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Effect of Water Organic Load and Total Ammonia Nitrogen on Broilers' Humoral Immune Response Against Newcastle Disease Virus Vaccination in Egypt

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ABSTRACT

Low-quality drinking water (DW) has a negative impact on poultry immunity raising the risk of emerging infections. The current trial used experimentally contaminated broilers' DW to study its effect on humoral immunity compared to 2 control groups. A total of 450 unsexed 1-day-old Cobb chickens were randomly allocated into 6 groups (75 birds in 5 replicates per group). DW of (T1) had 24 ppm chemical oxygen demand (COD), (T2) 12 ppm COD, (T3) 15 ppm total ammonia nitrogen (TAN), (T4) 5 ppm TAN, (T5) normal tap water (TW), (T6) TW with immunostimulant (IMU FORT®) a day before and after vaccination. Birds were subjected to the NDV DW vaccination program, serum was collected weekly, antibody (Ab) titers were measured along 6 weeks, lymphoid organs somatic indices (OSI) were evaluated after carcass evisceration at the end of the trial (42nd day), and Postmortem lesions were examined. Groups showed differences in Ab titers however, the 3rd and 6th weeks showed significant differences ($p \leq 0.05$), at the 3rd week T3 titers were significantly higher than T1, T2 but lower than T4, T6, and at the 6th week T6 titers were significantly higher than T1, T3, T4 but T5 was significantly lower than T3. The lowest OSI was in T3 which records 0.475, 0.133, 0.101 for thymus, bursa, and spleen, respectively while T6 was the highest. Broilers' DW which has a high organic load (COD) and TAN, significantly decreases Ab titers and OSI. Both good quality DW and immunostimulant supplementation have a positive effect on NDV vaccines' immune response.

Key words: Water quality, Chemical contaminants, Humoral immunity, Somatic indices, Poultry vaccination.

INTRODUCTION

The effects of drinking water pollutants and poor nutritional status on broiler chicken performance and immune function are highly relevant (Vodela et al. 1997). On a weight basis, birds consume roughly 1.6-2.0 times as much water as they do feed (Kellems and Church 2002). Water is only second to oxygen as a crucial ingredient for maintaining life and maximizing poultry growth and productivity. Drinking water provided 70 to 97 percent of the water required by animals. The kind of water source and the extent of contamination from abiotic and biotic sources, such as dissolved nutrients or directly deposited urine or feces, impact water quality (Kamal et al. 2019a).

The water oxygen demand organic pollutants, which is one of the most damaging characteristics affecting the drinking water quality, are among the primary aspects that are assessed. Biofilms' organic matter affects the efficacy of drinking water vaccination (Kamal et al. 2019b). Biological oxygen demand (BOD) was introduced as a measure of the total amount of organic pollutants in the water. Due to a long time it takes to detect BOD (at least five days incubation), another metric, chemical oxygen demand (COD), which can be examined rapidly, has become a substitute indication of organic components (Luo et al. 2019).

Organic nitrogen can be biologically converted to ammonium, then to nitrite and nitrate in nature. These nutrients can be utilized by algae and aquatic organisms to convert back to organic forms of nitrogen once they are in the nitrate or ammonium forms (Heiskary and Lindon 2010).

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One of the preventive measures against endemic ND has been reported to be vaccination through drinking water (Orajaka et al. 1999). Newcastle disease is a highly contagious viral disease caused by paramyxovirus which results in significant economic losses due to high mortality, morbidity, and stress (Suarez et al. 2019). The efficacy of vaccines and medicines given through water lines may be harmed by poor water quality (Van Der Sluis 2002).

When compared to non-filtered tap water, broiler chicks using filtered water showed a superior immune response and illness resistance. The use of filtered tap water vs. non-filtered tap water resulted in a considerable rise in bursa relative weight, which reflects the health, growth, and immune function of the bird (ElSaidy et al. 2015).

Immunomodulation becomes a high-priority topic in medical and veterinary science. Immunomodifiers are substances that can negatively (immunosuppression) or positively (immunostimulant) impact the immune system's reactivity (Abdel-Moneim et al. 2020). Immunostimulant derived from herbal extracts and antioxidants improves poultry immunity and immunological response, as well as the relative weights of lymphoid organs (Khaleghi Miran et al. 2010). Lectins and oregano essential oils also non-specifically augment the immune response and general performance of broilers (Alagawany et al. 2018; El Seedy et al. 2018).

