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Impact of dietary supplementation of organic acids on the growth performance and immunity in broilers fed low protein diets

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Abstract

The current work aimed to evaluate the effect of feeding organic acids (OA) on growth performance, carcass traits, meat quality, blood parameters and immune response of broilers fed with a low protein diet (LPD). A total number of 68 broiler chicks (one-day-old) were randomly distributed into 4 equal groups each of 17 chicks. The first group was fed the basal diet (100% NRC crude protein (CP)) free from OA and considered as a control (T1). The other three groups (T2, T3 and T4) were fed on diets with different protein levels (95% NRC, 90% NRC & 85% NRC, respectively) and supplemented with OA at a level of 0.45%. The results showed that body weight and gain during starter phase (days 1-21) were not significantly ($P = 0.7$, $P = 0.13$, respectively) influenced by the supplementation of OA. However, during finisher phase (days 22-42) body weight and gain were significantly decreased ($P < 0.05$) in OA groups (-18% and -24%, respectively). A significant ($P < 0.05$) reduction in abdominal fat content and the meat cholesterol, triglyceride, and fat mass of broiler breast and thigh meat, while protein content significantly ($P < 0.05$) increased in all OA supplemented groups. In conclusion, adding OA improved immune response through increased serum globulin, and an increase in bursa relative weight of broilers. Moreover, the addition of OA to broiler fed LPD has no effect on growth parameters and carcass traits but improves broiler immunity and produces healthy meat to consumers.

Keywords:
Broilers, Dietary crude protein, Growth Performance, Immunity, Meat cholesterol, Organic acids.

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Introduction

Broiler meat considered one of the most important protein sources in the global market, and it is currently the second most consumed meat worldwide (FAO, 2013). This may be due to many factors from which consumer acceptance is very crucial. However, consumer demands are changing and becoming more complex because consumers seek to satisfy their personal interest. Therefore, efforts are made to produce healthy and good quality meat (Arshad et al. 2016).When it comes to broiler diets, the main focus is on crude protein (CP), because protein is a major component of broiler diets, and it, together with the other main nutrients, is essential for life (Cheeke, 2005). In addition, protein and amino acid supplementation in the diet is crucial for maintaining proper immune function and protecting the host from a wide range of diseases (Beski et al., 2015). However, the high price of protein sources, as well as environmental concerns related to high nitrogen excretion, have resulted in increasing interest in using low protein diets (LPD) in broilers production (Ravangard et al., 2017). By lowering the CP content of broiler diets, the protein efficiency ratio linearly increases. In LPD, this favorable impact associated with lower feed cost (Cheng el al., 1997). The drawback of this strategy is the substantial effect on performance parameters as feed conversion ratio (FCR) and weight gain. Therefore, many nutritionists attempted to deal with this problem to achieve a better economic return and reduce the environmental pollution through using new approaches in poultry production (Awad et al., 2015). Disease prevention and improvement of growth and feed efficiency are critical factors in poultry production. For decades, antibacterial growth promoters (AGP) were widely used in broiler feed to improve performance and feed efficacy (Roth et al., 2019). The European ban the non-therapeutic use of AGP due to development of microbial resistance which consequently increased digestive disorders in broilers (Dalle Zotte et al., 2016). Thus, meat production from broiler chicken without application of AGP is important to protect human health against antimicrobial resistance (Haque et al. 2020). As a result of the ban, researchers have focused their efforts on developing natural feed additives that would improve intestinal health and productivity of poultry in the meantime (Ramigani et al., 2017). Because consumer demands and legislative pressure dictate the use of AGP, it is challengeable to find suitable, reliable and cost-effective alternatives to AGP for sustainable poultry production (Kocher et al., 2004). As summed up in a review of Dalle Zotte et al., (2016), the natural feed additives can be divided into probiotics, herbs, prebiotics and organic acids (OA). Because of its three-free characteristics (pollution- free, drug resistance and residual free), OA has been preferred by enterprises (Dittoe et al., 2018). With in-depth research, scholars have discovered that using OA inhibited the growth of pathogenic bacteria, yeasts and moulds. Due to their antimicrobial properties, OA could be suitable alternatives to AGP in poultry (Khan and Iqbal , 2016; Waseem et al., 2016). In addition, OA were reported to have several beneficial effects such as providing better intestinal health for the bird (Mousa, 2018), increased pancreatic secretion (Adil et al. 2010; Samanta et al. 2010), improved protein and energy digestibilities and reduced endogenous nitrogen losses (Suiryanrayna and Ramana, 2015). Because consumers are seeking for meat that can contribute to their personal contentment, OA supplementation can satisfy consumer demands via providing healthy and nutritious broilers meat (Lakshmi et al., 2016). Thus, nutritional intervention has been proposed as a viable strategy for improving meat quality and consequently consumer acceptance (Nieto and Ros, 2012). Considering the positive effects of OA on protein utilization, using LPD

