Effects of Drinking Diluted Deep Sea Water on Growth Performance and Immune Response in Broiler Chickens

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Abstract
Deep sea water (DSW) has emerged as an alternative source of drinking water, it worth to examine whether the DSW exerts any difference in animal responses upon drinking. This study aims to investigate the effect of drinking DSW in broiler chicken. A total of 400 Ross 308 broilers were allocated into 4 treatments with 5 replications (20 birds per pen per a treatment) during a 28-d feeding periods. Control birds were provided with fresh water for drinking. The DSW was diluted with deionized water (W) in the ratio of 1:80 (1DSW:80W), 1:40 (1DSW:40W) and 1:20 (1DSW:20W). The diets for both control and 1DSW:80W groups were supplemented with 0.18% of food-grade salt to satisfy the minimal salt needs. Water and feed were available for *ad libitum* consumption for all the groups. Total salt intake of birds drinking 1DSW:80W and 1DSW:20W was higher ($p<0.05$) than 1DSW:40W and control groups. Average daily water intake, water to feed intake ratio, mortality and serum IgG levels were not different ($p>0.05$) between treatments and control group. However, nutrient utilization of 1DSW:40W group was improved ($p<0.05$) when compared with the control. The relative weight of thymus and bursa of fabricius in 1DSW:40W and 1DSW:20W groups were greater ($p<0.05$) than 1DSW:80W and the control. The DSW might be successfully used as drinking water to broiler chickens and its serum IgG is slightly improved at the level of 1DSW:20W. However, a total salt intake equilibration is a pre-requisite for the effective DSW utilization.
Keywords: Deep sea water, broilers performance, lymphoid organs, serum IgG

Introduction

Deep sea water (DSW) is receiving an attention due to its global abundance as an alternative water resource. The DSW is also highlighted due to its mineral concentration as well as its characteristic of solubilization. The DSW, excavated from the depth of 300 meters below sea level contained 3 ~ 10 times higher amount of beneficial minerals compared to surface sea water (Matsubayashi et al., 1994). Keohavong et al. (2010) reported that DSW have relatively higher concentrations of Na, Cl, Mg, Ca, S, K, Br, Mn and Zn compared to fresh water. The amount of the beneficial minerals that can be obtained by ordinary water drinking is usually less than the need of modern poultry (McDowell, 2003).

In fact, the DSW has been recognized as a cleaner, safe water resource and no disease-causing bacteria have been investigated by Ogawa and Fujimoto, 2002. Moreover, The DSW is abundance in beneficial minerals content as it can be considered as a great source of good water quality. Since the water consumption by chicken as twice as compared to the feed consumption, chick between one week old and upto mature age, their body water content ranged between 85 to 55 %, respectively, while the most largest amount of the water remaining in the body of bird was found in the plasma of level of 25 % on a weight basis (Leeson and Summers, 2001). Due to the high level of water requirement of the birds and varied with their ages, the quality of drinking water as specially as some minerals concentration in drinking water may exert adverse effects on the growth performance of broilers.

In our knowledge, no study has assessed the effect of DSW drinking on poultry performance. In an *in vivo* study, both DSW and treated DSW were evaluated as mouth rinse and reported to be effective to sterilize the halitosis causing microorganisms (Kim et al., 2007). Kimata et al. (2002) reported that drinking desalted DSW decreased the skin allergy in humans. Tsuchiya et al., 2004 had evaluated that drinking desalted DSW and purified water showed equivalence in differences. However, there is no reliable information available so far on the beneficial effects of DSW on the performance of poultry upon drinking. Although some studies have examined the physico-chemical properties of DSW (Suzuki, 2000; Ueshima et al., 2003), the benefits of DSW upon drinking has not clearly been elucidated yet.

Due to the salinity of the DSW (~ 3.34%), the drinking amount by the animal might not be sufficient enough to impart any advantage. In addition, broiler chickens under three weeks of ages are relatively more susceptible to saline drinking water (Mirsalimi and Julian, 1993). Therefore, appropriate dilution is indispensable to enable substantial amount of drinking. As the appropriate concentration of mineral content in DSW is important to chick immune response and growth performance of broilers might be affected by drinking DSW. Therefore, this study was conducted to evaluate DSW drinking on growth performance, nutrient utilization, relative weight of lymphoid organs and the concentration of serum Immunoglobulin G (IgG) in broiler chickens.

