Physiological and Immunological Effects of Bacterially Contaminated Tigris River Water on Broiler Chickens

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Abstract

Water quality is important in chicken production as poor quality water may affect the health, productivity and immunity of the birds. The study examined the physiological and immunological effects of Tigris River bacterial contamination on chickens. In present study forty healthy Broiler Chickens were randomly divided into two equal groups. The control group was given purified water whereas the treatment group was given untreated water. Water samples were tested for total bacterial count, total coliforms, faecal coliforms and pathogenic bacteria. At the end of the trial blood samples were collected for hematological, biochemical and immunological tests.

The results revealed that Tigris River water was contaminated with Escherichia coli and Salmonella species. Birds drinking the contaminated water showed a significant decrease in red blood cells, haemoglobin and packed cell volume. However, white blood cells increased significantly compared to the control group. Also hepatic and renal stress was indicated by higher levels of AST and ALT and of urea and creatinine in the treated birds. Immunological testing revealed an increased heterophil count, a decreased lymphocyte count, an increased heterophil-lymphocyte ratio, and elevated IgG and IgM levels, all indicative of immune activation and physiological stress.

The study suggested that the bacterially contaminated river water could damage the physiological balance and immunity of Broiler Chickens. Improve poultry health, reduce disease risk and promote sustainable poultry production. This can be done through regular monitoring and treatment of drinking water.

Key words**:** Tigris river**,** poultry**,** water quality, bacterial contamination, physiological stress, immune response, hematological parameters.

Introduction

Water is a key ingredient in poultry production as it plays a key role in maintaining physiological balance, metabolic activities and immunological competence[1]. The quality of the feed must be adequate, but it is equally important that the quality of the water is adequate, as polluted water can be a vehicle for pathogenic microbes and poisonous compounds, and thus have a deleterious effect on bird health and production. Birds under intensive poultry system are more vulnerable to the infections by the pollutants carried by water because they drink a large amount of water daily[2].

Water pollution has become a major environmental issue worldwide mainly due to the rapid pace of industrialization, agricultural runoff and poor waste management [3]. Surface waters, in particular rivers, usually contain a mixture of pollutants, including organic matter, heavy metals and microbiological agents. Bacterial contamination is a major threat to animal and public health because it facilitates the transmission of harmful microorganisms through drinking water supplies [4].

The Tigris River is one of the most important water resources in Iraq and the surrounding area. It supports operations in agriculture, industry and domestic life. Much of the high level of bacterial pollution, including fecal coliform bacteria and other harmful germs, comes from the discharge of raw sewage, agricultural runoff and animal waste [5].

Bacterial pollution of water is related to a number of serious physiological and immunological effects in chickens. In the case of pathogenic bacteria, the integrity of the gut may be compromised, gut microbiota may be altered and nutrient absorption may be impaired [6]. In addition, the use of polluted water can lead to immunological responses such as modifications in leukocyte profiles, cytokine production, and antibody levels [7]. Such changes may lead to reduced growth performance, higher disease susceptibility and economic losses in chicken production [8].

In addition to the direct pathogenic effects, the birds drinking polluted water may be affected by oxidative stress and metabolic abnormalities . Such a stress may affect the activity of the liver and the kidneys , hematological markers and the general physiological balance . The effect of water quality on the health of chickens should be evaluated by physiological and immunological indicators. This is because immune function and environmental stressors interact [9].

There is an increasing number of studies concerning water pollution and poultry health, however, few studies have discussed the combined physiological and immunological consequences of the bacterially polluted river water especially from Tigris River. Information on integrated biological responses of domestic poultry is limited, with most existing research focusing on general water quality assessments or on individual health markers. Therefore, the objective of this investigation was to assess the physiological and immunological impact of drinking water contaminated with bacteria of Tigris River on domestic chickens. The present study aims to evaluate comprehensively the health indicators and contribute to the development of strategies for improvement of water quality management in chicken production systems.

Materials and Methods

Study Area and Water Sampling

Water samples were obtained from three different locations along the Tigris River in Wasit Province, Iraq. The sites were chosen to represent varying degrees of anthropogenic impact, from the vicinity of the discharge of domestic sewage, to outlets of agricultural drainage, to less polluted portions of the river. Nine water samples were obtained by taking three replicate samples from each location in sterile glass bottles. Samples were collected in early morning and transported to the laboratory in insulated ice boxes and processed for bacteriological analysis on the day of collection.

