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Effects of Organic Acid and Feed Restriction Intensity on the Performance and Immune Response of Broilers

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Key words
Globacid, Feed restriction, Humoral immunity, Performance, Broiler

ABSTRACT

This study was performed to evaluate the effects of organic acid and feed restriction intensity on the performance and immune response of broilers. For this purpose, 540 one-day-old male broilers of Ross 308 strain were grown in a 3×3 factorial experiment with three levels of Globacid (0, 0.5, and 1 mL/L) and three levels of feed restriction intensity (0, 10, and 20%) in a completely randomized design with 9 experimental treatments, 6 replicates with 10 chickens in each replicate up to 42 days of age. The group containing Globacid (1 mL/L) and the restriction intensity of 10% had the highest significant difference compared to the control (p <0.05). The highest thymus weight was observed in the treatments with Globacid levels of 0.5 and 1 mL/L, respectively (p < 0.05). Also, the highest humoral immune response among the groups was seen in the group containing Globacid (1 mL/L) and the restriction intensity of 10% (p < 0.05). The results of this study generally showed that increasing the level of organic acid together with reducing the intensity of dietary restriction can improve the performance and immune responses of broilers.

INTRODUCTION

In recent years, the growth rate of broilers has increased significantly due to advances in nutrition and genetics (Ahmadi *et al*., 2018).

Today, one of the most important successes in poultry feeding is the use of additives in the diet, which in addition to improving feed efficiency, maintains the health of chickens (Ahmadi *et al*., 2019; Poorghasemi *et al*., 2017). Organic acids are among the novel additives with positive effects. These effects include increasing digestion and absorption of nutrients, balancing the electrolytes of diet and intestine, creating a suitable bacterial population, and increasing the secretion of digestive enzymes (Adhikari *et al*., 2020).

Organic acids increase the antimicrobial activity of the intestine by decreasing the pH of the intestine, decreasing the action of various microorganisms, especially pathogenic microbes, and increasing the action of beneficial microorganisms (Mohamed and Abdel-Naeem, 2018). Through the competitive elimination of pathogenic bacteria, organic acids can stimulate the immune system of broilers (Ferrari *et al*., 2013).

Organic acids are also used to protect feed against micro-organisms and increase feed storage time, which can increase mineral intake (El-Baaboua *et al*., 2018). Zaki *et al*. (2015) showed that adding organic acids to the diet of broiler chickens can affect the type and number of intestinal microbes, increase growth, reduces mortality, improve absorption and feed conversion ratio and strengthen the immune system.

Although genetic selection has also made significant improvements in the growth rate of birds, this rapid growth has had adverse consequences such as a poor ratio of the growth rate of the breast and carcass to the digestive organs, lungs, and heart. This discordance is manifested in the form of metabolic abnormalities (Jahanpour *et al*., 2018). Applying dietary restriction is one of the methods that can improve this growth discordance to an acceptable extent. Dietary restriction reduces the heat produced in the body and thus reduces maintenance needs, the rate of basal metabolism and the dynamic effect of feed, leading to the reduction of oxygen consumption, which will help prevent the problems associated with rapid growth (Davoodi-Omam *et al*., 2019).

The most common dietary restriction is physical

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restriction. When using physical restriction, the amount of feed allocated to birds is quantitatively limited in different ways. Another method of dietary restriction is that the intensity of dietary restriction is determined as a percentage of free feed intakes. Broiler chickens consume less feed at short intervals after a period of dietary restriction (Camacho *et al*., 2004). This method of feed intake increases the secretion of the enzymes in the digestive system such as amylase, lipase, aminopeptidase, and dipeptidase which ultimately improves absorption and the conversion ratio (Plavnik *et al*., 1986). The compensatory growth in birds due to severe dietary restrictions can also be effective in reducing metabolic disorders such as various types of leg problems, sudden death syndrome, and ascites (Sahraei, 2014).

There have been numerous reports of the effects due to organic acids in broiler diets and the application of different intensities of dietary restrictions during the broiler breeding period. However, these two methods have rarely been used together in the management of broiler breeding. Therefore, the present experiment was performed to investigate the effects of organic acids together with the intensity of dietary restrictions and their interactive effect of combined uses on the performance and immune system of broilers.

