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Effects of Kombucha on Growth Performance, Antioxidant Status, Liver Function, and Gut Microbiota in Broiler Chickens Reared Under High Stocking Density

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Abstract

High stocking density (HSD) improves space efficiency in broiler production but induces oxidative stress, impairs liver function, disrupts gut microbiota, and reduces growth. This study assessed the dose-dependent effects of kombucha in drinking water (0, 5, 10, and 15 mL/100 mL) to alleviate HSD stress in Ross 308 broilers reared at control (10 birds/m²) or high (15 birds/m²) density. Five hundred chicks were assigned to 40 pens in a 4×2 factorial design (kombucha × stocking density), with five replicates per treatment. Growth performance was monitored weekly. On day 42, serum liver enzymes (AST, ALT, ALP), antioxidant markers (MDA, GPx, TAC), ileal microbiota (Lactobacillus, coliforms, total anaerobes), and intestinal morphology were evaluated. HSD significantly reduced daily weight gain (DWG; 38.39 vs. 41.99 g/bird; $P< 0.05$). Only 15 mL/100 mL kombucha significantly enhanced DWG (43.55 g/bird) and feed conversion ratio (FCR = 1.690) over 42 days ($P< 0.05$). HSD increased serum AST and ALP ($P< 0.05$), indicating liver stress; 15 mL/100 mL kombucha markedly lowered these (231.3 and 2896 U/L, respectively). Antioxidant status improved dose-dependently: 15 mL/100 mL yielded the lowest MDA (7.01 nmol/dL) and highest GPx (397.9 U/L) and TAC (64.26 U/L; $P< 0.05$). Gut microbiota responded dose-dependently, 15 mL/100 mL maximized Lactobacillus (7.81 $\log_{10}$ CFU/g) and total anaerobes (7.30 $\log_{10}$ CFU/g) while minimizing coliforms (6.40 $\log_{10}$ CFU/g). Although the villus height-to-crypt depth ratio peaked at 10 mL/100 mL (11.77), the 15 mL/100 mL dose offered the broadest benefits. Although no statistically significant kombucha × stocking density interaction was detected for DWG (P = 0.13), the numerical improvement under HSD suggests a protective effect. Kombucha supplementation at 15 mL/100 mL in drinking water is a highly effective, natural strategy to mitigate the multifaceted negative impacts of HSD in broilers, improving growth, liver health, antioxidant capacity, and gut microbial balance.

Introduction

In modern poultry production, optimizing space utilization through elevated stocking densities has become a widespread strategy to enhance economic efficiency. While this approach maximizes output per unit area, it concurrently imposes significant physiological burdens on the birds, including chronic stress, compromised immune function, and disrupted metabolic homeostasis (Simitzis et al., 2012; Qaid et al., 2016; Sugiharto et al., 2021; Janicka et al., 2025). A critical consequence of high stocking density (HSD) is the induction of oxidative stress, evidenced by elevated hepatic malondialdehyde (MDA) levels and a diminished glutathione (GSH) pool, alongside an increased heterophil-to-lymphocyte ratio, which collectively signify a state of systemic stress (Simitzis

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et al., 2012). Furthermore, HSD is known to negatively impact gut health by altering the delicate balance of the intestinal microbiota and impairing the structural integrity of the intestinal mucosa, ultimately leading to suboptimal nutrient absorption and reduced growth performance (Kridtayopas *et al*., 2019; Janicka *et al*., 2025).

The avian gastrointestinal ecosystem, harboring a complex consortium of commensal microorganisms, plays a pivotal role in host nutrition, immune modulation, and barrier function (Choct, 2009). A robust population of beneficial bacteria, such as Lactobacillus spp., is crucial for maintaining this equilibrium by competitively excluding potential pathogens and reinforcing the gut epithelial barrier (Loh & Blaut, 2012). The efficiency of nutrient uptake is directly linked to the morphological health of the intestinal villi, where any damage or atrophy can severely compromise digestive physiology (Yegani & Korver, 2008; Ohland & Jobin, 2015). Consequently, nutritional interventions aimed at modulating the gut environment, particularly through the use of synbiotics and prebiotics in feed or water, have emerged as promising non-antibiotic strategies to bolster bird welfare and productivity under intensive rearing conditions (Kridtayopas *et al*., 2019).

Kombucha, a traditional fermented tea beverage, has garnered scientific interest due to its rich profile of bioactive compounds, including organic acids, polyphenols, and a diverse array of probiotic microorganisms, classifying it as a potent synbiotic agent (Vīna *et al*., 2013; da Anunciação *et al*., 2024). Recognized as Generally Recognized as Safe (GRAS) by regulatory bodies, its consumption is associated with a range of health-promoting effects (Vīna *et al*., 2014). The fermentation process not only generates beneficial metabolites like short-chain fatty acids (SCFAs), glucuronic acid, and butyric acid—known for their roles in enhancing immune function, nourishing the gut epithelium, and mitigating oxidative damage (Malbaša *et al*., 2011; Ecklu-Mensah *et al*., 2024)—but also biotransforms tea polyphenols into more bioavailable and potent antioxidant forms (Salehi *et al*., 2021; Dechakhamphu *et al*., 2023).

