Organic Acids in Drinking Water Modulate the Humoral Immune Response and Performance of Broiler Chickens

Asmaa M. Ali[1]*, Abd Elrhman M. Gamal[1], Rania F. El Naggar[2], Mohamed A. Rohaim[3,4], Mohamed M. Hamoud[5], Asmaa S. Morsi[6], Shimaa A. E. Nasr[1], Mohamed M. Ali[1], Samah E. Laban[1]

1. Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
2. Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, Sadat, Egypt
3. Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
4. Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, UK
5. Department of Poultry and Rabbit Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
6. Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

* Corresponding author: Asmaa M. Ali, E-mail: dr_fulla_2007@cu.edu.eg

1. Abstract

The high density and genetic homogeneity of poultry flock populations may make them susceptible to infectious diseases, and dysbiosis of their microbiomes and viromes may negatively impact their immune system and health. Generally, the poultry immune system is always under pressure from a high growth rate, pathogen challenge and high stocking density. Therefore, the need for natural products to modulate the immune system is necessary. Our study tries to investigate the ability of different organic acid supplements either in single or mixed forms by drinking water to enhance the broiler immune response, Feed conversion ratio (FCR) and broiler wellbeing. A total of 1800 Arbor Acres plus broiler chicks were divided into eight treated groups (200 chicks each) that received four different organic acids either in single or mixture and a ninth on-treated group was kept as a negative control group. All groups were vaccinated against avian influenza (H5N1, H5N8), (H9N2) and Newcastle disease (NDV). At age days 17 and 25, broilers were injected with sheep red blood cells (SRBCs). Sera samples were collected at 7, 25 and 35 days for hemagglutination inhibition assay (HI) to determine sheep red blood cells (SRBCs), NDV, H5N1, H5N8, and H9N2 antibody titres. Our results revealed that using organic acids either alone or in combination had a significant effect on enhancing and modulating the immune antibody titres against NDV, H5N1, H5N8, H9N2 and SRBCs. Additionally, all treated groups had lower FCR and broiler mortality, compared to the negative control group, as organic acids act as cosurfactant. In conclusion, our findings indicate the ability of organic acids alone or in mixtures to improve the broiler immune response against ND, H5, H9 and SRBCs, body weight and lower chick mortality.

Keywords: Acidified drinking water; Antibody titre; Chick's mortality; FCR; H5N1, H5N8, H9N2, ND; Immune response; Organic acid.

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

2. Introduction

Over the last few decades, the Egyptian poultry industry has shifted from a meager backyard operation to a well-organized, fully integrated commercial sector. By increasing the global demand throughout the last 3 decades, broiler production has greatly increased 300% and in 2018 alone more than 128 million tons of broiler meat was produced, so the production and consumption of poultry meat and eggs have increased as well [1].

Usage of additives such as antimicrobial growth promoters, antibiotics, and other antimicrobial agents is common to enhance the well-being of poultry, leading to better performance. Consequently, the emergence of antibiotic resistant bacteria and increasing consumer concerns over food safety and health problems led the European Union in 2006 to counteract the dependence on adding antibiotics as feed additives for use as growth promoters. Nevertheless, their use was gradually restricted before a total ban was imposed in 2006 by EU Regulations [2].

Recent studies have aimed to find antimicrobial alternatives and prove their safety and beneficial effects on broiler performance. If faced with a high growth rate with a low feed conversion ratio (FCR), stress on the broiler immune system causes immunosuppression adversely affecting poultry performance **[3]**

Poultry immune modulation is a new alternative approach that strengthens the host natural mechanisms instead of anti-infective therapies. Immunomodulators enable poultry to utilize their innate immunity and boost their adaptive immune responses by developing rapid cross-protection against multiple pathogens **[4].** Immunostimulants can also be referred to as immunomodulators, adjuvants, or biological response modifiers. Immunostimulatory agents may be in the form of medicines, plant extracts or natural substances. Through stimulation of the monocyte-macrophage system, they modulate the body's immune system **[5]**.

Organic acids (OAs) are weak short-chain fatty acids that contain carboxyl functional group (-COOH), which is responsible for their acidic properties and reduction of gut pH. Oil in poultry feed interacts with cosurfactants to influence colloidal properties, which reflect bulky properties. **[6].**

Short-chain organic acids act as cosurfactants in microemulsions to stabilize colloidal nanodispersions of oil and water **[7].** Consequently, the low pH value caused by the addition of a short-chain organic acid creates an unfriendly environment for harmful bacteria **[8].** Therefore, OAs are qualified to be used as antimicrobial therapeutic/acidifier substances in poultry farms. The use of acidifiers (OAs) either in poultry feed or water is cost-effective for improving the growth performance, and nutrient

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

digestibility, reducing enteric disease infection rates and exerting immunomodulatory functions **[9].** In addition, many pathogenic and nonpathogenic intestinal bacteria decompose in the presence of organic acids, resulting in decreased colonization, infections, and inflammatory processes in the intestinal mucosa. In addition, OA stimulates gut immunity in both specific and nonspecific ways [10].

Dysbacteriosis may arise from environmental stress, viral or bacterial challenge, coccidiosis or in response to diet change. The treatment of dysbacteriosis can be achieved with antibiotics, but alternative treatment options such as probiotics or organic acids are preferred if a gut imbalance is suspected **[11,12,13]**. The gut microbiota can serve as a protective barrier against pathogenic bacteria colonization by attaching to the epithelial walls of enterocytes **[14].** In addition, they produce many protective substances, such as vitamins (e.g., vitamin K and vitamin B groups), short-chain fatty acid, organic acids (e.g., lactic acid, acetic acid, butyric acid, and propionic acid)) and antimicrobial compounds (e.g., bacteriocins), lower triglycerides, and induce nonspecific immune responses **[14, 15].** The mechanism by which OAs improve the efficacy of the broiler immune system relies on their ability to stimulate both specific and nonspecific gut immune functions. Likewise, OAs are able to increase the relative weight of primary lymphoid organs such as the thymus, spleen, and bursa of Fabricius, which are responsible for the production of T- and B cells and increase lymphocyte density in lymphoid organs **[16].** Although organic acids have been applied to poultry rations for some time, little is known about how they affect broiler chicks' immune systems **[17]**. Chicks fed butyric and citric acids may produce more antibodies against IBD disease and bronchitis virus compared to a control diet **[18]**. Laying hens supplemented with dietary organic acids had a significant increase in antibody titres against Newcastle disease, Also, in another study on broiler chickens, **[19]** found that treatment with 0.2% butyric acid produced an improvement in Newcastle antibody titres at 12 days post-vaccination, but not against bronchitis or Newcastle disease. Moreover, broilers supplemented with OAs and vaccinated with inactivated avian influenza subtype H9N2 vaccine showed a strong immune response against H9N2 **[20].**

