Research

Victory Osirimade Sumanu^{1*}, Tagang Aluwong¹, Joseph Olusegun Ayo¹, Ngozi Ejum Ogbuagu¹

Effect of probiotic and fisetin supplementation on performance, carcass characteristics and small intestinal morphology in broiler chickens

<https://10.1515/ovs-2020-0106>

Received Jan 21, 2021; accepted May 26, 2021

Abstract: We studied the dietary effects of probiotic and fisetin supplementation on performance parameters, carcass characteristics and small intestinal morphology in broiler chickens. Sixty (one-day-old) Arbo Acre breed of broiler chickens were allotted into 4 groups of 15 each randomly: Group I, Control was administered only distilled water; Group II, fisetin (5 mg/kg); Group III, probiotic (4.125×10^6 cfu/100 mL); and Group IV, probiotic and fisetin (4.125×10^6 cfu/100 mL and 5 mg/kg, respectively) for the first one week of life. All administrations were performed orally through gavage. Broiler chickens supplemented with probiotic and probiotic+fisetin had greater feed intake significantly ($P < 0.05$) at weeks 5 and 6, when compared respectively with those of the control and fisetin groups which had lesser feed intake values. Body weight gain was of great significance ($P < 0.05$) in the probiotic-supplemented broiler chickens, while that of the control was less. Carcass quality of the probiotic-supplemented group was higher in significance ($P < 0.05$) than any other treatment group which had lower qualities. The pH changes in the breast muscle was lesser significantly ($P < 0.05$) in the probiotic+fisetin group, when compared with those of the control group which had greater changes. The villus height/crypt height ratio was higher in significance ($P < 0.05$) in the treatment groups, while that of the control group was lower. In conclusion, dietary supplementation of probiotic may enhance

broiler chickens' productivity, by improving performance, carcass weight, pH, and small intestinal morphology as compared to fisetin supplementation.

Keywords: Performance indices; carcass characteristics; small intestinal morphology; broiler chickens; probiotic; fisetin.

1 Introduction

The production of broiler chickens is aimed at obtaining good-quality meat products from heavy meat breeds [1, 2], usually sold live or processed at five to six weeks of age [3, 4]. Poultry meat is very tender [5, 6] and broiler enterprises have short production cycles [7]. The key to a successful broiler production depends on a systematic [8, 9] and efficient management programme adopted by the farmer [10]. During production, broiler chickens are often exposed to stressful management practices and environmental factors [11, 12]. Stress exerts adverse effects on broiler chickens' production by reducing performance indices, including body weight gain, antioxidant status and their ability to combat diseases [13].

Antioxidants are molecules capable of preventing or slowing other molecules oxidation [14, 15] and they are used as dietary supplements [16, 17]. They provide beneficial effects [18] against stress-induced tissue damages [19, 20].

Probiotic are living organisms that are beneficial to their host when administered in adequate amount, they can either be fungi (yeast) or bacteria strains. Yeast probiotic can confer several growth enhancement and antioxidant benefits to both humans and animals based on the components of their cell wall [11]. Administration of yeast probiotic to broiler or laying chickens can prevent the excessive production of reactive oxygen species

*Corresponding author: **Victory Osirimade Sumanu**, Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria. email: devicpet@gmail.com

Tagang Aluwong, Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria. email: alywong_tagang@yahoo.co.uk

Joseph Olusegun Ayo, Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria. email: ayojo94@yahoo.com

Ngozi Ejum Ogbuagu, Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria. email: ogbuagungozi1989@gmail.com

above which the body can tolerate. It also enhances the feeding pattern of these chickens positively, evident by an increment in performance such as weight gain [21], feed conversion efficiency, egg production [22, 23] and lowering the incidence of disease and mortality rate [11, 24], all of which increases their productivity vis-à-vis profitability to the farmers.

Flavonoids commonly found in most plants [25], exhibits considerable biological activities namely: antioxidant, anticarcinogenic, antibacterial, anti-inflammatory, antiviral and immune-stimulation effects [26]. Flavonoids and their polymers are one of the largest groups of phytonutrients which exert beneficial health effects [27]. They are important polyphenolic compounds under the class of plant secondary metabolites, such as; fisetin [28]. Fisetin is a dietary supplement present in vegetables and fruits, including strawberries, onions, grapes and cucumbers which are readily available [29, 30]. It exerts antioxidant effect by scavenging free radicals and preventing or slowing their oxidation [25]. Fisetin exhibits, antioxidant, anti-inflammatory, anti-neoplastic and neuroprotective properties [31, 32]. It mitigates oxidative and inflammatory responses in microglial cells found in mice [33], but its effect on the improvement of performance in poultry has not been reported.

Because of previously published studies showing potential benefits of probiotic but not fisetin on broiler weight gain, we hypothesised that supplementation of either alone or in combination would be beneficial. To test this hypothesis, we assessed performance parameters, carcass characteristics, and small intestinal morphology in separate groups of chicks given these supplements alone or in combination. Our findings showed that probiotic alone or with fisetin but not fisetin alone increased feed intake and weight gain.

