Effects of Dietary Garlic Powder and Copper on Cholesterol Content and Quality Characteristics of Chicken Eggs

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ABSTRACT : This experiment was conducted to investigate the dietary effect of garlic powder (GP) and copper (Cu) on laying performances and the cholesterol content and quality characteristics of eggs during storage in laying hens. A total of one hundred and eighty, 50-wk-old, Hy-Line Brown layers were divided into 6 groups with 3 replicates per group (10 layers per replicate) and fed one of six diets containing GP 0%, GP 1%, GP 3%, GP 5%, Cu 200 ppm, or GP 3%-Cu 200 ppm for 5 wks. There were no differences in the laying performances and feed intakes bertween treatments. Eggshell strength, eggshell thickness and yolk color were also not affected by feeding of GP and Cu. With increasing dietary GP, Haugh unit was linearly increased after 2 wk of storage ($p<0.05$). The levels of serum total cholesterol in hens fed diets containing GP or Cu were lower than that of the control ($p<0.05$), but high density lipoprotein-cholesterol was not influenced by dietary GP or Cu. The content of egg yolk cholesterol from hens fed diets containing GP or Cu was significantly decreased from that of the control, except for the GP 1% group. Based on the results of this experiment, the decrease of Haugh unit during storage was alleviated by feeding of GP. The feeding of GP or Cu alone and in combination altered the cholesterol fractions in serum and reduced the content of egg yolk cholesterol in laying hens. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 4 : 582-586)***

Key Words : Garlic Powder, Cupric Sulfate, Haugh Unit, Serum Cholesterol, Egg Yolk Cholesterol, Layers

INTRODUCTION

Average consumption of eggs in the developed country has steadily decreased. It is partly due to a concern that dietary cholesterol may contribute to the incidence of coronary heart disease (Waldroup et al., 1986), although this concern is already under strong debate (Brisson, 1984). The negative concerns related to yolk cholesterol have stimulated the researches to decrease cholesterol content in egg yolk (Reddy et al., 1991).

Many researchers have paid interests on various natural substrates as a cholesterol lowering agent (Santoso et al., 2005). Garlic (*Allium sativum*) is widely spread and already used in all parts of the world as a spice and herbal remedy for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases (Konjufca et al., 1997). Plasma cholesterol as well as hepatic total lipid and cholesterol were reduced by dietary garlic in rats (Myung et al., 1982). Qureshi et al. (1983a, b) reported that the various garlic extracts reduced the level of serum cholesterol in chickens through the inhibition of the rate limiting enzymes in cholesterol and lipid synthesis. Egg yolk cholesterol was also reduced when layer were fed diet containing 1 or 3% garlic powder for 3 wks (Sharma et al., 1979). Chowdhury et al. (2002) reported that dietary garlic paste at various levels reduced egg yolk cholesterol in dose-dependent manner. Birrenkott et al. (2000), however, demonstrated that the levels of cholesterol in egg yolk and serum were not decreased significantly by 3% garlic powder after feeding of several months.

It has been known that copper (Cu) deficiency was shown to induce hypercholesterolemia in rats (Klevay, 1973). Dietary Cu, at pharmacological level, has demonstrated to alter the lipid metabolism of rats (Petering et al., 1977), swine (Amer and Elliot, 1973) and chicken (Bakalli et al., 1995). Many reports showed that dietary garlic or Cu had lowered the levels of cholesterol in plasma and liver in various species, including poultry. But only limited information is available on the combined effect of garlic and Cu on yolk cholesterol in avian species. The object of this study was to determine the dietary effects of the single or combination feeding of garlic and Cu on laying performances, the cholesterol content and quality characteristics of egg during storage.

