Arch. Anim. Breed., 60, 347–356, 2017
https://doi.org/10.5194/aab-60-347-2017

Open Access
Archives Animal Breeding

Effect of dietary supplementation with *Rhizopus oryzae* or *Chrysonilia crassa* on growth performance, blood profile, intestinal microbial population, and carcass traits in broilers exposed to heat stress

Sugiharto Sugiharto, Turrini Yudiarti, Isroli Isroli, Endang Widiastuti, and Fatan Dwi Putra

Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia

Correspondence to: Sugiharto Sugiharto (sgh_undip@yahoo.co.id)

Received: 9 March 2017 – Revised: 24 August 2017 – Accepted: 31 August 2017 – Published: 29 September 2017

Abstract. Dietary supplementation of additives has recently been part of strategies to deal with the detrimental effects of heat stress (HS) on the performance and carcass traits in broiler chicks. This study aimed to investigate the effect of dietary supplementation with the fungi *Rhizopus oryzae* or *Chrysonilia crassa* on growth, blood profile, intestinal microbial population and carcass traits in broiler chicks subjected to HS. *R. oryzae* and *C. crassa* are filamentous fungi isolated from the ileum of indigenous Indonesian chickens which exhibited probiotic and antioxidant properties. Two hundred and forty 21-day-old male broiler chicks were randomly allotted into six groups, including birds reared under normal temperature ($28 \pm 2\,^{\circ}C$) (CONT), birds reared under HS conditions ($35 \pm 2\,^{\circ}C$) (HS-CONT), birds reared under HS and provided with commercial anti-stress formula (HS-VIT), birds reared under HS and provided with *R. oryzae* (HS-RO), birds reared under HS and provided with *C. crassa* (HS-CC) and birds reared under HS and provided with rice bran (HS-RB). Body weight gain was highest ($P < 0.01$) and lowest ($P < 0.01$) in CONT and HS-CONT birds, respectively. The heart was heavier ($P < 0.05$) in CONT than in HS-CONT and HS-VIT birds. CONT birds had heavier duodenum ($P < 0.05$) and jejunum ($P < 0.01$) than other birds. Eosinophils was higher ($P < 0.05$) in HS-CC than in other birds. Low-density lipoprotein (LDL) was higher ($P < 0.05$) in HS-CONT than in CONT, HS-VIT and HS-CC birds. Total triglyceride was highest ($P < 0.05$) and lowest ($P < 0.05$) in HS-RB and HS-RO birds, respectively. Alanine aminotransferase (ALT) was higher ($P < 0.05$) in HS-CONT than in other HS birds. Total protein was lowest and highest ($P < 0.05$) in CONT and HS-CONT birds, respectively. Albumin was higher ($P < 0.05$) in HS-CONT and HS-VIT than in HS-RO birds. Globulin was lower ($P < 0.05$) in CONT than in HS-CONT, HS-VIT and HS-RB birds. Uric acid was lower ($P < 0.05$) in CONT than in HS-CONT and HS-VIT birds. The 2,2′-azino-bis(3-ethyl-benzthiazolin-6-sulfonic acid) (ABTS) percentage inhibition values of the serum were higher ($P < 0.01$) in CONT, HS-CONT and HS-VIT than those in HS-RO, HS-CC and HS-RB birds. In conclusion, dietary supplementation of *C. crassa* decreased serum LDL concentration and ALT activity and improved antioxidant status of broiler subjected to HS. Supplementation with *C. crassa* seemed beneficial in improving physiological conditions of HS birds.

Published by Copernicus Publications on behalf of the Leibniz Institute for Farm Animal Biology.

1 Introduction

The broiler industry is an important subsector of livestock production and plays an important role in the Indonesian economy. As a tropical country, Indonesia experiences high temperature and humidity throughout the year. This tropical environment can be a life-threatening factor to broilers (reared in housing without temperature control equipment), as it can induce heat stress (HS). A recent study has revealed the detrimental effects of HS on physiology, immunology and microbiology resulting in abnormalities and impaired performances in birds (Sugiharto et al., 2016a). HS also decreases carcass yield and increases fat content in broiler chicks (Zeferino et al., 2016).

Apart from providing the temperature-controlled housing facilities, nutritional strategies such as supplementation of probiotics or antioxidants in broiler diets has been suggested to alleviate some of the detrimental effects of HS. Sohail et al. (2010) reported that probiotics improved the physiological condition of HS birds. Likewise, probiotics prevented an intestinal microbial shift (Song et al., 2014; Jahromi et al., 2015) and improved humoral immunity in birds during HS (Sohail et al., 2010). With regard to antioxidants, supplementations of vitamin C, E and chromium in feed improved blood profile, immune responses, antioxidant status and performance (Khan et al., 2011, 2012; Tawfeek et al., 2014) and reversed the increased fat yield in HS broilers (Tawfeek et al., 2014).

In addition to bacteria and yeast, some fungi have been known to possess probiotic and antioxidant properties (Sugiharto et al., 2015, 2016b). In a recent study we isolated some filamentous fungi from the ileum of the Indonesian indigenous chickens, two of which were *Rhizopus oryzae* and *Chrysonilia crassa* (Yudiarti et al., 2012). Indeed, the fungi exhibited probiotic potentials as they were able to grow in the simulated gastric juice (pH 3 and 8) and bile solution (0.4 and 0.8 % of bile salt) and capable of inhibiting the growth of *Escherichia coli* and *Aspergillus flavus* in vitro (Yudiarti, 2012; Yudiarti et al., 2013). In the current study, the antioxidant capacities of *R. oryzae* and *C. crassa* were noted as indicated by the inhibitory potentials of the fungi against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethyl-benzthiazolin-6-sulfonic acid) (ABTS) free radicals. On the basis of their probiotic and antioxidant potentials, it was therefore interesting to exploit *R. oryzae* and *C. crassa* for ameliorating the detrimental effect of HS in commercial broilers. Therefore, the present study aimed to investigate the effect of dietary supplementation with the fungus *R. oryzae* or *C. crassa* on growth, blood profile, intestinal microbial population, and carcass traits in broilers subjected to HS. Both *R. oryzae* and *C. crassa* were grown in rice bran before being given to broilers. Rice bran was selected as a medium for growing the fungi as it contains prebiotic components (Komiyama et al., 2011) that can support the growth of the fungi.