2 Materials and Methods

2.1 Study design and animal management

All 60-day-old broiler chicks (Arbor Acre) were sourced from Zartech Farms, Ibadan, Nigeria. They were kept in an intensive management system. The broiler chicks were fed with broiler starter (weeks 1 to 4) and broiler finisher (weeks 5 to 6) (Hybrid Feeds, Kaduna, Nigeria) *ad libitum*. Table 1 shows the ingredients and proximate analysis of the feeds. The broiler chickens were randomly allotted into four groups, each comprising 15 chicks (based on prior studies) [11], as follows: Group I, control was administered with distilled water *ad libitum*; Group

Table 1 Composition and proximate analysis of broiler chicken diets

Feed Composition	Starter	Finisher
Ingredients (%)		
Crude protein	22.0	19.5
Fat	5.1	3.8
Crude fibre	4.3	3.0
Calcium	1.2	1.2
Bio-available phosphorus	0.5	0.4
Methionine	0.6	0.5
Lysine	1.3	1.2
Metabolizable energy (Kcal/kg)	3000.0	3100.0
Proximate analysis		
Crude protein (%)	22.0	21.0
Fat (%)	7.9	6.8
Crude fibre (%)	4.3	3.0
Calcium (%)	2.0	2.0
Bio-available phosphorus (%)	0.8	0.7
Methionine (%)	0.6	0.5
Lysine (%)	1.2	1.2
Metabolizable energy (Kcal/kg)	2900.0	2980.0

II, fisetin (Sigma Inc., New Orleans, Louisiana, USA) at a dose of 5 mg/kg; Group III, probiotic (Montajat Pharmaceuticals, Bioscience Division, Dammam, Saudi Arabia) at a dose of 4.125×10^6 CFU/100 mL, based on the method of competitive exclusion, and Group IV, probiotic (4.125×10^6 CFU/100 mL) and fisetin (5 mg/kg). All the administrations were performed orally through gavage on a daily basis for the first one week of life (at 7:00 h, for 1 week). Each broiler chicken was properly identified on the leg using a masking tape for accurate recordings. Biosecurity was adhered to during the period of study.

Ethical Approval: Protocol of the research was approved by the Ethical Committee on Animal Use and Care of the Ahmadu Bello University, Zaria, with reference number ABUCAUC/2018/021. The research related to animals' use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

2.2 Evaluation of feed intake

Daily feed intake of the broiler chickens was measured once a day at 07:00 hour during the entire period of the experiment. The mean value for each week of experiment was used as the daily feed intake values for each week

during the study period. The weights of the feed before placement were measured using a digital precision weighing balance, and the remaining feeds were measured again 24 hours later. The difference between the amount of feed supplied and the remnant after consumption gave the absolute feed intake [34].

2.3 Evaluation of water intake and body weight gain

Daily water intake of broiler chickens was measured once a day at 07:00 hour during the experiment. During the study period, the mean value for each experimental week was used as the daily water intake values. A graduated cylinder was used to measure the volume of water before placement, and the remaining water was measured again 24 hours later [34].

All the broiler chickens were weighed using a digital precision weighing balance, with a sensitivity of 0.01 g before and after each period of 7-days feeding and watering. The average values obtained for each week were considered as the body weight gain for that study period [34].

2.4 Evaluation of carcass and organ weights

Five broiler chickens were selected at random from each group and fasted for 12 hours, and each broiler chicken was reweighed. They were euthanised via cervical dislocation, after which the abdominal fat was collected separately and weighed after evisceration. The carcass, crop, proventriculus, gizzard, liver, gall bladder, spleen, heart, lungs, thigh and drumstick were weighed individually using a digital precision weighing balance with a sensitivity of 0.01 g.

2.5 Evaluation of pH changes in breast muscle

Twenty broiler chickens (five from each group) were randomly selected and euthanised by cervical dislocation at the end of the feeding trials. The breast muscles of each chicken was collected and analysed for pH changes. After collection, the breast muscles were stored in the refrigerator at 4 °C. The pH values of the breast muscles from the twenty broiler chickens selected were all measured at four post-mortem condition times (0, 24, 48 and 72 hours) using a portable pH meter (Jenweny PG 305, England). The pH meter was calibrated at pH 4.0 and 7.0

prior to each use. The electrode attached to the pH meter was directly placed at the breast muscle and the reading was taken. Each sample was measured in triplicate and the average pH value was calculated for each sample.

2.6 Evaluation of small intestinal morphometry

Five broiler chickens were selected at random from each group and euthanised by cervical dislocation. Exactly 3 cm of the jejunum was dissected at mid-point, flushed with sterile (cold) saline solution and was opened longitudinally. They were harvested and fixed in 10% formal saline and the tissues were histologically processed. In addition, the tissues were sectioned at 5 µm thickness via the Rotary Microtome Machine (Leica RT 25, Newcastle, UK). Sectioned tissues were mounted on slides, dried and stained using haematoxylin and eosin stain. Tissue-stained slides were photomicrographed using Amscope Digital Camera (Tokyo, Japan) for microscope version 2.0. Histomorphometric analysis was carried out using Digimizer image analysis software, version 4.5 (Medcalc Software, Oostende, Belgium) for measurement of villus width and height, crypt width and height. Villus height/crypt ratio was calculated. Height of the villus was measured from the villus tip to the junction of the villus crypt, and the depth of the crypt was measured from the base to the transition region between the villus and the crypt. Villus and crypt surface area were calculated using the following formulae [35]:

$$(2\pi) \times \left(\frac{CW}{2} \right) \times (CH), \text{ Where } CW = \text{crypt width and } CH = \text{crypt height}$$

2.7 Statistical analysis

For the statistical analysis, GraphPad Prism 5.03 (GraphPad Software, San Diego, CA, USA) was used. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison *post-hoc* test were used to determine the significance of the differences between the means of the treatment groups and control. Results were expressed as mean ± standard error of mean. Values of $P < 0.05$ were significant.

3 Results

3.1 Feed and water intake

The feed intake among the experimental groups was not significant when compared with those of the control group

at weeks 1 to 4, respectively. Feed intake in the probiotic and probiotic+fisetin experimental groups was significant ($P<0.05$; 1741.0 ± 103.6 g/bird and 1360.0 ± 38.1 g/bird) at week 5, and the probiotic group alone (2705.0 ± 49.5 g/bird) at week 6, compared with the control group (935.0 ± 62.3 g/bird and 1298.0 ± 114.1 g/bird, respectively). The overall mean feed intake was significantly higher ($P<0.05$) in the probiotic group (1268.0 ± 121.3 g/bird) than in the control, fisetin and probiotic+fisetin groups (905.6 ± 57.8 g/bird; 932.7 ± 62.1 g/bird and 991.0 ± 66.3 g/bird, respectively) (Table 2).

