

Effect of Graded Levels of Mint Leaf Meal on Growth Performance, Immune Competence Traits and Meat Composition of Coloured Chicken

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Abstract

Two hundred and ten, day old straight run coloured chicken (Chabro) were randomly distributed into seven dietary treatments: T1-basal diet, T2- T1+0.25% Mint leaf meal (MLM), T3- T1+0.5% MLM, T4- T1+0.75% MLM, T5- T1+1% MLM, T6-T1+1.25% MLM, T7- T1+1.5% MLM, having three replicates each. During 4-8 weeks, T4 birds had significantly higher ($P<0.01$) body weight gain and significantly better ($P<0.01$) FCR than T3, T5, T6 and comparatively better performance than T1. HA response to 1% SRBC (log₂ titre) and cell mediated response to PHA-P was significantly higher ($P<0.01$) in T4. Percent ether extract of breast and thigh muscle was significantly lower ($P<0.01$) in T6 than T1. Thus, dietary supplementation of 0.75% mint leaf meal resulted in significantly higher humoral and cell mediated immune response in coloured chicken. Further, dietary supplementation of 1.25% mint leaf meal resulted in decrease in percent crude fat of breast and thigh meat cuts in coloured chicken.

Keywords: Body Weight, Coloured Chicken, Immunity, Mint Leaf Meal

Introduction

Poultry is one of the fastest growing segments of the agricultural sector in India with around 8 percent growth rate per annum (Chatterjee and Rajkumar, 2015). Backyard poultry holds a key for sustainability of poultry production and plays a pivotal role in nutritional and livelihood security of the rural masses. Over the years, various improved genotypes of chicken have been developed to specifically cater the needs for backyard poultry farming. Chabro is a breed, developed by Central Poultry Development Organization (CPDO) especially for the farmers of our country as it is comparatively more adaptive to climatic fluctuations and variable conditions of the farm as compared to commercial broilers.

In recent years, efforts have been made to counteract the adverse effects of various levels of stress and augment the production potential in broilers by using herbs or phytochemicals possessing therapeutic potential. Mint is one of the oldest and most popular herbs that is grown throughout the world. Studies have been undertaken on mint leaf meal as dietary supplement in poultry (Loreto and Velikova, 2001; Sharkey, 2001; Loreto *et al.*, 2004; Ameri *et al.*, 2016; Nozhat *et al.*, 2014). However, comprehensive studies are needed to assess the efficacy of mint leaf meal as a dietary supplement in poultry. Hence, the present experiment was designed to study the effect of mint leaf meal at graded levels on the body weight gain, feed conversion ratio, immune competence traits and meat composition of coloured chicken.

Materials and Methods

Fresh mint leaves were harvested during early morning from the mint plant. The harvested leaves were thoroughly washed in running tap water twice to remove the presence of dirt or surface impurities if any. Fresh leaves were dried under shade in dust free environment to obtain fine powder. The dehydrated leaves were crushed to powder using dry grinder. The powder formed was kept in airtight container. Representative samples of ground mint leaves were analyzed for their nutrient composition *viz.* dry matter, crude protein, and total ash as per A.O.A.C (2005).

Birds and Feed

Two hundred and ten, day old straight run coloured chicks (Chabro) were randomly distributed into seven dietary treatments comprising three replicates and ten chicks in each replicate. The feed was offered *ad lib* in equal quantity in different experimental groups. The birds of the control group, T1 were fed basal or control diet; broiler starter diet till 4 weeks and thereafter broiler finisher diet till eight weeks), T2-T1+0.25% Mint leaf meal (MLM), T3-T1+0.5% MLM, T4- T1+0.75% MLM, T5- T1+1% MLM, T6-T1+1.25% MLM, T7- T1+1.5% MLM. The birds were housed in deep litter system. Water was offered *ad lib*.

Growth Performance

Weekly body weight of birds and group wise feed consumption was recorded. Thereafter, phase wise (0-4 weeks, 4-8 weeks and 0-8 weeks) body weight gain and phase wise feed conversion ratio were calculated at the end of the experiment.

Antibody Response to Sheep Red Blood Cells (SRBC)

The microtitre plate haemagglutination procedure as described by (Siegel and Gross, 1980) with slight modifications was followed to measure total HA antibody titres in chabro chicks on day zero and day 5 post injection. The procedure followed is described below.

Preparation of Sheep Red Blood Cells (SRBC) Suspension

Blood from jugular vein was collected from healthy sheep in Alsever's solution. The red blood cells were washed thrice in PBS (phosphate buffer saline, pH 7.2). Finally, 1% suspension of SRBC in PBS (V/V) was prepared.

