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Evaluation of Some Phytogenic Feed Supplements for Lean Meat Production in Commercial Broiler Chicken

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Authors' contributions

This work was carried out in collaboration among all authors. Author KR conceived the study and drafted the protocol. Authors ENM, KNRK and KS designed and performed the experiments and performed statistical analyses. Authors Su and BG prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Chicken meat tends to be high in polyunsaturated fatty acids and cholesterol, which may increase the risk of atherosclerosis, stroke and other associated diseases. A study was conducted for 42 days to evaluate the efficacy of dietary supplementation of two phytogenic feed supplements *viz.* AV/LMP/10 and AV/HLP/16 (*M/s* Ayurvet Limited, India) for their ability to produce lean meat in commercial broiler chicken. One hundred and fifty (150) one-day old Cobb-400 broiler chicks were randomly allocated to five equal groups. All the groups were fed standard basal ration. T0 was control group (without treatment), groups T1 and T2 were supplemented with AV/LMP/10 @ 500 g/ton and 1 kg/ton of feed, respectively. Groups T3 and T4 were supplemented with AV/HLP/16 @ 1 kg/ton and 1.5 kg/ton of feed, respectively. On day 42, significantly better (P<0.05) results were

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obtained in the treated groups (T1-T4) in terms of body weight (2118.10±0.61, 2159.20±0.34, 2098.90±0.41, and 2180.40±0.56 g), total protein (19.10±0.56, 19.93±1.18, 20.80±0.36, and 21.04±0.64%), crude fat (1.25 ±0.25, 1.25 ± 0.31, 0.92 ± 0.24, and 1.03 ± 0.03%), *pH* (6.01 ± 0.07, 5.93 ± 0.09, 5.78 ± 0.08, and 6.06 ± 0.09), TBARS value (1.11±0.07, 1.11±0.05, 1.06±0.06, and 1.08±0.06 mg/Kg), cholesterol (54.19±1.11, 35.72±1.71, 45.61±1.54, and 36.09±1.045 mg/dL), HDL cholesterol (16.70±0.56, 12.61±1.28, 14.16±1.02, and 15.59±0.57 mg/dL) and total lipids (1.60±0.13, 1.56±0.22, 1.64±0.15, and 2.03±0.12 mg/g) in comparison to the untreated group (2050.80±0.71 g, 18.15±0.41%, 1.66 ± 0.48%, 6.07 ± 0.08, 1.18±0.09 mg/Kg, 62.36±0.88 mg/dL, 24.25±0.54 mg/dL and 2.42±0.18 mg/g). FCR and slaughter parameters varied non-significantly among different groups. The meat: bone ratio was numerically higher in the treated groups T1-4 (2.01±0.20, 2.09 ±0.19, 2.16±0.20 and 1.89±0.35) as compared to the control group (1.80±0.18). The highest protein content was obtained in T4 (21.04%) followed by T3 (20.80%), T2 (19.93%), T1 (19.10%) and lowest in T0 (18.15%), while highest crude fat was obtained in the muscles of the control group T0 (1.66%) followed by T1 and T2 (1.25%), T4 (1.03%) and T3 (0.92). Overall sensory acceptability of meat also improved in the supplemented groups. From the present study, it could be concluded that the phytogenic formulations AV/LMP/10 and AV/HLP/16 were effective in lean meat production in broiler chicken.

Keywords: Lean meat; broilers; hypolipidemic; phytogenic; feed supplement; AV/LMP/10; AV/HLP/16.

1. INTRODUCTION

Meat is an excellent source of valuable nutrients, and meat fat acts as a key determinant of the texture and flavor of the product [1]. Concerningly, chicken meat has increased in fat content, probably due to the selection of broilers on the basis of live weight with little consideration given to other important criteria such as carcass composition. With excessive fat in the body there may be a variety of health and production problems encountered by both the bird itself and by the consumers. In birds, the incidence of leg weakness and reduced reproductive performance in the breeding stock has been observed with increased fat deposition in the body, and in consumers, increased risk of atherosclerosis, hardening of the arteries, stroke and cancer have been implicated with long term consumption of such products [2].

