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Research Article

Influence of Feeding *Moringa oleifera* Pods as Phytogenic Feed Additive on Performance, Blood Metabolites, Chemical Composition and Bioactive Compounds of Breast Meat in Broiler

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Abstract

This study was conducted to explore the effect of *Moringa oleifera* pods meal (MPM) on growth performance, blood metabolites, chemical composition, meat quality and immunity of broilers. For the purpose, two hundred (Hubbard classic) broiler birds having weight 40.4±3.02 g, were assigned to four treatments with five replicates and ten birds per replicate in a Completely Randomized Design. Starter and finisher diets were added with four levels (0, 0.5, 1.0 and 1.5% of MPM) over and above. Results of this study showed that growth performance was improved as feed conversion ratio (FCR) and feed intake (FI) were decreased with the increase in supplementation level (P≤0.05). Dressing percentage was lowered, whereas giblet weights were improved with the MPM supplementation levels (P≤0.05). β-carotene, Quercetin and Selenium content of breast meat was linearly increased resulting in higher values of di-phenyl picryl hydrazil (DPPH) radical scavenging and improved shelf life. Serum biochemical compounds like serum glutamic pyruvic transaminase (SGPT), Creatinine, Glucose and meat cholesterol level was significantly decreased and was recorded lowest in 1.5% MPM supplemented diet (P≤0.05). At the end of the trial it was concluded that Moringa pods may positively affect the growth and chemical composition of broiler meat.

Keywords: *Broiler, β-carotene, DPPH, Moringa pods, Quercetin, Selenium*

Gıda Katkısı Olarak *Moringa oleifera* Kabukları İle Beslemenin Broiler Tavuklarda Performans ve Kan Parametreleri İle Göğüs Etinin Kimyasal Kompozisyonu ve Biyoaktif Bileşiklerine Etkisi

Öz

Bu çalışma *Moringa oleifera* kabukları içeren yemin broiler tavuklarda büyüme performansı, kan metobolitleri, etin kimyasal kompozisyonu ve kalitesi ile bağışıklığa olan etkilerini araştırmak amacıyla yapıldı. Bu amaçla, 40.4±3.02 g ağırlığında toplam 200 broiler (Hubbard classic) 4 uygulama grubu ve 5 tekrar olmak üzere (her tekrar için 10 tavuk olacak şekilde) tamamıyla rastgele olarak kullanıldı. Başlangıç ve bitirme diyetlerine %0, 0.5, 1.0 ve 1.5 miktarlarında *Moringa oleifera* kabukları eklendi. Çalışma sonucunda, artan katkı maddesi miktarıyla orantılı olarak büyüme performansının iyileştiği, yem konversiyon oranın ve yem tüketiminin azaldığı tespit edildi (P≤0.05). Artan katkı maddesi miktarıyla orantılı olarak tüy yüzdesi düşerken sakatat ağırlığında artma gözlemlendi (P≤0.05). Göğüs etinde β-karoten, kuersetin ve selenyum miktarı doğrusal olarak artış göstererek daha yüksek değerlerde difenil pikril hidrazil (DPPH) radikal temizleme ve artmış raf ömrü gözlemlenmiştir. Serum glutamik piruvik transaminase, kreatinin, glukoz ve et kolesterol gibi serum biyokimyasal bileşiklerinin seviyeleri anlamlı derecelerde düşmüş ve en düşük olarak %1.5 *Moringa oleifera* kabukları içeren yemde kaydedilmiştir (P≤0.05). Çalışma sonunda Moringa oleifera kabuklarının broiler tavuklarda büyüme ve etin kimyasal kompozisyonuna pozitif etkisinin olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Broiler, β-karoten, DPPH, Moringa kabukları, Kuersetin, Selenyum*