MATERIALS AND METHODS

Birds and experimental diets

In the present study, 540 Ross 308 strain day-old male broilers were grown in a 3×3 factorial experiment with three levels of globacid (0, 0.5, and 1 mL/L) and three levels of feed restriction intensity (0, 10, and 20%). The design was completely randomized with 9 experimental treatments, 6 replicates with 10 chickens per each replicate up to 42 days of age. As can be seen in Table I, the experimental diets included the starter (1-21 days) and grower (22-42 days) diets, which were adjusted based on the requirements manual of Ross 308 strain using user friendly feed formulation done agai (UFFDA) software. During the breeding period, all the groups received the same diets based on corn-soybean. The experimental treatments included: 1- Control (without globacid and restriction intensity), 2- Globacid (0 mL/L)+10% restriction intensity, 3- Globacid (0 mL/L)+20% restriction intensity, 4- Globacid (0.5 mL/L)+without restriction intensity, 5-Globacid (0.5 mL/L)+10% restriction intensity, 6- Globacid (0.5 mL/L)+20% restriction intensity, 7- Globacid (1 mL/L)+without restriction intensity, 8- Globacid (1 mL/L)+10% restriction intensity and 9- Globacid (1 mL/L)+20% restriction intensity. The feed restriction intensities were applied to the chickens from 14 to 21 days of age. The chickens had free access to water and feed during the first to 14 days and also after the end of the period of restriction intensity.

The organic acid used in this study was from Globacid DW, a product of the French company Nutri-Concept, which contained acetic acid, propionic acid, lactic acid, and phosphoric acid. Globacid was mixed with the drinking water and given to the chickens continuously from 2 to 42 days of age.

Table I. The ingredients and chemical feed analysis of the experimental diets.

Ingredients (%)	Starter (1-21 days old)	Grower (22-42 days old)
Corn	54.49	61.25
Soybean meal	38.09	30.66
Soybean oil	2.50	4.19
CaO	1.71	1.42
Dicalcium phosphate	1.82	1.41
Salt (NaCl)	0.36	0.37
Mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.25	0.25
DL-methionine	0.33	0.20
L-sine	0.20	0.00
Chemical analyses		
Metabolizable energy (kcal/kg)	3150	3200
Crud protein (%)	20.00	19.00
Lysine (%)	1.28	1.12
Methionine + Cysteine (%)	0.95	0.86
Methionine (%)	0.50	0.38
Threonine (%)	0.91	0.82
Arginine (%)	1.61	1.43
Tryptophan (%)	0.29	0.26
Ca (%)	0.90	0.85
Available Phosphorus (%)	0.45	0.42
Na (%)	0.16	0.16
Cl (%)	0.18	0.17

[1]Mineral mixture per ton of diet: Mn: 48 g; Zn: 40 g; Fe: 16 g; Cu: 6.4 mg; Se: 0.12 g and I: 0.5 g. [2]Vitamin premix per ton of diet: vitamin A: 4000000 IU; vitamin D_3: 2000000 IU; vitamin E: 20000 IU; vitamin K_3: 1.2 mg; vitamin B_6: 1.6 g; vitamin B_{12}: 0.0064 g; Thiamine: 0.8 g; Riboflavin: 2.4 g; Pantothenate: 6 g; Nicotinic acid: 24 g; Folic acid: 0.7 g; vitamin H2: 0.04 g; Choline chloride (form Betafin): 300 mg and Antioxidant: 0.4 g.

Performance

The amount of weight gain was calculated by determining the additional weight of the birds in a specified period (the interval of 1 to 42 days of age), along with the weight of casualties in that period, divided by the number of live chickens. The feed intake for the entire period was obtained by weighing the amount of feed left at the end of the period and subtracting it from the given amount, and finally dividing by the number of live chickens. The feed conversion ratio was calculated by the feed intake divided by the weight gain, of the entire period (Poorghasemi *et al*., 2013a).

Lymphoid organs

At the end of the experiment (42 days of age), to investigate the effect of experimental treatments on the weight of lymphoid organs, three chickens were randomly selected from each replicate and after slaughtering, the three lymphoid organs of the thymus, spleen, and bursa of Fabricius were removed and weighed using a digital scale with an accuracy of ± 0.01 g (Poorghasemi *et al*., 2013b).