2 Materials and methods

2.1 Preparation of *R. oryzae* and *C. crassa*

R. oryzae and *C. crassa* inoculum were prepared by retrieving the fungal culture (maintained on a potato–dextrose–agar (PDA; Merck KGaA, Darmstadt, Germany) medium and stored at 4 °C), streaking on PDA medium and incubating at 38 °C for 2 days. The fungal mycelia was dislodged from the PDA and diluted in 100 mL of sterilized distilled water. The suspension was then used to inoculate 200 g of sterilized rice bran (85.9 % dry matter, DM). The suspension (inoculum) was standardized to contain ca. 1×10^{12} cfu mL^{-1} for each inoculation. After aerobic incubation at room temperature for 4 days, fungal colony grown in rice bran was enumerated based on the colony counting method. Samples of *R. oryzae*- and *C. crassa*-fermented rice bran were obtained for proximate analysis. The rest of the fermented rice bran was used for the vivo trial. The fungal fermented rice bran was produced in several batches, and the procedures and conditions were similar for the all of the batches.

2.2 Assay for antioxidant potentials of fungi

For the measurement of antioxidant potentials, fungi were inoculated onto potato dextrose broth in conical flask. After 2 days of aerobic incubation at 38 °C, the fungal colonies were transferred into conical tubes and then centrifuged at $2800 \times g$ for 10 min. The fungal pellets (1 g) were then homogenized, dissolved in 10 mL methanol and centrifuged at $4000 \times g$ for 5 min. Supernatants were obtained and further used for antioxidant assays. Antioxidant activities of the fungi were measured by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay (Sohaib et al., 2012) and 2,2′-azino-bis(3-ethyl-benzthiazolin-6-sulfonic acid) (ABTS) method (Petacci et al., 2010).

2.3 In vivo experiment

The rearing and handling of animals for the present study was approved by the Animal Ethics Committee of Faculty of Animal and Agricultural Sciences, Diponegoro University. A total of 300 male Lohmann MB 202-day-old chicks (body weight (BW) 47.3 ± 0.78 g; mean ± SD) purchased from a local hatchery were raised until day 21 according to the general practice of commercial broiler farming in Indonesia. They were placed in concrete floor pens on rice husk litter in temperature-controlled houses. The temperature at day 1 was set at 32 ± 2 °C and was decreased until it reached 28 ± 2 °C at day 21. Within this period, all birds were provided with commercial starter feed composed of yellow corn, rice bran, fish meal, soybean meal, copra meal, meat bone meal, peanut meal, canola, ground wheat grain, leaf meal, vitamins, dicalcium phosphate, and trace minerals. The feed contained (as dry basis) 13 % moisture, 23 % crude protein, 5 % crude fat, 5 % crude fiber, 7 % ash, 0.9 % calcium, 0.6 %

phosphorus and 3025 kcal kg^{-1} metabolizable energy (ME). The birds were vaccinated with Newcastle disease vaccine (NDV) through eye drops and drinking water at days 4 and 18, respectively. The birds were also vaccinated with infectious bursal disease (IBD) vaccine through drinking water at days 14 and 24. At day 20, the birds were weighed and 240 birds with uniform weight (845.21 ± 2.57 g) were randomly allotted into six groups of 40 chicks each and five replicates of eight chicks. These six treatment groups included (1) birds reared under normal temperature (28 ± 2 °C) (CONT), (2) birds reared under HS conditions (35 ± 2 °C) (HS-CONT), (3) birds reared under HS and provided with commercial anti-stress formula (CAS) (HS-VIT), (4) birds reared under HS and provided with rice bran containing *R. oryzae* (HS-RO), (5) birds reared under HS and provided with rice bran containing *C. crassa* (HS-CC), and (6) birds reared under HS and provided with rice bran (HS-RB). From day 21 onward, all birds were fed commercial finisher feed composed of yellow corn, peanut meal, soybean meal, fish meal, meat meal, rice bran, pollard, vitamins and trace minerals. The diet contained (as dry basis) 13 % moisture, 20 % crude protein, 5 % crude fiber, 5 % crude fat, 8 % ash, 0.9 % calcium, 0.6 % phosphorus (0.4 % available phosphorus) and 3225 kcal kg^{-1} ME. The diets and water were offered ad libitum. Treatments (HS and dietary supplementations) were applied from day 22 to 35. The fungi growing in rice bran (fermented rice bran containing *R. oryzae* or *C. crassa* ca. 1×10^7 cfu g^{-1}) or untreated rice bran were administrated in the diets by adding 10 g kg^{-1} of these supplements to the commercial grower feed just before feeding to broilers. The supplements were added at the expense of the commercial feed. The CAS (Vita Stress®; PT. Medion Farma Jaya, Bandung, Indonesia) was provided to the birds through drinking water according to the manufacturer's instructions (1 g per 2 L of drinking water). The CAS contained (per kg) 6 000 000 IU of vitamin A, 1 200 000 IU of vitamin D_3, 2500 IU of vitamin E, 3000 mg of vitamin K, 2000 mg of vitamin B_1, 3000 mg of vitamin B_2, 1000 mg of vitamin B_6, 2 mg of vitamin B_{12}, 20 000 mg of vitamin C, 15 000 mg of nicotinic acid, 5000 mg of calcium-D-pantothenate and 750 000 mg of electrolytes (Na, K, Ca, Mg). Temperature was maintained throughout the day until the end of the trial. Humidity in the broilers' house was maintained at 60–80 %. The BW gain and feed intake were recorded at days 28 and 35. Data of feed intake and BW gain were used to determine the feed conversion ratio (FCR).

On day 32, birds from each pen (five from each treatment group) were randomly selected for blood collection after fasting for 8 h. Blood was obtained from the birds' wing veins and placed in EDTA-containing Vacutainers for hematological analysis. For serum biochemical analysis, blood was placed in vacationer tubes containing no anticoagulant and allowed to clot for 2 h at room temperature. Serum was produced following centrifugation at 500 × *g* for 15 min. The same birds from which blood was sampled were slaughtered by means of halal neck cut after being weighed. The slaughtering method followed the standard stages of slaughtering (Alshelmani et al., 2016). The internal organs, carcass, breast and thigh muscles were immediately removed and weighed. Digesta samples from the ileum were aseptically obtained for microbiological analysis. Samples of breast and thigh muscles were also obtained for the measurement of pH and drip loss.

Microbial counts in the ileal digesta were determined according to Engberg et al. (2004) with few modifications. Total bacteria were enumerated on PDA medium (Merck KGaA) after aerobic incubation at 38 °C for 24 h. Coliform bacteria and lactose-negative enterobacteria were counted on MacConkey agar (Merck KGaA) following aerobic incubation at 38 °C for 24 h as red and colorless colonies, respectively. Enterobacteria were the number of coliform bacteria and lactose-negative enterobacteria. Lactic acid bacteria (LAB) were enumerated on de Man, Rogosa and Sharpe (MRS; Merck KGaA) agar after anaerobic incubation at 38 °C for 48 h.