Experimental Birds

The present study was conducted on forty healthy female Broiler Chickens of 3 weeks of age. The birds were obtained from a local commercial poultry farm in Wasit Province, Iraq. The birds had an average body weight of approximately 750 ± 50 g. The birds were clinically examined before the start of the experiment to ensure that they were free from any apparent signs of disease. All birds were reared under standard management conditions with adequate ventilation, controlled ambient temperature and uniform lighting schedule. During the experimental period, a commercial poultry diet was supplied ad libitum .

Experimental Design

The birds were randomly divided into two equal experimental groups (n = 20 birds per group). The control group was fed clean drinking water of poultry consumption and the treated group was fed untreated water collected from the Tigris River. The experiment went on for 30 consecutive days. The birds were monitored daily for feed intake, water consumption, body weight changes, general health status and any clinical or behavioural abnormalities during the study.

Bacteriological Examination of Water

Water samples were analyzed using conventional microbiological methodology for the determination of the level of bacterial contamination. Total bacterial count, total coliform and fecal coliform numbers were determined by specific culture media and serial dilution techniques. The use of biochemical and morphological properties were also applied to isolate and identify common harmful bacteria. The bacteria consisted of strains of Escherichia coli and Salmonella . Results were expressed as CFU/mL (colony-forming units/mL).

Physiological Measurements

When the experiment was over, blood samples were taken from the veins in the wings of birds in both groups, using sterile syringes. Hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb) and packed cell volume (PCV) were examined with the use of commonly employed laboratory techniques. During the biochemical analysis, the serum was separated to determine liver function markers like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and renal function indicators like urea and creatinine.

Immunological Assessment

The immune status of the birds was evaluated and analyzed by using different immunological markers. Differential leukocyte counts were used to determine the percentages of heterophils and lymphocytes and the ratio of heterophils to lymphocytes was calculated using these counts. The amounts of serum immunoglobulin (IgG and IgM) were measured as an indicator of the humoral immune response. These criteria were used to study the effect of bacterially polluted water on immunological function.

Statistical Analysis

The analysis of all the data collected from the experiment was performed using the required statistical software. Data are expressed as mean ± standard error. The analysis of variance (ANOVA) and Student's t-test were applied to identify the differences that existed between the control and treatment groups when it was required. A significance level of $P < 0.05$ was considered as statistically significant.

Results

Bacteriological Analysis of Water Samples

The bacteriological analysis of the drinking water showed obvious differences between the control water and the Tigris River water that was untreated and used in the treated group. The total bacterial count, total coliform count and fecal coliform count of the control water were 1.2×10^2 CFU/mL, 8.5×10^1 CFU/mL and 2.0×10^1 CFU/mL respectively indicating

a very low level of microbial contamination. In contrast, the water of Tigris River exhibited much higher degree of bacterial contamination with total bacterial count of 4.8×10^5 CFU/mL, total coliform count of 3.6×10^4 CFU/mL and fecal coliform count of 1.9×10^4 CFU/mL.

Microbiological isolation also provided further confirmation of the presence of dangerous bacteria, such as Escherichia coli and Salmonella species. However, these pathogens were not detected in the water samples used for the control group. These data suggest that the untreated river water was highly polluted and presented a serious microbiological risk to the birds that consumed it.

Table 1. Bacteriological Analysis of Water Samples

Parameter	Control Water	Tigris River Water
Total bacterial count (CFU/mL)	1.2×10^2	4.8×10^5
Total coliform (CFU/mL)	8.5×10^1	3.6×10^4
Fecal coliform (CFU/mL)	2.0×10^1	1.9×10^4
Microbiological isolation	Negative	*E. coli* & *Salmonella* spp.

Hematological Parameters

The hematological data showed that there were significant physiological changes in birds exposed to Tigris River water polluted with bacteria when compared to the control group. The count of red blood cells (RBC) was found to be considerably lower in the treatment group ($2.71 \pm 0.11 \times 10^6$/µL) compared to the control group ($3.25 \pm 0.14 \times 10$□/µL) with a statistically significant difference ($P < 0.05$). The concentration of hemoglobin was 11.8 ± 0.5 g/dL in control birds and 9.9 ± 0.4 g/dL in treated birds ($P < 0.05$). This means a significant decrease in hemoglobin concentration.

