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Immunomodulatory Effects of Moringa Oleifera Leaf Meal (MOLM) against Newcastle Disease in Broilers

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Etlik Piliçlerde Moringa Oleifera Yaprak Ununun (MOLM) Newcastle Hastalığına Karşı İmmünomodülatör Etkileri

Araștırma Makalesi	ÖZET
Tarihçe: Alınış: 16 Mayıs 2020 Kabul: 7 Ağustos 2020 Online Yayınlanma: Aralık 2020	Etlik piliçlerde immünomodülatör etkiler açısından Moringa oleifera yaprak ununun (MOLM) etkinliğini kontrol ettik. Çalışmada herbirinde 12 hayvan olan 4 grup oluşturulmuş, toplam 144 hayvan, 12 altgruba ayrılmıştır. Denemede Moringa oleifera yaprak ununun (MOYU) farklı düzeylerdeki (%0, 2, 4 ve 6)
Anahtar Kelimeler: Moringa Oliefera Bağışıklık Hematoloji Newcastle hastalığı Etlik piliç Farklı düzeyler	etkisi araştırılmıştır. Hayvanlar 14, 21. Ve 35.gün kesilmiş, hematolojik parametrelerin tespiti için kanları alınarak serumları çıkartılmıştır. Ayrıca HA / HI kullanılarak antikor titresinin bağışıklık sisteminin Newcastle Hastalığına karşı antikor üretmedeki etkinliğini kontrol edilmiştir. Çalışmada Diet b, maksimum canlı ağırlık kazancına ulaşırken; Diet D, en düşük canlı ağırlık kazancına sahip olmuştur. Maksimum yem tüketimi Diet A grubunda; en düşük yem tüketimi de Diet D grubunun yemiyle beslenen hayvanlarda görülmüştür. Diet B'de yemden yararlanma oranı iyileşirken; <i>Moringa oleifera</i> katılmayan grupta en yüksek yemden yararlanma oranı tespit edilmiştir. Yemlere Moringa katkısı, etlik piliçlerde bağışıklık parametrelerini artırken; hastalıkla mücadele de başarılı olmalarını sağlamış; kümes hayvanları için tavsiye edilen bir yem katkısı olmuştur. Denemede Moringa oleifera yaprak ununun karma yeme optimum

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katılma düzeyinin 4 g/kg olduğu da tespit edildi.

Research Article ABSTRACT We checked the efficacy of Moringa oleifera leaf meal (MOLM) for Received: 16 May 2020 immunomodulatory effects in broilers. 144 birds that were divided into 4 groups Accept: 7 August 2020 having 12 birds each that were further subdivided into groups of 12. Such that Available online: December 2020 treatment groups were D; receiving 0%, divided into replicates D1, D2, D3 while similar divisions of group A, B and C received 2, 4 and 6 % of MOLM infused in their feed as an additive supplement. The birds were slaughtered at 14th, 21th and Moringa Oliefera 35th day and blood as well as serum was collected for the sake of hematological parameters and to check the antibody titer using HA/HI checking for effectiveness Hematology of immune system at producing antibodies against Newcastle Disease, which the Newcastle disease Varying concentration

birds were vaccinated against. Diet B has maximum weight gain fed while Diet D has lowest weight gain, which was a control group. Maximum feed intake was with diet A while lowest feed intake with diet D. Improved FCR was with diet B whereas the highest feed conversion ratio (FCR) was observed in broilers fed diet without Moringa oleifera supplementations. It was concluded that Moringa infused in feed does enhance immune parameters of broilers, making them better at combating diseases, making *Moringa* a viable dietary supplement for commercial poultry. It was also found that the optimal dose of *Moringa* in MOLM is 4 g/kg feed.

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1. Introduction

Poultry industry is one of the wide spread poultry industry in all over the world. The poultry meat production is rapidly increasing up to 4% annually [1]. Medicinal plants have played an important role for the prevention of many poultry diseases. Almost 25% medicines used in the poultry industry are extracting from the plants as *Moringa oleifera* is usually used as herbal plant cultivated for purpose of medicine in all over the world [2].

