Veterinary Integrative Sciences 2024; 22(3): 1019 - 1028 DOI; 10.12982/VIS.2024.069

Vet Integr Sci

Veterinary Integrative Sciences

Faculty of Veterinary Medicine
Chiang Mai University

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Effect of fresh azolla *(Azolla pinnata)* feed replacement on growth performance, carcass quality, and oxidative stress in Thai native crossbred chicken

Montri Punyatong*, Patcharee Kanjak, Wanaporn Tapingkae, Chompunut Lumsangkul, Tossapol Moonmanee, Hien Van Doan and Pimporn Khamtavee

Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract

Azolla *(Azolla pinnata)*, an aquatic fern, has high amounts of crude protein and essential amino acids and thus could be an economical and plentiful alternative feed source for poultry. In this study we investigated how partial replacement of pelleted concentrate diet with fresh azolla affected growth performance, carcass quality, and oxidative stress in Thai native crossbred chicken. A total of 600 male, six week-old native crossbred chickens (Pradu Hang dum) were randomly assigned to six experimental groups that were for fed for four weeks with 100% commercial pelleted diet (control) or a diet having partial replacement of the control diet with 5%, 10%, 15%, 20%, or 25% fresh azolla (FA). The feed cost per weight gain was significantly decreased in chickens fed FA ($p<0.05$). No significant differences among the groups were seen in live body weight, defeather weight, carcass percentage and some internal organs, although the 15% and 20% FA groups had significantly lower ($p<0.05$) wing weight. Breast meat lightness and yellowness were significantly higher ($p<0.05$) for the 15% FA group, and the 25% FA group had significantly lower ($p<0.05$) meat redness relative to the other groups. The shear force of the breast meat was significantly lower ($p<0.05$) for the 10%, 15%, and 20% FA groups compared to the other groups. For oxidative stress parameters, the glutathione level (GSH) was similar among all the groups. The groups fed fresh azolla all had significantly lower ($p<0.05$) malondialdehyde (MDA) concentrations relative to the control group, although the MDA level in stored meat was similar among all the groups. Taken together, these results suggest that fresh azolla can be used as an alternative feed source to lower production costs for Thai native crossbred chicken

Keywords: Azolla, Carcass, Oxidative stress, Performance, Thai native crossbred chicken

***Corresponding author:** Montri Punyatong, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University 239, Huay Kaew Road, Mueang, Chiang Mai, 50200, Thailand. Tel. +66-62459-7474, E-mail: montri.pun@cmu.ac.th.

Article history; *received manuscript: 9 December 2023,*
revised manuscript: 5 January 2024,
accepted manuscript: 15 February 2024,
published online: 28 February 2024
Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

In northern Thailand, rural farmers raise chickens in their backyards as a source of both household protein and extra income. However, the cost of feed, especially commercial diets, is a major component of overall poultry production costs (~70% of total cost) and can constrain local poultry production (Joysowal et al., 2018). Replacing part of commercial concentrate diets, especially during the fattening period, can reduce production costs. Azolla *(Azolla pinnata)*, also known as mosquito fern, duckweed, fairy moss or water fern (Swain et al., 2022), is an aquatic fern that freely floats on water surfaces. Azolla plants can be inexpensively cultured in tropical habitats and used as a feed ingredient or supplement for farm animals to reduce feed costs (Mohamed et al., 2023). Previous studies showed that azolla has high nutritive value, particularly in terms of protein (20.4-28.5% crude protein on a dry matter basis), essential amino acids, and vitamins (beta-carotene and vitamin B12) (Khursheed et al., 2019; Bhatt et al., 2020). The content of minerals like iron, calcium, magnesium, potassium and manganese is also high in azolla, as is the concentration of bioactive substances such as phenols, tannins and glycosides that have natural antioxidant properties (Riaz et al., 2022).