MATERIALS AND METHODS

Garlic bulb harvested in May or June was provided by the Eui-Sung local National Livestock Co-operation Federation. The fresh garlic bulbs with husk were sliced and thinly spread on a mat under sunlight at 30 to 35°C for a day. And then they were dried under 50°C in drying oven and ground finely to powder. The dried garlic powder (GP) used in this study contained 99.2 g moisture/kg, 166.6 g crude protein/kg, 4.9 g crude fat/kg, 80.4 g crude fiber/kg

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Table 1. Formula and chemical composition of experimental diets[1]

Ingredients	Control	GP 1%	GP 3%	GP 5%	Cu	Cu+GP 3%
	---------- % ----------					
Yellow corn	57.21	57.21	57.21	57.21	57.21	57.21
Corn gluten meal	3.00	3.00	3.00	3.00	3.00	3.00
Soybean meal	17.34	17.34	17.34	17.34	17.34	17.34
Limestone	8.42	8.42	8.42	8.42	8.42	8.42
Dicalcium phosphate	1.13	1.13	1.13	1.13	1.13	1.13
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Lysine-HCl	0.19	0.19	0.19	0.19	0.19	0.19
DL-methionine	0.14	0.14	0.14	0.14	0.14	0.14
Choline-chloride	0.07	0.07	0.07	0.07	0.07	0.07
Mineral mixture[2]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mixture[3]	0.10	0.10	0.10	0.10	0.10	0.10
Wheat bran	10.00	9.00	7.00	5.00	10.00	7.00
Garlic powder	-	1.00	3.00	5.00	-	3.00
Copper	-	-	-	-	200 ppm	200 ppm
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
DM (%)			88.27			
CP (%)			16.00			
Ether extract (%)			4.73			
Crude fiber (%)			3.26			
Ash (%)			10.98			
Ca (%)			3.50			
Available P (%)			0.32			
TMEn (kcal/kg)			2,800.00			

[1] GP, garlic powder; Cu, copper.

[2] Mineral mixture provided following nutrients per kg of diet: Fe, 84 mg; Zn, 72 mg; Mn, 96 mg; Cu, 9 mg; I, 1.2 mg; Se, 0.2 mg .

[3] Vitamin mixture provided following nutrients per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,300 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3.5 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 12 mg; niacin, 30 mg; biotin, 0.12 mg; folic acid 0.7 mg.

and 143.6 g crude ash/kg. And cupric sulfate was purchase from Duksan Medicine Industrial Corporation as a copper source.

50-wk old Hy-Line Brown layers were randomly placed in three replicate pens with 10 birds each per treatment (total of 180 birds) in wire cage. The birds fed one of following six diets containing GP 0%, GP 1%, GP 3%, GP 5%, Cu 200 ppm, or GP 3%-Cu 200 ppm for 5 wks, respectively. The experimental diets were formulated to meet or exceed the nutrient requirements of NRC (1994). GP was substituted at the expense of wheat bran at 1, 3 or 5% levels on weight basis. A laboratory grade cupric sulfate pentahydrate was used as a Cu source (Table 1). Animal facilities and husbandry were similar to conditions described by An et al. (2003). The experimental diets and water were provided for *ad libitum* intake. A room temperature of 22±3°C and a photoperiod of 17/7 h light/dark cycle were maintained throughout the experimental period.

Egg production was recorded daily and experimental diets were freshly added. The eggs laid for the last three days of experiment were used for analysis of egg qualities according to the method of Hayirli et al.(2005), with some modification. The collected eggs were kept in storage temperature of 18°C during 7 or 14 days to observe the change of Haugh unit. The analysis of yolk cholesterol was measured by the modified method of Tompson and Merola (1993). In brief, two grams of egg yolk was homogenized with 40 ml of Folch solution (chloroform:methanol = 2:1, v/v) using Homogenizer (AM 77 model, Nissei, Japan) for total lipid extraction (Folch et al., 1957). Lipid extracts were dissolved by absolute alcohol and saponified with 1 ml of 5% NaOH for 1 h at 70°C. After cooling in room temperature, 5 ml of hexane was added and centrifuged and then 2 ml of upper layer was moved into silanized glass tube (Pyrex No., 13). The extracts were resolved with 1ml of dimethylformamide and mixed with 0.2 ml of hexamethydisilane and 0.1 ml of trimethylchlorsilane for derivatization. The turbid preparation obtained finally was mixed with 0.1% 5α-cholesterol as an internal standard. Egg yolk cholesterol was measured by gas-liquid chromatography (HP 5890 II Series, Hewlett Packard 6890 series injector, Hewlett-Packard, Atlanta, USA) using 0.25 mm I.D.×30 m silica capillary column (SACTM-5, Supelco Ltd., Pennsylvania, USA). The initial and terminal column temperature was programmed at 280°C without increased temperature**.** The detector was set at 250°C.

