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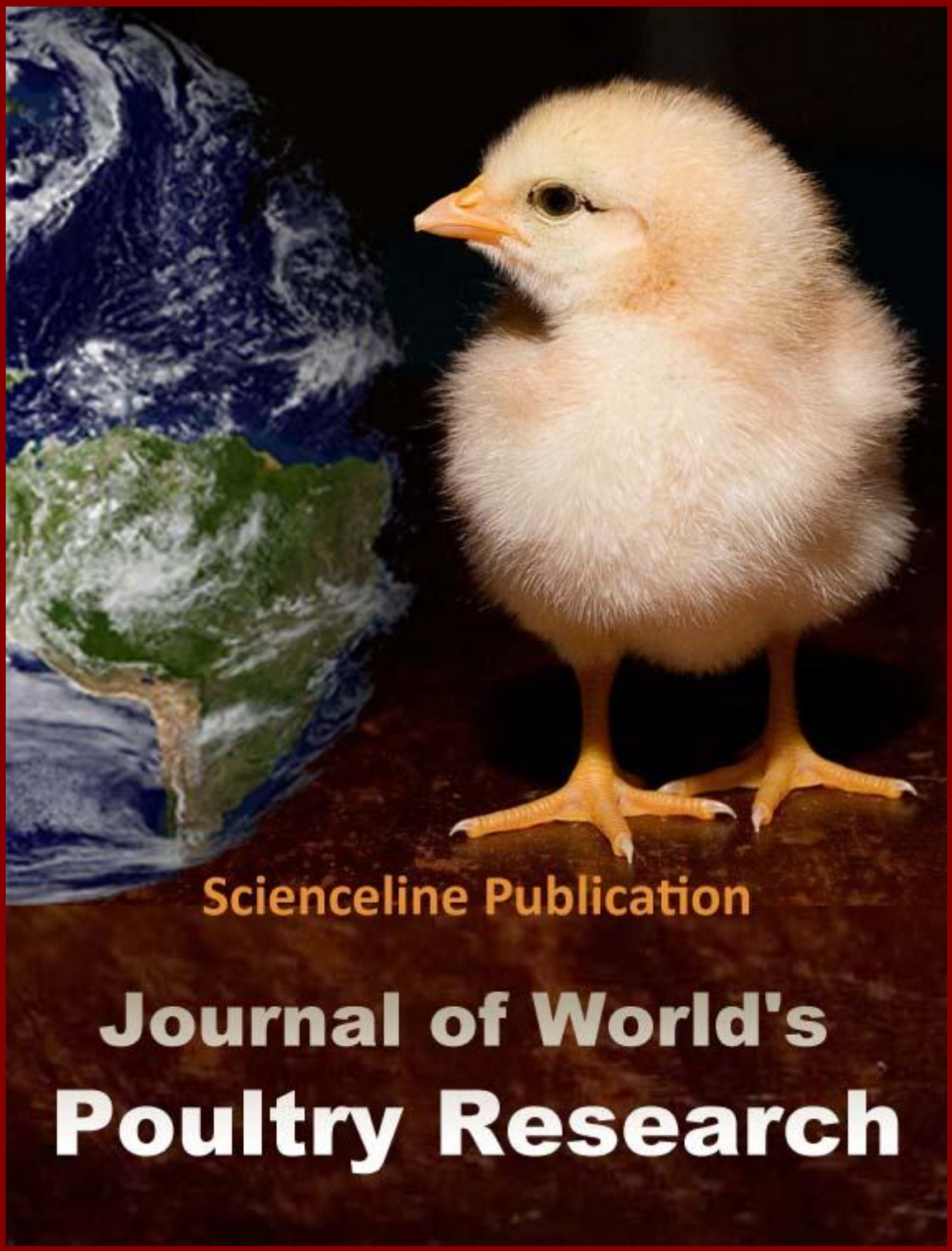

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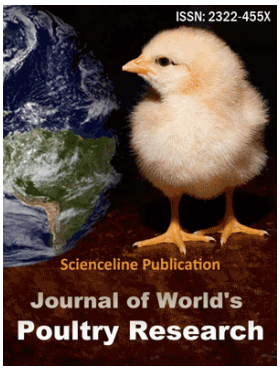

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Productive Performance and Immune Response of Two Broiler Breeds to Dietary Moringa Supplementation

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ABSTRACT

Antibiotic growth promoters were widely used to improve broiler performance however with the increased problems associated with its use such as their residues and subsequent resistance to bacteria has caused them to replace antibiotics for herbs and plant extract alternatives (phytogenics). One hundred and fifty Cobb500 chicks and 150 Ross 308 chicks were distributed from two to six weeks of age into three treatments (50 birds/ treatment) which included 2% *Moringa oleifera* supplemented ration (M 2%), 3% *Moringa oleifera* supplemented ration (M 3%) and control treatment for both breeds, moreover, chicks of each treatment were distributed into five replicates (10 birds/replicate). Ross breed achieved significantly higher ($P<0.05$) body weight, weight gain, feed intake, feed conversion ratio, carcass weight and breast muscle weight compared to Cobb breed. Moreover Ross breed responded better to dietary *Moringa oleifera* supplementation than Cobb. Firstly M(3%) was decreasing body weight and weight gain than M(2%) however with time the opposite occurred with carcass cuts and internal organs weights were not affected significantly ($P<0.05$) with dietary *Moringa oleifera* supplementation. Ross 308 breed showed an increase in HI titer against Newcastle disease virus than Cobb 500 breed. Finally we concluded that the Ross breed respond better to dietary *Moringa oleifera* supplementation. However, more future researches are required to study the response of different broiler breeds to different dietary Moringa levels.

