# The Effect of Adding Oxalic Acid and Phytic Acid to the Diet of Poultry on Calcium Contents of Eggshells

# Azhar Hussain QURESHI<sup>1</sup>, Mohammad Masood TARIQ<sup>2\*</sup>, Farhat Abbas BUKHARI<sup>2</sup>, Majed RAFEEQ<sup>2</sup>, Muhammad ATHER<sup>1</sup>, Yasir JAVED<sup>2</sup>, Kenneth WILSON<sup>3</sup>, Khalidullah KHAN<sup>4</sup>

<sup>1</sup>Livestock and Dairy Development Department, Quetta, Balochistan, Pakistan.

<sup>2</sup>Center for Advance Studies in Vaccinology & Biotechnology (CASVAB), University of Balochistan, Pakistan

<sup>3</sup>West Texas A&M University, Canyon, Texas, USA.

<sup>4</sup>Ministry of Education, Diversity and Equity Branch, Victoria BC V8W 9H3, Canada.

### Received (Geliş Tarihi) : 31.03.2011

# Accepted (Kabul Tarihi): 11.05.2011

ABSTRACT : The aim of this study was to evaluate the effect of adding either 0.2, 0.5 and 0.8% oxalic acid (Oa) or 1, 2, and 3% phytic acid (Pa) to the diet upon the quantity of calcium (Ca) deposited in the eggshell. Data of thirty White Leghorn laying hens at average 154 days of age were used. Five hens were randomly assigned to each of six group groups. Data were collected for 48 hours periods at the beginning and end of a seven day feeding period for two week. Numerical data collected included milli gram (mg) of calcium per gram (g) of eggshell (Es) and per egg, weight of oven dried eggshell, average daily feed intake, body weight change, and weight of egg yolk and albumen. No significant changes were determined for quantity of Ca per g of Es for either type of acid fed for the final 48 hr period. It was observed that initial to final period hens receiving Oa 0.2, 0.5 and 0.8% in their feeds had significant reduction in Ca per egg of 256, 195 and -116 mg, respectively. As the percent Oa was increased in the diet, a reduction of Ca per egg occurred (P<0.05). This change was not found with adding Pa to the diet. Hens receiving 1, 2, and 3% Pa increase of 329, 214 and 346 mg of Ca per Es. When combing the data of all levels of acid within a chemical treatment groups, those hens receiving Pa had significantly more Ca change per Es than those receiving Oa(P<0.05). The group receiving Oa had mean Ca increases per egg 176 mg and those receiving Pa had 297 mg (P<0.05). The highest percent of each chemical additive induced a significant depression of feed intake (P<0.05). In conclusion, adding Oa or Pa to the feed of hens had no significant effect on Ca content of eggshells during the final 48 hr period, however hen receiving Oa did have a reduction in Ca contents/Es from the initial to final period. As the quantity of Oa was increased in diet the Ca/Es decreased. However this level of effect was not found in groups receiving Pa. High percentages of each acid did decrease feed consumption. No significant effects were observed on body weight change, egg yolk, or albumen.

Key words: Eggshell, oxalic acid, phytic acid, calcium.

#### **INTRODUCTION**

The poultry industry in United States of America (USA), has earned from combined value of production from broilers, eggs, turkeys, and the value of sales from chickens in 2009 was \$31.6 billion, down 12 percent from the \$36.0 billion in 2008. Of the combined total, 69 percent was from broilers, 19 percent from eggs, 11 percent from turkeys, and less than 1 percent from chickens. Value of all egg production in 2009 was \$6.16 billion, down 25 percent from the \$8.22 billion in 2008. Egg production totaled 90.4 million eggs, up slightly percent from the 90.0 million eggs produced in 2008. In 2009, all eggs averaged 81.7 cents per dozen, compared with \$1.09 in 2008 (USDA 2010).

Eggshells quality is a major problem for laying hens. Poor shell quality is one of the most serious concerns facing the egg industry. Ronald (1984) reported that birds fed higher calcium levels produced eggs with improved shell quality. Roland (1977) estimated that 4.8% of eggs were lost because of eggshell problem. Hamilton (1982) reported that the annual loss to the American egg producer was \$100 million. Thin-shelled and shell-less egg are produced sporadically by most hens. This occurs more frequently during peak egg production and in older flocks (Peterson, 1965); the frequency varies among different genetic strains (Ronald, 1977). Most of the researchers have found that shell thickness decreased in summer as a result of the higher temperature. This effect also identifies in the second and third laying seasons (Jenkins and Tayler, 1960). It is well known that a calcium reserve is located in the avian egg shell. The reserve is located in millary layer and that chorioallantioc membrane of the embryo mobilizes the calcium reserve in the later phases of inoculation. In the domestic chicken, approximately 80% of calcium used during incubation comes from the eggshell (Dieckert et al., 1989). The three most common explanations (Paterson, 1965) given to explain the decline in shell quality (thickness of the eggshell) in hens include; ability to absorb calcium decreases with age, ability to mobilize skeletal calcium decreases with age genetic potential for production through years of genetic selection increases at a faster rate than hen's ability to maintain adequate shell deposition.

