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Role of glycerides of organic acids on broiler recovery challenged with the Newcastle disease virus (NDV) isolated from broiler chicken fields

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ABSTRACT

Newcastle disease virus is a highly contagious viral disease in poultry fields. Besides biosecurity and vaccinations, continuous outbreaks occur due to mutations in genomic sequence, resulting in new vaccine strains. This research hypothesized that using a mixture of short- and medium-chain fatty acids may protect the broiler chickens from this disease and improve the broiler performance. A sample from the NDV-like outbreak was collected from broiler chickens having high mortality and several lesion symptoms in the tracheae, lung, and gastrointestinal tract. Two hundred four broiler chicks were randomly divided into four treatments, including three replicates each. T1 negative control with no additives; T2 control in which the chicks received 2 ml/L of glycerides of organic acids; T3 2 ml/L of glycerides of organic acids + infected with NDV isolate; T4 infected with the NDV isolate. The results showed that the sample was NDV positive by Reverse Transcriptase Real-Time PCR. All the studied performance parameters were significantly improved in the group that had glycerides of organic acids with no infection. However, these compounds could not protect and minimize the mortality of chickens infected with a Newcastle disease virus field isolate. This study concludes that this mixture improves broiler performance in normal conditions.

1. Introduction

Organic acids are weak acids with an R-COOH carboxylic acid group that are intermediates in the breakdown of amino acids, carbohydrates, and lipids. Due to their nutritional value and antibacterial activity, these molecules are utilized in animal feedstuff (French, 2017, Chahardoli et al., 2020). For years to keep birds healthy and perform better, the feed industry has used organic acids as a feed preservatives and as an alternative to growth promoters. Sevral studies have indicated that organic acids can improve feed efficiency and subsequently increase growth performance (Adil et al., 2010, Polycarpo et al., 2017). Different results are obtained by various experiments regarding the uses of organic acids and this is because of general factors related to the type of organic acids, feed type, and bird's overall health status. Khan and Iqbal (2016) demonstrated that short-chain fatty acids benifit the health of the broiler's intestine. Whereas in other trials the benefits of medium-chain fatty acids have been stated (Zentek et al., 2011, Onrust et al., 2018). On the other hand, Kuang et al. (2015) and Huang et al. (2015) stated that the mixture of organic acids has greater positive effects on the performance, nutrient digestibility and absorption, and intestinal health of broiler than a single molecule of organic acids.

Because viruses, such as Newcastle disease virus (NDV), harm and result in massive economic losses to poultry, it has gained huge attention from the poultry farm industries (Ashraf and Shah, 2014, Rehan et al., 2019). Over time, the virus may have several mutations, and its virulence and biological characteristics have been changed. Therefore, this leads to ineffective vaccination and continuous outbreaks (Kapczynski et al., 2013, Bello et al., 2018, Absalón et al., 2019). Alternatives have been extensively studied by researchers and poultry industries to minimize and improve chicken gut health as well as boost immunity (M'Sadeq et al., 2015, Khan and Iqbal, 2016, Adhikari et al., 2020, Kumar et al., 2021). Numerous studies have shown that organic acids might boost chickens' natural immunological responses. Birds given an organic-acid-supplemented diet had bigger immunological organs (bursa of Fabricius and thymus), and a greater amount of globulin in their serum (Ghazalah et al., 2011). Antibody titer against Newcastle disease was significantly increased in broiler (Houshmand et al., 2012) and in layer (Abbas et al., 2013) when receiving different organic acids.

This research hypothesizes that providing the broiler with a combination of short-chain fatty acids (SCFA), propionic acid, and butyric acids, and medium-chain fatty acids (MCFA), such as caprylic, and capric acids will enhance the broiler immunity and protect them from viral diseases such as Newcastle Disease Virus (NDV). Subsequently, these combinations may improve the bird's gut conditions and enhance the performance of infected and non-infected with NDV. Therefore, this research aims to evaluate the influence of glycerides of organic acids on broiler performance, immunity, intestinal histology, and some serum biochemical tests.

2. Materials and Methods

2.1. Ethical approval and sample handling

The ethical approval was granted and monitored by the Animal Ethics Committee, Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, Iraq under the code AEC 280720231 following their procedure. The dead chickens were sanitized and bured in soil after clinical inspection and sample collection. The NDV-challenged groups were reared in different rooms from control and untreated groups to avoid cross-contamination or infection.

