MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Agricultural Science and Soil Sciences (ISSN: 2350-2274) Vol. 9(7) pp. 070-076, August, 2021
Available online http://meritresearchjournals.org/asss/index.htm

DOI: 10.5281/zenodo.5364431

Original Research Article

Effect of *Azolla pinnata* on the quality and cholesterol content of egg of laying hens

Mosammat Mahamuda Khatun and Mohammad Aminul Islam*

Department of Dairy and Poultry Science, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

***Corresponding Author's E-mail aminul_dgvc@yahoo.com**

Abstract

The experiment was carried out to evaluate the effect of Azolla on the quality and cholesterol content of chicken eggs. A total of 240 eggs from different dietary groups of D_1 (control), D_2 (diet with 4% Azolla), D_3 (diet with 8% Azolla) and D_4 (commercial farming eggs) at different ages of the birds; A_1 (168 days), A_2 (196 days), A_3 (224 days), A_4 (252 days) and A_5 (308 days) having 12 eggs/ diet/ age group were collected from an ongoing project "Using Azolla in the diet of laying hen", Bangabandhu Sheikh Mujibur Rahman Agricultural University and commercial farms to measure egg quality traits. Accordingly, 32 and 8 eggs were collected to measure the dry matter and cholesterol content of eggs, respectively. The highest egg weight, albumen weight, yolk height and yolk width but the lowest eggshell weight and eggshell thickness were observed in D_4, followed by D_3, D_2 and D_1, respectively. Diet D_3 and D_2 showed higher albumen height, eggshell weight and eggshell thickness compared to D_1 and D_4.Of the two diets, D_3 performed better than D_2 in terms of albumen height, eggshell weight and eggshell thickness. The deepest egg yolk color was observed in D_3, followed by D_2, D_4 and D_1, respectively. Egg weight, albumen width, albumen height, yolk weight, yolk width, yolk height, eggshell thickness and yolk color were increased with the increase of the age of the birds ($p<0.001$), but decreased eggshell weight and egg yolk-albumen ratio with the increase of the age of the birds ($p<0.01$). Azolla increased dry matter (DM) but decreased moisture content of egg ($p<0.01$). The highest cholesterol was measured in D_4 and the lowest in D_3 and D_2, and intermediate in D_1. Hence, a lower level of cholesterol was observed in D_3 compared to D_2. Therefore, 8% Azolla increased the quality and dry matter and yolk color of the egg as well as reduced cholesterol content in egg yolk.

Keywords: Azolla, cholesterol, egg quality, laying hen, yolk color.

INTRODUCTION

Poultry is one of the most important and profitable sectors of agriculture, which provides valuable animal protein source feed items; meat and egg essential for human consumption which is produced within the shortest possible time. Quality meat and egg production depend on quality feed. The quality feed with a reasonable price is a key factor for successful poultry operation (Basak et al., 2002). Feed cost accounts for about 65-70% of the total poultry production cost (Ahmed et al., 2012). There is constant competition between humans and poultry for feed ingredients. Hence, there is a necessity to find out the possibility of using alternate unconventional feed ingredients in the diet of poultry. Azolla (*Azolla pinnata*) is one of the cheapest and abundant unconventional plant protein source feed ingredients that can improve feed conversion efficiency, energy efficiency, and economic performance without any deleterious effects on birds, as well as, on the human body. Azolla is a small aquatic fern that flows on the water surface. It is grown abundantly in marshy lands particularly ponds, roadside ditches, lakes, fellow land and low-lying paddy fields with almost no agronomic care