supplemented with these additives in broiler diets may reduce the negative effects of LPD. We tested the hypothesis that using OA supplementation could be beneficial and ultimately compensate the reduction of CP content of the diet of broilers. The current study aimed at evaluating the potential modulatory role of OA supplementation during feeding LPD and its effects on performance, carcass traits, blood parameters and meat chemical composition.

Materials and methods

Ethical approval:

All procedures and protocols in this study was approved by Animal Care and Ethics Committee, Faculty of Veterinary Medicine, Assiut University, Egypt.

Birds, housing and feeding:

A total number of 68 bird one-day old unsexed broiler chicks (Ross 308 breed) were obtained from a local commercial source, weighed and randomly distributed to 4 equal groups each of 17 chicks. The initial average weight of the experimental chicks was (39.58±0.39g). Birds in all groups were housed in floor pens and kept under the same managerial system and environmental conditions. Birds were fed according to two phases feeding program: starter (0 – 21 days) and grower-finisher (22–42 days).

The first group was fed the basal (100% NRC CP) diet free from OA and considered as a control (T1). The other three groups (T2, T3 and T4) were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with OA (BACTICID DRY®, calcium format, calcium propionate, citric acid and calcium carbonate) at a level of 0.45% Birds in the four groups were fed ad libitum on their respective diets and given free access to fresh and clean water and the diets offered in a mash form (Table 1). Birds were vaccinated against New Castle viral disease (NCD) using Hitchner B1 strain at the age of 7day via eye drop, Lasota strain at the age of 17 day via eye drop and colon strain at the age of 27 day via eye drop. Infectious bursal disease vaccine was administrated to birds at the age of 10 day and 20 day in drinking water. Feed intake was recorded daily while live body weight was weekly recorded throughout the 6 weeks of the experimental period.

Feed analysis:

The chemical analysis was performed for the dietary ingredients to assess dry matter (DM), ether extract (EE), CP, and ash while nitrogen-free extract was calculated according to the methods of the association of Official Analytical Chemists (AOAC, 2011). In details, the DM was analyzed by drying samples in an oven at 100°C overnight, and the ash was analyzed by burning samples at 580°C overnight. Using a Soxhlet extractor, crude fat was evaluated as EE while CP was analyzed by the Kjeldahl's method. The metabolized energy (ME) content of the experimental diets was calculated based on chemical composition (NRC, 1994).

Sampling, carcass traits, chemical analysis of meat and meat composition:

At the end of the experiment, three birds were randomly taken from each group weighed and slaughtered to complete bleeding after fasting overnight. The weight of dressed carcass (the weight of slaughtered birds after removal feathers, head and feet but including all the edible offal's) was recorded. The absolute weights of some internal organs including (liver, gizzard and heart), abdominal fat pad and immune organs (bursa, spleen and thymus) were recorded. Internal, immune organ and abdominal fat pad weights were expressed as the relative weight of live body weight.

Meat samples from the breast and thigh of the slaughtered birds in all the

experimental groups were taken separately prepared (carefully minced and homogenized) and chemically analyzed for DM, CP, EE, and ash following AOAC (2011) official methods. Total cholesterol and triglycerides concentration in total lipid extracts obtained from breast and thigh meat samples were determined enzymatically using commercially available reagent kits, (Wako pure chemical industries, Ltd., Tokyo, japan) as described previously (Bligh and Dyer .1959; Naeemi et al., 1995 ; Afrose et al., 2009).