Key words: *Moringa oleifera*, Breed, Performance, immunity and Newcastle disease virus

INTRODUCTION

A lot of feed additives are being used in the poultry industry to maximize growth performance of broilers. Use of in-feed-antibiotics leads to residues in meat and eggs, increases the cost of production and develops microbial resistance to different antibiotics. However inhabit usage of Antibiotic Growth Promoters (AGPs) from poultry feed may affect their production performance and encourage regenerated pathogens leading to diseases and economic losses in farms (Yang et al., 2009)

Moringa is a genus from the plant family called Moringaceae. This genus comprises of 13 species and grow in tropical and subtropical climates (Yang, et al., 2006). All parts of the *Moringa oleifera* tree has beneficial properties. It is a multipurpose tree, various parts of which are used as feed stuff. Moringa contains high antioxidants and anti-inflammatory compounds (Yang, et al., 2006). Nutrient composition of *Moringa oleifera* leaves indicates a rich nutrient profile of important minerals, a good source of protein and amino acids, vitamins, ß -carotene and various phenolics with multiple feed additive purposes (Moyo et al., 2011).

Juniar et al. (2008) found that the inclusion of Moringa oleifera leaf meal at amounts up to 10% did not produce significant ($P> 0.05$) effects on feed consumption, body weight, feed conversion ratio and carcass weight in broiler chickens. Many researchers have reported a major effect of the genotype on live weight (Ojedapo et al., 2008; Razuki et al., 2011), feed conversion, carcass composition (Marcato et al., 2006; Nikolova and Pavlovski, 2009), carcass weight (Rondelli et al., 2003),

and abdominal fat (Barbato, 1992; Fontana et al., 1993). However, the question now is if the various broiler breeds will response differentially to Moringa supplementation?

Muhammd et al. (2016) observed that Moringa oleifera leaf meal may replace dietary soya beans meal up to 15%, with optimum level of 5% in growing Japanese quails, its effect on growth performance, immune function, and ileum microflora in broilers was studied by Yang et al. (2007) and they found a significant enhancement of duodenum traits, and enhancements of the immune system in broilers were observed.

Thus the objective of this study is to investigate the effect of inclusion different levels of dietary *Moringa oleifera* leaves on productive performance, carcass characters, blood antioxidants and immune response of two broiler breeds from 2 to 6 weeks age.

MATERIALS AND METHODS

Birds and experimental design

This work was applied in experimental poultry unit, faculty of veterinary medicine, Damanhour University, Egypt within August and November 2015. 150 Cobb500 chicks and 150 Ross 308 chicks were obtained from Arab poultry breeders company Ommat. The chicks of each strain were distributed into three treatments (50 birds/ treatment) which included 2% Moringa supplemented ration (M 2%), 3% Moringa supplemented ration (M 3%) and control treatment, moreover chicks of each treatment were distributed into five replicates (10 birds/replicate). Chicks were brooded under gas brooder supplied 33°C at the first week reduced 3°C per week till reaching 24°C. Light was supplied for 24 hours during the first 48 hours of life then lighting duration was reduced to 18 hours per day. Chicks were fed with starter ration (23% Crude Protein (CP) during first two weeks without the addition of Moringa. the Experiment was initiated at two weeks of age where chicks were fed on grower ration from two weeks till six weeks of age after the addition of moringa to treated groups at level of 2% and 3%. All chicks were vaccinated with HB1+H120 at eight days of age, Infectious Bursal Disease (IBD) at 12 days and La Sota at 18 days of age and all vaccines were applied through drinking water after following all precautions.

Moringa source and preparation

Moringa leaves used in this experiment were obtained as a powder product from the farm of Moringa friends at Sadat city, Menfoia, Egypt, then it was added to ration from two weeks till 6 weeks of age at two concentration 2% and 3%. The proximate analysis of Moringa leaves showed in the following table 1.

Table 1. Chemical composition (%) of Moringa leaves

	P %	Ca %	Ash %	EE %	Cp %	DM %
According to AOAC 2005	0.77	2.10	12.3	11.7	7.25	89.6

DM= Dry matter; Cp= Crude protein; Ca=calcium; P=phosphorus

Performance traits

Body weight: body weight measured to most exact gram weekly from two weeks till six weeks of age using sensitive scale.

Weight gain= W2 – W1 where W1 = is the weight at any week and W2 = the weight at the next week.

Feed intake/ bird/ week

Water intake/ bird/ week

Mortality/treatment/week

Feed Conversion Ratio was estimated according to Lambert et al. (1936).

$$FCR = \frac{\text{feed intake (g)/bird/week}}{\text{weight gain (g)/bird/week}}$$

Carcass traits

At six weeks of age three birds per replicate were slaughtered after starvation for 12 hours with continued water supply (Sadek et al., 2014). The birds were weighed before being slaughtered then weighing again after evisceration to calculate dressing percentage. Abdominal fat (including fat around gizzard) and internal organs (including intestine, liver, gizzard and heart) were weighed to the nearest gram using sensitive scale (0.0000). Carcasses were divided and the weight of thigh, shoulder and left breast were measured.

Chemical analysis

Blood samples were collected from the wing vein at the end of experiment (42 days), serum were separated through centrifugation at 3000 rpm for 15 minutes and preserved in a deep freezer at (-20°C) until the time of analysis.

Haemagglutination inhibition test

Newcastle Disease Virus (NDV) antigen, la Sota strain, was used to test serum samples collected at 35 days of age (10 samples per each group) for antibody titers against NDV as described by Allan et al. (1978). Here the Haemagglutination Inhibition (HI) titer was expressed as the reciprocal of the highest dilution that causes inhibition of agglutination and Gometric Man Titer (GMT) was calculated.

Lactobacillus count was done using Rogosa agar as a selective medium used for the isolation of lactobacilli and the typical colonies appeared after 48 hours of incubation at 37°C in 5% CO2. According to Rogosa et al. (1951) approximately 100 mg of intestinal digesta were collected three times after the end of essential oil treatment at 3, 7 and 14 days and mixed each time with 900µL of sterile saline solution (0.9% NaCl) and homogenized three minutes using a homogenizer. Each digesta homogenate was serially diluted from initial 10-1 to 10-9 and subsequently plated on selective agar media (Rogosa agar) and incubated anaerobically at 37°C for 48h for *Lactobacillus* count.

Ethical approval

This study was carried out in strict accordance with the recommendations in the guide for the care and use of laboratory animals of the national institutes of health. The protocol was approved by the committee on the ethics of animal experiments of Alexandria university, Egypt (Permit Number: 18261).