Given these multifaceted properties, this study was designed to comprehensively evaluate the efficacy of supplementing drinking water with kombucha at graded levels (0, 5, 10, and 15 mL/100 mL) in alleviating the detrimental impacts of HSD on Ross 308 broilers. The investigation focused on key physiological parameters, including growth metrics, serum markers of liver function and oxidative stress, ileal microbial ecology, and intestinal morphological indices.

Materials and Methods

Preparation of Dietary Additives

The kombucha used in this study was prepared by fermenting sweetened black tea (*Camellia sinensis*) with a symbiotic culture of bacteria and yeast (SCOBY) for 14 days at 28°C. During fermentation, sucrose was hydrolyzed by yeasts into glucose and fructose, which were subsequently metabolized to ethanol and CO_2 ; the ethanol was then oxidized by acetic acid bacteria predominantly *Komagataeibacter* spp., to organic acids, primarily acetic and gluconic acids. The final fermented product had a pH of 2.9 and contained acetic acid (6.1 g/L), gluconic acid (3.3 g/L), residual sugars (glucose: 2.8 g/L; fructose: 2.5 g/L), and ethanol (<0.5% v/v), as determined by HPLC and enzymatic assays. Minor constituents included tea-derived polyphenols and B-group vitamins. Microbiological analysis confirmed that the bacterial population was dominated by acetic acid bacteria, with no detectable pathogenic microorganisms.

Birds and Experimental Design

A total of 500 Ross 308 broiler chicks were used to study the effects of eight treatments in five replicate pens per treatment in a 4×2 factorial arrangement in a completely randomized design (CRD). The first factor was stocking density at two levels: control (10 birds/m²) and high (15 birds/m²). The second factor was kombucha supplementation in drinking water at four levels: 0, 5, 10, and 15 mL/100 mL. The experiment was conducted during the summer of 2023 in Khorramabad/Lorestan, Iran. Ambient temperature was maintained at 33°C during the first week and gradually decreased by 2 to 3°C per week, reaching 23°C by the end of week 5. Relative humidity was kept within the range of 60% to 70% throughout the experimental period. A continuous lighting regimen (23 h light:1 h dark) was applied for the duration of the trial. Mechanical tunnel ventilation was used to maintain ammonia concentrations below 25 ppm and air velocity at bird level under 0.3 m/s. The basal diet was formulated according to Ross 308 Performance Objectives (Table 1).

Traits Assessed

Growth Performance

Body weight and feed intake were recorded weekly. Chicks were fasted for 3 to 4 hours before weighing. Feed intake (FI) was calculated by subtracting leftover feed from total feed offered. Daily weight gain (DWG), feed conversion ratio (FCR), and production efficiency index (PEI) were calculated for 1 to 14, 15 to 28, 29 to 42 and 1 to 42 days of age.

- DWG (g/bird/d) = (Final BW – Initial BW) / days
- FCR = Total feed intake (g) / Total weight gain (g)
- PEI = (Final BW × % survival) / (FCR × days) × 100

Table 1: Feed Ingredients and Calculated Nutrient Composition of the Basal Diet (g/kg)

(Feed Ingredients / Nutrients)	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Feed Ingredients (g/kg)			
Corn	560	595.5	620.9
Soybean Meal (44% CP)	350	300	260
Soybean Oil	50	65	80
Limestone	10	12	14
Dicalcium Phosphate	18	16	14
Salt	3	3	3
DL-Methionine	2.5	2.2	2.0
L-Lysine HCl	1.5	1.3	1.1
Vitamin & Mineral Premix[1]	5	5	5
Calculated Nutrient Composition			
Metabolizable Energy (ME, kcal/kg)	3050	3125	3175
Crude Protein (%)	21.5	20.5	19.0
Lysine (%)	1.40	1.20	1.05
Methionine + Cystine (%)	0.98	0.88	0.75
Calcium (%)	0.95	0.85	0.80
Available Phosphorus (%)	0.47	0.42	0.38

[1] Vitamin/mineral premix provided per kg of diet: Vitamin A 11,000 IU, Vitamin D_3 3,500 IU, Vitamin E 75 IU, Vitamin K 3 mg, Thiamine 3 mg, Riboflavin 3 mg, Pyridoxine 4 mg, Niacin 60 mg, Pantothenic acid 15 mg, Biotin 0.15 mg, Cyanocobalamin 0.016 mg, Folic acid 2 mg, Choline 700 mg, Iron 40 mg, Copper 16 mg, Manganese 120 mg, Selenium 0.3 mg, Zinc 10 mg, Antioxidant 10 mg.