The current study was conducted to investigate the experimental effect of drinking water contaminated with organic matter (COD) and TAN on broiler humeral immune response, OSI and evaluate the added immunostimulant modulation effect.

MATERIALS AND METHODS

Ethical Approval of the study was obtained vide No. VET CU 8/03/2022/425, Cairo University Institutional Animal Care and Use Committee (Vet. CU. IACUC). Experiment was executed following all the guidelines of Bioethics Committee, Cairo University, Egypt.

Experimental Birds

The experiment was carried out for 6 weeks at the Poultry Management Unit in the Faculty of Veterinary Medicine at Cairo University, Egypt. A total of 450 unsexed day-old Cobb chickens purchased from a commercial hatchery at Badrashein city, Egypt, were randomly allocated into six treatments (75 birds in five replicates). Treatment one (T1); birds received tap water (TW) loaded with 24ppm COD (chemical oxygen demand), treatment two (T2); birds received TW loaded with 12ppm COD, treatment three (T3); birds received TW loaded with 15ppm TAN (total ammonia nitrogen), treatment four (T4); birds received TW loaded with 5ppm TAN, treatment five (1st control) (T5); birds received TW, and treatment six (2nd control) (T6); birds received TW fortified with immunostimulant (IMU FORT®) a day before and day after each vaccination (1cm/1 liter DW for 12 hours). Immunostimulant (IMU FORT®) composed of oregano oil (11.3%) containing thymol (3.8%) and carvacrol (7.5%), lectin (5%), vitamin E (5.27%), zinc (9.2%) and selenium (0.12%) (3A Pharma, MOA Reg. No.: 7560).

Housing and Management

The chickens were raised on a deep litter system and received the same standard management, hygienic, and environmental conditions. The stocking density was 11-12 birds/m^2. Feed and water were provided ad libitum using manual plastic feeders and drinkers. The ration was formulated to meet the nutrient requirements for broilers according to (NRC 1994). Chickens were vaccinated against NDV through the oral DW route at 7th day (HIPRAVIAR B1/H120, HIPRA, ND B1$\geq 10^{6.5}$ EID_{50}, H120 $\geq 10^{3.5}$ EID_{50}, batch: 50YT-1), 10th day (H5-ND divalent killed vaccine), 17th day (clone 30, Intervet, batch: 13630CJ01), 27th day (ND Clone 30, Intervet, A190A1J01). The light program was set as 24 h continuous light during the first 3 days and 23h light and 1 h dark (23L/1D) till the end of the experiment.

Preparation of Experimental Water

To prepare organic matter-loaded water, weighing known amount (5 gm/liter water) of endogenous dried feces, according to Chick and Martin (1908) taken from the same experimental floor litter, mixed well and dried at 105ºC for 24h then cooling in the desiccator. Add an appropriate amount of TW to obtain a COD level of 20-24mg COD/L (T1) and 2.5gm/ liter water to obtain 10-12mg COD/L (T2). The designed COD levels were according to WHO (2008). To prepare ammonia water, Ammonium chloride was added to TW (calculated from standard ammonium chloride solution preparation dissolving 3.819gm anhydrous NH_4Cl (dried at 105ºC) (M.W.53.49, LOBA Chemie, Lot # SL44771302) in distilled water and dilute to 1000mL with TW (1mL = 1mg N = 1.22mg NH3) according to Rice et al. (2017) then, calculate the required amount of NH_4Cl to make high concentration stock solution for (T3) 15mg ammonia/L water and for (T4) 5mg ammonia/L water.

Water Sampling and Analysis

Samples collection and Physico-chemical analysis were carried out according to Rice et al. (2017) for the determination of color, odor, taste, temperature, pH, EC, total hardness, TDS, ammonia, nitrite, nitrate, sulfate, and phosphate. Organic matter was determined by using chemical oxygen demand (COD) that was measured according to Rice et al. (2017). Total ammonia nitrogen (TAN) was estimated by ammonia nitrogen Nessler’s reagent spectrophotometry HJ 535-2009 (MEP 2009). The total bacterial count of water samples was carried out according to Ayandiran et al. (2014). Total coliform and fecal coliform count were enumerated by the three-tube MPN procedure (Rice et al. 2017).