in the tropical and sub-tropical regions. As it has rapid growth, Azolla can produce double biomass within a week (3-5 days) under suitable environmental conditions (Gopal, 1967). It forms a symbiotic relationship with the blue-green algae *Anaebena azollae* located in the cavity of Azolla leaf that can assimilate atmospheric nitrogen and convert it to plant protein. It contains essential amino acids (lysine, leucine, arginine and valine), vita-A, vita-B2, vita-B12, minerals (calcium, phosphorus, magnesium, potassium, iron, copper, manganese, zinc, sodium, etc.), carotene, Beta carotene, lutein, and zeaxanthin. This is why, it is rich in protein, vitamins, minerals, chlorophyll and carotenoid (Ali and Leeson, 1995; Pillai et al., 2005). Although the efficiency of leaf meals for broiler skin pigmentation has given less emphasis, it has been recognized for a long time for egg yolk pigmentation (Osei et al., 1990). Inclusion of aquatic plants at a lower level in the poultry diet showed better performance, especially when used as a source of protein or as a source of pigmentation for egg yolk and broiler skin (Maurice et al., 1984). Several studies have been done using Azolla meal (AZM) in the diet of broiler chickens, ducks and pigs by (Islam and Nishibori, 2017; Becerra et al., 1995 and Becerra et al., 1990). But limited works using Azolla in the diet of laying hens. Alalade et al. (2007) suggested using 15% Azolla in the diet of growing pullets without jeopardizing health, and subsequent laying performance and egg quality (eggshell thickness, eggshell and albumen) except for yolk weight. Egg yolk color is one of the most important traits of egg qualities preferred by consumers. Yolk color is not only the color preference but it has also a nutritional value. De-Groote (1970) and Fletcher (1999) reported that the egg yolk color is a major concern to consumers that affect their purchasing behavior (increasing attractiveness for egg yolk color and the presence of antioxidant). Egg yolk pigmentation is responsible for having an anti-oxidant (Vita-E, vita-C and beta- carotene) that can improve the immune modulator (system). Carotenoid pigments are potent natural antioxidants (Cho et al., 2013). They can neutralize the free radicals in the body that can help to protect the serious problem like cancer and cataracts, improve skin quality, etc. Nowadays, consumers are so alert to take safe and healthy foods which are enriched in carotene, omega-3 fatty acid and conjugated fatty acid that can reduce cholesterol in the body of human beings. Most of the consumers or processors of liquid, frozen and dried egg products desire to buy deep yellow yolk eggs (North and Bell, 1990). Pigmentation of egg yolk is influenced mostly by layer diet. It solely depends on the fat-soluble pigments present in the feed ingredients taken by birds. Few works have been done using Azolla in the diet of laying hens to assess the quality of the egg. No work was found in Bangladesh to assess the effect of Azolla on yolk color and cholesterol content of egg yolk. Therefore, the present study was planned to assess the effect of Azolla on the quality and cholesterol content of chicken eggs to produce safe, quality and natural anti-oxidant-rich eggs required for human beings.

MATERIALS AND METHODS

Animal care and data collection procedures for the present study were approved by the Institutional Committee on Animal Care and Use in Research (ICACUR) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (No.BSMRAU/DEAN/FVMAS/25/ ICACUR/19). EU standards are followed for the protection of animals used for scientific purposes.

The experiment was carried out at the lab of the Department of Dairy and Poultry Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, and at the lab of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka.

Egg quality traits

A total of 240 eggs from 4 dietary groups; D_1 (Control= 60 eggs); D_2 (4% Azolla = 60 eggs); D_3 (8% Azolla = 60 eggs), and D_4 (Commercial farm= 60 eggs) having 12 eggs/hen were collected from the research project “Using Azolla in the diet of laying hens” funded by RMC, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh to determine egg quality traits.

Data recording

The egg quality traits were measured using digital devices. Egg, albumen, yolk and egg shell weight were measured by using a digital balance. Yolk color was determined by comparing it with the DSM Yolk Color Fan. Albumen and yolk width were measured using digital slide calipers. Then albumen and yolk height were recorded with the help of a digital spherometer. Eggshell thickness was measured using a digital eggshell thickness meter/gauge. Finaly, the yolk-albumen ratio was calculated from yolk and albumen weight.

