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Original Article

Effect of supplementing magnesium picolinate in drinking water on growth performance, meat quality and cecal *E. coli* of broiler reared under tropical conditions

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Abstract

The effect of Mg supplementation in drinking water was evaluated on the growth performance, carcass quality, meat quality and cecal *E. coli* level of Broilers reared under Tropical Conditions. A sample of 192 male broiler chickens (Ross 308) were divided into 2 groups (each group consisting of 8 replicates of 12 chicks each) as: 1) control group, without Mg supplementation in the drinking water; and 2) Drinking Mg group, 10 mg of Mg/L was added into 1 L of their drinking water. It was shown that supplementing Mg in the drinking water significantly reduced body weight (P = 0.04) and tended to decrease feed intake (P = 0.08) between 1-21 days of age, and significantly reduced water intake (P = 0.02) and tended to reduce body weight (P = 0.06) between 21-35 days of age. For the overall period (1-35 days), water intake significantly decreased (P = 0.02) and the mortality rate tended to increase (P = 0.06) when Mg was supplemented in the drinking water. Carcass and meat quality were not significantly influenced by Mg supplementation. Furthermore, the *E. coli* population in the caecum was not significantly changed by the supplementation. In conclusion, supplementing Mg in drinking water at 10 mg/L showed negative effects on growth performance due to the reduction of water intake but had no significant effects on carcass and meat quality nor on *E. coli* in the caecum.

Keywords: Magnesium, Growth performance, Meat Quality, Microbial Ecology, Broiler

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Introduction

A decrease in feed intake, poor growth performance, low nutrients utilization and high mortality of chickens are commonly found under heat stress conditions (Siegel, 1995). Belay and Teeter (1996) reported that due to high excretion levels of minerals, broilers exposed to high temperature (35°C) reduced their retention of both Mg and Zn compared with birds housed at 24°C. Therefore, the reduction of feed intake and increased excretion of minerals adversely affected production performance, nutrient requirements, meat quality and the health status of broiler chickens.

Magnesium is required for cell metabolism and bone development (Shastak and Rodehutscord, 2015). Approximately 50% of Mg is found in bone, 49% is found in body tissue and organs, with only 1% of Mg in the blood (Rude, 1998). Although it has been suggested that the Mg requirements of broiler chickens should not exceed 0.6 g/kg diet (NRC, 1994), and Aviagen (2014) suggested the Mg requirement for ROSS 308 (all plant-protein based feeds) is around 0.05-0.30% of diet or 0.5-3.0 g/kg diet, the requirement may be increased due to stress. However, excess Mg intake affects the osmolarity of the intestinal contents and water reabsorption and leads to diarrhea (van der Hoeven-Hangoor *et al.*, 2013).

Since Mg deficiency is related to oxidative stress in chicks (Yang *et al.*, 2006), Mg supplementation may reduce the negative effect of stress in animals (Sahin *et al.*, 2005) and have beneficial effects on meat quality (D'Souza *et al.*, 1998). Yang *et al.* (2012) reported that dietary $MgSO_4$ supplementation prevents heat stress-induced oxidative damage and improves the growth performance in broilers due to the restoration of the activity of anti-oxidative enzymes. Normally, the volume of drinking water intake increases, while feed intake declines when chickens are exposed to heat stress. Hence, supplementation of Mg in drinking water to prevent some negative effects of heat stress may be more applicable than supplementation in feed. For example, increasing the water Mg concentration to 100 ppm during 1-3 weeks of age improves the feed efficiency and increases the incidence of swollen hocks and shortened tibia (Atteh and Leeson, 1983).

In addition, Mg is an inexpensive metal that can reduce the growth of aerobic bacteria (Robinson *et al.*, 2010). Robinson *et al.* (2010) in their *in vitro* study, using $MgCl_2$ as the source of Mg^{2+} ion reported an antibacterial effect of Mg on aerobic bacterial organisms (*E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). Tabassum *et al.* (2014) reported that the growth of bacteria is completely inhibited when a higher concentration (0.1 g /ml) of MgO nanoparticles is added to 100 ml of water sample with 5 ml of *E. coli* culture. Moreover, Stoimenov *et al.* (2002) stated that MgO nanoparticles and magnesium (Mg) nanoparticles are very effective biocides against Gram-positive and Gram-negative bacteria (*E. coli* and *Bacillus megaterium*) and bacterial spores (*Bacillus subtillus*).