Blood Sampling and HI Assay

Blood samples were collected from the wing vein weekly, then serum was separated after 8 to 10 hours as per the standard procedures and was stored at –20ºC. Detection of antibody (Ab) titers against NDV by hemagglutination inhibition (HI) test: It was performed every 7 days of the experiment to obtain log2 values (Thayer and Beard 1998).

Immune Organs Weight

At 42 days old, all birds are slaughtered, eviscerated, net carcass weights, organs weights (spleen, bursa, thymus) were recorded. Organ-somatic indices were determined from random samples from each replicate. Any dead birds have been examined for PM lesions.

The organ somatic index (OSI) was calculated by using the following formula: Organo-somatic index (OSI)=Organ weight (g)×100/Live body weight (g)) as per Chattopadhyay et al. (2011).

Statistical Analysis

Data and results were collected and computed using Microsoft Excel 2016. For analysis of data, Statistical Package for Social Sciences software, version 25.0 (SPSS Inc., Chicago, IL) was used. Initially, all information gathered was coded into variables. Normality of data was tested using Kolmogorov-Smirnov test. Both descriptive and inferential statistics involving the Kruskal Wallis H test and Friedman test were used to present the results. Post Hoc Dunn's test was done on the obtained results. For each test, a P-value of less than 0.05 was considered.

RESULTS

Results showed that water quality analysis of the used Tap water (TW) were within the acceptable range of poultry water quality standards (Watkins 2008) in physicochemical and bacteriological parameters (Table 1). Treatment water was TW with added 24ppm, 12ppm COD for T1, T2 and added 15ppm, 5ppm TAN for T3, T4. Control T5 and T6 received pure TW.

Table 2 presents the median and interquartile range (IQR) of NDV log2 titers every 7 days over 42 days of age. NDV log2 titer values are not normally distributed so Kruskal-Wallis H values (Table 3) were used to determine the significant difference between groups in each week. Table 3 shows a significant difference ($P \leq 0.05$) between treatment groups in both 21st day and 42nd day. POST HOC tests (Dunn's test) declare that on the 21st day, this significance was due to T3 that significantly ($P \leq 0.05$) higher than T1 and T2 but significantly ($P \leq 0.05$) lower than T4 and T6 (test statistics: 9.7, 17, -17, -12.7, respectively). On the 42nd day, this significance was due to T6 that significantly ($P \leq 0.05$) higher than T1, T2, T3, T4 (test statistics: -11.4, -10.2, -14.4, -11.4, respectively) and also due to T5 that significantly ($P \leq 0.05$) higher than T3 (test statistics: -12).

Fig. 1 presents NDV log2 titers trends with a major difference in each separate week alone along 6 water treatment groups. Table 4 shows a significant difference ($P \leq 0.05$) in NDV log2 titers over 6 weeks in each separate treatment group. Fig. 2 presents NDV log2 titers trends with minor differences in each separate treatment group over 6 weeks.

Fig. 3 shows lymphoid organs somatic indices (thymus, bursa, and spleen) at 42nd day in each group. Thymus gland records 0.42, 0.47, 0.40, 0.44, 0.49, 0.51%, bursa records 0.07, 0.13, 0.06, 0.08, 0.14, 0.16%, and spleen records 0.09, 0.1, 0.085, 0.097, 0.116, 0.123%, for the 6 groups, respectively.

The PM findings are recorded in Fig. 4 that shows PM lesions of the liver, breast muscles, and intestine in the T1 group at the 27th day old (a, b, c, d, e) and 40th day old (h, i). Fig. 4 also shows PM lesions of the spleen, liver, and intestine in the T2 group at the 27th day old (f, g) and 40th day old (j, k).

Table 1: Physico-chemical and bacteriological examination of Tap water (TW) source used in the experiment

Parameter	TW analysis	Maximum acceptable level*
Color	Colorless	
Odor	Inoffensive	
Taste	Palatable	
Temperature (ºC)	20.5	
pH	7.9	6 – 9
EC (µs/cm)	388.1	2500
TDS (ppm)	260	1500
Hardness (mg/L)	180	400
Chloride (mg/L)	113	350
Sulfate (mg/L)	35	400
Phosphate (mg/L)	1	5
COD (mg/L)	1	5
TAN (mg/L)	0.01	0.2
Nitrite-N (mg/L)	0.02	0.1
Nitrate-N (mg/L)	1	45
Total bacterial count (cfu/mL)	200	1000
Total coliform count (cfu/mL)	0	50
Fecal coliform count (cfu/mL)	0	0

*Adapted from Watkins (2008), EC=Electrical conductivity, TDS=Total dissolved solids, COD=Chemical oxygen demand, TAN=Total ammonia nitrogen.