Determination of dry matter content of eggs

A total of 32 eggs from experimental dietary groups and commercial farms were taken to determine the dry matter content of eggs.

Cholesterol content of egg yolk

A total of 8 eggs from experimental dietary groups; D_1 (Control), D_2 (4% Azolla), D_3 (8% Azolla) and Commercial

farm (D_4) were taken to determine the cholesterol content of eggs. The cholesterol content of egg yolk (mg/100 g) was measured by using the UV-Spectrophotometer method at the Lab of BCSIR, Dhaka.

Statistical Analysis

The collected data of egg quality traits and dry matter content of egg were analyzed in 4 diets X 5 age groups factorial design using the Statistix10 computer package program. The data of cholesterol content of egg yolk was subjected to t-test.

Statistical model for egg quality traits and dry matter content of egg

$$Y_{ijk} = \mu + D_i + A_j + (D \times A)_{ij} + e_{ijk}$$

Where, Y_{ijk} is the observation of k^{th} replication of the i^{th} dietary group and the j^{th} age group.
μ is the overall mean.
D_i is the fixed effect of the **i**th dietary groups (i= 1--------4).
A_j is the effect of the **j**th age groups (j= 1---------5).
$(D \times A)_{ij}$ is the interaction effect of the **i**th dietary group and the **j**th age group.
e_{ijk} is the random error.

RESULTS

Egg quality traits

The dietary groups (D_1= Control, D_2= diet with 4% Azolla, D_3= diet with 8% Azolla, and D_4= commercial farming egg) were significantly different for egg weight ($p<0.001$), albumen weight ($p<0.05$), albumen height ($p<0.001$), yolk width ($p<0.05$), yolk height ($p<0.001$), egg shell with membrane weight ($p<0.001$), egg shell with membrane thickness ($p<0.001$), egg shell thickness ($p<0.001$), yolk color ($p<0.001$) (Table 1). Albumen width, yolk weight and yolk albumen ratio were statistically similar among the dietary groups ($p>0.05$).The highest egg weight and albumen weight was observed in D_4, moderate in D_3, D_2 and the lowest in D_1. Evidently but not significantly, the lowest yolk weight was observed in D_4, followed by D_3, D_2 and D_1, respectively. The highest albumen height was observed in D_2, followed by D_3, D_1 and D_4, respectively. However, D_4 showed the highest yolk height followed by D_2, D_3 and D_1, respectively. Diet D_2 showed a lower yolk width compared to D_4, D_3, and D_1. The lower eggshell weight and eggshell thickness were observed in D_4 compared to D_3, D_2 and D_1.The value of eggshell weight and eggshell thickness were almost similar among the diets of D_3, D_2 and D_1. Of the three diets; D_3 performed better than D_2 and D_1in terms of egg weight and eggshell thickness. The deepest egg yolk color was observed in D_3, followed by D_2, D_4 and D_1, respectively. Therefore, Azolla increased the egg yolk color. No significant difference was observed among dietary groups for albumen width, yolk weight and yolk albumen ratio ($p>0.05$).

Egg quality traits differed significantly among the age groups, except for albumen weight. Egg weight, albumen width, albumen height, yolk weight, yolk width, yolk height, egg shell thickness and yolk color were increased with the increase of the age of the bird($p<0.001$). But egg shell weight and egg yolk-albumen ratio were decreased with the increase of the age of the bird ($p<0.01$). The albumen weight was almost similar among the age groups ($p>0.05$). Diet and age interacted for the traits of egg weight, albumen weight, albumen width, albumen height, yolk weight, yolk width, yolk height, yolk albumen ratio, egg shell with membrane thickness, egg shell thickness and yolk color ($p<0.001$), except for eggshell with membrane weight. There was no interaction between diet and age for eggshell with membrane weight ($p>0.05$).

