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EFFECT OF DRIED *MORINGA OLEIFERA* LEAVES ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILERS

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ABSTRACT

Rising prices for oilseed meals and expected feed crises due to rapid increase in human and livestock population motivate nutritionists to look for good quality alternative protein sources. For this purpose, a study was conducted to evaluate the effects of *Moringa oleifera* leaf meal on growth performance and immune response in broilers. Total 300-day-old broiler chicks were divided into 15 replicates having 20 chicks each. After procuring and determining the chemical composition of *Moringa oleifera* leaf meal, five iso-nitrogenous and iso-caloric experimental diets were formulated. Group A was considered as control and given 0% MOLM, while, group B, C, D and E were fed on 5% MOLM, 10% MOLM, 15% MOLM and 20% MOLM, respectively. Data on feed intake, body weight were recorded on weekly basis and feed efficiency was calculated. The obtained data were subjected to one-way analysis of variance (ANOVA) and means were compared through Duncan's Multiple Range (DMR) test. Results revealed that body weight of birds on diet 0%, 10% and 15% MOLM were significantly higher ($P \leq 0.05$) than others while, control group consumed significantly higher feed intake. On the other hand, 15% MOLM showed significantly improved mortality (%) and antibody titer against NDV while, feed efficiency was not affected by any treatment. It can be concluded that an inclusion level of 15% MOLM in diet improves the overall growth performance and immunity in broilers.

Keywords: Broilers, *Moringa oleifera*, Leaves, Growth performance, Immunity.

INTRODUCTION

The hike in the prices of conventional feed ingredients is a major factor affecting net return from the poultry business. This is because 70-75% of the total cost of poultry operation is incurred on feed (Mahmood *et al*., 2005). It has forced animal nutritionists to explore the options for utilizing non-conventional feedstuffs in poultry diets (Swain *et al.*, 2017; Elbashier and Ahmed, 2016). Their use in the diets not only helps reduce feed cost but also minimize the direct competition between man and the livestock industry for conventional feedstuffs (Onu and Aniebo, 2011).

Using leaf sources as a protein ingredient in broiler's diet is getting attention due to availability, abundance and relatively reduced cost (Onyimonyi and Onu, 2009). *Moringa oleifera* is the most widely cultivated species of a mono-generic family, the Moringaceae, inhabitant of sub-Himalayan tracts of Pakistan, India, Bangladesh and Afghanistan (Mughal *et al.*, 1999). Qaiser (1973) reported that in Pakistan, only two species of Moringa i.e. *Moringa oleifera and Moringa concanesis* are present which is locally known as "Sohanjna" and widely grown in Punjab and Sindh provinces of Pakistan. It is one of the most promising crops enriched in micronutrients with good antioxidant potential that ultimately strengthen immune system (Yang *et al.* 2006). Along with that it has high quality protein comprised of significant quantities of all the essential amino acids (Foidl and Paull, 2008). The present study was planned to explore nutritional potentials of *Moringa oleifera* leaf meal on the performance, mortality and immune response in broilers.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Unit of Poultry Production, University of Veterinary and Animal Sciences, Ravi Campus Pattoki. Five experimental diets were formulated comprised of 0% *Moringa oleifera* leaf meal (MOLM) which served as the control, while, the other four diets contained 05%, 10%, 15% and 20% MOLM. Ingredients and nutrient composition of the ration is presented in Table 1. Three hundred-day-old (Hubbard) broiler chicks were purchased from the local market and were divided randomly into five groups having 60 birds each. Experimental diets were allocated to the groups according to Completely Randomized Design (CRD). Each group was further subdivided into three replicates of 20 birds each. Birds were housed on deep litter floor system and given optimal conditions of temperature, humidity and ventilation in accord to breed recommendations. Experimental diets and clean drinking water were supplied *ad-libitum* throughout 42 days of the study. The data regarding different growth parameters:

feed intake, body weight gain, feed efficiency and mortality percentage was maintained on weekly basis.

