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## Effect of amla, ashwagandha, turmeric, *Aloe vera* and giloy based (Sreeni herbal and Vasanthu Sanjeevani Plus) herbal supplementation on serum protein, uric acid, creatine, cholesterol and breast meat composition of broilers

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### Abstract

The aim of this experiment was to study the effect supplementation of Sreeni herbal (SH) and Vasanthu Sanjeevani plus (VSP) on serum biochemical profile and breast meat composition of broiler chicken. For this, 400-day old broiler chicks individually weighed and randomly distributed into 5 dietary treatments with 16 replicates per treatment and each replicate had 5 birds. The first group was kept as a control (T<sub>1</sub>) and given the basal diet without antibiotic, second group (T<sub>2</sub>) was fed T<sub>1</sub> with antibiotic, T<sub>3</sub> BD + SH Solid gel form thoroughly mixed in feed, T<sub>4</sub>-BD + VSP Liquid gel form added to water and T<sub>5</sub> BD + VSP + SH (Moring Sreeni Herbal in feed and evening VSP in water). It was observed that supplementation of all herbal groups (T<sub>3</sub> to T<sub>5</sub>) significantly lowered the total serum cholesterol (mg/dL) concentration, triglycerides, LDL-C and VLDL-C, uric acid, creatinine levels and increased the serum total protein, albumin and serum HDL-C concentration compared to control and antibiotic groups. Moisture percentage, dry matter (%), crude fat (%), crude fiber (%), total ash (%) and NFE (%) of breast muscle did not show any significant ( $p>0.05$ ) difference among different treatments, while crude protein (%) content of breast muscle was increased significantly in sreeni herbal (T<sub>3</sub>), vasanthu sanjeevani plus (T<sub>4</sub>) and sanjeevani + sreeni combination (T<sub>5</sub>) groups when compared to control and antibiotic groups. Hence, amla, ashwagandha, turmeric, *Aloe vera* and giloy based herbal mixtures (sreeni herbal and vasanthu sanjeevani plus) may be used to reduce serum cholesterol and increase breast muscle protein of broilers.

**Keywords:** Broilers, Sreeni herbal, Sanjeevani plus, cholesterol, breast muscle, uric acid

### 1. Introduction

Herbal feed additives are plant derived products which are also known as phytochemicals or phytochemicals added to feed in order to improve animal performance. They encompass a wide range of substances further classified as herbs, spices, essential oils, oleoresins etc., according to botanical origin, processing and composition. Use of plant derivatives in the animal feed industry has been in the rise due to their GRAS (generally recognized as safe) status (Over *et al.*, 2010) [19]. Some of the most commonly used plant or plant derivatives as feed additives in poultry industry are turmeric, amla, giloy, ginger, cinnamon, coriander seeds etc. Turmeric, ashwagandha, amla, *Aloe vera* and giloy have wide variety of bio active compounds like polyphenols, sesquiterpenes, diterpenes, triterpenoids, sterols and alkaloids. Curcumin, one of the major phytochemical components of turmeric have been reported to stimulate the digestive enzymes of the pancreas (Platel and Srinivasan, 2004) [20], inhibition of enzyme responsible for cholesterol synthesis in the liver i.e. hepatic-3-hydroxyl-3-methylglutaryl CoA reductase (Al-Kassie *et al.*, 2011) [3]. supplementation of amla in broilers decreased the serum total lipids, cholesterol, uric acid and creatinine (Abo *et al.* 2023) [1]. Sreeni herbal (SH) and Vasanthu Sanjeevani plus-VSP is a unique blend of natural herbs which consists of ashwagandha, turmeric, *Aloe vera*, giloy, amla, tulasi, fenugreek, bhringraj,

karanja, cinnamon etc. Sreeni Herbal & Vasanthu Sanjeevani Plus are ammonia free, chemical free and known to improve the performance of poultry.

## 2. Materials and Methods

The study was conducted for a period of 6 weeks in the Poultry Experimental Station, Department of Poultry Science, College of Veterinary Science, Rajendranagar, Hyderabad. To conduct the proposed study, 400 day-old commercial (Ven cobb) broiler chicks were procured, wing

banded and individually weighed. The birds were categorized into different class intervals according to body weights. 16 replicate groups were randomly allotted to one of the 5 treatment groups. The chicks were reared in battery brooders under standard managerial conditions and fed with iso caloric and iso nitrogenous corn soya-based diets from day old to 42 d of age. Growth trial was conducted in a completely randomized design comprising 5 dietary treatments; each with 16 replicates having 5 chicks in each. The five diets were given in Table 1