Modern broilers minimally contain about 100 g of fat per kg body weight, which is physiologically inessential and causes great economic loss due to high feed cost [3]. The increasing concerns over health have led to efforts [1] for developing lean meat alternatives, which are a good source of protein for those following a low-calorie diet and good source of selenium and vitamin B3 and B6, all of which help in the conversion of glucose. Lean meat helps in the production of feel-good hormones, in the reduction of stress, and provides the body with choline that improves nervine functions and reduces inflammation [4].

AV/LMP/10 is a non-hormonal blend of phytogenic constituents, useful for obtaining low fat meat with better organoleptic properties. The herbal constituents in AV/LMP/10 are rich in phytochemicals like diosgenin, which are scientifically reputed to reduce levels of circulating cholesterol and saturated fats, lower fat deposition, and reduce the synthesis of fat [5]. AV/HLP/16 is a phytogenic hypolipidemic premix for poultry. The herbal constituents in AV/HLP/16 are rich in phytochemicals known for their hypolipidemic action, allicin, for example [6]. AV/HLP/16 is recommended for reducing fat and cholesterol content and for improving omega-3 fatty acid level in eggs and chicken meat. We report the results of a study undertaken to determine the efficacy of herbal formulations AV/LMP/10 and AV/HLP/16 in lean meat production in broilers.

2. MATERIALS AND METHODS

The present study was undertaken at the NTR College of Veterinary Science, Gannavaram, Andhra Pradesh. One hundred and fifty (150) one-day old Cobb-400 broilers of nearly similar live weight were procured and maintained under standard managemental conditions under deep litter system. The birds were randomly allocated to five equal groups as shown in Table 1 below:

Table 1. Treatments assigned to different groups under the study

Group	Treatment	Dose rate
Control (T0): Basal diet (Control)	No treatment	-
Treatment (T1): Basal diet supplemented with AV/LMP/10	AV/LMP/10	500 g/tonne of feed
Treatment (T2): Basal diet supplemented with AV/LMP/10	AV/LMP/10	1.0 Kg /tonne of feed
Treatment (T3): Basal diet supplemented with AV/HLP/16	AV/HLP/16	1.0 Kg/tonne of feed
Treatment (T4): Basal diet supplemented with AV/HLP/16	AV/HLP/16	1.5 Kg/tonne of feed

The birds were kept in open-sided houses in battery brooders with provision of a space of one square feet per bird using 4×4 cell per replicate, 24 hrs white color light with 60W intensity at a temperature of 21 to 28°C with RH of 55%. Isocaloric and isonitrogenous rations were prepared as per NRC [7]. Parameters such as body weight, feed intake, feed conversion ratio, carcass characteristics, cut-up parts yield, proximate analysis, *pH*, TBARS values, lipid profile and sensory attributes were assessed. Body weight was measured with electronic balance (Essae DS415) with an accuracy level of 5g, and feed intake and feed conversion ratio were calculated. Broilers were slaughtered in semi-automatic poultry processing plant of the Department of Livestock Products Technology, NTR CVSc, Gannavaram. Broilers were kept in the restraining cones and both carotid arteries and at least one jugular vein were manually severed with knife. The birds were bled for 90 seconds, scalded at 54.4°C for 120 seconds and mechanically defeathered. Feet were removed and carcasses were manually eviscerated, washed and allowed to drip for 5 min. The weights of head and shank, feathers, skin, intestines and giblets were determined using electronic balance. Dressing percentage was calculated by dividing the warm carcass weight by the shrunk live weight of the animal and expressing the result as a percentage. Cut-up parts were made mechanically using portioning machine. The neck was weighed and fore quarters were cut into wings and breast by severing the wings from the fore quarters at the proximal ends of humeri. Leg quarters were further made into thighs and drumsticks. The weights of the cut-up parts were recorded using electronic digital balance. In physico-chemical properties, *pH* was determined through digital *pH* meter (Oakton Instruments, USA) following the method of Troutt *et al.* [8] and lipid peroxidation (TBARS value) through distillation method of Tarladgis *et al.* [9], per cent moisture, total protein and per cent crude fat were estimated by the procedures of Association of Analytical Chemists (A.O.A.C.) [10] and were expressed as percentage. The pectoralis muscle was used for the assessment of lipid parameters namely total lipids, cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. The samples were collected from birds of all group of slaughter in triplicates. Lipid extracts were prepared following the method of Bligh and Dyer [11]. From the above lipid extracts, triglycerides, LDL cholesterol and HDL cholesterol were estimated using commercial Erba-Manheim kits. Total lipid was estimated gravimetrically [12] and total cholesterol was estimated following the method of Eswarapragada *et al.* [13]. The meat was cooked and subjected to sensory analysis by a consumer panel with a ranking test. The data was subjected to statistical analysis by ANOVA and *post-hoc* Duncan's multiple range test using SPSS version 15.0; unless stated otherwise, all statistical inferences were drawn at $P \leq 0.05$.