Preparation of Leaf Meal: The green leaves of *Moringa oleifera* were collected from different areas of South Punjab, Pakistan. The leaves were air dried in shady place to avoid the bleaching and vitamin C losses (depletes in sun-drying). After drying, ground and stored in airtight polythene bags in dark cool place to use for chemical analysis and biological trials.

Chemical Analysis: The following chemical analyses of MOLM were performed at University of Veterinary and Animal Sciences, Nutrition and WTO laboratories, following the standard procedures (AOAC, 2005).
Proximate analysis. Mineral analysis (Atomic absorption spectrophotometer; Perkin Elmer AA400), Gross energy (Bomb Calorimeter; Ika-werke-c2000). Metabolizable energy by applying equation (NRC, 1994) and Amino Acid profile (Perkin Elmer series 200).

Antibody titers against Newcastle disease: At the end of trial 3 birds per replicate were randomly picked for blood collection. 5ml blood was collected in syringes containing anticoagulant and then centrifuged at 1500 rpm for 15 minutes to get plasma. The hemagglutination inhibition test was performed (Ghahramani *et al.*, 2014) to examine the effects of different MOLM levels on antibody titers against Newcastle disease.

Statistical Analysis: The data thus collected on various parameters were subjected to one-way analysis of variance (ANOVA) under Completely Randomized Design (CRD) through SAS (2004). The difference in treatment means were separated using Duncan's Multiple Range (DMR) Test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of MOLM: Proximate analysis showed that MOLM contained 89% dry matter, 23% crude protein, 3.7% ether extracts, 11% crude fiber, 15% ash, 4215 kcal/kg gross energy and 1880 kcal/kg metabolizable energy (Table 2). The dry matter value for the *Moringa oleifera* leaves was lower than the values 93.70 and 96.70 % reported by Olugbemi *et al.* (2010b) and Affiku and Obge (2012), respectively. Yameogo *et al.* (2011) reported that *Moringa oleifera* leaves contained 27.20% protein, 5.90% moisture, 17.10% fat, and 38.60% carbohydrates on dry matter basis, while, Affiku and Obge (2012) reported 17.01% crude protein, 3.11% carbohydrates and appreciable amount of crude fiber 7.09%, ash 7.93% in *Moringa oleifera* leaf meal. Differences observed between the results of present and previous studies could be attributed to variation in geographical conditions, soil composition, cultivation climate, drying method of leaves and the extraction methods used.

The mineral profile of *Moringa oleifera* leaf meal (Table 2) showed that it contained calcium 1.768%, phosphorus 0.067%, sodium 0.368%, potassium 0.880%, iron 0.098% and zinc 0.0456%. Affiku and Obge (2012) reported that MOLM contained calcium 1.91%, phosphorus 0.03%, sodium 0.192%, potassium 0.97%, iron 0.107% and zinc 0.060%. Moyo *et al.* (2011) observed that dried *Moringa oleifera* leaves contained calcium 3.25%, phosphorus 0.3%, sodium 0.164%, potassium 1.50%, iron 0.049% and zinc 0.003%. Anjorin *et al.* (2010) reported very high levels of calcium 3.463% and 3.827% in lamina, 2.801% and 3.734% in petiole whereas iron was founded lower in lamina i.e. 0.041% and 0.0788% but higher in petiole in one of the two regions i.e. 0.047% and 0.144%. Variation in mineral composition of MOLM observed in the present and other researchers could be attributed to different origin and sources of MOLM.

The amino acid profile of *Moringa oleifera* leaf meal (Table 2) showed presence of essential amino acids such as methionine (0.37%), lysine (1.32%), leucine (1.95%) and iso-leucine (0.825%) which are lower than that reported by Moyo *et al.* (2011) in dried *Moringa oleifera* leaves as methionine (0.297%), lysine (1.63%), leucine (1.96%) and iso-leucine (1.17%) level.