The numbers of erythrocytes and leukocytes were determined based on the dilution flask method and a Bürker chamber was used to count corpuscles. Sahli's method was employed to estimate the level of hemoglobin. The hematocrit values were determined by means of the microhematocrit technique. The differential leukocytes of broilers were determined using a light microscope with an immersion lens. Coverslip technique was applied when preparing blood smears. Data for the numbers of heterophils and lymphocytes were used to calculate heterophils to lymphocytes (H/L) ratio.

Serum total triglyceride was measured based on the enzymatic colorimetric method using glycerol-3-phosphateoxidase (GPO). Total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were determined according to enzymatic colorimetric method using cholesterol oxidase (CHOD/PAP). The concentration of glucose in blood was determined based on enzymatic colorimetric method using glucose oxidase (GOD-PAP). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were measured based on the International Federation of Clinical Chemistry (IFCC) method without pyridoxal phosphate (37 °C). The measurement of total protein in the serum was conducted by means of photometric test based on the biuret method with the kit. The concentration of albumin in serum was measured by means of photometric test using bromocresol green. Globulin was obtained by subtracting albumin values from total serum protein. The assay of uric acid in serum was conducted based on the enzymatic color test. All biochemical assays in the serum were performed in duplicate and according to manufacturer's instructions (DiaSys Diagnostic System GmbH, Holzheim, Germany). The antioxidant activities in serum were measured by DPPH (Sohaib et al., 2012) and ABTS (Petacci et al., 2010) free-radical scavenging assay methods as mentioned above. For the entire assays in the whole blood and serum, the intra-assay and inter-assay

Table 1. Percentage inhibition values of the fungi towards DPPH or ABTS free radicals[1].

Composition (g/100 g)	*R. oryzae* ($n = 3$)	*C. crassa* ($n = 3$)	Ascorbic acid[2] ($n = 3$)
DPPH (% inhibition)	95.3 ± 2.08	75.1 ± 7.68	93.1 ± 1.55
ABTS (% inhibition)	78.6 ± 4.46	65.2 ± 7.88	96.1 ± 0.99

[1] Data are presented as means ± SD. [2] Ascorbic acid was used as a standard antioxidant. *n*: number of samples analyzed; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis(3-ethyl-benzthiazolin-6-sulfonic acid).

Table 2. Chemical composition of the *R. oryzae* or *C. crassa*-fermented rice bran (as dry basis)[1].

Composition (g/100 g)	*R. oryzae*-fermented rice bran ($n = 2$)	*C. crassa*-fermented rice bran ($n = 2$)	Untreated rice bran ($n = 2$)
Moisture	37.8 ± 2.91	39.8 ± 2.10	14.1 ± 0.90
Crude ash	12.4 ± 0.20	11.8 ± 0.34	10.7 ± 0.23
Crude fat	14.0 ± 0.51	8.08 ± 0.76	12.3 ± 0.54
Crude protein	14.7 ± 0.21	14.8 ± 0.34	14.7 ± 0.67
Crude fiber	19.2 ± 0.43	12.1 ± 0.67	23.9 ± 0.56

[1] Data are presented as means ± SD. *n*: number of samples analyzed.

coefficients of variation of the control samples were 1.5–4.8 and 2.7–5.4 %, respectively.

Drip loss of meat was measured according to Wang et al. (2015) with a few modifications. Around 45 min after slaughter, the breast or thigh muscles were weighed and the pH was measured. The meats were then placed in a Whirlpak bag, stored in a refrigerator (5 °C) and reweighed for the final pH measurement. The drip loss of meat was measured based on the weight loss and expressed as a percentage.

2.4 Data analysis

Data obtained in this study (except data of mortality) were analyzed according to a completely randomized design by ANOVA using the general linear models procedure in SAS (SAS Inst. Inc., Cary, NC, USA). The pen was treated as the experimental unit. Results are presented as least squares means (LSMEANS) and standard error of the mean (SEM). Significant differences among dietary treatments were further analyzed with Duncan's multiple-range test. A significance level of $P \leq 0.05$ was applied.

3 Results

3.1 Antioxidant activity of fungi and chemical composition of fungal fermented rice bran

The antioxidant activities of the fungi are presented as percentage inhibition (Table 1), at which the higher percentage inhibition towards DPPH or ABTS indicated the higher antioxidant potentials of the fungi. Based on DPPH assay, the fungus *R. oryzae* had antioxidant activity corresponding to ascorbic acid and was higher than that of *C. crassa*. Concomitant results were found when ABTS method was applied. Fermentation with the fungi especially *C. crassa* lowered the fiber and crude fat contents of rice bran (Table 2).

3.2 Performance of broilers

Data on the performance of broilers are presented in Table 3. Birds in CONT group had higher ($P < 0.01$) and lower ($P < 0.05$) BW gain and FCR, respectively, than in other birds during days 22 to 28. Body weight gain and FCR was lower ($P < 0.05$) and higher ($P < 0.05$), respectively, in HS-CONT group than in other groups during days 29 to 35. Throughout the treatment period (days 22 to 35), BW gain was highest ($P < 0.01$) and lowest ($P < 0.01$) in CONT and HS-CONT birds, respectively. FCR was lower ($P < 0.05$) in CONT than in HS-CONT and HS-RO birds, but not different ($P > 0.05$) when compared with HS-VIT, HS-CC or HS-RB. Feed intake and mortality were not different ($P > 0.05$) across the treatment groups throughout the experimental period, though numerically feed intake and mortality were higher and lower, respectively, in CONT than in other birds.

3.3 Internal organ weight

Data on internal organs of broilers are presented in Table 4. The relative weight of heart was higher ($P < 0.05$) in CONT than in HS-CONT or HS-VIT birds, but the difference was not significant compared to HS-RO, HS-CC and HS-RB

Table 3. Effect of dietary supplementation on BW gain, feed intake, FCR and mortality of broilers reared under normal or HS conditions.