Materials and Methods

Preparation of the deep sea water and experimental design

Deep sea water (DSW) was excavated at a depth of 300 m below sea level was obtained from the K-water. The saline concentration of DSW was 3.34%. Control birds were provided with fresh water for drinking. The DSW was diluted with de-ionized water (W) in the ratio of 1:80 (1DSW:80W), 1:40 (1DSW:40W) and 1:20 (1DSW:20W) for the three treatment groups. The mineral composition of fresh water and DSW were determined (Table 1). The experiment lasted for 28 days with two growth phases, i.e, the phase 1 (d 1 to 14) and phase 2 (d 15 to 28).

Experimental birds and diets

A total of 400 one-day old Ross 308 broiler chicks were used in this study. After 5 days acclimatization, the study started on day 6 of age, while the average initial body weight was 92.83±2.71 g/. A total of 20 chicks were randomly allocated to make a replicate pen, the pen size was 1.2 m²/pen. There were 5 replicate pens per treatment. All birds were reared on the floor pens with rice husk as a litter material in a thermostatically controlled house. The temperature was ranged from 22 °C to 34 °C during experiment and relative humidity was controlled at 60%. Light was provided for 24 h from d 1 throughout the experiment. All the care for the experimental birds followed the protocol approved by the Laboratory Animal Care and Use Committee of Kangwon National University.

The ingredients and chemical composition of both phase 1 and phase 2 diets are shown in Table 2, and the diets met or exceeded the nutrient requirements according to the AVIAGEN recommendations for Ross 308 broilers. To the basal diet, 0.18% of dietary salt was added to make control and 1DSW:80W diets to equilibrate the total salt supply. All the diets were processed as crumble. Both drinking water and feed were available for *ad libitum* consumption.

Growth trial and sample collections

The DSW dilution was prepared every single day to supply as fresh as possible. Experimental drinking waters were supplied through the bell drinkers. Both the DSW and fresh drinking water (control) were added daily into the waterers and the volume was recorded. Body weights of the birds were measured on d 1, d 14 and d 28 of the experimental period. The left over feed and water were recorded at the end of each phase (on d 14 and d 28) to calculate average daily feed intake and daily water intake in each replicate pen. The feed intake was recorded and corrected for mortality (mortality was also recorded, if any). Feed intake was used to calculate total salt intake for the control and 1DSW:80W group, and total water intake was used to calculate total salt intake for all experimental treatments (1DSW:80W, 1DSW:40W and 1DSW:20W).

On d 29, two birds per replicate pen was randomly selected and transferred to metabolic cages in the same housing conditions. One day acclimatization period was allowed to empty their digestive tracts. Weighed quantities of the diets were supplied and excreta were collected once a day (over 4 days) according to the total collection method. Care was taken during the collection of excreta samples to avoid contamination from feathers and other foreign materials. The excreta samples were dried in an electric oven with forced aeration at 60°C for the constant weight (at least 72 h). The dried excreta and phase 2 diet samples were weighed and then ground to pass through a 0.5 mm sieve and kept at room temperature for further analysis. The nutrients contents in the excreta and diets were measured to determine the nutrient utilization.

Table 1 Mineral profiles of fresh water and diluted deep sea water

Elements (mg/L)	Fresh water[1]	1DSW:80W[2]	1DSW:40W[2]	1DSW:20W[2]
Cl	478.00	926.62	1375.23	2272.47
Na	55.10	231.66	408.21	761.32
Mg	14.30	42.39	70.48	126.67
S	135.90	154.43	172.96	210.02
NO_3	0.00	7.14	14.29	28.58
Ca	57.10	61.26	65.42	73.73
K	4.30	12.49	20.69	37.07
Br	0.00	1.86	3.73	7.46
F	0.00	0.92	1.84	3.67
Sr	0.00	0.08	0.17	0.33

Table 1 (Con.)

