Original study

Impact of chlorine dioxide as water acidifying agent on the performance, ileal microflora and intestinal histology in quails

Asad Sultan, Irshad Ullah, Sarzamin Khan, Rifat Ullah Khan and Zahoor ul Hassan

Faculty of Animal Husbandry & Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

Abstract

The present study was planned to investigate the effect of different levels of chlorine dioxide on the performance, gut microbiota and intestinal histology of quails. For this purpose, 300 dayold healthy quail chicks were randomly placed in 20 specially designed pens (15 birds/pen) with water troughs containing chlorine dioxide at the level of 0.00 (DW-0.00), 0.3 (DW-0.3), 0.4 (DW-0.4) and 0.5 ppm (DW-0.5) in replicated fashion (5 replicate/treatment) for 28 days. Weight gain, feed conversion ratio and dressing percentage increased significantly (*P*<0.05) in DW-0.5 group. Similarly, liver, gizzard and heart weight increased significantly in treated groups linearly with increasing levels of treatment at day 21 and 28. The results showed that population of *Salmonella* and *E. coli* decreased linearly at day 21 and 28 of age. Villus height and goblet cells at day 21 and 28 were significantly higher in DW-0.5 group. The results of the present study suggested that the treatment of chlorine dioxide linearly increased the performance and gut morphology and decreased microbial population in quails.

Keywords: quails, chlorine dioxide, performance, Salmonella, E. coli, gut morphology

Abbreviations: CD: chlorine dioxide, DW-0: control, DW-0.3: 0.3 ppm chlorine dioxide, DW-4: 0.4 ppm chlorine dioxide, DW-5: 0.5 ppm chlorine dioxide

Archiv Tierzucht 57 (2014) 31, 1-9 doi: 10.7482/0003-9438-57-031

Corresponding author: Rifat Ullah Khan; rifatullahkhhan@gmail.com KPK Agricultural University Peshawar, Pakistan Received: 22 April 2014 Accepted: 27 August 2014 Online: 14 November 2014

© 2014 by the authors; licensee Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. This is an Open Access article distributed under the terms and conditions of the Creative Commons Attribution 3.0 License (http://creativecommons.org/licenses/by/3.0/).

Introduction

Water is one of the most important and essential nutrients in poultry production. Requirement of water is about two times greater than feed under optimum environmental conditions. Onset of mortality is possible if birds remain deprived of water even for a short period of time compared to feed deprivation (Watkins & Tabler 2009). Water acts as a solvent in various biological processes e.g. assimilation, digestion and absorption and regulation of body temperature. Water also provides the essential medium for almost all necessary chemical reactions required for the formation of useful products like eggs and meat. It also helps in the process of secretion, excretion and lubrication (Koelkebeck *et al.* 1993). Any deviation of standards in water quality may ultimately affect health status and production of poultry birds (Zimmermann & Douglass 1998).

Water is supplied in ample amounts in commercial poultry farms without giving any proper consideration to water quality (Narkis *et al.* 1995). Many management factors, when disturbed in a poultry production system, can compromise bird performance but this condition could be more devastated with bad water quality (Leung *et al.* 2007). Physical, chemical and biological properties of water should be considered seriously in any poultry operation. Transmission of viral, protozoal and bacterial diseases increases with contaminated drinking water (Hinton *et al.* 1990). Poor quality of drinking water provides a favourable environment for the growth of harmful pathogens (Andrews *et al.* 1995). Vaccines, vitamins and other medicines were effective and readily absorbed by the body with good quality of water (Zimmerman & Hilton 1995).

Presence of organic materials in drinking water promotes the growth of bacteria, especially when poultry droppings are accidentally get access to the drinking water system (Blake & Hess 2001). There were various other factors that can affect the water quality, including water source. The biofilm formed on the surface of water acts as a nest for microbes especially bacteria (Hancock *et al.* 2007). Water-born diseases in broiler production can be prevented by the protection of supply sources and water disinfection (Belluati *et al.* 2007). Moreover, factors of quality control like chemical, physical and microbiological characteristics are essential to prevent disease occurrence (Belluati *et al.* 2007). Water is a fundamental nutrient for avian, therefore its quality preservation is of prime importance to have a good flock performance (Wabeck *et al.* 1994).