3. RESULTS

3.1 Growth Performance

At 6^{th} week, 2180.40 g mean body weight of the group T4 fed with feed supplemented with phytogenic feed supplements was significantly higher ($P<0.05$) in comparison to the untreated control group T0 (2050.80) as given below in Table 2. Cumulative feed intake was significantly higher in group T4 (3679.20) followed by groups T2 (3561.80), T3 (3474.70) and T1 (3434.30) in comparison to the unsupplemented control group T0 (3260.00) (Table 3). FCR varied non-significantly among the different groups (Table 4).

3.2 Carcass Characteristics

Slaughter parameters (Table 5) varied non-significantly among different groups. Live weight was more in groups T2 and T4 *i.e.* 2.12 Kg, followed by groups T3 and T1 *i.e*. 2.08 Kg in comparison to T0 *i.e*. 2.01 Kg. Head and shanks (%) followed the order T2 (6.41) > T3 (6.40) > T0 (6.20) > T4 (5.90) > T1 (5.23). Bones (%) followed the order T1 (2.98) > T2 = T4 (2.94) > T0 (2.90) > T3 (2.88). Feathers (%) followed the order T1 (8.68) > T2 (7.42) > T3 (6.30) > T0 (6.15) > T4 (5.65). Similarly, skin (%) followed the

Table 2. Weekly body weight (g) of broilers fed with phytogenic lean meat supplements

Group	Week					
	1	2	3	4	5	6
T0	162.81±0.12	452.31±0.31^{a}	932.81±0.48^{ab}	1418.80±0.54	1924.10±1.21	2050.80±0.71^{a}
T1	156.00±0.09	423.81±0.31^{ab}	874.19±0.30^{bc}	1377.80±0.51	1928.90±1.09	2118.10±0.61^{b}
T2	164.12±0.23	454.56±0.22^{a}	958.44±0.21^{a}	1441.70±0.42	2020.40±0.92	2159.20±0.34^{b}
T3	153.81±0.47	444.12±0.17^{a}	907.38±0.57^{abc}	1381.00±0.33	1970.40±0.78	2098.90±0.41^{b}
T4	156.75±0.09	398.38±0.19^{b}	850.56±0.41^{c}	1359.80±0.21	1986.70±0.62	2180.40±0.56^{b}

Values bearing a common superscript within the same column do not differ significantly

Table 3. Weekly feed intake (g) of broilers fed with phytogenic lean meat supplements

Group	Week						Cumulative
	1	2	3	4	5	6	
T0	150.26±0.71^{a}	338.26±0.51^{e}	707.37±0.23^{a}	660.05±0.31^{b}	1114.70±0.54^{b}	889.27±0.33^{a}	3260.00±0.82^{a}
T1	174.38±0.98^{b}	294.25±0.71^{a}	805.12±0.42^{c}	594.81±0.22^{a}	1179.90±0.81^{d}	907.10±0.58^{b}	3434.30±0.51b^{a}
T2	175.94±0.57^{c}	313.56±0.29^{c}	804.560±0.85^{b}	769.62±0.72^{d}	1155.50±0.56^{c}	920.40±0.25^{c}	3561.80±0.67^{ba}
T3	176.50±0.61^{d}	309.12±0.33^{b}	852.06±0.39^{e}	794.76±0.41^{e}	1076.90±0.81^{a}	971.09±0.36^{c}	3474.70±0.79^{ba}
T4	177.88±0.39^{e}	315.75±0.22^{d}	816.33±0.72^{d}	746.93±0.51^{c}	1204.60±0.78^{e}	944.66±0.41^{c}	3679.20±0.39^{b}

Values bearing a common superscript within the same column do not differ significantly

Table 4. Weekly feed conversion ratio of broilers fed with phytogenic lean meat supplements