Sheep red blood cells (SRBCs) are considered natural nonspecific, nonpathogenic, multideterminant and T-cell dependent antigens **[21].** Researchers frequently depend on sheep red blood cells (SRBCs) to study humoral immunity. SRBCs can be counted to examine bird humoral immunity against any discovered antibiotic alternatives, growth promoters and new medical plants **[22].** Since SRBCs are secondary tools to study humoral immunity, it was revealed that measuring humoral immune responses in broilers is a precursor in

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

recent years. SRBCs trigger the immune system and drive cytosolic recognition of SRBC RNA, which serves as a danger signal, triggering immunity to a similar chain of reactions within immune cells [23].

Previous studies revealed positive correlations between antibody responses to SRBCs and enhanced responsiveness to bacterial vaccines **[24],** viral vaccines **[25],** protozoan infections **[26],** parameters of innate immunity complement levels **[27]**, and natural antibodies **[28, 29]**

Recently, the scientific community has been interested in natural immunomodulator products, that can be used safely. There is very limited data on the immunological effects of OAs. Hence, more research is needed to emphasize the studies currently published and identify the modes of action producing such benefits.

Consequently, the objective of this study was to declare the role of OA supplementation in either single or blended form in broiler welfare in term of FCR , and mortality % and to explore their efficacy in stimulating powerful and sustained immune responses against disease -causing agents (humoral immunity), which is important for protecting against viral diseases, overcoming the immunosuppressive effects of stress and environmental pollution, and enhancing the duration and level of the immune response following vaccination.

3. Materials and Methods

3.1. Broiler management

A total of 1800 day-old chicks (Arbor Acre plus) were used for the current experiment. Initial body weight of (0 day) 40-42 grams. Chicks were housed in a well-ventilated clean/disinfected room. The floor area forming stocking ratio (SR) was 10 chicks/m^2 forming 25kg/m^2 at the age of slaughter/end of experiment (35 days). The floor was bedded by fresh clean wood shavings as litter material (5 cm depth) and an adequate number of feeders and drinkers were provided for each compartment. Temperature, lighting, ventilation and other environmental conditions were provided according to the recommended standards [30]. On the day of arrival, the chicks were provided with starter ration while water-soluble vitamins and electrolytes were added to the drinking water for the first 3 days. After 24 hours, crop test and vent temperature were applied to ensure good vitality, feeding behavior, and standard welfare management procedures were applied. Feeding ration was provided by Cairo poultry company® (CPC), Egypt; starter ration (23% protein) until 10 days old (weight approximately 300 grams), then grower ration (21% protein) until 25 days old (weight approximately 1 kg) and finally finishing ration (18.5% protein) until the end of the experiment. Feed and water were continuously provided *ad libitum* daily throughout the trial period. No antibiotics, growth promoters,

probiotic or feed enzymes were provided in the water or basal diet. Strict biosecurity and hygienic measures have been applied for both employees and researchers.

3.2. Experimental Design:

Plain normal drinking water was provided to all chicks for the first 7 days, and then chicks were randomly divided into eight treated groups while the ninth was left as nontreated group (200 chicks; each). Groups 1- 4 received single organic acid by drinking water continuously for 12 hours/daily till end of the trial (35 days) at a concentration of 1%. Group 1 received formic acid, group 2 received lactic acid, group 3 received propionic acid and group 4 received citric acid (Figure 1). On the other hand, groups 5- 8 were treated with an organic acid mixture that was prepared at an equal ratio (0.3:0.3:0.3) and administered continuously for 12 hours/daily until the end of the experiment (35 days) as follows: group 5 (mixture 1: formic + lactic + propionic), group 6 (mixture 2: formic + lactic+ citric), group 7 (mixture 3: formic +propionic +citric) and group 8 (mixture 4: lactic +propionic + citric) (Figure 1). However, group 9 (Gr 9) received tap drinking water during the whole experiment and was considered nontreated negative control group (Figure 1). The vaccination regime that was used during the experiment is summarized in Table 2.

3.3. Humoral immune response assessment

3.3.1 Humoral immune response for NDV, H5N1, H5N8, and H9N2

Blood samples were collected at 7, 25 and 35 days of age, and the serum was separated following standard procedures **[32]** and stored at -20^{o} C until use. The antibody titres for NDV, and AI H5N1, H5N8, and H9N2 were measured by hemagglutination inhibition (HI) assay according to the standard protocols with Newcastle disease virus (NDV) genotype VII as the antigen (KF709445) **[32]**. Moreover, the antibody titres against SRBCs were determined as described by **[33]**. The antibody titres were expressed as the $\log^2$ of the reciprocal of the highest serum dilution giving complete agglutination.

3.3.2 Humoral Immune Response for sheep red blood cells (SRBCs)

The sheep red blood cells (SRBCs) were freshly prepared according to the method described by **[31]**. Briefly, sheep blood was obtained in a tube containing heparin solution and centrifuged at 3000 rpm for 10 min. the supernatant (containing serum and buffy coat) was discarded, and the RBCS pellet was washed 3 times with physiological saline (0.9% NaCl). SRBCs were administered at 17 days of age and then boosted at 25 days of age by injecting 0.5 ml of SRBC suspension in phosphate buffered saline (10% V: V).

3.4. Feed conversion ratio (FCR) calculation

Feed conversion was calculated at the end of the trial by dividing the average total feed intake by the average final body weight using the following equation:

$$\text{Feed conversion ratio} = \frac{\text{average feed intake}}{final\ body\ weight}$$

3.5. Statistical analysis

Pairwise comparisons of treated and control groups were performed using Student's t test. All statistical analyses and figures were conducted in GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

4. Results

4.1 Humoral immune response for NDV, H5N1, H5N8, and H9N2

HI assay showed that broilers supplemented with single OAs had high antibody titres (HI) of NDV, H5N1, H5N8 and H9N2 at 25 days old, which increased gradually to record high significant titers at 35 days old ($P<0.05$) compared to the nontreated group (Table 3). In addition, using different OA combination mixtures resulted in a high antibody titre (HI) against NDV, H5N1, H5N8 and H9N2 at 25 days of age (nonsignificant), which increased progressively to record a significant titre at 35 days of age ($P<0.05$) compared to the control group (Table 4).

