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Effect of Dietary Supplementation of Arjuna (*Terminalia arjuna*) Bark and Sahjan (*Moringa oleifera*) Leaf Powder on the Egg Quality and Egg Composition of Uttara Chicken

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ABSTRACT

This study was conducted during February, 2022 to February, 2023 to evaluate effect of dietary supplementation of *Terminalia arjuna* bark and *Moringa oleifera* leaf powder on external and internal egg quality parameters and egg composition of Uttara chicken. Day old chicks (n=72) were distributed into six treatment groups, having three replicates of 4 birds each. Birds were fed T_0 (control diet), T_1 (Basal diet+1% Arjuna bark powder+1% Sahjan leaf powder), T_2 (Basal diet+1% Arjuna+2% Sahjan), T_3 (Basal diet+1% Arjuna+3% Sahjan), T_4 (Basal diet+1% Arjuna+4% Sahjan) and T_5 (Basal diet+1% Arjuna+5% Sahjan). Eggs from the T_3 group consistently exhibited higher (p<0.01) egg weight, thicker egg shell, greater egg shell weight, and improved albumen quality, as evidenced by higher albumen height, albumen index, and Haugh unit values. Yolk weight and yolk color score were also improved (p<0.01) in treatment groups, notably T_2 and T_3 as compared to control group. The eggs from the T_3 group had significantly higher (p<0.05) crude protein content and lower yolk cholesterol levels compared to other groups. There was no significant difference in moisture (%) and ether extract (%) content of egg. Thus, it may be concluded that Arjuna bark @ 1% and Sahjan leaf @ 3% powder have positively influenced egg quality and egg composition of Uttara chicken indicating its potential as a beneficial dietary supplement in poultry diet.

KEYWORDS: Albumen, crude protein, egg shell, yolk cholesterol

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Medicinal herbs, spices, and aromatic plants are major sources of flavour, aroma, and colour for both human and animal food in addition to their beneficial effects (Abd El-Hack and Alagawany, 2015; Abd El-Hack et al., 2018, 2020a, 2020b). It was prohibited to use AGP as growth promoters in animal nutrition due to the advent of antibiotic-resistant organisms (Arif et al., 2019; Gado et al., 2019; Ashour et al., 2020; Batiha et al., 2020). Thus, it is imperative to identify alternate growth promoters. Several herbs have been utilised as alternatives to antibiotics in poultry. These plants are also utilised as alternatives to antiviral, anticocidiosis, antiparasite, and immunomodulatory medicines (Dhama et al., 2015). It can also have a beneficial, harmful, or fatal effect depending on the quantity consumed (Durmic and Blache, 2012). The bioactive components of antibiotics, such as isothiocyanates, thymoquinone, allicin, and azadirachtin (Adegbeye et al., 2018), determine their function, while the ability of these plants to boost feed intake, increase feed digestibility by stimulating digestive enzymes, and prevent pathogen colonisation in the gut or possibly affect the development of villi to aid in absorption, determines growth promotion (Sanjyal and Sapkota, 2012). Supplementation with herbal plant extracts can improve flavour and appetite that leads to increase in feed intake. Consumption and demand for medicinal plants have increased in many countries due to their low cost, easy availability, affordability, good antimicrobial nature, reduced disease-related risks, lowering blood cholesterol level, and diverse functions in improving growth performance in birds (Lewis et al., 2003).

Terminalia arjuna is found in Indo-sub-Himalayan regions including Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi, and Deccan (Chopra et al., 1958). It is used as a heart tonic to keep the heart healthy, blood pressure normal and lower cholesterol levels in Ayurvedic medicine. Its bark powder possesses substantial antioxidant activity comparable to vitamin E, as well as anti-platelet activity. Furthermore, scientists and researchers are attempting to battle fatal diseases in chickens by using medicinal herbs with the most potent compounds to boost growth, weight gain and immunostimulant. The main phytoconstituents of *Terminalia arjuna* include arjunic acid, arjunolic acid, arjungenin, arjunone, arjunolone, and luteolin, gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), and phytosterols (Bone, 1996; Kapoor, 1990). *Moringa oleifera* contain significant concentrations of vital nutrients, vitamins, minerals, fatty acids, and fibre (Razis et al., 2014). There have been claims that *M. oleifera* possesses therapeutic qualities (Ghasi et al., 2000; El-Tazi and Tibin, 2014). *M. oleifera* has anti-oxidant properties that reduce the generation of reactive oxygen species (ROS) and free radicals, contributing to its therapeutic effects (Ogbunugafor et al., 2011). *M. oleifera* has a crucial role against inflammatory and oxidant effects (Yang et al., 2006). Supplementation of *M. oleifera* leaf extracts inhibit the development of pathogenic gram-positive and gram-negative bacteria. It has many vitamins (A, E, B_2, B_5, B_6, folic acid) and minerals (Ca, Fe) (Biel et al., 2017). Moringa has antihypertensive, diuretic and cholesterol-lowering activities, antifertility, antimicrobial and antifungal activities (Caceres et al., 1992), hepatoprotective, antiulcer, antispasmodic, antitumor, anticancer and antioxidant activities (Jimenez et al., 2017). A variety of diseases and deficiencies can be cured by the attributes and substances of Moringa (Fordl et al., 2001). Both rural and urban areas are home to Moringa. Studies related to *Moringa oleifera* leaves supplemented with other herbal plant in the poultry are very much but with Arjuna bark is limited in poultry. In this view, the present study is undertaken in indigenous chicken to evaluate the effects of dietary supplementation of Arjuna bark and *Moringa oleifera* leaf powder on production performance, external and internal egg quality and egg composition of Uttara chicken.