There was no significant difference in water intake of the experimental groups when compared with the control group at weeks 1 to 5. Water intake in the probiotic-supplemented group was significant ($P<0.05$; 4003.0 ± 110.6 ml), when compared with those of the control (3272.0 ± 116.1 ml) at week 6. No significant difference was observed in the overall mean water intake between the experimental groups and the control group (Table 3).

3.2 Body weight gain

Broiler chickens in the treatment groups at weeks 1 to 4, showed no significant difference in body weight gain when compared with the values obtained in the control

group. At weeks 5 and 6, body weight gain differed significantly ($P<0.05$) in the probiotic group (1001.0 ± 51.4 g and 1242.0 ± 63.4 g, respectively), compared with the values of the control group (810.7 ± 42.0 g and 983.9 ± 59.2 g, respectively). There was no significant difference in mean body weight gain between the treatment and control groups (Table 4).

3.3 Carcass and organ weights

Organ weights such as the crop, proventriculus, gizzard, liver, gall bladder, spleen, heart and lungs were not different significantly in the treatment and control groups. The weight of abdominal fat of the fisetin group was lower in significance ($P<0.05$; 3.2 ± 1.1 g), compared with those of the control, probiotic and the co-administered groups (14.0 ± 3.2 g, 15.4 ± 2.1 g and 8.0 ± 2.6 g, respectively). The weights of the thigh and drumstick of the probiotic-supplemented (189.6 ± 4.8 g and 165.0 ± 4.3 g, respectively) group were significant ($P<0.05$), compared with the values obtained in the control group (126.2 ± 22.5 g and 116.0 ± 22.6 g, respectively). Live weights of broiler chickens in the probiotic-supplemented group were heavier in significance ($P<0.05$; 1650.0 ± 67.0 g), when compared with

Table 2 Effects of probiotic and/or fisetin on feed intake (g) of broiler chickens

Week	Control	Fisetin	Probiotic	Probiotic+Fisetin
1	278.6 ± 50.6	264.7 ± 43.8	284.1 ± 49.3	273.0 ± 40.8
2	755.4 ± 64.1	731.3 ± 76.0	768.3 ± 70.7	731.0 ± 75.9
3	1067.0 ± 56.0	1059.0 ± 64.7	1078.0 ± 55.6	1062.0 ± 56.5
4	1156.0 ± 37.0	1130.0 ± 71.1	1239.0 ± 101.0	1122.0 ± 34.4
5	935.0 ± 62.3^a	1068.0 ± 56.8^a	1741.0 ± 103.6^b	1360.0 ± 38.1^b
6	1298.0 ± 114.1^a	1413.0 ± 75.2^a	2705.0 ± 49.5^b	1467.0 ± 61.7^a
Mean $\pm$ SEM	905.6 ± 57.8^a	932.7 ± 62.1^a	1268.0 ± 121.3^b	991.0 ± 66.3^a

^{a,b} = Means with different superscript letters across the rows are significantly different ($P<0.05$), (n=15).

Table 3 Effects of probiotic and/or fisetin on water intake (ml) of broiler chickens

Week	Control	Fisetin	Probiotic	Probiotic+Fisetin
1	702.9 ± 139.4	670.0 ± 132.9	707.1 ± 136.5	650.7 ± 126.6
2	1471.0 ± 56.6	1418.0 ± 65.6	1479.0 ± 53.7	1400.0 ± 72.3
3	1819.0 ± 157.4	1803.0 ± 165.9	1874.0 ± 120.6	1745.0 ± 174.4
4	2414.0 ± 110.2	2297.0 ± 259.3	2303.0 ± 133.4	2264.0 ± 114.1
5	2364.0 ± 191.1	2225.0 ± 203.4	2890.0 ± 152.9	2502.0 ± 188.1
6	3272.0 ± 116.1^a	3473.0 ± 121.6^a	4003.0 ± 110.6^b	3552.0 ± 117.1^a
Mean $\pm$ SEM	1976.0 ± 135.7	1945.0 ± 148.1	$2165.0 \pm 169.9.0$	1982.0 ± 150.2

^{a,b} = Means with different superscript letters across the rows are significantly different ($P<0.05$), (n=15).

the control group (1001.0 ± 68.1). Carcass weight in the probiotic-supplemented group was significant ($P < 0.05$; 1490.0 ± 67.5 g) than in the control group (924.6 ± 66.2 g) (Table 5).

3.4 The pH changes of the breast muscle

At slaughter (0:0 hour), there was no significant difference in pH of the breast muscles obtained in the experimental groups compared with the control group. At 24 hours post-slaughter, pH was significant ($P < 0.05$) in the breast muscles obtained from the probiotic and probiotic+fisetin groups (6.3 ± 0.1 and 6.3 ± 0.1), compared with the values obtained from the control and fisetin groups (5.9 ± 0.1 and 5.9 ± 0.1 , respectively). At 48 hours post-slaughter, there was a significant ($P < 0.05$) difference between pH of the probiotic and probiotic+fisetin groups (6.0 ± 0.1 and $6.1 \pm$

0.1), compared with the fisetin (5.7 ± 0.1) or control groups (5.6 ± 0.1), respectively. Finally, at 72 hours post-slaughter, pH values in the probiotic and probiotic+fisetin groups (5.9 ± 0.1 and 6.0 ± 0.1 , respectively) did not differ, but the values obtained in the control group (5.5 ± 0.0) was slightly lower than that of the fisetin group (5.6 ± 0.0) (Table 6).

