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RESEARCH ARTICLE

Influence of *Moringa oleifera* Decorticated Seed Meal on Broiler Performance and Immunity

Kaleem Ul Hassan[1], Anjum Khalique[1], Talat Naseer Pasha[1], Muhammad Akram[2], Shahid Mahmood[2], Abdul Waheed Sahota[2], M. Saeed Imran[3] and Gulbeena Saleem[3]*

[1]Department of Animal Nutrition; [2]Department of Poultry Production, [3]Department of Pathology, University of Veterinary and Animal Sciences, Pakistan
*Corresponding author: gulbeena.saleem@uvas.edu.pk

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A B S T R A C T

The present study was conducted to evaluate the performance of broilers by providing different dietary levels of *Moringa oleifera* seed meal (MOSM). A total of 300 a-day-old Hubbard broiler chicks were maintained in 5 different treatment groups (control, 10, 15, 20 and 25%) with 3 replicates of 20 birds each. Feed and water offered *ad-libitum* throughout 42 days. Data on weekly basis were recorded for feed intake, body weight gain, feed conversion ratio, mortality and immune response. For statistical analysis ANOVA in Completely Randomized Design was used and means were compared using Duncan's Multiple Range test. Significant ($P<0.05$) differences were observed in feed intake, body weight, FCR and immune response of the broilers. Birds from control group exhibited significantly ($P<0.05$) better FCR by gaining more weight and consuming less feed and showed lower mortality percentage with better antibody titer against Newcastle Disease. A negative correlation between the higher levels of MOSM and performance of broilers was observed.

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INTRODUCTION

In developing countries, average daily protein intake in human diets is well below recommended standards. Poultry production is playing a major role to fill this gap. However, the poultry production in the tropics and developing countries has been challenged by the shortage and high prices of poultry feeds which makes up to 60-80% of the total production cost for broilers (Wilson and Beyer, 2000; Khatoon *et al*., 2016). Researchers have great interest in finding natural growth promoters to enhance poultry production and to reduce feed cost.

Plant products have been used for centuries by humans as food and to treat ailments. Natural medicinal products originating from herbs and spices have also been used as feed additives for farm animals (Guo, 2003). Among the ingredients, protein supplements are very expensive; therefore, it is necessary to look for alternative sources available locally for use as a protein supplement in poultry feed.

In many tropic and sub-tropic countries *Moringa oleifera* is a plant that possesses multiple advantages, because different parts of tree (leaves, fruits, immature pods and flowers) are edible and entered in traditional diets (Siddhuraju and Becker, 2003; Anhawange *et al.*, 2004; Ijaz *et al*., 2016; Olasehinde *et al*., 2016). The *Moringa oleifera* pods are pendulous, brown, triangular, splitting lengthwise into 3 parts when dry, 30 – 120 cm long, 1.8 cm wide, containing about 20 seeds embedded in the pith, pod tapering at both ends, 9-ribbed; seeds dark brown, with 3 papery wings (Chatterjee and Prakash, 1994). Anwar and Rashid (2007) reported that on the basis of dry matter, *Moringa oleifera* seeds contained ether extract 34.80%, protein 31.65%, fiber 7.54%, moisture 8.90% and ash contents 6.53%. Makkar and Becker (1997) noticed that the amount of sulfur containing amino acids of kernel were higher than the amino acid pattern of the FAO reference protein, but other essential amino acids of the kernel were deficient. Compaore *et al*. (2011) reported that *Moringa oleifera* seeds are good source of fats, proteins and minerals. *Moringa* seed meal after oil-extraction contains up to 58.6% crude protein on dry matter basis (Fuglie, 2001) which improves the growth of young broilers (Toye *et al*., 2013).