Table 3: Mean ranks and difference Kruskal-Wallis H values in each week with their significance of NDV log2 titers in 6 drinking water treatment groups

Mean Rank	7th day	14th day	21st day	28th day	35th day	42nd day
T1	18	16.8	14.4	11.8	12.4	12.4
T2	12.5	16.1	21.7	18.5	16.5	13.6
T3	17	22.1	4.7	10.9	9.9	9.4
T4	18	12.2	21.7	16	13	12.4
T5	19.5	9	13.1	18.5	18.7	21.4
T6	8	16.8	17.4	17.3	22.5	23.8
Test Statistics (Kruskal Wallis Test)						
Kruskal-Wallis H	7.75	7.41	15.76	4.26	7.26	11.15
Asymp. Sig.	0.170	0.192	0.008	0.513	0.202	0.048

Results are significantly different at $P \leq 0.05$.

Table 2: Effect of quality diversity of drinking water used for NDV vaccination on HI titer log2 along 6 weeks

Groups	T1	T2	T3	T4	T5	T6
	Median (IQR)					
7th day	6.0 (1.5)	6.0 (1.0)	6.0 (1.0)	6.0 (1.5)	7.0 (1.0)	6.0 (1.0)
14th day	5.0 (1.5)	4.0 (1.0)	5.0 (1.0)	4.0 (1.0)	3.0 (1.5)	5.0 (1.5)
21st day	3.0 (1.0)	3.0 (1.0)	2.0 (1.0)	3.0 (1.0)	3.0 (1.0)	3.0 (1.0)
28th day	2.0 (1.0)	3.0 (1.0)	1.0 (2.0)	2.0 (1.0)	3.0 (1.0)	2.0 (2.0)
35th day	6.0 (5.5)	7.0 (1.5)	5.0 (2.5)	6.0 (2.5)	7.0 (3.0)	8.0 (1.0)
42nd day	4.0 (2.0)	4.0 (4.0)	4.0 (1.0)	4.0 (1.0)	6.0 (1.0)	7.0 (2.0)

IQR=Interquartile range

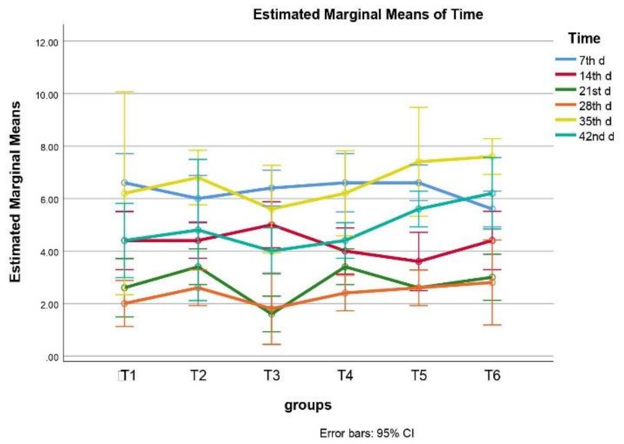

Fig. 1: Trends of NDV log2 titers in each week along 6 drinking water treatment groups.

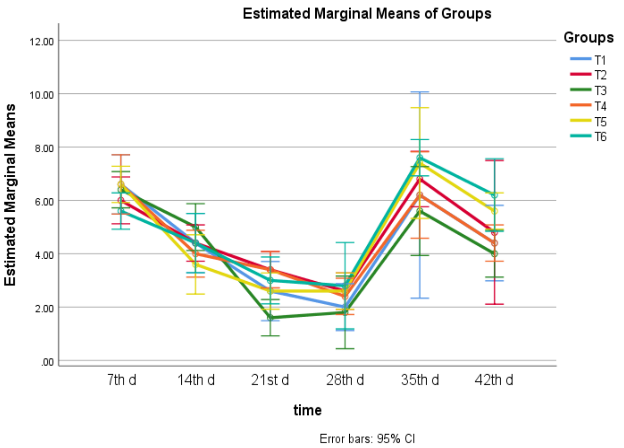

Fig. 2: Trends of NDV log2 titers in each water treatment group over 6 weeks.