3.5 Small intestinal morphometric parameters

As shown in Table 8, villus heights in fisetin (1000.0 ± 0.1 μm), probiotic (1100.0 ± 0.1 μm) and probiotic+fisetin (1200.0 ± 0.1 μm) groups were significant ($P < 0.05$) than in the control group (800.0 ± 0.0 μm). Villus widths in fisetin (100.0 ± 0.0 μm), probiotic (100.0 ± 0.0 μm) and probiotic+fisetin (100.0 ± 0.0 μm) experimental groups did not differ, compared with that of the control (100.0 ± 0.0 μm). Crypt heights

Table 4 Effects of probiotic and/or fisetin on the body weight gain (g) of broiler chickens

Week	Control	Fisetin	Probiotic	Probiotic+Fisetin
1	100.4 ± 3.1	99.4 ± 2.0	99.5 ± 2.3	99.9 ± 3.0
2	262.7 ± 7.4	268.8 ± 10.9	293.3 ± 23.8	289.9 ± 21.5
3	535.2 ± 12.2	540.3 ± 10.8	529.8 ± 24.4	524.5 ± 21.7
4	783.7 ± 27.2	750.4 ± 23.2	785.3 ± 36.0	735.6 ± 33.1
5	810.7 ± 42.0^a	846.9 ± 31.4^a	1001.0 ± 51.4^b	879.3 ± 52.4^a
6	983.9 ± 59.2^a	1003.0 ± 32.8^a	1242.0 ± 63.4^b	1060.0 ± 68.3^a
Mean $\pm$ SEM	579.4 ± 35.6	584.8 ± 34.8	658.4 ± 44.6	598.2 ± 38.5

^{a,b} = Means with different superscript letters across the rows are significantly different ($P < 0.05$), ($n = 15$).

Table 5 Effects of probiotic and/or fisetin on carcass and organ weights of broiler chickens

Parameters (g)	Control	Fisetin	Probiotic	Probiotic+Fisetin
Crop	29.2 ± 2.9	5.4 ± 0.5	5.4 ± 0.6	5.4 ± 0.4
Proventriculus	6.4 ± 24.0	6.0 ± 0.3	6.0 ± 0.3	7.2 ± 0.8
Gizzard	30.4 ± 1.6	31.8 ± 1.2	33.0 ± 0.9	34.6 ± 2.5
Abdominal fat	14.0 ± 3.2^a	3.2 ± 1.1^b	15.4 ± 2.1^a	8.0 ± 2.6^a
Liver	29.2 ± 2.9	27.2 ± 0.8	31.8 ± 1.4	29.4 ± 1.6
Gall bladder	3.0 ± 0.5	2.0 ± 0.8	2.4 ± 0.2	2.0 ± 0.2
Spleen	2.0 ± 0.3	2.0 ± 0.5	1.8 ± 0.4	1.8 ± 0.2
Heart	6.2 ± 1.0	4.8 ± 0.4	7.2 ± 0.2	6.4 ± 0.5
Lungs	7.4 ± 0.7	7.4 ± 0.5	7.8 ± 0.8	10.2 ± 0.5
Thigh	126.2 ± 22.5^a	108.4 ± 4.0^a	189.6 ± 4.8^b	160.8 ± 22.8^a
Drumstick	116.0 ± 22.6^a	88.2 ± 32.6^a	165.0 ± 4.3^b	152.4 ± 12.1^b
Live weight	1001.0 ± 68.1^a	1016.0 ± 42.8^a	1650.0 ± 67.0^b	1586.0 ± 60.8^b
Carcass weight	924.6 ± 66.2^a	954.6 ± 33.3^a	1490.0 ± 67.5^b	1383.0 ± 50.0^a

^{a,b} = Means with different superscript letters across the rows are significantly different ($P < 0.05$), ($n = 5$).

Table 6 Changes in pH of the breast muscle in broiler chickens administered with probiotic and/or fisetin

	Hour	Control	Fisetin	Probiotic	Probiotic +Fisetin
At slaughter	0:0	6.5 ± 0.1 ^a	6.5 ± 0.2 ^a	6.4 ± 0.2 ^a	6.5 ± 0.1 ^a
Post- slaughter:	24:0	5.9 ± 0.1 ^a	5.9 ± 0.1 ^a	6.3 ± 0.1 ^b	6.3 ± 0.1 ^b
	48:0	5.6 ± 0.1 ^a	5.7 ± 0.1 ^a	6.0 ± 0.1 ^b	6.1 ± 0.1 ^b
	72:0	5.5 ± 0.0 ^a	5.6 ± 0.0 ^b	5.9 ± 0.1 ^c	6.0 ± 0.1 ^c
Mean ± SEM		5.7 ± 0.1 ^a	5.7 ± 0.1 ^a	6.1 ± 0.1 ^b	6.1 ± 0.1 ^b

^{a,b,c} = Means with different superscript letters across the rows are significantly different ($P < 0.05$), ($n = 5$).

Table 7. Effect of probiotic and/or fisetin on small intestinal morphometric parameters in broiler chickens

Parameters	Control	Fisetin	Probiotic	Probiotic+Fisetin
Villus height (μm)	800.0 ± 0.0 ^a	1000.0 ± 0.1 ^b	1100.0 ± 0.1 ^b	1200.0 ± 0.1 ^b
Villus width (μm)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Crypt height (μm)	300.0 ± 0.0	400.0 ± 0.0	400.0 ± 0.0	400.0 ± 0.0
Crypt width (μm)	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Villus height/Crypt height	2.9 ± 0.2 ^a	3.0 ± 0.2 ^a	3.3 ± 0.3 ^b	3.6 ± 0.3 ^b
Villus surface area (μm ²)	6.0 ± 0.0	3.0 ± 0.0	4.0 ± 0.0	5.0 ± 0.0
Crypt surface area (μm ²)	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0

^{a,b} = Means with different superscript letters across the rows are significantly different ($P < 0.05$), ($n = 3$).

in fisetin ($400.0 \pm 0.0 \mu\text{m}$), probiotic ($400.0 \pm 0.0 \mu\text{m}$) and probiotic+fisetin ($400.0 \pm 0.0 \mu\text{m}$) groups were not significant when compared with the control group ($300.0 \pm 0.0 \mu\text{m}$). Villus height/crypt height ratio in probiotic+fisetin (3.6 ± 0.3) group was significant ($P < 0.05$) than in the control group (2.9 ± 0.2). Villus surface areas in fisetin ($3.0 \pm 0.0 \mu\text{m}^2$), probiotic ($4.0 \pm 0.0 \mu\text{m}^2$) and probiotic+fisetin ($5.0 \pm 0.0 \mu\text{m}^2$) groups did not differ significantly compared with that of the control ($6.0 \pm 0.0 \mu\text{m}^2$).