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INTRODUCTION

The broiler meat is one of the most abundant and cheapest sources of quality protein for human consumption. But unfortunately, broiler industry is facing multiple infectious threats caused by different pathogenic microbes. Therefore, safe and healthy chicken production needs proper microbial control for quality meat production [1]. Currently, different synthetic preparations including antibiotics are being used to control the microbial infection. But at the same times, abundant use of antibiotics resulted in hazardous outcomes in the form of emergence of antibiotic resistance in susceptible microbial populations, drug residues in meat and environmental pollution [2,3]. Due to the reason, this irrational use of antibiotics is being discouraged/ banned all over the world [4,5]. Currently, various phytogenic feed additives are encouraged in animal feed industry as a replacement to antibiotic growth promoters [6]. Plants are a rich source of bioactive compounds with diverse biological and pharmacological activities [7]. The functional properties of these bioactive compounds vary with the gradual partitioning of the whole plant starting from leaves, flowers, pods, fruits, stem, bark and roots [7,8]. Bioactive compounds route through modification of pancreatic activity by potentiating hydrolysis and decreasing the cellular damage in the intestine which consequently results in better nutrient utilization by improving metabolism and absorption and thus resulting in better growth performance and feed conversion ratios [9,10]. These compounds also enhance immune status of birds by reducing endotoxins and proliferation of pathogenic microbes [7]. Modern concept of phytogenic feed additives is based on the plant secondary metabolites like carotenoids, flavonoids and essential oils which help in fighting against multiple diseases in human when enriched in animal products through animal feed [11]. These compounds express antioxidant activities by scavenging the free radicals and becomes part of meat and eggs [12-14]. Various plant species including oregano, cinnamaldehyde, *Capsicum oleoresin*, garlic, turmeric and *Moringa (M.) oleifera* are rich in such bioactive compounds [9]. In this regard, *M. oleifera* is easily available in tropical and subtropical countries of the world including Pakistan, Bangladesh, India, Africa, Ethiopia, Kenya and many other countries. Poultry industry in developing countries facing the ingredients shortage, which enhances the importance of non conventional feed resources which could replace the costlier feed ingredients like proteins [15]. The *M. oleifera* pods contain fairly high quantities of essential amino acids enriched proteins, fat, minerals, vitamins and other bioactive compounds [15-17]. Keeping in view the nutritional quality of *M. oleifera* pods, this study was conducted to evaluate the effect of *M. oleifera* pods meal (MPM) in improving the growth performance and antioxidant attributes of broiler meat.

MATERIAL and METHODS

Moringa pods were collected from the fields of central and southern Punjab, Pakistan. After washing, cleaning and grading the pods were shade dried to a moisture level of ≤12% to keep the bioactive compounds intact in the plant material [18]. The dried pods were ground to fine powder, stored in sealed air tight containers at 4°C till further use and subjected to proximate analysis *(Table 1)* for determination of different nutrients by using the methodology described by Association of Official Analytical Chemist [19]. Four iso-caloric and iso-nitrogenous diets A, B, C & D were formulated having crude proteins 20.5 and 19% and ME levels 2850 and 2875 kcal/kg for both starter and finisher phases *(Table 2, Table 3)*, respectively as recommended by National Research Council [20]. Two hundred day old broiler (Hubbard) chicks with authentic hygienic and bio-security standards were randomly assigned to four dietary treatments viz. A (MPM-0%), B (MPM-0.5%), C (MPM-1.0%) and D (MPM-1.5%) with five replicates for every treatment for a duration of six weeks. The birds were reared on floor system in a modern environment controlled poultry house under standard management conditions. All birds were vaccinated according to local vaccination schedule [21]. Experimental diets and water were offered *ad-libitum* to all the birds.