The packed cell volume (PCV) of birds that consumed polluted water was also significantly decreased to 29.2 ± 1.1 %. In comparison, the control group exhibited $34.6 \pm 1.3\%$ ($P < 0.05$). Such decreases indicate the development of moderate anemia and physiological stress, both of which are associated with prolonged exposure to microbial pollutants.

However, it is worth noting that the white blood cell (WBC) count showed a remarkable increase in the treatment group ($28.9 \pm 1.5 \times 10^3$/µL) compared to the control group ($21.4 \pm 1.2 \times 103$/µL) ($P < 0.05$). This increase is an expression of the immunological activation and inflammatory response triggered by bacteria exposure.

Table 2. Hematological Parameters of Broiler Chickens

Parameter	Control Group (n = 20)	Treated Group (n = 20)	P-value
RBC ($\times 10^6$/µL)	3.25 ± 0.14	2.71 ± 0.11	<0.05
WBC ($\times 10^3$/µL)	21.4 ± 1.2	28.9 ± 1.5	<0.05
Hemoglobin (g/dL)	11.8 ± 0.5	9.9 ± 0.4	<0.05
PCV (%)	34.6 ± 1.3	29.2 ± 1.1	<0.05

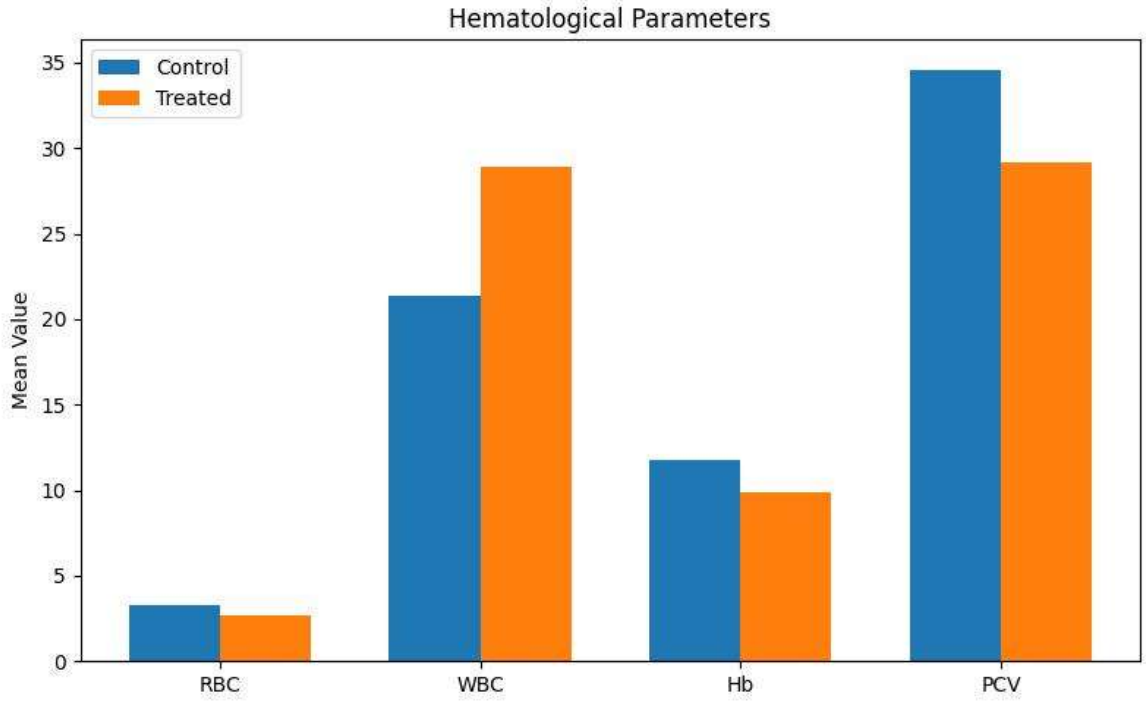

Figure (1) Hematological Parameters of Broiler Chickens

Biochemical Parameters

Serum biochemical examination revealed that birds exposed to polluted river water had significant changes in liver and kidney function indicators. The activity of aspartate aminotransferase (AST) in the treatment group was significantly higher than the control

group. The activity of AST increased significantly from 145.2 ± 6.3 U/L in the control group to 198.7 ± 8.1 U/L in the treatment group ($P < 0.05$). Similarly, alanine aminotransferase (ALT) levels were increased from 18.4 ± 1.1 U/L to 29.6 ± 1.7 U/L ($P < 0.05$).