Different methods can be used to eliminate or at least to minimize the impurities in drinking water. Various kinds of antibiotics, organic acids and sanitizers were used in the treatment of bad quality water to avoid its hazardous effects (Belluati *et al.* 2007). The use of these and other chemicals in huge quantity for water treatment can affect bird's performance negatively and is proven to be expensive (Gagnon *et al.* 2005). In broiler production, one of the efficient control strategies is the addition of acids or chlorine to drinking water (Tian *et al.* 2010).

Chlorine dioxide (CD) is a yellowish green oxidizing agent, which is one of the potential disinfectants among those unveiled earlier in this century (Clark *et al.* 2003). The use of CD for the purification and disinfection of drinking water has been approved by the United States Environmental Protection Agency (EPA) along with other application (Ji *et al.* 2008). Chlorine dioxide is becoming increasingly used for water purification and called the »ideal« biocide because of its unique combination properties (Vicuña-Reyes *et al.* 2008). At extremely low dosages, it rapidly kills pathogenic microbes, reduces the formation of biofilms with no bad taste, odour and taint problems. Moreover, it is economical and environmentally friendly

(Korn *et al.* 2002). It has the potential to kill different types of spores (Veschetti *et al.* 2002), yeasts, moulds and viruses (Bichai & Barbeau 2008).

To the best of our knowledge, reports dealing with the effect of CD on the production of quails are not available in the literature. Therefore, we planned to investigate the effect of CD on performance traits, *Salmonella* and *E. coli* population and gut morphology in Japanese quails.

Material and methods

Prior permission was obtained from the Ethical Committee on care and welfare of animals, Faculty of Animal Husbandry & Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan to conduct this study.

Quail husbandry and experimental design

Two hundred and forty day-old healthy quail chicks were purchased from a commercial supplier of poultry birds at District Peshawar, Pakistan. These birds were randomly placed in 20 specially designed pens and were allotted to four water mixed CD treatments at the rate of 0.00 (DW-0), 0.3 (DW-0.3), 0.4 (DW-0.4) and 0.5 ppm (DW-0.5) in replicated fashion (5 replicates/treatment) 15 quail chicks each. Quail birds were given optimal environmental conditions with standard managemental practices (Table 1).

Ingredients, %	Starter	Grower	Finisher
Maize	35.40	32.9	25.0
Soybean meal	27.50	20.5	17.0
Sunflower meal	11.0	15.1	11.05
Wheat	9.90	13.05	33.1
Wheat bran	-	3.70	-
Meat-bone meal	6.50	5.55	4.95
Vegetable oil	7.39	8.55	7.35
Limestone	1.35	-	-
Mineral-vitamins premix*	0.35	0.31	0.35
Sodium chloride	0.31	0.25	0.25
L-lysine	0.04	-	0.01
DL-Methionine	0.16	0.01	0.17
Calculated chemical composition, per kg of diet	**		
ME, MJ	13.2	13.4	13.4
Crude Protein, g	231.2	212.0	189.8
Calcium, g	15.0	9.0	8.0
Available phosphorus, g	5.0	4.7	3.9
Lysine, g	12.0	10.0	8.5
Methionine, g	5.6	4.0	5.2
Methionine + cystine, g	9.3	7.6	8.4
Sodium chloride, g	3.4	2.9	2.9

Table 1 Ingredients and composition of basal diet

*provides per kg of diet: Mn 80 mg; Zn 60 mg; Fe 60 mg; Cu 5 mg; Co 0.2 mg; I 1 mg; Se 0.15 mg; choline chloride 200 mg; vitamin A 12 000 IU; vitamin D3 2 400 IU; vitamin E 50 mg; vitamin K3 4 mg; vitamin B1 3 mg; vitamin B2 6 mg; niacin 25 mg; calcium-d- pantothenate 10 mg; vitamin B6 5 mg; vitamin B12 0.03 mg; d-biotin 0.05 mg; folic acid 1 mg, **calculated from NRC values (1994)

Chlorine dioxide was offered to the birds in three different treatment groups at the dose rate of 0.3 ppm, 0.4 ppm, and 0.5 ppm for 28 days. Chlorine dioxide was obtained from the Duka Production Ltd (Ommen, The Netherlands). Four grams of CD tablet were dissolved in one litre of drinking water to obtain 2 000 ppm stock solution. The stock solution was kept in dark to protect from direct sunlight. From the stock solution, 0.14, 0.17 and 0.2 ml per litre of drinking water were used to obtain the required concentration of 0.3, 0.4 and 0.5 ppm of CD in drinking water respectively.

