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Effect of supplementing germinated sprouts of millets on performance, carcass variables, immune and anti-oxidant responses in commercial broiler chickens

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ABSTRACT

Germination of millets (sprouts) increases activity of enzymes in grains and availability of amino acid, vitamins and trace minerals to chicken. These nutrients are required for growth, immunity and anti-oxidant system in biological system. An experiment was conducted to study the effects of feeding sprouts of *korra*, *ragi*, *bajra* and sorghum at 5% of expected feed intake from day one to 42 d of age. Total phenolic content among the millets was higher in sprouts of *bajra* followed by *ragi*, *korra* and sorghum in order (148, 110, 95 and 90 mg GAE/g, respectively). A group without feeding sprouts was kept as control. Maize-soybean meal diets having recommended concentrations of nutrients for starter (1–21 d) and finisher (22–42 d of age) phases were fed to all the groups. Maximum and minimum temperature and humidity during the 6-week experimental period were 32.17 and 25.1°C and 60.8 and 33.4%, respectively. Body weight gain and feed efficiency at 42 d of age, slaughter variables (ready to cook yield, relative weights of liver, breast, abdominal fat) and immune responses (antibody titres against ND vaccine, CMI response to PHA-P) were not affected due to feeding of sprouted grains. Lipid peroxidation and activities of glutathione peroxidase, glutathione reductase and super oxide dismutase were significantly less in broilers fed sprouted millets compared to the control group. Based on the results, it is concluded that oxidative stress could be reduced in broiler chicks reared during summer season in open sided poultry house without affecting performance, carcass variables and immune responses.

Key words: Broiler chicken, Immune responses, Oxidative stress, Performance, Sprouted millets

Fluctuations in environmental variables (temperature and humidity) will have direct impact on performance besides inducing stress and immune suppression in chicken reared in open sided poultry house. Dietary modulations are one of the proven approaches to minimize the negative effects of stress on chicken. Supplementation of vitamins (Yang *et al.* 2001), organic trace minerals (Rama Rao *et al.* 2016) and osmolytes (Rama Rao *et al.* 2011) in diet are known to reduce the ill effects of heat stress on chicken. Feed supplements from plant origin are gaining wider acceptability in livestock production owing to several benefits associated with the natural compounds. Germination triggers the enzymatic activity in sprouting grains, leading to the hydrolysis of complex proteins, carbohydrates and lipids molecules into simpler forms (Elkhalifa and Bernhardt 2010). Vitamin content increases (up to 20 times) and minerals will be chelated during the process of germination (Cuddeford 1989). Considering the potential benefits of supplementing germinated sprouts of millets for chicken, an experiment was conducted to study the possible beneficial effects of supplementing sprouted millets on anti-oxidant and immune responses, production performance and carcass variables in commercial broilers reared in open side poultry house during natural hot and humid season in tropical environment.

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MATERIALS AND METHODS

An experiment was conducted to study effects of feeding 4 varieties (*korra, ragi,* sorghum and *bajra*) of sprouted millet grains on performance, carcass variables, immune responses and anti-oxidant variables in broiler chicken.

Grains of millets were soaked in distilled water for 16 h for production of germinated sprouts (Badau *et al.* 2005). About 78.9% of grains of millets germinated. Sprouted millets were estimated for total phenolic content (TPC) using Folinciocalteus reagent method and anti radical activity (ARA) by estimating the per cent discoloration of DPPH (1,1-Diphenyl-2-picrylhydrazyl) as suggested by Bhatti *et al.* (2015).

A total of 225 Cobb 400 broiler male chicks were randomly distributed in to 45 raised wire floor stainless steel battery brooder pens (610 × 762 × 457 mm) containing 5 chicks in each in open side poultry house. Maize-soybean meal based diets for starter (1–21 d) and finisher (22–42 d of age) phases were prepared (Table 1). The diets were

Table 1. Ingredient and nutrient composition of diets (g/kg)

Ingredient	Starter (1–21 day)	Finisher (22–42 day)
Maize	531.70	603.06
Soybean meal	393.90	312.20
Vegetable oil	32.40	44.80
Common salt	4.00	4.00
Dicalcium phosphate	17.33	16.55
Shell grit	13.00	11.00
DL methionine	2.60	1.95
L lysine HCl	0.46	1.46
L threonine	1.01	1.38
Vitamin premix[A]	0.30	0.30
Choline chloride	0.60	0.60
Toxin binder[B]	1.00	1.00
Trace mineral premix[C]	1.20	1.20
Coccidiostat[D]	0.50	0.50
Nutrients		
Metabolizable energy (kcal/kg)	3000.00	3150.00
Crude protein (%)	22.50	19.50
Lysine (%)	1.26	1.15
Methionine (%)	0.58	0.48
Threonine (%)	0.945	0.863
Calcium (%)	0.90	0.80
Non phytatephosphorus (%)	0.45	0.42