Makker and Becker (1997) also reported about the anti-nutritional factors and found that whole kernels, ground kernels (meal), extracted kernels and extracted meal had low levels of saponins, whereas, trypsin and amylase inhibitors were absent. However, hemolytic activity was observed in kernels and extracted kernels. Talha and Ahmed (2012) published the research with *Moringa oleifera* un-decorticated seed powder in broilers and Walter *et al*. (2011) noticed that *Moringa oleifera* n-hexane extracted seeds created inhibitory effects on *Salmonella typhii* and *Escherichia coli*. In Pakistan, the *Moringa oleifera* is surplus available which can be easily utilized in poultry feed, however this plant has never been tested in Pakistan in past, therefore, this study was conducted to examine the effects of *Moringa oleifera* decorticated and de-oiled seed meal on the growth performance and the immune response of broilers.

MATERIALS AND METHODS

The study of 6-week duration was conducted at Poultry Research and Training center (PRTC), at University of Veterinary and Animal Sciences (UVAS), Ravi Campus Pattoki. A total of 300-day-old commercial (Hubbard) broiler chicks were allocated to 5 experimental groups; a control group (balanced diet-A) and 4 treatment groups with different levels of *Moringa oleifera* seed meal (MOSM) in feed i.e. groups B, C, D at 10, 15, 20 and 25 percent MOSM levels in feed (Table 1). These groups were further sub-divided into 3 replicates of 20 birds in each. They were maintained on deep litter system under optimum conditions of temperature, humidity and ventilation according to the recommendations of Hubbard management guide (2014). Birds were vaccinated against ND on days at 3rd, 13th, 24th and 30th as practiced in commercial operations due to heavy disease load in the area. Feed and water were provided *ad-libitum* throughout the experiment. The data regarding different growth performance such as feed intake, body weight gain, feed conversion ratio (FCR) and mortality percentage were recorded on weekly basis, along with the cumulative performance at the end of experiment.

Analysis seed meal: Samples of *Moringa oleifera* pods were collected from the trees spread in different areas of South Punjab, Pakistan. These pods were separated from leaves and opened to get seeds. These seeds were unshelled to get kernels. Oil from all the kernels was extracted through solvent extraction using hexane as solvent (AOAC, 2005).

Chemical analysis: The following chemical analysis of MOSM were performed following the standard procedures (AOAC, 2005) before the inclusion of seed meal in feed;

i. Proximate analysis
ii. Mineral analysis (Atomic absorption spectrophotometer; Perkin Elmer AA400)
iii. Gross energy (Bomb Calorimeter; Ika-werke-c2000)
iv. Metabolizable energy by applying equation (NRC, 1994)
v. Amino Acid profile (Perkin Elmer series 2000)

Table 1: Broiler feeds with different dietary *Moringa oleifera* seed meal levels

Ingredients	Control group	10% MOSM	15% MOSM	20% MOSM	25% MOSM
Maize	61.11	61	61	61	56.59
Soybean meal	25.00	14	7.9	1.8	0
Sunflower meal	7.78	8	9	10	5
DCP	1.17	1.2	1.2	1.2	1.2
L-Threionine	0.11	0.28	0.365	0.45	0.54
Lysine sulfate	0.64	0.6	0.575	0.55	0.47
Premix	0.32	0.44	0.42	0.4	0.38
Salt	0.33	0.25	0.25	0.25	0.1
MHA	0.32	0.13	0.115	0.1	0
MOSM	0.00	10	15	20	25
Chips	1.11	1.2	1.225	1.25	1.25
Molasses	2.11	2.8	2.85	2.9	9.2
Soda Bi-carbonate	0	0.1	0.1	0.1	0.27

Note: All the feeds were formulated based on energy/protein ratio ME=2800 kcal/kg and CP=19%