Elements (mg/L)	Fresh water[1]	1DSW:80W[2]	1DSW:40W[2]	1DSW:20W[2]
B	0.00	0.04	0.07	0.14
Si	0.00	0.01	0.02	0.05
Li	0.00	0.00	0.00	0.01
P	0.09	0.09	0.09	0.08
Zn	51.8	51.15	50.51	49.22
Se	0.02	0.02	0.02	0.02
Mn	29.40	29.03	28.67	27.93
Cu	13.80	13.63	13.46	13.11
Fe	43.90	43.35	42.80	41.71

[1] These values are taken from NRC (2005).
[2] Deep sea water (DSW): deionized water (W)

Determination of lymphoid organs

A total of 40 birds (two birds/pen with five pens per treatment) were randomly selected at the end of feeding trial (d 28). Birds were weighed individually and then slaughtered. The lymphoid organs (thymus, spleen and bursa of fabricius were removed. The collected thymus, spleen and bursa of fabricius were immediately kept in phosphate buffered saline solution (pH 7.4). Each organ was stripped to remove adhering fat tissue and then wet weights were taken. The values of lymphoid organs were expressed as percentage of body weight.

Determination of serum immunoglobulin G

Blood samples were collected at the end of the trial period (d 29), a total of 40 birds (two birds/pen with five pens per treatment) were randomly selected. Blood samples (5 ml) was collected from wing vein, in a plastic tube (BD Vacutainer, 5.0 ml, PL6 7BP, UK) and immediately transferred to laboratory for the separation of serum. The blood samples were centrifuged at 2000 × g for 20 min at 4°C, the serum was separated and stored at -78°C until analysis. Immunoglobulin G (IgG) determination was done by ELISA kit (Catalog No. E30-104) using the BioTek analyzer (SN 216766, USA), with slight modification. Briefly, serum samples were diluted with blocking solution (1% BSA diluted with TBS-T) in the ratio of 1:75,000. Diluted serum sample was tested in duplicate, the test repeated 3 times and the mean values of optical density (OD) were obtained. The concentration of IgG was expressed in mg/mL.

Table 2 Formula and chemical composition of basal diets

Ingredients	Phase 1 (d 1-14)	Phase 2 (d 15-28)
Corn	51.86	54.15
Wheat	10.00	10.00
Soybean meal	22.80	20.60
Rapeseed meal	1.52	1.52
Meat meal	4.00	4.00
Feather meal	2.00	2.00
Ground limestone	0.55	0.58
Tri calcium phosphate	1.32	1.11
Tallow	4.72	5.00
Choline	0.14	0.16
DL- Methionine	0.25	0.22

Table 2 (Con.)

Ingredients	**Phase 1 (d 1-14)**	**Phase 2 (d 15-28)**
L- Lysine	1.43	0.29
Threonine	0.05	0.01
Vit. Premix[1]	0.15	0.15
Min. premix[2]	0.12	0.12
Enramycin	0.05	0.05
Clinacox	0.05	0.00
Maduramycin	0.00	0.05
Salt[3]	-	-
Total (%)	100.00	100.00
Calculated chemical composition		
Protein (%)	21.00	20.01
Dry Matter (%)	88.29	88.27
Moisture (%)	11.71	11.73
Fat (%)	7.48	7.78
Fiber (%)	3.37	3.30
Ash (%)	5.12	4.84
Ca (%)	0.90	0.85
TME (Kcal/kg)	3150.00	3200.00

[1] The vitamin premix contains the following per kg of diet: vit.A, 18.000IU; vit.D_3, 4.500IU; vit.E, 31.5IU; menadione (K_3), 3.6mg; thiamin (B_1), 1.8mg riboflavin (B_2), 4.8mg; pyridoxine (B_6), 3.6mg; cobalamine (B_{12}), 0.03mg; niacin, 22.5mg; pantothenic acid, 15mg; folic acid, 0.45mg.

