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Effect of Fatty Acids on Production and Immunological Status of Vaccinated Broiler Chickens

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***Abstract*—This study was conducted on 400 one day-old male broiler chicks (Ross-308) randomly divided to 2 main groups, 1st main group (GA) feeding basal diet with medium chain fatty acid (MCFA) at rate of 0.15% and divided to four subgroups, 3 subgroups vaccinated with different routes with Newcastle Disease (ND) and non-vaccinated group. The 2nd main group (GB) feeding basal diet without MCFA and divided as the same 1st main gruop. The parameters used in this study included: body weight (BW), phagocytic index (PI), stress index (SI) and weight of spleen and bursa of Fabricious. The aim of this study to evaluate the effect of (MCFA) on these parameters. The experiment lasted for 42 days. Results indicated that additional dietary fatty acid (FA) significantly increased average body weight during the different weeks of the experiment over the basal diet group of birds. Data of PI revealed that birds fed FA supplemented diet had lower values of PI than the basal diet on 2W and the reverse was true on 4W of the experiment. The non-vaccinated birds, on the other hand, showed lowest PI values on 2W and 4W of age. As for, SI a pronounced difference was found due to the two types of diet, and the vaccination methods, as well.**

***Index Terms*—Broiler, fatty acid, newcastle disease, phagocytic index, stress index.**

I. Introduction

Newcastle disease (ND) is a highly contagious avian disease and one of the major causes of economic loss in the poultry industry (Rasolia, et al., 2014). Although all Newcastle disease virus (NDV) isolates belong to a single serotype, significant genetic diversity has been described among NDV isolates (Zhang, et al., 2014). Newcastle disease (ND) remains a constant threat to poultry producers' worldwide, in spite of the availability and global employment of ND vaccinations since 1950s (Kapczynski, Afonso and Miller, 2013). The clinical signs seen in affected birds due to this disease vary widely and are dependent on factors like the virus strain, host species, age of birds, immune status, environmental stress and concurrent infection (Al-Habeeb, Mohamed and Sharawi, 2013). Various approaches have been used for identifying the specific components of the immune system involved in protection (Al-Shahery, Al-Zubeady and Al-Baroodi, 2008). Feed additives are often used to improve physical diet characteristics, feed acceptability and bird health according to (Leeson and Summers, 2008).

Currently, it has been found that natural additive such as herb and medical plants have some properties as growth enhancement to replace synthetic drugs. The antimicrobial effect of the medical plants is well documented by (Mahmmod, 2013). EOs enhance production of digestive secretions, stimulate blood circulation, exert antioxidant properties, reduce levels of pathogenic bacteria and may enhance immune status (Brenes and Roura, 2010). Using these medicinal plant oil in the diet showed significant effects on performance, carcass quality, feed conversion ratio FCR and body weight gain of treated chicks (Ashan, 2011). Many nutrients are capable of modulating the immune system as stated by (Korver, 2012).

Different types of dietary fatty acids have been shown to have variable effects on bacterial clearance and disease outcome through suppression or activation of immune responses (Harrison, Balan and Babu, 2013). By using Aromatic herbal extract, an increase broiler performance (body weight gain, feed conversion, and carcass quality), and enhanced of the immunological performance. HI titer of Newcastle disease virus was significantly higher with addition of aromabiotic, and weight of lymphoid organs (thymus, Bursa of Fabricius) were increased a combined with improvement of leukocytes (heterophil, lymphocyte and eosinophil were noticed by (Tollba, Shahbaan and Abdel-Mageed, 2012). Supplementation the feed with Aromabiotic poultry lead to better growth performances, where average

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daily gain was significantly better in the starter and grower period of broiler chicks (Isaac, et al., 2013).