Collection of Data, Serum and Meat Samples

Birds handling and collection of samples was performed according to the procedure approved by advance studies and research board (ASRB) of the University of Veterinary and Animal Sciences, Lahore, Pakistan in the meeting (DAS/1948) held on 23 September 2013. Feed intake and mortality were recorded on daily basis; whereas, cumulative weight gain (CWG) and FCR were calculated

Table 1. *Chemical composition of Moringa oleifera pods meal*

Chemical Composition	Proportion	Unit
Moisture	8.05	g/100 g
Crude Protein	18.98	g/100 g
Ether Extract	2.34	g/100 g
Ash	7.88	g/100 g
Minerals		
Sodium	805	mg/100 g
Potassium	2815	mg/100 g
Calcium	291	mg/100 g
Magnesium	251	mg/100 g
Phosphorus	9456	mg/100 g
Selenium	25.71	mg/100 g
Bioactive Compounds		
Quercetin	114	mg/100 g
β-carotene	2.76	mg/100 g

Table 2. *Experimental broiler starter diets and their chemical compositions*

Ingredients	Proportions in Experimental Diets			
	A	B	C	D
Maize (kg)	50.00	50.00	50.00	50.00
Soybean meal (kg)	22.13	22.13	22.13	22.13
Canola meal (kg)	15.00	15.00	15.00	15.00
Rice polish (kg)	6.54	6.54	6.54	6.54
DCP (kg)	2.34	2.34	2.34	2.34
Soy oil (kg)	1.76	1.76	1.76	1.76
Limestone (kg)	0.60	0.60	0.60	0.60
L-lysine sulphate (kg)	0.55	0.55	0.55	0.55
DL-methionine (kg)	0.25	0.25	0.25	0.25
Soda bicarb (kg)	0.35	0.35	0.35	0.35
(Vit.& Min Premix) (kg)	0.20	0.20	0.20	0.20
Salt (kg)	0.15	0.15	0.15	0.15
L-threonine (kg)	0.12	0.12	0.12	0.12
Total (kg)	100	100	100	100
Moringa pod (%)	0	0.5	1.0	1.5
Chemical Composition	**Nutrients Proportion in Diets**			
Dry matter (%)	90.20	90.20	90.20	90.20
Crude Protein (%)	20.50	20.50	20.50	20.50
ME (kcal/kg)	2850	2850	2850	2850
Fat (%)	5.05	5.05	5.05	5.05
CF (%)	4.74	4.74	4.74	4.74
Ash (%)	6.27	6.27	6.27	6.27
Dig. lysine (%)	1.2	1.2	1.2	1.2
Dig. threonine (%)	0.78	0.78	0.78	0.78
Dig. meth + Cysteine (%)	0.88	0.88	0.88	0.88
Sodium (%)	0.18	0.18	0.18	0.18
Calcium (%)	0.9	0.9	0.9	0.9
Available phosphorus (%)	0.44	0.44	0.44	0.44
Se (mg/kg)	0.13	0.31	0.42	0.65
β-carotene (mg/kg)	0.34	0.57	0.70	0.79
Quercetin (mg/kg)	0.48	7.98	15.81	22.87

Table 3. *Experimental broiler finisher diets and their chemical compositions*

Ingredients	Proportions in Experimental Diets			
	A	B	C	D
Maize (kg)	50.00	50.00	50.00	50.00
Soybean meal (kg)	17.79	17.79	17.79	17.79
Canola meal (Kg)	15.00	15.00	15.00	15.00
Rice polish (kg)	11.29	11.29	11.29	11.29
DCP (kg)	2.22	2.22	2.22	2.22
Soy oil (kg)	1.62	1.62	1.62	1.62
Limestone (kg)	0.62	0.62	0.62	0.62
L-lysine sulphate (kg)	0.38	0.38	0.38	0.38
DL-methionine (kg)	0.24	0.24	0.24	0.24
Soda bicarb (kg)	0.37	0.37	0.37	0.37
Vit & Min Premix (kg)	0.20	0.20	0.20	0.20
Salt (kg)	0.12	0.12	0.12	0.12
L-threonine (kg)	0.14	0.14	0.14	0.14
Total (kg)	100	100	100	100
Moringa pod (%)	0	0.5	1.0	1.5
Chemical Composition	**Nutrients Proportion in Diets**			
Dry matter (%)	90.20	90.20	90.20	90.20
Crude protein (%)	19.18	19.18	19.18	19.18
ME (kcal/kg)	2875	2875	2875	2875
Fat (%)	5.67	5.67	5.67	5.67
CF (%)	5.16	5.16	5.16	5.16
Ash (%)	6.18	6.18	6.18	6.18
Dig. lysine (%)	1.15	1.15	1.15	1.15
Dig. threonine (%)	0.75	0.75	0.75	0.75
Dig. meth + Cysteine (%)	0.84	0.84	0.84	0.84
Sodium (%)	0.18	0.18	0.18	0.18
Calcium (%)	0.84	0.84	0.84	0.84
Available phosphorus (%)	0.43	0.43	0.43	0.43
Se (mg/kg)	0.13	0.31	0.42	0.65
β-carotene (mg/kg)	0.34	0.57	0.70	0.79
Quercetin (mg/kg)	0.48	7.98	15.81	22.87