**Table 1:** Dietary treatment groups

Groups	Group Details	Dose and route
T <sub>1</sub>	Basal diet (BD) without additive	---
T <sub>2</sub>	BD + Antibiotic	BMD @ 500 gm/ton in feed
T <sub>3</sub>	BD + Sreeni Herbal Solid gel form	<b>Direction for use: with feed Pre-Mix</b> Day 1. Morning: 25 gms/100 chicks: Evening: 25 gm/100 chicks Day 7. Mor: 50 gms/100 chicks: Eve.50 gms/100 chicks Day 14. Mor: 75 gms/100 chicks. Eve.75 gms/100 chicks Day 21. Mor: 120 gms/100 chicks; Eve.120 gms/100 chicks Day 28.Mor: 150 gms/100 chicks. Eve.150 gms/100 chicks Day 35. Mor: 200 gms/100 chicks.Eve.200 gms/100 chicks. Till slaughter.
T <sub>4</sub>	BD + Vasanthu Sanjeevani plus-VSP Liquid gel form	<b>Direction for use : with water</b> Day 1-20 ml/100 chicks Day 7-30 ml/100 chicks Day 14-45 ml/100 chicks Day 21-65 ml/100 chicks Day 28-90 ml/100 chicks Day 35-120 ml/100 chicks
T <sub>5</sub>	BD + Vasanthu Sanjeevani plus + Sreeni Herbal	Moring sreeni herbal in feed (Half of the dose of T <sub>3</sub> ) and evening Sanjeevani plus in water (Half of the dose of T <sub>4</sub> )

### 2.1 Blood Collection and Serum Biochemistry

On 42<sup>nd</sup> day, blood samples were collected from one bird from each replicate. Blood samples were collected aseptically from wing vein with the help of sterilized needles and placed in a clean sterilized vacutainer and kept in incubator at room temperature for serum collection. Further, blood samples were centrifuged at 3000 rpm for 5 minutes to separate the serum and serum was transferred to labeled 5 ml Eppendorf tubes which were stored at -20 °C until analysis. The serum is used for estimation of cholesterol, total protein, albumin, by using spectrophotometer with commercially available kits (ERBA diagnostic Mannheim-gmbh transasia bio-medicals Limited) and triglycerides, LDL, HDL, VLDL, uric acid and creatinine by using M/s. Span Diagnostics Private Limited.

### 2.2 Serum Biochemical Constituents

**2.2.1 Total Protein (g/dL):** Total protein in serum was estimated by Biuret Method (Reinhold, 1953) [21] using a commercial kit in spectrophotometer (Metstar MUV-61 PCS UV Double Beam). In this method, proteins bind with copper ions in the alkaline medium of the biuret reagent and produce a blue colored complex, whose absorbance is proportional to the protein concentration when read at 550 nm.

**Procedure:** To the test tubes labeled as Blank (B), Standard (S), and Test (T) and the reagents were added as follows.

Addition sequence	Blank (ml)	Standard (ml)	Test (ml)
Biuret Reagent	1.0	1.0	1.0
Distilled water	0.02	-	-
Protein standard	-	0.02	-
Serum sample	-	-	0.02

The test tubes were mixed well in vortex mixer and incubated at room temperature for 20 minutes. Absorbance (Abs) of T and S, against B was measured in a spectrophotometer (Metstar MUV-61 PCS UV Double Beam) at 550 nm. Total protein concentration in serum was calculated as

$$\text{Total protein (g/dl)} = \frac{\text{Abs of test sample}}{\text{Abs of standard}} \times 8$$

**2.2.2 Cholesterol (mg/dL):** The enzyme cholesterol esterase was used to hydrolyze the cholesterol esters present in the serum to free cholesterol and free fatty acids. The enzyme cholesterol oxidase in the presence of oxygen, oxidizes the cholesterol to cholest-4-en-3 one and hydrogen peroxide. Hydrogen peroxide oxidizes phenol and 4-aminoantipyrine to produce red color that can be measured spectrophotometrically at 505 nm (Allain *et al.*, 1974) [4]. Commercial kit supplied by M/s. Coral Clinical Systems, Goa was used for analysis in spectrophotometer (Metstar MUV-61 PCS UV Double Beam).