II. Material and Methods

A. Experimental Birds and housing

Four hundred one day-old male broiler chicks (Ross-308) were obtained from a commercial (VANO) hatchery in Erbil city. The chicks represented a very homogenized sample in the initial where it ranged from 43-44 gm. This was achieved by weighing 400 birds of the sexed male broiler chicks individually and only those lied within aformentioned range was kept for running experiment. Thereafter the 400 chicks were divided into two groups named GA and GB, where the GA (200 birds) group of chicks reared on FA supplemented 0.15% starting, grower, and finisher pellet diet from factory (Agree land) in Erbil city, Table I. The added FA is characterized by being medium chain fatty acid (Aromabiotic) produced by Vitamix Belgium Company. The other GB (200 birds) group of chicks were reared on the same basal diet without FA added. Each of GA and GB chicks where subdivided to 4 groups, 50 birds each, and was subjected to different methods of vaccination against ND, orally, oculonasal, S/C, and control (non-vaccinated). Vaccination was applied when birds were 10 days-old. The sub groups were symbolically named G1, G2, G3 and G4 for birds of GA, according to the three vaccination methods and control, respectively. By the same talking G5, G6, G7 and G8 were referred to the birds of GB. The treatment chicks were reared in floor pen (2.5×1m) on chicken paper liter allowed the access of water and subjected to 24 hour light. The electrically heated house was furnishing the birds with a temperature schedule consist of an initial temp of 34ċ was reached on day of the experiment. Feed was given ad-libitum with feed through space held constant for all birds. Extra care was taken to secure biosecurity during the course of the experiment.

B. Body Weight

At the end of each of the last 5 experiment weeks, body weight were determent for each treatment replicate.

C. Blood Collection

Blood samples (2ml) from a wing vein of six birds of each treatment were collected at 2W and 4W of age after starting the experimental diet and vaccination program on day 10 of age. The blood samples were placed onto labeled slides and smears were fixed to determine the Stress index, from the data H/L ratio according to (Redmond et al., 2011) and the Phagocytic index according to (Park, Fikrig and Smithwick, 1968).

D. Immune Organs

Immune organs, represented by Bursa of Fabricius and Spleen were excised from 6 slaughter birds of each of the experimental treatment. Percent of the organs were determent base on live body weight of the birds according to this formula {(organ weight/body weight) × 100} at 2 and 4 weeks of age, for the purpose organ mass was normalized for body weight as somatic index (Keil, et al., 2008).

TABLE I
Composition and Chemical Analysis of the Basal Diet Feed to the Experimental Birds

No	Ingredients	Starter % (1-2wks)	Grower % (3-4wks)	Finisher % (5-6wks)
1	Corn	380	390	450
2	wheat	160	200	200
3	bran	85	80	70
4	Soybean	324	270	218
5	Oil	10	19	23
6	Lysine	1	1.5	1.5
7	Methionine	1	1.25	1.25
8	Colin	1	1	1
9	Calcium	15	14	13
10	Di-calcium hosphate	15	14	14
11	Vitamin	3	3	3
12	minerals	0.2	0.2	0.2
13	Anticoccidia	0.5	0.5	0.5
14	Enzyme	0.75	0.75	0.75
15	Antifungal	1	2	1
16	Salt	2.55	2.8	2.8
	Chemical analysis			
1	Crude protein	22.06%	20.12%	18.04%
2	Energy	2817.4	2916.45	3011.97
3	Methionine	0.45	0.45	0.42
4	Methionine and cysteine	0.74	0.72	0.68
5	Lysine	1.28	1.18	1.04
6	Calcium	0.99	0.92	0.87
7	Available phosphate	0.43	0.41	0.40
8	Sodium	0.16	0.16	0.16
9	Crude fiber	2.96	2.87	2.73
10	Crude fat	3.26	4.18	4.69

Supplied per Kg of diet: Vit. A, 10 000 IU; Vit. D3, 2 000 IU; Vit. E,10 mg; Vit. K3,2 mg; Vit. B1, 2mg; Vit. B2, 6 mg; Vit. B6, 2 mg; Vit. B12, 10 mcg; Niacin, 30mg; Pantothenic acid, 10mg; Folic acid,0.75mg; Biotin, 50mcg; Choline,300mg; Copper, 4 mg; Iron, 40mg; Manganese, 70mg; Zinc,40mg; Iodin

III. Statistical Analysis

Data for each parameter was analyzed by a tow way general linear model analysis of variance (SigmaStat Ver. 31. 2012) with source of variation being affected of dietary FA, vaccination methods and their inter actions. The statistical analysis of trait (BW, SI, PI, Bursa and Spleen percent) was done based on tow way analysis of variance being the effect of FA, vaccination method and their interaction are the main affecting factors, within each age period. The dunncans test used for comparing the means.