Statistical analysis

Body weight data were analyzed using a two way analysis of co-variance for initial body weight data (two weeks body weight) as there is a significant difference of initial weight between the two breeds, however other productive and carcass traits absolute weight data were analyzed using the two way analysis of variance by SAS (2002), Proc GLM (P<0.05).

RESULTS AND DISCUSSION

Effect of breed and *Moringa oleifera* leaves supplementation and their interaction on broiler performance are shown in table 2. Concerning the effect of breed, there are significant increases (P<0.05) in body weight and weight gain of Ross breed than Cobb allover experimental period (2172.89 g vs. 1784.86 g). Hascik et al. (2010) found that the Ross 308 chicks responded most positively to the feed commercially manufactured compound feed as compared with hybrid Cobb500 and Hubbard JV also, they were the most adaptable in the current farming environment.

Cobb chicken showed significant reduced body weight and body weight gain when fed on different levels of *Moringa oleifera* as opposed to unsupplemented groups. In contrast to Ross broiler which showed higher body weight and weight gain with Moringa supplemented groups when compared with the control group. However, the differences were not significant. Rashid et al. (2012) found that Ross strain got the highest significant (p<0.05) live body weight gain in comparison with Cobb and Hubbard strains under heat stress and different dietary protein level. These results may be referring to higher ability of Ross breed on acclimatization and adaptation on the new environmental condition or dietary composition than the Cobb breed.

Table 2. Effect of breed, Moringa supplementation and their interactions on weekly body weights of broilers from two to six weeks

Item		Week2	Week3	Week4	Week5	Week6
Breed						
	Cobb	451.25±5.44^{b}	691.7±8.51^{b}	1061.82±14.16^{b}	1454.64±21.6^{b}	1784.86±27.56^{b}
	Ross	483.11±5.02^{a}	756.67±8.23^{a}	1204.71±13.72^{a}	1761.73±20.71^{a}	2172.89±25.25^{a}
Moringa (%)						
	M(2%)	474.35±6.87	733.56±10.42^{a}	1128.69±17.4^{ab}	1600.6±26.39	1921.19±33.4
	M(3%)	470.97±5.96	702.11±9.57^{b}	1099.96±15.85^{b}	1592.59±24.39	1990.86±31.1
	Control	461.14±7.1	736.89±9.82^{a}	1171.15±16.45^{a}	1631.37±24.76	2024.56±29.96
Breed*Moringa (%)						
	M(2%)	445±8.93	692.13±16.13	1059.13±26.71^{c}	1440.74±39.92^{bc}	1709.86±51.8^{c}
Cobb	M(3%)	463.62±5.7	671.57±13.37	997.53±22.58^{c}	1389.17±35.69^{c}	1741.74±44.7^{c}
	Control	444.52±11.78	711.41±13.63	1128.79±22.55^{b}	1534.01±34.2^{b}	1902.99±43.52^{b}
	M(2%)	497.83±7.86	774.99±13.69	1198.25±23.03^{a}	1760.46±35.49^{a}	2132.53±43.86^{a}
Ross	M(3%)	477.42±9.98	732.65±13.92	1202.38±22.65^{a}	1796.01±33.93^{a}	2239.99±43.79^{a}
	Control	475.86±7.71	762.38±14.34	1213.5±24.26^{a}	1728.73±36.25^{a}	2146.14±41.52^{a}

Means within the same column under the same category carry different superscripts are significant (P<0.05)

There is no significant difference in the final body weight of different groups fed diets supplemented with different levels of *Moringa oleifera* leaves (2%, 3% and 0%). These results are in agreement with Onunkwo and George, (2015) who found that there was no significant difference (P >0.05) in growth performance parameter in broiler chickens when fed graded levels (0%, 5.0%, 7.5% and 10%) of *Moringa oleifera* leaves meal for seven weeks (49 days. There is no significant difference in feed intake between different experimental groups. Chicken fed with diets containing *Moringa oleifera* leaves at level 3% showed significant increase in FCR at age 28 and 45 day when compared with those fed basal diets. But those of group fed diets supplemented with *Moringa oleifera* leaves at level 2% showed insignificant difference in FCR when compared with the control group all over experimental period (P<0.05).

These results are agree with those of Nkukwanaa et al. (2014) who found that no significant differences were observed in feed intake between treatments during periods from 0 to 35 d, FCR was the highest (P<0.05) in birds supplemented with *Moringa oleifera* leaf meal. However FCR1 from 2-3 weeks was lower on Ross breed than Cobb breed which mean higher weight gain acquired with feed intake in Ross breed however, the opposite occurred with FCR4 from 5-6 weeks where Cobb breed recorded significantly (P<0.05) lower FCR than Ross breed (table 3) which ensures our previous interpretation about the prolonged time required until the adaptation of Cobb breed to the new environmental conditions. Ross breed recorded significantly higher (P<0.05) feed intake than Cobb breed all over the experiment (table 4). Similar results were obtained with Rashid et al. (2012) who recorded significantly (P<0.05) higher feed intake and feed conversion ratio for Ross breed compared with Cobb one.

From our results we may be to conclude that Ross breed adapted more rapidly on new environmental condition than Cobb breed. Regard to water intake it was found that chicken which fed on diets supplemented with *Moringa oleifera* leaf meal drink significantly (P<0.05) more water than the control group table 4. This may be due to leaf meals are generally bitter in taste (Onunkwo and George, 2015), so, the inclusion of *Moringa oleifera* leaves in the diets could have resulted in increase water intake to overcome the bitter taste of the broiler diets.

Table 5 showed the impact of *Moringa oleifera* leaf meal at different levels (2, 3 and 0%) on carcass characters and dressing percentage. There were no significant differences in dressing percentage and other carcass characteristics of different experimental groups (table 5).

