The Pharma Innovation Journal 2019; 8(9): 492-495

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(9): 492-495

www.thepharmajournal.com
Received: 12-07-2019
Accepted: 16-08-2019

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Effect of Ashwagandha root powder supplementation on thigh and breast muscle parameters of broiler chicken

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Abstract
To study the effect of dietary supplementation of Ashwagandha root powder on muscle parameters of broiler chicken, a total of 300, one day-old commercial broiler chicks were randomly distributed into six dietary treatments with five replicates per treatment and each replicate has ten birds. Feeding trial was conducted for 42 days in two different growth phases i.e. starter (0-28d) and finisher (29-42d). The control group (T_1) was offered maize- soybean meal based diet which was formulated as per specifications to fulfill the metabolizable energy (ME) and crude protein requirements of broilers. The first group was kept as a control (T_1) and given the basal diet without antibiotic while second (T_2) basal diet with antibiotic, third (T_3), fourth (T_4), fifth (T_5) and sixth (T_6) groups were supplemented with Ashwagandha root powder @ 0.25, 0.5, 0.75 and 1%, respectively in the diet. At end of feeding trial (6 weeks), one bird from each replicate was selected and slaughtered. Samples of breast and thigh muscles were weighed and taken for analysis of moisture, protein and ether extract as per AOAC (2013). Highest thigh muscle weight percentage was observed in T_6 (10.26%) followed by T_5 (10.21%) and was significantly ($P< 0.05$) higher than control (9.70%) group. Breast muscle weight percentage ranged from 26.13% (T_1) to 28.29% (T_5) under different dietary treatments and significantly ($P< 0.05$) higher breast percentage was found in 0.75% Ashwagandha supplemented groups as compared to control and antibiotic supplemented group. Percent crude protein of thigh muscle and breast muscle was significantly ($P< 0.05$) higher in 0.75% and 1.0% Ashwagandha supplemented group as compared to control (T_1) and antibiotic (T_2) supplemented group. There was a significant ($P< 0.05$) decrease in ether extract percentage of thigh and breast muscle in T_4, T_5 and T_6 groups as compared to control group (T_1). Thus, supplementation of Ashwagandha root powder resulted in increased weight of thigh muscle and breast muscle, crude protein percentage, whereas ether extract in thigh and breast muscle was found decreased as compared to control.

Keywords: Ashwagandha root powder, broilers, thigh and breast muscle weight, crude protein, ether extract

Introduction
The poultry industry in the country in present era has grown rapidly on account of its low capital investment, early assured returns, short generation intervals and limited land requirements (Singh *et al.*, 2014) [14]. It becomes necessary to have an efficient programme with maximum utilization of the nutrients from the available feedstuffs. The modern development of biotechnology ensures the availability of different growth promoters like enzymes, probiotics, organic acids, antibiotics, yeasts, algae, etc. These can be incorporated in the ration for promoting their growth and performance. An ideal growth promoter should be readily biodegradable, free from environmental hazards, non-toxic, involved with transferable drug resistance and improve performance effectively and economically. Common feed additives used in poultry diets include antimicrobials, antioxidants, emulsifiers, binders, pH control agents and enzymes.

Use of synthetic growth promoters like antibiotics has led to success in limiting most of the prevalent bacterial diseases which affected man and animals in epidemic proportions. At the same time, inadvertent and overuse of antibiotics resulted in disadvantages like high cost of production, toxicity and development of resistance and environmental and health hazards. The use of antibiotics started to be seen as a risk factor to human health probably by induction of cross-resistance for pathogenic bacteria in humans (Costa *et al.*, 2007) [8]. To eliminate antibiotics, numerous substances, commonly known as natural growth promoters (NGPs) have been identified as effective alternatives to antibiotics.

Commonly used growth promoters are prebiotics, probiotics, synbiotics, enzymes, acidifiers and phytobiotics. Phytobiotics are NGPs, which have been growing in popularity as feed additives, due to their beneficial effect on gut health and immunity and growth performance. *Withania somnifera* (Ashwagandha) holds a celebrated position in the Indian *Materia medica*. Ashwagandha (*Withania somnifera*) is a plant of Solanaceae family. Ashwagandha plant constitutes alkaloids and steroidal lactones, but the withanine, the main alkaloid found in its roots and leaves is thought to be responsible for its biological activity. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII). It consists of following properties *viz*. adaptogenic, antidepressant, liver-tonic, antioxidant, immune modulator, immune adjuvant (Ziauddin *et al.*, *1996*) [18] and also improve feed intake, body weight gain, FCR, hematological profile and immunological status, neuro-protective and rejuvenate muscles (Ansari *et al.*, 2008) [4].