Magnesium picolinate has generated interest because it is very inexpensive and can easily be made into a liquid supplement (Hrkal, 2013). However, there have been no research trials supporting its specific growth performance, meat quality or its health benefits for livestock and poultry. Therefore, this study was conducted to investigate the effect of adding Mg picolinate to drinking water on the growth performance, meat quality and *E. coli* in the caecum of broiler chickens under tropical conditions.

Materials and Methods

Animals and Management: The experimental animals were kept, maintained, treated and handled in accordance with accepted standards for the humane treatment of animals under license number U1-07385-2561. The 192 male broiler chickens (Ross 308) used in this experiment were located in an open-house system from day 1 to day 35 of age. The average daily environmental temperature ranged from 26.14 ˚C to 33.48 ˚ C. The average temperature and relative humidity during the trial were 29.81 ˚C and 82.88 %, ***respectively***. The experimental chickens were divided into 2 groups with 16 replications (12 birds/replicate). Management and vaccination were provided according to commercial practices. Water and feed were offered *ad libitum* throughout the experiment.

Mg Supplementation in Drinking Water: In order to evaluate the effect of Mg in drinking water on the growth performance, carcass quality, meat quality and some bacterial content in the caecum, drinking water was provided until 35 days of age as follows:

1) Control group: Mg picolinate was not added to the drinking water
2) Drinking Mg group: Mg picolinate was added to the drinking water (10 mg/L or 10 ppm).

Under these conditions (10 ppm), the increase of Mg intake from drinking water would not be higher than 15% of the control group as long as feed intake was not affected.

Water intake was calculated from water consumption and water loss measurements. To avoid possible water loss via evaporation, water consumption was measured using a water meter from closed water tanks and water loss was regularly collected (four times daily) from a tray under the pen throughout the experimental period. Water intake was calculated from the water consumption and water loss measurements.

Experimental Diets: All the chicks were fed a starter diet (age 1-21 days) and a grower diet (age 22-35 days). The experimental diets were formulated to meet the recommended nutritional requirements of the Ross 308 broiler strain as shown in Table 1.

Determinations

Growth Performance: The body weight gain (BWG), feed intake (FI) feed conversion ratio (FCR) and water intake were determined. The birds were weighed at 1, 21 and 35 days of age. Feed and water consumption were determined during 1-21 and 22-35 days of age. Mortality was checked twice daily; the weight of any dead birds was used to adjust the FCR.

Carcass and Meat Quality: At 35 days of age, feed was removed for 12 hours before processing. Two broilers

from each replication (16 chickens) were killed using CO_2 asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO_2). The breast meat, thigh, wings and drumsticks were collected, weighed and calculated as a percentage of the live body weight.

The breast muscles were refrigerated overnight at 4 °C and then brought to room temperature before cooking. The breast muscle from each bird was cooked to an internal temperature of 70 °C measured using a digital thermostat water bath (HH-4, Jiangbo Instrument, Jiangsu, China). The end-point internal temperature was monitored using a thermometer. Cooked muscle was cooled to room temperature. Slices (1 cm × 1 cm) were cut perpendicularly to the fiber orientation of the muscle. Ten 1 cm × 1 cm cores about 3 cm. thick were removed parallel to the fiber orientation from the thickest portion of the cooked muscle. Warner-Bratzler shear force was determined using an Instron Universal Mechanical Machine (Instron model 4411, Instron Corp., Canton, MA). A Warner-Bratzler apparatus was attached to a 50 kg load cell and tests were performed at a cross head speed of 127 mm/min. Signals were processed using the Instron Series nine software package (Jiang *et al.*, 2007).