3.6 Discussion

The present study examined the dietary effect of probiotic and fisetin supplementation on performance indices, carcass characteristics and small intestinal morphology in broiler chickens. To the best of our knowledge, little or no information has been published on the use of fisetin both singly and in combination with probiotic on performance study in broiler chickens as at the time of this study. Our results showed that the supplementation of fisetin singly had no difference significantly on feed and water intake as compared with the control. This may be attributed to the fact that fisetin, a phytonutrient may not influence the lateral hypothalamic area, which is the feeding center located in the brain that stimulates hunger, thereby causing the broiler

chickens to feed and subsequently drink water to further aid digestion. Fisetin might only be effective in enhancing feed and water intake when administered in combination with a potent antioxidant or a growth promoting agent like probiotic.

Validating this hypothesis, we found that probiotic alone or in combination with fisetin increased feed and water intake. Importantly, yeast probiotic *Saccharomyces cerevisiae* possess the gut-brain connection ability both physically and chemically, which influences the lateral hypothalamic area, thereby stimulating feeding and subsequently drinking of water to help in the process of digestion, hence, the reason behind the increase in feed and water intake observed during this study in the probiotic supplementation group. Therefore, it could be stated that yeast probiotic might be efficient in stimulating feed and water intake when administered singly or in combination with other antioxidants or phytonutrients. The results agreed with Moataz et al. [22], who reported an increase in feed intake, which may concurrently increase water intake in the probiotic-supplemented group of layers, during his study. Similar findings were also reported in broiler chickens [36]. It furthermore agreed with that of Wang et al. [37] and Aluwong et al. [34], who both reported that the addition of dietary probiotic increased feed intake in broiler chickens. The results disagreed with the study

of Bai et al. [38], who recorded a decrease in feed intake in broiler chickens supplemented with probiotic, his finding was attributed to the strain of probiotic used.

The obtained results showed that the supplementation of probiotic and/or fisetin had no effect significantly on body weight gain of the broiler chickens within the first 4 weeks of life. However, from the 5th to 6th week, we observed a difference significantly in body weights between the control and probiotic groups, but not between the control and fisetin groups. This may be attributed to the time it took for the establishment of eubiosis by the probiotic in the digestive system of the broiler chickens [39-41]. The fact that the body weight was highest in the probiotic-supplemented broiler chickens at that age range (5 to 6 weeks) may be due to the enhanced absorptive area of the intestine clearly as increased villi length, and antigenic response of the gut mucosa. Also, feed intake in broiler chickens increased with a high dose of probiotic administration, and increased feed intake may exert a positive effect on water intake to facilitate digestion, which may further enhance an increase in body weight gain of the broiler chickens. Probiotic acts via the reduction of feed conversion ratio, which results in an increase in the body weight gain of the broiler chickens [38, 7]. This is achieved through the enhancement of digestibility by the microflora resident in the gut and the establishment of eubiosis [16].

The decrease in body weight gain observed in the fisetin supplementation group may be attributed to the poor feed and water intake which results in decrease weight gain of the broiler chickens as clear in this group. It could be speculated that fisetin is devoid of growth promoting ability as their administration singly is not of benefit to the chickens, although they have been reported to possess an antioxidant effect but they are not gut effective. Therefore, fisetin might be effective in enhancing growth rate when administered in combination with a potent growth promoting agents, e.g. yeast probiotic as seen in this study. Nevertheless, the decrease feed conversion ratio that led to an increase in body weight gain of the probiotic-supplemented broiler chickens in this study, agreed with Bai et al. [38], who reported that probiotic supplementation improves broiler chickens' performance. Also, Mahmood et al. [39], stated in his study that, increase feed intake is beneficial in increasing the body weight gain of broiler chickens. However, we observed that the co-administration of probiotic and fisetin did not exert synergistic effect on performance indices which might be attributed to fisetin not being effective as a growth promoter in broiler chickens.

The results of the carcass quality showed that broiler

chickens administered with probiotic were relatively heavier than those in the other treatment groups; as the group supplemented with probiotic showed an elevated body weight gain, followed by the co-administration of probiotic and fisetin group, as compared to the fisetin and control groups. This was evidenced by the organs weight, including the gizzards, heart, crop, liver, lungs, drum stick and thigh in the probiotic-supplemented group. The carcasses of broiler chickens administered probiotic were heavier after evisceration; this may be attributed to the adequately developed muscles and bone tissue formation that might have been enhanced by the yeast cell. Our results agreed with that of Berrama et al. [42], who reported a significant increase in the liver, pancreas and proventriculus of broiler chickens administered with cumin seeds, which is an antioxidant. The quantity of abdominal fat in the fisetin-supplemented group was lower when compared with the other experimental groups. This may be attributed to the lipolytic effect of fisetin in the body system [43, 44]. This effect may also be attributed to the decreased body and organ weights observed in broiler chickens administered with fisetin. Hence, it might not be suitable in enhancing their growth rate as every poultry farmer's goal is to maximise profits.