Materials and Methods

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC), 235/CPCSEA dated 1-8-2000 in the Department of Animal Nutrition, LUVAS. Three hundred, one day old broiler chicks, were purchased from a local commercial hatchery. The chicks were individually weighed, wing banded and randomly distributed into 30 subgroups means six dietary treatments with five replicates per treatment and each replicate has ten birds. Basal ration was formulated as per BIS (2007) [6] to fulfill the metabolizable energy (ME) and crude protein requirements of birds. Level of crude protein in starter (0-4weeks) and finisher (4-6weeks) ratio was 23 and 20%, respectively. The respective ME content was 3000 and 3200 Kcal/kg are presented in Table 1. Ingredient compositions of experimental diets during different phases of growth are presented in Table 2. The first group was kept as a control (T_1) and given the basal diet without antibiotic while second (T_2) basal diet with antibiotic, third (T_3), fourth (T_4), fifth (T_5) and sixth (T_6) groups were supplemented with Ashwagandha root powder @ 0.25, 0.5, 0.75 and 1%, respectively in the diet. The experimental birds were reared under deep litter system. The litter was regularly racked to avoid any lump formation. Birds were vaccinated against F_1 strain of Ranikhet disease on 3rd day and IBD on 14th day.

The weekly record of the feed offered and residual amount was maintained for each replicate to calculate the feed consumption per bird. The birds were weighed individually at fortnightly intervals and the body weights, feed intake were recorded up to 6 weeks of age. Feed Conversion Ratio (FCR) for each replicate was calculated. At the end of feeding trial (42 days), one bird from each replicate was selected. The birds were kept off feed and water was withdrawn three hours prior to their sacrifice. Immediately after recording their live weights, the birds were sacrificed by severing the jugular vein and allowed to bleed completely following scientific method of slaughtering. Their heads were removed at the atlanto-occipital joint and shank at hock joint. Thigh and breast muscles were collected and weight of thigh and breast were recorded after washing and bloating, from each slaughtered bird, and their relative weights (percentage of live weight) were then calculated. Samples of breast and thigh muscles were taken from each of the slaughtered birds and stored in deep-freeze separately for further analysis. These samples were analyzed for moisture, protein and ether extract as per AOAC (2013) [5]. The data were analyzed using general linear model procedure of statistical package for social sciences 20th version (SPSS) [15] and comparison of means tested using Duncan's multiple range test (DMRT) [9] and significance was considered at $P< 0.05$.

Table 1: Chemical composition of feed ingredients used in ration formulation

Ingredient	CP (%)	CF (%)	EE (%)	TA (%)	Lysine* (%)	Methionine* (%)	ME* (kcal/kg)
Maize	9.31	2.48	3.49	2.25	0.18	0.15	3300
Soyabean meal	45.40	3.96	3.16	8.47	2.57	0.76	2230
Fish meal	47.40	1.82	5.16	26.62	1.42	1.42	2210
Ashwagandha root powder	2.91	6.34	2.30	4.41	-	-	245

*Calculated values (Singh and Panda, 1992) [13].

Table 2: Ingredient composition of experimental diets during different phases of growth

Ingredient (kg /100 of feed)	0-4 wk	4-6 wk
Maize	58	60
Soybean meal	30	25
Fish meal	7	7
Vegetable oil	3	6
Mineral mixture	2	2
Feed additives (g/100 kg feed)		
Spectromix[1]	10	10
Spectromix BE[2]	20	20
Veldot[3]	50	50
Choline chloride[4]	50	50
Lysine[5]	50	50
DL-methionine[6]	150	150

1. Spectromix: Powder (Ranbaxy Animal Health, New Delhi). Each g. contained Vitamin A- 82,500 IU, Vit D3-12000 IU, Vit B2-50 mg and Vit.K-10 mg. Mixing rate: 10 g/100 kg of feed.
2. Spectromix BE: Powder (Ranbaxy Animal Health, New Delhi). Each g contained Vit.B1- 8 mg, Vit.B6- 16 mg, Vit.B12- 80 mg, niacin-120mg, calcium pentothenate-80 mg, Vit. E-160 mg, Lysine hydrochloride-10 mg, DL-methionine-10 mg and calcium 260mg. Mixing rate: 20g/100 kg of feed.
3. Veldot: Venkeys- Dinitro-O-Toluamide (Coccidiostat). Mixing rate: 50 g/100 kg of feed.
4. Choline chloride: Contain 60 percent choline. Mixing rate: 50g/100 kg of feed.
5. Lysine: Contained 98% lysine. Mixing rate: 50 g/100 kg of feed.
6. DL-methionine: Contained 98% methionine. Mixing rate: 150 g/100 kg of feed.