Table 1 Nutrient composition of experimental diets

Item	Starter 1-21 days	Grower 22-35 days
Ingredient		
Corn	52.27	60.04
Rice bran oil	4.05	5.65
SBM 48%	38.91	30.33
L-Lysine	0.30	0.22
L-threonine	0.13	0.06
DL-Methionine	0.41	0.33
MCP-22	1.75	1.37
Limestone	1.35	1.14
Vitamin and Mineral Premix	0.24	0.24
Antioxidant	0.06	0.06
Salt	0.54	0.56
Total	100	100
Nutrients by calculation		
ME for poultry Kcal/Kg	3000.00	3200.00
Crude protein, %	23.08	19.50
Dry mater, %	88.70	88.75
Fiber, %	3.64	3.32
Fat, %	6.63	8.33
Methionine, %	0.75	0.63
Methionine+Cystine, %	1.08	0.91
Lysine, %	1.44	1.16
Threonine, %	0.97	0.78
Tryptophan, %	0.26	0.21
Calcium, %	0.96	0.79
Total P, %	0.79	0.67
Available P, %	0.48	0.39
Na, %	0.25	0.25

Vitamin & mineral premix content (composition per kg): Vitamin A 12,000,000IU, Vitamin D 3,000,000IU, Vitamin E 15,000 IU, Vitamin K 1500 mg, Thiamine 1,500 mg, Riboflavin 5,000 mg, Pyridoxine 2,000 mg, Niacin 25,000 mg, Vitamin B 504 mg, Pantothenic acid 8,000 mg, Folic acid 3,000 mg, Biotin 120 mg, Choline chloride 160 mg, Antioxidant 30 g, Manganese 80 g, Zinc 60 g, Iron 40 g, Copper 8 g, Iodine 0.50 g, Selenium 100 mg, Cobalt 100 mg

Meat color was measured 45 mins postmortem using a chroma meter (CR-410, Minolta Co. Ltd, Suita-shi, Osaka, Japan) to measure the CIE LAB values (L^* measures relative lightness, a^* measures relative redness and b^* measures relative yellowness). A reading was taken at the surface of the sample, representing the whole surface of the muscle. A white tile (L^* 92.30, a^* 0.32, b^* 0.33) was used as the standard (Jiang *et al.*, 2007).

Caecum Bacterial Count: The contents in the caecum were collected immediately after exsanguination, placed into sterile centrifuge tubes, put on ice and transported (within one hour of collection) to the laboratory for bacterial enumeration.

DNA Extraction: DNA extraction was carried out according to the studies of Chen and Kou (1993); Yu and Morrison (2004); Chanyalew and Loongyai (2013). Approximately 0.5 g of digesta was used to extract DNA using a QIAamp DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany). Bacterial DNA was precipitated by adding 200 µl of ethanol and captured on a spin column. Then, washing buffer AW1 and AW 2 were added in the spin column consecutively and eluted in 50 µl of TE buffer. After DNA extraction, the DNA sample concentration was measured using a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnology GmbH, Erlangen, Germany). Finally, the DNA obtained was dissolved in 10–20 µl of distilled water.

Primers and real-time polymerase chain reaction (real-time PCR): The targeted populations were all bacteria and *E. coli*. All primer set sequences are shown in Table 2. The total volume (10 µl) of PCR reaction mixture

containing 1 µl of DNA template, 0.5 µl of each primer, 6 µl of deionized water and 2 µl of 5x HOT FIREPol® qPCR Mix Plus (no ROX). HOT FIREPol® qPCR Mix Plus (no ROX) comprised all the components necessary to perform qPCR including the HOT FIREPol® DNA polymerase, ultrapure dNTPs, $MgCl_2$ and EvaGreen® dye. The real-time PCR conditions for the *E. coli* were: denaturation for 15 minutes at 95° C followed by 40 cycles of denaturation at 95° C for 30 seconds, annealing at 62° C for 30 seconds and extension at 72° C for 30 seconds, with a final 10 minutes incubation at 72° C alloweing for the completion of primer extension after the last cycle. For the *E. coli* samples, the real-time PCR conditions were similar to those for the total bacteria except for the annealing temperatures which were 60 °C. When bacterial amplification finished, the threshold cycle* (CT) values were averaged and used to determine the number of bacteria. A standard curve was used for serial dilution of bacterial DNA in each strain at a known concentration. The genomic size of bacteria was used to calculate the corresponding number of bacteria (Dumonceaux *et al.*, 2006). Bacteria were reported as log CFU/g of digesta.