2.2. Virus sample collection

An NDV outbreak occurred in a broiler field with a capacity of 25000 birds located in Duhok province, Kurdistan region of Iraq, with more than 85% mortality in less than 72 hours. This field was vaccinated at the first and seventh days of age with B1 and Clone 30 NDV vaccines, respectively. The swabs (tracheal and air sacks) and tissues (trachea and proventriculus) were sampled from three NDV symptomatic dead birds and put in 3 ml of Viral Transport Medium (VTM) containing antibiotics (Penicillin 1000 IU/ ml and Nystatin 50 mg/ml) to inhibit the bacterial and fungal growth. The sample was sent to a diagnostic laboratory (Duhok vet Lab, Duhok province, Kurdistan region of Iraq) for infection confirmation by Reverse Transcriptase Real-Time PCR (RT-qPCR) (PowerAmp96 system, Kogenebiotech, Soul, South Korea) using Ready-To-Use specifc NDV diagnostic kit (PowerPrepTM NDV). A total of 20 µl of reaction was set up containing 4.2 µl of Primer/Probe Mix, 10 µl of 2X RT-PCR Reaction Buffer, 0.8 µl of 25X RT Enzyme Mix, 5 µl of template RNA. The heating program was as follows: Reverse transcription at 50 oC for 30 minutes, Initial denaturation at 95 oC for 10 minutes, cyclic denaturation at 95 oC for 15 seconds, and annealing and extension at 60 oC for 1 minute (Detection of flourescent was measured at this step). According to postmortem inspections, there was no secondary infection.

2.3. Experimental design

A total of 204 unvaccinated broiler chicks (Ross 308) aged three weeks were used in this experiment. They were randomly and equally divided into four treatments containing three replicates each in separated 1m*1m floor cages (17 birds/cage). After taking two birds for initial blood sampling each cage contained 15 birds. The first treatment (T1) was the negative control and did not receive organic acid supplementation and virus. The second group (T2) received the glycerides of organic acids in drinking water (2 ml/L) with no infection. Both groups were kept and reared in separate rooms to avoid cross-contamination and infection. The third group (T3) was challenged with isolated NDV and received the glycerides of organic acids (Nutriservice Company, France) in drinking water (2 ml/L). The final group (T4) was the positive control and the birds were infected with isolated NDV with no glycerides of organic acids administration. Glycerides of organic acid are composed of a mixture of propionic acid, butyric acid, caprylic acid, and capric acid (Nutriservice Company, France).

2.4. Newcastle Disease Virus (NDV) challenge

The initial collected sample from the broiler farm was confirmed positive for NDV by Real-Time PCR. Therefore, two groups of birds (T3 and T4) containing three replicates, each with 15 broiler chicks aged three weeks were challenged with the NDV isolated from the field. After ten serial dilutions of collected NDV VTM, a 500 µl/bird from the last dilution was orally given to birds. Directly after the NDV infection, T3 was provided with glycerides of organic acids in drinking water (2 ml/L). The T4 was provided with fresh water. Water and feed were provided freely during the entire project. At the first sign of NDV infection, two birds (dead or live) were selected for swabbing, tissue, and blood collection.

2.5. Production performance

Initial body weight for all treatments was recorded on the first day of the experiment. At the end of the project, the final body weight of T1 and T2 were measured. However, the final body weight of T3 and T4 were not recorded due to 100% mortality. The total consumed feed in T1 and T2 was recorded to measure the feed conversion ratio (FCR).

2.6. Intestinal histology parameters

On the 4th day of infection, two birds from each replicate within T1 and T2 (a total of six intestinal sections) were selected for intestine histology. A small section (2 cm) of the small intestine was washed several times with normal saline and put in a 10% formaline solution (stock concentration 38%). Staining was performed with eosin and Haematoxylin. Paraffin wax was used for tissue fixation, and the 10X magnification light microscope was used for readings. The measurements of surface area, villi height, villi apical width, villi base width, crypt depth, and villi height/crypt depth were recorded according to Iji et al. (2001). The sample preparation, fixation, and staining were performed at the Histology Center, Azadi Teaching Hospital, General Directorate of Health, Duhok Province, Kurdistan Region of Iraq, and histological measurements were taken in the Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok.

2.7. Immunity and serum biochemical parameters

On the first day of the trial, two birds from each replicate within each treatment (six samples in total) were used for blood sampling. This was used for the initial antibody titer against NDV by ELISA test (AccuSkan FC Filter-Based Microplate Photometer, Thermofisher, Germany) using Newcastle Disease Virus Antibody Test Kit (BioChek, UK). On the fourth day post-infection (4 dpi) two other birds were selected for blood collection for secondary antibody titer measurement of NDV and serum biochemical parameters. The serum biochemical measurements were carried out in Cobas machine (Cobas 6000, Germany) using specified kits.