Immunization and Harvest of Immune Serum

1 ml of 1% (V/V) of SRBC suspension was injected to 6 birds of each treatment group at 8 weeks of age. About 3

ml of blood on 0 and 5th day post immunization (dpi) were collected from jugular vein. The blood was endorsed to clot in an incubator having temperature of 37°C for 1 hour. The blood was endorsed to retract after detaching it from sides of its container and left at 4°C. Centrifugation of blood was carried out at 2000rpm for 5-10 minutes as it facilitated rapid collection of serum. The antibody titer was determined by HA methods (Vander Zijpp, 1983; Siegel and Gross, 1980). Antibodies were determined by means of a mercaptoethanol (ME) HA test as per the method described by Martin *et al.* (1989) with slight modification.

***In vivo* Cell Mediated Immune Response**

The cellular immune response was assessed by cutaneous basophilic hypersensitivity test *In vivo* by using PHA-P (Phytohaemagglutinin, lectin from *Phaseolus vulgaris*) (Corrier and De Loach, 1990). Coloured chicken were injected intra-dermally between 3rd and 4th toe of the right foot with 0.1 mg PHA-P in 0.1 ml of PBS (1 mg PHA-P/ml of PBS) at 8 weeks of age. The left foot received 0.1 ml of PBS and served as control. The thickness of inter-digital skin was measured using micrometer (AMES) at 0 and 24 hr after injection. The skin swelling was calculated by subtracting the skin thickness at 0 hr from that of after 24 hours of injection. The foot web index (FWI) was determined as the difference between inter-digital swelling values of PHA-P injected and control foot.

Analysis of Proximate Principles of Breast (*Pectoralis major*) and Thigh (*Iliotibialis*) Muscle of Coloured Chicken

After 8 weeks of age, 6 birds from each treatment group (3 male and 3 female) were sacrificed and there after fresh samples of breast (*Pectoralis major*) and thigh (*Iliotibialis*) muscles were processed and analyzed for moisture, crude protein (CP), ether extract (EE), total ash, calcium and phosphorous (A.O.A.C., 2005).

Statistical Analysis

Data were subjected to one-way analysis of variance in a completely randomized design (Snedecor and Cochran, 1994) using Statistical Package for the Social Sciences (S.P.S.S., 2011). Homogenous subsets were separated using multiple range test described by Duncan (1955). Differences among treatments were considered to be significant when $P \leq 0.05$.

Results and Discussion

Proximate Analysis of Feed and Mint Leaf Meal

The proximate composition of broiler starter and finisher feed and mint leaf meal have been presented in Table 1. The proximate values of mint leaf meal were in order as reported by Mainasara *et al.* (2018). Broiler starter and finisher feeds were adequate in all the nutrients as per B.I.S (2007).

Table 1: Proximate analysis of broiler starter feed, broiler finisher feed and mint leaf meal

Category	Dry Matter %	Total Ash %	Ether Extract %	Calcium %	Phosphorous %	Protein %	Crude Fibre %
Mint leaf meal	18.5	22	5	0.044	0.324	7.64	9
Broiler Starter feed	88.2	5.38	3.18	1.17	0.67	21.88	3.54
Broiler Finisher feed	88.2	4.9	2.9	1.11	0.57	17.62	3.9

Phase wise Body Weight Gain

The body weight gain of coloured chicken during 0-4 weeks, 4-8 weeks and 0-8 weeks of growth phases has been tabulated in Table 2. During 4-8 weeks, T4 group birds had significantly higher ($P < 0.01$) body weight gain than T3, T5 and T6 group birds and apparently higher body weight gain than T1, T2 and T7. During 0-8 weeks, T4 group birds had significantly higher ($P < 0.01$) body weight gain than T3, T5 and T6 and apparently higher body weight gain than T1, T2 and T7. Isha *et al.* (2018) reported that chickens fed diet supplemented with 2% spearmint had significantly lower body weight gain compared to control or fed spearmint at lower concentrations. Further, Ocak

et al. (2008) reported that 0.2% dry peppermint leaves had a higher growth promoting efficacy at an early stage of broilers' life.

Table 2: Effect of dietary supplementation of mint leaf meal on the body weight gain (g) of coloured chicken at different phases of growth

Treatment	0-4 weeks	4-8 weeks	0-8 weeks
T1	562.53	1121.60 ^{bcd}	1684.13 ^{cd}
T2	539.73	1119.48 ^{bcd}	1659.21 ^{bcd}
T3	530.6	1068.93 ^{ab}	1599.53 ^{ab}
T4	539	1179.60 ^d	1718.60 ^d
T5	543.46	1004.21 ^a	1547.68 ^a
T6	533.97	1091.05 ^{bc}	1625.02 ^{bc}
T7	532.46	1155.85 ^{cd}	1688.31 ^{cd}
Pooled SEM	3.2	14.09	14.25
Significance Level	NS	P<0.01	P<0.01

