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Effects of Drinking Reverse-osmosis Treated Deep Sea Water on Growth Performance and Immune Response in Broiler Chickens

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ABSTRACT

This study was executed to investigate the effects of drinking deep sea water treated by reverse osmosis process (RO-DSW) on growth performance, nutrient utilizability, relative weight of lymphoid organs and the concentration of serum immunoglobulin G (IgG) in broiler chickens. A total of 200 one day old broiler chickens (Ross 308) were equally and randomly distributed into 10 ground floor pens (20 chicks per pen, 5 pens per treatment) bedded with rice-husks. The broilers were offered either fresh tap water (Control) or RO-DSW for 28 days (from d 6 to d 33) as the drinking water. The same basal phase 1 diet for first 2 weeks and phase 2 diet for last 2 weeks were offered *ad libitum* to the birds. The RO-DSW was prepared by diluting 1:20 ratio with deionized water before offering to chickens. The diet for control birds was supplemented with 0.21 % of food-grade salt to satisfy salt need of the birds. Broiler feeding study resulted that there were no differences in amount of water consumption, mortality and FCR between RO-DSW and control chickens. However, feed intake and body weight gain were increased ($p<0.05$) by RO-DSW drinking. There was no ($p>0.05$) difference in nutrients utilizability between RO-DSW and fresh water drinking. There were no ($p>0.05$) differences in the immune response between the control and treatment group. The serum IgG levels were 3.01 vs 2.87 mg/ml and the relative weights of spleen, thymus and bursa of Fabricius were 0.23, 0.18 and 0.20 vs. 0.20, 0.17 and 0.14 for RO-DSW vs. control birds, respectively. The immune responses were tended to be improved by RO-DSW drinking. This study showed an improvement in weight gain and feed intake that could be induced by RO-DSW drinking, although it is difficult to explain the reasons of the improvement at this moment. This study implied that RO-DSW could be successfully used as drinking water to broiler chickens.

(**Key words :** RO-DSW, Performance, Nutrient utilizability, Lymphoid organs, IgG, Chicken)

INTRODUCTION

Water is known as an essential substance of blood and cellular fluid, and it mediate most of animal body functions, including the transport of nutrients, metabolites and waste products in the body. Due to this essential role of water, the source and quality of water has been a primary concern for the living animal as well as for poultry production. This is the background of the recent many approaches to seek for alternative and better quality water resources. Recently, deep sea water (DSW) is receiving public attention due to its global abundance as an alternative water resource. The DSW is also highlighted since it usually contains three to ten times more nutritional minerals compared to the surface sea water (Matsubayashi et al., 1994).

Reverse osmosis (RO) process has been used to treat or to desalinate the high salt water. The RO technology has been applied to remove relatively bigger particle constituent since the membranes are capable of rejecting practically all particles, bacteria and organics >300 Daltons molecular weight (Jaworski, 2008). Therefore, a question has arisen, whether RO treated deep sea water (RO-DSW) could be utilized as a drinking water and what would be its effect on animal. However, there are only limited numbers of scientific literatures available that had evaluated DSW with experimental animals. Although some studies have examined the characteristics of natural DSW (Suzuki, 2000; Kimata et al., 2002; Ueshima et al., 2003), the efficacy of DSW has not clearly been elucidated yet. Suzuki (2000) found that deep sea water had an effect of decreasing serum total carbohydrate levels in mice. Kimata et al. (2002) reported that drinking of treated deep sea water was effective to reduce skin allergy. But it is also reported that the desalted DSW can be evaluated just safe as the ordinary purified

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water for drinking (Tsuchiya et al., 2004).

Many chemical analyses of various deep sea waters have shown the presence of many essential minerals, at varying concentrations. In addition, the amount of these minerals that can be supplied by ordinary water drinking is observed to be less than their requirement by poultry (McDowell, 2003).