Moringa oleifera belonged to *Moringaceae* family which is also referred to as drum stick tree. Almost all medical plants have good properties like anticancer, cardiovascular, anti-asthmatic and antioxidant. Extract of *Moringa oleifera* has nutritive values [3].

Moringa oleifera seeds and leaves are rich sources of vitamins C and B and lysine [4]. Moringa oleifera is rich in iron. Seeds are used for several soap making and cosmetic purposes. Quality of Moringa oleifera oil extract is better than olive oil [5]. With sunflower meal, it enhances the feed intake, feed conversion ratio and growth rate of birds. Up to 20% Moringa oleifera used in the layer birds lead to increase in feed intake, layer performance and egg size [6]. It is drought resistant tree with rapid growing property. It also used as fodder for livestock an adjuvant, a natural micronutrient fluid [7]. Moringa oleifera grows in hot climate, loam soil 5-7,7 pH of soil, 30-48°C. Extract of Moringa oleifera has no significant effect on the broiler chick performance, feed utilization and weight gain. [8]. M. oleifera is used as a cheap, highly digestible and highly bioavailable protein source [9] that may have side effects if used in more than that of the required concentration [10].

M. oleifera has ability to improve hair and skin, lowering blood pressure and blood sugar level, aids in hypotension, skin disorders and diabetes. Seeds of *Moringa oleifera* are full of flocculants which have ability to enhance water quality.

These seeds are utilized for treating in many purposes such as hypertension, arthritis and

sexually transmitted disorders. And also oil extract of seeds used in enhancing milk quality and soil fertility [5]. Moringa oleifera also having antifungal and antibiotics properties against the staphylococcus aureus and Pseudomonas aeruginosa. It is used in the treatment of anemia. Its bark is used for cardiac problem. Its leaves have tocopherol and carotenoid which prevent free radical damage. M. oleifera is used for reduction of cholesterol level in eggs of lavers [9]. Moringa oleifera seeds and leaves boost up the immunity. It is also used as antimicrobial agent in ponds [4]. Moringa oleifera is an antibiotic growth promoter [11, 12]. Moringa oleifera increases numbering of phagocytic cells and used to decrease the levels of carbon from blood. It facilitates phytate breakdown by action of phytase enzymes which lead to absorption of phosphorus [13]. Moringa oleifera plants treats Hepatitis B infection in humans [14].

MOLM is also a good source of flavonoids, carotenoids and ascorbic acid [15]. *Moringa oleifera* leaf meal (MOLM) decreases intake but increases feed conversation ratio (FCR) in poultry [16]. It has high production rate [17]. *Moringa oleifera* extract up to ratio of 25% used in poultry feed did not cause any negative effect [18]. MOLM having the positive influences on growth performances and serum biochemistry of starter chicks and also could be integrated at 7,8% in broiler diets without any destructive consequences on blood chemistry and growth rate of chickens.

Newcastle disease not only affects the egg production but also reduces the quality of eggs and bird performance [19] and is a major threatening and demoralizing disease in the poultry production. We evaluated the efficacy and immunity of *Moringa oleifera* leaves against ND virus in broiler at different concentrations and to check effect of *Moringa oleifera* on hematology parameters.

2. Materials and Methods

2.1. Flock management and House preparation

Before the arrival of chicks, the flock shed was prepared to keep the birds under the standard condition. The shed undergo following practices, wall white wash, disinfection and wiring. The birds kept under recommended condition by providing proper floor space, ventilation, humidity, temperature and provision of feed and water. The trial was continued up to 35 days.

In each group representative birds were slaughtered at 14th, 21th and 35th day of trial and blood samples not including anticoagulant were obtained for serum used in HA/HI antibody titres and also for further hematological parameters. Thymus, bursa and spleen were taken for calculation of weight. In group (A, B, C, D) experimental feed was partially or completely replaced by normal feed in different ratios which are given below in the following table 1. This study protocol was allowed by graduate studies and research board (GSRB) of University of Agriculture, Faisalabad with letter number DGS/23525-28 Dated 21-05-2018.