Table 2. Dietary effects of the garlic powder and copper on the laying performances, egg and egg shell quality in laying hens[1]

	Control	GP 1%	GP 3%	GP 5%	Cu	Cu+GP 3%
Egg production rate (%)	74.38±4.30[2]	70.00±5.73	69.33±3.66	79.71±5.58	84.19±3.91	73.05±3.89
Egg weight (g)	58.14±0.80	57.10±0.85	56.81±1.23	56.66±0.50	57.29±0.87	57.17±0.51
Feed intake (g/hen/day)	100.33±4.61	104.57±2.40	102.85±7.16	102.63±3.84	104.12±1.95	99.82±5.88
Egg and eggshell quality						
Egg shell strength (kg/cm^2)	3.96±0.14	3.87±0.16	3.76±0.17	3.89±0.15	3.87±0.17	3.89±0.16
Egg shell thickness (0.01 mm)	35.76±0.42	34.40±0.75	34.33±0.41	35.06±0.43	34.85±0.46	34.35±0.50
Yolk color (RCF[3])	7.83±0.24	7.92±0.12	7.87±0.14	7.76±0.14	7.77±0.11	7.72±0.12

[1] GP: garlic powder; Cu: copper. [2] Means±SE. [3] RCF: Roche color fan.

Table 3. Dietary effect of the garlic powder and copper on the change of Haugh unit during storage [1]

	Control	GP 1%	GP 3%	GP 5%	Cu	Cu+GP 3%
7 day	61.72±0.77^{b,2}	70.35±0.62^{a}	70.65±0.97^{a}	69.22±1.30^{a}	67.62±1.97^{a}	69.37±2.21^{a}
14 day	56.59±1.60^{d}	64.21±1.96^{bc}	68.97±1.66^{ab}	70.27±1.53^{a}	64.19±1.91^{bc}	65.22±1.58^{b}

[1] GP: garlic powder; Cu: copper. [2] Means±SE.

[a-d] Mean values in the same row having different superscripts are significantly different ($p<0.05$).

At the end of experimental period, 8 birds from each group were randomly selected. The blood was drawn from wing vein using sterilized syringes for determination of the various blood profiles. At necropsy, the liver was immediately removed and weighed as described by Dong et al. (2005). The concentration of total cholesterol and high density lipoprotein-cholesterol (HDL-C), the activity of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were estimated according to the colorimetric method using cholesterol diagnostic kit (Cholesterol E kit, HDL-cholesterol kit, Youngdong Medical Corporation) and GOT-GPT assay kit (BCS GOT-GPT assay kit, Bio Clinical System Corporation), following the manufacturer's direction.

The main effects between treated groups were subjected to ANOVA using the general linear models procedure of SAS (2002), and significant differences were determined using Duncan's multiple range test at the level of $p<0.05$ (Duncan, 1955). Percentage data were transformed to arc sine percentages before square root percentages ANOVA was performed.

RESULTS AND DISCUSSION

There were no significant differences in egg production rate, egg weight and feed intake in laying hens fed experiment diets as shown in Table 2. The relative liver weight was not significantly different among the treatments (data were not shown). Reddy et al. (1991) reported that egg production, egg mass, feed intake, and feed efficiency were not affected through 8 wk feeding of 0.02% garlic oil. Chowdhury et al. (2002) also observed that there were no effect of dietary garlic on egg production rate, egg weight and feed consumption. Pesti and Bakalli (1998), however, reported that dietary Cu at the level of 250 ppm had positive effect on the feed intake and egg production.

