proposed synergistic action for a combination of *Lactobacillus* spp. and *Saccharomyces cerevisiae;* however, there was limited improvement for some parameters. Again, this observation underscores the proposed use of higher concentrations (10^6−10^9 CFU/kg) of probiotics. This proposed research with higher concentrations might indicate the first week in which improvements occur and are sustained thereafter. Research remaining for the present study includes identification of *Lactobacillus* spp. and *S. cerevisiae* in the microbiota of the digesta and ceca for each treatment, and to measure a potential immune response to probiotics, the serum heterophil: lymphocyte ratio should be determined.

Ultimately, proposed future work should identify the probiotics (*Lactobacillus* and *S. cerevisiae*) in present study that were within the digesta collected from the ileum and ceca. Additionally, further analysis including quantification of present microbes within the GI tract should be conducted to determine establishment of the probiotics. Following the quantification of microbes, the next phase of research should include how probiotics alter the serum heterophil:lymphocyte ratio to further examine potential immune responses. While higher concentrations of probiotics could have a stronger affect than in the present study, stepwise concentrations (10^6−10^9 CFU/kg) of probiotics using the same combination will ascertain the minimum inclusion needed to sustained reduction of NH_4^+ in manure and improvement of performance within the grow-out period.

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DISCLOSURES

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102525.

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