Elevated liver enzymes indicate stress on the liver and the possibility of liver tissue damage, both of which are associated with long term exposure to bacteria-laden water.

In addition, there were marked differences in indices of kidney function. The serum urea concentration was increased from 21.6 ± 1.4 mg/dL in the control group to 34.8 ± 2.0 mg/dL in the treatment group and the difference was statistically significant ($P < 0.05$). Likewise, creatinine levels were significantly elevated from 0.72 ± 0.05 mg/dL to 1.18 ± 0.08 mg/dL ($P < 0.05$). Based on the data it looks as if poor renal function and metabolic abnormalities are caused by persistent microbial stress.

Table 3. Biochemical Parameters of Liver and Kidney Functions

Parameter	Control Group (n = 20)	Treated Group (n = 20)	P-value
AST (U/L)	145.2 ± 6.3	198.7 ± 8.1	<0.05
ALT (U/L)	18.4 ± 1.1	29.6 ± 1.7	<0.05
Urea (mg/dL)	21.6 ± 1.4	34.8 ± 2.0	<0.05
Creatinine (mg/dL)	0.72 ± 0.05	1.18 ± 0.08	<0.05

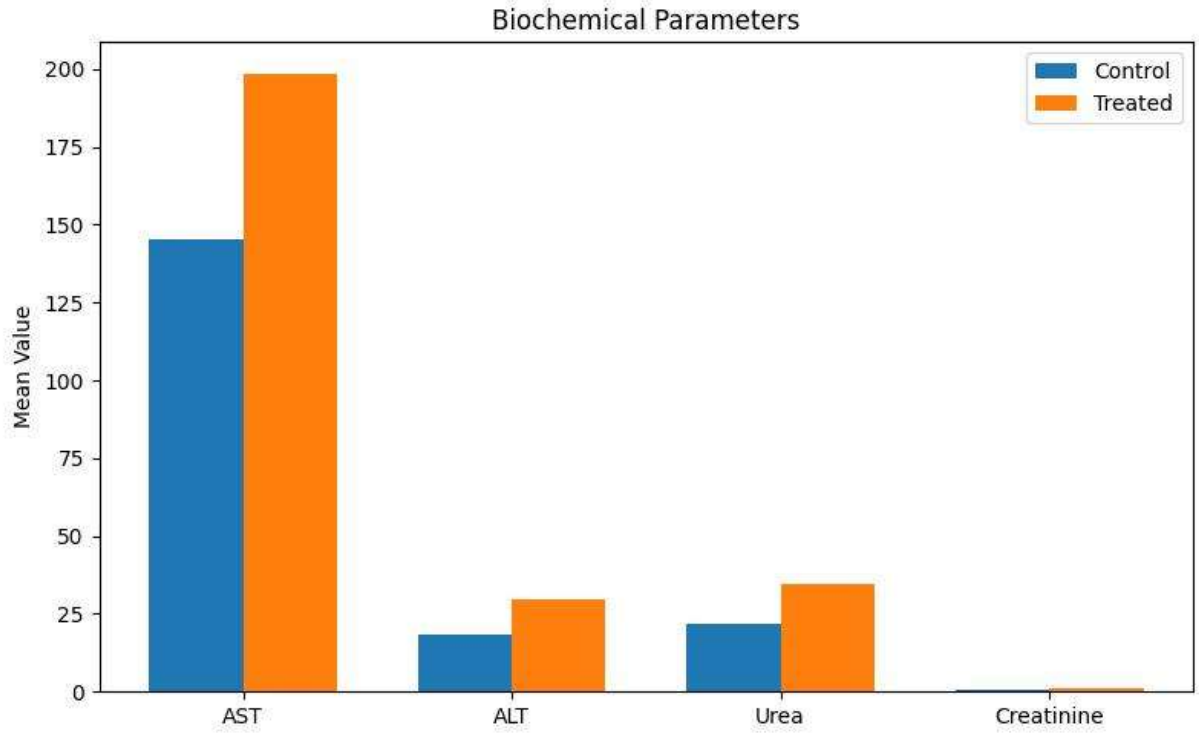

Figure (2) Biochemical Parameters of Poultry Birds

Immunological Parameters

Immunological studies were carried out on birds fed with contaminated water from the Tigris river and significant alterations were observed in immune response markers. The percentage of heterophils in the treated group was significantly higher ($P < 0.05$) than that in the control group (34.5 ± 2.1%) with the treated group (48.7 ± 2.8%). The lymphocyte percentage, on the other hand, decreased considerably, from 58.2 ± 2.6% in the control birds to 42.6 ± 2.3% in the treated birds ($P < 0.05$: statistical significance).

As a result, the ratio of heterophils to lymphocytes (H/L) was significantly higher in the treatment group (1.14 ± 0.07) than in the control group (0.59 ± 0.04) ($P < 0.05$), which suggests that the levels of physiological stress and immune system activation were increased.

The serum immunoglobulin analysis also showed marked changes. The immunoglobulin G (IgG) levels were elevated in the treatment group compared to the control group (973 ± 42 mg/dL in the treated group vs 842 ± 35 mg/dL in the control group, $P < 0.05$). Similarly, immunoglobulin M (IgM) levels increased from 176 ± 11 mg/dL to 214 ± 13 mg/dL ($P < 0.05$).

Table 4. Immunological Parameters of Poultry Birds

Parameter	Control Group (n = 20)	Treated Group (n = 20)	P-value
Heterophils (%)	34.5 ± 2.1	48.7 ± 2.8	<0.05
Lymphocytes (%)	58.2 ± 2.6	42.6 ± 2.3	<0.05
H/L Ratio	0.59 ± 0.04	1.14 ± 0.07	<0.05
IgG (mg/dL)	842 ± 35	973 ± 42	<0.05
IgM (mg/dL)	176 ± 11	214 ± 13	<0.05

