

Effects of Microwave-Treated Drinking Water on Growth and some Physiological Characteristics of Japanese Quail (*Coturnix coturnix japonica*)

Research Article

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ABSTRACT

Present preliminary scientific trial was designed to investigate of microwave effects on Japanese quails as an animal model. Thirty six three days-old quail chicks were randomly divided into 3 experimental groups and received: A) un-treated tap water, B) tap water boiled by electrical heater and then cooled and C) tap water treated by microwave oven and then cooled. The whole experimental period was 40 days. During the experiment body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured. At the end of the study, carcass, liver, spleen and bursa of fabricius weighed and small intestinal length, blood hemoglobin and hematocrit, small intestinal villus height and crypt depth were measured. The lowest body weight gain was observed in group C ($P < 0.01$). Haemoglobin and hematocrit values of group C were less than group A ($P > 0.01$). Carcass, liver, spleen and bursa of fabricius weights and small intestinal length were the lowest in group C ($P < 0.01$). Crypt depth of group C was higher than the group A ($P < 0.01$). Qualitative observations of reproductive organs such as: ovaries, oviducts, follicles and testis of group C showed less growth in comparison with the other groups. Treatment of drinking water by microwave had side effects on Japanese quails. Tease effects were not the simple effects of boiling since heat-boiled drank quails did not show such effects.

KEY WORDS health, Japanese quail, microwave, water.

INTRODUCTION

There is much information about microwave on the internet that people are not firm to accept or reject them. In many pages adverse affects of microwave have been showed. Microwave is an electromagnetic wave with wavelengths longer than those of terahertz waves, but shorter than radio waves (Wong *et al.* 2009). Microwave is not a form of heat, but a form of energy which manifests as heat through its interaction with materials (Wong *et al.* 2009). Transmission of microwave to an object results in vibration of molecules

by induced or permanent dipoles (Wong *et al.* 2009). Microwave radiation has many usages in different fields which are close related to food and health such as: long-term storage of vegetables (Abbasi and Azari, 2009), fruits (Motavali *et al.* 2011) and dairy industry such as yoghurt powder production (Ghaderi *et al.* 2010), through moisture removal, sterilization of surgery and medical equipment (Fais *et al.* 2009) and degradability of animal feedstuff (Ebrahimi *et al.* 2010). It has been reported that microwave cause to formation of cancer-causing substances, leakage of toxic chemicals from the packaging into the foods, destruc-

tion of nutrients, decreased bioavailability of vitamin B complex, vitamin C, vitamin E, essential minerals and lipotropic factors in all food tested and loss of natural food antioxidants.

Nervous disorders such as: headache, dizziness, sleeplessness, irritability, anxiety, nervous tension, inability to concentrate is symptoms of direct expose to microwave radiation (Lita, 2001).

Accidental exposure to high levels of microwave energy can alter or kill sperm, causing temporary sterility. Microwave radiation interferes with the signal from pacemakers.

Because pacemakers are electronic devices, interference from other electrical sources can cause the pacemaker to malfunction and thus send incorrect information to the heart muscles. Therefore, a study was designed to scheme a preliminary scientific trial to survey whether microwave oven is a harmful stuff by using Japanese quails as an animal model.

MATERIALS AND METHODS

Experimental groups, birds and feed

Thirty six three-day old Japanese quail were divided into three experimental groups with eight birds in each that kept individually as replicate (eight replicate for each group). Experimental groups were including: group A (control) drank untreated tap water during experimental period. Group B received electrical heater-treated tap water (boiled via laboratory electrical heater (Hiedolph, Germany) in 1 lit beaker (95 °C for 20 min) after that kept in room temperature).

Group C received microwave-treated tap water (treated via kitchen microwave oven (Butan-Model CE300S-TD, Iran) in 1 lit beaker until formation of bulbs, after going down in room temperature). The drinking water was prepared from a same tap due to omit uncontrolled source of variation. Chicks had free access to water and a commercial diet formulated to meet or exceed NRC requirements (NRC, 1994) specifications for Japanese quail for the entire experimental period (40 d) (Table 1).

Body weight gain and feed conversion ratio

Every ten day, individual body weight (BW), body weight gain (BWG) and feed intake (FI) was measured. Feed conversion ratio was calculated as FI/BWG.

Blood parameters

On 40th day, 1.5 mL of blood sample was collected from the left brachial vein of each bird for measuring of haemoglobin and hematocrit. The time of handling of birds and end of taking blood samples was less than 2 minutes.

Morphological and organs parameters

At the end of the experiment weight of liver, spleen, bursa of fabricius and length of duodenum, jejunum and ileum of small intestine were measured. For histomorphological analysis segments of approximately 2 cm were taken from the midpoint of the duodenum (duodenum), from the midpoint between the bile duct entry and meckel's diverticulum (jejunum) and midway between meckel's diverticulum and the ileo-cecal junction (ileum). Segments were gently flushed twice with 0.9% phosphate buffer saline (PBS) to remove the intestinal contents and were fixed in fresh 10% formalin. All samples were dehydrated, cleared and embedded in paraffin. Sections were deparaffinized in xylene and rehydrated in a graded alcohol series. Sections were cut at 5 µm and were placed on glass slides (McManus, 1948). Villus height, crypt depth, number of goblet cells along 100 µm of villus length was determined under light microscope (Carl ZEISS standard 20, Germany). The results of the morphometric determinations were from at least 10 well-oriented crypt villus structures from each chick. These data were pooled to calculate the mean value of each variable using Dinocapture software (Dino-lite, Ver. 3.3.0.0, Korea).