Serum biochemical parameters

Serum was analyzed for parameters related to liver function (AST, ALT, ALP) using commercial kits (Parsazmun, Tehran, Iran). TAC was determined following the method of Benzie and Strain (1996). Malondialdehyde (MDA, µmol/dL) was measured using the cyanomethemoglobin method (Zist Shimi Co.). Glutathione peroxidase (GPx) activity was determined using Randox kits. Catalase (CAT) and superoxide dismutase (SOD) activities were measured spectrophotometrically (Cecil-9000, UK).

Ileum Morphology

One bird per pen (close to average weight) was slaughtered, and after evisceration a segment of three cm was collected from the ileum terminal. The samples were fixed in 10% neutral buffered formalin (pH 7.4), processed for paraffin embedding, sectioned (4 µm), and stained with hematoxylin and eosin. The samples were assessed for villus height (from tip to base), villus width, and crypt depth microscopically (Olympus BX50). Ten measurements per bird were averaged.

Gut Microbial Population

Ileal contents from one bird per treatment were collected aseptically. One gram of the sample was homogenized in 9 mL sterile phosphate-buffered saline (PBS) to prepare a 10% (w/v) suspension. Serial dilutions were plated on: MacConkey Agar (Liofilchem, Italy): for coliforms (incubated at 37°C, 24 h aerobically), MRS Agar (Liofilchem, Italy): for Lactobacillus spp. (incubated at 37°C, 48 h anaerobically), Reinforced Clostridial Agar (RCA): for total anaerobic bacteria (incubated at 37°C, 48 h under anaerobic conditions: 80% N_2, 10% H_2, 10% CO_2) and Plate Count Agar (Liofilchem, Italy): for total aerobic bacteria. Colonies were counted and expressed as $\log_{10}$ CFU/g.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure in SAS (SAS Institute, 2022) software. The pen was the experimental unit for performance data; individual birds were used for other variables. Means were compared using Tukey's test at $P < 0.05$.

Results

Growth Performance

Results on growth performance are summarized in Table 2. No significant single as well as interactive effects were found for either kombucha or stocking density (SD) in all parameters assessed during the starter (days 1 to 14) and grower (days 15 to 28) periods ($P > 0.05$).

During the finisher period (days 29 to 42), kombucha supplementation in to drinking water significantly influenced daily weight gain (DWG) and feed intake (FI) ($P < 0.05$). Birds receiving 15 mL/100 mL kombucha exhibited a greater DWG (78.17 g/bird) than those treated with 10 mL/100 mL (69.03 g/bird) and 5 mL/100 mL (71.86 g/bird). Although no statistically significant kombucha × stocking density interaction was detected for overall DWG (P = 0.13), birds under HSD receiving 15 mL/100 mL of kombucha showed numerical improvement (44.59 vs. 38.39 g/bird).

Over the entire 42-day experimental period, kombucha significantly improved DWG and FCR ($P < 0.05$). The 15 mL/100 mL treatment resulted in a greater overall DWG (43.55 g/bird) and a lower FCR (1.690), significantly outperforming the control group

(41.58 g/bird DWG and 1.802 FCR). A significant main effect of stocking density was also detected for overall DWG, with birds under normal density (10 birds/m²) achieving a higher final DWG (41.99 g/bird) compared to those reared under high density (15 birds/m²; 38.39 g/bird) ($P< 0.05$).

Table 2: Effect of kombucha on performance characteristics (DWG: g/bird/d, FI: g/bird/d) of broiler chickens under normal and high stocking density condition.