Table 3. Effect of breed, Moringa supplementation and their interactions on weight gain and feed conversion ratios of broilers from two to six weeks

Items		WG1	WG2	WG3	WG4	FCR1	FCR2	FCR3	FCR4
Breed									
Cobb		234.58±8.5 5^{b}	385.13±12.69^{b}	479.03±20.25^{b}	392.07±15.07^{b}	2.87±0.15^{a}	2.19±0.07	2.01±0.08	1.63±0.1^{b}
Ross		293.13±9.38^{a}	500.17±14.23^{a}	615.65±19.73^{a}	452.77±14.85^{a}	2.3±0.17^{b}	2.23±0.07	2.05±0.08	2.21±0.09^{a}
Moringa(%)									
M(2%)		270.33±11.62	445±17.51	526.16±24.44	407.68±19.64	2.57±0.21	2.08±0.09^{b}	2.21±0.1	1.9±0.13a^{b}
M(3%)		243.11±10.81	417.72±16.41	556.4±25.36	417.68±18.12	2.7±0.19	2.39±0.09a	1.95±0.1	2.14±0.11^{a}
Control		278.13±10.52	465.24±15.56	559.46±23.62	441.90±17.12	2.47±0.19	2.15±0.08b	1.94±0.09	1.73±0.11^{b}
Breed*Moringa(%)									
Cobb	M(2%)	233.75±16.63	390±22.92	440.24±34.96	329.54±27.78^{c}	3.02±0.30	2.1±0.12	2.21±0.14	1.72±0.19
	M(3%)	208.97±13.81	348.4±22.46	479.47±36.75	440.00±26.60^{ab}	3.04±0.25	2.35±0.12	1.97±0.15	1.8±0.16
	Control	261.03±13.81	417±20.5	517.39±33.4	406.66±23.79^{b}	2.53±0.25	2.12±0.11	1.86±0.13	1.37±0.16
Ross	M(2%)	306.9±16.23	500±26.47	612.09±34.16	485.82±27.78^{a}	2.13±0.29	2.07±0.14	2.2±0.14	2.08±0.19
	M(3%)	277.3±16.63	487.1±23.94	633.33±34.96	395.36±24.63^{bc}	2.35±0.3	2.44±0.12	1.93±0.14	2.48±0.15
	Control	295.23±15.86	513.5±23.42	601.52±33.4	477.14±24.63^{a}	2.41±0.28	2.19±0.12	2.01±0.13	2.08±0.14

Means within the same column under the same category carry different subscript are significant (P<0.05); WG1= weight gain from 2-3weeks; WG2= weight gain from 3-4weeks; WG3= weight gain from 4-5weeks and WG4= weight gain from 5-6weeks. FCR1=feed conversion from 2-3weeks; FCR2=feed conversion from 3-4weeks; FCR3=feed conversion from4-5weeks and FCR4=feed conversion from 5-6weeks

Table 4. Effect of breed, Moringa, week and their interactions on weekly feed intake, water intake and mortality of broilers during two to six weeks

Level		Feed/bird/week	Water/bird/week	Mortality (%)
Breed				
Cobb		714.17±45.1[b]	1386.67±123.82[b]	1.5±0.4[a]
Ross		973.33±63.75[a]	1981.67±155.42[a]	0.33±0.19[b]
Treatment				
Moringa (2%)		825±94.79	1843.75±212.85[a]	1.13±0.48
Moringa (3%)		876.25±77.9	1716.25±183.31[a]	0.88±0.44
Control		830±79.31	1492.5±207.53[b]	0.75±0.41
Week				
Week3		600±22.36[a]	1031.67±73.73[c]	0±0[b]
Week4		918.33±66.2[b]	1788.33±149.56[b]	0.33±0.28[b]
Week5		1043.33±73.29[a]	2156.67±184.84[a]	1.67±0.56[a]
Week6		813.33±94.15[c]	1760±222.38[b]	1.67±0.49[a]
Moringa * Breed				
Moringa(2%)	Cobb	672.5±81.07	1555±197.08	1.5±0.87
	Ross	977.5±140.91	2132.5±342.04	0.75±0.48
Moringa(3%)	Cobb	772.5±74.2	1512.5±238.24	1.75±0.63
	Ross	980±125.03	1920±269.04	0±0
Control	Cobb	697.5±92.14	1092.5±171.68	1.25±0.75
	Ross	962.5±95.69	1892.5±254.64	0.25±0.25
Week * Breed				
Week3	Cobb	556.67±6.67	900±97.13[f]	0±0
	Ross	643.33±24.04	1163.33±20.28[ef]	0±0
Week4	Cobb	776.67±23.33	1480±116.76[d]	0.67±0.67
	Ross	1060±36.06	2096.67±56.08[bc]	0±0
Week5	Cobb	893.33±14.53	1830±167.03[c]	2.67±0.33
	Ross	1193.33±64.38	2483.33±190.29[a]	0.67±0.67
Week6	Cobb	630±100	1336.67±253[de]	2.67±0.33
	Ross	996.67±26.67	2183.33±63.6[b]	0.67±0.33

Means within the same column under the same category carry different subscript are significant (P<0.05); Feed/bird/week= feed intake per bird per week; Water/bird/week= water intake per bird per week

Table 5. Effect of breed, Moringa supplementation, and their interactions on carcass weight, dressing, thigh, breast and shoulder weights traits of broilers at 42 days

Item		Carcass Weight	Dressing (%)	Thigh	Breast	Shoulder
Breed						
Cobb		1348.06±54.89[b]	0.76±0.007	311.89±24.38	269.44±17.33[b]	76.11±3.2
Ross		1561.11±51.15[a]	0.74±0.004	365.44±15.59	319.44±21.27[a]	87.22±4.26
Moringa (%)						
	M(2%)	1440.83±108.98	0.76±0.009	338.17±24.77	304.17±39.25	83.33±6.28
	M(3%)	1396.25±60.51	0.75±0.009	320±21.17	275.83±17.48	80±6.06
	Control	1526.67±54.99	0.75±0.006	357.50±23.57	303.33±15.2	81.67±3.07
Breed*Moringa (%)						
	M(2%)	1221.67±25.87	0.77±0.018	286.67±3.33	233.33±13.33[c]	73.33±3.33
Cobb	M(3%)	1307.5±86.64	0.77±0.007	290±15.28	245±20.21[c]	73.33±7.26
	Control	1515±72.34	0.75±0.012	385.66±25.66	330±15.28[ab]	81.67±6.01
	M(2%)	1660±103.32	0.76±0.004	389.67±20.09	375±50.08[a]	93.33±9.28
Ross	M(3%)	1485±54.08	0.73±0.008	350±33.29	306.67±13.02[abc]	86.67±9.28
	Control	1538.33±98.76	0.74±0.003	356.67±30.87	276.67±14.53[bc]	81.67±3.33