[2] The mineral premix contains the followings per kg of diet: Mn, 86.4mg; Zn, 72mg; Fe, 57.6mg; Cu, 6mg; I, 1.5mg; Co, 0.288mg; Se, 0.216mg.

[3] To the diets of control and 1DSW:80W groups, 0.18% dietary salt was supplemented.

Chemical analysis

Proximate analyses of excreta and diets were executed according to AOAC (2005) to measure dry matter (DM), crude protein (CP), crude ash and crude fat. Gross energy was measured by bomb calorimeter (Model 1261, Parr Corp, USA).

Statistical analysis

All data and results generated from proximate analysis, performance parameters, nutrient utilization, weight of lymphoid organs and serum IgG levels were evaluated by the ANOVA procedure (SAS, 2004). Each replicate pen was considered as an experimental unit. The statistical significance was accepted at $p<0.05$ (Steel and Torrie, 1980).

Results

Body weight gain was lowest in 1DSW:40W ($p<0.05$), probably due to lowest ($p<0.05$) feed intake in the same group. The FCR was affected ($p<0.05$) by DSW dilution rate in both phases 1 and phase 2, but it was not different ($p>0.05$) among treatments and control when it counts overall phase (Table 3). Moreover, salt intake by broilers that received 1DSW:40W was lowest ($p<0.05$) than the birds received other waters. This result implied the less salt level caused less feed intake therefore exerted lowest body weight gain. However, this study

showed that DSW drinking did not affect (p>0.05) mortality, water intake and water to feed intake ratio at all phases (Table 3).

Table 3 Growth performance of broilers drinking fresh water and diluted deep sea water.

Items	**Fresh water**	**1DSW:80W[1]**	**1DSW:40W[1]**	**1DSW:20W[1]**	**SEM[2]**
Phase 1 (d 1-14)					
BW at d 1 (g/bird)	92.35	92. 45	93.65	92.85	2. 89
Feed intake (g/bird)	859.05^{a}	884.55^{a}	774.59^{b}	803.08^{b}	33.78
BW gain (g/bird)	604.40^{a}	612.66^{a}	473.85^{b}	624.96^{a}	34.83
FCR (g feed:g gain)	1.42^{b}	1.45^{b}	1.64^{a}	1.29^{c}	0.08
Water intake (ml/bird)	1409.40	1444.40	1372.40	1357.80	157.41
Salt intake (g/bird)	1.55^{b}	2.20^{a}	1.15^{c}	2.27^{a}	0.14
Mortality (%)	1.00	1.00	0.00	1.00	0.50
Phase 2 (d 15-28)					
BW at d 14 (g/bird)	696.75^{a}	705.11^{a}	567.50^{b}	717.81^{a}	34.80
Feed intake (g/bird)	1697.47^{ab}	1744.07^{a}	1623.50^{b}	1670.90^{ab}	68.44
BW gain (g/bird)	1032.74^{a}	1027.89^{a}	990.44^{ab}	946.61^{b}	60.25
FCR (g feed:g gain)	1.65^{b}	1.70^{ab}	1.64^{b}	1.77^{a}	0.08
Water intake (ml/bird)	3024.04	3230.58	3292.30	3138.77	322.63
Salt intake (g/bird)	3.06^{c}	4.49^{b}	2.75^{c}	5.24^{a}	0.37
Mortality (%)	2.05	1.00	1.00	2.05	0.61
Overall (d 1-28)					
Final BW (g/bird)	1729.49^{a}	1733.00^{a}	1557.94^{b}	1664.41^{a}	74.84
Feed intake (g/bird)	2556.52^{ab}	2628.62^{a}	2398.09^{c}	2473.99^{bc}	85.05
BW gain (g/bird)	1637.14^{a}	1640.55^{a}	1464.29^{b}	1571.56^{a}	74.67
FCR (g feed:g gain)	1.56	1.61	1.64	1.58	0.06
Water intake (ml/bird)	4433.44	4674.98	4664.70	4496.57	420.49
Salt intake (g/bird)	4.60^{c}	6.68^{b}	3.89^{d}	7.51^{a}	0.45
Mortality (%)	3.00	2.00	1.00	3.00	0.96