<sup>\*</sup>Corresponding author: Tariq, M.M., : tariqkianiraja@yahoo.com

26

It has been reported that phytate hydrolysis is affected by many factors such as dietary Ca, vitamin D<sub>3</sub>, fiber and Ca, P, type of dietary ingredients, feed processing, age, and genotype of birds (Tamim and Angel, 2003; Tamim et al., 2004). An organic acid found in certain leafy vegetables that binds with Ca and inhibits its absorption from these foods is called oxalic acid. It combines with Ca to form calcium Oxalate (insoluble) in digestive tract. The absorptive efficiency of most vegetable sources is a as good unless they have high concentrations o oxalic acid or phytic acid (Weaver 1992). Several vegetable though excellent sources of nutrition are also high in oxalic acid. If fed frequently, oxalic acid can cause health problems. When you feed a food high in oxalic acid and balance it with foods low in oxalic acid, it becomes less of a concern. Regardless, foods having high oxalic acid should not be fed on a daily basis. Although oxalic acid binds to calcium (producing calcium oxalate crystals), and making calcium unavailable for absorption, many vegetables that contain oxalic acid also contain calcium. The amount of oxalic acid is what determines how much calcium is bound. So if there is more calcium than oxalic acid present, the calcium that has not been bound will be available for use by the body. Phytates (salts of phytic acid), are formed due to combination of six phosphate molecule with inositol, a cyclic alcohol with six hydroxy radicals similar to hexose sugar. The availability of phytin P is influenced by alimentary tract pH and the level of vitamin D<sub>3</sub>, calcium, Ca to P ratio and Zn. Supplementation with adequate minerals, which are affected by Phytates, is usually practiced. Dietary supplementation of phytase enzyme (250-500 units/kg) is practiced to enhance the utilization of phytate P in poultry (Scholbe, 2002). The aim of this study was to evaluate the influence of the oxalic acid or phytic acid upon the egg shell density.

#### **MATERIAL and METHODS**

Data of thirty White Leghorn laving hens at average 154 days of age were used in a study conducted at West Texas A&M University, Canyon, Texas, USA. Five hens were weighed and randomly assigned to each of six different treatment groups. Hens were individually housed in wire-type cages. Hens were weighed at the start and end of the data collection periods. The six treatment groups consisted of adding two chemicals, oxalic acid (sodium oxalate), (0.2, 0.5 and 0.08%) or dehydrated phytic acids, (1, 2, and 3%) to the basal feed (Acadian Seaplants Limited, USA). Experiment was carried out for two week period. Egg data were collected for 48 hours after one week on basal diet prior to chemical inclusion and than the last 48 hours fallowing a one week feeding period with added chemicals. The numerical criteria included part per million (ppm) of Calcium (Ca) in the shell expressed as mg/g shell and mg/egg, weight of oven dried egg shell, average daily feed intake, body weight change, and weight of yolk and albumen. The differences between

the means of the first 48 hr and last 48 hr egg collection periods were used for statistical analysis for Ca contents per egg and body weight. Five hens were randomly assigned to each of six group groups.

Chemical analysis of Ca contents was determined with the selective Ca ion electrode and Orion 93-20 Model and Reference Electrode 90-01 Model Single Junction (Zhou et al., 1992). Egg shells were washed with distilled water, dried at 100 °C for 24hrs and dissolved in 25 ml concentration Hydrochloric acid (HCl). The solution was diluted into 1000 ml distilled water and maintains the pH at 5.0. Yolk and albumen were separated using a commercial separator. Yolk was blotted dry and weight on a Whatman filter paper. Albumen was collected in disposable plastic cup and weight. A commercial granular laying feed (Paladuro poultry feed) was provided as basal diet (Table 1). Seventy gram allotments were provided to each hen as needed. Feed remaining in the feeder at the end of trial weighed and subtracted from the data.

 Table 1. Nutrient composition of basal diet

 Sno.
 Item

Sno.	Item	Percent (%)
1	Crude protein	15
2	Crude fat	2.5
3	Crude fiber	8.00
4	Calcium	4.00
5	Phosphorus	0.60
6	NaCl	0.45
7	Metabolizable Energy (ME)	2750 Kcal/kg

Source: ACCO Feed, Inc. Minneapolis, MN.