Day	Treatment group						SEM	*P* value
	CONT ($n = 40$)	HS-CONT ($n = 40$)	HS-VIT ($n = 40$)	HS-RO ($n = 40$)	HS-CC ($n = 40$)	HS-RB ($n = 40$)		
BW gain (g bird^{-1})								
22–28	501^{a}	404^{b}	411^{b}	405^{b}	417^{b}	417^{b}	15.1	< 0.01
29–35	586^{a}	459^{b}	574^{a}	521$^{a, b}$	567^{a}	575^{a}	26.5	0.02
22–35	1087^{a}	905^{c}	985^{bc}	926^{bc}	985^{bc}	993^{b}	26.6	< 0.01
Feed intake (g bird^{-1})								
22–28	856	827	825	814	839	807	19.5	0.56
29–35	1221	1153	1152	1182	1144	1150	31.6	0.52
22–35	2076	1981	1977	1996	1984	1957	40.5	0.40
FCR								
22–28	1.72^{a}	2.05^{b}	2.01^{b}	2.02^{b}	2.02^{b}	1.93^{b}	0.06	0.01
29–35	2.09^{a}	2.54^{b}	2.02^{a}	2.33$^{a, b}$	2.04^{a}	2.00^{a}	0.13	0.03
22–35	1.91^{a}	2.16^{b}	2.01$^{a, b}$	2.17^{b}	2.02$^{a, b}$	1.97$^{a, b}$	0.06	0.04
Mortality[1]	0	2	2	1	2	3		0.91

[1] The number of dead chickens from 40 chickens per treatment. The data were subjected to χ^2 analysis. Numbers in the same row with different letters (a, b, c) are significantly different. CONT: birds reared under normal temperature (28 ± 2 °C); HS-CONT: birds reared under HS conditions (35 ± 2 °C); HS-VIT: birds reared under HS and provided with CAS; HS-RO: birds reared under HS and provided with rice bran containing *R. oryzae*; HS-CC: birds reared under HS and provided with rice bran containing *C. crassa*; HS-RB: birds reared under HS and provided with rice bran; *n*: number of birds per experimental groups; FCR: feed conversion ratio.

Table 4. Effect of dietary supplementation on internal organs of broilers reared under normal or HS conditions.

Items	Treatment group						SEM	*P* value
(% live weight)	CONT ($n = 5$)	HS-CONT ($n = 5$)	HS-VIT ($n = 5$)	HS-RO ($n = 5$)	HS-CC ($n = 5$)	HS-RB ($n = 5$)		
Heart	0.58^{a}	0.41^{b}	0.41^{b}	0.49$^{a, b}$	0.48$^{a, b}$	0.46$^{a, b}$	0.04	0.04
Liver	2.27	2.40	2.50	2.14	2.18	2.38	0.17	0.67
Spleen	0.10	0.09	0.10	0.09	0.09	0.07	0.01	0.49
Thymus	0.20	0.21	0.19	0.32	0.24	0.25	0.03	0.07
Bursa of Fabricius	0.16	0.11	0.07	0.12	0.09	0.08	0.02	0.06
Proventriculus	0.47	0.43	0.49	0.36	0.37	0.45	0.04	0.10
Gizzard	1.41	1.36	1.51	1.25	1.44	1.40	0.08	0.41
Gall bladder	0.10	0.10	0.11	0.10	0.11	0.10	0.01	0.92
Duodenum	0.91^{a}	0.75^{b}	0.72^{b}	0.70^{b}	0.67^{b}	0.75^{b}	0.05	0.02
Jejunum	1.42^{a}	1.15^{b}	1.08^{b}	1.23^{b}	1.07^{b}	1.08^{b}	0.06	< 0.01
Ileum	1.04	0.84	0.79	0.83	0.85	0.88	0.07	0.18
Caecum	0.53	0.39	0.38	0.40	0.44	0.49	0.04	0.09

Numbers in the same row with different letters (a, b) are significantly different. CONT: birds reared under normal temperature (28 ± 2 °C); HS-CONT: birds reared under HS conditions (35 ± 2 °C); HS-VIT: birds reared under HS and provided with CAS; HS-RO: birds reared under HS and provided with rice bran containing *R. oryzae*; HS-CC: birds reared under HS and provided with rice bran containing *C. crassa*; HS-RB: birds reared under HS and provided with rice bran; *n*: number of birds per experimental groups.

birds. Birds in CONT group had higher relative weights of duodenum ($P < 0.05$) and jejunum ($P < 0.01$) when compared with the birds in other groups. There was no difference ($P > 0.05$) in the relative weight of lymphoid organs (spleen, bursa of Fabricius and thymus) among the treatment groups.

3.4 Hematological and biochemical parameters

Data on hematological and biochemical parameters of broilers were presented in Table 5. The proportion of eosinophils was higher ($P < 0.05$) in HS-CC than in other birds. Significant difference was not observed in other hematological parameters. With regard to serum biochemistry, LDL cholesterol was higher ($P < 0.05$) in HS-CONT than in CONT, HS-VIT and HS-CC birds. Total triglyceride was highest

Table 5. Effect of dietary supplementation on hematological and biochemical parameters of broilers reared under normal or HS conditions.

Items	Treatment group						SEM	*P* value
	CONT ($n = 5$)	HS-CONT ($n = 5$)	HS-VIT ($n = 5$)	HS-RO ($n = 5$)	HS-CC ($n = 5$)	HS-RB ($n = 5$)		
Hematological parameters								
Hemoglobin ($g\,dL^{-1}$)	7.44	7.18	6.68	7.38	7.62	5.98	0.59	0.39
Erythrocytes ($10^{12}\,L^{-1}$)	2.12	2.28	2.32	2.07	2.14	2.06	0.20	0.91
Hematocrit (%)	21.8	21.2	19.6	21.8	22.4	17.6	1.74	0.41
MCV (fl)	103	91.4	92.1	101	105	85.9	7.72	0.35
MCH (pg)	35.1	31.0	31.4	34.3	35.6	29.2	2.38	0.36
MCHC ($g\,dL^{-1}$)	34.1	33.9	34.1	33.9	34.0	34.0	0.28	0.99
Leukocytes ($10^{9}\,L^{-1}$)	19.5	18.9	18.2	22.9	24.0	23.4	2.93	0.58
Heterophils (%)	29.2	34.2	36.8	48.0	42.0	32.2	5.27	0.17
Eosinophils (%)	2.60^{a}	3.00^{a}	1.40^{a}	1.60^{a}	6.60^{b}	2.80^{a}	1.07	0.03
Lymphocytes (%)	63.4	55.6	57.0	43.2	46.2	56.0	5.91	0.20
Monocytes (%)	4.80	7.20	4.80	7.20	5.20	9.00	1.72	0.44
H/L ratio	0.49	0.89	0.72	1.20	0.94	0.64	0.23	0.32
Serum biochemical parameters								
Total cholesterol ($mg\,dL^{-1}$)	118	135	128	113	131	136	8.10	0.27
HDL ($mg\,dL^{-1}$)	112	104	107	87.2	112	104	7.98	0.30
LDL ($mg\,dL^{-1}$)	11.2^{a}	38.3^{b}	12.5^{a}	$26.2^{a,b}$	16.9^{a}	$27.8^{a,b}$	5.71	0.02
Total triglyceride ($mg\,dL^{-1}$)	35.7^{bc}	$42.6^{a,b}$	$43.9^{a,b}$	29.4^{c}	38.9^{bc}	51.2^{a}	3.53	0.01
Glucose ($mg\,dL^{-1}$)	203	184	201	175	185	182	13.8	0.64
AST (U/L)	257	298	332	268	294	277	24.6	0.36
ALT (U/L)	$8.38^{a,b}$	9.30^{a}	6.48^{bc}	5.80^{dc}	4.46^{dc}	4.24^{d}	0.67	< 0.01
Total protein ($g\,dL^{-1}$)	2.75^{c}	3.83^{a}	$3.46^{a,b}$	3.10^{bc}	3.35^{abc}	$3.67^{a,b}$	0.21	0.02
Albumin ($g\,dL^{-1}$)	$1.00^{a,b}$	1.21^{a}	1.16^{a}	0.90^{b}	$1.10^{a,b}$	$1.09^{a,b}$	0.07	0.04
Globulin ($g\,dL^{-1}$)	1.74^{b}	2.62^{a}	2.30^{a}	$2.20^{a,b}$	$2.26^{a,b}$	2.58^{a}	0.17	0.02
Uric acid ($mg\,dL^{-1}$)	1.56^{b}	5.08^{a}	5.16^{a}	$2.94^{a,b}$	$2.42^{a,b}$	$3.81^{a,b}$	0.84	0.04