Means bearing different superscripts within a column differ significantly ($P<0.05$); NS: Not Significant ($P>0.05$), SEM: Standard Error of Means

Phase-wise Feed Conversion Ratio

The feed conversion ratio (FCR) of coloured chicken at different phases 0-4 weeks, 4-8 weeks and 0-8 weeks of growth phase have been tabulated in Table 3. During 0-4 weeks of age, FCR was significantly better ($P<0.01$) in control group compared to other treatment groups. During 4-8 weeks of age, FCR was significantly better ($P<0.01$) in T4 compared to T3, T5, T6 and comparatively better than T1, T2 and T7. However, during 0-8 weeks of age, FCR was significantly better ($P<0.01$) in T1 than T3, T5, T6, T7 and comparatively better than T2 and T4. These results clearly indicate that FCR was significantly better in the 0.75% mint leaf meal (supplemented) group compared to the control group after 4th week. Contrary to these results, Isha *et al.* (2018) reported that supplementation of different levels of spearmint to the diets of broiler chicks improved feed conversion ratio. However, Ocak *et al.* (2008) reported that there was no significant difference in FCR of broilers fed diets supplemented with 0.2% dry peppermint leaves compared to the control group.

Table 3: Effect of dietary supplementation of mint leaf meal on the FCR of coloured chicken at different phases of growth

Treatment	0-4 weeks	4-8 weeks	0-8 weeks
T1	1.89 ^a	2.32 ^{ab}	2.17 ^a
T2	2.06 ^b	2.37 ^{abc}	2.27 ^{ab}
T3	2.15 ^c	2.50 ^c	2.38 ^c
T4	2.16 ^c	2.20 ^a	2.25 ^{ab}
T5	2.17 ^c	2.68 ^d	2.50 ^d
T6	2.24 ^d	2.47 ^{bc}	2.40 ^c
T7	2.30 ^d	2.33 ^{ab}	2.32 ^{bc}
Pooled SEM	0.02	0.03	0.02
Significance Level	P<0.01	P<0.01	P<0.01

Means bearing different superscripts within a column differ significantly ($P<0.05$); NS: Not Significant ($P>0.05$), SEM: Standard Error of Means

Humoral Immune Response

Data on humoral immune response revealed that HA titre was significantly higher ($P<0.01$) in 0.75% MLM supplemented group as compared to other treatment groups (Table 4). Similarly, IgG response was better in T4 group as compared to the other treatment groups 8 weeks of age. Further, IgM response was significantly higher ($P<0.01$) in T4 compared to T1 and apparently higher than other treatment groups. These findings are in agreement with the results of Al Ankari *et al.* (2004), who reported that use of herbal mint (*Mentha ongifolia*) in broiler chicken diets increased antibody titre. This may be due to the various essential oils *viz.* menthol, mentone, cavone, methyl acetate and piperitone present in mint leaf meal. It has already been reported that peppermint oil protect the

structural integrity of immune cells due to its strong antioxidant action which protects cell membrane from free radical oxidants, thereby resulted in improved immune response (Nickels, 1996). According to Mekay and Blumberg (2006), peppermint oil has a significant antimicrobial, antitumor, antiviral, immunomodulating and chemo-preventive potential. Awaad *et al.* (2010) reported that peppermint oil has potent immunomodulatory impact in broiler chicks.

Cell Mediated Immune Response

The cell mediated immune response to PHA-P was significantly higher ($P < 0.01$) in 0.75% dietary supplemented group compared to other treatment groups (Table 4). Awaad *et al.* (2010) noted that peppermint oil has the ability to improve innate cell mediated immune response in broiler chicks.

Table 4: Effect of dietary supplementation of mint leaf meal on the humoral immune responses [antibody (log 2) values] to 1% SRBC and cell mediated immune response (Foot Web Index) to PHA-P in coloured chicken at 8 weeks of age

Treatment	Total immunoglobulins	IgG	IgM	Foot web index
T1	6.33 ^a	2.1	4.25 ^a	0.31 ^a
T2	10.00 ^b	2.25	7.75 ^b	0.39 ^b
T3	8.75 ^b	2.37	6.37 ^b	0.38 ^b
T4	10.75 ^a	3.59	7.16 ^b	0.49 ^c
T5	9.37 ^b	2.62	6.75 ^b	0.43 ^b
T6	10.25 ^b	3.12	7.12 ^b	0.45 ^b
T7	9.25 ^b	3.37	5.87 ^{ab}	0.37 ^b
Pooled SEM	0.23	0.19	0.26	0.01
Significance Level	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$

Means bearing different superscripts within a column differ significantly ($P < 0.01$); NS: Not Significant ($P > 0.05$), SEM: Standard Error of Means

Proximate Analysis of Breast (*Pectoralis major*) Muscle

Results pertaining to yield of proximate analysis of breast (*Pectoralis major*) muscle of the carcass at 8th week of age have been presented in Table 5. Percent ether extract was significantly lower ($P < 0.01$) in T6 than other treatment groups T1, T2, T3, T4, T5. Further, percent calcium and phosphorus were significantly lower ($P < 0.01$) in T5 than T1, T2, T3, T4, T6 and T7. Contrary to the results obtained in present study, Al-Ankari *et al.* (2004) reported that fat contents in breast and thigh muscles were not influenced by wild mint feeding in broiler diet.