on weekly basis. Blood samples were collected in EDTA coated vacutainers at 4th and 6th weeks of experiment and stored at -20°C for biochemical (SGPT, Glucose, Cholesterol and Creatinine) tests. The antibody titers against Newcastle disease and Infectious bursal disease vaccines were calculated using haemagglutination inhibition (HI) and enzyme-linked immunosorbent assay kit (Merck Microlab-300, country Germany), respectively. Three birds from each replicate were weighed, slaughtered and examined for dressing percentage and giblet relative weights at termination of experiment. Breast meat samples were taken at termination of experiment and analyzed for the detection and quantification of minerals (Na, K, Ca, Mg, Se, etc.) using atomic absorption spectrophotometry [19].

Breast Meat β-Carotene and Quercetin Analysis

High Performance Liquid Chromatography (HPLC) technique was used for the estimation of carotenoids (β-carotene) and flavonoids with standard methods used in previous studies [22,23]. Briefly, breast meat sample (1 g) was vortexed thrice for 5 min after addition of methanol (0.8 mL) and 1N HCl (0.2 mL) followed by centrifugation at 4000 rpm for 15 min. Supernatant was separated and dried on water bath set at 70°C. Extraction of organic compounds was performed by 0.1 mL mobile phase (70:20:10 v/v/v, Acetonitrile: Dichloromethane: Methanol) addition. The sample was vortexed for 5 min and filtered into HPLC vials by using 0.1 µm filter paper (Whatman No. 40) and

subjected to HPLC analysis for bioactive β-carotene. HPLC system having a diode array detector (DAD) at 450 nm with 5 µm C18 reverse phase column was opted. Samples were injected with a flow rate of 1.0 mL min^{-1} at 30°C and retention time 6.19 min. Standards were used to draw calibration curve, which helped in quantification of β-carotene. For this purpose the standard solution with a serial dilution ranging from 0.01 to 0.08 mg/L were used for the determination of β-carotene [22].

Estimation of quercetin was done with method used by Tokusoglu *et al*.[23]. Standards of quercetin (HPLC grade) were purchased from Sigma chemicals (St Louis MO, USA) through a local supplier. For sample preparation breast meat (1 g) was taken in a glass tube having acidified methanol containing 1% (v/v) HCl and 0.5 mg mL^{-1} TBHQ (Tertiary Butyl Hydroquinone). The temperature of the extract was lowered down to room temperature and centrifugation was done at 1500 g (5000 rpm). The supernatant was removed and sonicated for 5 min to remove air and finally filtered for injection into HPLC. Estimation of Quercetin was conducted with high-performance liquid chromatograph (HPLC) model (LC-10As) Shimadzu, Kyoto, Japan. Sample volume 20 µL was injected with flow rate of 1.0 mL min^{-1} at 30°C for chromatographic separation. Standards were used to draw calibration curve, which helped in quantification of quercetin. For this purpose the standard solution with a serial dilution ranging from 0.01 to 0.08 mgL^{-1} were used for the determination of quercetin.