Means within the same column under the same category carry different subscript are significant (P<0.05); M(2%)= moringa oleifera 2%; M(3%)= moringa oleifera 3%

Table 6. Effect of breed, Moringa supplementation, and their interactions on internal organs weight of broilers at 42 days

Item		Gizzard	Abdominal fat	Intestine	Liver	Heart
Breed						
	Cobb	30.56±1.45[b]	24.67±5.02	88.11±5.87[b]	38.11±1.4[b]	8.89±0.59
	Ross	35.33±1.29[a]	19.89±1.61	107.56±4.64[a]	49.67±3.23[a]	10.33±0.55
Moringa (%)						
	M(2%)	33±2.5	26±7.4	91.33±8.69	45.17±4.78	9.17±1.14
	M(3%)	33.67±1.31	18.33±2.01	93.83±6.3	39.83±2.36	9.83±0.65
	Control	32.17±2.07	22.5±2.32	108.33±6.51	46.67±4.01	9.83±0.31
Breed*Moringa (%)						
	M(2%)	29±1.73	35.67±13.38	73.33±4.41	36.67±1.45	7.33±0.33
Cobb	M(3%)	31.67±1.76	18.33±4.41	87.67±10.11	36±3	9.33±1.33
	Control	31±4.16	20±3.51	103.33±8.21	41.67±1.67	10±0.58
	M(2%)	37±3.51	16.33±1.2	109.33±5.81	53.67±6.33	11±1.73
Ross	M(3%)	35.67±1.2	18.33±0.88	100±7.64	43.67±2.03	10.33±0.33
	Control	33.33±1.67	25±2.89	113.33±10.93	51.67±7.26	9.67±0.33

Means within the same column under the same category carry different subscript are significant (P<0.05); M(2%)= moringa oleifera 2%; M(3%)= moringa oleifera 3%

Table 7. Newcastle disease virus HI titers for the collected blood samples from both breeds (Cobb 500 and Ross 308) at 42 days of age

Chickens Groups	Geometric mean (GM) of HI titers (Log 2)	
	Cobb	Ross
Moringa (2%)	3.2	3.5
Moringa (3%)	3.6	4
Control	2.9	3

Table 8. *Lactobacillus* Count of intestinal samples from both breeds (Cobb 500 and Ross 308) at 42 days of age

Chickens Groups	***Lactobacillus* count**	
	Cobb	Ross
Moringa (2%)	3×10^5	8×10^6
Moringa (3%)	25×10^5	1×10^7
Control	4×10^4	3×10^4

Regarding the breed effect, Ross 308 showed significant increase (P<0.05) in carcass weight and breast muscle weight compared to Cobb 500 (table 4) which may be attributed to higher final body weight of Ross than Cobb breed. Moreover, gizzard, liver and intestine weights were significantly (P<0.05) higher with Ross compared to Cobb breed this may be resulted from significantly (P<0.05) higher feed intake of Ross than Cobb breed which increased gizzard, intestine and liver weights.

The effect of *Moringa oleifera* on immune response, indicated that Ross 308 breed showed an increased immunity against NDV than Cobb 500 breed (table 6) and these data were a confirmation to Eze et al. (2013) who reported that *Moringa oleifera* extract increased ND HI titer in the vaccinated and un-vaccinated chicken groups with NDV vaccines.

The observed data indicated the better weight gain and FCR in Ross 308 chickens as it has a significant increase in *Lactobacillus* count inducing better feed digestion, absorption, increased digestive enzymes as well as reducing the bad effect of harmful bacteria in the intestinal tract. Also, Yang et al. (2007) indicated the positive effect of *Moringa oleifera* (3% dried leaves) on enhancement of duodenum traits, increased concentrations of total globulin, γ-globulin and IgA, lymphocyte ratio, reduced *E. coli* and increased *Lactobacillus* counts in

ileum improving the whole immune responses and improved intestinal health of broilers which helped in increasing the production of digestive secretions and nutrient absorption, reduced pathogenic stress in the gut, exert antioxidant properties and reinforce the animal's immune status, which help to explain the enhanced performance in poultry.

CONCLUSION

Ross breed responded better to dietary Moringa supplementation than Cobb. Also, Ross breed achieved significantly higher ($P<0.05$) body weight, weight gain, feed intake, FCR, carcass weight and breast muscle weight compared to Cobb breed. Ross 308 breed showed an increase in HI titer against NDV than Cobb 500 breed.

Competing interests

The authors declare that they have no competing interests.

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Effect of Probiotic *Lactobacillus* Species Supplementation on Productive Traits of White Leghorn Chicken

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ABSTRACT

Probiotics are live microbial food ingredients that have a beneficial effect on human health. Intake of probiotics improves feed intake, egg production and egg quality in laying breeds. The objective of this study was to evaluate the effect of the probiotic *lactobacillus* species supplementation on productive traits of White Leghorn chicken. For this purpose, 30 samples of cow milk were collected from Haramaya university dairy farm during the period from May to August 2015. The probiotic properties of each isolates were confirmed by simulating gastrointestinal tract conditions. Based on physiological and biochemical tests *Lactobacillus acidophilus* and *Lactobacillus plantarum* were isolated. The experimental design used in this experiment was single-factor Completely Randomized Design (CRD) with treatments basal feed (control), supplementation of *L. acidophilus* (T2), *L. plantarum* (T3) and their combination (T4) and a 5% ($P<0.05$) level significance was used. Supplementation of Lactobacillus species improved the Feed Intake (FI), Hen Day Egg production (HDEP) and egg weight. The FI recorded were 98.9 g/day/hen, 99.8 g/day/hen, 101.8 g/day/hen and 105.0 g/day/hen in control, T1, T2 and T3 respectively. HDEP of 0.31%, 0.33%, 0.33% and 0.34% were recorded at control, T1, T2 and T3 respectively. The egg weight of the control treatment, T1, T2 and T3 were 50.8g, 51.4 g, 51.4g and 51.9g respectively. Probiotic *Lactobacillus* species (*L. acidophilus and L. plantarum*) improves the productive traits of the laying flock. Chicken received the combination of probiotic *lactobacillus* species significantly perform best in FI, HDEP and egg weight.

