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Effect of dietary supplementation of Arjuna (*Terminalia arjuna*) Bark and Sahjan (*Moringa oleifera*) leaf powder on haematology and Serum Biochemicals of Uttara chicken

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Abstract
Due to the high cost of animal feeds, researchers are concentrating their efforts on unconventional feed sources. A total of 72 female Uttara chicks were distributed into six groups, each of which had three replicates with four chicks 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). At 20 and 52 weeks, Hb and PCV were significantly greater (p<0.05) in the T_1, T_2 and T_3. Six laying hens from each treatment were randomly chosen at the 20 and 52 week and blood was collected. At 52 weeks, the T_1, T_2, T_3 groups had significantly increased TEC, MCH, and MCHC values (p<0.05). TLC and MCV readings did not differ significantly at 20 and 52 weeks of age. In T_3, T_2 and T_1, there were substantial reduction (p<0.01) in serum glucose, total cholesterol, LDL-cholesterol, and atherogenic index. At 20 and 52 weeks, HDL-cholesterol, total protein, albumin, and the A/G ratio were greater (p<0.01) in T_3 and T_2. However, uric acid levels in T_3, T_2 and T_1 at 52 weeks were considerably low (p<0.01). No discernible variation in globulin. Therefore, it can be concluded that chicken can be supplemented with Arjuna bark at 1% and Sahjan leaf powder at 3% for improved health without experiencing any negative effects.

Keywords: Hb, glucose, cholesterol, powder, Uric acid

1. Introduction
In developing nations, the poultry sector has a number of difficulties, one of which is the high cost of feed brought on by the high cost of protein and energy sources (Abbas, 2013) [1]. Costs associated with feeding livestock in developing nations remain a problem. According to Mahmood *et al*. (2005) [20], feed makes up 65–70% of the total cost of production for broilers and 75–80% for layers. The expensive costs of animal feeds have compelled researchers to concentrate their efforts on unconventional feed sources, with a particular focus on protein substitutes. According to Safameher *et al*. (2012) [29], the various plant materials utilised as feed additives may enhance the health and productivity of birds.Due to their ease of use, preference, cheaper production costs, decreased risk of toxicity, minimum health risks, and environmental friendliness, herbs are likely to be employed as feed supplements (Devegowda, 1996) [6]. One plant that hasn't been studied in many years, Moringa, is currently being looked into because of its quick growth, superior nutritional content, and expanding use as a feed for livestock fodder crop (Nouman *et al*., 2013) [25]. According to the Pharmacopoeia of India (1955) and Vaidya (1994) [31], *Terminalia arjuna* L. is widely used as a heart tonic in Indian medicine. The flavonoids/phenolics component of *T. arjuna* is thought to give antioxidant activity as well as vascular amplification activity, hence verifying this plant's various actions for its cardio-protective function. The saponin glycosides in *T. arjuna* are thought to be responsible for its inotropic effects (Kapoor *et al*., 2014; Maulik and Talwar, 2012; Dwivedi, 2007) [17, 21, 7]. Since commercial poultry farming is only permitted in urban and semi-urban areas, the majority of India's population still lives in rural regions and has access to only 25% of all chicken products. According to Gumpha *et al*. (2018) [13], the number of chickens in rural areas increased somewhat over the past 40 years, from 63 million to 75 million.

Adapting poultry farming in rural areas is one way to increase the availability of poultry products and boost the financial well-being of rural residents because these breeds of chicken require little in the way of management and nutrition inputs while performing better. Prices for poultry products are 10–14% higher in rural locations than they are in urban and semi-urban ones. By establishing small-scale chicken farming in backyards or rural families, protein deficiencies can be decreased in vulnerable groups like children, women, pregnant women, and the elderly. The Kumaon region of Uttarakhand is home to the Uttara chicken, which is more resistant to disease than exotics there. Additionally resilient to the problems of severe winters and thriving in harsh conditions such as poor housing, bad management and poor food. Uttara chicken is popular among marginal farmers, tribals, and others in Uttarakhand's hilly regions and is raised using traditional methods. In order to assess an animal's health, haematological measures are routinely examined. Haematological parameters have been found to be good markers of an animal's physiological status, and their variations are significant in measuring the animal's response to diverse physiological settings (Khan and Zafar, 2005) [18]. This study sought to determine how adding Arjuna bark and Sahjan leaf powder to the diet influenced the haematological and serum biochemical parameters of Uttara chicken.