Table 5: Effect of dietary supplementation of mint leaf meal on the proximate analysis of breast (*Pectoralis major*) muscle of coloured chicken at 8 weeks of age

Treatment	Moisture (%)	Protein (%)	Total ash (%)	Ether extract (%)	Calcium (mg/100g)	Phosphorous (mg/100g)
T1	71.94	20.87	1.60 ^{ab}	0.23 ^e	3.30 ^b	171.95 ^c
T2	71.41	21.27	1.62 ^{ab}	0.21 ^d	4.33 ^d	192.09 ^d
T3	71.49	23.71	2.00 ^d	0.19 ^c	4.69 ^e	196.27 ^e
T4	72.17	22.3	1.88 ^c	0.17 ^b	4.57 ^e	217.77 ^f
T5	71.3	18.28	1.71 ^b	0.20 ^d	2.70 ^a	156.04 ^a
T6	71.83	20.75	1.68 ^b	0.16 ^a	3.45 ^c	168.02 ^b
T7	71.49	21.14	1.54 ^a	0.18 ^{bc}	4.41 ^d	283.27 ^g
Pooled SEM	0.18	0.6	0.03	0	0.13	7.64
Significance level	NS	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

Means bearing different superscripts within a column differ significantly ($P < 0.05$); NS: Not Significant ($P > 0.05$), SEM: Standard Error of Means

Proximate Analysis of Thigh (*Iliotibialis*) Muscle

Results pertaining to yield of proximate analysis of thigh (*Iliotibialis*) muscle at 8 weeks of age have been presented in Table 6. Percent ether extract in thigh (*Iliotibialis*) muscle of coloured chicken at 8 weeks of age was significantly higher ($P<0.01$) in T1, T2 and T3 as compared to T4, T5, T6 and T7 treatment groups. Percent calcium was significantly higher ($P<0.01$) in T1 than T4 and T5 groups. Percent phosphorus was significantly higher ($P<0.01$) in T1, T2, T3 and T4 as compared to T5 and T6. Since, studies on effect of dietary supplementation of mint leaf meal on the composition of breast and thigh muscle are limited; discussion on this aspect comparing reports of other studies was not feasible.

Table 6: Effect of dietary supplementation of mint leaf meal on proximate analysis of thigh (*Iliotibialis*) muscle of coloured chicken at 8 weeks of age

Treatment	Moisture (%)	Protein (%)	Total ash (%)	Ether extract (%)	Calcium (mg/100g)	Phosphorous (mg/100g)
T1	70.07	1.41	1.32 ^a	1.41 ^c	6.08 ^e	235.26 ^{bc}
T2	69.49	1.49	1.39 ^a	1.49 ^c	5.38 ^{cde}	254.80 ^c
T3	69.59	1.39	1.44 ^a	1.39 ^c	5.56 ^{de}	241.30 ^c
T4	71.01	1.26	1.39 ^a	1.26 ^b	4.32 ^{ab}	230.19 ^{bc}
T5	19.61	1.15	1.30 ^a	1.15 ^b	3.68 ^a	181.91 ^a
T6	20.25	1.01	1.65 ^b	1.01 ^a	4.99 ^{bcd}	183.67 ^a
T7	71.19	1.23	1.47 ^{ab}	1.23 ^b	4.47 ^{abc}	212.07 ^b
Pooled SEM	0.03	0.75	0.03	0.75	0.17	5.84
Significance level	NS	NS	$P<0.05$	$P<0.01$	$P<0.01$	$P<0.01$

Means bearing different superscripts within a column differ significantly ($P<0.05$); NS: Not Significant ($P>0.05$), SEM: Standard Error of Means

Conclusion

Thus, it may be concluded that dietary supplementation of 0.75% mint leaf meal in coloured chicken diet resulted in better growth performance. Further, dietary supplementation of 0.75% mint leaf meal resulted in significantly higher humoral and cell mediated immune response in coloured chicken. In addition, dietary supplementation of 1.25% mint leaf meal resulted in decrease in fat content of breast and thigh meat cuts in coloured chicken.

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Conflict of Interests

There is no conflict of interest.

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