Key words: Chicken, *Lactobacillus*, Probiotic, Productive trait, Supplement

INTRODUCTION

Probiotics are defined as live microbial food/feed a supplement which beneficially affects the host animal by improving its intestinal balance that prevent from the growth of pathogenic bacteria, help the growth, multiplication and establishment of beneficial microflora in the intestinal environment (Fuller, 1989). Feeding viable *Lactobacillus* improves feed consumption, size of egg, and mineral retentions and decreases intestinal length from 7 to 59 weeks of age (Nahanshon et al., 1996).

Probiotics supplementation into poultry diets improves feed intake and growth performance in poultry breeds (Sarangi et al., 2016). Similarly, inclusion of probiotics significantly influences feed conversion ratio, egg production performance and egg quality in laying strains (Lei et al., 2013;Inatomi, 2016).Commonly used microorganisms as probiotics in animal feed are mainly bacteria strains belonging to different genera, e.g. *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Bacillus* and microcopic fungi, including *Saccharomyces* yeasts (Guillot, 2009). Feeding viable *Lactobacillus* species increased daily feed consumption, egg size, and nitrogen and calcium retentions in laying breeds (Nahashon et al., 1996). Probiotics improve feed intake and body weight gain in chicken fed with probiotics compared with that in control group fed basal diet (Zhang and Kim, 2014).

Moreover, probiotics have several beneficial impacts, including stimulating appetite, improving intestinal microbial balance, stimulating the immune system, producing digestive enzymes and utilizing indigestible carbohydrates (Prins, 1977; Nahanshon et al., 1992; Nahanshon et al., 1993; Fuller, 1989; toms and Powrie, 2001; Gilliland and Kim, 1984; Saarela et al., 2000). The objective of this study was to evaluate the effect of the probiotic *lactobacillus* species supplementation on productive traits of White Leghorn chicken.

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MATERIALS AND METHODS

Study area and sample collection

The experiment was conducted at Haramaya university poultry farm, Ethiopia (Effect of probiotic supplementation) and microbiology laboratory (isolation, characterization and testing *Lactobacillus* species). A total of 30 samples of raw cow milk were collected from Haramaya university dairy farm during the study period May to August 2015. The raw cow milk samples were collected using sterile bottles and transported to the microbiology laboratory in icebox for analysis. Aseptic sampling was followed as described by the Health Protection Agency (HPA, 2014) and the Food and Drug Administration (FDA, 2003). After arrival at the laboratory, samples were kept at temperatures below 4^{o}C and were analyzed within 48 hours of collection.

Ethical approval

This research did not involve feeding of birds with pathogenic microorganisms, introduction of any intervention in/on birds, or direct collection of cells, tissues or any material from birds.

Isolation of lactic acid bacteria lactic acid bacteria

Lactic acid bacteria (LAB) were isolated from raw cow milk. A 0.1 ml of different dilution (10^{-2} to 10^{-8}) of samples was inoculated on De Man Ragosa Sharpe (MRS) agar medium (pH 6.2) plates and incubated at 37^{o}C for 24-36 hours anaerobically. The presence of acetate, citrate and tween-80 in MRS agar allows selective isolation of LAB, at the same time ensuring the removal of most fastidious organisms.

Physiological and biochemical characterization of lactic acid bacteria

Phenotypic properties of LAB such as cell morphology of all isolates were determined using a microscope by Gram staining (Bergey et al., 1989). Isolates were further tested for different tests including catalase test, CO_2 production form glucose, growth at different temperatures (15, 37 and 45^{o}C) as well as the ability to grow in different concentrations of sodium chloride, antibiotic resistance and pH in MRS agar. Sugar fermentation patterns of LAB isolates were determined using different sugars.

Feasibility tests of *Lactobacillus* probiotics

Feasibility tests of *Lactobacillus* was carried out using Gastrointestinal Tract (GIT) conditions of chicken including, antibiotic resistance, resistance to low pH, resistance to bile salt, bile salt hydrolysis and antimicrobial activity against pathogens were done using standard procedures.

Experimental animal management and design

A total of 120 White leghorn layers were used for the study. The feed ingredients used in the experiment were according to standard layers diet (basal diet) and probiotic bacteria were supplemented. Before the commencement of the actual experiment and placing the experimental animals in the pen, watering troughs, feeding troughs, laying nests and the pen itself were cleaned thoroughly, disinfected and sprayed. The birds were vaccinated for the common diseases.

The chickens were randomly distributed into the pens each having the capacity of 10 hens. The birds were fed in a group providing feed twice a day at 8:00 and 16:00 hours. Each pen was provided with laying nest, feeders and watering point. A regular 16 hours light was provided throughout the experimental period of 84 days (12 weeks). The birds were acclimatized for one week for the new feed treatment.

A completely randomized design with four treatments was used as in table 1. T1 was control without probiotic bacteria supplementation, T2 was supplementation of *Lactobacillus acidophilus* in the diet, T3 was supplementation of *Lactobacillus plantarum* in the diet and T4 was supplementation of both *Lactobacillus acidophilus* and *Lactobacillus plantarum* in the ration. Each treatment was replicated three times having 10 layers each replica. The probiotic bacteria used for the study were the isolated, characterized and cultivated probiotic bacteria in the Haramaya University, microbiology laboratory.

Response criteria

The parameters employed in this experiment were: Feed Intake (FI), Hen Day Egg Production (HDEP), egg weight and egg size. FI was calculated by subtracting the amount of feed refusal from the amount of feed offered/day. HDEP was calculated as the ratio of the number of eggs collected/day with the number of birds in the pen. Eggs collected during the experiment categorized as jumbo, extra-large, large, medium, small and pee wee based their size (table 2).

Data analysis

Collected data were analyzed using of SAS 9.1.3 and data on production and egg quality parameters were stratified into the main factor (probiotics). A 5% ($P<0.05$) level of significance was used to determine statistical significance.