Dry matter content of egg

Fresh egg weight, fresh albumen weight, dry egg weight, moisture and dry yolk weight differed significantly among the dietary groups. However, fresh yolk weight, fresh egg shell weight, dry albumen weight and dry eggshell weight were almost similar among the dietary groups ($p> 0.05$) (Table 2).There was no effect of age, and interaction of age and diet on the dry matter content of the egg ($p>0.05$).The highest dry matter but the lowest moisture content of the egg was estimated in D_2, followed by D_3, D_1 and D_4, respectively. Similar trend was observed in case of the dry yolk weight (%). Therefore, Azolla increased the dry matter content of the egg.

Cholesterol content of egg yolk

The cholesterol content of the egg yolk differed significantly among dietary groups ($p<0.001$) (Table 3). The highest amount of cholesterol was measured in the D_4, followed by D_1, D_2 and D_3, respectively. Therefore, Azolla reduced the cholesterol level in the egg yolk.

DISCUSSION

Egg quality traits

Few works were found on the egg quality traits of laying hens affected by Azolla. In the present study, Azolla performed the best in terms of albumen height, yolk

Table 1. Egg quality traits of experimental and commercial farming eggs of laying hens at different ages of the bird

Traits	Diets (D)	Age (A)						LSD value and level of significance +		
		A_1	A_2	A_3	A_4	A_5	Mean	D	A	D * A
Egg weight (g)	D_1	49.50	50.88	54.29	55.26	57.03	53.39	1.563***	1.747***	3.495***
	D_2	48.90	52.48	54.48	57.14	56.50	53.90			
	D_3	49.93	52.63	53.84	58.02	57.06	54.29			
	D_4	42.80	61.97	64.54	68.86	64.92	60.62			
	Mean	47.78	54.49	56.79	59.82	58.88	55.55			
Albumen weight (%)	D_1	61.41	62.09	60.74	62.11	61.88	61.64	1.201*	1.343NS	2.685***
	D_2	62.59	62.45	61.73	61.64	65.55	62.79			
	D_3	63.52	62.93	61.61	62.06	61.55	62.33			
	D_4	57.41	64.74	66.47	65.18	62.66	63.29			
	Mean	61.23	63.05	62.64	62.74	62.91	62.52			
Albumen width (mm)	D_1	73.20	75.80	81.53	83.47	80.70	78.94	2.274NS	2.543***	5.085***
	D_2	69.46	75.15	76.21	77.88	83.25	76.39			
	D_3	72.09	78.61	75.07	75.96	82.62	76.87			
	D_4	71.40	71.12	79.71	84.10	74.35	76.14			
	Mean	71.54	75.17	78.13	80.35	80.23	77.08			
Albumen height (mm)	D_1	10.28	9.51	9.57	9.76	9.87	9.80	0.474***	0.529***	1.059***
	D_2	11.09	10.96	11.58	11.37	10.41	11.08			
	D_3	9.99	10.07	11.58	11.27	10.08	10.60			
	D_4	5.45	10.04	10.68	9.04	8.54	8.75			
	Mean	9.20	10.14	10.85	10.36	9.73	10.06			
Yolk weight (%)	D_1	24.39	24.31	25.57	26.01	25.29	25.12	1.336NS	1.494**	2.988*
	D_2	23.28	23.65	24.83	25.13	28.47	25.07			
	D_3	23.22	23.18	24.74	24.78	24.99	24.18			
	D_4	26.74	22.97	21.04	22.97	25.55	23.85			
	Mean	24.41	23.53	24.05	24.72	26.08	24.56			
Yolk width (mm)	D_1	38.05	37.48	38.49	39.64	39.40	38.61	0.858*	0.959***	1.918*
	D_2	36.46	36.32	39.96	39.08	37.20	37.81			
	D_3	37.92	37.76	38.75	39.88	39.94	38.85			
	D_4	38.24	38.16	37.64	39.16	41.37	38.91			
	Mean	37.67	37.43	38.71	39.44	39.48	38.55			
Yolk height (mm)	D_1	14.35	14.73	14.24	14.69	14.83	14.57	0.269***	0.301***	0.602***
	D_2	14.28	14.88	15.16	15.23	14.59	14.83			
	D_3	14.58	14.97	15.11	14.98	14.25	14.78			
	D_4	14.24	17.92	17.64	17.33	17.50	16.93			
	Mean	14.36	15.62	15.54	15.56	15.29	15.28			
Yolk-Albumen Ratio	D_1	0.40	0.39	0.43	0.42	0.41	0.41	0.023NS	0.026*	0.051***
	D_2	0.37	0.38	0.40	0.41	0.43	0.40			
	D_3	0.37	0.37	0.40	0.40	0.41	0.39			
	D_4	0.47	0.36	0.32	0.35	0.41	0.38			
	Mean	0.40	0.38	0.39	0.40	0.41	0.40			
Eggshell with membrane weight (%)	D_1	13.14	12.92	12.85	12.74	12.51	12.83	0.387***	0.433**	0.865NS
	D_2	13.34	13.35	12.66	12.54	12.60	12.90			
	D_3	12.87	12.90	13.00	12.31	12.69	12.75			
	D_4	12.58	11.54	11.82	11.10	11.15	11.64			
	Mean	12.98	12.68	12.58	12.17	12.24	12.53			
Eggshell with membrane thickness (mm)	D_1	0.52	0.50	0.52	0.43	0.44	0.48	0.0229***	0.0256***	0.0512***
	D_2	0.47	0.53	0.51	0.38	0.48	0.47			
	D_3	0.58	0.51	0.49	0.36	0.50	0.49			
	D_4	0.30	0.30	0.36	0.50	0.45	0.38			
	Mean	0.47	0.46	0.47	0.42	0.47	0.46			