DPPH- Radical Scavenging Activity

Antioxidant activity of meat samples was quantified by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity as described by Singh *et al*.[24]. A known volume (25 µL) of Butylated hydroxyl anisole (BHA) was added to 50 µL of meat extract in a tube. The final volume was adjusted to 100 µL by adding methanol (MeOH) followed by the addition of methanolic solution of DPPH (5 mL). The tube was shaken vigorously and incubated at 27°C for 20 min. Same method with only difference of meat extract was used for preparation of control along with methanol for baseline correction. Samples were run on UV-visible and absorbance was taken at 517 nm. The parameters for radical scavenging activity was DPPH radical inhibition percentage [24].

Cholesterol Estimation of Breast Meat

Sample was prepared by using the methodology described by AOAC [19]. Briefly, acetone (1 mL) was added in broiler breast meat (1 g) and vortexed for two minutes after vigorous shaking. Acetone was decanted after centrifugation at 10.000 rpm for 10 min. This procedure was repeated thrice and the acetone fractions obtained were pooled down and allowed to evaporate. The acetone extract thus obtained was supposed to have cholesterol and was subjected to UV-visible spectrophotometer to get the absorbance at wavelength of 500 nm. The cholesterol contents were calculated by using the formula given below [25].

Relative standard deviation;

$$\%RSD = \frac{\text{Standard deviation of measurements}}{\text{Mean value of mesarumenets}} \times 100$$

Recovery;

$$\%Recovery = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

Amount found;

$$\text{Amount found (mg/mL)} = \frac{\text{Mean test absorbance}}{\text{Mean std. absorbance}} \times \text{Std. Conc.}$$

Amount added;

$$\text{Amount added (mg/mL)} = \frac{\text{Weight}}{\text{Volume}}$$

Limit calculation for stability of analytical solution study;

$$\text{Limit} = \frac{\text{Absorbance of Std. initial - Absorbance of Std. (at different time)}}{\text{Absorbance of Std. initial}} \times 100$$

Statistical Analysis

Data thus obtained were analyzed through one-way ANOVA technique using Generaliz Linear Model (Proc Glm, SAS 9.4) [26]. The differences between means were calculated through Duncan's Multiple Range test. The differences were considered significant at $P<0.05$.

RESULTS

Growth Performance

The results of present study showed significant ($P\leq0.05$) growth performance of the broiler birds fed on diet supplemented with *Moringa oleifera* pods meal (MPM). Linear decrease in feed intake was observed and highest feed intake was recorded in the control group whereas, lowest value was recorded in the diet D supplemented with MPM-1.5%. Similarly, carcass traits and organ weights were significantly ($P\leq0.05$) affected with supplementation of MPM. Organs weights like liver, gizzard and heart were significantly ($P\leq0.05$) improved with the MPM supplementation, however, decreased after optimum level of supplementation *(Table 4)*. Body weight and body weight gain showed a quadratic response and was decreased as the supplementation

Table 4. *Growth performance and carcass traits of broilers fed on different levels of Moringa oleifera pod meal at 42 days of age*

Parameter	Control	MPM 0.5 %	MPM 1.0 %	MPM 1.5 %
FI	5068±69.20^{a}	5005±100.19^{ab}	4817±81.48^{b}	4808±44.42^{b}
BW	2500±41.35^{ab}	2525±41.76^{ab}	2575±42.59^{a}	2433±40.24^{b}
BWG	2463±41.49^{ab}	2487±41.26^{ab}	2538±42.63^{a}	2394±39.75^{b}
FCR	2.03±0.04^{a}	1.98±0.04^{ab}	1.87±0.05^{b}	1.98±0.04^{ab}
Liv %	94.00±1.25	94.33±1.13	94.00±1.63	95.56±1.27
Carcass Traits				
Carcass Wt	1895±19.60^{a}	1852±23.54^{ab}	1921±29.00^{a}	1818±22.59^{b}
Dressing %	75.87±0.60^{a}	73.34±0.24^{c}	74.55±0.35^{bc}	74.73±0.49^{ab}
Liver %	52.50±0.58^{ab}	53.03±0.58^{a}	54.09±0.60^{a}	51.10±0.56^{b}
Gizzard %	30.00±0.33^{ab}	30.30±0.33^{a}	30.91±0.34^{a}	29.20±0.32^{b}
Heart %	15.00±0.17^{ab}	15.15±0.17^{a}	15.45±0.17^{a}	14.60±0.16^{b}