2.8. Statistical analysis

A one-way ANOVA was used to assess serum biochemical and histological parameters of jejunum among all treatments. A t-test was established for the broiler performance measurements between T2 and T3 due to complete mortality in T3 and T4.

For serum biochemical and histological parameters of jejunum. A one-way ANOVA was used among all four treatments. However, because of complete mortality in T3 and T4, the t-test was established for broiler performance measurements. These two analyses were performed using the SAS statistical software (version 2.0).

3. Results

3.1. Chicken antibodies titer against NDV

In the initial antibody titer (pre-experiment), all groups had no significant variations. However, the post-infection antibody titer significantly differed among treated and non-treated groups with organic acids. The T2 recorded the highest Ab titer, followed by T1, while the challenged groups were significantly lower (P<0.0001) (Table 1).

Table 1. The NDV antibody titters of broiler chickens under the impact of glycerides of organic acids during pre and post-NDV infection.

Antibody titer	T1	T2	T3	T4	SEM	*p*-value
Initial titre	1126.67	1121.83	1125.65	1123.33	11.94	0.99
4 dpi	126.60 [b]	555.83 [a]	26.16 [c]	27.83 [c]	45.74	0.0001

*Means with different subscript letter in the same row differ statistically

3.2. Clinical signs and postmortem lesions

In the first sampling (initial outbreak) all the NDV-like symptoms were evident in the chickens, such as difficulty in breathing, green feces, depression, and hemorrhagic lesions in the gizzard and small intestine. After the experimental challeng of the broiler chickens with the isolated NDV, almost all of the abovementioned symptoms were detected in 24-36 hours (Figure 1).

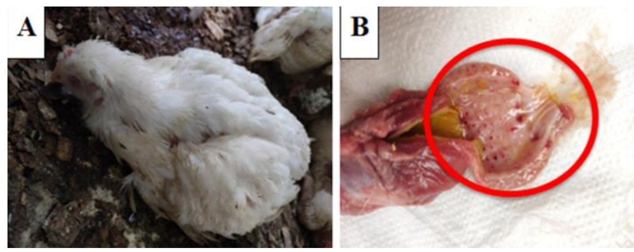

Figure 1: External and internal symptoms and lesions of broiler chicken challenged with isolated NDV. A. external symptom (depression, difficulty in breathing). B. hemorraghic dots in the gizzard due to infection with NDV (red circle).

3.2. Serum biochemical parameters

The results of serum biochemical measurements are shown in Table 2. The glucose level, uric acid, creatinine kinase, ALT, and AST in the challenged groups were significantly higher (P<0.013) compared to non-challenged groups. Thyroid hormones (T3 and T4), total protein, globulin, and serum electrolytes (Na, Ca, K, Cl, and P) were significantly higher (P<0.0001) in the non-challenged group that received the mixture of OAs. However, no significant variation was observed in T3/T4 ratio and albumin level.

Parameters of jejunum histology are shown in Table 3 (Figure 2). All the studied parameters, except the cryptic depth, were significantly increased in the non-challenged group that received the mixture of OA. The cryptic depth was significantly higher in the challenged group (positive control) compared to other groups. In most cases, these parameters in the challenged groups (T3 and T4) were significantly lower compared to other groups.

Table 2. Serum biochemical parameters of broiler chickens under the effect of glycerides of organic acids.