2. MATERIALS AND METHODS

2.1. *Experimental birds*

A total of 72 female day-old chicks of Uttara breed after sexing were kept at Instructional Poultry Farm of College of Veterinary & Animal Sciences, Pantnagar, Uttarakhand (263145), India. All the birds were individually weighed and randomly distributed into six treatment groups having three replicates with four chicks each.

2.2. *Housing and management*

All the birds in the experiment were housed under deep litter system in brooder house till 8 weeks of age. The fresh and dry wheat straw was used as bedding material. The day-old chicks were wing banded individually for identification and weighed individually before allocating to different dietary treatments. Adequate light for 24 hours and ventilation were provided during brooding phase. The weighed amount of feed was offered daily and clean and fresh drinking water would be provided ad-libitum. The body weights of individual birds and residual feed was recorded weekly replicate wise. After chick phase of 8 weeks, all the birds were separated to open side house under deep litter system and kept there for 52 weeks of age. The laying phase was divided into two phases; phase I (20–36 weeks) and phase II (36–52 weeks).

2.3. *Collection of arjuna (Terminalia arjuna) bark and sahjan (Moringa oleifera) leaf*

Arjuna bark and Sahjan leaves were collected from the surrounding areas of Pantnagar. Arjuna bark was shade dried to avoid nutrient loss and grounded to powder by an electric grinding machine at Department of Agronomy, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar. Sahjan leaves was also shade dried and grounded to powder by an electric grinding machine at Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar. Various levels of powdered Arjuna bark and Sahjan leaf were added to the feed of chicken.

2.4. Experimental diets

Standard chicken diets were prepared for chick (0–8 weeks), grower (9–20 weeks) and layer (21–52 weeks) phases of growth by mixing the ingredients to meet the nutrient requirements of layer chicken as per recommendations of Anonymous (2007). Proximate composition of experimental feed, Arjuna bark and Sahjan leaf was analyzed using Anonymous (2000) procedure.

2.5. Experimental design

Birds were fed T_0 (control diet), T_1 (Basal diet+1% Arjuna bark powder+1% Sahjan leaf powder), T_2 (Basal diet+1% Arjuna+2% Sahjan), T_3 (Basal diet+1% Arjuna+3% Sahjan), T_4 (Basal diet+1% Arjuna+4% Sahjan) and T_5 (Basal diet+1% Arjuna+5% Sahjan).

2.6. Egg quality parameters

Thirty-six eggs were taken in each phase for evaluating the external and internal egg quality characteristics. Egg weight (g), length and width of egg (cm), shape index, egg shell thickness (mm) and egg shell weight (g) were observed for determining external egg quality. Width and height of thick albumen (mm), width and height of yolk (mm), albumen weight (g), yolk weight (g), albumen index, haugh unit, yolk index and yolk color was observed for determining internal egg quality.

2.7. Egg composition

According to Anonymous (2000) the chemical composition of the egg (including its crude protein, crude fat and ash content) was also determined at the end of phase I and phase II. Two eggs per replication were randomly collected at the end of each phase to determine egg composition. The eggs were first boiled and then after it the shell and shell membranes were removed carefully. The boiled eggs were chopped, placed on aluminium foil that had been previously weighed and dried in a hot air oven at 100±2° C for 24 hours or until no weight change was noticed.