[A]Supplied per kilogram of diet: 1 mg of thiamin, 2 mg of pyridoxine, 0.01 mg of cyanocobalamine, 15 mg of niacin, 10 mg of pantothenic acid, 10 IU of α-tocopherol, 10 mg of riboflavin, 0.08 mg of biotin, 2 mg of menadione, 2.75 mg of retinol acetate, 0.03 mg of cholecalciferol, 650 mg of choline; [B]800 mg hydrated sodium calcium alumina silicates; [C]8 mg of copper, 45 mg of iron, 80 mg of manganese, 60 mg of zinc, 0.18 mg of selenium; [D]50 mg of monensinsodium (Venky's Pvt. Ltd., Pune, India).

offered *ad lib.* throughout the experiment. Weighed quantity of feed was offered twice in a day to ensure *ad lib.* feed availability throughout the day during the experiment. After completion of morning feeding, weighed quantities (5% of calculated daily feed intake) of sprouted grains was offered on top of the feed considering the number of birds in each pen on daily basis. Sprouts of each grain were produced daily as per the requirement of the day. The quantity of sprouted grains offered (5% of average daily feed intake) to broilers during week 1, 2, 3, 4, 5, and 6, respectively was 1.25, 2.75, 4.5, 6, 7.75 and 8 g/b/day. Each sprouted grain was offered to 9 replicates consisting of 5 chicks each.

Performance: Body weight (BW) and feed intake (FI) were recorded at day old, 21 and 42 days of age. Body weight gain (BWG) and feed intake per kg weight gain (FE) were calculated from day 1 to 21 and 1 to 42 d of age.

Carcass variables: One bird representing the mean BW of the treatment was slaughtered by cervical dislocation on day 43 to study ready to cook yield (RTC), relative weights of abdominal fat, liver and breast weight. The relative weights were expressed as g per kg pre-slaughter live weight of the respective bird.

Humoral immune response: Humoral response (titres against Newcastle disease vaccine) was recorded at 21 and 42 d of age. Broilers were vaccinated against Newcastle disease (ND) by ocular route at 7th and 28th d of age with Lasota strain (ND Lasota Vac-500, Indovax Pvt Ltd., Hyderabad, India). At 21 and 42 d of age, blood was collected from brachial vein and the serum was separated. Antibody titres against ND vaccine were measured with ELISA (Synbiotics, San Diego, CA, USA) technique.

Cell mediated immune (*CMI*) *response:* Coetaneous delayed type hypersensitivity response elicited in chickens by an intra dermal injection of phyto hemagglutinin phosphate (PHA-P, TC 226, HiMedia, Mumbai, India) was used to assess the *in vivo* cell mediated immune (CMI) response. 0.1 ml (100 µg) of PHA-P solution was inoculated into right toe web and 0.1 ml PBS into left toe web. Thickness was measured at 24 h after inoculation with digital thickness gauge (Mitutoto, Japan). The CMI response was measured as per cent increase in thickness due to PHA-P in relation to the initial thickness of the toe web (Miljkoviæ *et al.* 2007).

Stress parameters: Stress indices in terms of lipid peroxidation (LP), activities of glutathione peroxidase (GSHPx), glutathione reductase (GSHRx) and super oxide dismutase (SOD, EC 1.15.1.1) in serum were estimated.

Blood samples were collected from one bird per replicate for measuring lipid peroxidation (Ohkawa *et al.* 1979), activities of GSHPx (Paglia and Valantine 1967), GSHRx (Cohen *et al.* 1970) and SOD enzymes. Approximately 3 ml of blood from each bird was drawn from the brachial vein on 35th d of age into a centrifuge tube containing citrate (0.8 g/100 ml) buffer (1.5 ml/10 ml blood) for erythrocyte separation and enzyme estimation.

Statistical analysis: The variations in data of different parameters were analyzed using the general linear model procedure (SPSS software package, version 18, SPSS, Chicago, IL, USA). The model included different dietary treatments as source of variation. Treatment means were compared using Duncan's multiple range test (Duncan 1955) at $P<0.05$.

RESULTS AND DISCUSSION

The TPC among millets was higher in sprouts of *bajra* followed by *ragi*, *korra* and sorgum in order (148±4.91, 110±1.15, 95±2.08 and 90±7.04 mg GAE/g, respectively). The ARA in terms of DPPH reduction ranged between 78.2±1.38 to 81.4±1.02% among different sprouted millets.