For poultry production, diet replacement with 100 g azolla/day/bird was associated with increased profit and average egg production (Sinha et al., 2018). Addition of up to 15% azolla to broiler feed improved growth performance and nutrient digestibility (Samad et al., 2020). A corn-soybean-based diet containing 5% azolla powder improved daily weight gain and feed conversion ratio (FCR) in broiler chickens (Naghshi et al., 2014). Addition of azolla leaf meal to feed tended to improve body weight gain and increased feed intake while linearly decreasing the percentage of breast meat loss during cooking and increasing the propionate production in the cecum of broilers (Abdelatty et al., 2020). In turkeys, choice-feeding with azolla and a basal diet improved FCR without affecting blood biochemistry or immune competence (Shukla et al., 2018).

Production of native crossbred chickens is popular in Thailand because the meat has a more desirable texture and flavor while being lower in fat, cholesterol, and triglycerides than meat from broiler chickens (Phianmongkhol et al., 2012). Most crossbred chickens in Thailand are raised by small-scale farmers. Azolla could a potential feed source for this type of chicken to reduce costs and increase sustainable production. In this study we evaluated how partial replacement of a concentrate diet with fresh azolla affected growth performance, carcass quality, and oxidative stress of Thai native crossbred chickens during the fattening period.

MATERIALS AND METHODS

Animal and experimental design

All experiments were conducted according to the guidelines established for the care and use of laboratory animals, and were approved by Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Thailand. The ethical approval number was AG02002/2566.

A total of 600 male, six week-old Thai native crossbred Pradu Hang dum chicks from Chiang Mai province were used in this study. The chicks were weighed and assigned to treatment groups so that the different groups had similar initial weights. The experimental design was randomized using a CRD (completely randomized design) to include six groups, which were given 100% commercial pelleted diet (control; corn-soy based diet, 18% protein and 2,900 kcal/kg ME) or replacement of the commercial diet with 5%, 10%, 15%, 20%, or 25% (wt:wt) fresh azolla (FA). The chemical composition of the control diet and diets with FA replacement is presented in Table 1. Feed and water were supplied to all chicks ad libitum for the four-week experimental period.

Table 1 Chemical composition of control commercial pelleted diet and diet with partial replacement with fresh azolla (*Azolla pinnata*) (% dry matter basis)

Chemical composition	Diet					
	CON	5%FA	10%FA	15%FA	20%FA	25%FA
Dry matter; DM (%)	90.06	87.33	83.23	80.66	77.49	75.43
Ash (%)	9.45	10.78	11.12	12.34	14.67	15.02
Crude protein; CP (%)	18.00	17.25	16.70	15.55	14.89	14.03
Crude fiber; CF (%)	3.40	5.07	6.15	7.22	8.03	9.37
Ether extract; EE (%)	4.23	3.89	3.65	3.21	3.19	3.02
Nitrogen free extract; NFE (%)	52.06	49.79	44.85	41.21	38.81	35.59
Metabolizable energy; ME (kcal/kg)	2,900	2,637	2,580	2,520	2,460	2,395

CON = control diet, 5% FA = replacement of control diet with 5% fresh azolla, 10% FA = replacement of control diet with 10% fresh azolla, 15% FA = replacement of control diet with 15% fresh azolla, 20% FA = replacement of control diet with 20% fresh azolla, and 25% FA = replacement of control diet with 25% fresh azolla.

Growth performance and carcass quality evaluation

Feed intake (FI) was recorded daily. Body weight (BW) was measured weekly for performance parameters, which were calculated as: weight gain (WG) = final BW - initial BW, feed conversion ratio (FCR) = FI/WG, average daily gain (ADG) = WG/day on test, and feed cost per gain (FCG) = (FI x cost of feed)/WG (baht/kg). At the final timepoint, 10 birds were randomly selected from each treatment and weighed before slaughter. Chickens were slaughtered by hand using a conventional neck cut for bleeding. The carcasses were scalded at 60 °C for 120 seconds and manually eviscerated. The hot carcasses were processed with a Thai-style retail cut and body parts including the skeletal frame, drumstick with thigh, wing, breast with fillet, liver, gizzard, spleen and heart were weighed. The hot carcass percentage was calculated as a percentage of live body weight. The percentage weight of cuts was calculated as a percentage of the hot carcass weight (Pripwai et al., 2014).