2.8. Determination of egg yolk cholesterol

During the 52nd week, assessment of egg yolk cholesterol was done. By rolling the unbroken egg over filter paper, the yolk was initially entirely separated from albumen and the adhering membrane. Folch et al. (1957) described a method for extracting yolk lipids. Following homogenization of the yolk, 0.5 g of the homogenised egg yolk sample was placed in a centrifuge tube along with 7.5 ml of a 2:1 solution of chloroform and methanol, and vortexed for 30 seconds. 2.5 ml of pure water was added, thoroughly combined, and centrifuged for 10 minutes at 2500 rpm. The chloroform layer was evaporated to dryness over a water bath at 80°C after the aqueous methanol layer was removed by suction. The manufacturer's instructions were followed to use a cholesterol kit to estimate total cholesterol after reconstituting the dried residue with 4ml glacial acetic acid. The total cholesterol content in the egg yolk was calculated using the following formula:

Total cholesterol (mg/dl)=(Absorbance of test/Absorbance of standard)×Concentration of standard (mg dl^{-1})

2.9. Statistical analysis

The experimental data collected for the current study was statistically analysed using one-way ANOVA (for more than two groups of data) with the aid of SPSS software version 21 (Snedecor and Cochran, 1994)) and Duncan's multiple range test (Duncan, 1955) was used to determine the difference between the treatment means.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of experimental diets

Table 1 shows the proximate composition of chicken diets fed to chicks, growers and layers. Chemical composition of Arjuna bark and Sahjan leaf powder is presented in Table 2.

3.2. Egg quality parameters

During phase I, the egg weight (g) was significantly higher (p<0.01) in T_3 followed by T_2 and T_1 groups than T_0, T_4 and T_5 groups. Similar results were found in phase II and overall period (Table 3, 4 and 5). There was no significance difference found in egg width, egg length and shape index among treatment groups. Few studies which are

Table 1: Chemical composition (%) of basal feed given to birds during different phases of growth

Particulars	Moisture	Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract	Ca	P
Chick feed (0–8 weeks)	11.64	20.22	2.26	7.34	8.75	61.43	0.95	0.46
Grower feed (8–20 weeks)	11.41	16.25	2.11	8.76	9.06	63.82	1.03	0.40
Layer feed (20–52 weeks)	11.16	18.00	2.18	8.72	9.43	61.67	3.09	0.42

Table 2: Chemical composition (%) of Arjuna (*Terminalia arjuna*) bark and Sahjan (*Moringa oleifera*) leaf powder

Particulars	Moisture	Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract	Ca	P
Arjuna bark (*Terminalia arjuna*)	6.12	4.07	4.35	15.45	27.65	48.47	3.05	0.03
Sahjan leaf (*Moringa oleifera*)	6.43	24.50	2.84	9.76	9.82	53.09	2.64	0.39

Table 3: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on external egg quality of Uttara chicken during phase I (20–36 weeks)

Treatment	Egg weight (g)	Egg width (cm)	Egg length (cm)	Shape index	Egg shell thickness (mm)	Egg shell weight (g)	Shell %
T_0	45.43 ± 0.12^a	3.55±0.016	4.76±0.019	74.56±0.044	0.40 ± 0.004^a	5.46 ± 0.017^a	12.03±0.062
T_1	46.10 ± 0.20^b	3.57±0.018	4.78±0.021	74.65±0.057	0.42 ± 0.003^{bc}	5.55 ± 0.008^b	12.05±0.051
T_2	46.33 ± 0.29^b	3.57±0.019	4.79±0.021	74.71±0.056	0.43 ± 0.003^{bc}	5.59 ± 0.020^b	12.06±0.048
T_3	46.42 ± 0.29^b	3.58±0.017	4.79±0.022	74.71±0.021	0.43 ± 0.002^c	5.60 ± 0.015^b	12.06±0.052
T_4	45.32 ± 0.10^a	3.54±0.016	4.75±0.019	74.58±0.044	0.40 ± 0.004^a	5.46 ± 0.014^a	12.05±0.049
T_5	45.26 ± 0.10^a	3.54±0.018	4.74±0.021	74.63±0.049	0.39 ± 0.004^a	5.45 ± 0.014^a	12.04±0.046
Sig. level	$p<0.01$	NS	NS	NS	$p<0.01$	$p<0.01$	NS

Means bearing different superscripts within a column differ significantly ($p<0.05$); NS: Not significant

Table 4: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on external egg quality of Uttara chicken during phase II (36–52 weeks)

Treatment	Egg weight (g)	Egg width (cm)	Egg length (cm)	Shape index	Egg shell thickness (mm)	Egg shell weight (g)	Shell %
T_0	53.98 ± 0.14^a	4.54±0.009	6.14±0.027	73.88±0.18	0.45 ± 0.004^a	6.62 ± 0.028^a	12.26±0.022
T_1	54.68 ± 0.18^b	4.56±0.026	6.17±0.026	73.99±0.2	0.47 ± 0.003^b	6.72 ± 0.020^b	12.3±0.005
T_2	54.83 ± 0.14^b	4.57±0.025	6.17±0.025	74.08±0.19	0.48 ± 0.002^{bc}	6.73 ± 0.018^b	12.28±0.002
T_3	54.96 ± 0.14^b	4.58±0.027	6.18±0.022	74.10±0.25	0.49 ± 0.002^c	6.75 ± 0.014^b	12.29±0.007
T_4	53.82 ± 0.11^a	4.52±0.015	6.12±0.021	73.88±0.02	0.45 ± 0.005^a	6.61 ± 0.018^a	12.29±0.013
T_5	53.78 ± 0.12^a	4.51±0.012	6.11±0.03	73.79±0.17	0.44 ± 0.005^a	6.59 ± 0.012^a	12.26±0.01
Sig. level	$p<0.01$	NS	NS	NS	$p<0.01$	$p<0.01$	NS