Parameters	T1	T2	T3	T4	SEM	*p*-value
Glucose (mg/dl)	173.83 b	169.50 b	183.50 a	169.33 b	1.87	0.013
Uric acid (mg/dl)	2213.17 b	1961.02 c	3218.33 a	3109.33 a	119.9	0.0001
Creatine kinase (U/L)	2.14 c	2.65 b	3.82 a	3.65 a	0.16	0.0001
AST (U/L)	199.02 a	162.50 b	200.67 a	228.33 a	7.36	0.008
ALT (U/L)	2.38 b	2.60 b	2.71 ab	3.05 a	0.097	0.016
T3 (nmol/L)	2.46 b	2.79 a	1.70 c	1.30 d	0.127	0.0001
T4 (nmol/L)	3.47 b	4.11 a	2.60 c	2.05 d	0.175	0.0001
T3/T4	0.71	0.68	0.67	0.64	0.020	0.720
TP (mg/dl)	2.24 b	3.17 a	1.55 c	1.28 c	0.158	0.0001
Albumin (mg/dl)	1.10	1.02	1.04	1.05	0.013	0.278
Globulin (mg/dl)	1.13 b	2.14 a	0.50 c	0.22 c	0.161	0.0001
Na (mEq/L)	125.83 b	135.50 a	103.67 c	105.72 c	2.87	0.0001
Calcium (mg/dl)	6.04 b	7.03 a	4.20 c	4.34 c	0.25	0.0001
Potassium (mEq/L)	2.15 b	3.18 a	1.66 c	1.57 c	0.135	0.0001
Chloride (mg/dl)	60.83 b	70.50 a	38.67 c	40.61 c	2.87	0.0001
Phosphorus (mg/dl)	11.78 b	13.69 a	8.20 c	8.46 c	0.49	0.0001

*Means with different subscript letter in the same row differ statistitically

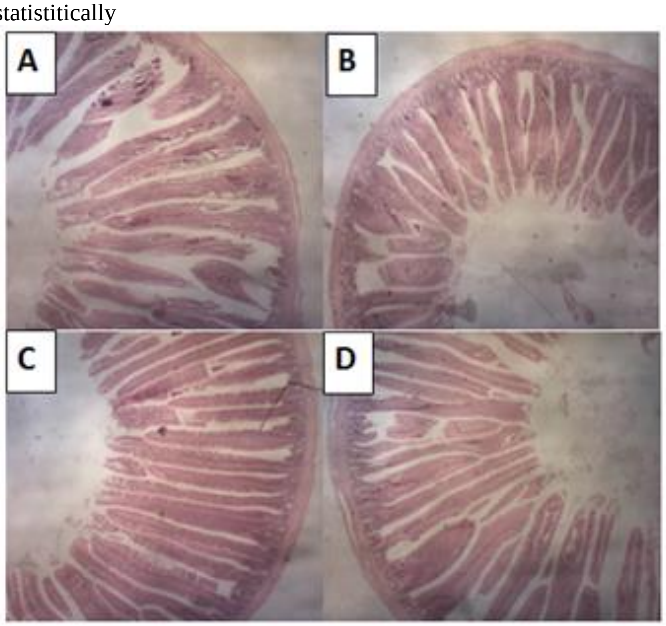

Figure 2: Histological sections of jejunum. A. Treatment 1 (negative control). B. treatment 1 (2 ml/L & no infection). C.Treatment 2 (2 ml/L & NDV infection). D. Treatment 3 (positive control).

Table 3. Jejunum histological parameters of broiler chickens under the effect of glycerides of organic acids.

Histological parameters	T1	T2	T3	T4	SEM	*p*-value
Villi Height (µm)	864.49 b	1145.53 a	610.18 c	603.70 c	20.99	0.0001
Crypt depth (µm)	142.64 b	136.54 b	136.54 b	237.54 a	4.87	0.0001
Villi apical width (µm)	67.65 b	95.11 a	62.25 c	63.75 c	1.33	0.0001
Villi base width (µm)	102.11 b	158.11 a	87.75 c	77.62 d	2.88	0.001
Villi Height /crypt depth	6.35 b	8.55 a	4.80 c	2.59 d	0.22	0.0001
Surface area (µm^2)	1682.56 b	2520.42 a	924.17 c	960.20 c	61.30	0.001

*Means with different subscript letter in the same row differ statistically

3.3. Broiler performance

The performance measurements for broilers in T1 and T2 are shown in Table 4. Because there was a complete mortality in challenged groups (T3 and T4), the t-test was used for the remaining two groups to check for any differences. The two groups had no significant variations in initial body weight on day one. However, the live body weight (final body weight) was significantly higher ($P<0.001$) in the broilers that received a mixture of glycerides of organic acids. Similarly, the weight gain and daily weight gain were significantly increased ($P<0.008$) in T2 compared to T1. Although the feed intake was not statistically significant between the two groups, the FCR was significantly improved ($P<0.01$) in the T2. Due to zero mortality in both treatments (T1 and T2) which were not challenged, there was no significant difference in livability percentage.

Table 4. Effect of glycerides of organic acids on broiler chicken performance.