2. Materials and Methods

2.1 Collection of Arjuna (*Terminalia arjuna*) bark and Sahjan (*Moringa oleifera*) leaf

The nearby areas of Pantnagar were used to obtain Sahjan leaves and Arjuna bark. To prevent nutrient loss, Arjuna bark and Sahjan leaves were shade-dried and then processed into a fine powder using an electric grinder.

2.2 Location

In the foothills of the Himalayas at 29°N latitude, 79.3°E longitude, and 243.8 m above mean sea level, the experiment was carried out at the Instructional Poultry Farm (IPF), Nagla, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar. This area experiences frigid winters and hot, humid summers due to its subtropical climate. The hottest temperature throughout the summer time is 42 °C.

2.3 Experimental bird and design

After being sexed, 72 female Uttara breed chicks were reared for 52 weeks at the instructional poultry farm of the Pantnagar. Each bird was weighed separately before being divided into six distinct treatment groups, each of which contained three replicates and four chicks each. According to the guidelines of BIS (2007), standard chicken diets were made by combining the ingredients to suit the nutrient needs of layer chicken during the chick (0–8 weeks), grower (9–20 weeks), and layer (21–52 weeks) phases of growth. Experiment was divided into different treatment groups as T_0 (Control, basal diet), T_1 (Basal diet + 1% Arjuna bark powder + 1% Sahjan leaf powder), T_2 (Basal diet + 1% Arjuna bark powder + 2% Sahjan leaf powder), T_3 (Basal diet + 1% Arjuna bark powder + 3% Sahjan leaf powder), T_4 (Basal diet + 1% Arjuna bark powder + 4% Sahjan leaf powder) and T_5 (Basal diet + 1% Arjuna bark powder + 5% Sahjan leaf powder).

2.4 Housing of birds

The experiment's birds were kept in deep litter systems. The bedding material was made of dried, fresh wheat straw. The day-old chicks were weighed and provided individual wing bands for identification. During the brooding phase, adequate lighting and ventilation were provided. Daily feeding of the weighed amount of feed was made, and unlimited access to clean, fresh water was guaranteed. For the first eight weeks, a record of each bird's weekly body weight and remaining feed was kept. They were moved to open-sided housing with a deep litter system after an 8-week brooder phase. From 10 to 20 weeks of age, measurements of each bird's biweekly body weight and residual feed were made.

2.5 Hematological analyses

Six laying hens from each treatment were randomly chosen at the 20 and 52 week and had their blood collected. Standard methods were used to analyse the blood samples that had been drawn and placed in anticoagulant vials for haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

2.6 Biochemical analyses

At 20 and 52 weeks, wing vein blood was taken. Collected blood was centrifuged at 3000 rpm for 10 minutes to extract the serum, which was then preserved for further examination of the following parameters: total protein, albumin, A/G ratio (albumin/globulin ratio), uric acid, glucose, cholesterol, HDL, LDL, and atherogenic index (LDL/HDL) (Conkbayir *et al*., 2015) [5].

2.7 Statistical analysis

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

3. Results and Discussion

3.1 Hematological parameters

Table 1 shows the haematological parameters of Uttara chickens at 20 weeks of age in various treatment groups, and Table 2 shows the parameters at 52 weeks of age. At 20 weeks, it was discovered that the T_1, T_2, T_3 groups had significantly greater ($p<0.05$) Hb (g/dl) and PCV (%) values than the other groups. At 20 weeks of age, there was not a significant difference in the TEC ($\times 10^6$/µl), TLC ($\times 10^3$/µl), MCV (fl), MCH (pg), MCHC (%) values across the groups. Hb (g/dl), PCV (%) and TEC ($\times 10^6$/µl) were all substantially greater ($p<0.01$) in the T_1, T_2, T_3 than in the other groups at 52 weeks of age. When compared to other groups, MCH (pg) and MCHC (%) were substantially greater ($p<0.01$) in the T_1, T_2, T_3 groups. At 52 weeks of age, there was no significant change in the TLC ($\times 10^3$/µl) and MCV (fl) values across the treatment groups. Our findings are consistent with a few earlier investigations. When compared to hyperlipidemic rats, *Terminalia arjuna* administration caused a significant ($p<0.05$) elevation in PCV value, according to Mojarradgandoukmolla *et al*. (2021) [23]. Hussein and Jassim (2019) [14] looked at the values of red blood cell (RBC), packed cell volume (PCV %), and haemoglobin (Hb) in all groups given *Moringa oleifera* and found that they were all significantly higher ($p<0.05$). Elbashier and Ahmed (2016) [8] found an increase in RBC, PCV, and Hb levels. Higher levels