Means bearing different superscripts within a column differ significantly ($p<0.05$); NS: Not significant

Table 5: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on external egg quality of Uttara chicken during overall period (20–52 weeks)

Treatment	Egg weight (g)	Egg width (cm)	Egg length (cm)	Shape index	Egg shell thickness (mm)	Egg shell weight (g)	Shell %
T_0	49.70 ± 0.12^a	4.04±0.013	5.45±0.023	74.22±0.068	0.42 ± 0.004^a	6.04 ± 0.010^a	12.14±0.023
T_1	50.39 ± 0.19^b	4.06±0.022	5.47±0.023	74.32±0.122	0.44 ± 0.003^b	6.14 ± 0.012^b	12.17±0.028
T_2	50.58 ± 0.21^b	4.07±0.022	5.48±0.023	74.40±0.113	$0.45 \pm 0.001b^c$	6.16 ± 0.017^b	12.17±0.023
T_3	50.69 ± 0.21^b	4.08±0.022	5.48±0.022	74.41±0.137	0.46 ± 0.002^c	6.17 ± 0.013^b	12.17±0.029
T_4	49.57 ± 0.10^a	4.03±0.015	5.44±0.020	74.23±0.013	0.42 ± 0.004^a	6.04 ± 0.009^a	12.17±0.023
T_5	49.52 ± 0.10^a	4.02±0.015	5.43±0.025	74.21±0.064	0.42 ± 0.004^a	6.02 ± 0.007^a	12.15±0.027
Sig. level	$p<0.01$	NS	NS	NS	$p<0.01$	$p<0.01$	NS

Means bearing different superscripts within a column differ significantly ($p<0.05$); NS: Not significant in agreement with the findings of egg weight which was improved during present experiment in Arjuna bark @1% and Sahjan leaf powder @ 3% supplemented group. N'nanle et al. (2020) reported that diet supplemented with MOL resulted in significant increase (p<0.01) in egg weight as compared to control. Antara et al. (2019) found that extract fermented *Moringa oleifera* by probiotics *Saccharomyces spp* (MLF) in drinking water treatment improved (p<0.05) total weight of eggs compared to control group. Ruelas et al. (2023) noticed that there was improvement in whole egg weight (p<0.05) when laying hens supplemented with MOL @ 2.5% and 4.5%. There was no significant change in egg width in present study. Abubakar et al. (2021) found that there was no significant difference in egg width between diet supplemented control group and group supplemented 2.5% *Moringa oleifera* leaf meal (MOLM) and between 2.5% *Moringa oleifera* and 2.5% Baobab leaf meal groups. There was also no significance difference found in egg length among treatment groups which is in agreement with Abubakar et al. (2021) as they found that there was no significant difference in egg length between diet supplemented with *Moringa oleifera* and Baobab leaf meal and diet of control group. In agreement with these results, Swain et al. (2017) found that there was no significant difference found in shape index among various treatment groups on dietary supplementation of *Moringa oleifera* leaf meal (MOLM). In another study conducted by Ebenebe et al. (2013) showed that supplementation of MOLM (2.5 to 7.5%) resulted in no significant difference (p>0.05) in egg shape index. Abou-Elkhair et al. (2020) examined that egg shape index did not significantly change on addition of *M. oleifera* seed powder in the diet. Ashour et al. (2020) reported no significant difference in egg shape index among the groups (p>0.05) in response to *M. oleifera* leaves in quail diet. Also, when diet supplemented with 0, 2, 4, 6% MOL resulted no significant difference in egg shape as reported by Bidura et al. (2020).