Table 1 Composition of the basal diets

| Ingredient (%) | |
|---|-------|
| Corn | 42.32 |
| Soybean meal | 40.20 |
| Vegetable oil | 7.48 |
| Fish meal | 7.30 |
| CaCO ₃ | 1.21 |
| Di-calcium phosphate | 0.01 |
| Sodium chloride | 0.28 |
| Mineral and vitamin premix ¹ | 0.50 |
| DL-methionine | 0.03 |
| Washed sand | 0.67 |
| Total | 100 |
| Calculated value² | |
| ME (kcal/kg) | 3130 |
| CP (%) | 25.90 |
| Lys (%) | 1.40 |
| Met + Cys (%) | 0.81 |
| Calcium (%) | 0.86 |
| Non-phytate phosphorus (%) | 0.32 |

¹ Supplied the following per kilogram of diet: Retinyl acetate: 9000 IU; Cholecalciferol: 2000 IU; DL- α -tocopheryl acetate: 12.5 IU; Menadione sodium bisulfite: 1.76 mg; Biotin: 0.12 mg; Thiamine: 1.2 mg; Riboflavin: 3.2 mg; Calcium d-pantothenate: 6.4 mg; Pyridoxine: 1.97 mg; Nicotinic acid: 28 mg; Cyanocobalamin: 0.01 mg; Choline chloride: 320 mg; Folic acid: 0.38 mg; MnSO₄.H₂O: 60 mg; FeSO₄.7H₂O: 80 mg; ZnO: 51.74 mg; CuSO₄.5H₂O: 8 mg; Iodized NaCl: 0.8 mg; Na₂SeO₃: 0.2 mg.

² Calculated from NRC (1994).

Statistical analyses

Data was analyzed by 1-way ANOVA using the GLM procedure of statistical analysis system (SAS, 2001).

Differences among treatment means were measured by Duncan's multiple range test and considered significant at (P<0.05).

RESULTS AND DISCUSSION

Control group had significantly lower BW and BWG than the other groups ($P < 0.01$). Average of feed intake of each group per bird was 703.94, 709.12 and 683.21 for control, heat-treated and microwave-treated groups respectively. FCR was slightly higher in microwave-treated group than the others ($P > 0.01$) (Table 2). Blood haemoglobin and hematocrit of microwave-treated group was slightly lower ($P > 0.01$) than other groups (Table 3).

All measured internal organs were significantly different between microwave-treated and control groups ($P < 0.01$) except for bursa of fabricius (Table 4). Small intestinal values are shown in Table 5.

Villus height of every three segments was higher in control group than the other groups. Crypt of duodenum and ileum was deeper in microwave-treated group than control ($P > 0.05$) whereas there was no difference in jejunum. In general, Japanese quail's maturity and appearance of first egg are started around day 40.

In this study, females of control and heat-treated groups started to lay at 37 and 39 d of experiment respectively, but the birds drank microwave-treated water did not lay until end of the experiment.

At the time of slaughter, qualitative observations of reproductive organs such as: ovaries, oviducts, follicles and testis of microwave-treated group showed less growth in comparison with the other groups.

Table 2 The effect of treated drinking water on BW, BWG and FCR of Japanese quails

| Treatment | BW (g) | | | | BWG (g) | FCR |
|----------------|--------|------|------------------|------------------|------------------|--------|
| | 10 d | 20 d | 30 d | 40 d | 3-43 d | 3-43 d |
| A ¹ | 69.0 | 131 | 210 ^a | 276 ^a | 261 ^a | 2.70 |
| B ² | 67.4 | 129 | 188 ^b | 266 ^a | 251 ^b | 2.83 |
| C ³ | 65.4 | 125 | 172 ^b | 250 ^b | 234 ^c | 2.92 |
| SEM | 1.41 | 2.98 | 4.79 | 4.45 | 13.5 | 0.070 |
| P-value | NS | NS | ** | ** | ** | NS |

A¹, B² and C³: un-treated tap water, tap water boiled by electrical heater then cooled and tap water treated by microwave oven then cooled.

The means within the same column with at least one common letter, do not have significant difference ($P > 0.05$).

* ($P < 0.05$) and ** ($P < 0.01$).

NS: non significant.

SEM: standard error of the means.

Table 3 The effect of treated drinking water on haemoglobin and hemotocrit of Japanese quails

| Treatment | Haemoglobin (g/dL) | Hemotocrit (%) |
|----------------|--------------------|----------------|
| A ¹ | 10.4 | 39.0 |
| B ² | 11.0 | 39.2 |
| C ³ | 10.1 | 28.3 |
| SEM | 0.346 | 1.20 |
| P-value | NS | NS |

A¹, B² and C³: un-treated tap water, tap water boiled by electrical heater then cooled and tap water treated by microwave oven then cooled.