$^{a, b, c, d}$ Different superscripts in the same row are significantly different (p <0.05)

[1] Deep sea water (DSW): deionized water (W)

[2] SEM: Pooled standard error of the means

The utilization of crude protein, ether extract, crude ash, energy, and dry matter were significantly (p<0.05) higher in 1DSW:40W group compared with the control (Table 4). Drinking 1DSW:20W also exerted higher (p<0.05) crude protein and ether extract utilization than that by control. This study showed all the DSW drinking group showed significantly or at least tenderly improvement in nutrient utilization compared to control group.

The relative weights of thymus and bursa of fabricius in broilers that received DSW in 1:40 and 1:20 dilution were greater (p<0.05) than 1:80 and control groups (Table 5). Only the weight of spleen in treatments 1DSW:40W and 1DSW:20W was lower (p<0.05) than control and 1DSW:80W group. Besides, the values of serum immunoglobulin G (IgG) did not significantly (p>0.05) differ between DSW and control groups.

Table 4 Nutrients utilization in broilers drinking fresh water and diluted deep sea water.

Parameters	Fresh water	1DSW:80W[1]	1DSW:40W[1]	1DSW:20W[1]	SEM[2]
Nutrients utilization (%)					
Dry Matter	86.93[b]	88.50[ab]	91.08[a]	89.51[ab]	2.07
Energy	88.87[b]	90.54[ab]	92.44[a]	91.26[ab]	1.72
Crude protein	78.25[b]	80.80[ab]	84.93[a]	83.12[a]	2.60
Ether extract	93.91[b]	95.54[ab]	97.21[a]	96.16[a]	1.27
Crude ash	65.17[b]	67.55[ab]	74.69[a]	70.41[ab]	4.53
Total-CHO	82.20[a]	79.28[ab]	87.34[a]	85.00[a]	2.95

[a, b] Different superscripts in the same row are significantly different ($p<0.05$)
[1] Deep sea water (DSW): deionized water (W)
[2] SEM: Pooled standard error of the means

Table 5 Effects of drinking fresh water and diluted deep sea water on lymphoid organs and serum immunoglobulin G levels in broilers.

Parameters	Fresh water	1DSW:80W[1]	1DSW:40W[1]	1DSW:20W[1]	SEM[2]
Relative weight of lymphoid organs (%, of body weight)					
Thymus	0.20[c]	0.16[c]	0.29[b]	0.35[a]	0.08
Spleen	0.17[a]	0.15[a]	0.11[b]	0.12[b]	0.04
Bursa of fabricius	0.14[b]	0.14[b]	0.20[a]	0.21[a]	0.06
Serum Immunoglobulin G (mg/ml)					
IgG	11.92	12.99	11.17	16.98	6.43

[a, b, c] Different superscripts in the same row are significantly different ($p<0.05$)
[1] Deep sea water (DSW): deionized water (W)
[2] SEM: Pooled standard error of the means

Discussion

According to the Aviagen recommendation (Aviagen, 2009), expected body weight of Ross 308 broilers at age of 19th and 33rd day should be 741 and 1843 g/bird, respectively. The present study indicated that mean body weight of Ross 308 broilers was with the range of the expected values. FCR in this study were slightly better than the expected values (Aviagen, 2009; Manning et al., 2007). This study did not show significant benefit of DSW drinking on growth performances. Similar results were reported by Kimura et al. (2004) who found the slightly positive effect of deep sea water drinking in rats. They demonstrated only non-significant improvement in efficiencies of deep sea water. However, Tsuchiya et al. (2004) reported mean water intake was significantly ($p<0.01$) higher in mice given desalted deep sea water diluted with purified water at 20%. Since DSW in this study was not completely desalted, it is speculated that salt content in DSW affect water intake. Water intake pattern of broilers were closely related to ambient temperature within daily cyclic temperature range of 24 to 35°C (May and Lott, 1992), and drinking water intake was increased with age although its consumption per unit weight was decreased with age (North and Bell, 1990). Therefore, salt intakes in the 1DSW:80W and 1DSW:20W groups were increased with increasing water intake and water intake of birds increased with age, the birds will consume 2 to 7 times more amount of water as compared to the amount of feed and the amount of salt added to the ration should seldom be over 0.25% to 0.35% (Leeson and Summers, 2001).