Performance traits

The birds were offered a weighed amount of feed daily from which refused feed was subtracted to get daily feed intake. Daily feed intake was used to work out the weekly feed intake. Weekly weight gains were calculated by subtracting initial weight from final weight of every week. Weekly feed conversion ratio was calculated by dividing weekly feed consumed by weekly weight gain. Dressed weight of body after slaughtering was determined and expressed in term of percentage of whole body weight. Visceral organs i.e., liver, heart, spleen were weighed separately at the end of experiment.

Lower ileal micro flora

At day 21 and 28, four birds were randomly selected from each replicate and were slaughtered to collect the sample from lower part of intestine, aseptically. The samples were transported at 4°C to the Laboratory of Microbiology, Department of Animal Health, University of Agriculture, Peshawar, Pakistan. A loop full of broth was streaked on plates of Salmonella-Shigella agar (Oxoid, Basingstoke, UK). The plates were incubated at 37 °C for 24 h. Suspected colonies of *Salmonella* and *E. coli* from each plate were collected for presumptive identification based on their morphological characteristics and various biochemical tests that included catalase, oxidase, indole and methyl red test. The bacteria were counted on bacterial counting machine. The bacterial population was measured as log10 cfu/g.

Gut morphology

Four birds per replicate on day 21 and 28 were randomly selected and intestine was removed. One cm segment of the midpoint of the duodenum, jejunum and ileum were removed, placed in physiological saline solution and fixed in 10% buffered-formalin. Each segment was then embedded in paraffin and a 2 μ m section of each sample was placed on a glass slide and stained with haematoxylin and eosin for examination (Sakamoto *et al.* 2000). Histological sections were examined with a microscope. Villus height was measured from the top of the villus to the top of the lamina propria. Fifteen measurements were taken per bird for this variable for purpose of statistical analysis.

Data analysis

Data generated from field and lab work were statistically analysed using standard procedure of the analysis of variance (ANOVA) in a completely randomized design (CRD) as suggested by Steel & Torrie (1980). *P*-value less than 0.05 was considered as statistically significant.

Results

Feed intake, weight gain, feed conversion ratio and dressing percentage are given in Table 2. It is obvious that feed intake was not significant, although numerically highest feed intake was recorded in control group. Weight gain, feed conversion ratio and dressing percentage increased significantly (P<0.05) in DW-0.5 group. The results also indicated that these parameters increased linearly with increasing the level of CD level. Weight of liver, gizzard and heart are given in Table 3. Similarly, liver, gizzard and heart weight increased significantly (P<0.05) in treated groups linearly with increasing levels of treatment at day 28. The highest weight gain in liver, gizzard and heart were observed in DW-0.5 group.

Salmonella and E. coli population are given in Table 4 and 5 respectively. The results showed that that the population of Salmonella and E. coli decreased linearly (P<0.05) at day 21 and 28 of age. The least populations of both bacteria were recorded in the group having a CD level of 0.5 ppm. The Villus height of control and treated groups are given in Table 6. The results showed that villus height increased significantly (P<0.05) in DW-0.5 on day 21 and 28. The number of goblet cells at day 21 and 28 were significantly higher (P<0.05) in DW-0.5 group (Table 7).

Table 2

Effect of Dutrion (chlorine dioxide) on feed intake, weight gain, feed conversion ratio and dressing percentage of Japanese quails

Groups	Feed intake, g	Weight gain, g	Feed conversion ratio, g/g	Dressing percentage
DW-0	68.01	17.25 ^d	3.93ª	59.09 ^d
DW-0.3	67.98	17.85°	3.80 ^b	59.67°
DW-0.4	67.93	18.14 ^b	3.74 ^c	59.9 ^b
DW-0.5	67.78	18.6ª	3.63 ^d	60.21ª
SEM	0.08	0.05	0.03	0.04
P-value	0.5	0.0001	0.001	0.0001

Means in column carrying different superscripts are significantly different at P<0.05.