Determination of antibody titer produced against New-castle disease and influenza viruses

To measure antibody titers against Newcastle disease and influenza, after receiving Newcastle vaccine at 8 days of age and influenza vaccine at 18 days of age, the first blood sampling was taken ten days after the injection and the second blood sample was taken 10 days after the first blood sampling (Poorghasemi *et al*., 2015). To take the blood samples, three birds were randomly selected from each replicate and 2 ml blood was taken from the wing vein. Blood samples were transferred to the laboratory and centrifuged for 15 minutes at 3000 rpm to extract the serum. The antibody titers were then determined in the laboratory as described by Shabani *et al*. (2019).

Determination of antibody titer produced against SRBC

To measure the titer of immunoglobulins produced against sheep red blood cells (SRBC) on 28 and 35 days of age, 4 chickens per experimental unit were injected with 0.2 ml of sheep red blood cell suspension (5%) washed in sterile phosphate buffer in the chest muscle and marked with colour on the feathers. Seven days after each injection, on days 35 and 42, about 2 ml of blood was drawn from the wing vein of the same birds. Blood samples were kept at laboratory temperature for 6 h to separate the blood clot from the serum. The obtained serum was centrifuged at 4000 rpm for 15 min. Then, microtiter hemagglutination method was used to determine the immunoglobulin M (IgM) and immunoglobulin G (IgG) titers as well as the total titers of immunoglobulins produced against sheep red blood cells (Poorghasemi *et al*., 2015).

Statistical analysis

The data obtained from this experiment were analyzed using a statistical model that included the effect of Globacid, restriction intensity, and their interactive effects, by SAS statistical software and general linear modeling (GLM) method. The means of treatments were compared using Duncan's test at 5% probability level ($p < 0.05$). The statistical model of the design was as $Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$ where Y_{ijkl} is the value of each observation for the studied trait, μ is the average of the observations, A_i is the effect of Globacid, B_j is the effect of restriction intensity, $(AB)_{ij}$ is the interactive effect of Globacid and restriction intensity and e_{ijk} is the effect of experimental error.

RESULTS

Performance

The results of the effect of the experimental treatments on the performance of broilers are presented in Table II. Weight gain over the entire period in the treatments containing different levels of organic acid showed a significant difference compared to the control ($p < 0.05$). The highest weight gain was related to the treatment containing 1 ml of Globacid and the lowest was related to the control. Weight gain in the treatments having restriction intensity was not significantly different from the control ($p < 0.05$). Weight gain in birds having Globacid and feed restriction intensity showed that the diets containing different levels 0.5 and 1 ml of Globacid alone or along with dietary restriction had the highest significant difference compared to the control ($p < .05$). Feed intake in the entire period was significantly increased for Globacid-containing treatments compared to the control ($p < 0.05$). By applying feed restriction, no significant difference was observed in the feed intake of the treatments compared to the control ($p > 0.05$). Comparing the experimental groups, although the use of 1 ml Globacid with a restriction intensity of 10% had the highest amount of feed intake among the groups, this amount was not significantly different from the control ($p > 0.05$). By using organic acid in the diet, the feed conversion ratio of the birds was significantly reduced compared to the control ($p < 0.05$). The restriction intensity applied in the treatments had no significant effect on improving the feed conversion ratio of broilers ($p > .05$). The feed conversion ratio of the birds was significantly reduced by the application of Globacid along with restriction intensity compared to the control ($p < 0.05$).

Lymphoid organs

Table III shows the effect of the experimental groups on the lymphoid organs of broilers at 42 days of age. The difference between the weights of the spleen and the bursa of Fabricius for different levels of Globacid did not show a significant difference ($p < 0.05$) but thymus weight had a significant increase in all levels of Globacid compared to the control ($p < 0.05$). Lymphoid organ weights in the treatments having feed restriction were not significantly different from the control ($p < 0.05$). Also, the results on the interactive effects of using organic acid and feed restriction on the weight of thymus, spleen, and bursa showed that there was no significant difference between the experimental groups ($p < 0.05$).

Table II. The effects of experimental diets on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR).