4.2 Humoral immune response for sheep red blood cells (SRBCs)

This study detects that there was a highly significant increase in the non-specific antibody titres against SRBCs in broilers supplemented with a single organic acid ($P<0.05$) and a mixture of OAs ($P<0.05$) compared to the nontreated control group (Tables 5 and 6 as well as figures 3 and 5).

4.3 Feed conversion ratio (FCR) and mortality rate

Our results revealed that the broiler groups supplemented with OAs (single or mixture) recorded a low (non-significant) FCR when compared with control group (Tables 5 and 6 as well as figures 3 and 5). Besides, they had a lower mortality rate (2 – 6%) than nontreated control group (8%) (Tables 3 and 4 as well as figures 3& 5).

5. Discussion

Our results assert that modulation of broiler immune response issues, including the nonspecific immune response against SRBCs and humoral immunity against NDV, H5N1, H5N8, and H9N2, could be boosted by using OAs either alone or in combination in drinking water for 12 hours/daily. Earlier studies reported that continuous dietary supplementation with 0.5% citric acid increased the serum globulin and consequently enhanced the specific immunity (antibody titres) for NDV [34]. Likewise, [35] reported that continuous

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

dietary supplementation with 0.5% formic acid was able to increase the immune response to NDV. Prior studies showed the positive effect of organic acids on the immune system represented by antibody production against NDV [36,37]. [38] did not notice any effect of continuous supplementation with acidified water on the humoral immunity of broilers supplemented with a mixture of lactic acid, formic acid, propionic acid, sorbic acid and citric acid. Additionally, [39] found no significant effect of a water acidifier with lactic acid on the immune organs and thus the immune response. Moreover, [40] recorded no significant effect of dietary acidifier product 0.1% containing citric, acetic, propionic, and lactic acid on the NDV antibody titres.

Our results illustrated in (Tables 3 and 4) come in agreement with [41, 42, 43] who found that a strong immune response can be achieved in broilers supplemented with OAs as a result of increasing the size of immune organs, and increasing immunoglobulin levels [41, 42, 43]. Additionally, immune system enhancement can also be associated with positive changes of organic acids in the gut by decreasing colonization of pathogenic microbes [44], improving nutrient digestion and absorption, and increasing the bioavailability of nutrients that are important for the immune system [45]. Former findings can express the smallest mortality rate of the group treated with organic acids compared to the control group, which may be explained by the antimicrobial effect of OAs [44, 46]. Our results showed in (Tables 3 and 4 as well as figures 3 and 5) revealed that the broiler groups supplemented with OAs (single or mixture) had a lower mortality rate (2 – 6 %) than non-treated control group (8%).

The injection of SRBCs to produce an antibody response in chickens revealed a good immune response to SRBCs, influenced by multiple factors, such as dose, route of injection, term of estimation and age [47]. In the present study, there was a highly significant increase in the antibody titres in broilers supplemented with a single organic acid ($P<0.05$) and a mixture of OAs ($P<0.05$) compared to the nontreated control group. In addition, a high immune response expressed by antibody titres for SRBCs in broilers can be achieved by supplementation with organic acids either in single form: (0.5% formic acid, 0.5% propionic acid) or mixed form [43,48]. Additionally, dietary supplementation with organic acids is able to enhance lymphocytes density in lymphoid organs, and consequently enhance nonspecific immunity [49]. Chickens with the ability to produce high antibody titres against sheep red blood cells exhibited stronger antibodies against Newcastle disease, and were more resistant to *Mycoplasma gallisepticum, Eimeria necatrix*, a splenomeglia virus, and feather mites [50]. Organic acids were able to improve the digestion, absorption and availability of nutrients and minerals required for host immunity [45]. Previous studies reported that continuous administration of water

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

acidifiers from hatching to 42 days old can improve FCR and the immune response [51, 52, 53], even under heat-stressed conditions [54]. Body weight is a direct reflection of growth, and it influences the production and reproduction traits of birds. The correlation between the broiler FCRs and their immune response against NDV, H5N1, H5N8, H9N2 and SRBCs is described in figures 2 and 3. Selection for the time and level of antibody response in meat type birds has been successfully conducted, and resistance to infectious disease has been tested [55]**.** A high antibody response to SRBCs has been associated with a larger bursa size [56]. Furthermore, there is a clear association between non-MHC genes and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines [57]**.** High antibody production was positively correlated with resistance to parasites and viruses. To summarize, organic acid supplementation as immunomodulators either in single or mixtures can enhance and modulate the broiler immune system, achieving highly significant antibody titres against NDV, improving the nonspecific immune response for SRBCs, reducing the mortality rate, and improving the FCR.

OAs had a beneficial effect on both humoral and cellular immune responses against conventional killed and live vaccines as well as the HVT-NDV-IBD recombinant vaccine.

6. Conclusion

The use of organic acids either (single or mixture) is a safer alternative to regular antibiotics or growth promoters that can be used in the poultry business. The need to solve the problems of antibiotic residue in poultry products and antibiotic resistance cannot be overemphasized. The different broilers were treated with four organic acids either alone or in mixtures by drinking for 12 hrs. per day gave positive results since the broilers were able to forestall infections due to increased SRBCs immune response, HI against NDV, H5N1, H5N8 and H9N2 as well as improved feed conversion and lower mortality rate.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Cairo poultry company for their support and providing ration for our experiment.

Ethics approval

All experiments were carried out in accordance with all relevant guidelines, regulations and animal ethics permits issued by the Faculty of Veterinary Medicine, Cairo University, Egypt and approved by the Institutional Animal Care and Use Committee, Cairo University (CU-IACUC) under approval number: CU-IIF-2019.

7. References

1. Food and agriculture organization (FAO). online datasets. 2018 http://www.fao.org/faostat/en

2. European commission. Regulation (EC) no 1831/2003 of the european parliament. additives for use in animal nutrition. Off. J. Eur. Union. 2003; 268: 29–43

3. Andreopoulou M, Tsiaris V, Georgopoulou I. Effects of organic acids on the gut ecosystem and on the performance of broiler chickens. Journal of the Hellenic veterinary medical society. 2014; 65**:**289-302

4. Kogut M. Issues and consequences of using nutrition to modulate the avian immune response. Journal of applied poultry research. 2017; 26**:** 605-612

5. Ganguly subha, Indira Paul, Sunit K Mukhopadhyay. Application and Effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture. a review. Israeli journal of aquaculture. 2010; 62 (3): 130 – 138.