Oxidative stress determination

Blood was collected from 10 chicks in each group at 6, 8, and 10 weeks of age. The serum was separated to measure malondialdehyde (MDA) (Santos et al., 1980) and glutathione (GSH) (Boyne and Ellman, 1972) levels. MDA levels were determined by adding 0.45 ml normal saline solution (NSS), 0.2 ml thiobarbituric acid reagent (TBA) and 1 ml trichloroacetic acid reagent (TCA) to a 0.1 ml serum sample in a test tube. The solution was boiled at 100 °C in a water bath for 30 minutes. Then, 2 ml distilled water was added and the mixture was centrifuged at 3,000g for 10 minutes. The supernatant was collected and absorbance was measured at 532 nm. The measured values were

compared against an MDA standard and the amount of MDA in each sample was calculated. GSH levels were measured by adding 1.6 ml distilled water to a 0.4 ml serum sample. After precipitation of the proteins with 3 ml precipitation solution, the mixture was centrifuged at 2,000g for 10 minutes. Supernatants were collected and 4 ml phosphate buffer and 0.5 ml dithiobisnitrobenzoic acid (DTNB) was added before measurement of absorbance at 412 nm, which was compared with a GSH standard to calculate the amount of GSH in the sample.

Statistical analysis

Data were statistically analyzed using SPSS software (SPSS Inc., Chicago, IL, USA). A completely randomized design was assessed by analysis of variance (ANOVA). Differences between the mean values of the treatments were determined using a Duncan's multiple-range test. Differences were considered significant with $P<0.05$. All mean values for each parameter of the different treatments were reported as a pooled standard error of the means (SEM).

RESULTS

The effects of partial replacement of concentrate diet with fresh azolla on growth performance were measured for Thai native chickens fed for four weeks with either a control diet (no replacement) or replacement with 5%, 10%, 15%, 20% and 25% fresh azolla (FA; wt:wt; Table 2). No significant difference in any of the performance parameters relative to the control was seen ($p>0.05$), except for a significant decrease in feed cost per gain (FCG) exhibited by the 10%, 15%, 20% and 25% FA replacement groups ($p<0.05$).

Live body weight, defeather weight, carcass percentage and the weight of some internal organs also showed no significant differences between the control and FA groups ($p>0.05$; Table 3). The weight of the wings was significantly lower ($p<0.05$) for the 15% and 20% FA groups relative to the control group and other FA groups. The breast meat color expressed as lightness (L*) and yellowness (b*) was significantly higher ($p<0.05$) for the 15% FA group relative to the other groups, but the redness (a*) of the meat for the 25% FA replacement group was significantly lower ($p<0.05$) than the other groups. The shear force (N) of breast meat from the 10%, 15%, and 20% FA groups was also significantly lower than the other groups.

We also measured the blood serum content of molecules related to oxidative stress (Table 5). The malondialdehyde (MDA) concentration was significantly lower ($p<0.05$) in all groups that had fresh azolla replacement compared to the control group. However, in stored meat samples, there were no significant differences in MDA levels among the experimental groups ($p>0.05$). Glutathione (GSH) levels also showed no significant differences among any of the groups (Table 6).

Table 2 Growth performance parameters for Thai native crossbred chicken (Pradu Hang dum) fed commercial pelleted diet with partial replacement by fresh azolla (*Azolla pinnata*).