Minerals and their combination in DSW were also proposed to be responsible for several bio-modulations in living organism. *In vivo* human study by Kim et al. (2007) reported that a mouth rinse made of RO-DSW and DSW were effective for oral hygiene. This report demonstrated a potential benefit of RO-DSW drinking on biological and physiological level modulation even in animal body. Although the drinking water sources were not DSW products, salt content in drinking water along with its amount of drinking have been evaluated in views of ameliorating heat stress (Teeter et al., 1985; Branton et al., 1986) and improving survival rate in broiler chickens (Deyhim and Teeter, 1991). However, there have been only little *in vivo* animal studies that have evaluated treated DSW drinking in the view of biological and physiological efficacy. In our knowledge, there has been so far no poultry study that has assessed the effect of RO-DSW drinking.

Therefore, the objective of this study was to investigate the effect of drinking deep sea water treated by reverse osmosis process (RO-DSW) on the growth performance, nutrient utilizability, relative weight of lymphoid organs and the concentration of IgG in broiler chickens.

MATERIALS AND METHODS

1. Experimental design

The experimental design and allocation of the birds was done according to the completely randomized design principle. The deep sea water treated by reverse osmosis process (RO-DSW) was obtained from the Korea Institute of Water and Environment, Republic of Korea. The salinity concentration of RO-DSW was 5.31% as pure salt and the mineral composition of RO-DSW are shown in Table 1. The experiment was carried out for 28 days, with two growth phases, i.e. the phase 1 (d 1 to 14) and phase 2 (d 15 to 28). In the control group the birds were provided with fresh drinking water, whereas, birds in the treatment group were provided with RO-DSW diluted with deionized water in 20:1 ratio (deionized water : RO-DSW). The diet for control birds was supplemented with 0.21% of food-grade salt to satisfy salt need of the birds. to provide equilibrate salt supple of 0.27%, There were 5 replicate pens for each treatment. All the care of the experimental birds followed the protocol approved by the Laboratory Animal Care and Use Committee of Kangwon National University. This experiment was carried out during September, when the ambient temperature was relatively high.

2. Animals, housing and diets

A total of 200 one day old broiler chickens (Ross 308) were employed to compare the two drinking waters. A five day acclimatization period was allowed before initiating the experiment. The experiment was started on day 6 of age by allocating birds to have similar average initial body weight (82.43±2.21 g/bird) per pen. The chicks were randomly selected to make a pen (20 birds in each pen) and they were placed into a squarely fenced rice-husk floored pen (1.2 m^2/pen), with 5 replicate pens per treatment. All birds were reared on the ground floored thermostatically controlled house after proper cleaning and disinfection. During the experimental period, birds were raised under 24 h/d manipulated lighting and ventilation controlled at 34.46 ± 1.71°C in-house temperature.

The basal diets are corn and soybean meal based diet. The diets were prepared for two growth phases. Diets for phase 1 (1-14 d) were formulated to contain 21% CP and 3150 kcal/kg ME and phase 2 (15-28 d) diets were formulated to contain 20% CP and 3200 kcal/kg ME. To the basal diet, 0.21% of dietary salt was added to make control diet to equilibrate the total salt supply. All diets were processed as crumble. Both drinking water and feed were provided *ad libitum*. The ingredients formula and chemical composition of both phase 1 and phase 2 diets are shown in Table 2. The diets are formulated to meet or slightly exceed the nutrients recommendation by Aviagen for Ross 308 broiler. Proximate analyses of excreta and diets, which are dry matter (DM), crude protein (CP), gross energy, crude ash and crude fat were executed according to AOAC standard procedures (AOAC, 1990).

3. Growth performance, nutrients utilizability and lymphoid organ weight

The RO-DSW dilution was done every day to supply it as

fresh as possible. Both RO-DSW and fresh drinking water (control) were filled daily into the waterer (bottles) and the filled volume was recorded. The mortality of the birds and chicken house temperature were recorded daily. Body weights for each replicate pen were weighed on d 1, 14 and 28 of the experimental period. The remaining amounts of given experimental diet and water were measured just before next