Numbers in the same row with different letters (a, b, c) are significantly different. CONT: birds reared under normal temperature ($28 \pm 2\,°C$); HS-CONT: birds reared under HS conditions ($35 \pm 2\,°C$); HS-VIT: birds reared under HS and provided with CAS; HS-RO: birds reared under HS and provided with rice bran containing *R. oryzae*; HS-CC: birds reared under HS and provided with rice bran containing *C. crassa*; HS-RB: birds reared under HS and provided with rice bran; *n*: number of birds per experimental groups; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; H/L ratio: heterophils to lymphocytes ratio; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AST: aspartate transaminase; ALT: alanine aminotransferase.

($P < 0.05$) and lowest ($P < 0.05$) in the serum of HS-RB and HS-RO birds, respectively. ALT activity was higher ($P < 0.05$) in HS-CONT when compared with other HS birds. Total protein was lowest and highest ($P < 0.05$) in the serum of CONT and HS-CONT birds, respectively. Albumin was higher ($P < 0.05$) in the serum of HS-CONT and HS-VIT than in HS-RO birds. Globulin was lower ($P < 0.05$) in CONT birds than in HS-CONT, HS-VIT and HS-RB birds. Uric acid was lower ($P < 0.05$) in CONT birds than in HS-CONT and HS-VIT birds, but the difference was not pronounced when compared with HS-RO, HS-CC and HS-RB birds.

3.5 Serum antioxidant activity

The ABTS percentage inhibition values of the serum were higher ($P < 0.01$) in CONT, HS-CONT and HS-VIT as compared to those in HS-RO, HS-CC and HS-RB birds. The DPPH percentage inhibition values were not different ($P > 0.05$) among the treatment groups (Table 6).

3.6 Intestinal microbial population

No significant difference ($P > 0.05$) was observed in the populations of total bacteria, enterobacteria, coliform bacteria, lactose-negative bacteria and LAB in the digesta collected from the ileum of broilers (data not presented).

3.7 Carcass characteristics

There was no difference ($P > 0.05$) in carcass traits, pH and drip loss of breast meats (data not presented) from broilers reared under normal temperature or HS conditions.

Table 6. Effect of dietary supplementation on the percentage inhibition values of serum towards DPPH or ABTS free radicals in broilers reared under normal or HS conditions.

Items	Treatment group						SEM	*P* value
	CONT ($n = 5$)	HS-CONT ($n = 5$)	HS-VIT ($n = 5$)	HS-RO ($n = 5$)	HS-CC ($n = 5$)	HS-RB ($n = 5$)		
DPPH (% inhibition)	28.1	65.6	54.5	34.2	26.0	42.6	11.0	0.09
ABTS (% inhibition)	55.2[a]	56.8[a]	49.5[a]	37.3[b]	34.4[b]	37.4[b]	3.15	< 0.01

Numbers in the same row with different letters (a, b) are significantly different. CONT: birds reared under normal temperature (28 ± 2 °C); HS-CONT: birds reared under HS conditions (35 ± 2 °C); HS-VIT: birds reared under HS and provided with CAS; HS-RO: birds reared under HS and provided with rice bran containing *R. oryzae*; HS-CC: ,birds reared under HS and provided with rice bran containing *C. crassa*; HS-RB: birds reared under HS and provided with rice bran; *n*: number of birds per experimental groups; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis(3-ethyl-benzthiazolin-6-sulfonic acid).

4 Discussion

The HS has been associated with the depressed performance in broiler chickens (Sohail et al., 2013). Accordingly, our result showed that birds reared under normal temperature gain more weight than HS birds throughout the trial period. In this study, dietary supplementations failed to alleviate the adverse effect of HS on the performances of birds, though BW gain and FCR were better in the supplemented HS birds as compared to HS-CONT birds during day 29 to 35. To date, a definite explanation for the lack of effect by dietary supplementations on growth performance in HS birds remains unclear. Salami et al. (2015) reported that several factors may be responsible for the efficacy of dietary supplementations in broiler chickens under HS, including dose and duration of supplementations, diets, age of birds and the level of stress.

In this study, HS was associated with the reduced weight of active organs such as heart and intestine. This result was similar to that reported by Sohail et al. (2013) and Zeferino et al. (2016). Apart from the physiological adjustment derived from depressed feed intake (Zeferino et al., 2016), the increased corticosterone level in HS broilers may be responsible for the lower percentage of the organs (Hu et al., 2010). Corticosterone may decrease glucose transport to a variety of tissues/organs, resulting in tissue regression and weight loss (Yang et al., 2015). Interestingly, the percentage of heart was not different between CONT and HS birds receiving fungi or rice bran in the present study. Although we did not measure corticosterone in this study, it is likely that probiotics (in the fungal supplements) and prebiotics (in untreated rice bran) reversed the increased level of corticosterone in HS birds (Sohail et al., 2012). Moreover, the antioxidant capacities in fungi (according to the data above) and rice bran (Jung et al., 2017) may also help to ameliorate oxidative stress, and hence the increased level of corticosterone and tissue atrophy in chickens could be prevented (Lin et al., 2004). In the present study, treatments with the fungi or rice bran were associated with the improved antioxidant status and, possibly, the decreased corticosterone level in birds. Indeed, the correlation between oxidative status and corticosterone level in birds has been demonstrated (Lin et al., 2004).