Superscripts on different means within row show significant difference (P≤0.05); FI: Feed Intake (g); ***BW:*** *Body weight (g);* ***BWG:*** *Body Weight Gain (g);* ***FCR:*** *Feed Conversion Ratio;* ***Liv:*** *Liveability %;* ***Wt:*** *weight (g)*

Table 5. *Bioactive compounds and selenium experimental feeds and breast meat broilers fed on different levels of Moringa oleifera pod meal*

Parameter	Control	MPM 0.5 %	MPM 1.0 %	MPM 1.5 %
Diet Sample				
β-carotene	0.34±0.01^{d}	0.57±0.01^{c}	0.70±0.02^{b}	0.79±0.01^{a}
Quercetin	0.48±0.02^{d}	7.98±0.04^{c}	15.81±0.09^{b}	22.87±0.10^{a}
Selenium	0.13±0.00^{d}	0.31±0.00^{c}	0.42±0.00^{b}	0.65±0.00^{a}
Breast Meat Sample				
β-carotene	0.00±0.00^{d}	0.06±0.00^{c}	0.07±0.00^{b}	0.08±0.00^{a}
Quercetin	2.47±0.20^{d}	43.58±0.23^{c}	86.34±0.47^{b}	124.89±0.54^{a}
Selenium	19.42±0.26^{d}	45.15±0.26^{c}	71.02±0.26^{b}	95.86±0.26^{a}
Cholesterol	65.50±0.45^{a}	64.85±0.45^{a}	64.20±0.45^{a}	61.63±0.43^{b}
DPPH	20.92±0.41^{d}	25.65±0.45^{c}	29.06±0.43^{b}	32.26±0.48^{a}

Superscripts on different means within row show significant difference (P≤0.05); β-carotene, Quercetin and Selenium in diet: mg/kg; β-carotene, Quercetin and Selenium in breast meat: µg/100 g, Cholesterol: mg/100 g, DPPH: (1, 1-Diphenyl -2-picrylhydrazyl) %

was increased above 1.0% MPM group, offered 1.5% MPM *(P*≤0.05).

Bioactive Compounds and Proximate Profile of Meat

The group supplemented with highest level of Moringa pods was enriched to best levels of bioactive compounds i.e. β-carotene, quercetin, cholesterol, selenium and DPPH contents of broiler breast meat samples were 0.08 µg/100 g, 124.89 µg/100 g, 95.86 mg/100 g, µg/100 g 61.63 and 32.26%, respectively *(Table 5)*. The cholesterol level was also significantly decreased with the increasing supplementation levels. Breast meat samples showed a significant linear decrease (*P*≤0.05) in Ash and EE content with the supplementation *(Table 6)*. Crude protein (CP) content of broiler breast meat samples was significantly increased with the supplementation level and highest value was observed in the diet D supplemented with 1.5% MPM, which increase the lean meat and lower ether extract *(Table 6)*.

Serum Biochemical and Antibody Titers

Blood serum of the broilers fed on *Moringa oleifera* pods meal as a feed additive was analyzed for biochemical indices especially SGPT, glucose, creatinine and cholesterol and significant difference in the treatment groups was recorded (*P*≤0.05). The control group showed highest values of all biochemical indices whereas lowest levels of SGPT, Glucose, and cholesterol were observed in the group fed maximum level of MPM *(Table 7)*. However, the lowest Creatinine was recorded in MPM-1.0% group. Immune response of commercial broiler birds was estimated by evaluating the antibody titers against Newcastle disease (ND) and Infectious bursal disease (IBD). Significantly higher titers of both the viruses ND as well as IBD were recorded in the groups supplemented with *Moringa oleifera* pods meal (MPM) when compare with control groups *(Table 7)*. Highest titers of ND were recorded in the group supplemented with 1.0% MPM during starter phase and 1.5% MPM during finisher phase. Moreover highest