The egg shell thickness in phase I, phase II and overall period was significantly higher (p<0.01) in T_3 than T_0, T_4, T_5 and comparatively higher than T_1 and T_2 groups (Table 3, 4 and 5). Egg shell thickness is also depicted graphically in Figure 1. Egg shell thickness in present study was significantly higher in Arjuna bark @1% and Sahjan leaf powder @3% supplemented group. This result is agreed by Abou-Elkhair et al. (2020) in which they reported that there was higher (p<0.01) egg shell thickness on supplementation of *M. oleifera* seed powder in quail. Bidura et al. (2020) discovered that adding *Moringa leaves* leaf powder at 4 and 6% to diets resulted in a significant (p<0.05) increase in shell thickness of birds than the control. Siti et al. (2019) reported that that 4–6% the Moringa leaves powder in diet showed significant increase (p<0.05) in shell thickness. This could

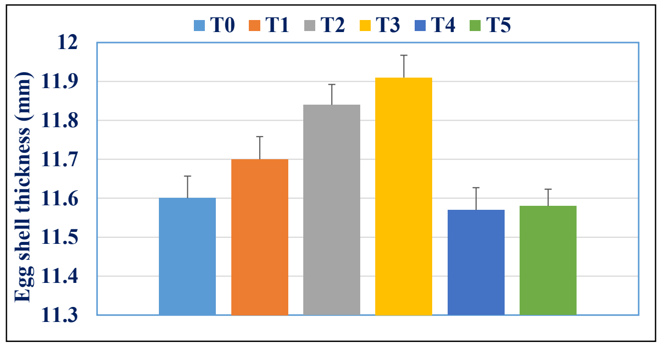

Figure 1: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on egg shell thickness (mm) of Uttara chicken during overall period (20–52 weeks)

be attributed to the presence of nutrients such as calcium, which is essential for proper eggshell formation. Arjuna bark and Moringa leaves both contains calcium, as well as other minerals like phosphorus and magnesium. Ruelas et al. (2023) noticed that MOL @ 2.5% resulted in higher (p<0.01) eggshell weight. Abubakar et al. (2021) reported that control and *Moringa oleifera* leaf meal (MOLM) @ 2.5% groups had lower shell weight (p<0.05) as compared to groups of 2.5% Baobab leaf meal (BLM) and group of 2.5% MOLM and 2.5% BLM of diet. Egg shell weight increased in present experiment might be due to its correlation with increased eggshell thickness.

It was noticed that T_3 had significantly higher (p<0.01) albumen height (mm) than T_1, T_0, T_4 and T_5 and comparatively higher than T_2 in phase I, phase II and overall period (Table 6, 7 and 8). There was no significant difference in albumen width (mm) in all phases of production. T_3 had significantly higher (p<0.01) albumen index than all other experimental groups in all phases of production. T_3 had significantly higher (p<0.01) haugh unit than T_0, T_4, T_5 and comparatively higher in T_1 and T_2 in phase I, phase II and overall period. T_3 followed by T_2 and T_1 groups had significantly higher albumen weight than (p<0.05) than T_0, T_4 and T_5 treatments in all phases of production. Albumen quality was improved in Arjuna bark @ 1% and Sahjan leaf powder @ 3% supplemented group in present experiment. The results are complementary with few other previous studies. Lu et al. (2016) reported that as level of MOL in diet increased the albumen height was also increased significantly (p<0.05) when eggs stored at 4°C and 28°C for 4 weeks. Lal et al. (2020) reported that albumen index was higher in 1% MLM-supplemented group compared to control and 0.25% MLM at 30 weeks of age. Lal et al. (2020) revealed that haugh unit was higher in 1% MLM–supplemented group compared to control and 0.25% MLM at 30 weeks of age. Lu et al. (2016) reported that as level of MOL in diet increased the haugh unit was also increased significantly (p<0.05) when eggs stored at 4°C and 28°C for 4

Table 6: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on internal egg quality of Uttara chicken during phase I (20–36 weeks)

Treatment	T_0	T_1	T_2	T_3	T_4	T_5	Sig. Level
Albumen height (mm)	6.48±0.034^{b}	6.69±0.028^{c}	6.97±0.031^{d}	7.07±0.040^{d}	6.32±0.023^{a}	6.3±0.040^{a}	p<0.01
Albumen width (mm)	72.36±0.197	72.46±0.205	72.51±0.196	72.55±0.216	72.34±0.188	72.32±0.191	NS
Yolk height (mm)	16.23±0.026	16.24±0.026	16.26±0.029	16.28±0.028	16.22±0.026	16.21±0.024	NS
Yolk width (mm)	41.9±0.023	41.91±0.026	41.94±0.026	41.94±0.032	41.89±0.023	41.88±0.02	NS
Albumen Index	8.96±0.023^{b}	9.23±0.013^{c}	9.62±0.018^{d}	9.74±0.027^{e}	8.73±0.009^{a}	8.71±0.034^{a}	p<0.01
Haugh unit	84.99±0.17^{b}	86.00±0.10^{c}	87.61±0.09^{d}	88.14±0.14^{e}	84.00±0.11^{a}	83.94±0.22^{a}	p<0.01
Yolk Index	38.73±0.04	38.75±0.03	38.78±0.04	38.81±0.09	38.71±0.04	38.7±0.04	NS
Albumen weight (g)	25.51±0.07^{a}	25.88±0.12^{b}	26.01±0.16^{b}	26.06±0.16^{b}	25.45±0.05^{a}	25.41±0.05^{a}	p<0.01
Yolk weight (g)	14.45±0.05^{ab}	14.66±0.07^{bc}	14.73±0.10^{c}	14.75±0.10^{c}	14.41±0.04^{ab}	14.39±0.04^{a}	p<0.01
Yolk colour	8.4±0.052^{a}	8.87±0.049^{b}	8.97±0.092^{b}	9.61±0.093^{c}	8.53±0.049^{a}	8.5±0.047^{a}	p<0.01