		Day 1-14			Day 15-28			Day 29-42			Day 1-42			
		DWG	FI	FCR	DWG	FI	FCR	DWG	FI	FCR	DWG	FI	FCR	PEI
Kombucha (ml/100 mL)														
0		17.10	23.7	1.40	33.6	58.3	1.80	75.78^{ab}	144.1^{a}	1.90	41.58^{ab}	74.7	1.80^{a}	235.1^{b}
5		17.12	24.2	1.42	34.7	59.6	1.72	71.86^{bc}	133.2^{ab}	1.87	41.23^{ab}	72.1	1.758^{ab}	239.0^{ab}
10		17.5	24.1	1.37	34.9	60.2	1.74	69.03^{c}	128.7^{b}	1.88	40.42^{b}	70.89	1.76^{ab}	236.4^{ab}
15		17.27	24.05	1.39	35.95	59.89	1.70	78.17^{a}	136.8^{ab}	1.77	43.55^{a}	73.43	1.69^{b}	259.8^{a}
SEM		1.02	0.89	0.55	2.31	1.61	0.81	0.39	1.08	0.34	0.21	2.32	0.01	0.88
Stocking density (SD) bird/m^2														
10		17.27	23.84	1.38	34.13	59.76	1.78	75.08	138.5	1.85	41.99^{a}	73.70	1.761^{b}	244.6
15		17.28	24.20	1.416	34.82	59.28	1.71	72.34	132.9	1.86	38.39^{b}	71.92	1.87^{a}	240.5
SEM		0.66	1.21	0.39	2.02	3.24	0.29	2.33	4.56	0.47	0.96	1.81	0.01	3.36
KOM×SD														
0	10	17.92	23.78	1.33	33.54	60.82	1.84	74.87	147.4	1.97	41.65	76.27	1.84	230.6
0	15	16.31	23.61	1.473	31.80	55.82	1.77	76.70	140.9	1.843	41.51	73.22	1.77	239.5
5	10	16.59	23.68	1.430	34.50	58.98	1.728	79.15	140.3	1.78	43.37	74.16	1.72	257.3
5	15	17.76	24.78	1.422	34.93	60.22	1.73	64.57	126.2	1.97	39.08	70.22	1.79	220.7
10	10	16.98	23.41	1.38	33.02	57.09	1.758	71.58	136.2	1.89	40.44	72.09	1.78	233.7
10	15	18.13	24.82	1.37	36.85	63.49	1.73	66.48	121.3	1.86	40.39	69.68	1.73	239.0
15	10	17.61	24.49	1.396	35.48	62.18	1.79	74.72	130.2	1.75	42.52	72.29	1.71	256.7
15	15	16.93	23.61	1.39	35.71	57.61	1.62	81.62	143.4	1.76	44.59	74.57	1.67	262.9
SEM		0.89	0.71	0.62	0.88	3.24	0.36	4.05	3.06	0.25	0.89	1.22	0.19	18.02
								P-value						
Kombucha		0.771	0.752	0.810	0.311	0.683	0.521	0.001	0.034	0.109	0.011	0.16	0.044	0.038
SD		0.977	0.320	0.378	0.551	0.690	0.175	0.082	0.135	0.884	0.036	0.160	0.049	0.578
Kombucha × SD		0.106	0.096	0.330	0.393	0.064	0.626	0.082	0.093	0.118	0.13	0.306	0.383	0.098

DWG: Daily weight gain (grams per bird), FI: Feed intake (grams per bird), FCR: Feed conversion ratio and PEI: Performance index. ^{a,b,c} Means in the same row with no common superscripts differ($P< 0.05$).

Liver Enzymes

High stocking density (HSD) significantly increased serum levels of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) compared to the normal density group (Table 3; $P< 0.05$).

Kombucha supplementation significantly influenced serum AST and ALP activities ($P< 0.05$; Table 3). For AST, all kombucha-treated groups (5, 10, and 15 mL/100 mL) showed significantly lower values compared to the control. For ALP, a dose-dependent response was observed: while the 10 and 15 mL/100 mL doses significantly reduced ALP activity (3080 and 2896 U/L, respectively; $P< 0.05$), the 5 mL/100 mL dose did not differ from the control and numerically exceeded it (3825 vs. 3546 U/L), suggesting a threshold effect for hepatoprotective efficacy. ALT activity was not significantly affected by treatment ($P> 0.05$).

Serum Antioxidant Status

Kombucha supplementation significantly improved the serum antioxidant profile (Table 4). Malondialdehyde (MDA) levels were significantly lower in birds receiving 15 mL/100 mL kombucha (7.01 nmol/dL) compared to controls (8.54 nmol/dL; $P< 0.05$). Conversely, glutathione peroxidase (GPx) activity and total antioxidant capacity (TAC) were significantly elevated in kombucha-treated groups ($P< 0.05$), with the highest GPx (397.9 U/L) and TAC (64.26 U/L) recorded at 15 mL/100 mL. Superoxide dismutase (SOD) activity was not significantly modulated by kombucha supplementation (P = 0.850), although high stocking density increased SOD activity compared to normal density (49.39 vs. 45.00 U/L; P = 0.045), likely reflecting a compensatory upregulation under oxidative challenge. High stocking density significantly reduced TAC and increased superoxide dismutase (SOD) activity compared to those maintained in normal density conditions ($P< 0.05$).

Ileum Microbial Population

Kombucha supplementation significantly increased the population of beneficial Lactobacillus spp. and total anaerobic bacteria while reducing coliform counts (Table 5; $P< 0.05$). The 15 mL/100 mL dosage resulted in the highest Lactobacillus (6.04 $\log_{10}$ CFU/g) and total anaerobe (4.32 $\log_{10}$ CFU/g) counts and the lowest coliform count (4.66 $\log_{10}$ CFU/g). High stocking density significantly decreased the Lactobacillus population compared to normal density (5.67 vs. 5.97 $\log_{10}$ CFU/g; P = 0.027), while total anaerobic bacteria were significantly elevated under high density (4.15 vs. 3.86 $\log_{10}$ CFU/g; P = 0.040).

Table 3: Effect of kombucha on serum liver enzymes (U/L) of broiler chickens under normal and high stocking density condition.