**Procedure:** The reagent was added to tubes labeled as Blank (B), Standard (S), and Test (T) in the following sequence

Addition sequence	Blank (ml)	Standard (ml)	Test (ml)
Working Reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Cholesterol standard	-	0.01	-
Serum sample	-	-	0.01

The tubes were mixed well, incubated at room temperature for 5 minutes and optical density of colour developed was read at 505 nm in spectrophotometer (Metstar MUV-61 PCS UV Double Beam). The absorbance of the Standard and Test was measured against the Blank, within 60 minutes of colour development. Cholesterol concentration in serum was calculated as.

$$\text{Cholesterol (mg/dl)} = \frac{\text{Abs of test sample}}{\text{Abs of standard}} \times 200$$

**2.2.3 Albumin (g/dL):** Bromocresol green (BCG) method (Doumas *et al.*, 1971) [12] was used to estimate albumin content of serum in which albumin in the presence of BCG under acidic medium produces a green coloured complex whose absorbance is proportional to the albumin concentration when read at 630 nm. Commercial kit supplied by M/s. Coral Clinical Systems, Goa was used for analysis in spectrophotometer (Metstar MUV-61 PCS UV Double Beam).

**Procedure:** To the test tubes labeled as Blank (B), Standard (S), and Test (T) and the reagents were added as follows

Addition sequence	Blank (ml)	Standard (ml)	Test (ml)
BCG Reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Albumin standard	-	0.01	-
Serum sample	-	-	0.01

The test tubes were mixed well in vertex mixer and incubated at room temperature for 20 minutes. Absorbance (Abs) of T and S, against B was measured in a spectrophotometer (Metstar MUV-61 PCS UV Double Beam) at 630 nm. Albumin concentration in serum was calculated as.

$$\text{Albumin (g/dl)} = \frac{\text{Abs of test sample}}{\text{Abs of standard}} \times 4$$

### 2.3 Proximate Analysis

The proximate analysis of meat samples was performed as per the procedures described by AOAC (2012) [7].

### 2.4 Statistical Analysis

The data were analyzed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS, version 20) by one way analysis of variance and comparison of means was done using Duncan's multiple range test and significance was considered at  $p < 0.05$ . All the statistical procedures were carried out as per the procedures of Snedecor and Cochran (1989) [25] by programming and processing in computer.

## 3. Results and Discussion

### 3.1 Total protein, albumin, uric acid and creatine

The data on serum biochemicals parameters like serum total protein, albumin, globulin, and uric acid and creatine were presented in Table 2. The serum total protein content ranged from 3.56 to 4.22 g/dl among different treatments. The serum total protein and albumin content was significantly higher in diets supplemented with sreeni herbal (T<sub>3</sub>), sanjeevani plus (T<sub>4</sub>) and sanjeevani + sreeni combination (T<sub>5</sub>) groups compared to control and antibiotic groups.

Abou *et al.* (2014) [1] found significant increase in total protein and globulin in the broiler serum sample supplemented with black pepper, turmeric and coriander seeds mixture. Chaudhary *et al.* (2014) [10], Singh *et al.* (2018) [24] and Almayali and Alshukri (2021) [5] in their investigations found significantly increased serum total protein content and Ahsan *et al.* (2022) [2] observed significant increase in serum albumin content in broilers supplemented with turmeric. Khetmalis *et al.* (2018) [17] and Bharambe and Garde (2020) [9] studied the effect of amla and giloy respectively and stated that there was significant improvement in serum total protein, albumin and globulin content of broilers. Liver functional status is assessed by serum proteins and various enzymes secreted from hepatocytes into the blood circulation. Broilers due to their rapid growth undergo a lot of physiological stress which results in decreased serum protein and increase in enzymes which are required to remove or neutralize the oxidative ions or radicals. Increase in serum total protein and albumin indicates hepatoprotective and antioxidant property of the chosen botanical feed additives for the investigation i.e. turmeric, amla and giloy.

The serum creatinine content ranged from 1.14 to 1.35 mg/dl among different treatments. The serum creatinine content decreased significantly ( $p < 0.01$ ) in sanjeevani plus (T<sub>4</sub>), sanjeevani + sreeni combination (T<sub>5</sub>) groups followed by sreeni herbal (T<sub>3</sub>) compared to control and antibiotic groups. Supplementation of sreeni herbal (T<sub>3</sub>), sanjeevani plus (T<sub>4</sub>) and sanjeevani + sreeni combination (T<sub>5</sub>) groups significantly reduced the serum uric acid levels compared to control and antibiotic groups. Almayali and Alshukri (2021) [5] observed significantly reduced uric acid levels in the serum of broilers supplemented with turmeric powder. Abo *et al.* (2023) [1] revealed that there was significant reduction in uric acid and creatinine levels in the birds serum sample supplemented with amla extract.