Table 3	
Effect of Dutrion (chlorine dioxide) on liver weight, gizzard weight and heart weight at o	day 28

Groups	Liver weight, g	Gizzard weight, g	Heart weight, g
DW-0	3.16 ^b	2.87 ^b	1.16 ^c
DW-0.3	3.29ª	2.99ª	1.23 ^b
DW-0.4	3.31ª	3.01ª	1.28 ^{a b}
DW-0.5	3.33ª	3.03ª	1.31ª
SEM	0.02	0.01	0.02
P-value	0.07	0.01	0.001

Means in column carrying different superscripts are significantly different at P<0.05.

	,	(<i>S</i> , 1	, ,
Groups	Day 0	Day 21	Day 28
DW-0	2.72	2.72ª	2.13ª
DW-0.3	2.80	1.77 ^b	1.62 ^b
DW-0.4	2.72	1.60 ^c	1.45°
DW-0.5	2.75	1.42 ^d	1.28 ^d
SEM	0.03	0.03	0.04
P-value	0.6684	0.0001	0.0001

Table 4

Effect of Dutrion (chlorine dioxide) on total Salmonella count (cfu/g) of quails at different days of age

Means in column carrying different superscripts are significantly different at P<0.05.

Table 5

Effect of Dutrion (chlorine dioxide) on total E. coli count (cfu/g) of quails at different age of life

Groups	Day-0	Day-21	Day-28
DW-0	2.82	2.42ª	2.27ª
DW-0.3	2.85	1.87ª	1.77 ^b
DW-0.4	2.82	1.70°	1.55°
DW-0.5	2.78	1.52 ^d	1.38 ^d
SEM	0.03	0.03	0.05
P-Value	0.82	0.0001	0.0001

Means in column carrying different superscripts are significantly different at P<0.05.

Table 6

Effect of Dutrion (chlorine dioxide) on villus height (µm) of lower ileum of quails at different age of life

Groups	Day-21	Day-28	
DW-0	74.25 ^b	76.25 ^b	
DW-0.3	77.75 ^{ab}	79.25 ^{ab}	
DW-0.4	80.25 ^{ab}	82.25ª	
DW-0.5	81.75°	83.75ª	
SEM	1.23	1.3	
P-value	0.0397	0.0278	

Means in column carrying different superscripts are significantly different at P<0.05.

Table 7

Effect of Dutrion (chlorine dioxide) on goblet cells count per unit area of villus at lower ileum of quail

Groups	Day-21	Day-28	
DW-0	81.25°	84.75°	
DW-0.3	92.00 ^b	100b	
DW-0.4	95.75 ^b	103.50 ^b	
DW-0.5	101.25°	108.75ª	
SEM	1.21	1.23	
P-value	0.0001	0.0001	

Means in column carrying different superscripts are significantly different at P<0.05.

Discussion

In the present study different levels of CD improved weight gain, feed conversion ratio and dressing percentage linearly with increasing level of the treatments. We also found out that by increasing the dose of CD, the pH decreased. Thus it is an alternative to any acidic agent. Studies regarding the use of CD are scarce to compare our results. Desai et al. (2007) reported that combination of formic and propionic acids in the drinking water increased weight gain and improved feed conversion ratio in broilers. The positive effect of treating water with some organic acids on digestion may be due to better absorption of nutrients (van der Sluis 2002). Our result can be correlated to the work of Pesti et al. (2004) who observed that acidified drinking water increased body weight in comparison to normal drinking water given to broilers. The positive effects of CD on birds' performance could be related to its potency to combat harmful microbes and to maintain the gut pH in acid range for activation of digestive enzymes. As a result, the intestinal tract remains healthy and expresses its function fully in terms of more nutrients absorption and assimilation. These findings can be justified by the findings of Chaveerach et al. (2004) who reported that birds offered chemically treated water with organic acids responded better in term of body weight gain. Similarly, Bahnas (2009) also reported on significant increase in body weight gain of Japanese guails by malic acid supplementation.

We found a significant increase in the weight of liver, gizzard and heart in the DW-0.05 group. Results of the present study could be justified by the findings of Islam *et al.* (2008) who reported on a positive effect on liver weight by supplementing various acidifiers in the diet of boilers. Similarly, in line with present outcomes, Islam *et al.* (2008) observed that quail birds, given acid treated water, showed a significant effect on the weight of the visceral organs.