Table 1. Feed Formulation

Ingredients	0%	2%	4%	6%			
Moringa Leaves	0,00	2,00	4,00	6,00			
Corn/ Maize	58,43	57,14	55,85	54,57			
Canola Meal	6,00	6,00	6,00	6,00			
Soybean Meal 44 %	25,09	24,10	23,10	22,10			
Fish Meal < 50	5,00	5,00	5,00	5,00			
Oil	1,75	2,04	2,33	2,62			
Chips/Limestone	0,63	0,60	0,57	0,53			
Soda/Sodium Bi Carbonate	0,20	0,20	0,20	0,20			
DCP	1,23	1,25	1,27	1,29			
Salt/NaCl	0,21	0,21	0,21	0,22			
Lysine Sulphate	0,48	0,47	0,47	0,47			
DL- Methionine	0,33	0,34	0,36	0,37			
L- Threonine	0,14	0,14	0,14	0,13			
Premix	0,50	0,50	0,50	0,50			
Total	100	100	100	100			

Lincomycin 4.4% 0.100g, Mineral-Mix 0.250g, (Packing of 25kg with: Iron 95,940mg, Copper 20,250mg, Zinc 123mg, Manganese 153mg, Selenium 401mg, Iodine 2,032mg, Sepiolite (binder) 100,000mg, Calcium 6.3% and Magnesium 1.5%), Vitamix 0.250g (Packing of 25kg with: Vit. A 20,000,000 U.I, Vit D3 4,000,000 U.I, Vit E 60,000mg, Vit K3 8,640mg, Vit B1 4,000mg, Vit B2 12,000mg, Niacin 80,290mg, Vit B5 20,000mg, Vit B6 6,000mg, Vit B8 200mg, Vit B9 2,000mg, Vit B12 20mg, Calcium 27.8%, and Antioxidants (BHA 60mg and Ethoxyquin 60mg) and M.Tox® 0.500g per 100 kg of feed.

2.2. Experimental birds

In this study, 144 experimental Hubbard birds in good health were purchased from Big $Bird^{\textcircled{R}}$ hatchery and were brooded at farm. After that,

chicks were shifted on experimental pen and these birds were kept in 23-37°C with humidity of 60-70%. Fresh and clean fresh water along with the basal diet were provided ad libitum to the birds during this period and divided randomly into 4 groups and 3 replications with 12 birds in each replicate. Feed intake and refusal was recorded for all groups on daily basis. One group was kept as control named as group D and was offered feed without Moringa oleifera. Other groups were A, B and C and were fed with experimental diet of Moringa oleifera @2 g/kg, 4 g/kg and 6 g/kg respectively. The birds were vaccinated against the diseases including Infectious Bronchitis, Newcastle disease, with other important diseases which were Hydropericardium Syndrome and Infectious Bursal Disease.

Table 2. Chemical composition of formulated feed

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Ingredients	Analysis			
Dry Matter %	88	88	88	89
Metabolizable Energy	2975	2975	2975	2975
Crude Protein	21	21	21	21
Crude Fat	5,15	5,56	5,97	6,38
Crude Fiber	3,52	3,87	4,23	4,59
Ash	5,58	5,68	5,78	5,87
Linoleic Acid	2,24	2,36	2,48	2,61
Calcium	0,90	0,90	0,90	0,90
Available Phosphorous	0,45	0,45	0,45	0,45
Sodium / Na	0,16	0,16	0,16	0,16
Chloride/ Cl	0,23	0,23	0,23	0,23
Potassium	0,95	0,92	0,90	0,87
DEB	248	238	233	225
Digestible Lysine	1,22	1,22	1,22	1,22
Digestible Methinone	0,65	0,65	0,65	0,65
Digestible M+C	0,91	0,91	0,91	0,91
Digestible Threonine	0,83	0,83	0,83	0,83
Digestible Tryptophane	0,23	0,23	0,23	0,23
Digestible Isoleucine	0,76	0,76	0,75	0,74
Digestible Valine	0,85	0,84	0,84	0,83
Digestible Arginine	1,19	1,18	1,17	1,16

2.3. Performance characteristics

Body weight of all birds was measured after every one week. At the time of slaughtering weight of internal organs like spleen, bursa and thymus were measured. Relative weight was also measured by following equation:

Relative weight
$$\% = \frac{\text{organ weight}}{\text{body weight}} \times 100$$

2.4. Blood picture

During slaughtering of broiler chick's blood was collected and serum was also separated after collected in EDTA tube for measuring values of hematological parameters demonstrated by [20]. There were following hematological parameters like pack cell volume and hemoglobin concentration. Pack cell volume was determined by standard procedure of micro-Hematocrit method [20]. Sahile's and Drabkin method was adopted to calculate the hemoglobin concentration.