Table 1. Mineral compositions of freshwater, RO-DSW and DSW

Elements (mg/L)	Fresh tap water[a] (Control)	RO-DSW[b]	DSW[b] (Reference)
Cl	478.000	35704.635	36367.357
Na	55.100	13100.805	14179.546
Mg	14.300	2163.406	2261.631
S	135.900	1516.728	1618.226
NO_3	nd	550.661	571.586
Ca	57.100	679.904	389.772
K	4.300	1222.032	659.751
Br	nd	126.418	149.109
F	nd	66.301	73.480
Sr	nd	11.286	6.679
B	nd	3.578	2.849
Si	nd	2.936	0.944
Li	nd	0.138	0.102
P	0.087	0.036	0.013
Ba	nd	0.007	0.005
Mo	nd	0.008	0.006
As	nd	0.006	0.006
V	nd	0.030	0.030
Ti	nd	0.001	0.001
Zn	51.800	0.162	0.128
Ni	nd	0.004	0.002
Al	nd-	nd	0.057
Cr	nd	nd	nd
Sb	nd	nd	nd
Se	0.016	nd	nd
Mn	29.400	0.001	0.001
Cd	nd	0.003	0.003
Cu	13.800	nd	nd
Fe	43.900	0.002	0.001

[a] The values from NRC (1974).
[b] The values were analyzed by the Kangwon National University. nd denote not detected.

Table 2. Formula and chemical composition of broiler basal diets for each phase

Items	Phase 1 (d 1-14)	Phase 2 (d 15-28)
Ingredients (%)		
Corn	60.91	62.95
Soybean meal	25.28	23.20
Meat meal	3.00	3.00
Feather meal	2.00	2.00
Poultry meat meal	1.00	1.00
Limestone	0.55	0.61
Dicalcium phosphate	1.54	1.26
Tallow	4.52	4.80
Glucose	0.00	0.20
Choline chloride	0.14	0.16
DL-methionine	0.25	0.22
L-lysine	0.43	0.25
Threonine	0.03	0.00
Vitamin mix[a]	0.15	0.15
Mineral mix[b]	0.12	0.12
Anticoccidial	0.05	0.05
Antioxidant	0.03	0.03
Salt[c]	—	—
Total (%)	100.00	100.00
Chemical compositions, calculated (%)		
Dry matter	88.19	88.17
Crude protein	21.01	20.00
Fat	7.55	7.86
Fiber	3.30	3.23
Ash	5.12	4.83
Ca	0.90	0.85
Na	0.20	0.15
Methionine	0.65	0.61
Lysine	1.54	1.31
ME (Kcal/kg)	3150.06	3200.82

[a] The vitamin premix contains the followings per kg of diet: vit. A, 18.000IU; vit. D_3, 4.500IU; vit. E, 31.5 IU; menadione (K_3), 3.6 mg; thiamin (B_1), 1.8 mg riboflavin (B_2), 4.8 mg; pyridoxine (B_6), 3.6 mg; cobalamin (B_{12}), 0.03 mg; niacin (B_3), 22.5 mg; pantothenic acid (B_5), 15 mg; folic acid (B_9), 0.45 mg.
[b] The mineral premix contains the followings per kg of diet: Mn, 86.4 mg; Zn, 72 mg; Fe, 57.6 mg; Cu, 6 mg; I, 1.5 mg; Co, 0.288 mg; Se, 0.216 mg.
[c] To the portion of diet, 0.21% dietary salt was additionally mixed to equilibrate salt supply for control diet.

feeding and watering in the morning of d 14 and 28 to calculate average daily feed intake (ADFI) and daily water intake of each replicate pen. The ADFI was calculated after correcting any mortality. The ADFI was used to calculate total salt intake for control group, whereas total salt intake for RO-DSW treatment was calculated by water intake.

Six birds were randomly selected from each treatment and transferred to metabolic cages for the collection of excreta samples on d 29, for the nutrient utilizability trial. One day acclimatization period was allowed to empty their digestive tracts. Weighed quantities of the diets were supplied and excreta were collected over 72 h on plastic polyethylene sheets placed under wire mesh floor cage 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 to the constant weight (at least 72 h). The dried excreta and finisher diet samples were weighed and then ground to pass though a 0.5 mm sieve and kept at room temperature for further analysis. The nutrient utilizability (%) was expressed as 100 [(total nutrient intake – total nutrient excretion)/total nutrient intake].