Table 1. Continue

Eggshell thickness (mm)	D_1	0.48	0.47	0.49	0.41	0.40	0.45	0.0238***	0.0266**	0.0532***
	D_2	0.40	0.50	0.47	0.35	0.45	0.43			
	D_3	0.54	0.48	0.46	0.33	0.47	0.46			
	D_4	0.24	0.23	0.32	0.47	0.40	0.33			
	Mean	0.42	0.42	0.44	0.39	0.43	0.42			
Yolk color (DSM)	D_1	5.75	5.00	5.17	7.42	6.50	5.97	0.4287***	0.4793***	0.9585***
	D_2	11.08	10.58	12.10	10.67	12.08	11.30			
	D_3	10.83	13.33	14.25	12.92	14.00	13.07			
	D_4	7.33	6.25	6.67	6.50	7.00	6.75			
	Mean	8.75	8.79	9.54	9.38	9.86	9.27			

+ NS, p> 0.05; *, p<0.05; **, p<0.01; ***, p<0.001; D_1=Control diet (No Azolla); D_2= Diet with 4% Azolla; D_3= Diet with 8% Azolla; D_4= Commercial farming eggs;A_1=168 days; A_2= 196 days; A_3= 224 days; A_4= 252 days; A_5= 308 days.

Table 2. Dry matter content of experimental and commercial farming eggs of laying hens at different ages of the bird