Maximum and minimum temperature and humidity during the 6-week experimental period were 32.17±1.42 and 25.1±2.36°C and 60.8±5.30 and 33.4±4.69%, respectively.

Performance and carcass variables: Body weight gain (BWG) was not affected at day 21 due to supplementation of sprouted millets, while FI and FCR increased significantly ($P<0.05$) in groups fed sprouted millets compared to those of control group (Table 2). The increased FCR could be due to higher FI in broilers fed sprouted grains compared to the control group. Though the FI in sprouts fed groups was

Table 2. Performance of commercial broilers fed sprouts of various millets at fixed level of intake (5% of feed intake)

Treatment	Week 3			Week 6		
	BWG (g)	FI (g)	FI/BWG	BWG (g)	FI (g)	FI/BWG
Control	794.9	1056^{b}	1.330^{b}	2393	3739^{b}	1.566
Bajra#	773.2	1095^{a}	1.417^{a}	2355	3849^{a}	1.636
Ragi#	766.4	1086^{a}	1.419^{a}	2435	3857^{a}	1.586
Korra#	781.2	1083^{a}	1.380^{a}	2434	3867^{a}	1.590
Sorghum#	782.5	1089^{a}	1.392^{a}	2340	3839^{a}	1.642
P	0.230	0.002	0.002	0.106	0.051	0.137
N	9	9	9	9	9	9
SEM	4.050	3.477	0.008	14.27	15.51	0.011

BWG, body weight gain; FI, feed intake; #Supplemented at the rate of 5% of FI. abcMeans having common superscripts in a column do not vary significantly (P<0.05).

significantly higher such reduction in FE was not noticed in broilers fed sprouts at the end of experiment (42 d of age). Contrarily, reduced FI was observed in literature (Abbas and Musharaf 2008) when the birds were fed with sprouted grains, which was attributed to increased concentration of tannins due to germination. The reduced FI was probably due to reduced palatability, taste or smell due to tannin production. According to Oduguwa and Farolu (2004), FI was reduced in broilers because sprouting caused bitterness in taste. Production of tannins during sprouting might be responsible for the bitter taste and reduced FI (Anganga and Adogla-Bessa 1999). However, few studies reported no significant difference in FI due to feeding of sprouts (Fafiolu *et al.* 2006). The variation in FI was also attributed to difference in germination time. The longer the germination time, the higher the concentrations of tannins was reported in the sprouted grains (Abbas and Musharaf 2008).

Similar to the current study, few authors reported that BWG of chicken was not affected due to feeding of water treated grains (Oduguwa and Farolu 2004). Abbas and Musharaf (2008) determined the effect of days of germination of sorghum grain on the growth performance of broiler birds. They reported that 3 days germinated grains had no effect on the growth performance of broiler birds but when days of germination increased growth depression was reported due to increased tannin contents due to longer germination period.

Though germination/sprouting of grains was reported to increase enzyme activities in grains (Shipard 2005) and *in vivo* digestibility of nutrients (Elkhalifa and Bernhardt 2010), the benefits of improved nutrient digestibility was not reflected on broiler performance in the current study, which could be due to lower inclusion rate (5% of feed intake) of sprouted grains, which might have failed to influence the bird performance. Similar to our results, Abbas and Musharaf (2008) also did not found any significant improvement in growth when germinated sorghum grain was fed to the broiler birds.

Slaughter variables and immune responses: Slaughter variables (Table 3) and immune responses (Table 4) were also not affected with feeding of sprouts to commercial broiler chicken at the rate of 5% of FI. The authors did not get any reference in literature on either slaughter variables or immune parameters in broilers fed sprouted grains.

Antioxidant responses: At both day 21 (Table 5) and 42 of age (Table 6), LP was significantly reduced in broilers

Table 3. Slaughter variables (g/kg pre-slaughter body weight) of commercial broilers fed sprouts of various millets at fixed level of intake (5% of feed intake)

Treatment	RTC	Breast	Liver	Fat
Control	785.8	254.4	18.03	6.546
Bajra#	796.0	257.8	18.75	6.396
Ragi#	789.2	248.3	19.84	7.272
Korra#	797.1	257.8	18.66	7.882
Sorghum#	794.1	255.9	18.70	8.825
P	0.668	0.754	0.829	0.493
N	9	9	9	9
SEM	2.728	2.469	0.466	0.479

RTC, ready to cook yield; #Supplemented at the rate of 5% of FI.