A total of 24 birds (12 birds per treatment) were randomly selected at the end of the trial (d 29). Chickens were weighed individually then slaughtered by severing the carotid arteries. The thymus, spleen and bursa of Fabricius were removed from the abdomen. The collected thymus, spleen and bursa of Fabricius were immediately kept in the solution of phosphate buffered saline (PBS, pH 7.4) in polypropylene conical tube (Falcon 50 ml, USA). Each organ was stripped to remove adhering fat tissue and then weighed individually. Relative organ weights were calculated as percentages of body weight = [(organ weight/body weight) × 100].

4. Blood collection and IgG quantification

At the end of the trial, a total of 16 birds (8 birds per treatment) were randomly selected from each treatment for blood collection (via wing vein), of approximately 5 ml per sample in a plastic tube (5.0 ml, BD Vacutainer, PL6 7BP, UK) containing anticoagulant (EDTA) and immediately transferred to Laboratory for the separation of serum. The serum was isolated by centrifuge at 2000 × g for 15 min at 4°C and stored at −78°C until used for analysis. The determination of IgG concentration was done by ELISA quantification kit (Catalog No. E30-104) as described below.

The concentration of IgG antibody optical density (OD) titers were measured by indirect ELISA. Coating solution was prepared with the dilution of Goat anti-Chicken IgG-Fc (A30-104A-11) (10 µl/ml 0.05 M Carbonate-Bicarbonate, pH 9.6), the solution at 100 µl /well was used to coat an ELISA plate (Apogent Co, Denmark) and incubated at 4°C overnight. After washing it five times with PBS-T (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0), each well was added with 200 µl of blocking solution (1% BSA diluted with PBS-T) and kept for incubation at 37°C for 30 min. Wells were washed five times, again with PBS-T. Then, each well was added with 100 µl of samples (serum dilution of 1/75000 blocking solution) and standards (Chicken Reference Serum, RS10-102-3). Incubation was carried out at 37°C for 60 min. Wells were again washed with PBS-T for five times, then each of them were added with 100 µl of freshly prepared HRP conjugate (A30-104P) solution (1/40000 PBS-T). After incubation at 37°C for 60 min, wells were washed again with PBS-T for five times, then each well was added with 100 µl of substrate solution (prepared as 2 µl of hydrogen peroxide 30% + 1 tablet of TMB (3.3',5.5'-tetramethylbenzidine) + 1 ml of DMSO (dimethyl sulphoxide) + 9 ml of phosphate-citrate buffer). Then, 100 µl of stopping solution (2 M H_2SO_4) was applied to each well to stop the reaction after a 30 min incubation period at room temperature. The color developed was read at 450 nm with an ELISA reader (BioTek, USA). Each serum sample was tested in duplicate, the test repeated 3 times and their mean ODs were obtained. The concentration of IgG was expressed in mg/ml.

5. Statistical Analysis

Data processing was done using Microsoft excel 2003. For the growth performance parameters and nutrient utilizability, mean value of each pen was considered as an observation unit. For thymus, spleen and bursa of Fabricius measurement, individual birds were considered as an observation unit. For the IgG concentration, mean value of each sample was considered as an experimental unit. All the data and results taken from proximate analysis, growth performance (BW, BWG, FI and FCR), mortality and water consumption were subjected to statistical analysis by the General Linear Model (GLM) procedure of SAS program, version 9.1.2 (2004). Statistical difference of the values were expressed at $p<0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

1, Amount of water consumption

Results of total and average daily water intake, water to feed intake ratio (WFIR) and salt intake by chicken are shown in Table 3. The result showed that RO-DSW did not affect ($p>0.05$) the amount of water intake of birds at both growth phases. However, the WFIR of broilers received RO-DSW in phase 1 was lower ($p<0.05$) than that of the control. But the WFIR was not different ($p>0.05$) during phase 2 and overall phase. Total salt intake by broilers that received RO-DSW was significantly ($p<0.05$) higher than that by the control birds. The salt intake in RO-DSW group was increased with increasing water intake with age as similarly shown by North and Bell (1990) who reported increased drinking water intake with age. They also suggested that the amount of salt added to the ration should seldom be over 0.25 to 0.35%.