of these indicators could be the result of consuming *Moringa oleifera* leaves, which are rich in nutrients including protein and minerals. The PCV and RBC of the birds varied significantly (p<0.05) between groups, according to Onu and Aniebo (2011) [26]. The *Moringa oleifera* leaf contains Fe (23 mg/100 g), which is necessary for several bodily functions which is required for the synthesis of myoglobin and haemoglobin. Animals can digest fibrous food due to a natural enzyme discovered in *Moringa oleifera* (Gaia, 2010) [10].

Table 1: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on haematological parameters of Uttara chicken at 20 weeks of age

Treatment	Hb (g/dl)	PCV (%)	TEC (×10^6/µl)	TLC (×10^3/µl)	MCV (fl)	MCH (pg)	MCHC (%)
T_0	8.68±0.049^a	27.73±0.06^a	2.70±0.049	22.28±0.073	102.64±1.64	32.11±0.40	31.29±0.11
T_1	9.10±0.079^b	29.01±0.13^b	2.82±0.058	22.36±0.075	102.81±1.65	32.26±0.39	31.38±0.13
T_2	9.27±0.110^{bc}	29.43±0.23^{bc}	2.86±0.065	22.39±0.078	102.84±1.64	32.39±0.39	31.50±0.13
T_3	9.43±0.118^c	29.77±0.35^c	2.89±0.073	22.23±0.074	102.98±1.57	32.62±0.37	31.68±0.03
T_4	8.61±0.062^a	27.55±0.12^a	2.69±0.055	22.36±0.065	102.62±1.65	32.06±0.43	31.25±0.09
T_5	8.55±0.061^a	27.39±0.11^a	2.67±0.052	22.32±0.021	102.53±1.64	31.99±0.39	31.19±0.12
Sig. Level	p<0.01	p<0.01	NS	NS	NS	NS	NS

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

Table 2: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on haematological parameters of Uttara chicken at 52 weeks of age

Treatment	Hb (g/dl)	PCV (%)	TEC (×10^6/µl)	TLC (×10^3/µl)	MCV (fl)	MCH (pg)	MCHC (%)
T_0	9.31±0.067^b	28.63±0.055^b	2.81±0.055^{ab}	22.74±0.079	102.08±1.81	33.19±0.42^b	32.52±0.17^a
T_1	10.17±0.087^c	30.12±0.061^c	2.93±0.061^{abc}	22.71±0.081	102.99±1.93	34.76±0.43^a	33.76±0.22^b
T_2	10.43±0.089^d	30.57±0.067^d	2.95±0.060^{bc}	22.75±0.084	103.59±1.85	35.34±0.44^a	34.12±0.22^b
T_3	10.58±0.092^d	30.92±0.127^e	2.99±0.065^c	22.71±0.044	103.61±1.82	35.46±0.47^a	34.23±0.16^b
T_4	8.84±0.064^a	27.00±0.044^a	2.76±0.038^a	22.76±0.087	97.86±1.18	32.05±0.21^b	32.75±0.19^a
T_5	8.83±0.061^a	26.99±0.041^a	2.75±0.032^a	22.75±0.085	98.16±0.99	32.11±0.15^b	32.72±0.18^a
Sig. Level	p<0.01	p<0.01	p<0.05	NS	NS	p<0.01	p<0.01