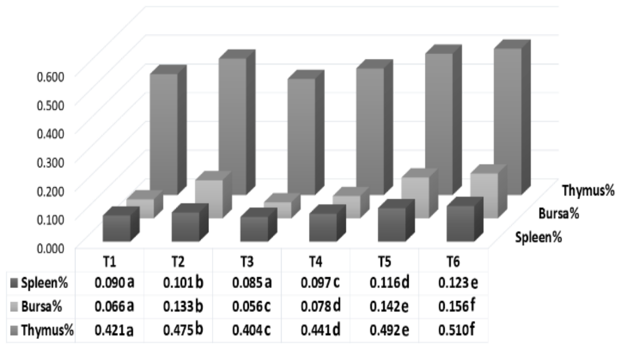

Fig. 3: Means of lymphoid organs' somatic indices (thymus, bursa, and spleen) of 4 drinking water treatment groups (T1, T2, T3, T4) compared to control groups (T5, T6) at 42nd day of age (means with different letters within the same raw are significantly different at P≤0.05).

DISCUSSION

Drinking water (DW) has different contaminants that affect poultry immunity and performance (Kamal et al. 2019a). The present study has focused on finding the negative effect of two DW quality parameters on both humoral immune response, lymphoid organs somatic indices, and how to correct this effect using a novel herbal medicine additive.

Table 4: Mean ranks and Chi-Square difference values in each group with their significance of NDV log2 titers in 6 weeks

Mean Rank	T1	T2	T3	T4	T5	T6
7th day	5.6	4.9	5.7	5.7	5.3	4.1
14th day	3.8	3.6	4.1	3.1	2.6	3
21st day	2	2.1	1.5	2.4	1.7	1.8
28th day	1.3	1.4	1.5	1	1.7	1.5
35th day	4.6	5.5	4.9	5.2	5.5	5.9
42nd day	3.7	3.5	3.3	3.6	4.2	4.7
Test Statistics (Friedman Test)						
Chi-Square	18.88	19.16	22.49	23.41	22.76	22.01
Asymp. Sig.	0.0020	0.0018	0.0004	0.0003	0.0004	0.0005

Results are significantly different at P≤0.05.

Used tap water (TW) analysis is within permissible limits (Table 1) for poultry drinking water standards (Watkins 2008). Medians of NDV titers log2 in 6 groups, shown in Table 6 over 6 weeks, and to detect a significant difference between groups in each week, the Kruskal Wallis test was applied (Table 3). Results showed no significant difference (P≤0.05) in the first 2 weeks (7th, 14th day) as the water contaminants effect was probably still minor and it took a time to make physiological and immunological changes as shown in Fig. 1 which presents each week separately. Results of the Friedman test (Table 4) showed a significant difference (P≤0.05) in NDV titers trends in all groups over 6 weeks as shown in Fig. 2 which presents each group separately.

Maternally derived antibodies (MDAs) are important for chick survivability during the first days of life until its immune system can start producing sufficient immunoglobulins (Hasselquist and Nilsson 2009). Baby chicks receive different MDAs residues, which progressively decrease to approximately 50% on the 14th day age (Gelb Jr and Jackwood 1998), but the result in Table 3 showed that MDAs levels did not have a significant difference till the 7th day age. These results are in agreement with Rose and Orlans (1981) who stated that chickens can produce their antibodies by 5 days post-hatch, but they depend on passive immunity from breeder IgY for the first 14 days post-hatch.

Birds were primed on the 7th day with NDV vaccine (Hitchner B1) via drinking water. NDV antibody titers progressively decreased till the 21st – 28th day, but the difference between groups began to be significant (P≤0.05) on the 21st day, as titers of T3, were significantly (P≤0.05) higher than T1 and T2, but lower than T4 and T6. High total ammonia nitrogen (TAN) in T3 affects birds' immune status which indicated the negative effect of TAN in water on antibodies titer post 1st vaccination as well on neutralizing MDAs residues. TAN's negative effects on antibody titers post vaccines coincided with records of Gentry and Braune (1972) and AL-Mayah et al. (2009). Both levels of TAN (T3 vs. T4) have different trends of antibody titers (Table 3 and Fig.2). High TAN in T3 vaccination water may have a direct effect on live vaccine virus titer during dilution. These results coincided with Van Der Sluis (2002) who mentioned that poor water quality may reduce the efficacy of vaccines and medications administered through water and AL-Mayah et al. (2009) who stated that no immunity would develop if the water contained a substance that inactivates live vaccine virus. One of the most important reasons for