Traits	**Age(A)**	**Diet (D)**					**LSD value and level of significance+**		
		D_1	**D_2**	**D_3**	**D_4**	**mean**	**D**	**A**	**D*A**
Fresh egg weight (g/egg)	A_1	56.97	60.54	58.58	49.97	56.51	5.539*	4.188NS	7.253NS
	A_2	56.35	59.20	58.06	53.15	56.69			
	Mean	56.66	59.87	58.32	51.56	56.60			
Dry egg weight (%)	A_1	30.87	34.27	33.17	28.84	31.79	1.757**	1.328NS	2.300NS
	A_2	31.15	31.29	31.91	29.76	31.03			
	mean	31.01	32.78	32.54	29.30	31.41			
Moisture (%)	A_1	69.13	65.74	66.83	71.16	68.21	1.757**	1.328NS	2.300NS
	A_2	68.85	68.71	68.09	70.24	68.97			
	mean	68.99	67.22	67.46	70.70	68.59			
Fresh albumen weight (%)	A_1	62.86	61.81	61.56	67.39	63.40	2.961*	2.131NS	3.876NS
	A_2	61.37	63.13	62.99	65.01	63.12			
	mean	62.11	62.47	62.27	66.20	63.26			
Fresh yolk weight (%)	A_1	25.48	25.61	25.49	21.97	24.64	3.062NS	2.203NS	4.009NS
	A_2	25.95	23.99	24.20	23.98	24.53			
	mean	25.71	24.80	24.84	22.98	24.58			
Fresh egg shell weight (%)	A_1	12.59	12.26	12.85	12.30	12.49	1.709NS	1.230NS	2.238NS
	A_2	11.62	12.00	12.37	12.08	12.02			
	mean	12.11	12.11	12.61	12.19	12.25			
Dry albumen weight (%)	A_1	8.23	8.66	7.76	8.55	8.30	1.180NS	0.893NS	1.546NS
	A_2	8.84	7.94	7.89	8.30	8.24			
	mean	8.53	8.30	7.82	8.42	8.27			
Dry yolk weight (%)	A_1	12.99	15.82	14.77	10.24	13.46	2.246*	1.698NS	2.940NS
	A_2	12.52	13.24	13.77	11.02	12.64			
	mean	12.76	14.53	14.27	10.63	13.05			
Dry egg shell weight (%)	A_1	9.65	9.96	10.64	10.02	10.07	1.303NS	0.985NS	1.706NS
	A_2	9.78	10.09	10.24	10.42	10.13			
	mean	9.71	10.03	10.44	10.22	10.10			

+NS, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001; D_1= Control-No Azolla; D_2=4% Azolla; D_3=8% Azolla; D_4= Commercial farming egg; A_1=252 days; A_2=308 days.

Table 3. Cholesterol content of the egg yolk of the experimental and commercial farming eggs of laying hens at 287 days age of the bird.

Diets (D)	**Cholesterol (mg/ 100g)**	**t- value and level of significance+**
D_1	237.12	16.338***
D_2	210.95	
D_3	201.93	
D_4	263.98	

+ ***, p<0.001; D_1=Control diet; D_2= Diet with 4% Azolla; D_3= Diet with 8% Azolla; D_4= Commercial farming eggs

height, eggshell weight, eggshell thickness, and yolk color compared to the control and commercial farming eggs. Of the two diets, D_3 performed better than that of D_2 for egg quality traits. Azolla increased the freshness of egg, as well as the quality of eggs because of increasing albumen height. Eggshell thickness is the most important trait of laying hens which were increased using Azolla in the diet of laying hens. It is required to form the structure and handling of eggs. It is also effective for the hatchability of eggs of the breeder flock. For this, Azolla plays an important role to form the structure of the egg. The better quality of egg was observed in the present study when the diet was formulated using Azolla, which performed better than the previous findings reported by several authors (Khatun et al., 1999; Alalade et al., 2007; Boitoi et al., 2018). The present study also showed that the 8% of Azolla was far better than that of the 4% Azolla in terms of egg quality traits of laying hens. Commercial farming eggs showed the lowest quality of egg in terms of albumen height, yolk height, eggshell thickness and egg yolk color. The commercial farming egg was even a lower performer than the control diet for these traits. But it had the highest egg weight as well as the albumen weight. Therefore, the commercial farming egg was in the poorest grade among the dietary groups in terms of egg quality traits, dry matter and cholesterol content of the egg of laying hens. The yolk-albumen ratio was almost similar among the dietary groups.