6. Fasolin L h, Santana R C, Cunha R L. Influencie of organic acids on surfactant self assemblies in surfactant / oil/ wáter systems. Colloids and surfaces a physicochemical and engineering aspects. 2014; 459: 290-297.

7. Spernath A, Aserin A, Garti N. Fully dilutable microemulsions embedded with phospholipids and stabilized by short-chain organic acids and polyols. Journal of colloid and interface science. 2006; 299: 900–909.

8. Hui Zhang, Yan Shen, Peifang Weng, Guoqun Zhao, Fengqin Feng, Xiaodong Zheng. Antimicrobial activity of a food-grade fully dilutable microemulsion against *Escherichia coli* and *Staphylococcus aureus.* International Journal of Food Microbiology. 2009; 135 (3). 211-215.

9. Adhikari P, Yadav S, Cosby D E, Cox N A, Jendza J A, Kim w K. Research Note: Effect of organic acid mixture on growth performance and *Salmonella* Typhimurium colonization in broiler chickens. Poultry science symposium. 2020; 99 (5): 2645-2649

10. Friedman, A., Bar-shira, E., Effects of nutrition on development of immune competence in chicken's gut-associated lymphoid system. In proceedings of the 15th european symposium on poultry nutrition, balatonfüred, hungary, 25-29 September, World's poultry science association. 2005; 247-255.

11. Silva, C R, Uso de probiotico em rac, Oes de frangos de corte. Desempenho, digestibilidade e energia metabolizavel. Phd diss. Univ. Federal de vic ´ ¸osa. Vic¸osa, minas gerais, brasil. 2009. .http://locus.ufv.br/handle/123456789/5570

12. Donoghue A M, Farnell M B, Cole K, Donoghue d J. Mechanisms of pathogen control in the avian gastrointestinal tract. avian gut function in health and disease. Perry, G.C, ed., department of clinical veterinary science, university of Bristol, UK. 2006; 9:138–155

13. Bailey R A. Gut Health in Poultry: The World within–Update. The Poultry

Site.2021.http://en.aviagen.com/assets/Tech_Center/Broiler_Breeder_Tech_Articles/English/AviagenBrief-GutHealth-2019-EN.pdf

14. Yegani M, Korver. Factors affecting intestinal health in poultry. J. Poult sci. 2008; 87 (20): 52–63.

15. Apajalahti, J. Comparative gut microflora, metabolic challenges, and potential opportunities. Journal of Applied Poultry Research. 2005; 14 (2): 444-453.

16. Al-Mutairi H M, Hussein E O, Jar El Nabi A R, Swelum A A, Abd El-Hack M E, Taha, A E *et.al*. Does the consumption of acidified drinking water affect growth performance and lymphoid organs of broilers? Sustainability. 2020; 12 (8): 30-93.

17. Dehghani N, Jahanian R. Effects of dietary organic acid supplementation on immune responses and some blood parameters of broilers fed diets with different protein levels. World's poultry science journal. 2012; 39: 569-575.

18. Kazempour F, jahanian, R. Effect of different supplemental organic acids on immunocompetence and some blood metabolites in laying hens fed varying nonphytate phosphorus levels. In 18th. European symposium on poultry nutrition. Izmir, Turkey. 2011; 665-67.

19. Ganguly S. Supplementation of prebiotics, probiotics and acids on immunity in poultry feed: a brief review. World's poultry science journal. 2013; 69 (3): 639-648.

20. Lee I K, Bae S, Gu M J, You S J , kim G, Park S M, *et.al.* H9N2-specific igg and CD4+ CD25+ T cells in broilers fed a diet supplemented with organic acids. Poultry science symposium. 2017; 96 (5)**:** 1063-1070.

21. Saxena, V. k., Singh, H., Pal, S. k., Kumar, s., Genetic studies on primary antibody response to sheep erythrocytes in guinea fowl. British poultry science. 1997; 38: 156–158.

22. Ali Maghsoudi, Esmaeil Vaziri, Mansoureh Feizabadi, Mehran Mehri. Fifty years of sheep red blood cells to monitor humoral immunity in poultry: a scientometric evaluation. poultry science. 2020; 99 (10): 4758 – 4768.

23. Claudia Loetsch, Joanna Warren, Adrienne Laskowski, Rodrigo Yazquez-lombardi, Christoph Jandl, David B, *et.al.* cytosolic recognition of RNA drives the immune response to heterologous erythrocytes cell reports. 2017; 21 (6): 1624–1638.

24. Heller E D, Leitner G, Friedman A, Uni Z, Gutman M, Cahaner A. Immunological parameters in meat-type chicken lines divergently selected by antibody response to Escherichia coli vaccination. J Veterinary immunology and immunopathology. 1992; 34 (1-2): 159-172.

25. Parmentier H K, Nieuwland M G B, Rijke E, Reilingh G D V, Schrama j W. Divergent antibody responses to vaccines and divergent body weights of chicken lines selected for high and low humoral responsiveness to sheep red blood cells. J Avian diseases. 1996; 634-644.

26. Parmentier H k, abuzeid S Y, Reilingh G D, Nieuwland M G, Graat E A. Immune responses and resistance to *Eimeria acervulina* of chickens