Parameter	Treatment						SEM	*P*-value
	CON	5%FA	10%FA	15%FA	20%FA	25%FA		
Initial weight (g)	426.00	446.80	424.95	433.10	431.15	450.30	3.21	0.082
Final weight (g)	1,046.23	1,053.97	1,022.88	1,015.57	1,019.58	1,024.10	7.91	0.689
Weight gain; WG (g)	620.23	607.17	597.93	582.47	588.43	573.80	7.40	0.524
Average daily gain; ADG (g/bird/day)	22.17	21.27	21.83	20.75	21.81	20.84	0.53	0.719
Average daily feed intake; ADFI (g/bird/day)	64.32	63.61	64.60	64.36	64.51	64.38	0.20	0.806
Feed conversion ratio; FCR	2.91	2.87	3.00	3.03	3.06	3.04	0.04	0.690
Feed cost per gain; FCG* (Baht/kg)	26.88^{a}	24.35^{b}	23.37^{b}	21.85^{b}	20.37^{c}	19.07^{c}	0.58	*<0.001*

$^{a, b, c}$different letters in the same row indicates significant difference in mean values *($p<0.05$)*.
SEM = pooled standard error of the mean.
CON = control diet, 5% FA = replacement of control diet with 5% fresh azolla, 10% FA = replacement of control diet with 10% fresh azolla, 15% FA = replacement of control diet with 15% fresh azolla, 20 % FA = replacement of control diet with 20% fresh azolla, and 25% FA = replacement of control diet with 25% fresh azolla.

Table 3 Carcass quality and internal organ weight of Thai native crossbred chicken (Pradu Hang dum) fed commercial pelleted diet with fresh azolla (*Azolla pinnata*) replacement.

Parameter	Treatment						SEM	*P*-value
	CON	5%FA	10%FA	15%FA	20%FA	25%FA		
Live weight; lw (kg)	1.09	1.05	1.07	1.04	1.06	1.06	0.02	0.973
Defeather weight (kg)	0.78	0.71	0.80	0.76	0.70	0.71	0.01	0.137
Carcass percentage (%)	63.22	65.12	61.42	63.85	64.00	63.53	1.54	0.260
Inner organ (%lw)	15.89	15.71	14.64	15.03	16.31	15.89	0.22	0.253
Breast (%lw)	7.64	6.94	7.86	7.28	6.95	7.25	0.21	0.764
Thigh (%lw)	10.36	9.77	10.70	9.72	9.28	9.04	0.22	0.234
Liver (%lw)	2.69	2.35	2.71	2.46	2.53	2.50	0.07	0.610
Spleen (%lw)	0.44	0.41	0.49	0.39	0.37	0.48	0.03	0.848
Kidney (%lw)	0.83	0.66	0.75	0.66	0.67	0.64	0.02	0.127
Heart (%lw)	0.56	0.52	0.56	0.52	0.52	0.50	0.01	0.653
Proventriculus (%lw)	0.99	1.15	1.12	1.13	1.02	1.04	0.03	0.593
Gizzard (%lw)	4.62	4.50	4.51	4.21	4.69	4.86	0.12	0.734
Intestine (%lw)	6.16	6.03	5.62	6.16	5.99	6.62	0.17	0.690
Wing (%lw)	10.01^{ab}	9.70^{ab}	10.81^{a}	8.58^{b}	8.67^{b}	9.29^{ab}	0.22	*0.024*
Leg (%lw)	10.44	10.31	11.04	10.16	9.92	9.92	0.22	0.707

$^{a, b}$different letters in the same row indicates significant difference in mean values *($p<0.05$)*.
SEM = pooled standard error of the mean.
CON = control diet, 5% FA = replacement of control diet with 5% fresh azolla, 10% FA = replacement of control diet with 10% fresh azolla, 15% FA = replacement of control diet with 15% fresh azolla, 20% FA = replacement of control diet with 20% fresh azolla, and 25% FA = replacement of control diet with 25% fresh azolla.

Table 4 Meat quality of Thai native crossbred chicken (Pradu Hang dum) fed commercial pelleted diet with fresh azolla (*Azolla pinnata*) replacement.