Table 2: Chemical analysis of *Moringa oleifera* seed meal

Sr. No.	Proximate analysis	%age	Mineral analysis	%age
1.	Dry Matter	92	Calcium	0.075
2.	Crude protein	44.14	Phosphorus	0.529
3.	Ether extract	18.9	Sodium	0.024
4.	Crude fiber	3.9	Potassium	0.049
5.	Ash	4.6	Iron	0.013
6.	Gross energy	4575 kcal/kg	Zinc	0.305
7.	Metabolizable energy	2140 kcal/kg		
Sr. No.	Essential amino acids analysis	%age	Non-essential amino acids analysis	%age
1	Valine	1.63	Aspartic acid	1.70
2	Methionine	0.8	Tyrosine	3.00
3	Iso-leucine	1.38	Serine	0.85
4	Leucine	2.33	Glutamic acid	3.48
5	Therionine	0.82	Proline	2.08
6	Phenylalanine	2.96	Glycine	2.14
7	Histidine	2.98	Alanine	1.70
8	Lysine	0.67	Cysteine	1.82

Antibody titers against Newcastle disease: On termination of trial, 3 birds per replicate were selected for blood collection. 5ml blood was collected in syringes without anticoagulant and then centrifuged at 1500 rpm for 15 minutes to get serum. The hemagglutination inhibition test was performed (Allan and Gough, 1974) to examine the effects of different MOSM levels on antibody titers against Newcastle disease.

Statistical analysis: The data produced from the experiment was analyzed using analysis of variance according to SAS Institute (1990). To further assess the significance between means, Duncan's multiple range tests was used as described by Little and Hills (1978).

RESULTS AND DISCUSSION

Chemical composition of MOSM: Proximate analysis of MOSM showed that there was 92% dry matter, 44.14% crude protein, 18.9% ether extracts, 3.9% crude fiber, 4.6% ash and gross energy was 4575 kcal/kg with metabolizable energy 2140 kcal/kg (Table 2). Bridgemohan and Knights (2012) reported that de-hulled *Moringa oleifera* seed meal contained crude fiber (3.01%), ash (4.3%) and crude protein (45.28%). Similarly, Compaore *et al*. (2011) reported that seeds of *Moringa oleifera* are particularly rich in proteins (35.37%), lipids (43.56%), ash (4.98%) and crude fiber

Table 3: Feed intake (g) of broilers as affected by different ***Moringa oleifera* seed meal** levels

Age (weeks) \ Levels	Control	10% MOSM	15% MOSM	20% MOSM	25% MOSM
1	142.4±1.16^{b}	147.6±1.02^{a}	146.8±1.88^{ab}	145.4±1.20^{ab}	145.2±2.03^{ab}
2	382±2.50	386.6±1.91	385.6±2.18	385.2±2.41	384.8±1.39
3	690.2±1.28^{b}	700.2±1.31^{a}	555.3±1.72^{c}	560.8±2.0^{c}	556.4±2.78^{c}
4	976.2±2.13^{a}	881.3±3.17^{b}	784.6±2.99^{c}	686.3±0.72^{d}	684.0±1.49^{d}
5	1049.6±2.61^{a}	946.9±2.02^{b}	843.2±1.80^{c}	682.5±1.82^{d}	684.5±0.45^{d}
6	1198.6±3.26^{a}	1083.6±2.32^{b}	967.2±2.16^{c}	780.6±2.12^{d}	783.6±0.56^{d}

Values (mean±SD) in a row with different letters differ significant (P<0.05).

Table 4: Body weight gain (g) of broilers as affected by different *Moringa oleifera* seed meal levels

Age (weeks) \ Levels	Control	10% MOSM	15% MOSM	20% MOSM	25% MOSM
1	119±1.09^{a}	116.8±0.86^{ab}	115.4±1.46^{ab}	116±1.30^{ab}	114.4±0.92^{b}
2	291±1.51	292.4±2.95	290.4±1.07	290±1.00	293.6±1.80
3	451.4±1.36^{a}	451.8±1.65^{a}	358.4±1.77^{b}	332.1±0.76^{c}	327.9±0.97^{d}
4	516.2±1.97^{a}	455.4±1.82^{b}	398.8±1.68^{c}	357.7±0.82^{d}	353.2±1.05^{d}
5	466.6±0.92^{a}	423.3±0.83^{b}	372±1.21^{c}	278.5±0.79^{d}	275.3±0.83^{e}
6	446.2±2.15^{a}	387.0±2.91^{b}	357.92±1.44^{c}	263.8±1.83^{d}	266.4±3.5^{d}

Values (mean±SD) in a row with different letters differ significant (P<0.05).