Treatment		BWG (g/hen/ period)	FI (g/hen/ period)	FCR
Globacid (mL/L)				
0		57.73 [c]	108.68 [b]	1.88 [a]
0.5		63.7 [b]	111.19 [a]	1.74 [b]
1		68.58 [a]	113.37 [a]	1.65 [c]
	SEM	0.423	0.652	0.007
	P-value	0.0001	0.0001	0.0001
Restriction intensity (%)				
		64.16	112.109	1.75
0		63.1	111.42	1.76
10		62.75	110.72	1.77
20	SEM	0.423	0.652	0.007
	P-value	0.0665	0.169	0.2255
Globacid (mL/L)- Restriction intensity (%)				
0 - 0		58.77 [c]	109.8 [abc]	1.86 [a]
0 - 10		57.43 [c]	107.78 [c]	1.87 [a]
0 - 20		57 [c]	108.47 [bc]	1.9 [a]
0.5 - 0		64.23 [b]	112.11 [abc]	1.74 [b]
0.5 - 10		63.75 [b]	111 [abc]	1.74 [b]
0.5 - 20		63.11 [b]	110.47 [abc]	1.75 [b]
1 - 0		68.11 [a]	112.47 [abc]	1.65 [c]
1 - 10		69.48 [a]	114.41 [a]	1.64 [c]
1 - 20		68.15 [a]	113.23 [ab]	1.66 [c]
	SEM	0.733	1.13	0.013
	P-value	0.001	0.02	0.0001

The means within the same column with at least one common letter, do not have significant difference (P<0.05). SEM, standard error of the means.

Antibody titer produced against Newcastle disease and influenza viruses

The results of the experimental groups on the titers of the antibodies produced by Newcastle disease and influenza are shown in Table IV. In the first and second times, the difference between Newcastle disease and influenza titers in the Globacid group had a significant increase compared to the control ($p < 0.05$). No significant difference was observed in the first and second times for Newcastle disease and influenza titers in diet-restricted treatments ($p < 0.05$). In the case of interactive effects, the antibody titer produced against Newcastle disease in the first and second stages for the groups fed by 1 ml Globacid in di*et al*ong with restriction intensity had a significant increase compared to the control and the other treatments ($p < 0.05$). There was no significant difference among the groups in the interactive effects for the antibody titer produced against influenza in the first stage, but in the second stage, only the antibody titer produced against influenza in the treatment containing 1 ml Globacid with 10% restriction intensity showed a significant increase compared to the control ($p < 0.05$).

Table III. The effects of experimental diets on weight of lymphoid organs.

Treatment		Thymus (g)	Spleen (g)	Bursa (g)
Globacid (mL/L)				
0		0.260 [b]	0.122	0.142
0.5		0.366 [a]	0.133	0.154
1		0.369 [a]	0.123	0.16
	SEM	0.154	0.005	0.008
	P-value	0.0001	0.33	0.317
Restriction intensity (%)				
0		0.369	0.128	0.157
10		0.359	0.123	0.143
20		0.368	0.126	0.156
	SEM	0.014	0.005	0.008
	P-value	0.848	0.836	0.44
0 - 0		0.343	0.113	0.14
0 - 10		0.372	0.126	0.133
0 - 20		0.366	0.126	0.152
0.5 - 0		0.381	0.143	0.16
0.5 - 10		0.352	0.13	0.146
0.5 - 20		0.376	0.127	0.157
1 - 0		0.382	0.13	0.17
1 - 10		0.352	0.114	0.15
1 - 20		0.364	0.124	0.16
	SEM	0.024	0.01	0.014
	P-value	0.739	0.529	0.961

The means within the same column with at least one common letter, do not have significant difference (P<0.05). SEM, standard error of the means.

Table IV. The effects of experimental diets on immune responses of broiler chickens after Newcastle and influenza vaccination.