In the present study, birds in HS-CC group had higher proportion of eosinophils than in CONT and other HS birds. The exact rationale for this condition remains unclear, but the probiotic activity of *C. crassa* seemed to be responsible as Chuka (2014) reported that probiotic yeast (*Saccharomyces cerevisiae*) was able to increase eosinophil count in broilers. With this taken into account, *C. crassa* seemed to be beneficial for improving the resistance of HS chickens to pathogens. The HS has been attributed to the increase in LDL concentration in broilers (Habibian et al., 2014). Accordingly, our result showed higher LDL level in HS-CONT than in CONT and HS birds provided with either CAS or *C. crassa*. Earlier studies reported a reduction in plasma LDL in broilers treated with either antioxidant vitamins (Ahmed et al., 2015) or probiotics (Hosseini et al., 2013). Our finding may therefore suggest that CAS and *C. crassa* were able to reverse the increased LDL level in HS broilers. The mechanisms through which CAS and *C. crassa* lowered the LDL level of HS birds are largely unknown, but antioxidants in CAS and probiotic and antioxidant properties in the fungus seemed to control corticosterone secretion (Sugiharto et al., 2016a) resulting in lowered LDL level in HS birds. Indeed, Yuan et al. (2008) reported that plasma LDL as well as triglyceride increased with the increased level of corticosterone. In the current study, total triglyceride was lower in the serum of HS birds provided with fungi (especially *R. oryzae*) than in HS-CONT and HS-VIT broilers, confirming that probiotic fungi had a triglyceride-depressing effect in broilers (Idoui and Karam, 2016). Similar to a previous study (Hosseini-Vashan et al., 2012), ALT activity was higher in HS-CONT as compared with that in HS birds receiving the supplements. In broilers, serum ALT level generally increases with the liver dysfunction following HS (Hosseini-Vashan et al., 2012). Hence, supplementation with either CAS, fungi or rice bran was able to ameliorate the liver disorder in HS broilers.

In the present study, total protein in serum was lower in CONT than in HS-CONT, HS-VIT and HS-RB birds. In

some cases, the increased serum total protein was associated with inflammation (Shen et al., 2010) and tissue damage/protein breakdown (Kim et al., 2015). In this study, HS seemed to contribute to the inflammatory condition in broilers as indicated by the higher H/L ratio in HS than in CONT birds (though statistically not significant). It is known that HS was associated with the increased corticosterone level (Sohail et al., 2012) leading to tissue damage in birds (Xie et al., 2015). Interestingly, there was no significant difference in serum total protein between CONT and HS birds provided with fungi. This may suggest that fungi (with probiotic and antioxidant properties in it) could alleviate the HS-induced muscle proteolysis resulting in lower serum total protein. It has been suggested that globulin concentration increased during inflammatory processes (Neto and de Carvalho, 2009). Concomitant with this, globulin level was lower in CONT than in HS-CONT, HS-VIT and HS-RB, but not different when compared with HS birds receiving fungi. Again, the fungi may control the inflammatory process and thus elevation of serum globulin during HS could be alleviated. It has been reported in broilers that plasma level of albumin increased with HS (Akfiit et al., 2006). In this study, serum albumin was lower in HS-RO than in HS-CONT and HS-VIT broilers but did not differ from CONT, HS-CC and HS-RB birds. In this case, probiotic activity of the fungus seemed to be responsible. In this study, concentration of uric acid was lower in CONT than in HS-CONT and HS-VIT birds. Lin et al. (2004) reported that increased uric acid was contributed by the increased corticosterone, leading to muscle proteolysis. Such a condition is often seen in birds exposed to HS. Indeed, no significant difference was observed in serum uric acid between CONT and HS birds receiving fungi or rice bran in the current study, suggesting that the fungi and rice bran were able to alleviate muscle proteolysis in HS birds.

The HS has been attributed to the oxidative stress in broiler chicks (Sugiharto et al., 2016a). As a natural protective response against oxidative stress (Sohail et al., 2011; Akbarian et al., 2016), serum total antioxidants have been reported to be increased in HS birds (Sohail et al., 2011). Conversely, HS did not significantly increase the serum antioxidant activity of broilers in the present study. The chronic HS applied in the current study seemed to permit acclimatization of birds to HS (Al-Fataftah and Abu-Dieyeh, 2007; Akbarian et al., 2016). This acclimatization may alleviate the oxidative stress (Al-Fataftah and Abu-Dieyeh, 2007), thus preventing further increased antioxidant activity in the serum of HS birds. Irrespective of the HS effect, antioxidant activity was lower in the serum of HS birds supplemented with the fungi or untreated rice bran when compared with that in CONT, HS-CONT and HS-VIT birds. As oxidative stress may be accompanied by the increase in antioxidant activity (Akbarian et al., 2016), the lower serum antioxidant activity in the fungal- or rice-bran-supplemented HS birds may therefore be attributable to the lower oxidative stress in the respective birds. The mechanism by which the fungi or rice bran reduced the serum antioxidant activity of birds remains unclear, but the antioxidant properties in the fungi and rice bran might improve the antioxidant defense systems, thus alleviating the oxidative stress in birds.

Our present data showed that neither HS nor additive supplementations had changed any of the intestinal microbial population of birds. This finding was in accordance with Sohail et al. (2013, 2015), who reported that neither chronic HS nor probiotic treatments affected the intestinal microbial communities of broilers. Nevertheless, our finding was in contrast to Song et al. (2014), who showed an improvement of microbial communities of the intestine in HS broilers fed probiotics. With regard to the HS effect, it was most likely that the acclimatization of birds to chronic HS may ameliorate the adverse effect of HS on the intestinal microbial population in HS birds. The neutral effects of fungal and other supplements on the intestinal microbial population in HS birds were not expected and cannot be confirmed at the moment by the published data as the effectiveness of the supplements may depend on several factors, including dose and type of supplements, application method, diets, bird age, farm hygiene and stress (Sohail et al., 2013).

In the present study, HS had no effect on the carcass traits in broilers. Concomitant with our finding, Sakomura et al. (2013) reported no difference in carcass, leg and breast yield and drip loss in broilers reared under thermoneutral and HS conditions. Moreover, Hosseini-Vashan et al. (2016) reported no effect of HS on the abdominal fat content of broilers. Nevertheless, our data were in contrast to Zeferino et al. (2016), who showed negative effects of HS on the carcass composition and meat quality in broilers. The exact reason for the lack influence of HS on carcass traits in broilers is unclear, but the acclimatization of birds to chronic HS seemed to improve their heat tolerance (Al-Fataftah and Abu-Dieyeh, 2007), resulting in less alteration of metabolism and thus carcass composition.