Experimental data were statistically analyzed using General Liner Model procedure of SAS (1985). Duncan's Multiple Range test was utilized to detect significance mean differences (Steel and Torrie, 1980).

The following mathematical model was adopted: Where;

Yijkl = Ui + Tj + Ck + Tj + (Ck) + eijkl

Yijkl = Calcium concentration (mg),

- Ui = the over mean,
- $T_i = treatment (i = 1, 2),$

Ck= level of each treatment (k = 1, 2, 3)

Tj (Ck) = level within treatment,

Eijkl = the experimental error.

#### **RESULTS and DISCUSSION**

The results of the effect of adding oxalic acid or phytic acid to the poultry feed on calcium deposit in the hen eggshell are presented in table 2. Final mean calcium per gram of shell for the group receiving oxalic acid at 0.2, 0.5, and 0.8% were 440. 437, and 407 mg and those receiving 1, 2, and 3% phytic acid had 420, 444, and 414 mg/g of shell. No significant differences were observed (P>0.05).

Hens do lay eggs during a clutch which have greater amounts of shell per egg. It was reasoned that the addition of chemicals to the feedstuff might alter the calcium content of the total shell from the initial to final feeding period. Neither of the chemicals changed the quantity of calcium/g of dried egg shell (Khan and Elahi 1991). However, when expressed as change from initial to final period a significant difference was noted for calcium per egg shell (P<0.05). The findings of the present study were also supported by some authors

(Zhou *et al.*, 1992; Tamim and Angel, 2003). Hens receiving oxalic acid had significant decrease in Ca/egg as the percent acid was increased in their feed, those receiving 0.02, 0.5and 0.8% had mean changes of 256, 195 and -16 mg/egg (P<0.05). This trend was not observed at the various levels of phytic acid (Table 3).

Table 2. Mean Ca Content of Eggshell for the Final 48 hr Feeding Period (mg)

Replicates	Oxalic acid	1		Phytic Aci	d	3.0% 430			
_	0.2%	0.5%	0.8%	1.0%	2.0%	3.0%			
1	430	460	388	440	410	430			
2	420	440	410	392	460	392			
3	460	473	400	420	450	410			
4	440	392	448	460	460	400			
5	450	420	388	388	440	440			
Mean	440	437	407	420	444	414			

Table 3. Mean Total Calcium Change per Egg from Initial to Final Day on feed (mg)

Replicates	Oxalic acid		-	Phytic Aci	d	
-	0.2%	0.5%	0.8%	1.0%	2.0%	3.0%
1	102	708	-117	225	1	766
2	151	295	81	290	388	220
3	665	332	2	338	71	171
4	90	295	-52	218	195	520
5	274	276	-169	537	417	57
Mean	256 <sup>a</sup>	195 <sup>b</sup>	-16 °	329 <sup>a</sup>	214 <sup>b</sup>	346 <sup>a</sup>
Treatment		176.3 <sup>a</sup>			296.7 <sup>b</sup>	
Mean						

abc Values with in row having different superscript differ (P<0.05).

Table 4. Mean Weight of Dried Eggshel	for the Hens Receiving	Oxalic Acid and Phytic	Acid for the Final 48 hr period (g)

Replicates	Oxalic acid		Phytic Acid				
	0.2%	0.5%	0.8%	1.0%	2.0%	3.0%	
1	4.07	5.64	4.19	4.79	4.99	4.83	
2	4.86	6.27	5.35	5.51	5.34	5.52	
3	4.85	5.58	5.21	6.42	3.53	5.90	
4	5.54	4.63	5.00	3.44	4.61	5.49	
5	5.54	5.86	5.23	6.41	4.52	4.92	
Mean	4.97	5.60	5.00	5.31	4.60	5.37	

The results of the present study were consistent with findings of many researchers who (Dieckert *et al.*, 1989; Tamim and Angel, 2003; Tamim *et al.*, 2004) reported that birds receiving phytic acid had significantly effect on Ca increase per egg.

No significant differences were detected among the level or treatment means for the weight of the complete eggshell obtained during the final 48 hr feeding period. Means for oxalic acid and phytic acid were estimated as: (4.97, 5.60 and 5.00 g) and (5.31, 4.60 and 5.37 g), respectively (Table 4).

Less feed was consumed by the hens receiving the highest percent of each acid added (P<0.05). Continued decline in the feed consumption would be expected to eventually decrease the quantity of all egg components, similar findings were observed by Tamim and Angel 2003. This indicates that the feeding period for this test has been extended.