Group	Week						Cumulative
	1	2	3	4	5	6	
T0	1.32±0.082^{a}	1.17±0.045^{a}	1.50±0.057^{a}	1.52±0.011	2.27±0.019	1.14±0.058	1.59±0.048
T1	1.63±0.063^{b}	1.12±0.096^{a}	1.85±0.069^{b}	1.41±0.029	2.20±0.035	1.17±0.003	1.63±0.058
T2	1.54±0.014^{b}	1.09±0.024^{a}	1.68±0.014^{ba}	1.75±0.037	2.64±0.065	1.12±0.069	1.67±0.042
T3	1.65±0.011^{b}	1.08±0.036^{a}	1.82±0.036^{ba}	1.56±0.007	2.20±0.069	1.19±0.074	1.69±0.039
T4	1.63±0.042^{b}	1.34±0.058^{b}	1.91±0.029^{b}	1.50±0.012	2.18±0.062	1.53±0.038	1.72±0.008

Values bearing a common superscript within the same column do not differ significantly

Table 5. Carcass characteristics of broilers fed with phytogenic lean meat supplements

Group	Live wt (Kg)	Head and shank (%)	Bone (%)	Feathers (%)	Skin (%)	Giblets (%)	Intestines (%)	Dressing Percentage (%)
T0	2.01±0.11	6.20±0.11	2.90±0.45	6.15±0.68	13.76±0.67	5.91±0.71	6.36±0.31	60.95±2.48
T1	2.08±0.11	5.23±0.43	2.98±0.33	8.68±2.82	10.85±1.88	6.03±0.98	6.27±0.66	59.41±4.99
T2	2.12±0.15	6.41±0.10	2.94±0.47	7.42±0.85	13.22±1.56	6.17±0.57	6.50±0.61	61.82±0.39
T3	2.08±0.11	6.4± 0.50	2.88±0.44	6.30±0.64	12.12±1.11	5.94±0.58	5.83±0.29	63.60±0.85
T4	2.12±0.15	5.90±0.22	2.94±0.46	5.65±0.68	13.07±1.77	6.78±0.78	5.79±0.23	59.98±3.62

Table 6. Cut-up parts of broiler carcasses fed with phytogenic lean meat supplements

Group	Neck%	Wings%	Drumsticks%	Thigh%	Breast%	Bone%	Meat (Kg)	Bone (Kg)	Meat:Bone
T0	3.47±0.57	10.71±0.63	14.37±0.48	16.67±0.61	33.30±1.52	21.48±1.81	0.79±0.08	0.44±0.02	1.80±0.18
T1	3.89±0.51	10.31±0.79	12.14±0.30	17.75±0.27	35.23±1.99	20.68±1.83	0.83±0.10	0.42±0.05	2.01±0.20
T2	4.24±0.33	11.26±1.12	14.20±0.26	17.77±1.48	32.63±4.05	19.90±1.41	0.88±0.07	0.43±0.04	2.09 ±0.19
T3	4.02±0.29	10.29±0.54	12.79±0.25	17.38±0.63	37.53±1.35	17.99±1.34	0.90±0.06	0.42±0.04	2.16±0.20
T4	3.62±0.27	9.88±0.28	13.26±0.27	16.89±0.41	35.69±1.00	20.66±0.64	0.82±0.11	0.45±0.04	1.89±0.35

Table 7. Proximate analysis, *pH* and TBARS values in meat of broilers fed with phytogenic lean meat supplements

Group	Moisture (%)	Total Protein (%)	Crude Fat (%)	*pH*	TBARS (mg malonoldehyde/ Kg meat)
T0	71.77±1.36	18.15±0.41^{a}	1.66 ± 0.48^{b}	6.07 ± 0.08^{b}	1.18±0.09^{b}
T1	72.28±0.86	19.10±0.56^{b}	1.25 ±0.25^{a}	6.01 ± 0.07^{a}	1.11±0.07^{a}
T2	73.40±0.47	19.93±1.18^{b}	1.25 ± 0.31^{a}	5.93 ± 0.09^{a}	1.11±0.05^{a}
T3	73.04±0.68	20.80±0.36^{b}	0.92 ± 0.24^{a}	5.78 ± 0.08^{a}	1.06±0.06^{a}
T4	73.46±0.87	21.04±0.64^{b}	1.03 ± 0.03^{a}	6.06 ± 0.09^{a}	1.08±0.06^{a}