Statistical analysis: A t-test was used to compare measurement values obtained from the two independent groups on growth performance, microbial ecology and meat quality of broiler chickens. The microbial populations were log transformed before statistical analysis. Statements of statistical significance were based on P<0.05.

Table 2 PCR primer sets for real-time PCR

Target species	Primer sequence (5′ – 3′)	Size (bp)	Reference
Total bacteria	F: CGG YCC AGA CTC CTA CGG G R: TTA CCG CGG CTG CTG GCA C	200	Lee *et al.* (1996)
E. coli	F: GCG AAA ACT GTG GAA TTG GG R: TGA TGC TCC ATA ACT TCC CTG	252	Cebula *et al.* (1995)

Results

The effects of supplementing Mg picolinate in the drinking water on the growth performance of broiler chicks are presented in Table 3. During the starter period (age 1-21 days), the growth rate of the chicks was significantly decreased by adding Mg in drinking water (P<0.05), and feed intake also tended to decrease (P=0.08). The Mg intake significantly increased with supplemental Mg picolinate in the drinking water (P<0.05). The FCR, water intake and mortality were not significantly affected by adding Mg to the drinking water during this period. During the grower period (22-35 days), the volume of water intake was significantly reduced (P=0.02), the water intake: feed intake ratio decreased (P<0.05) and the Mg intake increased (P<0.05) by adding Mg to the drinking water. Moreover, weight gain tended to decrease (P=0.06). The FCR, growth rate and mortality were not significantly affected by adding Mg to the drinking water. For the overall period (age 1-35 days), adding Mg picolinate to the drinking water significantly increased the Mg intake (P<0.05), decreased the water intake (P=0.02) and tended to increase the mortality rate (P=0.06), while other parameters were not significantly different.

The effects of adding Mg picolinate to the drinking water on the carcass and breast meat quality of broiler chicks are presented in Table 4. The carcass weight of the control group was significantly heavier than that of the group with Mg added to the drinking water (P = 0.03), while the carcass yield was not significantly different. There were no significant differences between the two groups for the percentage of breast meat, wing and thigh. However, adding Mg to the drinking water seemed to decrease the drumstick yield (P = 0.06). There were no significant effects of Mg supplementation in the drinking water on drip loss, cooking loss, thawing loss, shear force and color of the breast meat ($P > 0.05$).

The effects of adding Mg to drinking water on the caecum bacterial count of broiler chicks are presented in Table 5. The supplementation of Mg picolinate in the drinking water tended to increase the population of total bacteria (P = 0.08), while there was no effect on the *E. coli* level.

Discussion

Mg is an important cofactor in major metabolic pathways in the body (NRC, 2005) such as oxidative phosphorylation (Vitale *et al.*, 1957) and the formation of ATP (Leeson and Summers, 2001). Supplemental Mg picolinate in the drinking water retarded the growth rate of chicks during the starter period (1-21 days of age), reduced water intake and reduced the water/feed intake ration during the grower period. Although high ambient temperatures adversely influence mineral metabolism (Belay and Teeter, 1995), adding Mg in water failed to improve the performance of broiler chicken in the current study. The requirement of Mg of ROSS 308 strain has been recommended widely (starter period=59.15-355 mg/day/bird; grower period=106.75-640.50 mg/day/bird) (Aviagen, 2014). However, it seems that 238 mg/day/bird (starter period) and 365 mg/day/bird (grower period) of Mg intake (+15% from dietary Mg intake) negatively affected the growth rate and water intake when the Mg picolinate concentration in the drinking water was 100 ppm.