Egg shell strength, egg shell thickness and yolk color in layers fed experimental diets are shown in Table 2. Supplementation of GP and Cu did not affect the egg shell strength, egg shell thickness and yolk color, which was in agreement with results of study with layer, especially egg yolk color (Birrenkott et al., 2000).

The changes of Haugh unit during storage are shown in Table 3. The Haugh unit, an indicator of the most widely accepted measure of internal egg quality, tended to be decreased according to the elapsed time of storage as found (Williams, 1992). The Haugh unit of group fed diet containing 5% GP was highest among the eggs stored for 14 days. With increase in dietary GP, Haugh unit was linearly increased during storage ($p<0.05$). General nutrients in layer feed did not appear to have any beneficial effect on Haugh unit (Naber, 1979), but it suggested that certain natural antioxidants such as vitamin C, vitamin E and selenium being beneficial to albumen quality by it's antioxidant property (Keshavarz, 1996; Sahin et al., 2003). Rabinokov et al. (2000) reported that allicin in garlic is converted into various disulfide derivative exerting the anti-oxidative activity by reaction with sulfur containing compound. This mechanism could be suggested as a possible mechanism for the increased Haugh unit due to antioxidant effect from derivatives of allicin in garlic. The exact reason for the change of Haugh unit by feeding of GP requires further investigation, including the change of mineral and moisture contents in albumen.

The levels of serum GOT in all treated groups were significantly increased as compared with that of control group. The level of GOT in bird fed diet containing GP 3%-Cu 200 ppm was highest (Table 4). But GPT level was not affected by feeding of GP or Cu. Further studies are required to clarify whether the increase of serum GOT level is associated with the possible negative effects by dietary GP or Cu.

Table 4. Dietary effects of the garlic powder and copper on the GOP and GPT in serum, cholesterol fractions in serum and egg yolk in the laying hens[1]

	Control	GP 1%	GP 3%	GP 5%	Cu	Cu+GP 3%
GOT (IU/L)	110.40±1.87[c, 2]	126.45±3.09[b]	123.21±4.06[b]	125.18±2.25[b]	131.95±3.44[b]	142.63±3.82[a]
GPT (IU/L)	3.47±0.42	3.70±0.42	3.69±0.46	3.79±2.19	3.95±0.51	5.41±1.32
Serum						
Total-C (mg/dl)	170.41±6.15[a]	135.56±4.76[cd]	142.98±7.05[bc]	154.71±6.85[ab]	131.27±5.41[cd]	123.53±6.22[d]
HDL-C (mg/dl)	73.04±11.93	68.18±17.72	67.10±6.42	69.55±11.25	74.59±3.98	74.21±9.33
Egg yolk						
Cholesterol (mg/g)	14.79±0.27[a]	14.03±0.38[ab]	13.31±0.18[b]	13.79±0.12[b]	13.66±0.49[b]	13.12±0.28[b]
Total lipid (%)	30.53±0.73	30.72±0.45	29.16±1.06	29.77±0.55	30.24±0.82	30.06±0.92

[1] GP: garlic powder; Cu: copper; GOT: gluatmic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase. Total-C: total cholesterol; HDL-C: high density lipoprotein-cholesterol.

[2] Means±SE. [a-d] Mean values in the same row having different superscripts are significantly different ($p<0.05$).

Serum total cholesterol concentration did not responded linearly with increase levels of GP, but was decreased by single feeding of Cu or GP and combination of copper and GP (Table 4). There was further reduction in serum cholesterol concentrations when layers fed 3% GP with supplementation of 200 ppm of Cu. This is in agreement with that of Chowdhury et al. (2002) who reported that dietary 2, 4, 6 and 8% garlic paste reduced the levels of serum total cholesterol on average by 15, 28, 33, and 43%, respectively. Qureshi et al. (1983a) also showed that a various levels of dietary garlic paste linearly reduced serum cholesterol in male broiler chickens by dose-dependent manner. However 0.02% garlic oil did not affect serum total cholesterol (Reddy et al., 1991). It may be due to differences in supplemental levels, feeding duration or preparation method (e.g., organic solution extraction, alcohol extraction, simple drying, and etc). Pesti and Balkalli (1998) demonstrated that serum total cholesterol was reduced by feeding of 125 ppm or 250 ppm Cu during 4-wk and 8-wk. The concentration of High density lipoprotein-cholesterol (HDL-C), however, was not affected by feeding of Cu or GP as shown in Table 4. This result agree with Qureshi et al. (1983b) who have suggested that the level of HDL-C was not influenced by dietary garlic extracts in chickens.