Treatment		ATAN1 (log_2)	ATAN2 (log_2)	ATAI1 (log_2)	ATAI2 (log_2)
Globacid (mL/L)					
0		4.33 [b]	5.25 [b]	2.91 [b]	3.5 [c]
0.5		4.75 [b]	5.5 [b]	3.33 [b]	4.58 [b]
1		6.5 [a]	7.41 [a]	4.58 [a]	5.83 [a]
	SEM	0.144	0.166	0.266	0.27
	P-value	0.0001	0.0001	0.0004	0.0001
Restriction intensity (%)					
0		5.25	6.16	3.83	4.91
10		5.16	6	3.58	4.58
20		5.16	6	3.41	4.41
	SEM	0.144	0.166	0.266	0.27
	P-value	0.8952	0.7194	0.5457	0.4247
Globacid (mL/L) - Restriction intensity (%)					
0 - 0		4.5 [b]	5.25 [b]	3.25	3.75 [bcd]
0 - 10		4.25 [b]	5 [b]	2.75	3.5 [cd]
0 - 20		4.25 [b]	5.5 [b]	2.75	3.25 [d]
0.5 - 0		4.75 [b]	5.75 [b]	3.5	4.75 [abcd]
0.5 - 10		4.5 [b]	5.5 [b]	3.5	4.5 [abcd]
0.5 - 20		5 [b]	5.25 [b]	3	4.5 [abcd]
1 - 0		6.5 [a]	7.2 [a]	4.75	5.75 [ab]
1 - 10		6.75 [a]	7.5 [a]	4.5	6.25 [a]
1 - 20		6.25 [a]	7.25 [a]	4.5	5.5 [abc]
	SEM	0.25	0.288	0.461	0.468
	P-value	0.001	0.002	0.97	0.003

ATAN1, antibody titre against Newcastle at first time; ATAN2, antibody titre against Newcastle at second time; ATAI1, antibody titre against Influenza at first time and ATAI2, antibody titre against Influenza at second time. The means within the same column with at least one common letter, do not have significant difference (P<0.05). SEM, standard error of the means.

Antibody titer produced against SRBC

Tables V and VI are related to the results of two stages of measuring IgG, IgM as well as total immunoglobulins produced after SRBC injection. IgG titer response, as well as total Ig, produced in both stages after SRBC injection in the treatments containing 0.5 and 1 ml Globacid had a significant increase compared to the control ($p < 0.05$). According to the results, the response of immunoglobulin titers produced after injecting the first and second stages of SRBC in the restricted experimental groups was not significantly different from the control ($p < 0.05$).

Table V. The effects of experimental diets on immune responses of broiler chickens after injection of the first stage of SRBC.

Treatment		IgG (log_{10})	IgM (log_{10})	Total Ig (log_{10})
Globacid (mL/L)				
0		1.66 [b]	1.75	3.41 [b]
0.5		3.66 [a]	2.33	6 [a]
1		4.16 [a]	2.5	6.66 [a]
		0.248	0.223	0.253
		0.0001	0.0618	0.0001
Restriction intensity (%)				
0		3.33	2.41	5.75
10		3.16	2.08	5.25
20		3	2.08	5.08
	SEM	0.248	0.223	0.253
	P-value	0.6423	0.4873	0.0894
Globacid (mL/L) - Restriction intensity (%)				
0 - 0		1.75 [bc]	1.75	3.5 [b]
0 - 10		1.75 [bc]	1.75	3.5 [b]
0 - 20		1.5 [c]	1.75	3.25 [b]
0.5 - 0		3.75 [ab]	2.5	6.25 [a]
0.5 - 10		3.75 [ab]	2.25	6 [a]
0.5 - 20		3.5 [abc]	2.25	5.75 [a]
1 - 0		4 [a]	3	6.25 [a]
1 - 10		4.5 [a]	2.25	7.5 [a]
1 - 20		4 [a]	2.25	6.25 [a]
	SEM	0.43	0.387	0.44
	P-value	0.001	0.86	0.001

The means within the same column with at least one common letter, do not have significant difference (P<0.05); SEM, standard error of the means.

In the interactive effects of combined uses, IgG concentration after the first stage injection only in the treatments containing 10 ml Globacid showed a significant increase compared to the control ($p < 0.05$). The difference in total Ig concentration in the first stage and also the difference in IgG and total Ig concentrations in the second stage in the treatments containing Globacid had a significant increase compared to the control ($p < 0.05$).

DISCUSSION

The results of the present experiment showed that the use of organic acid in the diet of broilers can have a significant improvement in weight gain. Fascina *et al.* (2012) stated in their results that organic acids in broiler chickens increase digestion and absorption of proteins by eliminating the deficiency of gastric hydrochloric acid secretion and increasing the activity of pepsin enzyme which is consistent with the results shown in the present study.

Table VI. The effects of experimental diets on immune responses of broiler chickens after injection of the second stage of SRBC.