Values bearing a common superscript within the same column do not differ significantly

Table 8. Meat lipid profile of broilers fed with phytogenic lean meat supplements

Group	Triglycerides mg/dL*	Cholesterol mg/dL*	HDL cholesterol mg/dL*	LDL cholesterol mg/dL*	Total lipids mg/g of fresh meat
T0	159.94±0.91^{c}	62.36±0.88^{c}	24.25±0.54^{c}	38.11± 0.64	2.42±0.18^{b}
T1	112.93±1.47^{a}	54.19±1.11^{b}	16.70±0.56^{b}	41.66± 3.96	1.60±0.13^{a}
T2	121.03±1.54^{a}	35.72±1.71^{a}	12.61±1.28^{a}	22.11±1.23	1.56±0.22^{a}
T3	137.56±1.95^{b}	45.61±1.54^{b}	14.16±1.02^{b}	31.44±2.28	1.64±0.15^{a}
T4	180.82±5.39^{d}	36.09±1.045^{a}	15.59±0.57^{b}	20.50±1.16	2.03±0.12^{a}

Values bearing a common superscript within the same column do not differ significantly;
**mg/dL of lipid extract obtained from 10 g of pectoralis muscle*

Table 9. Sensory attributes of meat of broilers fed with phytogenic lean meat supplements

Group	Color	Flavor	Tenderness	Juiciness	Overall acceptability
T0	7.23±0.09	7.15±0.13	7.03±0.18	7.15±0.09	7.14±0.12
T1	7.35±0.31	7.28±0.15	7.23±0.15	7.33±0.07	7.29±0.17
T2	7.48±0.11	7.38±0.19	7.30±0.11	7.40±0.09	7.39±0.12
T3	7.33±0.09	7.25±0.23	7.38±0.17	7.48±0.14	7.36±0.16
T4	7.33±0.03	7.38±0.19	7.25±0.25	7.20±0.09	7.29±0.14

order T0 (13.76) > T2 (13.22) > T4 (13.07) > T3 (12.12) > T1 (10.85). Giblets (%) were higher in group T4 (6.78), followed by groups T2 (6.17), T1 (6.03) and T3 (5.94) in comparison to the unsupplemented control group T0 (5.91). Percent intestines were higher in group T2 (6.50) followed by T0 (6.36), T1 (6.27), T3 (5.83) and T4 (5.79). Dressing percentage was higher in group T3 (63.60) followed by T2 (61.82). Dressing percentage of groups T4 (59.98) and T1 (59.41) varied non-significantly from the unsupplemented group T0.

3.3 Cut-Up Parts

Neck (%) followed the order T2 (4.24) > T3 (4.02) > T1 (3.89) > T4 (3.62) > T0 (3.47). Wings (%) followed the order T2 (11.26) > T0 (10.71) > T1 (10.31) > T3 (10.29) > T4 (9.88). Drumsticks (%) followed the order T0 > T2 > T4 > T3 > T1. Thigh (%) followed the order T2 > T1 > T3 > T4 > T0. Breast (%) followed the order T3 > T4 > T1 > T0 > T2. Bone (%) followed the order T0 > T1 > T4 > T2 > T3. Meat yield followed the order T3 > T2 > T1 > T4 > T0 >. Bone yield followed the order T4 > T0 > T2 > T1=T3. Meat to bone ratio (Table 6) was highest for group T3 (2.16) followed by groups T2 (2.09), T1 (2.01) and T4 (1.89) in comparison to T0 (1.80). However, all of these differences were statistically non-significant.

3.4 Physico-Chemical Characteristics of Meat

Moisture content of meat from the supplemented groups varied non-significantly from each other though the values were higher than the unsupplemented group T0. Total protein content varied significantly in the supplemented groups from the unsupplemented group and followed the order T4 (21.04) > T3 (20.80) > T2 (19.93) > T1 (19.10) > T0 (18.15). Crude fat content varied significantly in the order T3 (0.92) < T4 (1.03) < T1 = T2 (1.25) < T0 (1.66). *pH* differed significantly between control and treatment groups but the differences between treatments were non-significant. Thiobarbituric acid reactive substance was significantly lower in the supplemented groups in comparison to the unsupplemented group and followed the order T3 (1.06) < T4 (1.08) < T1 = T2 (1.11) < T0 (1.18) (Table 7).