The measurement of pH changes in breast muscles of broiler chickens is a factor used to determine the consumption quality of meat even when refrigerated [45, 46]. The supplementation of probiotic and probiotic+fisetin prevented the sharp decrease in pH of the breast muscle at 24 and 48 h post-slaughter, as compared to that of the control and fisetin groups. The results from the current study demonstrated that the supplementation of broiler chickens with probiotic and/or fisetin improved the quality of the breast muscles by retarding the reduction rate of pH hence becoming less acidic. A decline in the quality of meat is associated with high acidity of the muscle [47], which may also be unfit for consumption. These findings agreed with that of Bai et al. [38], who reported that the administration of dietary probiotic increases the meat quality of broiler chickens by preventing rapid decrease in pH, which may enhance the quality of preservation of the broiler chicken meat. But fisetin alone had no significant effect on pH changes of the breast muscles. This may be attributed to its poor meat protection effect, therefore, the need for it to be administered concurrently with potent antioxidants or phytonutrients that possess an increase in meat preservation quality.

The findings of the present study showed that probiotic administration, both singly and in combination with fisetin significantly increased the small intestinal villus height, and villus height/crypt height ratio in

broiler chickens. This may be attributed to the fact that probiotic enhanced the growth rate of the broiler chickens gut. Impaired intestinal morphology can be ameliorated by the administration of propolis-rich flavonoid [48] and probiotic [49]. But in this study, fisetin a flavonoid was not effective to achieve this purpose. Our findings agreed with that of Mehaisen et al. [48], who reported that the intestinal morphology and barrier integrity of broiler chickens were impaired by heat stress. Shokryazdan et al. [50], also reported a similar finding of an increased villus height in broiler chickens administered probiotic as feed additive. An increase in surface area is proportionate to an increase in villus height of the intestinal epithelium, thereby enhancing the absorption of bio-available nutrients effectively as observed in the probiotic-supplemented group [51, 52, 53]. Broiler chickens administered with fisetin singly did not enhance the intestinal epithelium for adequate absorption rate, due to the decreased height, width and surface area observed in the epithelium as compared to the group administered with probiotic alone. This may be attributed to the poor gut effect of fisetin.

Based on our findings, the supplementation of probiotic singly is hereby recommended to improve broiler chickens' productivity thus, enhancing profitability to the poultry farmers. Further studies should better define how the nutrient transporters could perform their functions, especially in the probiotic-administered group, in improving the performance of the broiler chickens.

4 Conclusion

Dietary supplementation of probiotic singly, increased performance, carcass quality, and surface area of small intestinal villi and crypts of the broiler chickens. On the other hand, fisetin supplementation either singly or in combination with probiotic had no synergistic effect in the enhancement of productivity.

Acknowledgements: We acknowledge the technical assistance rendered by Iliya Saleh during the period of the experiment. Furthermore, Drs Friday O. Zakari and Abdullahi A. Bada are appreciated for their advice regarding the data analysis.

Authors Contributions: Concept- J.O.A., T.A.; Design- J.O.A., T.A.; Supervision- T.A., J.O.A.; Resources- V.O.S., T.A., J.O.A., N.E.O.; Materials- V.O.S., T.A.; Data Collection and/or Processing- V.O.S., T.A., J.O.A., N.E.O.; Analysis and/or Interpretation- V.O.S., T.A., J.O.A., N.E.O.; Literature Search- V.O.S., T.A., J.O.A.; Writing Manuscript- V.O.S., J.O.A., T.A., N.E.O.; Critical Review- V.O.S., J.O.A., T.A.

Funding information: Authors state no funding involved.

Conflict of interest: The authors state no conflicts of interest.

Data availability statement:

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

References

- [1] Zawadzki A, Alloo C, Grossi AB, do Nascimento ES, Almeida LC, Bogusz Junior S, et al. Effect of hop β -acids as dietary supplement for broiler chickens on meat composition and redox stability. *Food Res Int.* 2018 Mar;105:210–20.
- [2] Darmani Kuhi H, France J. Modelling cumulative egg production in laying hens and parent stocks of broiler chickens using classical growth functions. *Br Poult Sci.* 2019 Oct;60(5):564–9.
- [3] Johnson TJ, Youmans BP, Noll S, Cardona C, Evans NP, Karnezos TP, et al. A consistent and predictable commercial broiler chicken bacterial microbiome in antibiotic-free production displays strong correlations with performance. *Appl Environ Microbiol.* 2018 May;84(12):e00362–18.
- [4] Qaid MM, Al-Mufarrej SI, Azzam MM, Al-Garadi MA, Albaadani HH, Alhidary IA, et al. Growth performance, serum biochemical indices, duodenal histomorphology, and cecal microbiota of broiler chickens fed on diets supplemented with cinnamon bark powder at pre-starter and starter phases. *Animals (Basel).* 2021 Jan;11(1):94.
- [5] Ren T, Qiao M, Zhang L, Weese J, Huang TS, Ren X. Antimicrobial activity of N-Halamine coated materials in broiler chicken houses. *J Food Prot.* 2018 Feb;81(2):195–201.
- [6] Saleh AS, Ragab MM, Ahmed EA, Abudabos AM, Ebeid TA. Effects of dietary zinc-methionine supplementation on growth performances, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature. *J Appl Anim Res.* 2018;46(1):820–7.
- [7] Ölmez M, Şahin T, Karadağoglu Ö, Yörük MA, Kara K, Dalğa S. Growth performance, carcass characteristics, and fatty acid composition of breast and thigh meat of broiler chickens fed gradually increasing levels of supplemental blueberry extract. *Trop Anim Health Prod.* 2021 Jan;53(1):109.
- [8] Stadig J, Lisanne M, Rodenburg T, Bas A, Bart R, Bert Y, et al. Effect of free range access, shelter type and weather conditions on free-range use and welfare of slow-growing broiler chickens. *Appl Anim Behav Sci.* 2016;12:1–6.
- [9] Wilson FD, Cummings TS, Barbosa TM, Williams CJ, Gerard PD, Peebles ED. Comparison of two methods for determination of intestinal villus to crypt ratios and documentation of early age-associated ratio changes in broiler chickens. *Poult Sci.* 2018 May;97(5):1757–61.
- [10] Kalia S, K Bharti V, Gogoi D, Giri A, Kumar B. Studies on the growth performance of different broiler strains at high altitude and evaluation of probiotic effect on their survivability. *Sci Rep.* 2017 Apr;7:46074.