Parameter	Treatment						SEM	*P*-value
	CON	5%FA	10%FA	15%FA	20%FA	25%FA		
pH 45 min	5.76	5.62	5.65	5.70	5.54	5.80	0.04	0.453
pH 24 h	5.35	5.53	5.41	5.52	5.38	5.46	0.02	0.057
Breast Meat Color								
Lightness (L*)	37.91^{b}	37.39^{b}	37.15^{b}	42.04^{a}	38.19^{b}	41.92^{a}	0.43	*<0.001*
Redness (a*)	2.6^{a}	2.49^{b}	2.84^{a}	2.68^{a}	2.71^{a}	2.20^{c}	0.09	*<0.001*
Yellowness (b*)	3.23^{c}	3.23^{c}	2.43^{c}	5.44^{a}	3.74^{b}	4.08^{b}	0.23	*<0.001*
Water holding capacity (%)								
Thawing loss	13.35	12.43	11.22	12.05	10.03	14.25	2.18	0.540
Drip loss	7.11	6.49	8.54	8.63	7.05	8.25	0.62	0.887
Shear force (N)	2.99^{a}	2.92^{c}	2.85^{e}	2.95^{b}	2.89^{d}	2.94^{bc}	0.01	*<0.001*

$^{a, b, c, d, e}$different letters in the same row indicates significant difference in mean values *($p<0.05$)*.
SEM = pooled standard error of the mean.
CON = control diet, 5% FA = replacement of control diet with 5% fresh azolla, 10% FA = replacement of control diet with 10% fresh azolla, 15% FA = replacement of control diet with 15% fresh azolla, 20% FA = replacement of control diet with 20% fresh azolla, and 25% FA = replacement of control diet with 25% fresh azolla.

Table 5 Oxidative stress in Thai native crossbred chicken (Pradu Hang dum) fed commercial pelleted diet with fresh azolla (*Azolla pinnata*) replacement.

Parameter	Treatment						SEM	*P*-value
	CON	5%FA	10%FA	15%FA	20%FA	25%FA		
Malondialdehyde (MDA (µg/ml))								
Week 6	18.88	14.68	14.92	10.36	17.48	12.20	1.10	0.227
Week 8	20.12^{a}	16.96^{b}	12.88^{c}	8.68^{d}	16.32^{b}	12.52^{c}	1.01	*0.016*
Week 10	19.36	16.36	13.60	9.60	16.20	12.60	0.96	0.052
Glutathione reduced form (GSH (µg/ml))								
Week 6	97.55	90.88	99.24	82.97	87.24	86.18	1.85	0.590
Week 8	95.61	80.52	94.76	85.97	89.55	82.24	2.11	0.186
Week 10	96.58	85.70	97.00	84.47	88.39	84.21	1.86	0.126

$^{a, b, c, d}$different letters in the same row indicates significant difference in mean values *($p<0.05$)*.
SEM = pooled standard error of the mean.
CON = control diet, 5% FA = replacement of control diet with 5% fresh azolla, 10% FA = replacement of control diet with 10% fresh azolla, 15% FA = replacement of control diet with 15% fresh azolla, 20% FA = replacement of control diet with 20% fresh azolla, and 25% FA = replacement of control diet with 25% fresh azolla.

Table 6 Oxidation of stored meat from Thai native crossbred chicken (Pradu Hang dum) fed commercial pelleted diet with fresh azolla (*Azolla pinnata*) replacement.

Parameter	Treatment						SEM	*P*-value
	CON	5%FA	10%FA	15%FA	20%FA	25%FA		
Malondialdehyde (MDA (µg/ml))								
Day 1 of storage	16.54	16.98	16.62	16.50	16.57	16.68	0.10	0.593
Day 3 of storage	16.94	17.06	16.70	17.34	16.94	16.82	0.10	0.202
Day 6 of storage	16.74	16.98	16.50	17.30	16.98	16.58	0.12	0.386
Day 9 of storage	17.46	17.30	16.70	17.34	16.94	16.82	0.32	0.532

SEM = pooled standard error of the mean.
CON = control diet, 5% FA = replacement of control diet with 5% fresh azolla, 10% FA = replacement of control diet with 10% fresh azolla, 15% FA = replacement of control diet with 15% fresh azolla, 20% FA = replacement of control diet with 20% fresh azolla, and 25% FA = replacement of control diet with 25% fresh azolla.