Results and Discussion

Thigh and breast percentage

Results showing mean values of thigh and breast percentage are presented in Table 3. Dietary supplementation of Ashwagandha root powder showed a significant increase in thigh percentage of the experimental birds. Highest thigh

percentage was observed in T_6 (10.26%) followed by T_5 (10.21%), T_4 (10.16%), T_3 (10.15%) and was significantly ($P< 0.05$) higher than control (9.70%) group. Breast percentage ranged from 26.13% (T_1) to 28.29% (T_5) under different dietary treatments and significantly ($P< 0.05$) higher breast percentage was found in 0.75% followed by 1.0% and 0.50% Ashwagandha supplemented groups as compared to control and antibiotic supplemented group. Our result findings are in corroboration with Brzoska *et al.* (2010) [7] who found that mannan oligosaccharide and fumaric acid with (WHE) and without (NHE) herb mixture significantly increased the weight of carcasses compared to the control group (CON; $P< 0.01$). The treatment factors had no effect on dressing percentage. The herb mixture (WHE) significantly increased gizzard weight compared to the other groups ($P< 0.01$). Also Saini *et al.* (2015) [12] reported that the carcass traits studied in terms of dressed weight and eviscerated weight revealed highly significant ($P< 0.01$) effect of Ashwagandha as feed additive. This increase in thigh and breast percentage may be related with improvement in body weight and FCR due to main active constituent withanine and withanolide of *Withania somnifera* root powder that could act not only as antibacterials and antioxidants but as a stimulant of digestive enzymes in the intestinal mucosa and pancreas that improve the digestion of dietary nutrients and feed efficiency, subsequently increasing the growth rate (Ali, 2011) [2]. In contrast to our findings, Aditya *et al.* (2018) [1] observed that carcass percentage and organ weight were not affected by addition of grape pomace as feed supplement to broiler diets.

Table 3: % Mean values of thigh and breast weight percentage of the experimental birds under different dietary treatments

Treatments	Thigh%	Breast%
T_1	$9.70^{a}\pm0.18$	$26.13^{a}\pm0.31$
T_2	$9.90^{ab}\pm0.15$	$26.15^{a}\pm0.15$
T_3	$10.15^{bc}\pm0.12$	$26.27^{a}\pm0.24$
T_4	$10.16^{bc}\pm0.04$	$27.58^{b}\pm0.18$
T_5	$10.21^{bc}\pm0.03$	$28.29^{c}\pm0.13$
T_6	$10.26^{c}\pm0.02$	$27.69^{bc}\pm0.26$

[a, b, c] Means bearing different superscripts in a column differ significantly ($P< 0.05$)

Composition of breast meat

Data pertaining to composition of Breast meat of the experimental birds under different dietary treatments are presented in Table 4. Moisture percentage of breast muscles was obtained in the range of 71.24% (T_5) to 71.40% (T_1) and did not differ significantly ($P< 0.05$) among treatment groups. No particular trend was observed in moisture content of breast meat. Crude protein percentage of breast muscles was significantly ($P< 0.05$) increased in Ashwagandha supplemented group T_5 and T_6 as compared to control and ranged from 21.88% (T_3) to 22.12% (T_6) under different dietary treatments. Significantly ($P< 0.05$) higher crude protein percentage was observed in T_5 and T_6 Ashwagandha supplemented group as compared to other treatments. Ether extract percentage significantly ($P< 0.05$) decreased in Ashwagandha supplemented groups as compared to control group and maximum reduction in fat was observed at 0.75% and 1.0% level of inclusion. The inclusion of *W. somnifera* root powder in a basal diet of broilers improved the protein content of the breast muscle which could be due to enhanced absorption of amino acids in the gut (Thorat *et al.*, 2015 [17]). According to Thakur *et al.* (2017) [16] the proximate composition of broiler breast meat revealed that significantly ($P< 0.05$) higher mean CP (dry matter basis) was in the broiler group supplemented with 1.5% *W. somnifera*. However, comparable performance was observed in T_8 group supplemented with 0.5% *W. somnifera* + 0.05% synbiotic. Mean ether extract of breast meat also exhibited significantly downward trend with increasing levels of *W. somnifera* supplementation. The results indicated the health promoting synergistic effect of herb *W. somnifera* and synbiotic substances in reduction of fatty substances. Significantly higher ether extract value was observed in the control group.