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

divergently selected for antibody responses to sheep red blood cells. Poultry science. 2001; 80: 894-900
27. Parmentier H K, Baelmans R, Nieuwland M G, Dorny P, Demey F. Haemolytic complement activity, C3 and factor B consumption in serum from chickens divergently selected for antibody responses to sheep red blood cells. Veterinary immunology and immunopathology. 2002; 90 (1-2): 91-100.
28. Parmentier H K, Van den kieboom w J, Nieuwland M G, Reilingh G D, Hangalapura B N, Savelkoul H F, *et.al.* Differential effects of lipopolysaccharide and lipoteichoic acid on the primary antibody response to keyhole limpet hemocyanin of chickens selected for high or low antibody responses to sheep red blood cells. Poultry science. 2004; 83 (7): 1133-1139.
29. Yi T, Li J, Chen H, Wu J, An J, Xu Y, *et.al* . Splenic dendritic cells survey red blood cells for missing self-cd47 to trigger adaptive immune responses. Immunity and ageing. 2015; 43 (4): 764-775.
30. Aviagen 2018. Arbor acers broiler management handbook. http://eu.aviagen.com/assets/tech_center/aa_broiler/aa-broilerhandbook2018-en.pdf.
31. Van der Zijpp A, Leenstra F. Genetic analysis of the humoral immune response of white leghorn chicks. Poultry science.1980; 59 (7)**:** 1363-1369.
32. OIE 2019, Manual of diagnostic tests and vaccines for terrestrial animals chapter 3.3.14.**,** pp: 971.
33. Ebrahimi H, Rahimi S, Khaki P, Grimes J L, kathariou S. The effects of probiotics, organic acid, and a medicinal plant on the immune systemand gastrointestinal microflora in broilers challenged with campylobacter jejuni. Turkish journal of veterinary and animal sciences. 2016; 40 (3)**:** 329-336.
34. Das S, Islam k, Islam M. Efficacy of citric acid in diet contains low levels of protein and energy on the performance and immunity of broiler. 7th international poultry show and seminar-2011 world's poultry science association-bangladesh branch. 2011; 25-27.
35. Sohail R, Saeed M, Chao S, Soomro R, Arain M, Abbasi I, *et.al.* Comparative effect of different organic acids (benzoic, acetic and formic) on growth performance, immune response and carcass traits of broilers. Animal production advances. 2015; 5**:** 757-764.
36. Houshmand M, Azhar k, Zulkifli I, Bejo M, kamyab A. Effects of nonantibiotic feed additives on performance, immunity and intestinal morphology of broilers fed different levels of protein. South african journal of animal science. 2012; 42 (1)**:** 23-32.
37. Emami N K, Naeini S Z, Ruiz-feria C. Growth performance, digestibility, immune response and intestinal morphology of male broilers fed phosphorus deficient diets supplemented with microbial phytase and organic acids. Livestock science. 2013;157 (2-3): 506-513.
38. Eftekhari A, Rezaeipour V, Abdullahpour R. Effects of acidified drinking water on performance, carcass,

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

immune response, jejunum morphology, and microbiota activity of broiler chickens fed diets containing graded levels of threonine. Livestock science . 2015; 180**:** 158-163.

39. Šamudovská A H, Demeterová M, Skalická M, Bujňák l, Naď P. Effect of water acidification on some morphological, digestive and production traits in broiler chickens. Bulg. J. Vet. Med. 2018; 21**:** 269-278.

40. Hedayati M, Manafi m, khalaji S, yari M, Esapour A, Nazari A, *et.al*. .Combination effect of probiotic and organic acids on blood biochemistry and immunity parameters of broilers. International journal of agriculture innovations research and exploration. 2015; 3 (4)**:** 1288-1293.

41. Tyler J. W, Hancock D, Parish S.M , Rea D E, Besser T E, Sanders S G , *et.al.* Evaluation of 3 assays for failure of passive transfer in calves. Journal of veterinary internal medicine. 1996; 10(5)**:** 304-307.

42. Abdel-Fattah S, El-sanhoury M, El-Mednay N, Abdel-Azeem F. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. International journal of poultry science. 2008; 7 (3)**:** 215-222.

43. Taherpour k and Ghasemi H. Single and combinative effects of fructo-oligosaccharide and organic acid on growth performance and immune function of broiler chickens. Agriculture communication. 2014; 2**:** 67-72.

44. Rahmani H and Speer W. Natural additives influence the performance and humoral immunity of broilers. International journal of poultry science. 2005; 4 (9)**:** 713-717.

45. Rafacz-livingston K, parsons C, jungk R. The effects of various organic acids on phytate phosphorus utilization in chicks. Poultry science symposium. 2005; 84 (9)**:** 1356-1362.

46. Brzóska F, Śliwiński B, Michalik-rutkowska O. Effect of dietary acidifier on growth, mortality, postslaughter parameters and meat composition of broiler chickens/wpływ zakwaszacza diety na masę ciała, śmiertelność, wydajność rzeźną i skład mięsa kurcząt rzeźnych. Annals of animal science.2013; 13(1)**:** 85-96.

47. Kundu A., Singh D. P., Mohapatra S. C., Dash, B. B., Moudgal R P, Bisht g S. Antibody response to sheep erythrocytes in indian native vis-à-vis imported breeds of chickens. Br. Poult. Sci. 1999; 40 (1): 40-43

48. Fathi R, Samadi M S, Qotbi A A, seidavi A, martínez-marín A L. Effects of feed supplementation with increasing levels of organic acids on growth performance, carcass traits, gut microbiota and ph, plasma metabolites, and immune response of broilers. Animal science papers reports and studies. 2016; 34 (2)**:** 195-206.

49. Haque M, Islam k M, Akbar M, Chowdhury R, khatun M, karim M, *et.al.* Effect of dietary citric acid, flavomycin and their combination on the performance, tibia ash and immune status of broiler. Canadian journal of animal science. 2010; 90 (1): 57-63.

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

50. Gross W G, Siegel p B, Hall R W, Domermuth C H, Duboise R T. production and persistence of antibodies in chickens to sheep erythrocytes. 2. Resistance to infectious diseases virginia polytechnic institute and state university. poultry science.1980; 59 (2): 205-210

51. Ali A M, Elagrb H M, Hamoud M M, Gamal A M, Mousa M R, Nasr S A, *et.al*. Effect of acidified drinking water by organic acids on broiler performance and gut health. Adv. Anim. Vet. Sci. 2020; 8 (12):1301-1309.

52. Marín-flamand E, vázquez-durán A, méndez-albores A. Effect of organic acid blends in drinking water on growth performance, blood constituents and immune response of broiler chickens. The journal of poultry science**.** 2014; 51 (2): 144-150

53. Mohamed M A, Daly E F, Azeem N A, Youssef A W, Hassan H M . Growth performance and histological changes in ileum and immune related organs of broilers fed organic acids or antibiotic growth promoter. International journal of poultry science. 2014; 13 (10): 602-610.

54. Kammon A ,Alzentani S, Tarhuni O, Asheg A. Research article effect of some organic acids on body weight, immunity and cecal bacterial count of chicken during heat stress. International journal of poultry science. 2019; 18 (6): 293-300

55. Yonash N, Leitner G, Waiman R, Heller E D, Cahaner A. Genetic differences and heritability of antibody response to *Escherichia coli* vaccination in young broiler chicks. Poultry science. 1996; 75 (6): 683-690.