The contents of total lipid and cholesterol in egg yolk are shown in Table 4. Dietary Cu or GP did not significantly reduce total lipid in egg yolk. However, the content of egg yolk cholesterol in all treated groups were significantly decreased as compared with that of control group ($p<0.05$). The cholesterol content in egg yolk was lowest when the layers fed diet contained the 3% of GP and 200 ppm of Cu. The yolk cholesterol was reduced by 4.1 or 5.5% when laying hens were fed 1 or 3% garlic powder for 3 wk (Sharma et al., 1979). Youn et al. (1998) reported that egg yolk cholesterol was reduced by feeding of 0.5% or 1.0% garlic paste. Chowdhury et al. (2002) also reported that cholesterol concentrations per gram of yolk decreased linearly with increasing levels of sun-dried dietary garlic paste. Pesti and Bakalli (1998) reported that feeding of 125 or 250 ppm of Cu significantly reduced yolk cholesterol as compare with that of control. Present study showed that the cholesterol concentration per gram of egg yolk in group fed diet containing 3% of GP and 200 ppm of Cu was lower than those of single feeding of same content of Cu or GP. It was concluded that sun-dried GP with Cu supplement at higher level can be used as a hypocholesterolemic material in layer diets without having a significant effect on overall laying performances and the quality characteristics of egg.

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REFERENCES

Amer, M. A. and J. I. Elliot. 1973. Effects of level of copper supplement and removal of supplemental copper from the diet on the physical and chemical characteristics of porcine depot fat. Can. J. Anim. Sci. 53:139-145.

An, B. K., K. H. Shinn, Y. Kobayashi, K. Tanaka and C. W. Kang. 2003. Excessive dietary conjugated linoleic acid affects hepatic lipid content and muscular fatty acid composition in young chicks. Asian-Aust. J. Anim. Sci. 16:1171-1176.

Bakalli, R. I., G. M. Pesti, W. L. Ragland and V. Konjufca. 1995. Dietary copper in excess of nutritional requirement reduces plasma and breast muscle cholesterol of chickens. Poult. Sci. 74:360-350.

Birrenkott, G., G. E. Brockenfelt, M. Owens and E. Halpin. 2000. Yolk and blood cholesterol levels and organoleptic assessment of eggs from hens fed a garlic-supplemented diet. Poult. Sci. 79(Suppl. 1):75.

Brisson, G. J. 1984. Fats and human health : A review of research. Feedstuffs Feb 20:30-35.

Chowdhury, S. R., S. D. Chowdhury and T. K. Smith. 2002. Effects of dietary garlic on cholesterol metabolism in laying hens. Poult. Sci. 81:1856-1862.

Dong, N. T. K., K. Elwinger, J. E. Lindberg and R. B. Ogle. 2005. Effect of replacing soybean meal with soya waste and fish

meal with ensiled shrimp waste on the performance of growing crossbred ducks. Asian-Aust. J. Anim. Sci. 18:825-834.

Duncan, D. B. 1955. Multiple range and multiple F test. Biometr. 11:1-42.

Folch, G., M. Lees and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Hayirli, A., N. Esenbuga, M. Macit, E. Lacin, M. Karaoglu, H. Karaca and L. Yildiz. 2005. Nutrition practice to alleviate the adverse effects of stress on laying performance, metabolic profile, and egg quality in peak producing hens: I. The humate supplementation. Asian-Aust. J. Anim. Sci. 18:1310-1319.

Keshavarz, K. 1996. The effect of different levels of vitamin C and cholecalciferol with adequate or marginal levels of dietary calcium on performance and eggshell quality of laying hens. Poult. Sci. 75:1227-1235.