Productive Performance: The data on growth performance of chickens fed different levels of MOLM (Table 3) showed that final body weight in control, 10% and 15% MOLM groups were highest and non-significant ($P>0.05$) among the groups whereas 5% and 20% MOLM were significantly ($P<0.05$) lower.. Similar results have been reported by Younis *et al.* (2016) and Onunkwo and George (2015) where they found no significant difference in growth performance when MOLM different levels were offered to broilers. The reduced weight gain of birds fed 20% MOLM in comparison to other diets could be ascribed somewhat to the higher crude fiber content which can impair nutrient digestion and absorption (Olugbemi *et al.*, 2010a). The lower weight gain of birds in the highest MOLM group, despite its higher protein content, might also be due to the negative effect of the anti-nutritional factors prevailing in it. *Moringa oleifera* contains 1-23g of tannin per kilogram of leaves and tannins interfere with the biological utilization of protein (Tijani *et al.,* 2016; El-Tazi, 2014;). This conforms to general observations made by Zanu *et al.* (2011) and Olugbemi *et al.* (2010a) that inclusion of leaf meal in broiler diets at higher levels results in depressed growth performance. The lower body weight in 5% MOLM group was probably a result of some mistake during data collection, since all the reviews had concluded an improved weight gain (Ayo-Ajasa, 2016; Elbashier and

Ahmed, 2016; Mahmud *et al.,* 2016) or non-significant differences (Alnidawi *et al.,* 2016) between control and 5% MOLM groups.

Results of the present trial showed a significant (P<0.05) variation in the broiler feed intake among different treatments. The birds in control group consumed more feed, while those having 15% MOLM consumed significantly lower feed as compared to other treatments. Tesfaye *et al.* (2012) reported a decrease in feed intake when broilers were fed rations in which soybean meal was replaced with MOLM. They concluded that the decrease in feed intake might be due to the un-palatability of experimental diets. Better growth rate and feed efficiency even with lower feed intake may attribute to its good nutritional profile having all essential amino acids and superior protein content which were efficiently metabolized for growth (El-Tazi, 2014). In accordance with the findings of present study, Portugaliza and Fernandez (2012) also reported higher feed intake in control group as compared to those supplemented with *Moringa oleifera* aqueous extract in broilers. This pattern could be due to the considerable amount of Vitamin C in MOLM (Ayssiwede *et al.*, 2011), which is also in agreement with Rajput *et al.* (2009) who reported the significant lower feed consumption in broilers supplemented with vitamin C in feed. Whereas, 20% MOLM group showed higher feed intake than 15% especially when feed intake was linearly going down with increasing levels of MOLM. This can be due to slightly lower energy levels in 20% MOLM group. Although feeds were formulated to be iso-calroic, but due to forced inclusion with 20% MOLM, metabolizable energy for this group was 30-40 kcal short. Therefore, birds consumed more feed to fulfill their energy requirement. Infante-Rodriguez *et al.* (2016) did a trial and found that feeds having lower energy levels increases the feed intake. The findings of present study are in contrast with those of Moustafa *et al.,* (2015) and Melesse *et al.* (2011) who reported higher feed intake with increasing levels of *Moringa* leaf meal in the diet of Quails and Rhode Island Red chicks. This could be due to the use of different breeds, *Moringa* leaf meal of different origins and using different inclusion levels in the feed. As far as feed efficiency is concerned non-significant (P>0.05) differences were observed when birds are fed at different levels of MOLM.

Antibody titers against Newcastle disease: The experiment results revealed that the inclusion of MOLM in broiler diets had a significant (P<0.05) effect on the antibody titer against NDV (Table 3). Among dietary treatments, the highest antibody titer against NDV was recorded in birds that received diet with 15%MOLM. It has been reported that *Moringa oleifera* leaf meal supplementation in broiler diets can boost antibody titer in birds against diseases (Du *et al.*, 2007). Increased antibody titer in the birds using MOLM may be due to the presence of lectin in the leaves of Moringa that also transform the body's defense system (Fuglier, 1999) and therefore *Moringa oleifera* is claimed to be an immune-boosting plant, which helps the body to build up its defense against diseases.