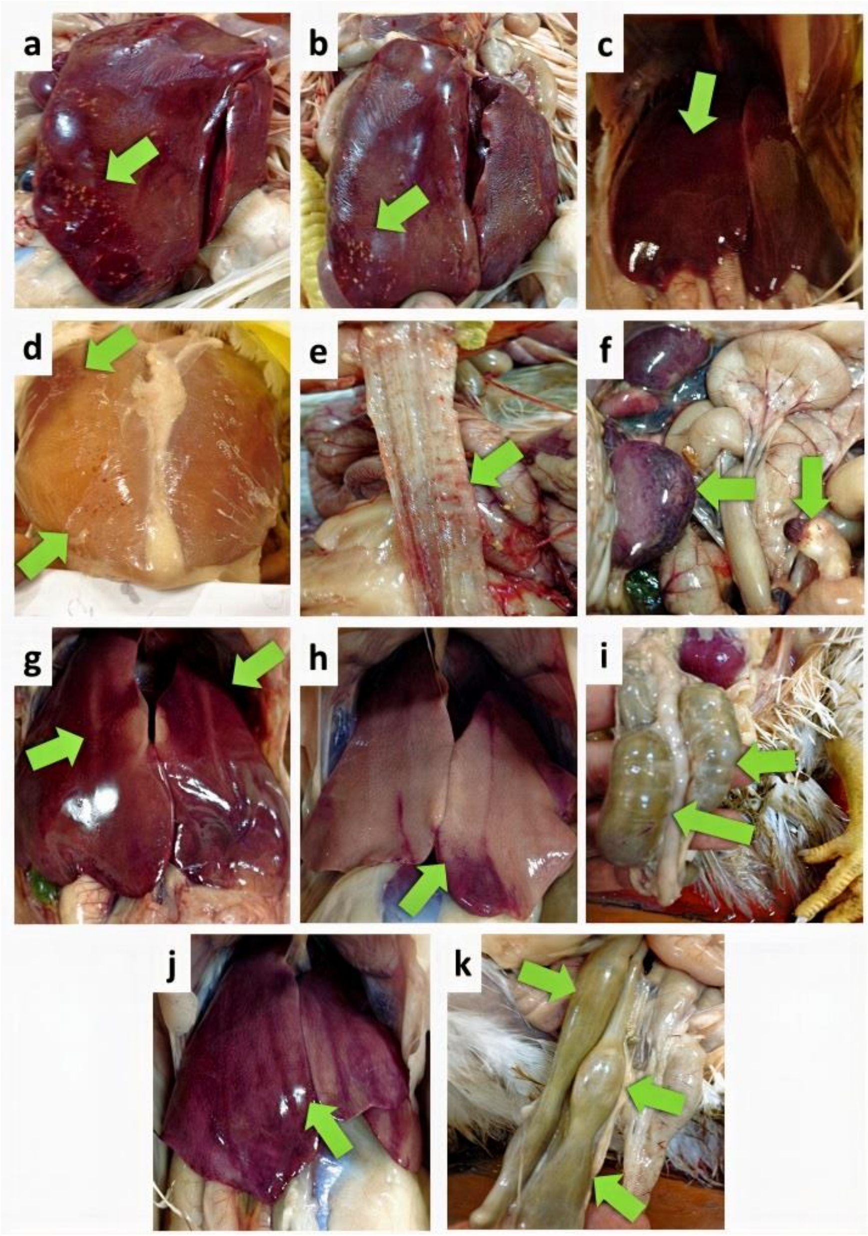

Fig. 4: PM lesions of the dead birds. T1 at 27 days old: Liver congested, friable and has petechial hemorrhage (a, b, c), petechial hemorrhage and congestion in breast muscles (d), hemorrhagic rings in the rectum (e). T2 at 27 days old: Congested mottled spleen and unabsorbed yolk sac (f), congested liver with white streaks (g). T1 at 40 days old: Pale fatty liver with hemorrhagic areas (h), ballooning of the 2 Ceci (i). T2 at 40 days old: Congested liver with hemorrhagic streaks(j), ballooning of the 2 Ceci (k).

drinking water vaccination failure and NDV outbreaks is bad quality drinking water that is considered a bad diluent for live vaccines.