Means bearing different superscripts within a row differ significantly (p<0.05); NS: Not significant

Table 7: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on internal egg quality of Uttara chicken during phase II (36–52 weeks)

Treatment	T_0	T_1	T_2	T_3	T_4	T_5	Sig. Level
Albumen height (mm)	6.98±0.049^{a}	7.25±0.049^{b}	7.44±0.122^{b}	7.69±0.098^{c}	6.87±0.035^{a}	6.82±0.037^{a}	p<0.01
Albumen width (mm)	75.07±0.172	75.15±0.18	75.25±0.196	75.62±0.19	75.03±0.178	74.98±0.176	NS
Yolk height (mm)	17.04±0.056	17.07±0.057	17.1±0.063	17.12±0.06	17.01±0.049	16.97±0.047	NS
Yolk width (mm)	43.53±0.053	43.57±0.052	43.6±0.052	43.65±0.043	43.49±0.056	43.46±0.044	NS
Albumen index	9.30±0.044^{a}	9.65±0.042^{b}	9.88±0.138^{b}	10.16±0.143^{c}	9.16±0.025^{a}	9.09±0.029^{a}	p<0.01
Haugh unit	85.24±0.25^{a}	86.63±0.24^{b}	87.67±0.66^{b}	89.05±0.5^{c}	84.59±0.18^{a}	84.30±0.19a	p<0.01
Yolk index	39.14±0.08	39.19±0.08	39.23±0.09	39.22±0.10	39.11±0.07	39.05±0.08	NS
Albumen weight (g)	28.73±0.07^{a}	29.08±0.09^{b}	29.17±0.09^{b}	29.25±0.10^{b}	28.67±0.04^{a}	28.61±0.03^{a}	p<0.01
Yolk weight (g)	18.62±0.03^{a}	18.87±0.07^{b}	18.92±0.03^{b}	18.95±0.04^{b}	18.53±0.05^{a}	18.57±0.07^{a}	p<0.01
Yolk colour	8.80±0.087^{a}	9.21±0.038^{b}	9.45±0.029^{c}	9.78±0.05^{d}	8.75±0.085^{a}	8.71±0.081^{a}	p<0.01

Means bearing different superscripts within a row differ significantly (p<0.05); NS: Not significant

weeks. In contrast, Ashour et al. (2020) found no significant difference in haugh units among various treatment groups (p>0.05) in response to *M. oleifera* leaves (ML) and *M. oleifera* seeds (MS) and their combination in diet of quails. Bidura et al. (2020) reported that there was no significant difference found in haugh unit on diet supplementation with MOL powder @ 0, 2, 4, 6% of the diet. Ruelas et al. (2023) noticed that MOL @ 2.5% and 4.5% resulted in improved (p<0.01) egg albumen weight. Abou-Elkhair et al. (2020) reported there was higher albumen (%) (p<0.01) on supplementation of *M. oleifera* seed powder @ 0.1, 0.2, 0.3% of diet as compared to control diet in quail. In contrast, Bidura et al. (2020) found no significant difference (p>0.05) among groups in the albumen on supplementation of diet with 0, 2, 4, 6% MOL powder.

There was no significant difference reported in yolk height (mm), yolk width (mm) and yolk index in all phases of production (Table 6, 7 and 8). T_3 followed by T_1 and T_2 groups had significantly higher yolk weight than (p<0.05) than T_0, T_4 and T_5 treatments in all phases of production. The yolk colour score was significantly higher (p<0.01) in T_3 group than T_0, T_4, T_5 groups and comparatively higher than T_1, T_2 groups in all phases and overall production. Result indicated that there was no significant difference in yolk index in all phases of production in present study. Ashour et al. (2020) found yolk index was not affected by the dietary supplementation of *M. oleifera* leaves (ML) and *M. oleifera* seeds (MS) and their combination in quail. Ahmad et al. (2017) reported that egg yolk index remained unchanged on supplementation of *Moringa oleifera* (Lam.) pod meal in commercial layers. In present study significantly higher yolk weight was found in Arjuna bark @ 1% and Sahjan

Table 8: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on internal egg quality of Uttara chicken during overall period (20–52 weeks)