Table 5: Feed conversion ratio of broilers as affected by different *Moringa oleifera* seed meal levels

Age (weeks) \ Levels	Control	10% MOSM	15% MOSM	20% MOSM	25% MOSM
1	1.196±0.011^{b}	1.264±0.014^{a}	1.272±0.017^{a}	1.253±0.007^{a}	1.269±0.020^{a}
2	1.312±0.013	1.322±0.017	1.327±0.014	1.328±0.009	1.310±0.009
3	1.529±0.006^{b}	1.549±0.007^{b}	1.549±0.007^{b}	1.688±0.008^{a}	1.696±0.011^{a}
4	1.901±0.015	1.935±0.012	1.957±0.011	1.918±0.004	1.936±0.008
5	2.249±0.006^{cd}	2.236±0.008^{d}	2.266±0.006^{c}	2.540±0.011^{b}	2.486±0.006^{a}
6	2.686±0.011^{c}	2.801±0.020^{b}	2.702±0.005^{c}	2.958±0.019^{a}	2.943±0.037^{a}

Values (mean±SD) in a row with different letters differ significant (P<0.05).

Table 6: Mortality (%) of broilers as affected by different *Moringa oleifera* seed meal levels

Age (weeks) \ Levels	Control	10% MOSM	15% MOSM	20% MOSM	25% MOSM
1	1.044±0.005^{c}	1.082±0.003^{ab}	1.068±0.003^{b}	1.088±0.013^{ab}	1.092±0.006^{a}
2	0.424±0.016	0.430±0.011	0.434±0.022	0.436±0.013	0.428±0.014
3	0.368±0.026	0.370±0.012	0.362±0.009	0.368±0.024	0.366±0.040
4	0.290±0.014	0.318±0.016	0.306±0.015	0.330±0.016	0.306±0.014
5	0.262±0.014	0.254±0.019	0.262±0.014	0.262±0.017	0.268±0.012
6	0.376±0.015	0.355±0.018	0.362±0.016	0.336±0.014	0.368±0.014

Values (mean±SD) in a row with different letters differ significant (P<0.05).

Table 7: Cumulative feed intake (g), body weight (g), FCR and mortality (%) of broilers as affected by different *Moringa oleifera* **seed meal** levels

Age \ Levels	Control	10% MOSM	15% MOSM	20% MOSM	25% MOSM
Cum. FI	4439.0±4.25^{a}	4146.6±5.10^{b}	3678.8±6.08^{c}	3240.8±1.91^{d}	3238.6±4.52^{d}
Cum Wt.	2330.6±19.38^{a}	2164.7±6.53^{b}	1932.8±1.95^{c}	1677.4±2.89^{d}	1670.3±5.57^{d}
FCR	1.905±0.013^{b}	1.915±0.006^{b}	1.903±0.001^{b}	1.932±0.003^{a}	1.939±0.008^{a}
Mortality %	2.764±0.041	2.809±0.051	2.794±0.040	2.820±0.032	2.828±0.036

Values (mean±SD) in a row with different letters differ significant (P<0.05).

Table 8: Antibody titer against Newcastle disease of broilers as affected by different *Moringa oleifera* seed meal levels

Levels	N.D Titer level
Control	5.150±0.020^{a}
10% MOSM	5.114±0.021^{ab}
15% MOSM	5.110±0.021^{ab}
20% MOSM	5.094±0.016^{ab}
25% MOSM	5.080±0.010^{b}

Values (mean±SD) in a row with different letters differ significant (P<0.05).