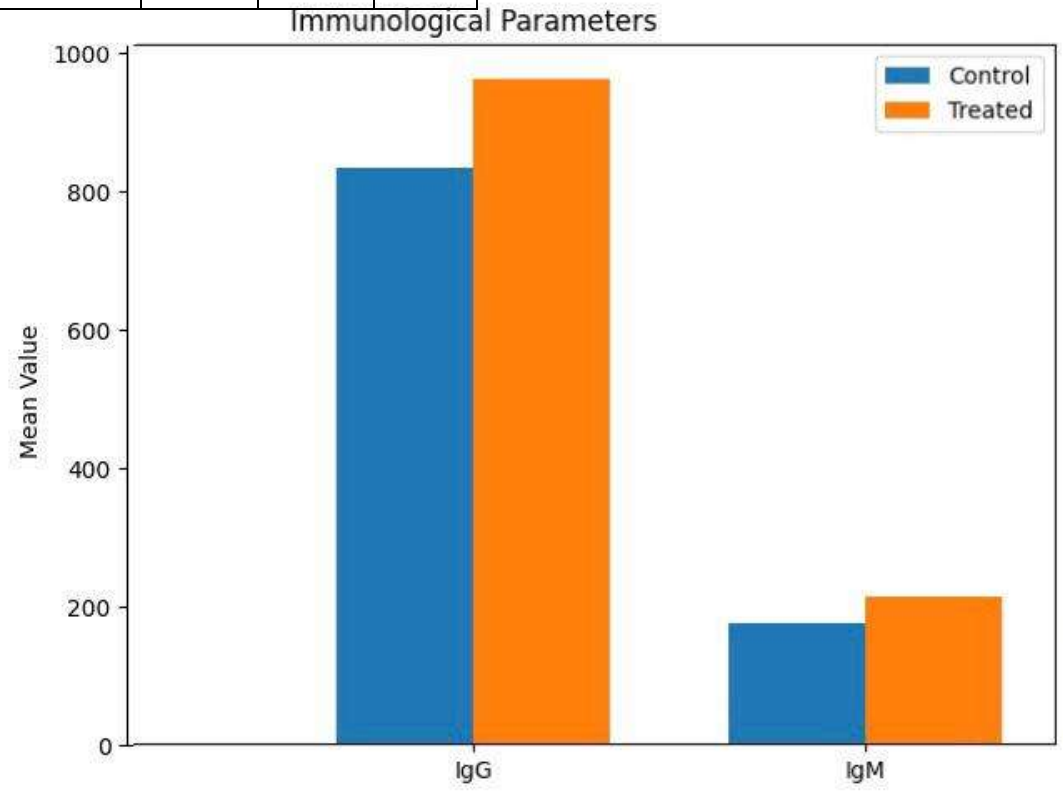

Figure (3) Immunological Parameters of Broiler Chickens

Clinical and General Health Observations

During the trial, the birds in the treatment group showed a noticeable decrease in both their activity level and the amount of feed they consumed compared to the birds in the control group. Mild digestive abnormalities including moist droppings and reduced appetite were commonly observed.

No significant mortality was recorded during the trial, but it was clear that the birds that had been given water from the Tigris River that had not been treated had a lower general vitality. These clinical observations were in substantial accord with those of the laboratory showing the detrimental effects of bacterially contaminated water on the physiological and immunological health of the domestic chicken.

Discussion

The data presented here suggest that the use of untreated Tigris River water by domestic poultry resulted in some physiological and immunological changes. This would imply that these results are associated with the microbiological quality of the water, especially the elevated levels of bacterial contamination observed in the present investigation. Similar results have been found in recent work emphasizing the role of water as a direct route of pathogen exposure in poultry systems [7]. Investigations have been conducted in recent years.

The high bacterial load found in the river water including the presence of Escherichia coli and Salmonella species is a reflection of the effect of environmental pollution sources such as sewage discharge and agricultural runoff. This type of contamination is recognized as a contributor to the transmission of diseases and the development of subclinical illnesses in chicken production-related environments [4].

The presence of reduced numbers of red blood cells, hemoglobin concentration and packed cell volume in the treated birds indicates the establishment of physiological stress and impairment of oxygen transport during treatment.

However, the significant increase in the number of white blood cells observed in the treated group indicates the activation of the immune system. This reaction indicates that the immune system is constantly challenged by microbial exposure [10]. Biochemical detoxification analysis showed increased levels of liver enzymes (AST and ALT), which indicated that the liver was under stress due to processes and metabolic load [11]. Also, Ali, and Karim (2026) found elevated kidney function indicators, such as urea and creatinine, which suggests that prolonged exposure to pollutants may be linked to the development of renal impairment [12].