DISCUSSION

Fresh azolla, an aquatic fern, has high nutrient content and can be fed to layers or broilers. Azolla plants have high levels of crude protein and all essential amino acids, with particularly high levels of leucine and lysine (0.47-0.53%), methionine (0.11-0.17%), threonine (0.53-0.55%) and tryptophan (0.14-015%) (Bhatt et al., 2020). The content of the vitamin A precursor beta-carotene and vitamin B12 (Gouri et al., 2012) in azolla is also good. The rapid growth and minimal production cost thus make azolla a cheap and plentiful alternative plant protein source that improves FCR, energy efficiency, and performance without negative effects on livestock or humans (Alalade and Lyayi, 2006; Kathirvelan et al., 2015; Anitha et al., 2016; Abd El-Ghany, 2020). A study in India involving chickens in the growth phase concluded that fresh azolla could replace 20% of commercial feed diets without compromising performance (Katole et al., 2017), and another study showed that diets containing up to 7.5% azolla were associated with improved performance and nutrient utilization as well as reduced feed cost without any negative effects on carcass quality (Mishra et al., 2016).

In this study, we examined the effect of diet replacement with fresh azolla on performance parameters of Thai native crossbred chickens, which are frequently produced by small scale farmers. We found that azolla replacement for fattening native crossbred chickens had no effect on growth parameters relative to control birds fed 100% pelleted commercial diet. However, the feed cost per gain (FCG) was significantly decreased in chickens fed fresh azolla (10%, 15%, 20%, and 25% FA replacement), particularly those fed diets with 20% and 25% FA, which contained lower amounts of commercial feed.

Other studies also noted decreased cost of production with azolla supplementation of feed, yet observed beneficial effects in poultry performance as evidenced by increased feed intake and tendency toward increased body weight gain (Shukla et al., 2018; Sinha et al., 2018; Rengma et al., 2019). In this study, the feed intake of birds in the FA groups did not differ from that of the control group, and this result was consistent with previous studies showing no adverse effects on feed consumption by chickens fed azolla (Mishra et al., 2016; Samad et al., 2020)

Abdelattly et al. (2020) reported that dietary supplementation of broiler chicken feed with azolla leaf meal had increased activation of p70S6 kinase in breast muscle and breast meat redness, whereas the meat lightness was within the normal range (L* = 50-60). In our study, the breast meat redness for the 10%, 15%, and 20% FA groups was not different from that of the control, which was similar to a report showing that the carcass components of azolla-fed chickens were similar to that for the control group that was not fed azolla (Hassen et al., 2019). We also found that the group with 25% FA had decreased breast meat redness and the shear force (N) of the breast meat was lower for all azolla groups than for the control.

The MDA concentration in blood serum was lower in all groups with fresh azolla replacement but GSH levels were similar among the groups. This result suggests that feed replacement with of azolla improved natural antioxidant potency, which is consistent with a study by Kamel and Hamed (2021) showing that dietary azolla increased catalase and glutathione in broiler chickens. This result may be associated with the high phenolic and flavonoid content of azolla that would act as antioxidant agents. Moreover, erythrocyte catalase activity was reportedly higher in chickens with azolla supplementation whereas malondialdehyde levels were not affected (Chichilichi et al., 2015).

CONCLUSIONS

In conclusion, this study showed that feeding of Thai native crossbred chickens (Pradu Hang dum) for four weeks with a commercial pellet diet having partial replacement with fresh azolla did not affect performance parameters relative to the control except for a significant decrease in the feed cost per gain (FCG) for birds fed FA diets. Some carcass and meat characteristics did differ among the experimental groups, but the oxidative status was improved by azolla supplementation as evidenced by decreased serum levels of malondialdehyde (MDA). Together, these results indicate that fresh azolla can be an alternate feed source to reduce production costs for Thai native crossbred chickens.