(4.70%). The protein value of MOSM was greater in the present study than reported by Abdulkarim *et al*. (2005) 38.8% and Anwar *et al*. (2006) ranging from 29.6-31.3%. Seeds are a potentially good source of proteins which should be exploited to determine their commercially viability. Nzikou *et al*. (2009) have indicated crude fiber value of 3.2% while Anwar *et al*. (2006) have reported a range of 6.6-9%. Although crude fiber does not contribute to nutrients or energy, it is a source of dietary fiber. This value of fiber might be helpful in terms of maintaining positive effects on intestine and colon physiology (McPherson, 1982). Differences observed between the results of this and other studies could be attributed to variation in geographical conditions, soil composition, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction methods used.

Mineral analysis of *Moringa oleifera* seed meal showed that there was 0.075% calcium, 0.529% phosphorus, 0.024% sodium, 0.049% potassium, 0.013% iron and 0.305% zinc (Table 2). Anjorin *et al*. (2010) reported calcium (1.029%) and Iron (0.075%) levels in *Moringa oleifera* seed kernel powder. Compaore *et al*. (2011) reported calcium 0.078%, sodium 0.025% and potassium 0.048% in seeds of *Moringa oleifera* and Nzikou *et al*. (2009) reported calcium, potassium and sodium contents as 0.083%, 0.036% and 0.022%, respectively. Variation in mineral composition of MOSM observed in the present and other researchers could be attributed to different origin and sources of MOSM.

Essential amino acid profile of *Moringa oleifera* seed meal showed that it contained 1.63% valine, 0.8% methionine, 1.38% iso-leucine, 2.33% leucine and 0.67% lysine (Table 2). These results are in agreement with those of Bridgemohan and Knights (2012) who reported a little higher valine 1.65%, methionine 0.84%, iso-leucine 1.36%, leucine 2.36% and slightly lower lysine 0.64% in the de-hulled *Moringa oleifera* seed meal.

Growth performance: Weekly as well as Cumulative values for feed intake, body weight and feed conversion ratio were significantly ($P<0.05$) affected by different MOSM levels in feed (Table 7). Birds from the control group showed significantly ($P<0.05$) higher feed intake and body weight followed by 10, 15, 20 and 25% MOSM level in feed which resulted into significantly ($P<0.05$) improved FCR with less mortality percentage numerically. A negative correlation was observed within bird's performance and the levels of MOSM in feed; as the level of MOSM in feed increased, feed intake and body weight of the birds decreased. These findings are in line with those of Talha and Ahmed (2012) who reported reduction in body weight and depression in feed efficiency by addition of higher levels of *Moringa oleifera* undecorticated seed meal (MOUSM) to broiler diets due to the presence of phytate considered as anti-nutritional factor reducing the bioavailability of minerals and digestibility of starch and protein (Thompson, 1993).

Antibody titers against Newcastle disease: Non-significant ($P>0.05$) differences were observed among control and 10%, 15% and 20% MOSM levels in feed (Table 8), however, 25% MOSM level in feed showed significantly ($P<0.05$) lower antibody titers against Newcastle disease. Walter *et al*. (2011) suggested that MOSM has beneficial effects on *Salmonella* and *Escherichia* so, it should increase overall immunity. Variation between present study and hypothesis of other researcher might be due to *Salmonella* and *Escherichia* free feed and water offered during whole experiment. So, probable reason behind poor antibody titers against ND might be the presence of certain anti-nutritional factors of seed meal as reported by Makkar and Becker (1997).

Conclusions: The results of this study concluded that *Moringa oleifera* leaf meal usage in feed up to 10% level have no detrimental effects on broiler growth and this can be used as substitute protein source in replacement of expensive protein meal sources used in poultry feeds.

Author's contribution: AK, TNP and MA conceived and design the experiment, KUH and SM executed the experiment, MSI and GS analyzed the data, while AWS was involved at the time of write up. All authors approved the final version of the manuscript.

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