IV. Results and Discussion

The effects of feed additives FA on body weight, immune organs weight and (phagocytic and stress indexes) of the broilers during the different phases of experiment are shown in Table Π, Table Ш, Table IV, Table V and Table VI.

A. Body Weight

Chicks fed diet containing 0.15% FA were significantly

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heavier ($p<0.05$) in weekly body weight than chicks fed the basal diet as showed in Table Π, this is due to MCFA act as an alternative of antibiotic and high energy diet. These results are agreement with (Kessler at el., 2009) who showed that the broilers fed the fat-supplemented diets presented higher weight gain as compared to those fed diets with no FA addition, it could be inhibit the excessive growth of a harmful intestinal microorganism, with the result may positively affect poultry health and productivity. Elagib, et al. (2012) stated that aromatic plants and their oil extracts are becoming more important in poultry production as growth promotants. Also (Ashan, 2011) showed that significant effect of medical plant oil on Body Weight (BW) and Body Weight Gain (BWG) and carcass quality. As for the effect of vaccination method, the 2 weeks body weight was significantly the least among birds subjected to oral vaccine, whereas 3rd and 4th week body weight were heavier for birds orally vaccinated and the control groups than those received vaccine via oculonasal an S/C groups. Data of 5th and 6th week body weight show some kind of superiority for birds received no vaccine over dose subjected to vaccination against ND. Data of interaction indicated that within each of two dietary groups, the control chicks showed heavier body weight at 6th week of age than the bird subjected to the ND vaccine through oral, oculonasal and S/C methods, these results are in accordance with those of (Kogut, 2009) who found that a vigorous immune response (vaccination) reduce bird growth, it may be due to use large amount aminoacids to produce Abs in birds against ND vaccine instead of growth Performanc. (Miller, et al., 2010) reported that broilers selected the high fat and energy diets since the first days of age, which leads to better poultry production.

TABLE II
BODY WEIGHT (MEAN ± SE) OF BROILER CHECKS AS AFFECTED BY DIETARY (FA) AND VACCINATION METHOD AT DIFFERENT AGES.

	Treatment	Age, Week				
		2W	3W	4W	5W	6W
Diet	FA	330.2±1.4a	902.3±6.4a	1521.1±6.1a	2331.4±6a	2849±14.4a
	without FA	322.3±1.8b	835.8±7.1b	1285.9±5.5b	2176.5±5.6b	2705.4±6.34b
Vaccination	Orall	325.1±2.52c	881.2±4.1a	1480.8±18b	2245.2±18 c	2761.9±17.3bc
	Oculonasal	330.7±3.8ab	828.5±11b	1423.5±15c	2228±24.9b	2746.5±23.4c
	subcutaneous	330.6±4.32a	859.3±16c	1456±24.8a	2267.8±25a	2793.5±35.5ab
	Control	334.5±1.1ab	907±11.9d	1453.5±24.8a	2274±27.6a	2806.8±24.7a
Interaction(GA)	G1	331.7±3.2b	890±2.9a	1538.1±4.2ab	2300.5±3.9b	2813.7±10.9a
	G2	342.2±0.3a	863.7±3.6b	1473.3±6.2d	2309.97±4b	2821.5±10.2a
	G3	344.6±0.5a	911±4.87c	1537.8±3.3bc	2350.6±5.9a	2879±50.9a
	G4	333.5±1.4b	944.3±2.2d	1535.2±2.9ac	2364.6±4.9a	2881.8±13.2a
Interaction(GB)	G5	318.5±0.7bc	872.5±6.1a	1423.5±10.3d	2190±15.7b	2710.2±11a
	G6	319.3±3.2ab	793.4±4.3b	1373.7±3.1bc	2146±4.4b	2671.6±8a
	G7	316.6±1.9ac	807.5±4.2c	1374.6±5.2ab	2165±4.15a	2708±7.4a
	G8	334.7±1.9d	869.7±8.1d	1371.5±4.6ac	2183.3±7.1a	2730.±14.4a