- [11] Aluwong T, Sumanu VO, Ayo JO, Ocheja BO, Zakari FO, Minka NS. Daily rhythms of cloacal temperature in broiler chickens of different age groups administered with zinc gluconate and probiotic during the hot-dry season. *Physiol Rep*. 2017 Jun;5(12):e13314.
- [12] Kikusato M, Xue G, Pastor A, Niewold TA, Toyomizu M. Effects of plant-derived isoquinoline alkaloids on growth performance and intestinal function of broiler chickens under heat stress. *Poult Sci*. 2021 Feb;100(2):957–63.
- [13] Del Vesco AP, Khatlab AS, Goes ES, Utsunomiya KS, Vieira JS, Oliveira Neto AR, et al. Age-related oxidative stress and antioxidant capacity in heat-stressed broilers. *Animal*. 2017 Oct;11(10):1783–90.
- [14] Ogbuagu NE, Aluwong T, Ayo JO, Sumanu VO. Effect of fisetin and probiotic supplementation on erythrocyte osmotic fragility, malondialdehyde concentration and superoxide dismutase activity in broiler chickens exposed to heat stress. *J Vet Med Sci*. 2018 Dec;80(12):1895–900.
- [15] Sumanu VO, Aluwong T, Ayo JO, Ogbuagu NE. Evaluation of changes in tonic immobility, vigilance, malondialdehyde, and superoxide dismutase in broiler chickens administered fisetin and probiotic (*Saccharomyces cerevisiae*) and exposed to heat stress. *J Vet Behav*. 2019;31:36–42.
- [16] Al-Nasrawi MA, Al-Kassie GA, Lateef Ali NA. Role of yeast (*Saccharomyces cerevisiae*) as a source of probiotics in poultry diets. *Eur J Mol Clin Med*. 2020;7(7):6611–7.
- [17] Zhang M, Wang D, Geng Z, Li P, Sun Z, Xu W. Effect of heat shock protein 90 against ROS-induced phospholipid oxidation. *Food Chem*. 2018 Feb;240:642–7.
- [18] Qwele K, Hugo A, Oyedemi SO, Moyo B, Masika PJ, Muchenje V. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. *Meat Sci*. 2013 Mar;93(3):455–62.
- [19] Sun H, Jiang R, Xu S, Zhang Z, Xu G, Zheng J, et al. Transcriptome responses to heat stress in hypothalamus of a meat-type chicken. *J Anim Sci Biotechnol*. 2015 Feb;6(1):6–14.
- [20] Zhang P, Yan T, Wang X, Kuang S, Xiao Y, Lu W, et al. Probiotic mixture ameliorates heat stress of laying hens by enhancing intestinal barrier function and improving gut microbiota. *Ital J* 375. *Anim Sci*. 2017;16:292–300.
- [21] López-Andrés JJ, Aguilar-Lasserre AA, Morales-Mendoza LF, Azzaro-Pantel C, Pérez-Gallardo JR, Rico-Contreras JO. Environmental impact assessment of chicken meat production via an integrated methodology based on LCA, simulation and genetic algorithms. *J Clean Prod*. 2018 Feb;174:477–91.
- [22] Fahti M, Al-Homidan I, Al-Dokhalil A, Ebeid T, Abou-Emera O, Alsagan A. Effects of dietary probiotic (*Bacillus subtilis*) supplementation on productive performance, immune response and egg quality characteristics in laying hens under high ambient temperature. *Ital J Anim Sci*. 2018 Jan;17(3):804–14.
- [23] Hanchai K, Trairatapiwan T, Lertpatarakomol R. Drinking water supplemented with wood vinegar on growth performance, intestinal morphology, and gut microbial of broiler chickens. *Vet World*. 2021 Jan;14(1):92–6.
- [24] Hag S, Poondla N. Genetically engineered probiotics. In: Kaur IP, Deol PK, editors. *Probiotic Research in Therapeutics*. Singapore: Springer; 2021. pp. 295–328. [Erratum in: Kaur IP, Deol PK, editors. *Probiotic Research in Therapeutics*. Singapore: Springer; 2021. pp. C1].
- [25] Singh S, Singh AK, Garg G, Rizvi SI. Fisetin as a caloric restriction mimetic protects rat brain against aging induced oxidative stress, apoptosis and neurodegeneration. *Life Sci*. 2018 Jan;193:171–9.
- [26] Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: a dietary antioxidant for health promotion. *Antioxid Redox Signal*. 2013 Jul;19(2):151–62.
- [27] Nan W, Fang Y, Ke L, Lanlan Z, Guo Y, Mingjun D, et al. Fisetin regulates astrocyte 408 migration and proliferation in vitro. *Int J Mol Sci*. 2017;18:852–9.
- [28] Youns M, Abdel Halim Hegazy W. The natural flavonoid fisetin inhibits cellular proliferation of hepatic, colorectal, and pancreatic cancer cells through modulation of multiple signaling pathways. *PLoS One*. 2017 Jan;12(1):e0169335.
- [29] Chen PY, Ho YR, Wu MJ, Huang SP, Chen PK, Tai MH, et al. Cytoprotective effects of fisetin against hypoxia-induced cell death in PC12 cells. *Food Funct*. 2015 Jan;6(1):287–96.
- [30] Antika LD, Dewi RM. Pharmacological aspects of fisetin. *Asian Pac J Trop Biomed*. 2021;11(1):1–9.
- [31] Prakash D, Gopinath K, Sudhandiran G. Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity. *Neuromolecular Med*. 2013 Mar;15(1):192–208.
- [32] Chuang JY, Chang PC, Shen YC, Lin C, Tsai CF, Chen JH, et al. Regulatory effects of fisetin on microglial activation. *Molecules*. 2014 Jun;19(7):8820–39.
- [33] Yuan Z, Yao F, Hu Z, Sun S, Wu B. Quercetin inhibits the migration and proliferation of astrocytes in wound healing. *Neuroreport*. 2015 May;26(7):387–93.
- [34] Aluwong T, Kawu M, Raji M, Dzenda T, Govwang F, Sinkalu V, et al. Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. *Antioxidants*. 2013 Nov;2(4):326–39.
- [35] [Solis de los SF, Tellez G, Farnell MB, Balog JM, Anthony NB, Pavlidis HO, et al. Hypobaric hypoxia in ascites resistant and susceptible broiler genetic lines influences gut morphology. *Poult Sci*. 2005 Sep;84(9):1495–8
- [36] Mohammed AA, Jacobs JA, Murugesan GR, Cheng HW. Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. *Poult Sci*. 2018 Apr;97(4):1101–8.
- [37] Wang X, Farnell YZ, Peebles ED, Kiess AS, Wamsley KG, Zhai W. Effects of prebiotics, probiotics, and their combination on growth performance, small intestine morphology, and resident *Lactobacillus* of male broilers. *Poult Sci*. 2016 Jun;95(6):1332–40.
- [38] Bai K, Huang Q, Zhang J, He J, Zhang L, Wang T. Supplemental effects of probiotic *Bacillus subtilis* fmbj on growth performance, antioxidant capacity, and meat quality of broiler chickens. *Poult Sci*. 2017 Jan;96(1):74–82.
- [39] Mahmood T, Mirza MA, Nawaz H, Shahid M. Exogenous protease supplementation of poultry by-product meal-based diets for broilers: effects on growth, carcass characteristics and nutrient digestibility. *J Anim Physiol Anim Nutr (Berl)*. 2018 Feb;102(1):e233–41.
- [40] Hafez A, Nassef E, Fahmy M, Elsabagh M, Bakr A, Hegazi E. Impact of dietary nano-zinc oxide on immune response and antioxidant defense of broiler chickens. *Environ Sci Pollut Res Int*. 2020 Jun;27(16):19108–14.