In the present study, the population of *Salmonella* and *E. coli* decreased linearly with the increasing level of CD. Chlorine dioxide is considered as more efficient disinfectant globally over a wide range of pH than other disinfectants and is more potent to inactivate resistant pathogens due to high penetration and absorption of the CD into the bacterial plasma membrane. The mechanism of action of CD is its capability to destroy microbes by increasing bacterial cell membrane permeability where it dissociates into anions and cations upsetting the electron balance inside the bacterial cell and kill it (Huang *et al.* 1997). It may produce acidic conditions that eradicate the coliform of the gastrointestinal tract by lowering the pH, which is unfavourable for the growth of the acid-intolerant species such as *E. coli* and *Salmonella*.

In the present study, villus height and goblet cells increased linearly in a significant fashion in the treated groups compared to control. It was assumed that CD has similar mechanism of action to organic acid reducing bacterial and fungal quantity in gut which improves villus height and helps in better nutrient utilization (Paul *et al.* 2007). The increase in the goblet cell count indicates that gut efficiency to protect itself and to utilize nutrients efficiently was maximized. Goblet cells produce mucus that forms a protective cover on the outer lining of gut (Paul *et al.* 2007). As discussed earlier there was an increase in the villus height with supplementation of CD that reflects increase in the number of goblet cells. Ferket *et al.* (2002) reported that turkeys, when fed substances that enhance and improve gut microbial profile, improved goblet cell number in the gut lumen. Similar findings were reported by Smirnov *et al.* (2005) that coincide with current outcomes. From the present results, we concluded that treating water with chlorine dioxide increased the production performance, improved gut morphology and decreased the intestinal micro flora.

References

- Andrews WH, June GA, Sherrod P (1995) *Salmonella*. In: Bacteriological Analytical Manual. U.S. Food and Drug Administration, 8th ed., AOAC International, Gaithersburg, MD, USA
- Bahnas MS (2009) Effect of using malic acid on performance of Japanese quail fed optimal and sub-optimal energy and protein levels. Egypt Poult Sci 29, 263-286
- Belluati M, Danesi E, Petrucci G, Rosellini M (2007) Chlorine dioxide disinfection technology to avoid bromate formation in desalinated seawater in potable waterworks. Desalination 203, 312-318
- Bichai F, Barbeau B (2006) Assessing the Disinfecting Power of Chlorite in Drinking Water. Water Qual Res J Can 41, 375-382
- Blake JP, Hess JB (2001) Evaluating Water Quality for Poultry. Alabama A & M University and Auburn University, Alabama Cooperative Extension System, Publication ANR-1201, Auburn and Huntsville, AL, USA
- Chaveerach P, Keuzenkamp DA, Lipman LJA, Van Knapen F (2004) Effect of Organic Acids in Drinking Water for Young Broilers on *Campylobacter* Infection, Volatile Fatty Acid Production, Gut Microflora and Histological Cell Changes. Poult Sci 83, 330-334
- Clark RM, Sivaganesan M, Rice EW, Chen J (2003) Development of a Ct equation for the inactivation of *Cryptosporidium* oocysts with chlorine dioxide. Water Res 37, 2773-2783
- Desai DN, Patwardhan DS, Ranade AS (2007) Acidifiers in poultry diets and poultry production. In: Lückstädt C (ed.) Acidifiers in Animal Nutrition: A Guide for Feed Preservation and Acidification to Promote Animal Performance. Nottingham University Press, Nottingham, UK, 63-69
- Ferket PR, Parks CW, Grimes JL (2002) Benefits of dietary antibiotic and mannanoligosaccharide supplementation for poultry. Multi-State Poultry Meeting 2002 http://www.feedinfo.com/files/multi 2002-ferket.pdf [last accessed 27.08.2014]
- Gagnon GA, Rand JL, O'Leary KC, Rygel AC, Chauret C, Andrews RC (2005) Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. Water Res 39, 1809-1817
- Hancock A, Hughes J, Watkins S (2007) In Search of the Ideal Water Line Cleaner. Avian Advice 9, 1-4, University of Arkansas Cooperative Extension Service, Fayetteville, AR, USA http://www.avianadvice.uark.edu/AA%20 PDFs/avianadvice_sp07.pdf [last accessed 27.08.2014]
- Hinton Jr A, Corrier DE, Spates GE, Norman JO, Ziprin RL, Beier RC, DeLoach JR (1990) Biological Control of *Salmonella* typhimurium in Young Chickens. Avian Dis 34, 626-633
- Huang J, Wang L, Ren N, Ma F, Li J (1997) Disinfection effect of chlorine dioxide on bacteria in water. Water Res 31, 607-613
- Islam MZ, Khandaker ZH, Chowdhury SD, Islam KMS (2008) Effect of citric acid and acetic acid on the performance of broilers. J Bangladesh Agril Univ 6, 315-320
- Ji Y, Huang JL, Fu J, Wu MS, Cui CW (2008) Degradation of microcystin-RR in water by chlorine dioxide. J China Univ Mining Technol 18, 623-628
- Koelkebeck KW, Harrison PC, Madindou T (1993) Research Note: Effect of Carbonated Drinking Water on Production Performance and Bone Characteristics of Laying Hens Exposed to High Environmental Temperatures. Poult Sci 72, 1800-1803
- Korn C, Andrews RC, Escobar MD (2002) Development of chlorine dioxide-related by-product models for drinking water treatment. Water Res 36, 330-342
- Leung YHC, Zhang LJ, Chow CK, Tsang CL, NG CF, Wong CK, Guan Y, Pieris JSM (2007) Poultry Drinking Water Used for Avian Influenza Surveillance. Emerg Infect Dis 13, 1380-1382