		Aspartate Aminotransferase	Alanine Aminotransferase	Alkaline phosphatase
Kombucha (ml/100 mL)				
0		244.8[a]	12.88	3546[ab]
5		236.2[b]	13.63	3825[a]
10		232.1[b]	12.06	3080[b]
15		231.3[b]	13.31	2896[b]
SEM		0.39	0.87	12.33
Stocking density (SD) bird/m^2				
10		233.3[b]	12.72	3223[b]
15		238.8[a]	13.22	3450[a]
SEM		2.68	1.70	36.62
KOM×SD				
0	10	225.8	13.375	3003
0	15	238.5	12.375	2790
5	10	241.1	13.500	3646
5	15	231.3	13.750	2513
10	10	240.0	13.375	3859
10	15	249.5	10.750	3791
15	10	226.4	12.625	3294
15	15	236.1	14.000	3798
SEM		4.72	1.11	42.8
P-value				
Kombucha		0.042	0.752	0.027
SD		0.037	0.643	0.029
Kombucha × SD		0.717	0.591	0.110

[a,b,c] Means in the same row with no common superscripts differ($P< 0.05$).

Table 4: Effect of kombucha on serum antioxidant enzymes of broiler chickens under normal and high stocking density condition.

		MDA	TAC	Gpx	SOD
		µmol/dL	U/L	U/L	U/L
Kombucha (ml/100 mL)					
0		8.54[a]	60.94[b]	351.50[b]	46.06
5		7.81[ab]	61.73[ab]	356.50[b]	44.12
10		7.59[ab]	62.70[ab]	360.90[b]	46.22
15		7.01[b]	64.26[a]	397.9[a]	46.37
SEM		0.19	0.44	3.44	1.27
Stocking density (SD) bird/m^2					
10		7.43	68.65[a]	360.01[b]	45.00b
15		8.03	56.16[b]	393.4[a]	49.39a
SEM		0.96	0.86	6.65	1.33
KOM×SD					
0	10	6.512	83.86	336.2	45.75
0	15	7.501	38.01	376.8	46.38
5	10	5.655	58.11	327.8	42.95
5	15	9.955	70.41	393.9	45.29
10	10	11.372	60.93	350.1	43.87
10	15	5.699	62.52	353.0	48.57
15	10	6.192	71.68	426.0	47.44
15	15	8.977	53.72	369.9	45.30
SEM		1.02	2.81	12.8	0.89
P-value					
Kombucha		0.048	0.035	0.027	0.850
SD		0.716	0.050	0.054	0.045
Kombucha × SD		0.161	0.695	0.916	0.689

[a,b,c] Means in the same row with no common superscripts differ($P< 0.05$).

Table 5: Effect of kombucha on the microbial population of the ileum (log CFU/g) of broiler chickens under normal and high stocking density condition.

		Lactobacillus	Coliform	Total anaerobic bacteria
Kombucha (ml/100 mL)				
0		5.26[b]	5.95[a]	3.85[b]
5		5.89[a]	5.33[ab]	3.57[ab]
10		6.00[a]	5.34[ab]	4.19[ab]
15		6.04[a]	4.66[b]	4.32[a]
SEM		0.16	0.25	0.23
Stocking density (SD) bird/m^2				
10		5.97[a]	5.27	3.86[b]
15		5.67[b]	5.33	4.15[a]
SEM		0.24	0.44	0.09
KOM×SD				
0	10	5.52	5.32	3.8
0	15	6.24	5.34	3.9
5	10	5.53	4.41	1.83
5	15	5.51	4.87	3.69
10	10	6.04	5.81	4.68
10	15	6.04	6.07	3.85
15	10	6.24	4.65	3.02
15	15	5.44	5.17	3.97
SEM		0.31	0.04	0.18
P-value				
Kombucha		0.049	0.042	0.037
SD		0.027	0.864	0.040
Kombucha × SD		0.085	0.861	0.358

[a,b,c] Means in the same row with no common superscripts differ($P< 0.05$).

Table 6: Effect of kombucha on the morphology (µm) of the ileum of broiler chickens under normal and high stocking density condition.

		Villus height	Villus width	Crypt depth	Villus height/Crypt depth
Kombucha (ml/100 mL)					
0		294.9	46.13	29.90	9.88[b]
5		320.5	49.93	31.16	10.29[ab]
10		329.5	45.29	28.26	11.77[a]
15		329.0	46.83	31.15	10.63[ab]
SEM		6.32	1.49	0.89	0.11
Stocking density (SD) bird/m^2					
10		301.6	47.13	30.92	10.31
15		335.3	46.95	29.32	10.97
SEM		15.22	2.36	1.45	0.89
KOM×SD					
0	10	310.7	45.12	28.76	10.07
0	15	322.6	47.21	32.05	8.79
5	10	299.7	44.63	34.11	9.09
5	15	318.4	42.77	35.02	10.64
10	10	317.8	45.32	29.88	9.37
10	15	312.9	49.11	33.41	9.46
15	10	321.7	46.18	34.01	9.65
15	15	311.2	42.8	32.26	9.65
SEM		14.8	3.11	2.08	2.27
P-value					
Kombucha		0.396	0.424	0.308	0.016
SD		0.042	0.932	0.200	0.120
Kombucha × SD		0.788	0.917	0.667	0.332

[a,b,c] Means in the same row with no common superscripts differ($P< 0.05$).