56. Ubosi C O, Gross W B, Hamilton P B, Ehrich M, Siegel P B. Aflatoxin effects in white leghorn chicken selected for response to sheep erythrocyte antigen: 2. Serological and organ characteristics. Poultry science. 1985; 64 (6): 1071-1076.

57. Zhang H M, Hunt H D, Kulkarni G B, Palmquist D E, Bacon I D . Lymphoid organ size varies among inbred lines 63 and 72 and their thirteen recombinant congenic strains of chickens with the same major histocompatibility complex..Poultry science. 2006; 85 (5): 844 – 853.

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2024.296320.1033

Table 1: The Organic acids used in the study

Organic acids	Chemical formula	Concentration	Manufacture
Formic acid	HCOOH	99%	Alpha Chemicals®
Lactic acid	CH3 CH OH COOH	95%	Alpha Chemicals®
Propionic acid	CH_3 CH_2 COOH	99%	Sigma ALDRICH®
Citric acid monohydrate	COOH CH2 C(OH) (COOH) CH2 COOH	99.5%	AVI-CHEM® laboratories, India

Table 2: Vaccination program applied

Age of vaccination	Disease	Vaccine/Route of application
0-day-old (on hatchery)	NDV and IBD	Innovax ND-IBD Vaccine® (SC)
3 days old	NDV and IBV	Clone 30 Ma5® and Var 2 (Eye drop)
9 days old	Geno type VII	Dulgoban G7 live vaccine (Eye drop)
11 days old	H5N1+H5N8+ND (Geno Type VII)	ME Vac multivalent killed vaccine Injection (SC)
14 days old	H9N2	ME VAC Injection (SC)

Table 3: Usage of Single OAs on Modulation Immune Response & Mortality

Organic acid 1%	Vaccination Immune Response (HI) Antibody titres (log^2)												Total Mortality rate
	At 7 days (before treatment)				At 25 days				At 35 days				
	H9N2	H5N8	H5N1	ND	H9N2	H5N8	H5N1	ND	H9N2	H5N8	H5N!	ND	
Formic	3.1	4	4.4	3.4	4.3	5.8[a]	4.6	4.9	6.0[a]	6.1[a]	7.1[a]	7.4[a]	2%
Lactic	2.9	3.8	4.1	3.6	3.9	5.2[a]	4.6	[a]5.7	5.2[a]	6[a]	7.5[a]	7.5[a]	6%
Propionic	3.1	4.0	4.3	3.4	4.0	5.1[a]	5.1	5.4[a]	5.8[a]	5.9[a]	7.3[a]	7.9[a]	4%
Citric	3.0	4	4.4	3.5	4.5	4.9	4.9	5.6[a]	5.6[a]	6.3[a]	6.1[a]	7.3[a]	4%
Control	3.1	3.9	4.3	3.4	4	4.3	4.5	4.6	4.2	4.9	4.6	5.1	8%

*[a] Indicates a significant difference at $P < 0.05$.

Table 4: Usage of OA mixtures to modulate the immune response and mortality

Organic acid 1% (0.33+0.33+0.33) mixtures	Vaccination Immune Response (HI) Antibody titer (log^2)												Total Mortality rate
	At 7 days (before treatment)				At 25 days				At 35 days				
	H9N2	H5N8	H5N1	ND	H9N2	H5N8	H5N1	ND	H9N2	H5N8	H5N!	ND	
Mixture1 (Formic + Lactic + Propionic)	3.1	4	4.4	3.4	4	5.4	5	7.8*	5.8*	6*	7*	7*	2.0%.
Mixture 2 (Formic + Lactic+ Citric)	2.9	3.8	4.1	3.6	4.1	5	4.9	7.2*	5.3*	5.5*	5.6*	7*	4.0%
Mixture 3: (Formic +Propionic +Citric)	3.1	4.0	4.3	3.4	3.9	4.5	5.1	6.9*	4.9*	5.9*	6.5*	6.8*	2.0%
Mixture 4 :(Lactic +Propionic + Citric)	3.0	4	4.4	3.5	4.2	4.3	4.8	6*	5.5*	5.6*	7*	7.1*	6.0%
Control	3.1	3.9	4.3	3.4	4	4.3	4.5	4.6	4.2	4.9	4.5	5.1	8.0%

*[a] Indicates a significant difference at $P < 0.05$.

Table (5): Effect of single OAs on the Non-specific immune response against SRBCs. and FCR

Organic acid 1%	SRBCs antibody titre (log^2)	FCR
Formic acid	5.4 *	1.45
Lactic acid	8.4*	1.42
Propionic acid	8.2 *	1.41
Citric acid	6.6 *	1.4
Control	3.8 *	1.51

*Indicates a significant difference at $P < 0.05$

Table (6): Effect of OA mixtures on specific immune response against SRBCs and FCR

Organic acid 1% (0.33+0.33+0.33) mixtures	SRBCs antibody titre (log^2)	FCR
Mixture1(Formic + Lactic + Propionic)	6.6 *	1.4
Mixture 2 (Formic + Lactic+ Citric)	8.0 *	1.39
Mixture 3: (Formic +Propionic +Citric)	7.4 *	1.36
Mixture 4 :(Lactic +Propionic + Citric)	6.2*	1.43
Control	3.8 *	1.51