Accordingly, Atteh and Leeson (1983); Gaal *et al.* (2004) suggested that Mg should not be supplemented with very young animals. Feeding excessive Mg (1.4% $MgCO_3$ of diet) lead to skeletal abnormalities (Lee and Britton, 1980). Lee and Britton (1980) also found that feeding excess Mg (0.9% of diet) reduced the growth and bone development of broilers. Similarly, increasing the Mg intake (from 0.15 to 0.91% diet) reduced the productive performance and eggshell quality of laying hens (Hess and Britton 1997). These reports suggest that supplementation of Mg at levels higher than that recommended could have negative

effects on the productive performance of young chickens (1-3 weeks of age). The reduction in drinking water intake and water intake: feed intake ratio may increases chickens susceptibility to high environmental temperatures (increased mortality rate). This may illustrate that high Mg intake depresses water intake that may be related to the excess Mg intake negatively influencing water reabsorption, digesta osmolarity and the gut digesta transit time (van der Hoeven-Hangoor *et al.*, 2013; Vu *et al.*, 2000; Etheridge *et al.*, 1984; Lee and Britton 1983).

Table 3 Effects of Mg supplementation in drinking water on growth performance of broiler chicks

Item	Control group	Added Mg to drinking water group	*P*-value	SEM
Starter (age 0-21 d)				
Initial weight (g/bird)	47.00 ±0.10	47.00 ± 0.10	1.00	0.00
Final weight (g/bird)	1193.21 ± 22.82	1161.82 ± 30.71*	0.04	7.69
Body weight gain (g/bird)	1146.21 ± 22.82	1114.82 ± 30.72*	0.04	7.69
Feed intake (g/bird)	1375.95 ± 35.02	1339.33 ± 42.77	0.08	10.56
Total Mg intake (mg/bird)	206.39 ± 5.25	238.00 ± 6.43*	<0.01	4.32
Feed conversion ratio	1.20 ± 0.03	1.20 ± 0.03	0.87	0.01
Water intake (ml/bird)	3808.56 ± 191.4	3710.89 ± 103.34	0.22	39.24
Water/feed intake ratio	2.76 ± 0.11	2.77 ± 0.11	0.90	0.02
Mortality rate (%)	2.08 ± 3.86	5.21 ± 6.20	0.25	1.31
Grower (age 21-35 d)				
Final weight (g/bird)	2353.88 ± 60.52	2264.01 ± 107.69	0.06	26.87
Body weight gain (g/bird)	1160.66 ± 66.18	1102.19 ± 111.45	0.22	26.48
Feed intake (g/bird)	2044.08 ± 63.21	2036.83 ± 151.00	0.90	27.97
Total Mg intake (mg/birds)	306.61 ± 9.48	365.06 ± 3.22*	<0.01	8.67
Feed conversion ratio	1.76 ± 0.09	1.86 ± 0.12	0.10	0.03
Water intake (ml/bird)	6719.74 ± 480.92	5954.39 ± 693.17*	0.02	174.71
Water/feed intake ratio	3.29 ± 0.24	2.93 ± 0.38*	0.04	0.09
Mortality rate (%)	2.18 ± 4.04	5.49 ± 8.01	0.31	1.59
Overall (age 1-35 d)				
Final weight (g)	2353.88 ± 60.52	2264.01 ± 107.69	0.06	26.87
Body weight gain (g/bird)	2296.88 ± 76.10	2209.13 ± 120.69	0.10	26.87
Feed intake (g/bird)	3420.03 ± 59.67	3355.01 ± 145.53	0.26	2.14
Total Mg intake[a] (mg/bird)	513.00 ± 8.94	599.90 ± 9.53*	<0.01	11.80
Feed conversion ratio	1.49 ± 0.04	1.52 ± 0.06	0.28	0.01
Water intake (ml/bird)	10528.30 ± 621.82	9665.28 ± 705.98*	0.02	195.51
Water/feed intake ratio	3.07 ± 0.15	2.89 ± 0.28	0.12	0.06
Mortality rate (%)	4.17 ± 6.30	11.46 ± 7.63	0.06	1.93

Values presented as mean ±SD

*Means within a row with different letters indicate a significant difference (P<0.05)

[a]Total Mg intake = dietary + watery Mg intake

Table 4 Effects of magnesium supplementation in drinking water on carcass and breast meat quality of broiler chicks