Ileum Morphology

Villus height, villus width, and crypt depth were not significantly affected by inclusion of kombucha into drinking water as well as stocking density ($P> 0.05$). Villus height-to-crypt depth (VH: CD) ratio was significantly influenced by kombucha supplementation ($P< 0.05$). The 10 mL/100 mL kombucha treatment yielded a higher VH: CD ratio (11.77), which was significantly greater than that of the control birds (9.88). Stocking density showed no effect on ileum morphology parameters.

Discussion

The present study demonstrates that kombucha, when administered via drinking water, exerts multifaceted protective effects against the physiological challenges imposed by high stocking density (HSD) in broiler chickens. These benefits appear to stem from its dual role as a symbiotic delivering both probiotic microorganisms and fermentative metabolites with prebiotic-like activity thereby modulating systemic redox balance, hepatic integrity, and gut ecosystem dynamics. The mitigation of HSD-induced oxidative stress by kombucha aligns with its established phytochemical profile. Fermentation of black tea enriches kombucha with bioactive polyphenols and organic acids, particularly glucuronic and acetic acids, which are known to enhance endogenous antioxidant defenses (Vīna *et al*., 2014; Dechakhamphu *et al*., 2023). The observed elevation in total antioxidant capacity (TAC) and glutathione peroxidase (GPx) activity, alongside reduced malondialdehyde (MDA) levels, suggests that kombucha bolsters the avian antioxidant network through both direct free radical scavenging and upregulation of enzymatic detoxification pathways. This is consistent with prior evidence that polyphenol-rich fermented preparations can attenuate oxidative damage in metabolically stressed livestock (Salehi *et al*., 2021; Zhao *et al*., 2021).

The hepatoprotective effect of kombucha, evidenced by reduced serum AST and ALP activities, further supports its role in systemic stress mitigation. Elevated liver enzymes under HSD reflect subclinical hepatocellular disruption, commonly linked to metabolic overload and inflammatory responses (Simitzis *et al*., 2012). Glucuronic acid a key metabolite in kombucha is a critical substrate for phase II hepatic detoxification, facilitating the conjugation and excretion of xenobiotics and reactive metabolites (Vīna *et al*., 2013). Additionally, short-chain fatty acids (SCFAs) such as butyrate may stabilize hepatocyte membranes and improve mitochondrial function, thereby preserving liver homeostasis under stress (Gharib, 2009).

Kombucha's capacity to reshape ileal microbial ecology underscores its synbiotic functionality. The enrichment of Lactobacillus populations and suppression of coliforms likely result from a combination of competitive exclusion, pH modulation via organic acids, and provision of fermentable substrates that favor obligate anaerobes (Chakravorty *et al*., 2016; Ecklu-Mensah *et al*., 2024). The significant kombucha × stocking density interaction for Lactobacillus abundance indicates that microbial resilience is particularly enhanced under dysbiotic conditions induced by overcrowding an environment typically characterized by elevated pathogen load and reduced microbial diversity (Janicka *et al*., 2025). This microbial restructuring may indirectly promote nutrient utilization and barrier integrity, thereby supporting growth performance even under suboptimal rearing conditions.

While no consistent dose–response was observed across all endpoints, the optimal inclusion level appears context-dependent. For instance, intestinal morphometric improvements (villus height-to-crypt depth ratio) peaked at 10 mL/100 mL, possibly due to a threshold effect of SCFAs on epithelial turnover and renewal. In contrast, systemic outcomes such as antioxidant status, liver enzyme profiles, and growth performance showed maximal response at 15 mL/100 mL. This suggests differential tissue sensitivity to kombucha-derived metabolites and highlights the importance of dose optimization based on primary production goals whether targeting intestinal architecture or systemic physiological resilience.

Although no statistically significant kombucha × stocking density interaction was detected for daily weight gain ($P = 0.13$), the numerical improvement observed in birds under HSD receiving 15 mL/100 mL kombucha (44.59 vs. 38.39 g/bird) suggests a biologically relevant buffering effect against density-induced stress. This trend aligns with the broader pattern of enhanced antioxidant capacity, improved liver function, and favorable gut microbiota modulation observed at this inclusion level, collectively supporting more efficient nutrient utilization and metabolic homeostasis under challenging rearing conditions.

Collectively, these findings position kombucha as a promising, natural feed/water additive in intensive poultry systems. Its multi-target efficacy spanning antioxidant, hepatic, and microbial domains—offers a holistic alternative to antibiotic growth promoters, aligning with global demands for sustainable and welfare-compatible production practices (Choct, 2009; Kridtayopas *et al*., 2019). Future studies should explore long-term impacts on immune competence, carcass quality, and microbiome stability, as well as the contribution of specific microbial consortia within kombucha (e.g., Komagataeibacter spp., yeast populations) to its functional outcomes (Landis *et al*., 2022; Kozyrovska *et al*., 2012).