Blood collection and analysis:

Blood samples were collected from the selected birds, allotted to clot at ambient temperature, centrifuged for 15 minutes at 4000 rpm and serum from each sample was extracted. the sera were transferred into aseptic vials and saved at -20 °C until further analysis. The log Newcastle virus serum antibody titer determined by Hemagglutination Inhibition (HI) assay was performed as previously described (OIE, 2013). Total protein and its fractions (albumin and globulin), triglycerides, and cholesterol were measured by spectrophotometer using commercial test kits (Spectrum, Cairo, Egypt).

Statistical analysis:

Data were subjected to analysis of variance using SPSS 20 statistical software (SPSS Inc., Chicago, IL, USA). All values are reported as least square means and standard error of the mean (SEM). Significance was declared at $P < 0.05$ and a trend was set at $0.05 < P < 0.10$.

Table 1: Physical and chemical composition (%) of the experimental diets

Groups[1]	Starter diet (0-21d)				Grower Finisher diet (22-42 d)			
	T1	T2	T3	T4	T1	T2	T3	T4
Feed stuff (%)								
Yellow corn, ground	48.95	53.37	57.83	62.21	60.94	64.77	68.64	72.37
Soybean meal	39.85	36.02	32.18	28.40	30.31	26.98	23.66	20.40
Sunflower oil	7.25	6.50	5.74	5.00	5.20	4.57	3.89	3.30
Limestone, ground	1.71	1.75	1.75	1.76	1.68	1.70	1.70	1.72
Mono calcium phosphate	1.38	1.39	1.42	1.45	1.00	1.01	1.05	1.07
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix[2]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.16	0.18	0.20	0.21	0.08	0.10	0.11	0.12
L-lysine	0.10	0.19	0.28	0.37	0.19	0.27	0.35	0.42
Total	100	100	100	100	100	100	100	100
Chemical composition (calculated)								
ME (Kcal/Kg)	3191.47	3191.51	3191.76	3192.10	3197.09	3198.85	3197.31	3200.05
Crude Protein %	23.00	21.85	20.70	19.55	20.00	19.00	18.00	17.00
Calcium %	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Available phosphorus%	0.45	0.45	0.45	0,45	0.35	0.35	0.35	0.35
Lysine %	1.30	1.30	1,30	1,30	1.16	1.16	1.16	1.16
Methionine %	0.50	0.50	0.50	0.50	0.38	0.38	0.38	0.38
EE %	8.80	8.13	7.45	6.79	6.97	6.41	5.80	5.28
CF %	2.83	2.75	2.67	2.60	2.66	2.59	2.52	2.45

[1]T1, control group fed basal diet without supplementation of organic acids ; T2, T3 and T4 were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with organic acids [2]Each 1.5 kg contains: Vit. A, 12000000 IU; Vit. D3, 4000000 IU; Vit. E, 50000 mg; Vit. k3, 4000 mg; Vit. B1, 5000 mg; Vit.B2, 8000mg; Vit. B6, 5000 mg; Vit. B12, 35 mg; Vit. B3, 70000 mg; selenium, 250 mg; Pantothenic acid, 20000 mg; Folic acid 1000 mg; Biotin, 250 mg; Manganese 100000 mg; Copper, 15000 mg; Iron, 50000 mg; Zinc 50000 mg; Cobalt, 250 mg; Iodine, 1500 mg.

Results

Growth performance

The total feed intake was 4165, 3734, 3732, and 3780 g/bird for T1, T2, T3 and T4, respectively with no significance differences between the experimental groups. The result indicated that starter (days 1-21) body weight and gain were not significantly influenced by the addition of OA to the different protein levels (95%, 90 % and 85% NRC). However, finisher (days 22-42) body weight and gain were significantly decreased in OA groups ($P < 0.05$). So, the birds had a lower final body weight compared to 100% NRC (control). Similarly, overall (days 1- 42) body weight gain was lower in OA supplemented groups than in the control. Moreover, the total feed intake of birds fed 95%, 90% and 85% NRC protein diet supplemented with OA (T2, T3 and T4) were decreased by 431.07, 433.28 and 384.97 g/bird respectively during the experiment. From the obtained results, it was clear that the inclusion of OA to LPD increased FCR compared with the control one (Table 2).

