Journal of Animal Research: v.4 n.1, p. 141-153. June 2014

DOI Number 10.5958/2277-940X.2014.00499.9

Effect of Sodium Butyrate Supplementation on Performance, Egg Quality and Bacterial load in the Excreta of Laying Hens

Chou Khong, Sinol Sen, Suhyup Lee, Yuhan Choi, Kwang-Yul Kim, Santosh Ingale, Ill-Kyong Kwon and Byung-Jo Chae*

College of Animal Life Sciences, Kangwon National University, Chuncheon, 200-701, Republic of Korea

**Department of Animal Resources Science, College of Animal Life Science, Kangwon National University, Chuncheon 200-701, Republic of Korea*

**Corresponding author: Prof. B. J. Chae; E-mail: bjchae@kangwon.ac.kr*

Received: 18 April 2014; Accepted: 21 May 2014

ABSTRACT

An experiment was conducted to investigate the effects of sodium butyrate supplementation on egg production performance, egg quality, nutrient retention, excreta microflora and blood metabolites in laying hens during late laying cycle. A total of 320 Hy-Line Brown layers (65 wk old) were randomly allotted to 4 treatments on the basis of laying performance. Each treatment had 8 replicates with 10 birds each (80 birds per treatment). Two hens were confined individually with the cage size as 0.2 x 0.2 m. and 10 birds (5 cages) shared a common feed trough between them forming one experimental unit. Dietary treatments were basal diet supplemented with 0 (control), 0.05, 0.10, and 0.20% sodium butyrate. Supplementation of increasing levels of sodium butyrate showed linear reduction ($P<0.05$) in broken egg percentage. Egg shell strength was linearly improved ($P<0.05$) with increase in dietary sodium butyrate. Dietary supplementation of increasing levels of sodium butyrate had greater (linear, $P<0.05$) retention of DM, CP and GE. Hens fed diet supplemented with increasing levels of sodium butyrate had increased (linear, $P<0.05$) total anaerobic bacteria and *Lactobacillus spp*. population. However, dietary supplementation of sodium butyrate had no effect ($P>0.05$) on feed intake, egg production, egg weight, egg mass, egg shell thickness, yolk color and serum metabolites. These results indicates that dietary supplementation of sodium butyrate had beneficial effects on egg shell strength, nutrient retention and fecal microflora and can be used as feed additive for laying hen during late laying cycle.

Keywords: Egg quality, Excreta microflora, Laying hens, Nutrient retention, Sodium butyrate

Profitability of egg production is mainly affected by egg shell quality. It is estimated that 6-10% of total egg produced have poor egg shell quality which results in tremendous economic losses (Roland, 1986). The top concern is a decrease in egg shell quality as the hen ages, because egg weight increase without an increase the proportion of calcium carbonate deposited in the shells. As a result, during final phase of laying cycle, the numbers of cracked eggs reaches up to 20% (Nys, 2001). Presently, dietary manipulation is the primary means to correct egg shell quality problems and the role of organic acids and their salts has received great attention.

Most of studies were conducted to investigate the effects of nutrition on eggshell quality in layers have been centered on macro-mineral and vitamin D (Nys, 1999). Inclusion of optimal calcium to laying hens diet is the most important in order to stimulate the proper calcification of the eggshell. However, it has reported that increasing the amount of calcium to laying hen diet above 3.8% seems no beneficial effect on quality of the eggshell. Limestone is considered as an effective source of calcium in term of rehabilitating the shell breaking strength (Koreleski and Swiatkiewicz, 2004). Several feeding trials carried out on pigs, rats, broiler chickens reported that organic acids improve the utilization of the consumed minerals (Boling *et al.,* 2000; Omogbenigun *et al.,* 2003; Liem *et al.,* 2008). Organic acids play role as an agent to reduce the intestinal pH, activates the digestive enzymes and solubility of minerals. For instance, older broiler breeder hens were used to conduct some feeding trails to indentify the in fluency of organic acids on laying performance and eggshell quality; the output of those experiments uniquely showed positive effect when hens consumed organic acids compared with control group that had no supplemented (Sengor *et al*., 2007; Soltan, 2008; Park *et al.,* 2009). Therefore, the present study was conducted to investigate the efficacy of dietary supplementation of sodium butyrate (sodium salt of butyric acid) on laying performance, egg quality, excreta microbial population and serum metabolites of older laying hens.

MATERIALS AND METHODS

The protocol for this experiment was approved and birds were cared according to the guidelines of the Institutional Animal Care and Use Committee of Kangwon National University, Chunchon, Republic of Korea. The birds were provided daily *ad libitum* feed and clean drinking water during 8 week feeding period. Laying hens were exposed to a 16-h incandescent light throughout the experimental period.