Table 4: Composition of Breast meat of the experimental birds under different dietary treatments

Treatments	Moisture%	CP%	EE%
T_1	71.40 ± 0.02	$21.92^{ab}\pm0.02$	$6.22^{c}\pm0.03$
T_2	71.36 ± 0.04	$21.98^{b}\pm0.03$	$6.20^{bc}\pm0.02$
T_3	71.36 ± 0.02	$21.88^{a}\pm0.03$	$6.19^{bc}\pm0.03$
T_4	71.29 ± 0.03	$21.94^{ab}\pm0.03$	$6.11^{b}\pm0.02$
T_5	71.24 ± 0.11	$22.09^{c}\pm0.02$	$5.93^{a}\pm0.04$
T_6	71.28 ± 0.10	$22.12^{c}\pm0.03$	$5.92^{a}\pm0.02$

[a, b, c] Means bearing different superscripts in a column differ significantly ($P< 0.05$)

Composition of thigh meat

The mean values of data regarding composition of thigh meat of experimental birds under different dietary treatments are presented in Table 5. Moisture percentage of experimental birds under different dietary treatments ranged from 75.29% (T_2 and T_6) to 75.33% (T_2) and no particular trend was observed in moisture content of thigh meat also. Percent crude protein of thigh muscle ranged from 16.70% (T_1) to 17.43% (T_6) and was significantly ($P< 0.05$) higher in 0.75% Ashwagandha supplemented group (T_5), 1.0% Ashwagandha supplemented group (T_6) followed by 0.50% (T_4) level of Ashwagandha root powder as compared to control (T_1) and antibiotic (T_2) supplemented group. Ether extract percentage of thigh muscle ranged from 6.98% (T_6) to 7.42% (T_1). As the level of inclusion of Ashwagandha root powder in diet increased, there was a significant ($P< 0.05$) decrease in ether extract percentage in T_4, T_5 and T_6 groups as compared to control group (T_1). Kale *et al.* (2014) [11] observed that the highest drumstick yield was found in treatment T_1 (0.25%) followed by T_2 (0.50%) indicating beneficial effect of feeding *Withania somnifera* root powder as herbal feed supplement. Our results were in agreement with the findings of Durrani *et al.* (2008) [10], who reported that increased weight of thigh and breast causing increased dressing percentage with the addition of dried habek mint in broiler diet was due to better feed utilization. However study conducted by Ansari *et al.* (2013) [3] indicated that the values of dressing percentage had no marked variation between *Withania somnifera* root powder supplementation and antibiotic as positive control at both 28 and 42 days of age.

Table 5: Composition of Thigh meat of the experimental birds under different dietary treatments

Treatments	Moisture%	CP%	EE%
T_1	75.29±0.03	16.70^{a}±0.06	7.42^{c}±0.02
T_2	75.33±0.03	16.71^{a}±0.05	7.39^{c}±0.03
T_3	75.32±0.02	16.77^{a}±0.08	7.34^{c}±0.03
T_4	75.32±0.03	17.21^{b}±0.02	7.18^{b}±0.02
T_5	75.31±0.02	17.38^{c}±0.01	7.04^{a}±0.03
T_6	75.29±0.02	17.43^{c}±0.02	6.98^{a}±0.02

[a, b, c] Means bearing different superscripts in a column differ significantly ($P< 0.05$)

Conclusion

Thus, in the light of these findings, it can be concluded that Ashwagandha root powder at the dietary level of 0.75% and 1.0% can be effectively supplemented as an alternative to antibiotic growth promoter in broiler ration which resulted in increased weight of thigh muscle and breast muscle, crude protein percentage, whereas ether extract in thigh and breast muscle was found decreased as compared to control. Ashwagandha root powder active ingredients: withanine and withanolide act as a stimulant of digestive enzymes in the intestinal mucosa and pancreas that improved the digestion of dietary nutrients and feed efficiency, subsequently increasing the growth rate and weight, better utilization of amino acids leading to increased crude protein content of muscles.

Acknowledgement

The authors would like to acknowledge the help and support received from Department of Animal Nutrition and College of Veterinary Sciences for providing the lab facility, guidance and financial assistance.

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