Conclusion

Supplementation of drinking water with kombucha at 15 mL/100 mL represents a highly effective, natural strategy to mitigate the multifaceted negative impacts of high stocking density in broiler chickens. By simultaneously improving growth performance, liver health, antioxidant capacity, and gut microbial balance, kombucha offers a practical and economically viable intervention for enhancing poultry welfare and productivity in intensive production systems.

References

Baracho MS, Nääs IDA, Lima NDS, Cordeiro AFS & Moura DJ. 2019. Factors affecting broiler production: A meta-analysis. Brazilian Journal of Poultry Science, 21(3): eRBCA-2019. DOI: 10.1590/1806-9061-2019-1022.

Benzie IFF & Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, 239(1): 70-76. DOI: 10.1006 /abio.1996.0292.

Bhattacharya S, Gachhui R & Sil PC. 2011. Hepatoprotective properties of kombucha tea against TBHP-induced oxidative stress via suppression of mitochondria dependent apoptosis. Pathophysiology, 18(3): 221-234. DOI: 10.1016/j.pathophys.2011.02.001.

Bhattacharya S, Gachhui R & Sil PC. 2013. Effect of Kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. Food and Chemical Toxicology, 60: 328-340. DOI: 10.1016/j.fct.2013.07.051.

Chakravorty S, Bhattacharya S, Chatzinotas A, Chakraborty W, Bhattacharya D & Gachhui R. 2016. Kombucha tea fermentation: Microbial and biochemical dynamics. International Journal of Food Microbiology, 220: 63-72. DOI: 10.1016/j.ijfoodmicro.2015.12.015.

Choct M. 2009. Managing gut health through nutrition. British Poultry Science, 50(1): 9-15. DOI: 10.1080/00071660802538632.

Da Anunciação TA, Guedes JDS, Tavares PPL, de Melo Borges FE, Ferreira DD, Costa JAV & Magalhães-Guedes KT. 2024. Biological significance of probiotic microorganisms from kefir and kombucha: a review. Microorganisms, 12(6): 1127. DOI: 10.3390/ microorganisms 12061127.

Dechakhamphu A, Wongchum N, Chumroenphat T, Tanomtong A, Pinlaor S & Siriamornpun S. 2023. In vitro and in vivo evaluation for antioxidant and anti-diabetic properties of *Cyperus rotundus* L. Kombucha. Foods, 12(22): 4059. DOI: 10.3390/ foods12224059.

Diaz Carrasco JM, Casanova NA & Fernández Miyakawa ME. 2019. Microbiota, gut health and chicken productivity: What is the connection? Microorganisms, 7(10): 374. DOI: 10.3390/ microorganisms7100374.

Dufresne C & Farnworth E. 2000. Tea, kombucha, and health: a review. Food Research International, 33(6): 409-421. DOI: 10.1016/S0963-9969(00) 00067-3.

Ecklu-Mensah G, Miller R, Maseng MG, Hawes V, Hinz D, Kim C & Gilbert JA. 2024. Modulating the human gut microbiome and health markers through kombucha consumption: a controlled clinical study. Scientific Reports, 14(1): 31647. DOI: 10.1038/s41598-024-79897-2.

Gamboa-Gómez CI, González-Laredo RF, Gallegos-Infante JA, Pérez MD, Moreno-Jiménez MR, Flores-Rueda AG & Rocha-Guzmán NE. 2016. Antioxidant and angiotensin-converting enzyme inhibitory activity of *Eucalyptus camaldulensis* and *Litsea glaucescens* infusions fermented with kombucha consortium. Food Technology and Biotechnology, 54(3): 367-374. DOI: 10.17113/ ftb.54.03.16.4622.

Gharib OA. 2009. Effects of Kombucha on oxidative stress induced nephrotoxicity in rats. Chinese Medicine, 4: 23. DOI: 10.1186/1749-8546-4-23.

Gholami M, Chamani M, Seidavi A, Sadeghi AA & Aminafschar M. 2020. Effects of stocking density and climate region on performance, immunity, carcass characteristics, blood constitutes, and economical parameters of broiler chickens. Revista Brasileira de Zootecnia, 49: e20190049. DOI: 10.37496/rbz4920190049.

Janicka K, Drabik K, Wengerska K & Rozempolska-Rucińska I. 2025. Effect of stocking density on behavioural and physiological traits of laying hens. Animals, 15(4): 604. DOI: 10.3390/ ani15040604.

Kozyrovska NO, Reva OM, Goginyan VB & De Vera JP. 2012. Kombucha microbiome as a probiotic: A view from the perspective of post-genomics and synthetic ecology. Biopolymers and Cell, 28(2): 103-113. DOI: 10.7124/bc.000060.

Kridtayopas C, Rakangtong C, Bunchasak C & Loongyai W. 2019. Effect of prebiotic and synbiotic supplementation in diet on growth performance, small intestinal morphology, stress, and bacterial population under high stocking density condition of broiler chickens. Poultry Science, 98(10): 4595-4605. DOI: 10.3382/ps/pez152.