Birds, diets and management

Hy-Line Brown layers (n = 320; 65-wk-old) were randomly allotted to four treatments based on body weight in a randomized completed block design. Each treatment were comprised of eight replicates with 10 birds each (80 birds per

Table 1. Ingredient and chemical composition of basal diet (as-fed basis) [1]

Item	Basal diet
Ingredients (%)	
Corn	35.19
Wheat	16.00
Soybean meal	13.28
Limestone	10.74
Distiller dried grains with soluble	8.00
Corn germ meal	4.00
Rice bran	4.00
Rapeseed meal	3.50
Tallow	2.31
Corn gluten meal	2.00
Salt	0.29
Dicalcium phosphate	0.26
$_{DL}$-Methionine (100%)	0.07
$_{L}$-Lysine	0.11
Mineral mix[2]	0.15
Vitamin mix[3]	0.07
Phytase	0.03
Chemical composition (%)	
Metabolizable energy (kcal/kg)	2,800
Crude protein	17.00
Total ash	13.95
Calcium	4.10
Available phosphorus	0.45
Lysine	0.79
Methionine + Cysteine	0.68

[1] Dietary treatments were basal diet supplemented with 0 (control), 0.05, 0.10 and 0.20% sodium butyrate.
[2] Supplied per kilogram of diet: 45 mg Fe as ferrous sulfate, 0.25 mg Co as cobalt sulfate, 50 mg Cu as copper sulfate, 15 mg Mn as manganous oxide, 25 mg Zn as zinc oxide, 0.35 mg I as potassium iodide and 0.13 mg Se as sodium selenite.
[3] Supplied per kilogram of diet: 9,000 IU vitamin A, 1,800 IU vitamin D_3, 30 IU vitamin E, 1.5 mg vitamin K_3, 1.5 mg vitamin B_1, 5 mg vitamin B_2, 4 mg vitamin B_6, 0.025 mg vitamin B_{12}, 15 mg pantothenic acid, 35 mg niacin, 0.15 mg biotin, 0.65 mg folic acid, 12 mg antioxidant.

treatment). Two hens were confined individually with cage size 35 x 35 x 40 cm and each 10 birds (5 cages) shared a common feed trough between them forming one experimental unit. Dietary treatments were basal diet supplemented with 0

(control), 0.05, 0.10 and 0.20% sodium butyrate (a sodium salt of butyric acid). All the birds were fed isocaloric and isoprotineous diet in mash form for 8 week. All the nutrients met or exceeded the nutrient requirements as recommended by NRC (1994).

Sampling and measurements

The laying hens were weighed at beginning and end of the experimental feeding. Daily egg production and egg weight per treatment group was recorded to determine the hen day egg production and the egg mass production (g/d/hen). Feed consumption was measured at the end of feeding. Laying rate and feed efficiency (kilograms of feed needed to produce a kilogram of eggs) were calculated at the end of the experiment. At weekly intervals, eggs were collected from each experimental unit for analysis of egg quality parameters. A digestibility trial was conducted during the last week of experimental feeding to determine retention of DM, CP, Ca, P, total ash and GE. Two birds from each replicate were allocated to individual cage (one bird/cage), to facilitate collection of excreta samples. The diets containing 0.25% chromic oxide (Cr) as an indigestible marker were fed during digestibility trials. Excreta samples (about 50 g/d per bird) were collected from each bird for 48 h. Then excreta samples collected were pooled and dried using a forced-air drying oven at 65^{o}C and stored for the analysis of DM, CP, Ca, P, ash and GE. The nutrients retention (%) by marker (chromic oxide) method was calculated as;

Nutrient retention (%) = 100- [100 x (% Cr in feed/ % Cr in excreta) x (% nutrient in excreta/ % nutrient in feed)]

To study microbial population, fresh excreta samples were directly collected from bird cloacae on last day of experimental feeding, stored in sterilized specimen cups, placed on ice and immediately sent to laboratory for further analysis. A 5 ml blood samples were collected from (2 birds per replicate) by wing venipuncture into disposable vacutainer tubes without anticoagulants (Becton Dickenson, Franklin, NJ). After centrifugation (3,000 × g for 15 mn), plasma samples were stored at -20^{o}C and later analyzed for concentrations GOT, GPT, BUN, CA, GLU and TCHO.