Treatment		IgG (log_{10})	IgM (log_{10})	Total Ig (log_{10})
Globacid (mL/L)				
0		3.08^{c}	1.75	4.83^{c}
0.5		5.33^{b}	1.91	7.25^{b}
1		6.33^{a}	2.16	8.5^{a}
	SEM	0.173	0.164	0.22
	P-value	0.0001	0.4728	0.0001
0		5.08	2	7.08
10		4.83	2	6.83
20		4.83	1.83	6.66
	SEM	0.173	0.164	0.22
	P-value	0.5091	0.8511	0.4166
0 - 0		3.25^{b}	1.75	5^{b}
0 - 10		3^{b}	1.75	4.75^{b}
0 - 20		3^{b}	1.75	4.75^{b}
0.5 - 0		5.5^{a}	2	7.5^{a}
0.5 - 10		5.25^{a}	2	7.25^{a}
0.5 - 20		5.25^{a}	1.75	7^{a}
1 - 0		6.25^{a}	2.25	8.5^{a}
1 - 10		6.5^{a}	2.25	8.75^{a}
1 - 20		6.25^{a}	2	8.25^{a}
	SEM	0.3	0.284	0.381
	P-value	0.0001	0.9967	0.001

The means within the same column with at least one common letter, do not have significant difference ($P<0.05$); SEM, standard error of the mean.

Organic acids can reduce the population of protein-degrading bacteria in the intestine and reduce the deamination of amino acids to eventually make more protein available to the animal for absorption, which in turn increases weight and improves performance. Ghanaatparast-Rashti *et al*. (2015) in a study on different levels of propionic acid in broiler diets reported that the use of different levels of propionic acid (0.2, 0.4, and 0.8%) had a significant effect on their feed intake. They stated that the reason for the increase in feed intake following the consumption of organic acids in broilers is related to the changes in the function of digestive enzymes, improvement of apparent digestibility, and improvement of intestinal microflora. The results of the present study showed that the use of organic acid increases feed intake, which is consistent with the results of Ghanaatparast-Rashti *et al*. (2015).

Contrary to the results of the present experiment, in a study on formic acid in the diet of broilers, the addition of 1% formic acid in the diet did not have a significant effect on feed intake compared to the control (Izat *et al*., 1990). They stated that having no increase in feed intake of broilers using organic acids is related to genetic differences, age, diet type, and the amount and type of organic acids. The results of other studies and the present experiment have shown that the feed conversion ratio in broilers is improved by the consumption of organic acids. Studies have shown that the addition of organic acids to the feed inhibits the microorganisms and improves the feed conversion ratio by improving digestion and absorption of nutrients, reducing the production of toxins, and decreasing the decomposition of nutrients in the intestine (Hassan *et al*., 2010; Oviedo-Rondón, 2019).

The results of this study showed that during the restriction period, with the application of restriction intensity, weight gain and average feed intake decreased insignificantly. The results of the present experiment are consistent with the results obtained by Jahanpour *et al*. (2018) that more intense restriction reduces the total feed intake. Also, the results of de Jong *et al*. (2002) showed that in a quantitatively specified restriction period, the average weight gain of chickens decreased with increasing restriction intensity, so that the average weight gain of the chickens with a restriction intensity of 20% was lower compared to the chickens with a quantitative restriction intensity of 10%, which is consistent with the results of the present experiment.

In general, the growth rate decreases during periods of quantitative feed restriction due to reduced feed intake. As growth slows, so does the size of the liver and digestive system. Decreased body size, decreased capacity of the digestive system, and possibly decreased metabolism due to reduced liver size may be the reasons for reduced feed intake and weight loss in the chickens under feed restriction (Tahamtani and Riber, 2020).

In this study, the conversion coefficient in the diet-restricted treatments increased insignificantly compared to the full-nutrition treatment. Consistent with the results of the present experiment, the study of Jalal and Zakaria (2012) showed that there is no significant difference in the total feed conversion ratio between feed restricted chickens and the control. They stated that weight loss in restricted treatments usually does not improve feed conversion ratio and performance.

As seen in the results, the interactive effects of the restriction intensity along with organic acid could increase the weight and decrease the feed conversion ratio and thus improve the performance of broilers. This improvement in the treatment containing 1 ml Globacid with the restriction intensity of 10% was higher than the other treatments. Little research has been done on the interactive effects of using organic acid and dietary restriction.