B. Phagocytic Index (PI)

Diet supplementing with FA cause lower PI on 2W than the basal diet group of birds and the reverse was true as bird aged to 4W, Table ΙΙΙ, may be the FA not influence the cell membrane of the heterophils at the early stage of life. (Kogut, 2009) showed that is largely due to a qualitative impairment of the avian innate host defenses characterized by a functional inefficiency of heterophils and macrophages for the first W to 2W days of life in chickens. On 2W of age the highest significant value of PI was noticed in birds subjected to ND vaccine via S/C method and the lowest was in the non-Vaccinated (control) birds. On the other hand, data of 4W of age revealed some statistical variation in PI values due to the different method of vaccination with the lowest values noticed in the control group, it is due to activation of immune cell after vaccination. As observed in the study of (Rue et al., 2011) that the host innate immune response to virus infection is an immediate reaction designed to retard virus replication and aid the host in developing specific protection from the adaptive immune responses. Data of inter action factors during both ages, indicated that with GA group none of the sub group treatment showed statistical different in PI means. However, with the GB group the S/C group of vaccination resulted in highest PI mean when birds 2W of age. (Baiao and Lara, 2005) showed that the biological point of view fatty acids, antioxidants are defined as compounds that completely protect the biological systems against the harmful effects or reactions that cause the oxidation of macromolecules or cellular structures.

C. Stress Index (H/L) Ratio

H/L ratio proved that within each age period, FA supplemented diet significantly ($p<0.05$) improved the SI parameter compared to the diet without FA groups of birds as shown in Table IV, it is due to direct transporting the FA into the immune cells. As indicated previously by (Gomes and Aoki, 2003) reported that the MCFA is transported through the mitochondrial membrane independently of the carnitine palmitoil-transferase (CPT) system. As for vaccination methods, no differences were detected in SI means during 2W and 4W of age due to different vaccination methods. In this regard, the control (non-vaccinated) birds showed statistically highest SI means over each of vaccination methods. (St-Onge

and Jones, 2002) observed that the MCFA absorbed directly into the portal circulation and transport to the liver for fast rate of oxidation leads to greater energy expenditure to immune cells. Some plant bioactive may play a role in the development of immune response in birds by protecting cells from oxidative damage and enhancing the function and proliferation of these cells which is supported by (Bozkurt at el., 2012). The data present here suggest an obvious involvement of FA in enhancing body weight a combined with improvement in healthy condition of the broiler chicks. Experimental investigations have confirmed that several fatty acids exert changes in the phospholipids of plasma membrane which affect the membrane fluidity and they also alter eicosanoid production (Pablo, at el., 2002).

D. Immune Organs

The ratios of both (Bursa of fabricius and spleen) showed no significant differences due to diet with FA verses diet without FA at 2W and 4W of age. Also, the different vaccination methods and interactions with types of diet had no effect the Bursa of Fabricius and spleen ratios, accept significant lower percent of spleen in birds vaccinated orally at 2W of age compared to the oculonasal, S/C, and control groups was recorded, as clarified in Table V and TableVI . These results supported by (Bozkurt, at el., 2012) who showed that the weight of the liver or bursa of Fabricius was not affected by fatty acid (P<0.05).

TABLE III
PHAGOCYTIC INDEX (MEAN ± SE) OF BROILER CHICKS AS AFFECTED BY DIETARY (FA) AND VACCINATION METHOD AT DIFFERENT AGES.

	Treatment	Phagocytic Index	
		2W	4W
Diet	FA	39.17±1.35b	46.58±1.15a
	without FA	47.29±2a	38.8±1.22b
Vaccination	Orall	41.42±2.44a	46.08±2.3ab
	Oculonasal	40.17±1.83a	43.17±2.11ac
	subcutaneous	51.67±3.12b	44.8±1.6bc
	Control	39.67±1.61a	37.42±2.44d
Interaction(GA)	G1	36.33±3a	51±1.57a
	G2	36.33±2.11a	48.7±1.65a
	G3	42.16±2.46a	47.17±2.75a
	G4	41.83±2.79a	39.5±4.5a
Interaction(GB)	G5	46.5±2.6a	41.17±3.59a
	G6	44±2.1a	37.67±2.01a
	G7	61.168±0.87b	41±0.82a
	G8	37.5±1.33c	35.33±2.03a

Means with no common superscripts within treatment period cell (age) are significantly different (p<0.05).