Immunological data reflected physiological stress and immune activation, with increased heterophil to lymphocyte ratio [8]. Repeated exposure to bacterial antigens leads to an increase in immunoglobulin levels (both IgG and IgM), which indicates stimulation of the humoral immune response [11].

In general, the findings suggest that the health and productivity of poultry can be influenced by untreated polluted water, highlighting the importance of devising and implementing effective water quality management strategies [7].

Conclusions

The present study has demonstrated that drinking water of the Tigris river contaminated with bacteria had a significant and adverse effect on the physiological and immunological state of the domestic chicken. Bacteriological examination revealed the river water to be heavily contaminated with microbes. The levels included total coliforms, fecal coliforms and presence of dangerous bacteria like Escherichia coli and Salmonella spp. ...a persistent source of biological stress for the birds.

Birds exposed to polluted water had significant reduction in red blood cells, hemoglobin concentration and packed cell volume. which means that the physiologically stress in birds were developing moderate anemia. The concomitant increase in white blood cells indicated a strong immunological response to the chronic bacterial exposure. These hematological alterations confirmed that polluted water has a direct impact on the overall health status of poultry. Also the biochemical analysis results showed significant disturbances of liver and kidney activities.

From the immunological perspective, the considerable increase in heterophil proportion, decrease in percentage of lymphocytes and increased heterophil to lymphocyte ratio were clear indicators of stress and immune system activation. The increase in IgG and IgM levels further demonstrated the continuous activation of the humoral immune system caused by the bacterial challenge. The immune system, when activated for a long time, can reduce resistance to subsequent infections.

Untreated river water, not routinely monitored for bacteriology, may lead to hidden economic losses. These losses may show up as poor growth performance, increased susceptibility to disease, and reduced agricultural efficiency.

Thus, it is highly recommended to monitor the water quality regularly, carry out regular bacteriological testing and use appropriate purification measures before using river water in poultry farms. Improvement of water hygiene should be considered as an important part of disease control techniques and sustainable chicken production systems.

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