Means body weight changes for initial to final period for groups receiving oxalic acid and phytic acid was determined non significant (P>0.05). No significant differences were also noted for the quantities of egg yolk and albumen produced per egg during the final 48 hr period. Egg yolk means for those receiving oxalic acid were (16.79, 18.19 and 16.54g) and phytic acid were (18.01, 17.10 and 17.34 g) respectively (Table 5). Albumen means those receiving oxalic acid were (33.01, 39.48 and 33.76 g) and for phytic acid (38.2, 33.43 and 38.81g), respectively (Table 6).

### CONCLUSION

In conclusion, no effect of adding oxalic or phytic acid to the feed of hens on Ca contents of eggshells during the final 48 hrs period was found. Hens receiving oxalic acid did have a reduction in Ca per eggshell from the initial to final period. As the quantity of oxalic acid was increased in the diet, the Ca per eggshell declined. However this level was not obtained in the groups receiving phytic acids. It is important to note that no significant differences were found for mg of Ca per gram of shell and total gram per complete shell for the final 48 hr period and no differences were observed for body weight change, eggs yolk, and albumen.

Table 5. Mean Weight of Egg Yolk for the Hens Receiving Oxalic Acid and Phytic Acid for the Final 48 hr period (g)

Replicates	Oxalic acid			Phytic Acid	ł		
	0.2%	0.5%	0.8%	1.0%	2.0%	3.0%	
1	14.39	18.50	16.46	17.47	18.12	15.98	
2	16.	17.50	16.98	18.35	16.24	16.96	
3	17.73	18.19	15.98	19.84	16.30	19.23	
4	16.42	18.80	16.54	14.86	17.36	16.31	
5	18.99	17.95	16.73	20.52	17.50	18.20	
Mean	16.79	18.19	16.54	18.01	17.10	17.34	

Table 6. Mean Weight of Albumen for the Hens Receiving Oxalic Acid and Phytic Acid for the Final 48 hr period (g)

Replicates	Oxalic acid	1		Phytic Acid	1		
	0.2%	0.5%	0.8%	1.0%	2.0%	3.0%	
1	28.12	39.92	35.65	32.15	29.65	31.52	
2	32.44	40.36	37.41	44.37	37.78	37.46	
3	36.77	39.48	26.63	40.93	30.88	39.19	
4	31.81	39.84	33.36	30.43	21.18	47.06	
5	35.91	37.78	35.74	43.10	36.64	38.80	
Mean	33.01	39.48	33.76	38.20	33.43	38.81	

#### REFERENCES

- Dieckert, J. W., Dieckert, M. C and Creger C. R. 1989. Calcium reserve assembly: A basic structural unit of the calcium reserve system of the egg shell. Poultry Science 68:1569-1584.
- Scholbe, G. (2002). Food Pyramid for Parrots www.holisticbirds.com/pages/foodpp1002.htm
- Hamilton, R. H. M. 1982. Method and factors affecting the measurement of eggshell quality. Poultry science 61:1192
- Zhou, J. R., Fordyce, E. J. Raboy, V., Dickinson, D. B., Wong, M.S., Burns, R. A. and Erdman, J. W. Jr. 1992. Reduction of Phytic Acid in Soybean Products Improves Zinc Bioavailability in Rats. Journal of Nutrition, 122: 2466-2473.
- Jenkins, N. K. and Tayler, C. 1960. Changes in egg shell thickness and white and yolk weight and composition over a period of a year. The Journal of Agricultural Science, 55: 323-331
- Khan, N., Zaman, R., Elahi, M. 1991. Effect of heat treatments on the phytic acid content of maize products. Journal of agricultural and food. 54:153-56

- Petersen, C.F. 1965. Factors influencing egg shell quality: A review. World's Poultry Science 21:110– 138.
- Production and Value 2009 Summary. 2010. Agricultural Statistics Board, United States Department of Agriculture, National Agricultural Statistics Service, USA.
- Roland, D.A. 1977. The extent of uncollected eggs due to inadequate shell. Poultry Science 56:1517–1521.
- Roland, D.A. 1984. Egg shell quality 1: The bodychecked egg. World's Poultry Science, 40:250–254.
- SAS. 1985. SAS user's guide: Statistics, 5th ed. SAS Institute, Inc Cary, N.C.
- Steel, N. G. D., and Torrie J. H. 1980. Principles and procedures of statistics: a biometrical approach, 2nd edition. McGraw-Hill, New York.
- Tamim, N. M., Angel, R. 2003. Phytate phosphorus hydrolysis as influenced by dietary calcium and micro-mineral source in broiler diets. Journal of agricultural and food chemistry. 51:4687–4693.
- Tamim, N. M., Angel, R. and Christman M. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poultry Science. 83:1358–1367.