Carcass traits and meat composition

The supplementation of OA had no significant effect on carcass traits and internal organs However, a significant reduction ($P < 0.05$) of abdominal fat content was observed in the OA supplemented groups by 24 % compared to control group (Table 3). On the other hand, there was a significant ($P = 0.02$) increase in bursa relative weight in broilers fed OA supplemented diet (Table 3).

An effect of dietary treatment on meat cholesterol and triglycerides contents was clear. The supplementation of OA significantly ($P < 0.05$) decreased the cholesterol content by 2%, 4.7% and 8.4% in breast meat (Fig. 1A) while 7.5%, 9% and 12 % in thigh meat (Fig. 1B) of broiler fed T2, T3 and T4 diets, respectively. Moreover, the reduction in triglycerides content was more prominent ($P < 0.05$) in both breast (8.29%, 10.62% and 16 %) and thigh meat (11.4%, 13 % and 18%) of broiler fed T2, T3 and T4 diets, respectively.

Table 2: Body weight development (g) and weight gain (g) of broilers fed different experimental diets

Item	Groups[1]				SEM	P Value
	T1	T2	T3	T4		
Body weight (g)						
0 week (Initial)	39.28	39.28	39.37	40.39	0.78	0.70
3 weeks	755.60	745.27	698.13	695.93	23.44	0.164
6 weeks	2411.67^{a}	1999.67^{b}	1981.67^{b}	1937.67^{b}	65.72	0.001
Weight gain (g).						
0-3 weeks	716.32	705.99	658.76	655.54	22.78	0.138
4-6 weeks	1656.07^{a}	1254.40^{b}	1283.54^{b}	1241.74^{b}	44.52	0.001
0-6 weeks	2372.39^{a}	1960.39^{b}	1942.30^{b}	1897.28^{b}	65.05	0.001
Feed conversion ratio (FCR)						
0-3 weeks	1.46	1.52	1.54	1.58		
4-6 weeks	1.88	2.12	2.11	2.21		
0-6 weeks	1.76	1.90	1.92	1.99		

[1]T1, control group fed basal diet without supplementation of organic acids ; T2, T3 and T4 were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with organic acids

Means within the same row with different superscripts are significantly different ($P < 0.05$).

Table 3: Carcass trait and relative weight of immune organs and abdominal fat bad of broilers fed different experimental diets

Item	Groups[1]				SEM	P value
	T1	T2	T3	T4		
Hot carcass %	86.70	85.64	86.49	87.48	0.45	0.116
Eviscerated carcass %	70.59	70.94	73.38	72.88	0.97	0.187
Dressed carcass %	74.58	74.92	77.21	76.51	0.98	0.248
Liver %	2.14	2.01	2.10	2.08	0.08	0.660
Heart %	0.49	0.45	0.53	0.49	0.04	0.556
Gizzard %	1.38^{ab}	1.42^{a}	1.21^{b}	1.22^{b}	0.05	0.001
Abdominal Fat %	1.52^{a}	1.11^{b}	1.11^{b}	1.25^{ab}	0.14	0.020
Spleen (%)	0.10	0.10	0.11	0.12	0.01	0.278
Thymus (%)	0.42	0.51	0.45	0.40	0.05	0.475
Bursa (%)	0.09^{b}	0.15^{a}	0.14^{a}	0.16^{a}	0.01	0.025

[1]T1, control group fed basal diet without supplementation of organic acids ; T2, T3 and T4 were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with organic acids. Means within the same row with different superscripts are significantly different ($P < 0.05$).