TABLE IV
STRESS INDEX (MEAN ± SE) OF BROILER CHECKS AS AFFECTED BY DIETARY (FA) AND VACCINATION METHOD AT DIFFERENT AGES.

	Treatment	Stress Index	
		2W	4W
Diet	FA	0.196±0.003a	0.173±0.003a
	without FA	0.202±0.002b	0.256±0.006b
Vaccination	Orall	0.194±0.003a	0.205±0.014a
	Oculonasal	0.196±0.002a	0.204±0.012a
	Subcutaneous	0.196±0.002a	0.208±0.011a
	Control	0.209±0.006b	0.242±0.016b
Interaction(GA)	G1	0.188±0.003a	0.163±0.003a
	G2	0.195±0.003a	0.167±0.005a
	G3	0.193±0.004a	0.172±0.004a
	G4	0.210±0.011a	0.190±0.001a
Interaction(GB)	G5	0.201±0.003a	0.247±0.001a
	G6	0.198±0.003a	0.242±0.007a
	G7	0.200±0.002a	0.243±0.006a
	G8	0.209±0.008a	0.293±0.005a

Means with no common superscripts within treatment period cell (age) are significantly different (p<0.05).

TABLE V
BURSA OF FABRICIUS OF BROILER CHICKS AS AFFECTED BY DIETARY (FA) AND VACCINATION METHOD AT DIFFERENT AGES.

	Treatment	Bursa	
		2W	4W
Diet	FA	0.168±0.014a	0.793±0.026a
	without FA	0.162±0.009a	0.732±0.029a
Vaccination	Orall	0.191±0.029a	0.791±0.034a
	Oculonasal	0.166±0.007a	0.819±0.034a
	subcutanius	0.143±0.009a	0.692±0.046a
	Control	0.159±0.01a	0.749±0.035a
Interaction(GA)	G1	0.192±0.057a	0.812±0.055a
	G2	0.168±0.008a	0.854±0.058a
	G3	0.144±0.009a	0.728±0.026a
	G4	0.166±0.01a	0.776±0.066a
Interaction(GB)	G5	0.189±0.02a	0.769±0.045a
	G6	0.163±0.013a	0.784±0.036a
	G7	0.142±0.016a	0.655±0.091a
	G8	0.153±0.018a	0.721±0.036a

Means with no common superscripts within treatment period cell (age) are significantly different (p<0.05).

TABLE VI
SPLEEN OF FABRICIUS OF BROILER CHICKS AS AFFECTED BY DIETARY (FA) AND VACCINATION METHOD AT DIFFERENT AGES.

	Treatment	Spleen	
		2W	4W
Diet	FA	0.088±0.004a	0.360±0.025a
	without FA	0.084±0.005a	0.312±0.021a
Vaccination	Orall	0.065±0.007b	0.325±0.020a
	Oculonasal	0.087±0.008a	0.313±0.021a
	Subcutanius	0.098±0.007a	0.361±0.052a
	Control	0.095±0.006a	0.344±0.031a
Interaction(GA)	G1	0.064±0.005a	0.328±0.030a
	G2	0.091±0.01a	0.349±0.037a
	G3	0.101±0.009a	0.407±0.091a
	G4	0.099±0.007a	0.355±0.011a
Interaction(GB)	G5	0.065±0.009a	0.323±0.03a
	G6	0.084±0.011a	0.277±0.07a
	G7	0.095±0.004a	0.315±0.051a
	G8	0.091±0.01a	0.333±0.062a

Means with no common superscripts within treatment period cell (age) are significantly different (p<0.05).

V. Conclusion

We can conclude from the results that feed additive given for Broiler chicks by supplying 0.15% MCFA to the basal diet significantly improves their performance. MCFA did not affect weight of spleen and bursa of Fabricuis at day 14 and 28 of their live. In addition, MCFA was not involved in enhancing innate immunity assessed by phagocytic stress index of 14 and 28 days old Broiler chicks.

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