2.5. Heamagglutination and Heamagglutination Inhibition Test

Birds were slaughtered at 14th, 21th and 35th day of age and also blood was collected for further immunological and hematological tests. Birds were opened to examine abdominal cavity and to collect the immune organs (Bursa, Thymus and Spleen) to record their weight. From collected blood serum was separated to check the antibody titer.

Data was collected through this trial that was based on Randomized control based design. It can be undergo further evaluation by Minitab Inc. 2010. Minitab Statistical Software (Release 17), State College, PA [21].

3. Results

The total body weight per broiler chick at the end of trial was 857, 1393, 1355 and 2038 grams fed diet A, B, C and D respectively and was significant (P<0.001) with maximum weight gain in broilers fed diet D while lowest weight gain in birds fed diet A (Table 3).

 Table 3. Growth performance of broilers fed experimental diets

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DAY	MOLM	MOLM	MOLM	MOLM	P-Value
	0 %	2%	4%	6%	
Bursa	weight (gran	ns)			
7	-	-	-	-	-
14	0,362	0,342	0,341	0,420	^{NS} 0,470
21	$0,094^{ab}$	0,067 ^b	0,110 ^{ab}	0,139 ^a	*0,015
28	-	-	-	-	-
35	0,066 ^a	0,073 ^a	0,633ª	0,099 ^a	^{NS} 0,413
BW (g	rams)				
7	189	201	218	217	^{NS} 0,129
14	538	455	543	490	*0,05
21	1035 ^{ab}	843 ^b	1116 ^a	1049 ^{ab}	*0,019
28	1414 ^a	836 [°]	1094 ^{bc}	1149 ^{ab}	0,002
35	2363 ^a	1032 ^c	1445 ^b	1375 ^b	***0,001
FCR					
7	1,23	1,04	0,95	0,83	^{NS} 0,209
14	1,6	1,29	1,21	1,39	^{NS} 0,069
21	2,92 ^a	1,73 ^b	1,48 ^b	1,66 ^b	**0,009
28	4,38 ^a	2,82 ^{ab}	2,30 ^c	2,65b ^c	**0,007
35	5,26	3,89	2,69	3,45	*0,148
Feed In	ntake (grams	.)			
7	229	209	204	179	*0,474
14	858 ^a	583 ^b	657 ^b	681 ^b	**0,006
21	2980 ^a	1452 ^b	1658 ^b	1732 ^b	**0,005
28	6250 ^a	2328 ^b	2509 ^b	3058 ^b	**0.003
35	12836 ^a	4002 ^b	3961°	4780 ^b	*0.048

The average feed intake in grams per broiler chick was 3089, 3575, 3785, and 3982 fed diet A, B, C and D respectively and was significant (P<0.001) with maximum feed intake in broilers fed diet D while lowest feed intake in birds fed diet A (Table 3).

The average feed conversion ratio per broiler chick was 1.79, 1.74, 1.73 and 1.76 for A, B, C and D, respectively and was significant (P<0.001) with diet C whereas the highest FCR in broilers was with diet without *Moringa oleifera* supplementations (Table 3).