Treatment	T_0	T_1	T_2	T_3	T_4	T_5	Sig. Level
Albumen height (mm)	6.73±0.04^b	6.97±0.03^c	7.2±0.07^d	7.38±0.06^e	6.59±0.02^{ab}	6.56±0.03^a	p<0.01
Albumen width (mm)	73.71±0.18	73.81±0.19	73.88±0.19	74.08±0.1	73.68±0.18	73.65±0.18	NS
Yolk height (mm)	16.63±0.04	16.66±0.04	16.68±0.04	16.7±0.04	16.61±0.03	16.59±0.03	NS
Yolk width (mm)	42.71±0.038	42.74±0.039	42.77±0.039	42.79±0.018	42.69±0.039	42.67±0.032	NS
Albumen index	9.13±0.03^b	9.44±0.02^c	9.75±0.07^d	9.95±0.08^e	8.94±0.01^a	8.90±0.03^a	p<0.01
Haugh unit	85.12±0.21^b	86.31±0.17^c	87.64±0.37^d	88.59±0.32^e	84.3±0.14^a	84.12±0.20^a	p<0.01
Yolk index	38.94±0.06	38.97±0.06	39±0.07	39.01±0.09	38.91±0.05	38.88±0.05	NS
Albumen weight (g)	27.12±0.07^a	27.48±0.10^b	27.59±0.12^b	27.66±0.13^b	27.06±0.05^a	27.01±0.04^a	p<0.01
Yolk weight (g)	16.54±0.04^a	16.76±0.07^b	16.82±0.06^b	16.85±0.06^b	16.47±0.04^a	16.48±0.06^a	p<0.01
Yolk colour	8.6±0.069^a	9.04±0.006^b	9.21±0.031^c	9.69±0.021^d	8.64±0.067^a	8.60±0.064^a	p<0.01

Means bearing different superscripts within a row differ significantly (p<0.05); NS: Not significant

leaf powder @ 3% supplemented group. Result is supported by Abou-Elkhair et al. (2020) in which they had reported there was higher yolk (%) (p<0.01) on supplementation of *M. oleifera* seed powder @ 0.1, 0.2, 0.3% as compared to control diet in quail. In contrast, Ashour et al. (2020) found that the yolk percent was not affected by the dietary supplementation of *M. oleifera* leaves (ML) and *M. oleifera* seeds (MS) and their combination in quail. Yolk color score was higher in Arjuna bark @ 1% and Sahjan leaf powder @ 3% supplemented group in present study. Lu et al. (2016) reported that there was deeper yolk colour (p<0.05) in 5% MOL supplemented group than those in control group. Abou-Elezz et al. (2012) noticed that yolk colour was significantly higher in ad libitum feed supplemented with MOL than both control and restricted feed amount (20% lower than control) with MOL. Antara et al. (2019) reported that laying hen given drinking water with extract fermented *Moringa oleifera* by probiotics *Saccharomyces spp* (MLF) resulted in significant increase (p<0.05) in egg yolk colour as compared to control group. Shen et al. (2021) found that yolk colour was significantly higher (p<0.05) in *Moringa oleifera* leaf powder groups than in the control group and gradually stabilised over time. The yellow color increase might be due to the carotenoid content present in Moringa leaf powder.

3.3. *Egg composition*

Egg composition has been depicted in Table 9. There was

Table 9: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on egg composition (%) of Uttara chicken

Parameter	Period	T_0	T_1	T_2	T_3	T_4	T_5	Sig. level
Moisture	Phase I	74.5±0.16	74.43±0.14	74.42±0.16	74.39±0.16	74.50±0.18	74.52±0.19	NS
	Phase II	74.15±0.09	74.11±0.08	74.07±0.07	74.06±0.1	74.19±0.08	74.21±0.08	NS
	Overall	74.33±0.085	74.27±0.077	74.24±0.084	74.22±0.091	74.35±0.09	74.37±0.093	NS
Crude protein	Phase I	11.55±0.04^a	11.64±0.05^{ab}	11.75±0.06^{bc}	11.83±0.08^c	11.53±0.05^a	11.55±0.03^a	p<0.05
	Phase II	11.65±0.065^a	11.77±0.065^{ab}	11.93±0.038^{bc}	11.98±0.035^c	11.61±0.063^a	11.6±0.064^a	p<0.05
	Overall	11.60±0.057^a	11.70±0.058^{ab}	11.84±0.052^{bc}	11.91±0.057^c	11.57±0.057^a	11.58±0.043^a	p<0.05
Crude fat	Phase I	9.48±0.034	9.43±0.037	9.39±0.046	9.39±0.025	9.5±0.032	9.51±0.037	NS
	Phase II	9.37±0.053	9.36±0.071	9.34±0.071	9.32±0.071	9.39±0.059	9.41±0.048	NS
	Overall	9.42±0.043	9.39±0.051	9.37±0.056	9.35±0.023	9.44±0.044	9.46±0.042	NS
Total ash	Phase I	3.28±0.034	3.34±0.032	3.31±0.037	3.33±0.037	3.26±0.031	3.25±0.02	NS
	Phase II	3.36±0.032	3.39±0.034	3.41±0.037	3.44±0.04	3.34±0.023	3.33±0.02	NS
	Overall	3.32±0.027	3.36±0.03	3.36±0.031	3.39±0.032	3.3±0.022	3.29±0.016	NS