In addition to prebiotics, rice bran has been reported to exhibit antioxidant activity (Jung et al., 2017). In this regard, rice bran supplementation seemed beneficial in alleviating the adverse effects of HS in broilers. By growing the fungi on rice bran, we previously expected to exert synergistic antioxidant effects of the fungi with rice bran on HS birds. However, the current data did not reveal such effects, as in general no notable differences between the effects of rice bran containing *R. oryzae*, rice bran containing *C. crassa* and rice bran itself could be observed (except for eosinophils and total triglyceride in serum). Owing to the latter condition, it was most likely that fermentation reduced the antioxidant activity in rice bran (Yoon et al., 2015), and that the synergistic antioxidant effects of the fungi with rice bran did not occur. This inference should, however, be interpreted with caution as we did not measure the antioxidant activity in fermented rice bran and untreated rice bran in the present study. Apart from the antioxidant activity in rice bran and fermented rice bran, the relatively moderate numbers of the

fungal colonies in the supplements may also be responsible for the lack of meaningful differences between the effects of fungal fermented rice bran and untreated rice bran on the growth and physiological responses in HS broilers.

5 Conclusions

Dietary supplementation with fungus, especially *C. crassa*, decreased serum LDL concentration and ALT activity and improved antioxidant status in broilers subjected to HS. Supplementation with *C. crassa* seemed beneficial in improving physiological conditions of HS birds.

Data availability. The original data of the paper are available upon request from the corresponding author.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. The study was funded by Diponegoro University through non-tax state revenue (*PNBP*), no. SP DIPA-042.01.2.400898/2016, 7 December 2015.

Edited by: Manfred Mielenz
Reviewed by: Bekir Hakan Köksal and one anonymous referee

References

Ahmed, S. Kh., Abdul-Abass, M. H., and Al-Hammed, S. A.: Effect of dietary supplementation of natural and synthetic antioxidants on broiler physiological and productive performance, Egypt Poult. Sci., 35, 93–105, 2015.

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., and De Smet, S.: Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals, J. Anim. Sci. Biotechno., 7, 37, https://doi.org/10.1186/s40104-016-0097-5, 2016.

Akşit, M., Yalçın, S., Özkan, S., Metin, K., and Özdemir, D.: Effects of temperature during rearing and crating on stress parameters and meat quality of broilers, Poultry Sci., 85, 1867–1874, 2006.

Al-Fataftah, A.-R. A., and Abu-Dieyeh, Z. H. M.: Effect of chronic heat stress on broiler performance in Jordan, Int. J. Poult. Sci., 6, 64–70, 2007.

Alshelmani, M. I., Loh, T. C., Foo, H. L., Sazili, A. Q., and Lau, W. H.: Effect of feeding different levels of palm kernel cake fermented by *Paenibacillus polymyxa* ATCC 842 on nutrient digestibility, intestinal morphology, and gut microflora in broiler chickens, Anim. Feed Sci. Tech., 216, 216–224, 2016.

Chuka, E.: Comparative study of the effects of probiotic and commercial enzyme on growth rate, haematology and serum biochemistry of broiler chicken, J. Food Process Technol., 5, 367, https://doi.org/10.4172/2157-7110.1000367, 2014.

Engberg, R. M., Hedemann, M. S., Steenfeldt, S., and Jensen, B. B.: Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract, Poultry Sci., 83, 925–938, 2004.

Habibian, M., Ghazi, S., Moeini, M. M., and Abdolmohammadi, A.: Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions, Int. J. Biometeorol., 58, 741–752, 2014.

Hosseini-Vashan, S. J., Golian, A., Yaghobfar, A., Zarban, A., Afzali, N., and Esmaeilinasab, P.: Antioxidant status, immune system, blood metabolites and carcass characteristic of broiler chickens fed turmeric rhizome powder under heat stress, Afr. J. Biotechnol., 11, 16118–16125, 2012.

Hosseini, S. A., Meimandipour, A., Alami, F., Mahdavi, A., Mohiti-Asli, M., Lotfollahian, H., and Cross, D.: Effects of ground thyme and probiotic supplements in diets on broiler performance, blood biochemistry and immunological response to sheep red blood cells, Ital. J. Anim. Sci., 12, e19, https://doi.org/10.4081/ijas.2013.e19, 2013.

Hosseini-Vashan, S. J., Golian, A., and Yaghobfar, A.: Growth, immune, antioxidant, and bone responses of heat stress-exposed broilers fed diets supplemented with tomato pomace, Int. J. Biometeorol., 60, 1183–1192, 2016.

Hu, X. F., Guo, Y. M., Huang, B. Y., Zhang, L. B., Bun, S., Liu, D., Long, F. Y., Li, J. H., Yang, X., and Jiao, P.: Effect of corticosterone administration on small intestinal weight and expression of small intestinal nutrient transporter mRNA of broiler chickens, Asian Austral. J. Anim., 23, 175–181, 2010.

Idoui, T. and Karam, N. E.: Effects of autochthonous probiotic feeding on performances, carcass traits, serum composition and faecal microflora of broiler chickens, Sains Malays., 45, 347–353, 2016.

Jahromi, M. F., Altaher, Y. W., Shokryazdan, P., Ebrahimi, R., Ebrahimi, M., Idrus, Z., Goh, Y. M., Tufarelli, V., and Liang, J. B.: Dietary supplementation of a mixture of *Lactobacillus* strains enhances performance of broiler chickens raised under heat stress conditions, Int. J. Biometeorol., 60, 1099–1110, 2015.

Jung, T.,-D., Shin, G.-H., Kim, J.-M., Choi, S.,-I., Lee, J.-H., Lee, S. J., Park, S. J., Woo, K. S., Oh, S. K., and Lee, O.-K.: Comparative analysis of γ-oryzanol, β-glucan, total phenolic content and antioxidant activity in fermented rice bran of different varieties, Nutrients, 9, 571, https://doi.org/10.3390/nu9060571, 2017.

Khan, R. U., Naz, S., Nikousefat, Z., Tufarelli, V., Javdani, M., Rana, N., and Laudadio, V.: Effect of vitamin E in heat-stressed poultry, World. Poultry Sci. J., 67, 469–478, 2011.

Khan, R. U., Naz, S., Nikousefaut, Z., Tufarelli, V., and Laudadio, V.: Effect of ascorbic acid in heat stressed poultry, World. Poultry Sci. J., 68, 477–490, 2012.

Kim, Y.-H., Kim, J., Yoon, H.-S., and Choi, Y.-H.: Effects of dietary corticosterone on yolk colors and eggshell quality in laying hens, Asian Australas, J. Anim. Sci., 28, 840–846, 2015.

Komiyama, Y., Andoh, A., Fujiwara, D., Ohmae, H., Araki, Y., Fujiyama, Y., Mitsuyama, K., and Kanauchi, O.: New prebiotics from rice bran ameliorate inflammation in murine colitis models through the modulation of intestinal homeostasis and the mucosal immune system, Scand. J. Gastroentero., 46, 40–52, 2011.

Lin, H., Decuypere, E., and Buyse, J.: Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus*

domesticus) 1. Chronic exposure, Comp. Biochem. Phys. B, 139, 737–744, 2004.