- Narkis N, Katz A, Orshansky F, Kott Y, Friedland Y (1995) Disinfection of effluents by combinations of chlorine dioxide and chlorine. Water Sci Technol 31, 105-114
- NRC (1994) Nutrient requirements of poultry. 9th rev. ed., National Research Council, National Academy Press, Washington, DC, USA
- Paul SK, Halder G, Mondal MK, Samanta G (2007) Effect of Organic Acid Salt on the Performance and Gut Health of Broiler Chicken. Poult Sci 44, 389-395
- Pesti GM, Bakalli RI, Vendrell PF, Chen HY (2004) Effects of organic acid on control of bacteria growth in drinking water for broilers. Poult Sci 83 (Suppl.), M303
- Sakamoto K, Hiros H, Onizuka A, Hayashi M, Futamura N, Kawamura Y, Ezaki T (2000) Quantitative Study of Changes in Intestinal Morphology and Mucus Gel on Total Parental Nutrition in Rats. J Surg Res 94, 99-106
- Smirnov A, Perez R, Amit-Romach E, Sklan D, Uni Z (2005). Mucin Dynamics and Microbial Populations in Chicken Small Intestine Are Changed by Dietary Probiotic and Antibiotic Growth Promoter Supplementation. J Nutr 135, 187-192
- Steel RGD, Torrie JH (1980) Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed., New York, NJ, USA
- Tian F, Qiang Z, Liu C, Zhang T, Dong B (2010) Kinetics and mechanism for methiocarb degradation by chlorine dioxide in aqueous solution. Chemosphere 79, 646-651
- Van der Sluis W (2002) Water quality is important but often overestimated. World Poult 18, 26-31
- Vicuña-Reyes JP, Luh J, Mariñas BJ (2008) Inactivation of *Mycobacterium avium* with chlorine dioxide. Water Res 42, 1531-1538Veschetti E, Cittadini B, Maresca D, Citti G, Ottaviani M (2005) Inorganic by-products in waters disinfected with chlorine dioxide. Microchem J 79, 165-170
- Wabeck CJ, Carr LE, Byrd V (1994) Broiler Drinker Systems and Seasonal Effects on Eviscerated Carcass and Leaf Fat Weights. J Appl Poult Res 3, 274-278
- Watkins S, Tabler GT (2009) Broiler Water Consumption. Avian Advice 11, 11-12, University of Arkansas Cooperative Extension Service, Fayetteville, AR, USA http://www.avianadvice.uark.edu/AA%20PDFs/ avianadvice_Vol11No2.pdf [last accessed 27.08.2014]
- Zimmermann NG, Hilton WR (1995) Influence of drinking water quality on laying hen performance. Poult Sci 74 Suppl. 1, 134
- Zimmermann NG, Douglass L (1998) A survey of drinking water quality and it' effects of broiler growth performance on Delmarva. Poult Sci 77 Suppl 1, 1-121