Daily water intake in broilers to 21 d of age was estimated to be 138 mL/bird/day as described by Brake et al. (1992) who found the following equation: 9.73 + 6.142 x d age for the determination of daily water intake of broilers under 21 d of age. The present study

showed daily water intake of broilers in phase 1 (to 21 d age) of the control and DSW groups were higher than the amount calculated by above equation and also greater than Aviagen recommendations (Aviagen, 2009), who suggested that Ross male to 21 days of age required daily water intake of 203 ml/bird/day at the same ambient temperature of 21°C with bell drinkers as used in this study. However, the results of the present study did not correct the amount of the spilled and evaporated water, although many previous researches had corrected for those values (Kalmar et al., 2007). Besides, Beker and Teeter (1994) reported that water consumption is positively correlated ($p<0.09$) to feed consumption, feed efficiency and growth rate of the birds.

Studies on the DSW drinking in avian species are scarce. The present results agreed with Kimura et al. (2004) who reported that mortality of the rats received deep sea water were not statistically different. Similarly, mortality of the mice received desalted deep sea water diluted to purified water with 6.7%, 10%, and 20% were not significantly different compared to purified water group (Tsuchiya et al., 2004). The DSW drinking may have positive effects on water intake and water/feed ratio in broilers at some instances and broilers become more resistant to saline water containing 0.20% sodium after three weeks of age (Mirsalimi and Julian, 1993). This study agreed well with the report of Tsuchiya et al. (2004) who demonstrated that DSW drinking in mice was as safe as purified drinking water in terms of growth. No differences in mortality in the present study indicated that the DSW might be successfully used as drinking water for broilers. Therefore, it is still early to say whether DSW drinking could improve growth performance of broiler chickens.

Increasing nutrients utilization of broilers are related to the increasing of some minerals intake, as broilers receiving low sodium and chloride had shown lower retention of protein and energy. The function of minerals are interrelated and balanced against each other and most often cannot be considered as single elements with independent and self-sufficient roles in the organized bodily processes (McDowell, 2003). Therefore, the birds in the 1DSW:20W group were received higher minerals (Table 1) through drinking water and its CP and energy retention was also resulted higher ($p<0.05$) in 1DSW:20W when compared with the control group. However, no differences were observed between control and 1DSW:80W group. Differences in nutrient utilization in the present study might be influenced by diluted DSW drinking and its mineral composition. This study suggested that specific minerals or microorganism-free status in DSW is presumed to be associated with the improved nutrient utilization.

Development of lymphoid organs indicates immune status of the birds. In addition, development of lymphoid organs was influenced by the nutrients and specific minerals consumption (Kwak et al., 1999). Relatively higher amount of some minerals in DSW was probably responsible for the increased organs weight in DSW drinking group. Moreover, some studies showed that zinc (Zn) is necessary for normal structure and function of the immune system in animals (Kidd et al., 1996; Sun et al., 1993). In spite, deficiency of Zn may decreased the weight and growth index of the thymus and spleen in lymphocytes (Wu et al., 1994). In vitro, increased immunoglobulin-G synthesis in lymphocytes was influenced by some minerals (Weetman et al., 1983). Overall, the present results showed that DSW drinking was not harmful to birds and might have improved the immune status of the birds.

Conclusion

It is still early to elucidate any nutritional and functional values to DSW drinking. But this study proved there is no problem to use DSW as a drinking water for broiler chickens. However, a total salt intake adjustment from both water and diet is critical to maximize the benefit of DSW drinking. The dilution level of DSW for drinking purpose should not exceed 1DSW:20W. Moreover, DSW might be successfully used as drinking water to broiler

chickens and its serum IgG is slightly improved at the level of 1DSW:20W. This result is deserved further studies to confirm the benefit of DSW drinking.

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