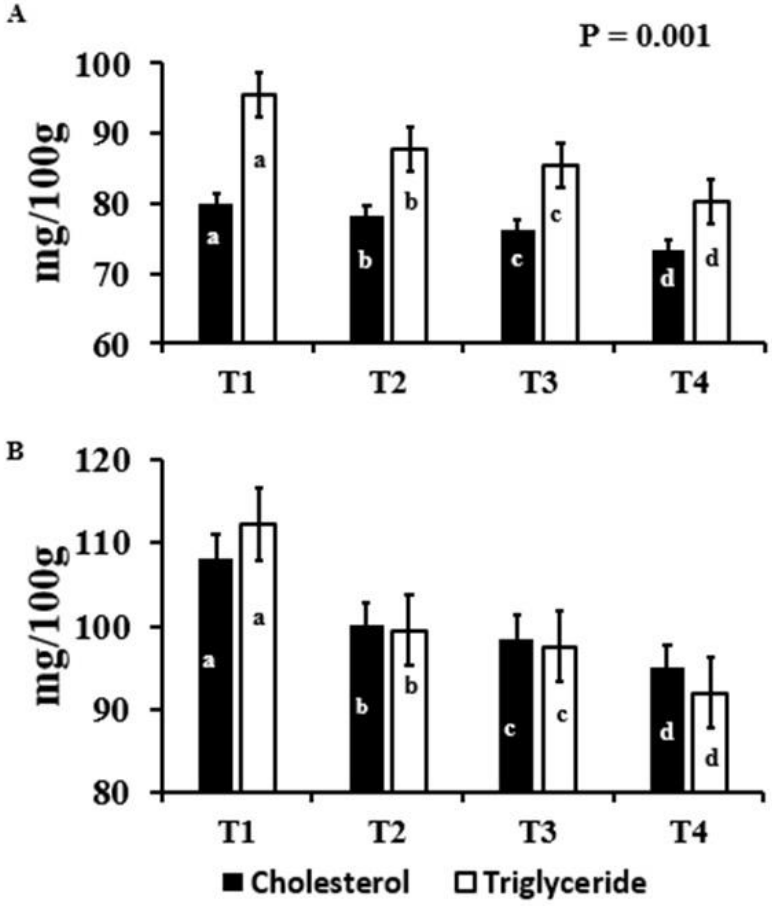

Fig. 1. **Cholesterol and triglycerides level (mg/100g) of breast (A) and thigh meat (B) of broilers chicken.** T1 control group fed basal diet without supplementation of organic acids; T2, T3 and T4 were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with organic acids.

The effect of OA supplementation to various dietary protein levels (T2, T3, T4) on the chemical composition of breast and thigh meat is shown in Table 4. The results indicated that supplementation of OA has no effect on DM and ash content of the breast or thigh meat. On the contrary, the CP content of breast and thigh meat of birds fed diets supplemented with OA increased ($P < 0.05$) compared to birds fed control diet (10.66%, 11.71%, and 14.6% and thigh meat by 9.75%, 11.62 and 15.35% in broiler fed T2, T3 and T4 respectively, Fig. 2) the content of EE was dramatically reduced ($P < 0.05$) in beast (32.65%, 36.73%, and 44.90%) and thigh meat (23.88%, 29.85%, and 38.80%) by supplementation of OA in broiler fed 95%, 90%, and 85% NRC protein diet respectively compared to control one.

Blood parameters

No significant ($P < 0.05$) differences in serum albumin, A/G ratio, cholesterol, and triglyceride content in broiler-fed organic acids were observed Table 5. There was an increase in serum total protein in T2 and T3 which fed 95% and 90% NRC protein diet, while there were no significant differences in total protein for broilers feed 85% NRC protein diet. Serum globulin increased by 21.76% and 15.88% in broiler-fed organic acids supplemented by 95% and 90% NRC protein diet (T2 and T3) respectively. On the other hand, the obtained data in the Table 5 showed no significant differences in the antibody titer of NC virus between different experimental groups at day 42 of age.

Table 4: Meat chemical composition (%) of broilers feed different experimental diets

Item	Groups[1]				SEM	P value
	T1	T2	T3	T4		
Chemical composition (%) of the breast meat:						
DM	26.19	29.55	27.00	26.39	1.16	0.225
EE (%DM)	4.90^{a}	3.30^{b}	3.10^{bc}	2.70^{c}	0.14	0.000
Ash (%DM)	3.27	3.80	3.65	4.47	0.58	0.551
Chemical composition (%) of the thigh meat:						
DM	26.34	29.70	26.80	27.27	1.16	0.247
EE (%DM)	6.70^{a}	5.10^{b}	4.70^{c}	4.10^{d}	0.12	0.000
Ash (%DM)	4.15	4.76	3.33	3.81	0.58	0.409

[1]T1, control group fed basal diet without supplementation of organic acids ; T2, T3 and T4 were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with organic acids. Means within the same row with different superscripts are significantly different ($P < 0.05$).