*Indicates a significant difference at $P < 0.05$

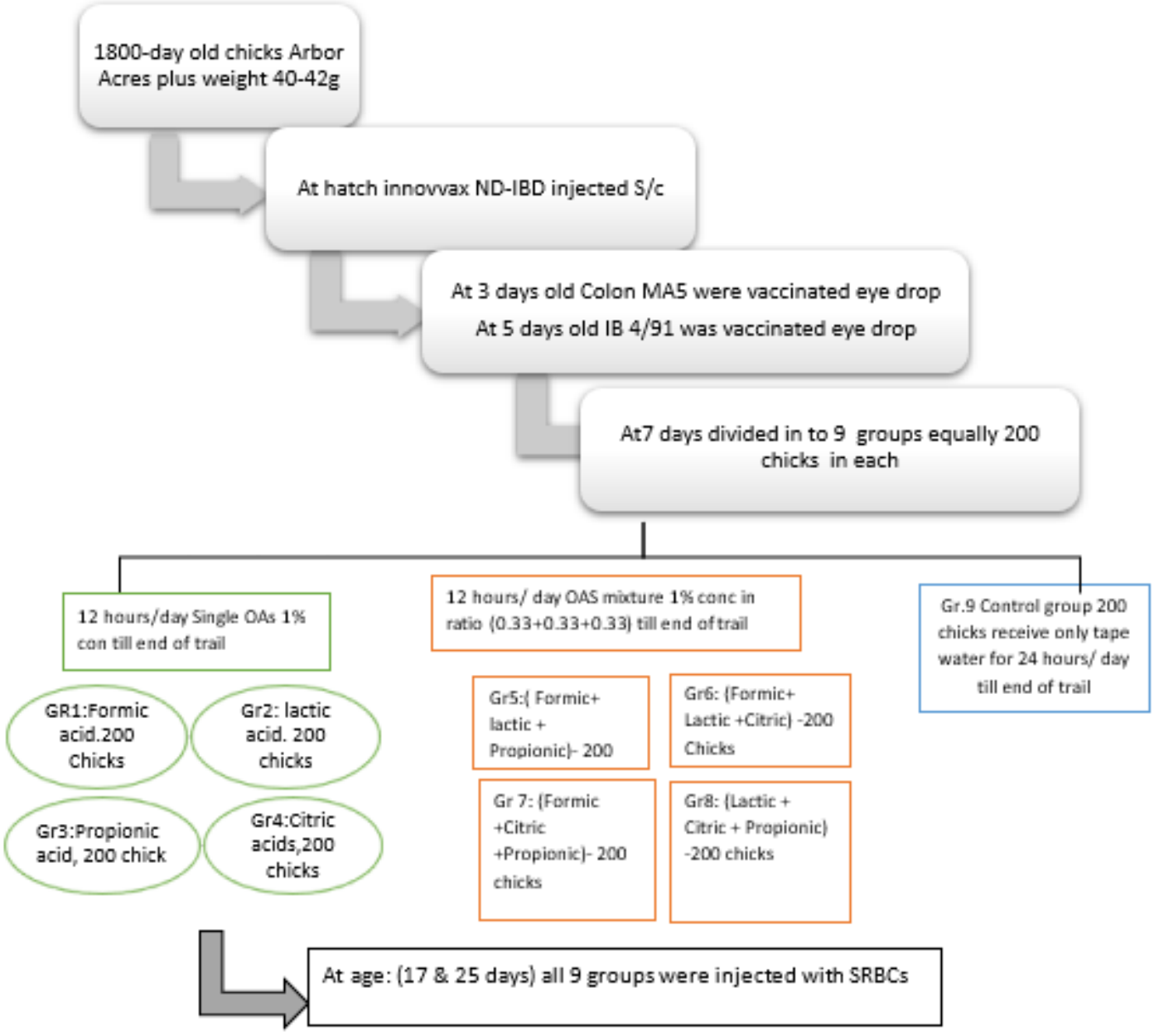

Fig. 1: Summary of the experimental design

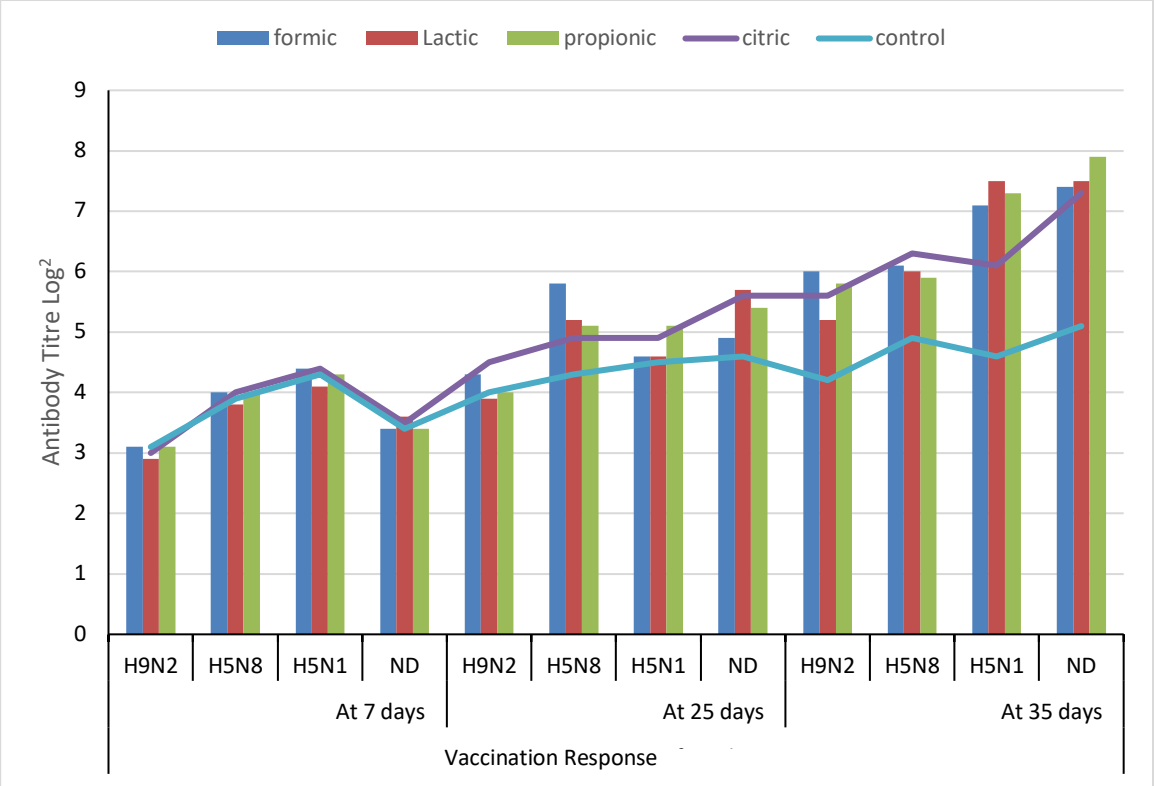

Fig. 2: Single usages of OAs on immune modulation

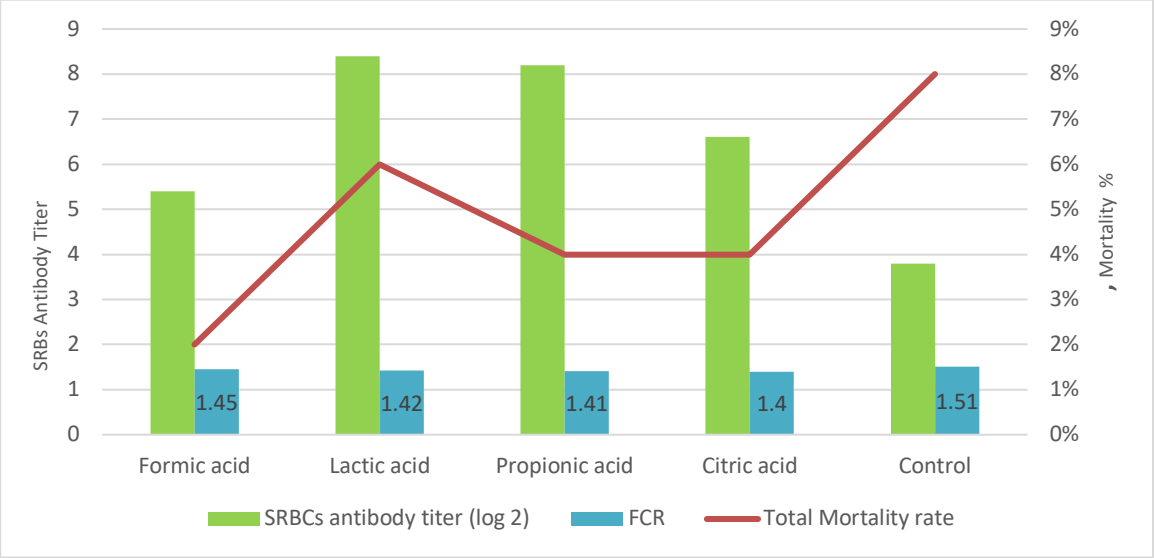