The inclusion of *Moringa oleifera* leaf meal in diets showed significantly (P<0.05) lower mortality percent than control (Table 3). These results are in line with Dey and De (2013) who noticed significantly reduced mortality in *Moringa* leaf meal supplemented diets. This improvement probably due to presence of antioxidants in the leaves which enhances the immunity (Yang *et al.,*2006). The findings of the present study suggest that dietary MOLM inclusion at level of 15 percent may prove more appropriate for obtaining optimum weight gain with better feed efficiency, less mortality percentage and better antibody response against NDV in broilers.

Table 1. Composition of Broiler feeds with and without different MOLM levels.

Ingredients	0% MOLM	5% MOLM	10% MOLM	15% MOLM	20% MOLM
Maize	61.11	57.22	57.78	55.20	51.80
Soybean meal	25.00	30.56	28.33	24.14	23.50
Dicalcium phosphate	1.17	1.28	1.25	1.35	1.39
Threonine	0.11	0.13	0.15	0.15	0.17
Oil	0.00	0.00	0.00	1.30	1.70
Lysine Sulfate	0.64	0.36	0.32	0.28	0.03
Premix	0.32	0.33	0.28	0.28	0.28
Salt	0.33	0.33	0.35	0.35	0.36
MHA liquid	0.32	0.36	0.35	0.35	0.39
MOLM	0.00	5.00	10.00	15.00	20.00
Chips	1.11	0.83	0.60	1.00	0.38
Sunflower meal	7.78	0.00	0.00	0.00	0.00
Molasses	2.11	3.61	0.60	0.60	0.00

Note: The rations were formulated on energy/protein ratio ME=2800 kcal/kg and CP=19%

Table 2. Chemical composition of MOLM.

Sr. No.	Proximate composition	%age	Mineral composition	%age
1.	Dry Matter	89	Calcium	1.768
2.	Crude protein	23	Phosphorus	0.067
3.	Ether Extract	3.7	Sodium	0.368
4.	Crude Fiber	11	Potassium	0.880
5.	Ash	15	Iron	0.098
6.	Gross Energy	4215 kcal/kg	Zinc	0.0456
7.	Metabolizable energy	1880 kcal/kg		
Sr. No.	**Essential Amino Acids composition**	**%age**	**Non-Essential Amino Acids composition**	**%age**
1	Valine	1.07	Aspartic acid	3.83
2	Methionine	0.37	Tyrosine	3.71
3	Iso-leucine	0.825	Serine	0.45
4	Leucine	1.950	Glutamic acid	2.97
5	Threonine	1.10	Proline	1.741
6	Phenylalanine	1.398	Glycine	2.569
7	Histidine	0.613	Alanine	3.16
8	Tryptophan	0.425	Cysteine	1.58
9	Lysine	1.32		
10	Arginine	1.34		

Table 3. Cumulative feed intake (g), body weight (g), feed efficiency and mortality (%) as affected by different MOLM levels in broilers.

Levels Performance	Control	05% MOLM	10% MOLM	15% MOLM	20% MOLM
Cum. Wt.	2310.2±1.15^{a}	2287.8±3.52^{b}	2305.6±2.44^{a}	2304.2±3.59^{a}	2285.6±2.87^{b}
Cum. FI	4476±1.87^{a}	4454±3.42^{b}	4448±5.61^{b}	4426.4±1.53^{c}	4453.2±4.39^{b}
Feed Efficiency	0.516±0.0007	0.513±0.0005	0.518±0.0002	0.520±0.0006	0.513±0.0004
Mortality (%)	2.876±0.03^{a}	2.82±0.01^{ab}	2.73±0.02^{c}	2.597±0.03^{d}	2.754±0.02^{c}

Means with different letters in rows differ significantly (P<0.05)

Table 4. N.D Antibody titers as affected by different MOLM levels in broilers

MOLM Levels	N.D Titers
Control Group (0 %)	4.612±0.032^{ab}
05 %	4.452±0.067^{c}
10 %	4.482±0.067^{bc}
15 %	4.676±0.016^{a}
20%	4.62±0.021^{ab}

Means with different letters in columns differ significantly (P<0.05)

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