Klevay, L. M. 1973. Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. Am. J. Clin. Nutr. 26:1060-1068.

Konjufca, V. H., G. M. Pesti and R. I. Bakalli. 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poult. Sci. 76:1264-1271.

Lumeiji, J. T. 1997. Avian Clinical Biochemistry. In: Clinical Biochemistry of Domestic Animals (Ed. J. J. Kaneko, J. W. Harvey and M. L. Bruss. 5th) Academic Press. pp. 857-883

Myung, S. C., T. K. Eunsook, and T. J. Stewart. 1982. Effects of garlic on lipid metabolism in rats fed cholesterol or lard. J. Nutr. 112:241-248.

Naber, E. C. 1979. The effect of nutrition on the composition of eggs. Poult. Sci. 58:518-528.

National Research Council. 1994. Nutrient Requirements of Poultry, 9th ed. National Academy Press, Washington, DC.

Rabinokov, A., T. Miron, D. Mirelman, M. Wilchek, S. Glozman, E. Yavin and L. Weiner. 2000. S-Allylmercaptoglutathione : the reaction product of allicin with glutathione possesses SH-modifying and antioxidant properties. Biochim. Biophys. Acta. 1499:144-153.

Pesti, G. M. and R. I. Bakalli. 1998. Studies on the effect of feeding cupric sulfate pentahydrate to laying hens on egg cholesterol content. Poult. Sci. 77:1540-1545.

Petering, H. G., L. Murthy and E. O'Flaherty. 1977. Influence of dietary copper and zinc on rat lipid metabolism. J. Agric. Food Chem. 25:1105-1109.

Qureshi, A. A., Z. Z. Dinc, N. Abuirmeileh, W. C. Burger, Y. Ahmad and C. E. Elson. 1983a. Suppression of avian hepatic lipid metabolism by solvent extract of garlic: Impact on serum lipids. J. Nutr. 113:1746-1755.

Qureshi, A. A., N. Abuirmeileh, Z. Z. Din, C. Elson and W. C. Burger. 1983b. Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fraction of garlic. Lipids 18:343-348.

Reddy, R. V., S. F. Lightsey and D. V. Maurice. 1991. Research note : Effect of feeding garlic oil on performance and egg yolk cholesterol concentration. Poult. Sci. 70:2006-2009.

Sahin, N., K. Sahin and M. Onderci. 2003. Vitamin E and selenium supplementation to alleciate cold-stress-associated deterioration in egg quality and egg yolk mineral concentrations of Japaness quails. Biol. Trace Elem. Res. 96:179-189.

Santoso, U. J. Setianto and T. Suteky. 2005. Effect of sauropus androgynus (Katuk) extract on egg production and lipid metabolism in layers. Asian-Aust. J. Anim. Sci. 18:364-369.

SAS. 2002. SAS User's Guide. Statistical Analysis System Institute. ver. 8.2 ed. Inc., Cary, NC.

Sharma, R. K., R. A. Singh, R. N. Pal and C. K. Aggarwal. 1979. Cholesterol content of chicken eggs as affected by feeding garlic, sarpagandha, and nicotinic acid. Haryana Agric. Univ. J. Res. 9:263-265.

Tompson, R. H. and G. V. Merola. 1993. A simplified alternative to the AOAC official method for cholesterol in multicomponent foods. J. AOAC inter. 76(5):1057-1068.

Waldroup, P. W., L. L. Ndife and H. M. Hellwig. 1986. Influence of probucol (4, 4'-Isopropylidine dithio)-bis(2, 6-di-t-butyl-phenol)) on egg yolk cholesterol content and performance of laying hens. Poult. Sci. 65:1949-1954.

Williams, K. C. 1992. Some factor affecting albumen quality with particular reference to Haugh unit score. World's Poult. Sci. J. 48:5-16.

Youn, B. S., H. S. Chae, S. C. Kim, D. W. Kim, J. N. Ahn and Y. K. Kim. 1998. Dietary effects of garlic to layers. Kor. J. Anim. Nutr. Feed. 22:357-362.