Neto, N. S. R. and de Carvalho, J. F.: The use of inflammatory laboratory tests in rheumatology, Braz. J. Rheumatol., 49, 413–430, 2009.

Petacci, F., Freitas, S. S., Brunetti, I. L., and Khalil, N. M.: Inhibition of peroxidase activity and scavenging of reactive oxygen species by astilbin isolated from Dimorphandra mollis (Fabaceae, Caesalpinioideae), Biol. Res., 43, 63–74, 2010.

Sakomura, N. K., Barbosa, N. A. A., Longo, F. A., da Silva, E. P., Bonato, M. A., and Fernandes, J. B. K.: Effect of dietary betaine supplementation on the performance, carcass yield, and intestinal morphometrics of broilers submitted to heat stress, Braz. J. Poult. Sci., 15, 105–112, 2013.

Salami, S. A., Majoka, M. A., Saha, S., Garber, A., and Gabarrou, J.-F.: Efficacy of dietary antioxidants on broiler oxidative stress, performance and meat quality: science and market, Avian Biol. Res., 8, 65–78, 2015.

Shen, Y. B., Piao, X. S., Kim, S. W., Wang, L., and Liu, P.: The effects of berberine on the magnitude of the acute inflammatory response induced by *Escherichia coli* lipopolysaccharide in broiler chickens, Poultry Sci., 89, 13–19, 2010.

Sohaib, M., Anjum, F. M., Khan, M. I., Arshad, M. S., and Shahid, M.: Enhancement of lipid stability of broiler breast meat and meat products fed on alpha lipoic acid and alpha tocopherol acetate supplemented feed, Lipids Health Dis., 11, 57, https://doi.org/10.1186/1476-511X-11-57, 2012.

Sohail, M. U., Ijaz, A., Yousaf, M. S., Ashraf, K., Zaneb, H., Aleem, M., and Rehman, H.: Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and *Lactobacillus*-based probiotic: dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity, Poultry Sci., 89, 1934–1938, 2010.

Sohail, M. U., Rahman, Z. U., Ijaz, A., Yousaf, M. S., Ashraf, K., Yaqub, T., and Zaneb, H.: Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers, Poultry Sci., 90, 2573–2577, 2011.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., Shabbir, M. Z., and Rehman, H.: Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress, Poultry Sci., 91, 2235–2240, 2012.

Sohail, M. U., Ijaz, A., Younus, M., Shabbir, M. Z., Kamran, Z., Ahmad, S., Anwar, H., Yousaf, M. S., Ashraf, K., Shahzad, A. H., and Rehman, H.: Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers, J. Appl. Poultry Res., 22, 485–491, 2013.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Shabbir, M. Z. Ijaz, A., and Rehman, H.: Molecular analysis of cecal and tracheal microbiome of heat-stressed broilers supplemented with prebiotic and probiotic, Avian Pathol., 44, 67–74, 2015.

Song, J., Xiao, K., Ke, Y. L., Jiao, L. F., Hu, C. H., Diao, Q. Y., Shi, B., and Zou, X. T.: Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress, Poultry Sci., 93, 581–588, 2014.

Sugiharto, S., Yudiarti, T., and Isroli, I.: Functional properties of filamentous fungi isolated from the Indonesian fermented dried cassava, with particular application on poultry, Mycobiology, 43, 415–422, 2015.

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., and Kusumanti, E.: Dietary supplementation of probiotics in poultry exposed to heat stress – a review, Ann. Anim. Sci., 17, 591–604, 2016a.

Sugiharto, S., Yudiarti, T., and Isroli, I.: Assay of antioxidant potential of two filamentous fungi isolated from the Indonesian fermented dried cassava, Antioxidants, 5, 6, https://doi.org/10.3390/antiox5010006, 2016b.

Tawfeek, S. S., Hassanin, K. M. A., and Youssef, I. M. I.: The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions, J. World's Poult. Res., 4, 10–19, 2014.

Wang, D., Huang, H., Zhou, L., Li, W., Zhou, H., Hou, G., Liu, J., and Hu, L.: Effects of dietary supplementation with turmeric rhizome extract on growth performance, carcass characteristics, antioxidant capability, and meat quality of Wenchang broiler chickens, Ital. J. Anim. Sci., 14, 3870, https://doi.org/10.4081/ijas.2015.3870, 2015.

Xie, J., Tang, L., Lu, L., Zhang, L., Lin, X., Liu, H.-C., Odle, J., and Luo, X.: Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders, Poultry Sci., 94, 1635–1644, 2015.

Yang, J., Liu, L., Sheikhahmadi, A., Wang, Y., Li, C., Jiao, H., Lin, H., and Song, Z.: Effects of corticosterone and dietary energy on immune function of broiler chickens, PLOS ONE, 10, e0119750, https://doi.org/10.1371/journal.pone.0119750, 2015.

Yoon, H. J., Lee, K. A., Lee, J. H., Jin, H. J., Kim, H. J., Kim, K.-T., and Paik, H.-D.: Effect of fermentation by *Bacillus subtilis* on antioxidant and cytotoxic activities of black rice bran, Int. J. Food Sci. Tech., 50, 612–618, 2015.

Yuan, L., Lin, H., Jiang, K. J., Jiao, H. C., and Song, Z. G.: Corticosterone administration and high-energy feed results in enhanced fat accumulation and insulin resistance in broiler chickens, Brit. Poultry Sci., 49, 487–495, 2008.

Yudiarti, T.: Potensi fungi filamentus sebagai probiotik dalam hubungannya dengan morfologi, komposisi mikroba saluran pencernaan dan produktivitas ayam kampung (*Gallus gallus domesticus*), PhD Dissertation, Diponegoro University, Semarang, Indonesia (article in Indonesian language), 2012.

Yudiarti, T., Yunianto, B. I., Isroli, I., and Murwani, R.: *In vitro* test of probiotic strain of seven species of fungi which isolated from gastrointestinal tract of indigenous chicken. Proceeding of the 6th International Seminar of Indonesian Society for Microbiology and the 11th Congress of Indonesian Society for Microbiology 2013 (ISISM-ISMCong), 17–19 October 2013, Pontianak, West Borneo, Indonesia.

Yudiarti, T., Yunianto, B. I., Murwani, R., and Kusdiyantini, E.: Isolation of fungi from the gastrointestinal tract of indigenous chicken, J. Indonesian Trop. Anim. Agric., 37, 115–120, 2012.

Zeferino, C. P., Komiyama, C. M., Pelícia, V. C., Fascina, V. B., Aoyagi, M. M., Coutinho, L. L., Sartori, J. R., and Moura, A. S. A. M. T.: Carcass and meat quality traits of chickens fed diets concurrently supplemented with vitamins C and E under constant heat stress, Animal, 10, 163–171, 2016.