AUTHOR CONTRIBUTIONS

The authors would like to thank the Department of Animal and Aquatic Sciences, the Faculty of Agriculture, Chiang Mai University, the Faculty of Animal Science and Technology, and Community Enterprise (Pradu Hang dum Raise Group), Ban Luang, Chom Thong, Chiang Mai, Thailand, for their assistance in the animal trial. This research work was supported by the National Research Council of Thailand, and partially supported by Chiang Mai University, Chiang Mai, Thailand and the Precision livestock farming, University of Phayao (FF67-UoE222/2567), Phayao, Thailand

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abd El-Ghany, W.A., 2020. A review on use of Azolla species in poultry production. J. World. Poult. Res. 10(2), 378-384.

Abdelatty, A.M., Mandouh, M.I., Al-Mokaddem, A.K., Mansour, H.A., Khalil, H.M.A., Elolimy, A.A., Ford, H., Farid, O.A.A., Prince, A., Sakr, O.G., Aljuaydi, S.H., Bionaz, M., 2020. Influence of level of inclusion of Azolla leaf meal on growth performance, meat quality and skeletal muscle p70S6 kinase α abundance in broiler chickens. Animal. 14(11), 2423–2432.

Alalade, O.A., Iyayi, E.A., 2006. Chemical composition and the feeding value of azolla (Azolla pinnata) meal for egg-type chicks. Int. J. Poult. Sci. 5(2), 137-141.

Anitha, K.C., Rajeshwari, Y.B., Prasanna, S.B., Shilpa Shree, J., 2016. Nutritive evaluation of azolla as livestock feed. J. Exp. Biol. Agric. Sci. 4(6), 670-674.

Bhatt, N., Singh, N.P., Singh, A.K., Kandpal, D., Chaudhary, P., Patoliya, P., 2020. Azolla – A potent unconventional feed and its effect of feeding on various livestock species –a review. J. Entomol. Zool. Stud. 8(2), 1693-1698

Boyne, A.F., Ellman, G.L., 1972. A methodology for analysis of tissue sulfhydryl components. Anal. Biochem. 46(2), 639-653.

Chichilichi, B., Mohanty, G.P., Mishra, S.K., Pradhan, C.R., Behura, N.C., Das, A., Behera, K., 2015. Effect of partial supplementation of sun-dried Azolla as a protein source on the immunity and antioxidant status of commercial broiler. Vet. world. 8(9), 1126-1130.

Gouri, M.D., Sanganal, J.S., Gopinath, C.R., Kalibavi, C.M., 2012. Important of azolla as a sustainable feed for livestock and poultry - a review. Agric. Review. 33(2), 93-103.

Hassen, W., Tafese, W., Amza, N., Gudeta, S., Beyene, A., Muleta, E., 2019. Effect of partial substitution of soybean meal (Glycine Max) by mosquito fern (Azolla pinnata) on growth performance and carcass characteristic of cobb500 broiler chickens. Ethiop. J. Appl. Sci. Technol. 10(2), 22-28.

Joysowal, M., Aziz, A., Mondal, A., Singh, S.M., Siddhnath, Boda, S., Chirwatkar, B., Chhaba, B., 2018. Effect of azolla (Azolla pinnata) feed on the growth of broiler chicken. J. Entomol. Zool. Stud. 6(3), 391-393.

Kamel, E.R., Hamed, E., 2021. Effect of dried azolla on growth performance, hematological, biochemical, antioxidant paprameters, and economic efficiency of broiler chickens. Adv. Anim. Vet. Sci. 9(11), 1886-1894.

Kathirvelan, C., Banupriya, S., M.R. Purushothaman, M.R. 2015. Azolla- an alternative and sustainable feed for livestock. Int. J. Environ. Sci. Technol. 4 (4), 1153-1157.

Katole, S.B., Shweta, R. Lende, Patil, S.S., 2017. A review on potential livestock feed: Azolla. Livest. res. int. 5(1), 1-9.