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

3.2 Biochemical parameters

Table 3 shows the biochemical parameters of Uttara chickens at 20 weeks of age in various treatment groups, and Table 4 shows the biochemical parameters at 52 weeks of age. The groups taking T_3, T_2 and T_1 supplements had significantly lower (p<0.01) glucose (mg/dl), total cholesterol (mg/dl), LDL-cholesterol (mg/dl), and atherogenic index values than the other groups at 20 weeks of age. The T_3 and T_2 supplemented group significantly exceeded (p<0.01) the other groups in terms of HDL-cholesterol (mg/dl) (P 0.01). Total protein (g/dl) and albumin (g/dl) were significantly greater (p<0.01) in T_3, T_2 and T_1 group over the other groups. At 20 weeks of age, there was no difference in globulin (g/dl) or uric acid (mg/dl) readings between the groups. The T3, T2, and T1 supplemented group significantly outperformed (p<0.01) the other groups in terms of A/G Ratio. Similar results were also found after 52 weeks. But when compared to other groups, uric acid levels in T_3, T_2 and T_1 supplemented group were significantly lower (p<0.01). Additionally, there was no discernible variation in globulin (g/dl) between various treatments. Other parameters were considerably different at 52 weeks of age. Our findings are consistent with a few earlier investigations. According to Voemesse *et al*. (2018), birds administered MOLM had significantly greater (p<0.05) levels of total protein and albumin than controls. According to Asha and Taju (2011) [3], *Terminalia arjuna* bark extract (6.75 mg/kg body weight) significantly increased HDL cholesterol levels while lowering serum total cholesterol, triglycerides, LDL cholesterol, and VLDL cholesterol. (Shaila *et al*., 1997) [30] reported that *Terminalia arjuna* has a hypolipidemic impact on rabbits with chow-induced hyperlipidemia. According to researchers, the presence of plant sterols, primarily beta-sitosterol, may have a role in the hypolipidemia and heart-protective effects (Becker *et al*., 1993) [4]. According to Gayathri *et al*. (2020) [11], dietary supplementation with MLM (moringa leaf meal) raised blood protein and albumin concentrations, but had no effect on serum globulin concentration. Comparing the 1% level of MLM dietary supplementation to either the lower level of supplementation or the control group, the blood creatinine and uric acid levels were significantly lower. According to Ahmad *et al*. (2017) [2], the addition of MLM to the feed of laying hens resulted in a greater blood protein concentration. They also saw a drop in blood total cholesterol as a result of including moringa pod meal (0.5–1.5%) in the diet. Similar to this, Riry *et al*. (2018) [28] observed higher total protein and lower A/G ratio in laying hens after adding MLM up to 7% to the diet. According to Mousa *et al*. (2017) [24], there was a significant rise in total protein value in both the *Moringa oleifera* plus *Cichorium intybus* and Moringa groups. However, there was a significant difference (p≤0.05) in serum total cholesterol values among groups and there was a lower value for triglycerides in the 0.75% *Moringa oleifera* plus *Cichorium intybus* powder supplemented group.

The LDL of birds decreased with the addition of Moringa due to the presence of numerous phytochemicals in *M. oleifera*. Several compounds with antioxidant, antibacterial, and anticancer activities are believed to be present in Moringa (Win and Jongen, 1996; Fahey, 2005; Mekonnen and Dräger, 2003) [15, 9, 22]. Similarly, *M. oleifera* extract displayed hypocholesterolemic properties, which were explained by the presence of B-sitosterol in Moringa (Ghasi *et al*., 2000; Kane and Malloy, 1982) [12, 16]. Additionally, Luqman *et al*. (2012) [19] confirmed the polyphenols and flavonoids in *M. oleifera* extract have antioxidant effects.

Table 3: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on serum biochemical parameters of Uttara chicken at 20 weeks of age

Parameters	Treatments						Sig. Level
	T_0	T_1	T_2	T_3	T_4	T_5	
Glucose (mg/dl)	228.76±0.86^{d}	224.98±1.18^{c}	216.84±1.34^{b}	202.69±1.44^{a}	229.55±0.67^{d}	229.65±0.56^{d}	p<0.01
Cholesterol (mg/dl)	162.29±1.46^{d}	153.77±1.51^{c}	148.34±1.40^{b}	142.80±1.64^{a}	161.77±1.69^{d}	161.87±1.62^{d}	p<0.01
HDL Cholesterol (mg/dl)	110.21±1.12^{a}	113.93±0.76^{b}	116.15±0.74^{bc}	117.59±0.69^{c}	109.16±1.21^{a}	109.21±1.12^{a}	p<0.01
LDL Cholesterol (mg/dl)	51.11±0.54^{c}	44.55±1.29^{b}	39.24±0.64^{a}	37.05±0.79^{a}	52.40±0.79^{c}	53.41±0.81^{c}	p<0.01
Atherogenic Index	0.46±0.001^{d}	0.39±0.011^{c}	0.34±0.006^{b}	0.32±0.007^{a}	0.48±0.003^{de}	0.49±0.002^{e}	p<0.01
Total Protein (g/dl)	4.58±0.026^{b}	4.93±0.019^{c}	5.13±0.021^{d}	5.19±0.019^{d}	4.51±0.018^{a}	4.51±0.019^{a}	p<0.01
Albumin (g/dl)	1.55±0.019^{a}	1.65±0.013^{b}	1.71±0.010^{c}	1.74±0.018^{c}	1.55±0.011^{a}	1.53±0.013^{a}	p<0.01
Globulin (g/dl)	2.36±0.023	2.38±0.024	2.39±0.022	2.41±0.022	2.35±0.018	2.34±0.015	NS
A/G Ratio	0.66±0.013^{a}	0.69±0.011^{b}	0.71±0.007^{b}	0.72±0.006^{b}	0.66±0.008^{a}	0.65±0.009^{a}	p<0.01
Uric Acid (mg/dl)	4.94±0.019	4.93±0.023	4.93±0.023	4.92±0.025	4.95±0.019	4.95±0.018	NS