The present findings showed the highest yolk color in D_3 and D_2 (DSM 11.3-13.1) and the lowest in D_4 and D_1 (DSM 5.97-6.75). Therefore, Azolla increased egg yolk color because of the presence of vita-A, carotene, Beta carotene in lutein and zeaxanthin located in the cavity of Azolla leaves. This is why the highest yellow yolk color was observed in the diet formulated using Azolla meal. Therefore, Azolla is responsible to increase the natural yellow color of egg yolk. This finding was supported by (Khatun et al., 1999; Sujatha et al., 2013; Swain et al., 2018). They reported that Azolla increased the egg yolk color. Of the two diets, 8% of Azolla was the best performer diet for the yolk coloration compared to the diet containing 4% Azolla. Therefore, the commercial farming eggs, as well as, the control diet performed the lowest for egg yolk coloration. It was the novelty of the present study.

Egg weight, albumen weight and yolk weight were increased but decreased eggshell weight and eggshell thickness with the increase of the age of the bird. In the present study, yolk color was increased with the increase of the age of the bird which corroborates the findings of Padhi et al. (2013).

Dry matter content of egg

No previous work was found on the dry matter content of the egg from the diet containing the Azolla meal. The present study recorded the increased dry matter and reduced moisture content of the egg in the diet containing Azolla meal. The highest amount of fresh albumen, dry albumen and yolk weight was measured in the diet of D_2 and D_3 compared to D_1 and D_4. The lowest amount of dry matter, albumen and yolk weight in commercial farming eggs. Therefore, Azolla influenced the dry matter content of the egg. Hence, the present findings investigated that Azolla improved the quality and quantity of the egg of laying hens. It was also noticeable that commercial farming eggs were inferior in quality and quantity to the experimental eggs especially for the eggs from the diet included the Azolla meal.

Cholesterol content of egg yolk

No previous work was found using Azolla in the diet of laying hen to assess the cholesterol content of egg yolk. It has been found in the present study that the lowest amount of cholesterol in D_3 and D_2 is compared to that of D_1 and D_4. It has also been found in the present study that commercial farming eggs (D_4) contained the highest amount of cholesterol. Of the two diets, D_3 was found to the lower in cholesterol content of the egg than the D_2. Therefore, Azolla affected reducing the cholesterol level of the egg yolk. Moreover, Azolla with a higher level (8%) in the diet of laying hen found a more reduction of cholesterol in egg yolk compare to the addition of a lower level of Azolla (4%) in the diet of laying hens. Islam and Nishibori (2017) reported that a lower amount of total cholesterol , triglyceride (TG), low-density lipoprotein (LDL) and a higher amount of high-density lipoprotein (HDL) in the blood of broiler chicken when added 5% and 7% Azolla in the diet compared to the control diet of broiler chickens.Therefore, D_3 may be the most suitable dietary group to reduce the cholesterol content of egg yolk. This is also the novelty of the present findings.

CONCLUSION

The present study reveals that Azolla improved the quality and dry matter (DM) of the egg, and reduced the moisture and cholesterol content of the egg compared to the control diet and commercial farming eggs.A higher level of the reduction of cholesterol of egg yolk was observed in diet D_3 compared to D_2. The highest yolk color was observed in D_3 and D_2 compared to D_1 and D_4. Therefore, Azolla increased egg yolk color. However, the diet with 8% of Azolla was better than that of the diet with 4% Azolla in terms of egg yolk coloration.The addition of 8% Azolla was more beneficial to 4% Azolla in the diet of laying hens in terms of egg quality, increasing egg yolk color and reducing the cholesterol level in egg yolk. Therefore, Azolla may be the most suitable and excellent feed item in the diet of laying hens. However, more

studies are needed before suggesting to use of Azolla in the Poultry Industry.

ACKNOWLEDGEMENT

The authors are grateful to the Research Management Committee (RMC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh for providing a fund to carry out research and prepare the scientific article.

REFERENCES

Ahmed MF, Nishibori M, Islam MA (2012). Production and price of indigenous naked neck and full feathered chicken reared under rural scavenging system in Bangladesh. J. Agric. Ext. Rural Develop. 4(4): 92-97.