RESULTS AND DISCUSSION

Isolation, testing and characterization of *lactobacillus* probiotic

Probiotic *Lactobacilli* species including *lactobacillus acidophilus* (hudf8) and *lactobacillus*

plantarum (hudf20) were the candidates of LAB species from raw unpasteurized cow milk samples (Table 3, 4 and 5).

Effects of probiotic *lactobacillus* species on productive traits

The effects of probiotic *L. acidophilus* and *L. plantarum* on FI, HDEP, egg weight and egg size are presented in (Table 6). Supplementation of probiotic *lactobacillus* species improved the FI, HDEP and Egg weight. However, there was no significant effect on egg size in layers supplemented with probiotic. Significantly higher FI, HDEP and egg weight was recorded at chicken supplemented the combination of the *lactobacillus* species (*L. acidophilus* and *L. plantarum*).

In this experiment, improvement in FI was recorded as a result of probiotic supplementation. Raka et al. (2014) reported a rise in feed and water consumption in laying hens fed with Liquid Probiotics Mixed Culture (LPMC) containing two type microorganisms, *Lactobacillus* and *Bacillus* species which is in agreement with the current study. Similarly, Nahashon et al. 1996, feeding viable *Lactobacillus* at 1100 mg kg^{-1}(4.4 $\times 10^7$ colony forming unit kg^{-1}) increased daily feed consumption, egg size, nitrogen and calcium retentions. Another study by Zhang and Kim (2014) reported an increase body in FI in chicken fed with multi-strain probiotics compared with that in control group fed basal diet. Similar results were observed with studies by Lei et al. (2013), Inatomi (2016) and Sarangi et al. (2016) in that Probiotics supplementation into poultry diets improves feed intake and growth performance in laying flocks. However, Inclusion of probiotic caused no significant increase in feed consumption, egg production and egg weight (P>0.05) (Mahdavi et al., 2005). Another study, Saadia and Nagla (2010) reported FI values of different treated groups were approximately similar and lacked significance with layer flock that fed with probiotics.

The study shows an increase in HDEP and average egg weight due to probiotic supplementation. Raka et al. (2014) reported the highest HDP and egg weight in layers supplemented with LPMC containing two type microorganisms, *Lactobacillus* and *Bacillus* species. Similarly, Yörük et al. (2004) reported that egg production in Hisex Brown layers fed with probiotics contained *L. plantarum* and *L. acidophilus*, showed greater egg production than the group fed with basal diet. Moreover, there were linear increases in egg production with increased supplemental probiotic. Moreover, significant improvement in egg production was observed in hens supplemented with a mixed culture of *L. acidophilus* and *L. casei* (Haddadin et al., 1996).

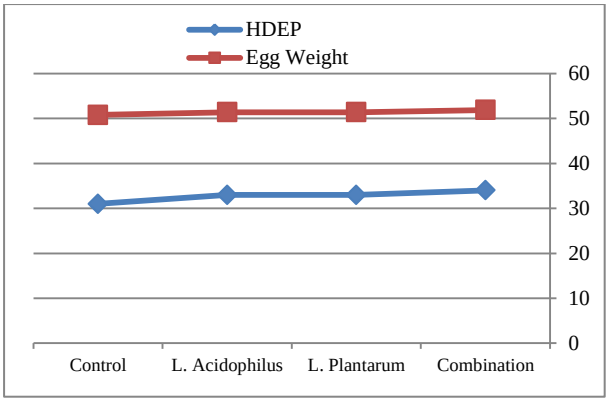

Figure 1.effect of *Lactobacillus acidophilus, Lactobacillus plantarum* and their combination on hen day egg production and egg weight in White Leghorn hens during the study period

A study by Davis and Anderson (2002) found no significant improvement in egg production of hens supplemented with Prima Lac, a commercial product containing *Lactobacillus* species. Similarly, Addition of probiotic had no significant effect (P>0.05) on shell hardness and shell thickness and these were expected which have already been reported (Haddadin et al., 1996 and Mohan et al., 1995). The same result was reported by Ramasamy et al. (2008) in which, supplementation of *Lactobacillus* cultures did not influence the egg production of hens throughout the experimental period and no significant difference in egg weight in hens fed with *L. acidophilus.*

Table1. Layout of the experiment on effect of probiotic *lactobacillus* species on productive traits in White Leghorn chicken during the study period

Treatments	Number of replication	Supplementation of lactic acid probiotic bacteria	Number of birds per replica	Total number of birds per treatment
T1	3	No probiotic bacteria (control)	10	30
T2	3	*Lactobacillus acidophilus*	10	30
T3	3	*Lactobacillus plantarum*	10	30
T4	3	*Lactobacillus acidophilus* and *Lactobacillus plantarum*	10	30

T1: treatment 1; T2: treatment 2; T3: treatment 3 and T4: treatment 4

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Table 2. Modern egg size chart for adult laying chicken used from May to August 2015

Size	Minimum weight (g)
Jumbo	70
Extra-large	63
Large	56
Medium	49
Small	42
Pee wee	<42

Table 3. Physiological and biochemical characteristics of *Lactobacillus* strains isolated from fresh cow milk

Characteristic	Isolates	
	***Lactobacillus acidophilus* (hudf8)**	***Lactobacillus plantarum*(Hudf20)**
Gas from glucose	+	-
Cell shape	bacillus	bacillus
Ammonia from arginine	-	-
Motility	-	-
Catalase test	-	-
Aerobicity	f.a	f.a
Growth at different temperature		
10^{o}C	-	-
15^{o}C	+	-
45^{o}C	v	+
Growth at different pH		
2.0	-	-
4.0	-	+
5.0	+	+
Growth in the presence of NaCl		
2%	+	-
4%	+	+
6.5%	-	-
Carbohydrate fermentation		
Lactose	+	+
Maltose	+	+
Glucose	+	+
Galactose	+	+
Mannose	+	+
Mannitol	+	-
Melezitose	+	-
Salicin	-	-
Melibiose	-	-
Cellulose	+	-
Rhamnose	-	-
Sucrose	-	+
Ribose	-	-

v=variable reaction; f.a=facultative anaerobic; n=2

Table 4. Probiotic feasibility test of *Lactobacillus* strains simulating under gastrointestinal tract conditions of adult layers