Khursheed, I., Masud, S., Khan, A., Khan, N., Kour, S., Dua, S., Khursheed, I., 2019. Proximate evaluation of Azolla pinnata as sustainable feed supplement for poultry. J. pharmacogn. phytochem. 8(3), 3157-3160.

Mishra, D.B., Roy, D., Kumar, V., Bhattacharyya, A., Kumar, M., Kushwaha, R., Vaswani, S., 2016. Effect of azolla (Azolla pinnata) meal on the performance, nutrient utilization and carcass characteristics of Chabro chicken. Indian J. Poult. Sci. 51(3), 259-263.

Mohamed, M.R., Ahmed, I.M., Osama, A.Z., Hamada, A.A., Janice A.R., Mohamed, S.H., 2023. Fresh Azolla, Azolla pinnata as a complementary feed for Oreochromis niloticus: growth, digestive enzymes, intestinal morphology, physiological responses, and flesh quality. Aquac Nutr. 2023, 1403704.

Naghshi, H., Khojasteh, S., Jafari, M., 2014. Investigation the effect of different levels of azolla (Azolla pinnata) on performance and carcass characteristics of cobb broiler chicks. Int. J. Farm. Alli. Sci. 3(1), 45-49.

Phianmongkhol, A., Wirjantoro, T., Chailungka, C., Prathum, C., Leotaragul, A., 2012. Public perception in Thai native chicken (Pradu Hang Dum Chiang Mai) via Food Contests. In Proceedings of the 2nd International Seminar on Animal Industry, Jakarta, Malaysia, 5-6 July 2012.

Pripwai, N., Pattanawong, W., Punyatong, M., Teltathum, T., 2014. Carcass characteristics and meat quality of Thai inheritance chickens. Thai. J. Agric. Sci. 6(2), 182-188.

Rengma, D.J., Savino, N., Vidyarthi, V.K., 2019. Effect of dietary inclusion of azolla powder on performance of broiler chicken. Livest. res. int. 7(2), 144-150.

Riaz, A., Khan, M.S., Saeed, M., Kamboh, A.A., Khan, R.U., Farooq, Z., Safdar Imran, S., Farid, M.U., 2022. Importance of Azolla plant in poultry production. Worlds. Poult. Sci. J. 78(3), 789-802.

Samad, F.A.A., Idris, L.H., Hassim, H.A., Goh, Y.M., Loh, T.C., 2020. Effects of Azolla spp. as feed ingredient on the growth performance and nutrient digestibility of broiler chicken. J. Anim. Physiol. Anim. Nutr. 104, 1704-1711.

Santos, M.T., Valles, J., Aznar, J., Vilches, J., 1980. Determination of plasma malondialdehyde-like material and its clinical application in stroke patients. J. Clin. Pathol. 33(10), 973-976.

Shukla, M., Bhattacharyya, A., Shukla, P.K., Roy, D., Yadav, B., Sirohi, R., 2018. Effect of Azolla feeding on the growth, feed conversion ratio, blood biochemical attributes and immune competence traits of growing turkeys. Vet. World. 11(4), 459-463.

Sinha, B.S., Kumar, S., Chudhary, G.K., 2018. Use of fresh azolla as dietary supplementation in backyard poultry. Int. J. Curr. Microbiol. App. Sci. 7, 1358-1361.

Swain, B.K., Naik, P.K., Beura, C.K., 2022. Nutritive value of azolla as poultry feed-A Review. Indian J. Anim. Nutr. 39(1), 1-11.

How to cite this article;

Montri Punyatong, Patcharee Kanjak, Wanaporn Tapingkae, Chompunut Lumsangkul, Tossapol Moonmanee, Hien Van Doan and Pimporn Khamtavee. Effect of fresh azolla (*Azolla pinnata*) feed replacement on growth performance, carcass quality, and oxidative stress in Thai native crossbred chicken. Veterinary Integrative Sciences. 2024; 22(3): 1019 - 1028