 Table 4. Immunological parameters of broilers fed experimental diets

HA/	HI titer				
7	170	213	256	256	^{NS} 0,219
14	18,667	69,33	29,33	98,667	^{NS} 0,571
21	341,333 ^{ab}	341,333ª	149,333 ^b	128,000 ^{ab}	*0,014
28	170,667 ^a	213,333ª	256 ^a	256 ^a	^{NS} 0,219
35	1024,00 ^a	554,67 ^{ab}	298,67 ^b	213,33 ^b	*0,016
Hemoglobin concentration					
7	-	-	-	-	-
14	6,5	9,4	7.03	7,53	^{NS} 0,137
21	9,8667 ^a	10,633 ^a	10.466 ^a	9,400 ^a	^{NS} 0,395
28	-	-	-	-	-
35	10,766 ^a	8,733ª	9,800 ^a	10,700 ^a	*0,041
Mor	tality %				
7	22,22ª	8,33 ^b	8,33 ^b	8,33 ^b	***0,001
14	47,22 ^a	19,44 ^b	16,67 ^b	19,44 ^b	***0,001
21	66,67 ^a	30,56 ^b	36,11 ^b	38,89 ^b	***0,001
28	75 ^a	38,89 ^b	38,89 ^b	47,22 ^b	**0,007
35	83,33ª	52,78 ^b	50 ^b	58,33 ^b	*0,034
PC\	1				
7	-	-	-	-	-
14	22	27	20	21	^{NS} 0,377
21	18,667 ^b	32,667 ^a	31,333 ^a	27,666 ^{ab}	*0,014
28	-	-	-	-	-
35	32,667 ^a	26,667 ^a	24,667 ^a	29,000 ^a	^{NS} 0,083
Sple	en weight (g	rams)			
7	-	-	-	-	-
14	0,482	0,324	0,279	0,32	^{NS} 0,321
21	0,104 ^a	0,0736 ^a	0,040 ^a	0,089ª	^{NS} 0,22
28	-	-	-	-	-
35	0,073 ^b	0,076 ^b	0,087 ^b	0,193 ^a	***0,001
	mus weight (grams)			
7	-	-	-	-	-
14	0,350	0,316	0,269	0,301	^{NS} 0,369
21	0,081ª	0,060 ^a	0,112 ^a	0,141ª	^{NS} 0,131
28	-	-	-	-	-
35	0,058ª	0,124 ^a	0,124 ^a	0,082 ^a	^{NS} 0,173

35 0,058 0,124 0,124 0,082 1.00,173 Different superscripts within the rows represents significant

difference (P < 0,05); NS: Non-significant; *: 0,01<P < 0,05; **: 0,001<P < 0,01; ***: P < 0,001

The average Log_2 value of ND HI titer against NDV were 7,00, 6,00, 7,44, 7,20 and 7,17 under different groups of A, B, C and D respectively and was significant (P<0.001) with highest antibody level in the birds of group C, while lowest level was found in the broilers of group B (Table 4).

The relative weight of spleen per broiler chick was 0,057, 0,328, 0,285 and 0,35 grams at Day

14; 0,057, 0,070, 0,091 and 0,130 grams at day 21; 0,087, 0,066, 0,097 and 0,057 grams at day 35 that were fed with diets A, B, C and D respectively, and were not significant (P>0,05) (Table 4).

The relative thymus weight per broiler chick was 0,406, 0,285, 0,209 and 0,457 grams at day 14; 0,065, 0,044, 0,082 and 0,115 grams at Day 21; 0,071, 0,145, 0,080 and 0,086 grams at Day 35 fed diets A, B, C and D respectively and were not significant (P>0,05) (Table 4).

The relative weight of Bursa of Fabricius per broiler chick was 0,460, 0,322, 0,367 and 0,457 grams at Day 14; 0,067, 0,094, 0,091 and 0,130 grams Day 21; 0,078, 0,038, 0,073 and 0,142 grams at Day 35 fed diets A, B, C and D respectively and was not significant (P<0.05) (Table 4).

Packed cell volume of blood serum of broiler was 16, 30, 18 and 19 % at Day 14; 21, 31, 33 and 28 % at Day 21; 29, 24, 23 and 30 % at Day 35, fed diets A, B, C and D respectively and was significant (P<0.001) (Table 4).

The hemoglobin (g/dL) of blood serum of broiler was 4,9; 8,3, 6,9 and 5,9 at Day 14; 7,9, 10,4, 10 and 8,9 at Day 21; 10,6, 7,2, 9 and 10,7 at Day 35, fed diets A, B, C and D respectively and was not significant (P>0.05) (Table 4).