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

Table 4: Effect of dietary supplementation of Arjuna bark and Sahjan leaf powder on serum biochemical parameters of Uttara chicken at 52 weeks of age

Parameters	Treatments						Sig. Level
	T_0	T_1	T_2	T_3	T_4	T_5	
Glucose (mg/dl)	238.28±0.83^{d}	230.18±1.22^{c}	223.21±1.19^{b}	205.55±1.83^{a}	240.15±0.55^{d}	239.98±0.45^{d}	p<0.01
Cholesterol (mg/dl)	153.79±1.76^{d}	142.79±1.28^{c}	132.71±0.98^{b}	126.71±1.09^{a}	153.06±1.53^{d}	153.22±1.52^{d}	p<0.01
HDL Cholesterol (mg/dl)	106.86±0.89^{a}	108.64±0.97^{a}	112.04±0.87^{b}	112.96±0.88^{b}	106.67±0.90^{a}	105.94±0.91^{a}	p<0.01
LDL Cholesterol (mg/dl)	48.26±0.61^{d}	42.54±0.63^{c}	36.75±0.59^{b}	33.41±1.07^{a}	49.49±0.67^{de}	50.84±0.81^{e}	p<0.01
Atherogenic Index	0.45±0.003^{d}	0.39±0.006^{c}	0.33±0.005^{b}	0.29±0.009^{a}	0.46±0.003^{de}	0.48±0.004^{e}	p<0.01
Total Protein (g/dl)	4.65±0.035^{a}	5.01±0.035^{b}	5.19±0.059^{c}	5.29±0.034^{c}	4.60±0.033^{a}	4.56±0.029^{a}	p<0.01
Albumin (g/dl)	1.60±0.031^{a}	1.7±0.027^{b}	1.87±0.047^{bc}	1.94±0.043^{c}	1.55±0.029^{a}	1.52±0.027^{a}	p<0.01
Globulin (g/dl)	2.38±0.026	2.40±0.031	2.43±0.027	2.45±0.025	2.37±0.023	2.35±0.0242	NS
A/G Ratio	0.67±0.014^{a}	0.73±0.014^{b}	0.77±0.022^{bc}	0.79±0.019^{c}	0.65±0.010^{a}	0.65±0.009^{a}	p<0.01
Uric Acid (mg/dl)	4.84±0.021^{cd}	4.79±0.025^{bc}	4.75±0.023^{ab}	4.71±0.023^{a}	4.85±0.021^{cd}	4.88±0.016^{d}	p<0.01

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

4. Conclusion

Dietary supplementation of Arjuna (*Terminalia arjuna*) bark @ 1% and Sahjan (*Moringa oleifera*) leaf powder @ 3% led to increase in Hb, PCV and total erythrocyte count in blood. Dietary supplementation of Arjuna (*Terminalia arjuna*) bark @ 1% and Sahjan (*Moringa oleifera*) leaf powder @ 3% resulted in significant decrease in serum glucose, cholesterol, LDL-Cholesterol, Uric acid and significant increase in HDL-cholesterol, total protein, albumin and A/G ratio. Thus, Arjuna (*Terminalia arjuna*) bark @ 1% and Sahjan (*Moringa oleifera*) leaf powder @ 3% may be supplemented to chicken for improvement of health without any adverse effect.

5. Acknowledgment

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6. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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