Table 5: Blood biochemical parameters and humeral antibody titers post-Newcastle vaccination broilers as influenced by different dietary treatment

Item	Groups[1]				SEM	P value
	T1	T2	T3	T4		
Total protein (g/dl)	2.47^{bc}	2.87^{a}	2.73^{ab}	2.30^{c}	0.09	0.009
Albumin (g/dl)	1.13	1.17	1.13	1.07	0.07	0.802
Globulin (g/dl)	1.33^{b}	1.70^{a}	1.60^{a}	1.23^{b}	0.08	0.010
A/G ratio	0.83	0.69	0.73	0.83	0.06	0.311
Cholesterol (mg/dl)	89.25	86.45	89.80	85.47	2.63	0.607
Triglycerides (mg/dl)	77.11	64.99	58.14	57.90	7.56	0.307
Antibody titer[2]	5.00	6.00	5.00	5.00	0.29	0.095

[1]T1, control group fed basal diet without supplementation of organic acids ; T2, T3 and T4 were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with organic acids. [2]Humeral antibody titers post-Newcastle vaccination. Means within the same row with different superscripts are significantly different ($P < 0.05$).

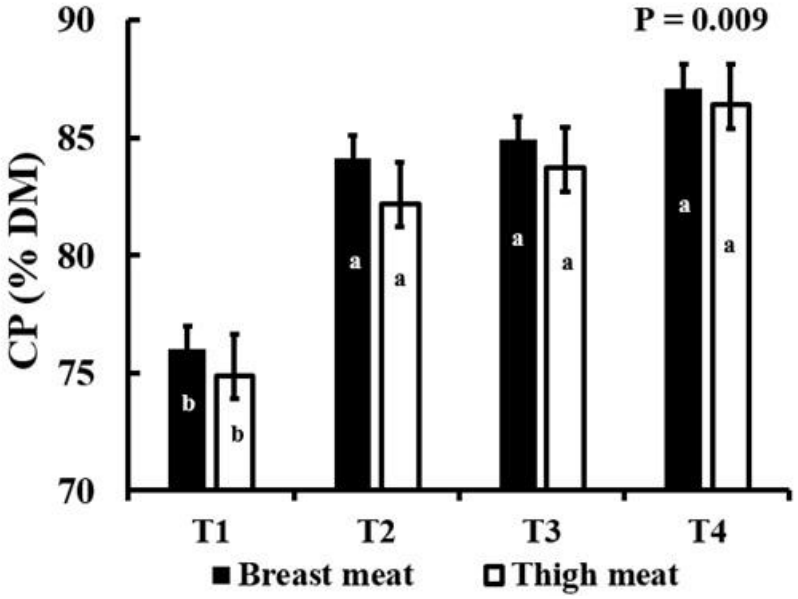

Fig. 2. Crude protein (CP % of DM) of breast and thigh meat of broilers chicken. T1 control group fed basal diet without supplementation of organic acids; T2, T3 and T4 were fed on diets with different protein levels (95% NRC, 90% NRC and 85% NRC) supplemented with organic acids.

Discussion

The importance of OA in livestock and poultry production has already been discussed (Khan et al., 2016; Gadde et al., 2017; Liu et al., 2018). However, the effect of OA on growth performance (Stamilla et al., 2020; Dai et al., 2021), immune-antioxidant ability (Emami et al., 2017; Choi et al., 2020), intestinal function (Kumar et al., 2020; Saleem et al., 2020), and intestinal microbiota of poultry (Sabour et al., 2019) were the focus of all previous studies. Dietary supplementation of OA is definitely useful to broiler health and productivity. Such beneficial impacts can be ascribed to a decrease in buffering capacity and enhancement of nutrient digestibility and also acidification of feed which in turn lower intestinal pH that acting as barrier against pathogens susceptible to low pH (Ghazalah et al., 2011). However, limited literature understands the effect of OA supplementation during feeding LPD. The present study deepened the knowledge of how OA supplementation in LPD affect broilers performance and immune response. In our findings, weight gain was not influenced by CP level only during starter phase which was similar to previous studies (Ravangard et al., 2017). On the other hand, feeding LPD did not improve weight gain or FCR during the grower-finisher phase. Thus, the deleterious effect of LPD on broiler performance appeared on grower-finisher phase. It is known that the intestinal function improves as the body grows leading to improvement of the digestibility and absorption of different nutrients thus improving broiler growth performance (Ma et al., 2021). One of the possible reasons for the reduction in performance at LPD is insufficient synthesis of non-essential amino acids to fulfill the need of fast-growing broilers (Ravangard et al., 2017). Although OA is known to enhance the weight gain and FCR in broiler chicken during the growing and final phases (Ma et