Significantly reduced uric acid and creatinine levels in serum samples of herbal feed additives supplemented birds than control might be attributed to nephrotoxicity modulation (Malik *et al.*, 2016) [18] and hepatoprotective effect of amla (Huang *et al.*, 2017) [14] and turmeric and tinosporin present in giloy is a natural diuretic agent which aid in the excretion of serum uric acid (Goel *et al.*, 2014) [13] likewise amla also contain diuretic property.

**Table 2:** Effect of sanjeevani plus and sreeni herbal supplementation on total protein, albumin, uric acid and creatine levels of broiler chicken

S. No	Diets	Total Protein (g/dL)	Albumin (g/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
T <sub>1</sub>	Control	3.63 <sup>b</sup>	1.83 <sup>c</sup>	1.61 <sup>c</sup>	1.35 <sup>d</sup>
T <sub>2</sub>	Antibiotic	3.56 <sup>b</sup>	1.97 <sup>b</sup>	1.50 <sup>b</sup>	1.24 <sup>c</sup>
T <sub>3</sub>	Sreeni Herbal	4.22 <sup>a</sup>	2.15 <sup>a</sup>	1.15 <sup>a</sup>	1.18 <sup>b</sup>
T <sub>4</sub>	Sanjeevani plus	4.13 <sup>a</sup>	2.20 <sup>a</sup>	1.11 <sup>a</sup>	1.14 <sup>a</sup>
T <sub>5</sub>	Sanjeevani plus + Sreeni Herbal	4.11 <sup>a</sup>	2.12 <sup>a</sup>	1.09 <sup>a</sup>	1.17 <sup>ab</sup>
	SEM	0.048	0.022	0.026	0.010
	N	16	16	16	16
	P-value	0.001	0.001	0.001	0.001

Values bearing different superscripts within a column are significantly ( $p < 0.05$ ) different

P value = probability value; N = number of replicates; SEM = Standard Error Mean.

### 3.2 Total cholesterol, Triglycerides, HDL, LDL and VLDL-C

The data on serum biochemical parameters like serum total cholesterol, Triglycerides, HDL, LDL and VLDL-C was presented in Table 3. Supplementation of all the test diets (T<sub>3</sub> to T<sub>5</sub>) significantly ( $p < 0.05$ ) lowered the total serum cholesterol (mg/dL) concentration compared to control and antibiotic groups. The lowest cholesterol levels were recorded in sreeni herbal (T<sub>3</sub>), sanjeevani plus (T<sub>4</sub>) and sanjeevani + sreeni combination (T<sub>5</sub>) groups.

The serum triglyceride content ranged from 66.46 to 85.02 mg/dl among different treatments. Supplementation of all test diets significantly reduced the serum triglyceride levels at 42 d of age. The lowest triglyceride levels were recorded in sreeni herbal (66.46), sanjeevani plus (69.99) followed by sanjeevani + sreeni combination (72.89). supplementation of sreeni herbal (T<sub>3</sub>), sanjeevani plus (T<sub>4</sub>) and sanjeevani + sreeni combination (T<sub>5</sub>) groups significantly increased the serum HDL-C levels when compared to control and antibiotic groups. The LDL Cholesterol content ranged from 43.93 to 58.88 mg/dl among different treatments. Dietary inclusion of sreeni herbal (T<sub>3</sub>), sanjeevani plus (T<sub>4</sub>) and sanjeevani + sreeni combination (T<sub>5</sub>) diets significantly

( $p < 0.05$ ) decreased the LDL and VLDL-C Cholesterol levels compared to control and antibiotic groups. The lowest LDL Cholesterol content recorded in sanjeevani plus (T<sub>4</sub>), sanjeevani + sreeni combination (T<sub>5</sub>) followed by sreeni herbal (T<sub>3</sub>).

Sahoo *et al.* (2018) [22], Dalal *et al.* (2018) [11] and B Harambe and Garde (2020) [9] reported significant decrease in total cholesterol and triglyceride levels in broilers supplemented with turmeric, amla and giloy respectively. Decrease in total cholesterol and triglyceride levels are attributed to the presence of pectin in amla; octadecanoic acid and stigmasterol in giloy, which possess hypocholesterolemic property and inhibition of hepatic 3-hydroxyl-3-methylglutaryl Co A reductase by curcumin, which is responsible for cholesterol synthesis in the liver (Al-Kassie *et al.*, 2011) [3]. Increase in HDL and decrease in LDL and VLDL attributed to curcumin, which might modify triglyceride metabolism in the liver and VLDL clearance in peripheral tissues without influencing triglyceride absorption in the intestine (Babu and Srinivasan, 1997) [8]; flavonoids present in amla (Anila and Vijayalakshmi, 2002) [6] and campesterol present in giloy (Jain, 2021) [16] possess anti atherosclerotic and hypocholesterolemic property.