Item	Control	Added Mg to drinking water group	P-value	SEM
Live weight (g)	2429.25±102.67	2329.25 ± 108.57	0.07	28.59
Carcass weight (g)	2054.94± 84.92	1933.34 ± 116.97*	0.03	29.25
Carcass yield %	84.61 ± 2.12	83.42 ± 1.83	0.26	0.52
Breast meat %	22.38 ± 0.84	21. 64 ± 1.67	0.27	0.33
Wing %	7.18 ± 0.19	7.16 ± 0.26	0.84	0.05
Thigh %	11.61 ± 0.37	11.51 ± 1.10	0.81	0.19
Drumstick %	10.36 ± 0.36	9.85 ± 0.59	0.06	0.13
Breast Meat Quality				
Drip loss (%)	2.61 ± 0.33	2.58 ± 0.50	0.88	1.10
Cooking loss (%)	29.72 ± 1.79	30.32 ± 1.72	0.50	0.43
Shear force (kg)	35.81 ± 11.81	36.31 ± 8.90	0.45	2.05
Thawing loss (%)	4.76 ± 0.93	4.50 ± 1.13	0.62	0.25
Color				
*L**	47.15 ± 2.24	46.30 ± 4.54	0.64	0.87
*a**	2.16 ± 0.80	2.02 ± 0.60	0.69	0.17
*b**	9.55 ± 0.94	9.46 ± 0.92	0.84	0.22

Values presented as mean ±SD

*Means within a row with different letters indicate a significant difference (P<0.05)

Table 5 Effects of Mg supplementation in drinking water on caecum bacterial count of broiler chicks (log CFU/g).

Item	Control	Added Mg to drinking water group	P-value	SEM
Total bacteria	12.48 ± 0.27	12.71 ± 0.25	0.08	0.06
E. coli	10.04 ± 0.79	10.31 ± 0.36	0.39	0.15

Values presented as mean ±SD

Several researchers have reported the beneficial effect of Mg supplementation on carcass and meat quality in pigs. Supplemental Mg decreases lipid and muscle tissue peroxidation and subsequently improves meat quality (Guo *et al.*, 2003), and furthermore, it reduces the stress-induced catecholamine secretion and then inhibits glycogen breakdown and glycolysis (D'Souza *et al.*, 1998 and Schaefer *et al.*, 1993). Chronic dietary Mg-fumarate (chelate form) supplementation improves meat quality as indicated by higher initial muscle pH and conductivity values and less pale pork meat (Otten *et al.*, 1992). Dietary Mg-aspartate supplementation can also greatly improve meat quality in "stressed" pigs, as evidenced by the reduced percentage drip loss and incidence of pale, soft, and exudative (PSE) carcasses in negatively handled pigs (Schaefer *et al.*, 1993) and reduced drip and cooking losses (Apple *et al.*, 2005). However, there has been no report of the effects of Mg picolinate on poultry meat quality and stability against peroxidation. Since supplemental Mg in the drinking water did not affect any carcass or meat quality parameters measured in the current study, it may be that there are differences in the effect of Mg on the meat quality of pork compared to chicken.

In the past, the growth of cells of *E. coli* was studied in media with varying amounts of Mg by Lusk *et al.* (1968). More recently, the *in vitro* study by Robinson *et al.* (2010) reported an antibacterial effect of $MgCl_2$ on aerobic bacterial organisms (*E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) where the mechanism appeared to be an alkaline pH (9.8-10.3) similar to fluoroquinlone**.** The growth of *E. coli* was completely inhibited when a higher concentration (0.1 g /ml) of MgO nanoparticles was added to the drinking water (Tabassum *et al,* 2014). However, in the current study, there was no significant effect of Mg picolinate on *E. coli* levels in the caecum of broiler chickens raised under tropical conditions. This may have been due to the addition of the Mg picolinate in the drinking water not having any alkalinity (pH = 7.5) and the particle size of this picolinate form dose not confering any activity against *E. coli.*

In conclusion, supplementation of Mg picolinate in the drinking water did not improve the growth performance or the carcass and meat quality and did not decrease the cecal *E. coli* level in broiler chickens under tropical conditions. Moreover, an excessive Mg intake from the drinking water reduced water consumption.

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