Fig. 3: Single usage of OAs on FCR, mortality and SRBs antibody titer

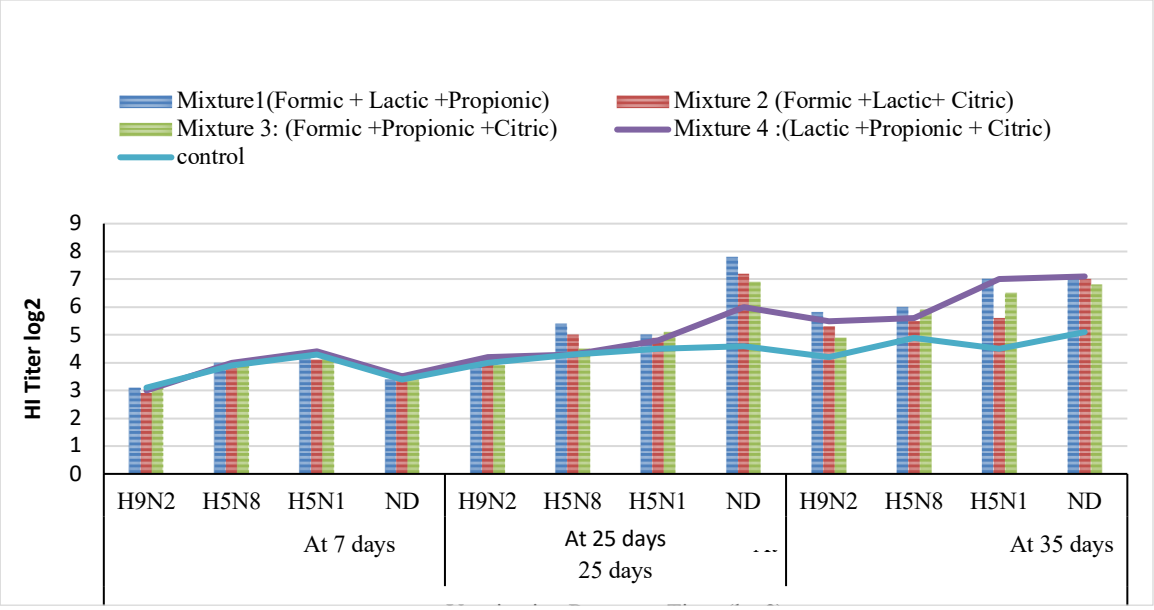

Fig. 4: Mixtures of OAs on immune modulation

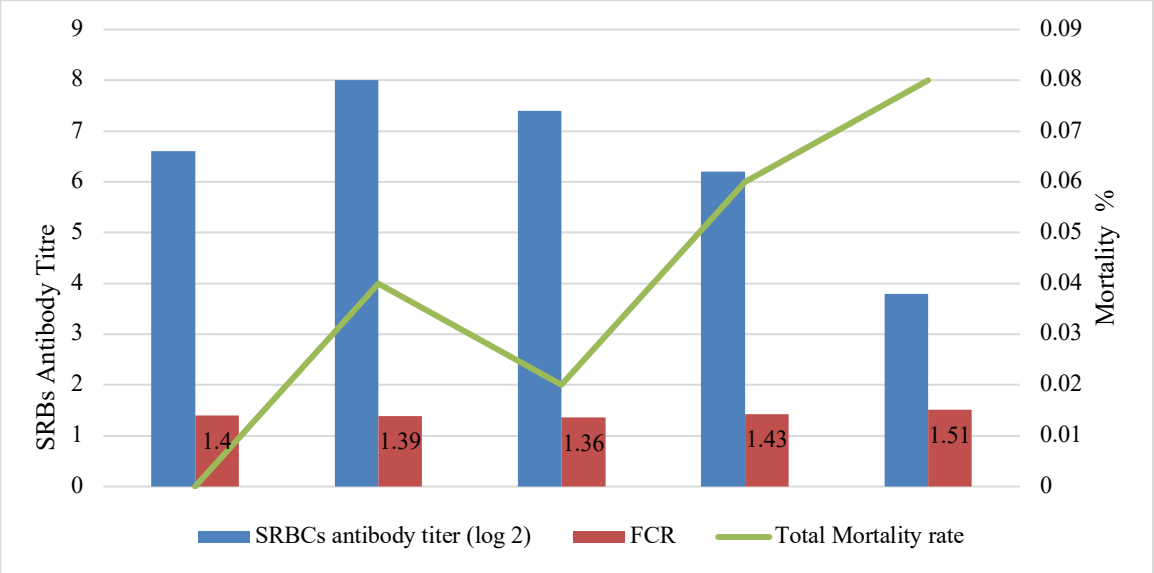

Fig. 5: Mixtures usage of OAs on non-specific immune response against SRBCs, FCR and mortality rate