al., 2021), this effect was absent in the current study. The reason could be attributed to using uncoated OA which is readily digested (Sugiharto, 2016) and thus could not effectively modulate the intestinal microflora and mucosal morphology in chickens (Hu and Guo 2007). These results suggest that OA supplementation OA could not overcome the adverse effect of LPD.

Our major key findings emphasize the efficacy of OA supplementation in reduction of abdominal fat during feeding LPD. Although feeding with LPD would increase lipogenesis in the liver of the birds, thereby caused in more liver weight and hence more abdominal fat deposition (Swennen et al., 2006), such an effect was not observed in the current study. The explanation probably lies in the fact that acidification of diets might stimulate glycogenesis by increasing the influx of glucose 6-phosphate into glycogen synthesis pathway (Hossain and Nargis 2016). In addition, this reduction in abdominal fat might be due to elevated serum triiodothyronine hormone concentration (Abdel-Fattah et al. 2008). This underlines a persisting effect and a strong implication of using OA for preventing the adverse effect of feeding LPD.

Because high fat intake is closely linked to increased risk of cardiovascular disease in humans, the level of cholesterol and triglycerides in meat should be considered as indicator for health parameters. In the current study, diets supplemented with OA decreased fat percentage in breast and thigh muscles. The reduction in fat percentage in meat could be correlated with the decrease in abdominal fat (Hossain and Nargis 2016; Khan and Iqbal, 2016; Lakshmi et al., 2016)). In line with our observations the acidification of the diet (either normal or LPD) increased meat protein percentage (Abd El-haliem et al., 2018) reflecting improved protein availability in these birds. Customers nowadays are focusing on minimizing cholesterol intake in order to reduce the risk of heart disease. In an effort to generate low-cholesterol poultry meat, researchers have investigated a number of nutritional supplements. However, knowledge about alternative production methods that can help reach this goal is yet limited. In the current study, cholesterol and triglycerides level was lower in all groups receiving OA than that of the control group. This result was in harmony with that found by Kalafova et al. (2014) and Elbaz et al. (2021) who recorded a numerical decreased in serum triglycerides in the citric acid group when compared with the control group.

The serum cholesterol, total protein and albumin levels were not affected by OA supplementation. These results are in harmony with Sugiharto et al. (2019) and Galli et al. (2021). Additionally, a higher serum globulin level than the control could indicate that broiler chickens fed acidified diets had a better immune response (Ghazalah et al., 2011). In contrast, Sugiharto et al. (2019) found that formic acid did not significantly affect serum globulin. The variance is most likely due to differences in the content and concentrations of mixed OA. Regarding the effect of LPD on antibody titer against ND virus, Zeng et al. (2015) reported that broilers fed diets containing 16.81% or lower CP had lower ND antibody titer. The authors reported that the minimum dietary CP requirement of broilers from 22 to 42 days of age was suggested as 17.63%. In contrast, Rao et al. (2011) demonstrated that antibody titers against ND virus were not affected by the reduction in CP level in the diet. Overall, the effect of OA supplementation on blood biochemical parameters and antibody titers against ND

virus is inconsistent among studies, which might be partially related to composition and concentration of OA as well as differences in experimental design.

Conclusion

In conclusion, adding OA improved immune response through increased serum globulin, and an increase in bursa relative weight of broilers. Moreover, the addition of OA to broiler fed 95%, 90% and 85% NRC CP has no beneficial effect on growth parameters and carcass traits but improves broiler immunity and produces healthy poultry products to the consumers.

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Conflict of interest statement

None to be declared.

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