Table 6. *Nutrients and mineral profile of breast meat of broilers fed on different levels of Moringa oleifera pod meal*

Parameter	Control	MPM 0.5 %	MPM 1.0 %	MPM 1.5 %
Proximate[1]				
Moisture	71.21±0.32[a]	63.77±0.16[c]	63.48±0.30[c]	66.41±0.46[b]
Crude Protein	21.89±0.18[b]	21.68±0.26[b]	22.15±0.35[b]	23.52±0.22[a]
Ash	0.46±0.00[a]	0.38±0.01[b]	0.29±0.01[c]	0.20±0.01[d]
Ether Extract	2.32±0.01[a]	2.06±0.02[b]	1.85±0.01[c]	1.69±0.01[d]
Mineral Profile[2]				
Sodium	1588±16.88[a]	1525±3.53[b]	1517±2.26[bc]	1499±3.75[c]
Potassium	3531±15.94[a]	3360±12.08[b]	3222±11.35[c]	3127±10.71[d]
Calcium	83.11±0.27[a]	78.96±0.26[b]	77.96±0.20[c]	75.39±0.18[d]
Magnesium	351.08±1.27[a]	331.13±2.02[b]	314.06±1.74[c]	302.52±0.98[d]
Phosphorus	3233±14.17[a]	3110±13.58[b]	3020±21.63[c]	2896±32.84[d]

Superscripts on different means within row show significant difference (P≤0.05); [1] Parameters for proximate analysis were expressed in g/100 g, [2] Parameters for mineral profile were expressed in mg/100 g

Table 7. *Serum chemistry and antibody response in broilers fed on different levels of Moringa oleifera pod meal*

Parameter	Control	MPM 0.5 %	MPM 1.0 %	MPLM 1.5 %
Blood metabolites and antibody response of serum sample (Starter phase; 0-4 weeks)				
SGPT	26.20±0.38[a]	20.13±0.69[b]	14.45±0.36[c]	12.41±0.33[d]
Glucose	268.07±1.60[a]	250.70±0.81[b]	239.65±0.76[c]	232.57±1.05[d]
Creatinine	1.68±0.01[a]	1.30±0.03[b]	1.12±0.01[d]	1.22±0.01[c]
Cholesterol	166.49±1.44[a]	150.10±1.24[b]	92.22±1.49[c]	86.35±1.20[d]
NDV titres	38.40±5.80[bc]	32.00±4.68[c]	57.60±3.42[a]	51.20±4.19[ab]
IBD titres	1465±110.94[b]	2860±226.12[a]	2842±239.10[a]	2882±106.35[a]
Blood metabolites and antibody response of serum sample (Finisher; 5-6 weeks)				
SGPT	24.72±0.36[a]	18.99±0.65[b]	13.64±0.34[c]	11.71±0.31[d]
Glucose	260.27±1.56[a]	243.40±0.79[b]	232.67±0.73[c]	225.80±1.02[d]
Creatinine	1.59±0.01[a]	1.23±0.03[b]	1.06±0.01[d]	1.16±0.01[c]
Cholesterol	157.07±1.36[a]	87.00±1.41[b]	87.00±1.41[c]	81.47±1.13[d]
NDV titres	44.80±4.19[b]	51.20±4.19[ab]	51.20±4.19[ab]	57.60±3.42[a]
IBD titres	1554±75.12[c]	2296±106.85[b]	2787±72.47[b]	3743±347.59[a]

Superscripts on different means within row show significant difference (P ≤ 0.05); SGPT: U/L; Glucose, Creatinine and cholesterol: mg/dL

values of IBD titers for both starter and finisher phases were recorded in 1.5% supplementation group *(Table 6)*. Moreover lowest values of titers for both ND and IBD were recorded in the control groups in all two phase of rearing.