It was reported that the water consumption was positively correlated ($p<0.09$) with bird growth rate, feed consumption, and feed efficiency (Beker and Teeter, 1994). Since the current study was carried out during the late summer season when the environmental temperature ranged 28 to 32°C, it could explain the relatively higher water consumption in this experiment. May and Lott (1992) also demonstrated that the water intake pattern of broiler is closely related to ambient temperature within daily temperatures of 24 to 35°C. Drinking water intake generally increased with age, although its consumption per unit weight decreased with age (Leeson and Summers, 2001). Brake et al. (1992) described daily water intake in broilers to 21 d of age by the following equation: $9.73 + 6.142 \times$ d age. Using this equation, broiler to 21 d of age was estimated to consume about 138.00 ml/bird/day of water. In the present study, daily water intake of broilers in grower phase (to 21 d age) of the control and RO-DSW groups were 162.45 and 159.72 (ml/bird/day), respectively (Table 3), that are higher than the amount calculated by the above equation. Aviagen (2009) also suggested that 21 day of age of Ross male broiler was able to drink 203 ml per bird per day at 21°C temperature (with bell drinkers as used in the current study). Water intake of RO-DSW group was slightly higher than the control, but the values were not statistically ($p>0.05$) different. Most of previous water intake determinations had corrected the amount of water spilled and evaporated (Kalmar et al, 2007).

Table 3. Effects of RO-DSW drinking on water and salt intake of broiler chickens

Items	Control	RO-DSW	SEM[1)]
Phase 1 (d 1-14)			
Daily water intake (ml/bird/d)	162.45	159.72	19.25
WFIR* (ml water : g feed)	2.12[a]	2.00[b]	0.08
Total salt intake (g/bird)	2.26[b]	5.71[a]	0.32
Phase 2 (d 15- 28)			
Daily water intake (ml/bird/d)	272.37	291.29	25.17
WFIR* (ml water : g feed)	2.40	2.47	0.07
Total salt intake (g/bird)	3.35[b]	9.52[a]	0.81
Overall (d 1-28)			
Daily water intake (ml/bird/d)	217.41	225.51	22.21
WFIR* (ml water : g feed)	2.28	2.28	0.06
Total salt intake (g/bird)	5.60[b]	15.23[a]	1.07

[1)] SEM : Pooled standard error of the mean (n = 10)
* WFIR : Water to feed intake ratio (ml water intake : g feed intake)
[a, b] The different superscripts within the same row are significantly different ($p<0.05$).

Since the present study did not correct the amount of the spilled and evaporated water, the reported value in this study could be a little bit over-estimated.

The present data demonstrated that any possible effect of RO-DSW to limit water drinking may be alleviated or disappeared with the age of broilers. Therefore, little differences in water intake of the present study may partly be due to the extended feeding period. In addition, the amount of salt in RO-DSW in this experiment was suggested not to be a limitation in drinking by the broilers. Similar study with rat reported the encouraging effect of treated deep sea water on drinking (Kimura et al, 2004). They reported that there was no significant improvement by treated deep sea water on water intake. However, water intake of mice, received desalted deep sea water diluted to purified water with 20% were significantly ($p<0.01$) lower than mean water intake of natural DSW (Tsuchiya et al, 2004). Mirsalimi and Julian (1993) demonstrated that broilers become more enduring after three weeks of age to saline water containing 0.20% sodium. Overall, the current study showed the RO-DSW can be used as a drinking water without any limitation in water intake. In addition, the older birds may able to consume more RO-DSW probably due to acclimatization or aging.