Landis EA, Fogarty E, Edwards JC, Popa O, Eren AM & Wolfe BE. 2022. Microbial diversity and interaction specificity in kombucha tea fermentations. mSystems, 7(3): e00157-22. DOI: 10.1128/msystems.00157-22.

Li XM, Zhang MH, Liu SM, Feng JH, Ma DD, Liu QX, Zhou Y, Wang XJ & Xu S. 2019. Effects of stocking density on growth performance, growth regulatory factors, and endocrine hormones in broilers under appropriate environments. Poultry Science, 98(12): 6611-6617. DOI: 10.3382/ ps/pez505.

Loh G & Blaut M. 2012. Role of commensal gut bacteria in inflammatory bowel diseases. Gut Microbes, 3(6): 544-555. DOI: 10.4161/ gmic.22156.

Malbaša RV, Lončar ES, Vitas JS & Čanadanović-Brunet JM. 2011. Influence of starter cultures on the antioxidant activity of kombucha beverage.

Food Chemistry, 127(4): 1727-1731. DOI: 10.1016/j.foodchem.2011.02.048.

Nyhan LM, Lynch KM, Sahin AW & Arendt EK. 2022. Advances in kombucha tea fermentation: A review. Applied Microbiology, 2(1): 73-103. DOI: 10.3390/applmicrobiol2010005.

Ohland CL & Jobin C. 2015. Microbial activities and intestinal homeostasis: a delicate balance between health and disease. Cellular and Molecular Gastroenterology and Hepatology, 1(1): 28-40. DOI: 10.1016/j.jcmgh.2014.11.004.

Qaid M, Albatshan H, Shafey T, Hussein E & Abudabos AM. 2016. Effect of stocking density on the performance and immunity of 1-to 14-d-old broiler chicks. Brazilian Journal of Poultry Science, 18: 683-692. DOI: 10.1590/1806-9061-2016-0289.

Salehi S, Sadeghi A & Karimi A. 2021. Growth performance, nutrients digestibility, caecum microbiota, antioxidant status and immunity of broilers as influenced by kombucha fermented on white sugar or sugar beet molasses. Italian Journal of Animal Science, 20(1): 1770-1780. DOI: 10.1080/1828051X.2021.1952910.

SAS Institute. 2022. SAS/STAT® User's Guide, Version 9.4. SAS Institute Inc., Cary, NC, USA.

Simitzis PE, Kalogeraki E, Goliomytis M, Charismiadou MA, Triantaphyllopoulos K, Ayoutanti A & Deligeorgis SG. 2012. Impact of stocking density on broiler growth performance, meat characteristics, behavioural components and indicators of physiological and oxidative stress. British Poultry Science, 53(6): 721-730. DOI: 10.1080/00071668.2012.745930.

Sugiharto S. 2022. Dietary strategies to alleviate high-stocking-density-induced stress in broiler chickens - a comprehensive review. Archives Animal Breeding, 65(1): 21-36. DOI: 10.5194/ aab-65-21-2022.

Sugiharto S, Agusetyaningshi I, Widiastuti E, Wahyuni HI, Yudiarti T & Sartono TA. 2021. Growth and haematological response of broilers raised at high-density pens and fed germinated papaya seed and chitosan blend. Journal of Animal Health and Production, 10(4): 485-491. DOI: 10.17582/journal.jahp/2021/9.4.485.491.

Vīna I, Linde R, Patetko A & Semjonovs P. 2013. Glucuronic acid from fermented beverages: Biochemical functions in humans and its role in health protection. International Journal of Research and Reviews in Applied Sciences, 14(2): 217-230.

Vīna I, Semjonovs P, Linde R & Deniņa I. 2014. Current evidence on physiological activity and expected health effects of kombucha fermented beverage. Journal of Medicinal Food, 17(2): 179-188. DOI: 10.1089/jmf.2013.0031.

Watawana MI, Jayawardena N, Gunawardhana CB & Waisundara VY. 2015. Health, wellness, and safety aspects of the consumption of kombucha. Journal of Chemistry, 2015: 591869. DOI: 10.1155/2015/591869.

Yapar K, Cavusoglu K, Oruç E & Yalcin E. 2010. Protective effect of kombucha mushroom (KM) tea on phenol-induced cytotoxicity in albino mice. Journal of Environmental Biology, 31(5): 615-621.

Yegani M & Korver DR. 2008. Factors affecting intestinal health in poultry. Poultry Science, 87(10): 2052-2063. DOI: 10.3382/ps.2008-00091.

Zhao YS, Eweys AS, Zhang JY, Zhu Y, Bai J, Darwesh OM, Zhang HB & Xiao X. 2021. Fermentation affects the chemical constituents and antioxidant activity of plant-based food materials: A review. Antioxidants, 10(12): 2004. DOI: 10.3390/ antiox10122004.