**Table 3:** Effect of vasanthu sanjeevani plus and sreeni herbal supplementation on total cholesterol, Triglycerides, HDL, LDL and VLDL-C levels of broiler chicken

S. No	Diets	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
T <sub>1</sub>	Control	207 <sup>b</sup>	84.52 <sup>c</sup>	125.56 <sup>c</sup>	58.88 <sup>c</sup>	33.50 <sup>c</sup>
T <sub>2</sub>	Antibiotic	199 <sup>b</sup>	85.02 <sup>c</sup>	129.56 <sup>b</sup>	55.93 <sup>c</sup>	31.00 <sup>c</sup>
T <sub>3</sub>	Sreeni Herbal	146 <sup>a</sup>	66.46 <sup>a</sup>	140.06 <sup>a</sup>	48.93 <sup>b</sup>	24.00 <sup>ab</sup>
T <sub>4</sub>	Sanjeevani plus	149 <sup>a</sup>	69.99 <sup>ab</sup>	139.36 <sup>a</sup>	43.93 <sup>a</sup>	26.30 <sup>b</sup>
T <sub>5</sub>	Sanjeevani plus + Sreeni Herbal	151 <sup>a</sup>	72.89 <sup>b</sup>	138.38 <sup>a</sup>	45.63 <sup>ab</sup>	22.10 <sup>a</sup>
	SEM	3.306	1.111	0.838	0.857	0.628
	N	16	16	16	16	16
	P-value	0.001	0.001	0.001	0.001	0.001

Values bearing different superscripts within a column are significantly ( $p < 0.05$ ) different

### 3.3 Proximate composition of breast meat:

Moisture percentage, dry matter (%), crude fat (%), crude fiber (%), total ash (%) and NFE (%) of breast muscle did not show any significant ( $p > 0.05$ ) difference among different treatments, while crude protein (%) content of breast muscle was increased significantly ( $p < 0.05$ ) in sreeni herbal (T<sub>3</sub>), sanjeevani plus (T<sub>4</sub>) and sanjeevani + sreeni combination (T<sub>5</sub>) groups compared to control and antibiotic groups (Table 4). the essential oils and phytochemical

constituents present in turmeric amla and giloy. Hussein (2013) [15] on supplementing turmeric to the broilers observed significant improvement in CP% of breast meat sample whereas dry matter, crude ash and crude fat remain unchanged as in agreement with the present finding. Sharma *et al.* (2019) [23] also observed significant improvement in CP% of broiler meat supplemented with phytochemical mixture containing amla, turmeric and ashwagandha.

**Table 4:** Effect of sanjeevani plus and sreeni herbal supplementation on proximate composition of breast meat sample in broiler chicken

S. No	Diets	Moisture (%)	Dry Matter (%)	Crude Protein (%)	Crude fat (%)	Crude fiber (%)	Total ash (%)	Nitrogen free extract (%)
T <sub>1</sub>	Control	74.34	25.30	21.84 <sup>b</sup>	9.49	0.33	5.39	2.26
T <sub>2</sub>	Antibiotic	73.97	25.77	21.94 <sup>b</sup>	9.50	0.33	5.30	2.25
T <sub>3</sub>	Sreeni Herbal	73.99	25.55	22.97 <sup>a</sup>	9.28	0.31	5.18	2.36
T <sub>4</sub>	Sanjeevani plus	74.20	25.50	23.03 <sup>a</sup>	9.67	0.38	5.44	2.33
T <sub>5</sub>	Sanjeevani plus + Sreeni Herbal	73.50	25.51	22.57 <sup>a</sup>	9.71	0.32	5.56	2.36
	SEM	0.148	0.243	0.149	0.228	0.014	0.088	0.033
	N	6	6	6	6	6	6	6
	P-value	0.495	0.990	0.001	0.987	0.680	0.789	0.764

Values bearing different superscripts within a column are significantly ( $p < 0.05$ ) different

### 4. Conclusion

Finally, it could be concluded that supplementation of sreeni herbal, sanjeevani plus individually and their combination reduced the serum cholesterol, triglycerides, LDL-C and

VLDL-C, uric acid, creatinine levels, and improved the breast meat protein of broilers. Hence, sreeni herbal and vasanthu sanjeevani plus may be used to low cholesterol and high protein meat in broilers.

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