DISCUSSION

The decrease in the feed intake was due to high density feed on account of essential amino acids, vitamins and minerals present in MPM which meet the body requirement even with smaller intake. During the experimental period best FCR and BWG was recorded in the group C supplemented 1.0% MPM. This might be due to rich availability of essential nutrients like amino acids, vitamins and antioxidant compounds present in *Moringa oleifera* pods which affect the overall health, production and FCR in experimental broilers. The increase in relative giblet weights can be attributed to the bioactive compounds (carotenoids, flavonoids) of Moringa pods meal, which interact with the metabolism and enhance the productive performance by improving digestibility. Some other studies also resulted that *Moringa oleifera* supplementation affect the giblet relative weight [27,28]. Similarly it has been reported in other studies that Moringa supplementation show positive impact on FCR of broiler birds [29-31]. Whereas some other scientist reported that growth performance was not affected by *Moringa oleifera* supplementation in the diet [32,33].

Carotenoids like β-carotene are bioactive chemicals which

are present in sufficient amounts in *Moringa oleifera* supplemented feed *(Table 5)* also reported in different previous studies on *Moringa oleifera* pods meal [34,35]. The flavonoids (quercetin) enrichment in breast meat of broilers fed on MPM also been reported in some studies [31,36]. The significant reduction of cholesterol in broiler meat due to supplementation of *Moringa oleifera* pods meal in feed might be attributed to phytosterols (β-sitosterol) present in the Moringa plant tissues, which decreases the absorption of cholesterol from the intestine with an immediate release in the feces. Similar results haven reported in many other studies where diet was manipulated with antioxidants and plant tissue material and cholesterol level was lowered [37-40]. The linear increase in the selenium level of breast meat samples in commercial broilers may be attributed to the higher selenium content in Moringa pod meal supplemented feed. Similarly it was reported in other studies that breast meat selenium content was increased by offering the birds selenium enriched feed [36,41,42]. The higher DPPH value may be attributed to the antioxidants (Quercetin, β-carotene, and selenium) enriched Moringa pods meal when supplemented in the diet of broilers. Same findings have been reported in some other studies which showed the strong free radical scavenging activity by using phytochemical enriched feeds [30,31].

Decline in moisture content of the breast meat samples could be due to the fact that moisture level is inversely proportional to the lipid content of the body and higher ash content of MLM [24]. Higher energy and protein values may be linked with decreased moisture levels and dense essential nutrients in MLM [43]. Increase in crude protein level may be attributed to higher bioavailability of essential amino acids present in Moringa, resulting in better tissue and muscle growth. Similar results were reported in other studies where chemical composition was significantly affected with *Moringa oleifera* leaf supplementation in the diets [33,38]. Present study showed decreased minerals levels in meat samples with the increase in supplementation level of *Moringa oleifera* pod. This response can be due to some anti-nutritional factor which decreases the feed intake and resulted in poor weight gain.

The bioactive compounds present in Moringa pods meal supplementation in the experimental diets increased efficiency of liver and kidneys which is evident from biochemical indicators showing functionality of kidneys and liver. However lowered cholesterol levels in the treatment groups can be attributed to β-sitosterol a plant sterol present in *Moringa oleifera* pods meal which lowers cholesterol due to its structural similarity to cholesterol, so decreases its absorption from intestine and increased the release in feces [44]. The results of the present study are in line with some previous researches to investigate the effect of Moringa on cholesterol and overall biochemical profile of commercial broilers [27,29,45]. The response of body towards antibody titers is attributed to bioactive compounds (antioxidant) vitamins, minerals and amino acid profile of *Moringa oleifera* pods meal supposed to be responsible for improved immune status of broiler birds. The findings of present study are in line with some earlier experiments which also reported that same response was observed while using *Moringa oleifera* as feed additive [29,46,47].

The *Moringa oleifera* pods meal supplementation in the experimental diets of broilers showed positive impact on the growth, immunity and serum biochemistry. In addition the meat quality of broilers improved due to lowering of cholesterol and enrichment of bioactive compounds in their meat.

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