Table 4. Immune responses in commercial broilers fed sprouts of various millets at fixed concentration (5% of feed intake)

Treatment	21 day of age		42 day of age	
	ND titre (log 2)	CMI response (%)	ND titre (log 2)	CMI response (%)
Control	3.89	106.0	8.667	103.7
Bajra#	4.00	133.0	9.111	97.7
Ragi#	3.89	126.8	8.778	108.8
Korra#	4.22	134.6	9.000	107.3
Sorghum#	3.67	119.8	8.889	107.4
P	0.839	0.511	0.803	0.966
N	9	9	9	9
SEM	0.147	5.651	0.120	5.148

#Supplemented at the rate of 5% of FI; ND, New castle disease; CMI, cell mediated immune response.

Table 5. Anti-oxidant variables in commercial broilers (21 day of age) fed sprouts of various millets at fixed level of intake (5% of feed intake)

Treatment	Unit/ml			
	LP#	GSHPx	GSHRx	SOD*
Control	1.484^{a}	857.9^{a}	892.2^{a}	5.899^{a}
Bajra#	1.209^{b}	707.0^{b}	678.9^{b}	4.597^{b}
Ragi#	1.218^{b}	655.1^{b}	698.7^{b}	4.027^{b}
Korra#	1.027^{c}	629.2^{b}	678.7^{b}	4.323^{b}
Sorghum#	1.180^{b}	658.3^{b}	729.1^{b}	4.133^{b}
P	0.001	0.001	0.001	0.027
N	9	9	9	9
SEM	0.033	16.70	16.53	0.167

#Nano moles of MDA; *% of inhibition; LP, lipid peroxidation; GSHPx, glutathione peroxidise; GSHRx, glutathione reductase; SOD, super oxide dismutase; #Supplemented at the rate of 5% of FI; abMeans having common superscripts in a column do not vary significantly (P<0.05).

Table 6. Anti-oxidant variables in commercial broilers (42 day of age) fed sprouts of various millets at fixed level of intake (5% of feed intake)

Treat	Unit/ml			
	LP#	GSHPx	GSHRx	SOD*
Control	1.518[a]	892.6[a]	840.6	3.473[a]
Bajra#	1.224[b]	678.9[b]	799.4	1.998[b]
Ragi#	1.169[b]	715.4[b]	805.1	2.573[b]
Korra#	0.892[c]	672.4[b]	758.3	2.410[b]
Sorghum#	1.146[b]	646.2[b]	730.7	2.136[b]
P	0.001	0.001	0.813	0.001
N	9	9	9	9
SEM	0.043	21.39	29.75	0.131

#nano moles of MDA; *% of inhibition; LP lipid peroxidation; GSHPx gluta thione peroxidise; GSHRx glutathione reductase; SOD super oxide dismutase; #supplemented at the rate of 5% of FI; abcmeans having common superscripts in a column do not vary significantly (P<0.05).

fed sprouted millets compared to the control group. Similarly, the activities of GSHPx and SOD at day 21 and 42d and GSHRx at day 21 were significantly lower in broilers fed sprouted millets compared to the control. Reduction in lipid peroxidation and activities of anti-oxidant enzymes are suggestive of reduced oxidative stress in broilers fed sprouted millets. The reduce stress indices in broilers fed sprouted grains indicate that germinated grains has stress alleviating effects in broilers. The anti stress effects of sprouts could be due to synthesis of tannins (Anganga and Adogla-Bessa 1999), increased availability of amino acids, trace minerals (Shipard 2005) and vitamins (Cuddeford 1989), which have pivotal roles in anti-oxidant defence mechanism in biological system including chicken.

Germination triggers activity of certain enzymes like phytase (Marshall *et al.* 2011) and protease (Elkhalifa and Bernhardt 2010). Phytase denature complex phytate molecule and release inositol, inorganic phosphorus, essential trace minerals like zinc, copper and manganese which are integral components of several anti-oxidant enzyme systems. Therefore supplementation of germinated/ sprouted millets in broiler diets reduced the concentration of MDA in serum, which is a potential marker of oxidative stress in chicken.

Based on the results, it can be concluded that supplementation of sprouted millets (*ragi, bajra, korra* and sorghum) at the rate of 5% FI significantly reduced lipid peroxidation and activities of GSHPx and SOD in serum of broilers without affecting performance (body weight gain and feed efficiency), carcass variables (ready to cook yield, relative weights of breast liver and abdominal fat) and immune responses (HI titre and CMI response to PHA-P) in commercial broilers reared in open sided poultry house during tropical summer.

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