However, T3 showed higher NDV titers than T1 and T2 which indicated the negative effect of organic matters (COD) on antibody titers post vaccines as well on the residual MDAs. Organic matter content affects overall broiler performance (Wernicke and Dott 1987) and increases coliform bacterial growth (Joret et al. 1991) which negatively affects cumulative birds' performance (Zimmermann and Douglass 1998).

Good quality TW in T5 showed improved titers levels, but T6 revealed the best titer levels due to the expected immunostimulant effects of Herbal medicine additive (IMU FORT®) which has oregano oils (thymol and carvacrol) that have positive impacts on both humoral immunity and lymphoid organs (Awaad et al. 2010; Adaszyńska-Skwirzyńska and Szczerbińska 2017). It acts along with lectin as a TLR agonist (St Paul et al. 2013). Antioxidants supply at the time of vaccinations also non-specifically augments immune response (Khaleghi Miran et al. 2010).

On the 17th and 27th days, birds got a booster dose of NDV vaccine (Clone 30) in DW. NDV antibody titers have no significant difference between groups on 28th and 35th but the difference returned to be significant on the 42nd day (Table 3). Birds were probably going through a period of adaptation till reached the 42nd day where T6 was significantly ($P \leq 0.05$) higher than T1, T3, T4 (test statistics: -11.4, -14.4, -11.4, respectively), also T5 was significantly ($P \leq 0.05$) higher than T3 (test statistics: -12). This finding was in accordance with Van Der Sluis (2002), AL-Mayah et al. (2009) and Suarez et al. (2019). High levels of certain chemicals in DW lead to changes in birds' behavior and performance, via decreased body weight and feed intake through the prevention of nutrients absorption from feed ingredients (ElSaidy et al. 2015). Broiler performance and feed conversion efficiency are closely related to the amount and type of gut microbial load, the morphological structure of the intestinal wall, and the immune system response which is affected by DW quality (Huyghebaert et al. 2010).

Increasing relative immune organ weight is considered an indication of immunological advances (Katanbaf et al. 1989). Assessing bursa weight and the bursa/body weight ratio (organ somatic indices or OSI) gave the most consistent and reliable indication of stress (Vahdatpour et al. 2009). The highest OSI was recorded in T5 and T6 which indicated the negative effect of COD and TAN levels on OSI in the other groups (Fig. 3). This finding is in agreement with Atef et al. (1991) who reported a decrease in bursa weights as a result of high nitrate or pathogenic bacteria challenges in poultry DW. On contrary, Graczyk (1993) figured out that COD in water didn't affect significantly bird's performance and organs somatic indices. Results showed a significant difference ($P \leq 0.05$) in OSI between groups as high COD and TAN in DW significantly decrease OSI (spleen, bursa, and thymus). However, Jenkins (2008) recorded that high ammonia in poultry DW is tolerated without effect on performance, body weight, and OSI.

Water Contaminants appear to affect internal organs (liver, intestine, and spleen) and breast muscles leading to the development of PM lesions (Fig. 4). These lesions appear to be linked with COD contamination of DW in T1 and T2 groups but need a further histopathological examination to examine the cellular changes.

Conclusion

Drinking water (DW) is a good route to administer live vaccines in poultry but is prone to water quality parameters' negative effects that must be considered and treated. Organic matters (COD) and total ammonia (TAN) are important DW parameters that affect the immune response, antibody titer curve, and lymphoid organs' somatic indices. COD has cumulative effects on poultry performance and immunity which lead to some pathological changes in critical organs.

Conflict of Interest

The authors of the current work declared no conflict of interest

Author Contributions

ZAMA, HMFEM, MAM, OA, ZEMA contributed to the conception, design, resources, and drafting of the manuscript, MAK and FM contributed to sample collection, lab analysis, produced data, and drafting of the manuscript, MAK and MAKh contributed to the designing of the study as well as analysis and interpretation of the data. All authors contributed to the final editing and approval of the manuscript.

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