Characteristics	Isolates	
	***Lactobacillus acidophilus* (hudf8)**	***Lactobacillus plantarum*(hudf20)**
Resistance to low pH		
2.0	1.03±0.02^{a}	0.98±0.00^{a}
3.0	1.25±0.00^{a}	1.05±0.01^{a}
4.0	1.31±0.00^{a}	1.32±0.00^{a}
Resistance to bile acids 0.3 % (w/v)		
0hr	1.23±0.03^{a}	1.23±0.00^{a}
1hr	1.01±0.01^{a}	1.13±0.01^{a}
2hr	0.93±0.00^{a}	0.98±0.02^{a}
3hr	0.87±0.00^{a}	0.89±0.00^{a}
Antibiotic resistance		
Streptomycin	R	R
Gentamycin	R	R
Tetracycline	R	R
Heamolytic test	-	-

^{a}Means bearing similar superscripts in the same column differs insignificantly (p>0.05); R=resistant; -=negative reaction, n=2

Table 5. Antimicrobial activity of *Lactobacillus* isolates from fresh cow milk from May to August 2015

Lactobacillus isolates	Means zone of inhibition zone (mm)			
	Streptococcus aureus	***Klebsiella pneumonia***	***Escherichia coli***	***Pseudomonas aeruginosa***
*L.acidophilus*hudf1	8±0.00[b]	11±0.00[b]	12±0.00[b]	8±0.00[b]
*L. plantarum*hudf3	9±0.01[b]	12±0.03[b]	9±0.00[b]	11±0.00[b]
*L.acidophilus*hudf8	21±0.02[a]	18±0.00[a]	17±0.02[a]	17±0.00[a]
*L.acidophilus*hudf12	11±0.02[b]	10±0.00[b]	13±0.00[b]	11±0.00[b]
*L. plantarum*hudf5	12±0.00[b]	10±0.01[b]	8±0.00[b]	9±0.00[b]
*L.acidophilus*hudf6	11±0.00[b]	11±0.03[b]	6±0.03[b]	10±0.00[b]
*L.plantarum*hudf20	19±0.03[a]	20±0.03[a]	18±0.00[a]	20±0.00[a]

[ab] Means bearing different superscripts in the same column differ significantly ($p<0.05$); n=2

Table 6.effect of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and their combination on productive traits in White Leghorn hens during May to August 2015

Parameter	Control	*Lactobacillus acidophilus*	*Lactobacillus plantarum*	Combination
FI (g/day/hen)	98.9 ± 1.16	99.8 ± 0.47	101.8 ± 2.12	105.0 1.00
HDEP (%)	0.31 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.34 0.01
Egg weight (G)	50.8 ± 0.40	51.4 ± 0.35	51.4 ± 0.25	51.9 0.15
Egg size (%)				
Jumbo	----	----	----	----
Extra-large	11.7 ± 0.76	12.8 ± 0.20	13.1 ± 0.25	13.5 ± 0.15
large	22.7 ± 1.06	23.3 ± 0.46	23.9 ± 0.25	24.5 ± 0.50
Medium	44.4 ± 1.24	44.8 ± 0.59	44.4 ± 0.40	44.7 ± 0.47
Small	17.0 ± 0.35	15.2 ± 0.96	14.8 ± 0.40	13.9 ± 0.36
Pee wee	4.0 ± 1.00	3.97 ± 0.15	3.8 ± 0.10	3.3 ± 0.21

CONCLUSION

Supplementation of probiotics into layers diet improves their production performance. In this study, supplementation of probiotics significantly improves FI, HDEP and egg weight. Mixture of probiotics (*L. acidophilus* and *L. plantarum*) is recommended as it significantly improves FI, HDEP and egg weight. However, there was no significant effect of probiotic supplementation on egg size. Despite the improvements in productive traits, further investigation is recommended to establish the optimum dosage and mode of inclusion for different classes of poultry.

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Competing Interests

The authors declare that they have no competing interests.

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Instructions for Authors

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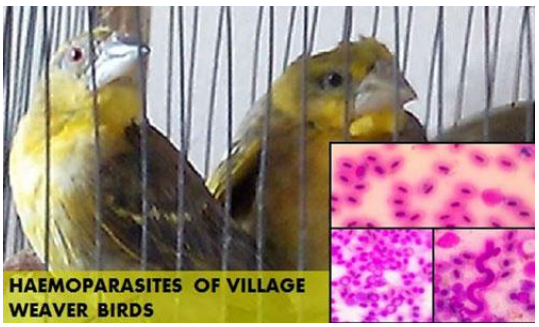

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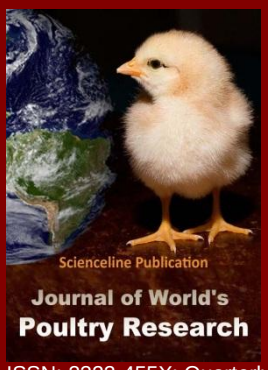

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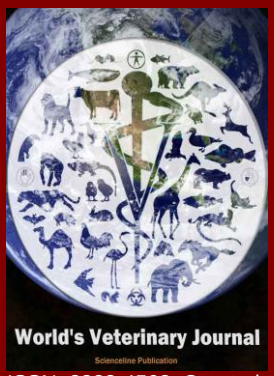

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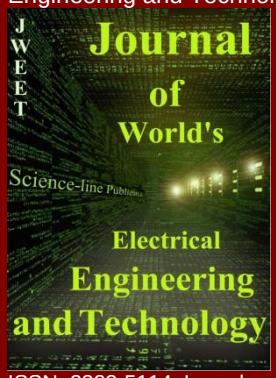

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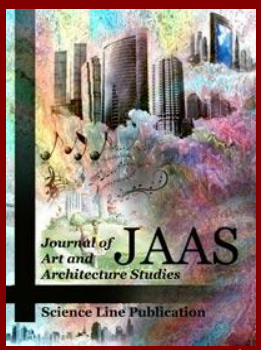

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