4. Discussion

Ayssiwede [18] studied that *Moringa oleifera* extract up to ratio of 25% used in poultry feed did not cause any negative effects in substitution of ground nut cake meal. Mahmood [22] studied *cichorium intybus*, *Azadirachta indica* and *Moringa oleifera* and concluded that the *Moringa oleifera* leaves indicate better immunomodulatory results as compared to the control group.

Similar results were found by Gadzirayi [23] study was investigated that *Moringa oleifera* leaf meal (MOLM) with soybean supplements used as a protein source in poultry. The total body weight data showed that there was significant difference (P<0.001) in weight gain of broilers between different groups at the end of trial.

Melesse [24] indicated that *Moringa stenopetala* leaf meal MSLM is a potential plant protein supplement and could be included to 6% in the diet of grower chicks to substitute expensive conventional protein sources and there was

significant difference (P<0.001) in feed conversion ratio of broilers fed diets A, B, C and D at the end of trial. Similar results were found by Banjo [25] whose results showed that Inclusion of *Moringa oleifera* significantly enhanced weight gain of birds at 4% level of inclusion the most. The inclusion of *Moringa oleifera* did not significantly enhance feed intake and feed conversion ratio despite the total body weight increments.

In the present study, the HI antibody titers against NDV were tested. The analysis of variance of the antibody titers amongst all groups in pair wise tests revealed that there was highly significant effect (P<0.001) of Moringa oleifera supplementation on ND HA/HI titers. The highest antibody level was found in the birds of group B, while lowest level was found in the broilers of group D. Similar results were found by a study of Mahmood [22], and they demonstrated that Feed intake, weight gain and FCR was better in group B fed respecting supplemented diet (P<0.05) whilst immune response against ND. There was no significant difference (P>0.05) in spleen weight of broilers among different groups in this experiment. An interesting observation in this study was that Moringa oleifera does increase the parameters but in a non-dose dependent manner and the effect seems to plateau out somewhere around 4 grams per kilogram of feed.

Supplementation of *Moringa oleifera* leaves with inactivated vaccine did not prevent the atrophy of bursa, spleen and harderian gland against the negative effect of vv IBDV 7 days post infection [27]. It was thus concluded that the immunomodulatory effect of Moringa oleifera does not exceed the physiological limitations while optimizing the immunological parameters and can be an exceptional ameliorant when added as a supplement to the commercial and backyard poultry.

In a nutshell, the aim of present study was to assess the effect of varying levels of immunogenic response of *Moringa oleifera* supplementation broiler against the Newcastle Disease vaccine. 144 day-old chicks (Male and Female mixed) with average weight of 46,78 g were selected for this experiment. Chicks were divided into four groups; A, B, C and D with 3 replicates consisting of 12 birds each in all groups using Completely Randomized Design as the driving statistical model. Diet D was without any supplementation of *Moringa oleifera*, diet A was supplemented with 2% *Moringa oleifera*, diet B was supplemented with 4% *Moringa oleifera*, diet C was supplemented with 6% *Moringa oleifera*. The study was conducted for a period of 35 days. Daily feed intake and fortnightly weight gain were recorded during the experimental period. The blood samples from each group were collected at day of 14, 21 and 35 after slaughtering experiment birds and weight of lymphoid organs and hematology parameters were observed.

During 1 to 35 days, best FCR was found for experimental diet C and Highest HI titer against NDV was observed for experimental diet A and B with supplemented with 2 % *Moringa oleifera*, diet B was supplemented with 4 % respectively. Among the weight of lymphoid organs, the weight of Bursa was highest for experimental diet C. No significant difference was observed for the weight of other body organs. At day 14th and 21th day, PCV was significant (P<0.001). Maximum PCV was in group B and lowest PCV was in group A. Maximum hemoglobin was in group B and C and minimum hemoglobin (g/dL) was in group A.

At day 35th, PCV data was significant (P<0.001) with maximum PCV in group D and lowest PCV was in group C and maximum hemoglobin in group D and minimum hemoglobin (g/dL) in group C. Form present study it may concluded that supplementation of *Moringa oleifera* imparts positive effect on growth performance of birds, hematological and immunological effects in birds and antibody titer against ND and it can be recommended as an ameliorative supplement.

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