Performance parameters	T1	T2	P value
Initial body weight (g)	1019.33±14.5	1037.67 ± 21.5	0.110
Live body weight (g)	1593.33 ± 8.81 b	1813.33 ± 25.87 a	0.001
Weight gain(g)	574.0 ± 20.23 b	739.6± 27.72 a	0.008
Daily weight gain (g)	82.00 ± 2.89 b	105.6 ± 3.96 a	0.008
Feed intake (g)	844.16 ± 44.2	929.52 ± 47.8	0.259
FCR	1.468 ± 0.025 a	1.256 ± 0.037 b	0.010
Livability%	100.0 ± 0.00	100.0 ± 0.00	0.11

4. Discussion

Newcastle Disease Virus (NDV) is considered the most challenging and contagious viral disease in the poultry industry, and it affects birds of all ages (Ganar et al., 2014). The most widely sensitive diagnostic technique is the Real-Time PCR assay (Isa et al., 2022, Al-Mubarak et al., 2024). Despite the implementation of various vaccination strategies and stringent biosecurity measures in broiler farms across Iraq, Newcastle disease virus (NDV) outbreaks continue to occur, causing significant losses in the poultry industry. Numerous commercial products have been employed in efforts to boost the immunity of chickens against this disease (Al-Hbiti and Al-Noaimy, 2022, Mohammad and Al-Mahmood, 2023). The potential benefit of glycerides of organic acids (short and medium-chain fatty acids) is to provide gut health and, therefore improve an animal's general health (Kumar et al., 2022). The results of the present study, in treatments 3 and 4, revealed that the fatty acids used in this experiment did not provide enough protection to broilers against the isolated NDV. In addition, the symptoms in broilers that were challenged with isolated NDV were stronger than expected. This may be due to the strong pathogenicity of the used NDV genotype. This may be because the glycerides of organic acids were provided to the chicken after the challenge. To answer this question at the end of the experiment, the chickens which were left from treatment 2 which had this product during the experimental period, were rechallenged with the same NDV isolate, and the results were similar as indicated in treatments 3 and 4 (data not shown).

The used organic acids did not show any improvement in the serum antibodies against the challenged NDV isolate. However, an improvement was found in the non-challenged group with organic acids administration. This may indicate the limitation of the activity of these OA in improving the broiler's health against some diseases. Alazawy and Al Ajeeli (2020) stated that maternal antibodies decrease with the increase of age. In addition, Gharaibeh and Mahmoud (2013) showed that the antibody titer decreased in vaccinated birds in 3 to 4 weeks post-vaccination. The broiler chicks that were used in this study were three weeks old, and they had not received any vaccination against NDV before the experiment. Negative indications in liver function (ALT and AST), metabolic hormones (Thyroid hormones), immunity parameters (total protein and globulin), and serum electrolytes in challenged groups reveal the pathogenicity of the studied NDV isolates in broiler chicken. On the other hand, most of these parameters were significantly improved in the second group (non-challenged + OAs) showing the beneficial effects of this organic material on the general health status of broilers.

These OAs significantly enhanced the histological parameters of the jejunum and therefore improved the general performance of the birds. However, this did not induce any improvement in the groups that were infected with the isolated NDV type. Additionally, the histological parameters were highly and negatively changed in challenged groups. An improvement in villus height affects the surface area which increases nutrient absorption, leading to an improvement in the bird's growth performance (Elnesr et al., 2020). Our findings are similar to those of Makowski et al. (2022) in which they obtained an increase in villus height with butyric acid administration in turkey birds.

Because the mortality rate was 100% in NDV-challenged groups, a t-test was applied to analyze the statistical variation among the control and OA groups. Therefore, the OAs supplement has significantly and positively improved the broiler performance. The increase in BW and reduction in FCR might be due to an absorptive surface area in the intestines, providing a better environment for nutrient absorption (Makowski et al., 2022). Similar findings were observed in broilers with OAs by several previous researchers (Smulikowska et al., 2009, Khan and Iqbal, 2016). Our results are in agreement with those found by Palupi et al. (2022), who concluded that the addition of 0.5% of propionic acid improved all the broiler performance parameters.

Conclusion

Overall, the hypothesis established in this research failed in that the used OA did not show any beneficial effect in immunizing the infected broiler with isolated NDV type and this may be due to the short duration after vaccination. However, these OAs have significantly improved the performance parameters, including BW, FCR, serum biochemical, and jejunum histology. Therefore, these organic materials are highly efficient in improving the general health of broilers and their production performance. Authors recommend that the circulating NDV should be isolated and sequenced to monitor the most prevalent strain in the area for a better vaccination program.

Conflict of interest

The authors declare that there is no conflict of interest.

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