Researchers have shown in their experimental results that by reducing feed restriction 276 intensity (from 20% to 10%), there is a possibility of better compensatory growth. However, the organic acids by affecting the microscopic structure of the small intestine, improving intestinal microflora and reducing toxic compounds in the intestine of broilers, increase the efficient use of dietary nutrients and improve performance (Fondevila *et al*., 2020; El-Baaboua *et al*., 2018; Adewole *et al*., 2021).

The results of the present experiment showed that the organic acid Globacid led to a significant increase in the relative weight of the thymus. Usually, the weight of the main organs of the immune system (thymus, spleen, and bursa of Fabricius) is used in several studies as an indicator to assess the bird's immune system. These organs are responsible for producing white blood cells to protect the birds against pathogens (Poorghasemi *et al*., 2015; Ahsan *et al*., 2021).

Research has shown that the addition of organic acids increases the number of the cells involved in immunity in thymus follicles of broilers and increases thymus weight (Broom, 2015), which is consistent with the results of the present experiment. Organic acids improve the immune system of birds and regulate their immune processes by destroying internal pathogens which leads to the anatomical evolution of thymus lymphatic tissues, as well as by increasing the size of mature immune cells (Kaczmarek *et al*., 2016).

As shown in the results, organic acid had a significant effect on the humoral immune system of the studied chickens. The results of this experiment are consistent with the results of similar experiments. According to a study by Aristimunha *et al*. (2020), the addition of 0.1% acid to the diet of broilers increased the antibody titer against Newcastle disease and influenza. They have suggested that the increase may be due to the stimulation of the immune system. Rahim *et al*. (2012) stated in their studies that organic acids improve the immune system of poultry and increase the serum IgG level of the chickens. They attributed this positive effect of organic acids on the immune system to a decrease in intestinal pH caused by the activity of organic acids increasing the beneficial bacteria in acidic environments, which leads to more digestion and absorption of nutrients and strengthens the immune system.

In the present experiment, the restriction intensity alone did not have a significant effect on the immune system of broilers. Some other experiments have also reported that dietary restriction does not affect the production of antibodies and immunoglobulin G (Liew *et al*., 2003). Research has shown that when applying dietary restriction, the intensity of the restriction, the duration of the restriction, the period allowed for re-feeding, the feed intake during the re-feeding period, the type and racial characteristics of the birds can be effective in strengthening the immune system (Turkyilmaz, 2008).

As seen in the results of interactive effects, the concentration of the antibodies produced against Newcastle disease and influenza, as well as the concentration of IgG and total immunoglobulins produced against sheep red blood cells, increased with increasing Globacid level and decreasing the intensity of the restriction, so that the highest significant difference with the control was for the groups fed by 1 ml of Globacid with 10% restriction intensity.

The interactive effects of organic acids and the intensity of dietary restriction in chickens are not clear. Research has shown that organic acids reduce the colonization of harmful bacteria and produce less toxic metabolites. These acids can act as substrates in metabolism and improve the digestion of protein, calcium, phosphorus, magnesium, and zinc, thereby reducing diseases and improving the immune system (Ragaa and Korany, 2016a). Organic acids also create acidic conditions in the digestive system by competitive elimination of pathogenic bacteria by reducing the sensitivity to diseases and having a positive effect on stimulating the immune system of broilers (Ragaa and Korany, 2016b). However, research has shown that restriction intensity or prolonged starvation can have adverse effects on the immune response following an increase in corticosterone levels.

Immunological mechanisms appear to improve properly with low restriction intensity because at high restriction intensity due to decreased amino acid intake and increased corticosterone secretion, expression of insulin-like growth factor-I (IGF-I) is reduced, which can impose a disorder in immune responses (Conlon and Kita, 2002). Therefore, the use of organic acids with low restriction intensity in broilers can improve and strengthen the immune system by activating proteolytic enzymes, increasing IGF-I, reducing the production of ammonia and growth-reducing microbial metabolites, by optimal absorption of minerals and reduction of subclinical infections (Butzen *et al*., 2013; Ragaa and Korany, 2016b).

CONCLUSION

The results showed that the use of organic acid had a significant effect on performance and also could improve the immune system response. Applying the intensity of dietary restriction had no significant effect on the performance and immune system of broilers compared to the control. In the interactive effects, the results showed that when a dietary restriction has been applied the use of organic acid can have positive and significant effects on performance and humoral immunity, which were enhanced by increasing the level of the organic acid and reducing restriction intensity.

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Statement of conflict of interest

The authors have declared no conflict of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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