- [41] Zheng XC, Wu QJ, Song ZH, Zhang H, Zhang JF, Zhang LL, et al. Effects of Oridonin on growth performance and oxidative stress in broilers challenged with lipopolysaccharide. *Poult Sci.* 2016 Oct;95(10):2281–9.
- [42] Berrama Z, Temim S, Souames S, Ainbaziz H. Growth performance, carcass and viscera yields, blood constituents and thyroid hormone concentrations of chronic heat stressed broilers fed diets supplemented with cumin seeds (*Cuminum cyminum L.*). *Kafkas Univ Vet Fak Derg.* 2017;23:735–42.
- [43] Zhao Y, Chen B, Shen J, Wan L, Zhu Y, Yi T, et al. The beneficial effect of quercetin, curcumin, and resveratrol in obesity. *Oxid Med Cell Longev.* 2017;2017:1459497.
- [44] Hassan FA, Roushdy EM, Kishawy AT, Zagloul AW, Tukur HA, Saadeldin IM. Growth performance, antioxidant capacity, lipid-related transcript expression and the economics of broiler chickens fed different levels of rutin. *Animals (Basel).* 2018 Dec;9(1):7–12.
- [45] Imik H, Ozlu H, Gumus R, Atasever MA, Urcar S, Atasever M. Effects of ascorbic acid and α -lipoic acid on performance and meat quality of broilers subjected to heat stress. *Br Poult Sci.* 2012;53(6):800–8.
- [46] Karadağoglu O, Şahin T, Olmez M, Yakan A, Ozsoy B. Changes in serum biochemical and lipid profile, and fatty acid composition of breast meat of broiler chickens fed supplemental grape seed extract. *Turk J Vet Anim Sci.* 2020;44(2):182–90.
- [47] Torki M, Soltani J, Mohammadi H. Effects of adding ethanol extract of propolis and cumin essential oil to diet on the performance, blood parameters, immune response and carcass traits of broiler chicks. *Iran J Appl Anim Sci.* 2015 Dec;5(4):911–8.
- [48] Mehaisen GM, Ibrahim RM, Desoky AA, Safaa HM, El-Sayed OA, Abass AO. The importance of propolis in alleviating the negative physiological effects of heat stress in quail chicks. *PLoS One.* 2017 Oct;12(10):e0186907.
- [49] Sugiharto S, Yudiarti T, Isroli I, Widiastuti E, Kusumanti E. Dietary supplementation of probiotics in poultry exposed to heat stress – a review. *Ann Anim Sci.* 2017;17(3):591–604.
- [50] Shokryazdan P, Faseleh Jahromi M, Liang JB, Ramasamy K, Sieo CC, Ho YW. Effects of a *Lactobacillus salivarius* mixture on performance, intestinal health and serum lipids of broiler chickens. *PLoS One.* 2017 May;12(5):e0175959.
- [51] Al-Sultan SI, Abdel-Raheem SM, El-Ghareeb WR, Mohamed MH. Comparative effects of using prebiotic, probiotic, synbiotic and acidifier on growth performance, intestinal microbiology and histomorphology of broiler chicks. *Jpn J Vet Res.* 2016;64:187–95.
- [52] Davoodi P, Ehsani A. Characteristics of carcass traits and meat quality of broiler chickens reared under conventional and free-range systems. *J World Poult Res.* 2020 Dec;10(4):623–30.
- [53] Al-Fataftah AR, Abdelqader A. Effects of dietary *Bacillus subtilis* on heat stressed broilers performance, intestinal morphology and microflora composition. *Anim Feed Sci Technol.* 2014;198:279–85.