3.5 Meat Lipid Profile

Triglycerides were significantly lower in group T1 followed by T2 and T3 in comparison to the unsupplemented control group T0. Cholesterol level was significantly lower in the supplemented groups in comparison to the unsupplemented group in the order T2 < T4 < T3 < T1 < T0. HDL cholesterol was lower in the supplemented groups than in the unsupplemented control group T0. It followed the order T0 > T1 > T4 > T3 > T2. LDL cholesterol level was lower in all supplemented groups in comparison to the unsupplemented group except in group T1 and followed the order T4 < T2 < T3 < T0 < T1. Total lipid content was significantly lesser in supplemented groups in comparison to the

unsupplemented group as T2 (1.56) < T1 (1.60) < T3 (1.64) < T4 (2.03) < T0 (2.42) (Table 8).

3.6 Sensory Attributes of Meat

Sensory attributes of meat (Table 9) varied non-significantly among all groups, yet overall acceptability improved in the supplemented groups and followed the order: Group T2 (7.39) > T3 (7.36) > T1 = T4 (7.29) > T0 (7.14).

4. DISCUSSION

AV/LMP/10 and AV/HLP/16 improved body weight gain in broilers. This may be attributed to the efficacy of constituent herbs of AV/LMP/10 and AV/HLP/16, namely *Commiphora mukul*, *Trigonella foenum graecum* and *Allium sativum* respectively, which are scientifically well-proven for improving growth, productivity & hepatoprotective action [14]. Improved body weight of broilers may be attributed to the presence of the fatty acids [15] or due to active components such as neurin, biotin and trimethylamine, which stimulate appetite by their action on the nervous system [16]. The results of the present study agree with the findings of Laguna *et al*. [17] who observed that the addition of 0.2-2.0% diosgenin prevented hypercholesterolemia, rise of serum *β*-lipoproteins and phospholipids as well as reduced liver fat and cholesterol. Irrespective of the type of feed additive added, the treatment groups exhibited lower *pH* and TBARS values when compared to the meat from control group. This might be due to the nature of herbal constituents in the feed supplements which are rich in phytochemicals like diosgenin and allicin. Our findings on moisture and fat content of meat are in alignment with the fact that as the fat percentage increases, the percentage of water decreases because fat is non-polar (no water holding) and it decreases the relative amount of protein available for attracting and holding water [18].

The results of the present study are upheld by previous findings, where it was reported that chicks fed on diets supplemented with powder of *Trigonella foenum graecum* seeds (FSP) consumed more feed, gained higher body weight and achieved better feed conversion. Further, the supplementation of FSP in the broiler diets significantly decreased serum cholesterol, and, resulted in higher profits [19]. Our findings are also in agreement with those of Alloui *et al.* [15] who noted that addition of fenugreek seeds in broiler diets increased live body weight, feed intake and feed conversion ratio in broilers. Similarly, Mahmood reported improved body weight, feed intake, feed conversion ratio, dressing percentage, and reduced blood serum triglycerides and total cholesterol concentrations in broiler chicken supplemented with allicin [20]. Increases in the carcass yield of the broiler chicken were also observed by Azouz [21] and Abdel-Azem [22] with *Trigonella foenum graecum* and by Ahmad [23] and Mahmood *et al*. [20] with *Allium sativum*.

5. CONCLUSION

Dietary supplementation with phytogenic feed supplements AV/LMP/10 and AV/HLP/16 improved feeding and growth performance, carcass characteristics, physico-chemical characteristics, lipid profile and sensory attributes of meat in commercial broiler chicken in a dose-dependent manner. On the basis of the findings of the study, the phytogenic feed supplements AV/LMP/10 and AV/HLP/16 were found efficacious for lean meat production in commercial broiler chicken.

DISCLAIMER

M/s Ayurvet Limited intends to manufacture AV/LMP/10 and AV/HLP/16 commercially and funded this study. Sunidhi, KR and BG are employees of M/s Ayurvet Limited. However, the nature of this affiliation did not influence the outcomes of the study in any manner.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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