Alalade OA, Iyayi EA, Alalade TO (2007). The nutritive value of Azolla (Azolla pinnata) meal in diets for growing pullets and subsequent effect on laying performance. J. Poultry Sci. 44(3): 273-277.

Ali MA, Leeson S (1995). Nutritional value and aquatic weeds in the diet of poultry. World's Poulty Sci. J. 50: 239-251.

Basak B, Pramanik MA, Rahman MS, Tarafdar SU, Roy BC (2002). Azolla (Azolla pinnata) as a feed ingredient in broiler ration. Int. J. Poultry Sci. 1(1): 29-34.

Becerra M, Murgueitio E, Reyes G, Preston TR (1990). Azolla filiculoides as partial replacement for traditional protein supplements in diets for growing-fattening pigs based on sugar cane juice. Livestock Research and Rural Development 2(2): 15-22.

Becerra M, Preston TR, Ogle B (1995). Effect of replacing whole boiled soya beans with azolla in the diets of growing ducks. Livestock Research and Rural Development 7(3): 32-38.

Boitai SS, Babu LK, Panda AK, Mohapatra L, Sahoo B (2018). Effect of dietary incorporation of azolla meal on production performance and egg quality of Vanaraja laying hens. Int. J. Livestock Res. 8(5): 264-270. doi: 10.5455/ijlr.201708 28054121

Cho JH, Zhang ZF, Kim IH (2013). Effects of canthaxanthin on egg production, egg quality, and egg yolk color in laying hens. J. Agric. Sci. (Toronto) 5(1): 269-274.

De Groote G (1970). Research on egg yolk pigmentation and its practical application. World's Poultry Sci. J. 26(1) : 435-441. http://dx.doi.org/10.1079/WPS19700005

Fletcher DL (1999). Broiler breast meat color variation, pH, and texture. Poultry science 78(9): 1323-1327.

Gopal B (1967). Contribution of Azolla pinnata R. Br. to the productivity of temporary ponds at Varanasi. Tropical Ecology 8:126-130.

Islam MA, Nishibori M (2017). Use of multivitamin, acidifier and Azolla in the diet of broiler chickens. Asian-Australian J. Animal Sci. 30(5): 683.

Kamalasanana Pillai P, Premalatha S, Rajamony S (2005). Azolla: A sustainable feed for livestock. LEISA Magazine 26-27.

Khatun A, Ali MA, Dingle JG (1999). Comparison of the nutritive value for laying hens of diets containing azolla (Azolla pinnata) based on formulation using digestible protein and digestible amino acid versus total protein and total amino acid. Animal Feed Science and Technology 81: 43-56.

Maurice DV, Jones JE, Dillon CR, Weber JM (1984). Chemical composition and nutritional value of Brazilian elodea (Egeria densa) for the chick. Poultry Science 63(2): 317-323.

North MO, Bell DD (1990). Commercial chicken production manual. 4th Edition, Van Nostrand Reinhold, New York, U.S.A. Pp. 913.

Osei SA, Opoku RS, Atuahene CC (1990). Gliricidia leaf meal as an ingredient in layer diets. Animal Feed Science and Technology 29: 303-308.

Padhi MK, Chatterjee RN, Haunshi S, Rajkumar U (2013). Effect of age on egg quality in chicken. Indian J. Poultry Sci. 48(1): 122-125.

Sujatha T, Kundu A, Jeyakumar S, Kundu MS (2013). Azolla supplementation: Feed cost benefit in duck ration in Andaman Islands. Tamilnadu J. Vet. Animal Sci. 9(2): 130-136.

Swain BK, Naik PK, Sahoo SK, Mishra SK, Kumar D (2018). Effect of feeding of Azolla (Azolla pinnata) on the performance of white pekin laying ducks. Int. J. Livestock Res. 8: 248. doi: 10.5455/ijlr.20180117114123