Means bearing different superscripts within a row differ significantly (p<0.05); NS: Not significant no significant difference in moisture (%) of egg. Result is agreed with Abubakar et al. (2021) who found that dietary treatments of *Moringa oleifera* and *Adansonia digitata* (Baobab) leaf meals did not affect (p>0.05) the moisture % of egg. Crude protein (%) of egg was significantly higher (p<0.05) in T_3 than T_0, T_4, T_5 and comparatively higher than T_1 and T_2 in phase I, phase II and overall period of production. Result is in agreement with a study conducted by Sharmin et al. (2021) in which they found that those birds supplemented 0.5 and 1.5% MOL had a higher crude protein content than control group. Ahmad et al. (2014) also reported that there was increase in crude protein content in egg on supplementation of *Moringa oleifera* (Lam.) pod meal.

There was no significant difference in ether extract (%) of egg in present study. This result is supported by Abubakar et al. (2021) as they found that dietary treatments of *Moringa oleifera* and *Adansonia digitata* (Baobab) leaf meals did not affect (p>0.05) the ether extract % of egg of layer. Total ash (%) of egg was also non-significant in present study. In the above mention study by Abubakar et al. (2021), they also found that dietary treatments of *Moringa oleifera* and *Adansonia digitata* (Baobab) leaf meals did not affect (p>0.05) the total ash % of egg of layer. Sharmin et al. (2021) found no significant change in crude ash content in yolk of egg when *Moringa oleifera* leaf was given in diet.

Egg yolk cholesterol has been presented in Table 10. Result indicated that egg yolk cholesterol was significantly lower (p<0.01) T_3 than T_0, T_1, T_4 and T_5 groups. There are few studies which are in agreement with our results. Bidura et al. (2020) reported that adding 2–6% Moringa leaf powder to diets significantly lowers yolk cholesterol levels. Antara et al. (2019) found that the egg yolk cholesterol was significantly decreased (p<0.05) on addition of extract fermented *Moringa oleifera* by probiotics *Saccharomyces* spp (MLF) in drinking water of laying hen. According to Patil et al. (2010), the reduction of cholesterol by alkaloids present is partly due to a decrease in lipogenic enzyme activity and a rise in bile acid excretion in the faeces. The ability of β-carotene to lower cholesterol is linked to the hydroxymethyl glutaril CoA enzyme. This enzyme contributes to the production of mevalonics during the biosynthesis of cholesterol. The mevalonic pathway, which is derived from acetyl CoA, allows for the simultaneous production of cholesterol and beta-carotene. If β-carotene consumption exceeds saturated fatty acid consumption, the HMG-CoA enzyme will guide the biosynthesis process to β-carotene synthesis, preventing saturated fatty acids from being converted to cholesterol (Adriani et al., 2017; Rao et al., 2003; Godinez–Ov et al., 2016). Mevalonic is required for the process of cholesterol production because it prevents the development of cholesterol by inhibiting enzymes (Syahruddin et al., 2013)

Table 10: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on egg yolk cholesterol (mg g^{-1}) of Uttara chicken at 52 weeks of age

Treatment	Egg yolk cholesterol (mg g^{-1})
T_0	9.01±0.011^{d}
T_1	8.74±0.011^{c}
T_2	8.21±0.014^{b}
T_3	8.02±0.014^{a}
T_4	9.02±0.011^{d}
T_5	9.02±0.012^{d}
Sig. level	p<0.01

Means bearing different superscripts within a column differ significantly (p<0.05)

4. CONCLUSION

The dietary supplementation of Arjuna bark powder @ 1% and Sahjan leaf powder @ 3% resulted in increased egg weight, shell thickness, shell weight, albumen height, albumen weight, albumen index, haugh unit, yolk weight, yolk colour, crude protein of egg and lowered egg yolk cholesterol of chicken.

5. FURTHER RESEARCH

Further studies may be done to determine the effect of Arjuna bark powder @1% and Sahjan leaf powder @3% of diet on expression of growth and immunity genes. Studies can be done on effect of Arjuna bark powder and Sahjan leaf powder on the performance of commercial layers.

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