2. Growth performance

Performance of broilers expressed as body weight (BW),

average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and mortality are presented in Table 4. RO-DSW drinking did not affect ($p>0.05$) growth performance in phase 1 (d 1-14). Both FCR and mortality were not significantly different ($p>0.05$) between treatments. However, the ADG of broilers that received RO-DSW drinking during phase 2 and overall phases were significantly ($p<0.05$) greater than control group. Considering overall phase, both final BW and ADFI of broilers received RO-DSW were significantly ($p<0.05$) higher than control. Since there was no significant difference in F/G between control and RO-DSW group (Table 4), the improvement in gain was presumed to be induced by increase in feed intake by RO-DSW. In addition, since the amount of drinking was not different (Table 3), the evident difference that could affect feed intake in this experiment was suggested as difference in mineral intake between fresh water and RO-DSW drinking groups.

Even though, the growth performance in phase 1 was not statistically different ($p>0.05$), broilers taken RO-DSW showed slightly higher values than the control. During overall phase (d 1-28), broilers received RO-DSW as drinking water showed respectively 3.04%, 3.92% and 3.22% greater final BW, ADFI and ADG than those values of the control. Mortality occurred only in control birds during early stage (phase 1) in this study. Therefore, it is early to tell whether RO-DSW has imparted a benefit on survival. The result of this study is in agreement with Kimura et al. (2004) who reported non-significant effect on the mortality of the rats by treated deep sea water drinking. Moreover, Tsuchiya et al (2004) also reported no difference in mortality of mice received desalted deep sea water compared to mice received purified water. No differences in mortality in the present study indicated that the RO-DSW can be successfully used as drinking water for broiler.

Matsubayashi et al. (1994) showed that deep sea water can be used for the culture of various species of marine micro-algae. Many works have examined the short-term effects of the salt (NaCl) addition to the feed or water in terms of the amelioration of acute and chronic heat exposure (Teeter et al, 1985; Branton et al, 1986) in broiler chickens. One study (Barton, 1996) has examined the water intake in the view of feed conversion, body weight, livability, and condemnation. Deyhim and Teeter (1991) indicate improved performance of heat stressed broilers by supplementing salt in the drinking water where their survival rate was closely related with the change in water consumption. This study showed the RO-DSW drinking could exert beneficial effect on growth performance of broiler chickens during hot humid weather by not affecting water consumption as well as salt intake. Further study with DSW drinking under different extreme climate condition may be needed to understand any benefit of salt in water as well as RO-DSW as drinking water.

Table 4. Effect of RO-DSW drinking on growth performance

Items	Control	RO-DSW	SEM[1)]
Phase 1 (d 1-14)			
Initial BW (g/bird)	81.46	82.48	1.89
ADFI (g/bird/d)	76.78	80.13	2.92
ADG (g/bird/d)	52.10	52.28	0.88
FCR (g feed:g gain)	1.48	1.53	0.05
Mortality (%)	0.16	0.00	0.15
Phase 2 (d 15-28)			
BW at d 14 (g/bird)	810.84	814.40	11.42
ADFI (g/bird/d)	113.71	117.84	3.33
ADG (g/bird/d)	67.98[b]	71.66[a]	1.50
FCR (g feed:g gain)	1.67	1.64	0.05
Mortality (%)	0.00	0.00	0.00
Overall (d 1-28)			
Final BW (g/bird)	1762.51[b]	1817.71[a]	26.81
ADFI (g/bird/d)	95.25[b]	98.98[a]	2.51
ADG (g/bird/d)	60.04[b]	61.97[a]	1.00
FCR (g feed:g gain)	1.59	1.60	0.03
Mortality (%)	0.16	0.00	0.15

[a, b] The different superscripts within the same row are significantly different ($p<0.05$).
[1)] SEM : Pooled standard error of the mean (n = 10)

3. Nutrient utilizability

The results of this study did not find any significant adverse effect of RO-DSW drinking on nutrient utilizability as shown in Table 5. The utilizability of crude protein (CP), crude fat, crude ash, gross energy and dry matter (DM) of broilers received RO-DSW drinking were not statistically