NS: non significant.

SEM: standard error of the means.

Table 4 The effects of treated drinking water on body internal parameters of Japanese quails

| Treatment | Carcass (g) | Internal organs (g) | | | Small intestinal segments (cm) | | |
|----------------|------------------|---------------------|--------------------|--------------------|--------------------------------|-------------------|--------------------|
| | | Liver | Spleen | Bursa of fabricius | Duodenum | Jejunum | Ileum |
| A ¹ | 188 ^a | 7.72 ^a | 0.151 ^a | 0.246 ^a | 11.4 ^a | 29.6 ^a | 25.0 ^a |
| B ² | 183 ^a | 7.90 ^a | 0.149 ^a | 0.174 ^b | 11.1 ^a | 27.5 ^a | 22.8 ^{ab} |
| C ³ | 172 ^b | 5.56 ^b | 0.120 ^b | 0.232 ^a | 9.82 ^b | 25.8 ^b | 19.7 ^b |
| SEM | 1.76 | 0.255 | 0.005 | 0.008 | 0.180 | 0.449 | 0.607 |
| P-value | ** | ** | * | ** | ** | ** | ** |

A¹, B² and C³: un-treated tap water, tap water boiled by electrical heater then cooled and tap water treated by microwave oven then cooled.

The means within the same column with at least one common letter, do not have significant difference ($P > 0.05$).

* ($P < 0.05$) and ** ($P < 0.01$).

SEM: standard error of the means.

Table 5 The effects of treated drinking water on small intestinal morphology of Japanese quails

| Treatment | Villus height (μm) | | | Crypt depth (μm) | | |
|----------------|---------------------------------|------------------|------------------|-------------------------------|---------|-------------------|
| | Duodenum | Jejunum | Ileum | Duodenum | Jejunum | Ileum |
| A ¹ | 868 ^a | 437 ^a | 253 ^a | 24.0 ^b | 14.2 | 10.7 ^b |
| B ² | 806 ^b | 357 ^b | 262 ^b | 24.3 ^{ab} | 14.6 | 11.2 ^a |
| C ³ | 761 ^c | 313 ^c | 203 ^c | 25.1 ^a | 15.0 | 11.2 ^a |
| SEM | 8.8 | 10.4 | 3.80 | 0.160 | 0.220 | 0.080 |
| P-value | ** | ** | ** | * | NS | ** |

A¹, B² and C³: un-treated tap water, tap water boiled by electrical heater then cooled and tap water treated by microwave oven then cooled.

The means within the same column with at least one common letter, do not have significant difference ($P > 0.05$).

* ($P < 0.05$) and ** ($P < 0.01$).

NS: non significant.

SEM: standard error of the means.

Water molecule is composed of two hydrogen and one oxygen atoms via covalent bond. Water structure consists of water clusters, involving up to hundreds of bonded with each other by hydrogen bonds (Eisenberg and Kauzman, 1969) and create a crystalline-like structure that is called water cluster. The free spaces between clusters are fulfilled by un-bonded, single water molecules (Eisenberg and Kauzman, 1969). Molecules that participate in water cluster are not capable of movement but those that are not a part of cluster-like structure can easily move. The solubility of solids in water is accompanied by free solute molecules moving from one empty cavity to another within a liquid lattice (Wong *et al.* 2009). The process of dissolution proceeds through migration of molecules into the cavity of liquid dissolution medium and subsequent formation of bonding between drug and water molecules (Aulton, 2002).

It has been assumed that microwave irradiation caused breaking of the hydrogen bonds between water molecules in water clusters, which lead to the destruction of large water clusters and generation of smaller clusters and free un-bonded water molecules. Technically, a higher fraction of free cavity may be formed in bulk water following the transmission of microwave to water which results in mobility of water molecules, local superheating and lost of crystalline structure of water network (Pan *et al.* 2001). It has been reported that some chemical reactions carried out under microwave treatment run even 200 times faster (Wong *et al.* 2007), thus this could be the responsible of microwave radiation on water structure.

Increasing motility in microwave treated water cause breaking of hydrogen bonds between water molecules, thereafter decreasing of clusters size, increasing of number of free molecules and number of hydroxyl ions (Walczak and Dziuban, 2004). This mechanism could be the responsible of electrical conductivity reported by Wong *et al.* (2009). They also mentioned that treatment of water by microwaves lead to higher drug solubility maybe due to the reduction of cluster size and increasing of number of free molecules and their motility (Wong *et al.* 2007). Water cluster has dipolar structure, mean one side positive and the other side has negative charge. Solubility of water especially for polar substances is related to this structure (Lehninger *et al.* 2008). Those molecules which participate in structure of cluster are not dipolar, microwave treatment cause free and non-polar molecules.

CONCLUSION

According to some remarkable changes observed in microwave-treated drinking water drank birds but not in heat-treated drinking water drank group, one consider that these findings are not simple effects of heat to boil water and

treatment by microwave may has the effects beyond simple heat on water to boil. Approval or disapproval of such findings needs to be further study considering physiological and biochemical aspects.

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