Table 5. Effects of RO-DSW drinking on nutrients utilizability in broilers

Utilizability (%)	Control	RO-DSW	SEM[1)]
Crude protein	54.42	58.33	5.58
Crude fat	86.46	88.41	2.65
Crude ash	24.24	28.12	7.10
Gross energy	75.74	78.52	3.53
Dry matter	71.24	74.78	3.76

[1)] SEM : Pooled standard error of the mean (n = 20).

different (p>0.05) between treatments. However, all the nutrients utilizability of the diet of broilers received RO-DSW drinking was numerically higher than those of the control. The slightly higher nutrients utilizability in RO-DSW group may be mediated by minerals in RO-DSW since Na and Cl are major imparter of salinity of the RO-DSW as indicated in Table 1. The present result agreed well with McDowell (2003) who demonstrated that Na and Cl often help the passage of nutrients into the cells and waste products out, thereby the lack of Na lowers the utilization of digested protein and energy. Magnesium is the third major element of the salinity of RO-DSW (Table 1) and Mg has been suggested to have many diverse physiological functions (Al-Ghamdi et al., 1994). Moreover, the function of minerals are interrelated and balanced each other and most often cannot be considered as a single element having individual role (McDowell, 2003). The present result showed that there was no significant improvement in nutrient utilizability by RO-DSW drinking compared to fresh water although there were differences in mineral composition of both waters.

4. The relative weight of the lymphoid organs and serum immunoglobulin G

The relative weight of lymphoid organs, thymus, spleen, bursa of Fabricius and the concentration of serum immunoglobulin G (IgG) are shown in Table 6. Both lymphoid organs and IgG values did not significantly (p>0.05) differ between the RO-DSW and control group. However, both the relative weight of the lymphoid organs and the concentration of IgG in RO-DSW group were numerically higher than the values in the control birds. This result is similar to Kwak et al. (1999) who reported that either enhanced or depressed immune responses are depended on the nutrients level, more specifically the level of specific minerals that are also required for optimal development of immune organs. The development of the thymus and bursa of Fabricius as the part of lymphoid tissue has been described (Payne, 1971; Rose, 1979).

Tsuchiya et al. (2004) has compared desalted DSW with desalted surface sea water and purified water by measuring hematological values in mice. They found no significant differences in those values between the desalted DSW and purified water groups. However, Kimata et al. (2002) found that natural DSW drinking can be used to reduce skin allergy in humans. In addition, Weetman et al. (1983) reported that iodine could increase IgG synthesis in human lymphocytes *in vitro*. Tai et al. (2000) also reported that drinking DSW in healthy volunteers showed significant decrease in both whole blood flow time and blood pressure. Kimata et al. (2002) suggested that DSW intake decreases serum total and allergen-specific Ig E levels by reducing Ig E-inducing cytokines production. Although there was no statistical difference, the current result proposed there could be RO-DSW induced immune stimulator. Since RO-DSW contains various minerals in higher quantity than fresh tap water as shown in Table 1, it could be suggested that specific minerals in RO-DSW may affect the development of the lymphoid organs and IgG concentration. To understand the precise mode of action by RO-DSW on the possible immune stimulation, it certainly needs further investigation with animals under stress.

Table 6. Effects of RO-DSW drinking on relative weight of lymphoid organs and serum IgG concentration

Items	Control	RO-DSW	SEM[1)]
Lymphoid organs (%, of body weight)			
Thymus	0.20	0.23	0.08
Spleen	0.17	0.18	0.06
Bursa of Fabricius	0.14	0.20	0.05
Serum Immunoglobulin G (mg/ml)			
Serum IgG	2.87	3.01	0.54

[1)] SEM : Pooled standard error of the mean (n=24).

CONCLUSIONS

In conclusion, considering the results of this study as well as the reported literature, it is early to conclude any nutritional benefit and immuno-modulating effect of RO-DSW drinking. But the present study proved there is no adverse effect of using RO-DSW as a drinking water for broiler chickens. In addition, this study implied the mineral profiles of RO-DSW could be critical to impart any effect on broilers. Therefore, it certainly deserves further animal studies with RO-DSW containing higher minerals but less salt.

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