Chemical and microbial analyses

Experimental diet was analyzed in triplicate for DM (Method 930.15), CP (Method 990.03), ash (Method 942.05), Ca and P (Method 985.01) using AOAC (2007). Gross energy of experimental diet was measured by a bomb calorimeter (Model 1216, Parr Instrument Co., Moline, IL), while chromium was measured with an atomic absorption spectrophotometer (Model AA-680G, Shimadzu, Japan) according to the procedure of Fenton and Fenton (1979). The fecal microflora was analyzed by using culture technique as described previously (Choi *et al.,* 2009).

One gram of mixed content was diluted with 9 ml of Butterfields phosphate buffer solution, followed by further serial dilutions in the same. Samples were inoculated (0.1 ml/plate) and incubated in duplicate. The microbial groups analyzed were total anaerobic bacteria (Tryptic soy agar), Lactobacillus spp. and Bifidobacterium spp. (MRS agar + 0.02% NaN3 + 0.05% L-cystine hydrochloride monohydrate), Clostridium spp. (Genus name should be in italic font) (TSC agar) and coliforms (violet red bile agar). The anaerobic conditions during the assay of total anaerobic bacteria and Clostridium spp. were created by using gas-pak anaerobic system (BBL, No. 260678, Difco, Detroit, MI, USA). The microbial populations were log transformed before statistical analysis. Diagnostic kits (Fujifilm Corporation, Japan) were used for blood metabolites.

Statistical analysis

Data generated in the present study was subjected to statistical analysis using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) in a randomized complete block design. When significant differences were identified among treatment means, they were separated using Tukey's Honestly Significant Difference test. The orthogonal polynomials were used to evaluate the linear and quadratic effects of dietary sodium butyrate levels (0, 0.05, 0.10 and 0.20 %). Probability was considered significant at ($P<0.05$).

RESULTS

Production performance

Dietary supplementation of increasing levels of sodium butyrate had no effects (linear or quadratic; $P>0.05$) on feed intake, egg production, egg weight, and egg mass in laying hens during their late laying cycle (Table 2). However, dietary supplementation of increasing levels of sodium butyrate reduced (linear, $P<0.05$) broken egg percentage. Hens fed 0.05, 0.10 and 0.20% sodium butyrate supplemented diets had lesser broken eggs percentage than that of hens fed the control diet. Moreover, broken eggs percentage in hen fed 0.20% sodium butyrate supplemented diet was lesser ($P<0.05$) than that of hens fed 0.05% sodium butyrate supplemented diet; whereas broken eggs percentage of hens fed 0.10% sodium butyrate remained comparable ($P>0.05$) to hens fed 0.05 and 0.20% sodium butyrate supplemented diets.

Egg quality

Dietary supplementation of increasing levels of sodium butyrate improved (linear, $P<0.05$) egg shell strength (Table 2). In addition, hens fed 0.05, 0.10 and 0.20 % sodium butyrate supplemented diets had greater ($P<0.05$) egg shell strength than that of hens fed the control diet. However, dietary treatments had no effects (linear or quadratic; $P>0.05$) on eggshell thickness, yolk color and haugh unit.

Table 2. Effects of sodium butyrate supplementation on feed intake, egg production and their quality attributes in laying hens.

Parameters	Sodium butyrate (%)				SEM[1]	P-values	
	Control	0.05	0.10	0.20		Linear	Quadratic
Production performance							
Feed intake (g/hen/day)	122.17	122.26	122.82	123.40	0.226	0.084	0.193
Egg production (%)	77.29	77.73	78.78	79.57	0.634	0.237	0.520
Egg weight (g/egg)	68.00	68.37	68.30	68.20	0.187	0.613	0.623
Egg mass[2] (g/d/hen)	52.48	53.14	53.76	54.27	0.469	0.195	0.666
Broken egg (%)	3.82^{a}	2.54^{b}	2.00^{bc}	1.58^{c}	0.205	<0.001	0.546
Egg quality							
Egg shell strength (kg/cm^2)	3.02^{c}	3.30^{b}	3.41^{ab}	3.55^{a}	0.045	<0.001	0.311
Egg shell thickness (mm)	0.383	0.386	0.390	0.391	0.002	0.103	0.717
Yolk color, RCF	9.13	9.26	9.20	9.15	0.052	0.723	0.385
Haugh unit	86.14	86.19	87.43	86.91	0.307	0.238	0.749

^{abc} Values with different superscripts in the same row differ significantly (P<0.05).
[1] Standard error of means
[2]Egg mass = egg production x egg weight/100

Table 3. Effects of supplementation of sodium butyrate on nutrient retention (%) in laying hens (d 49-56)[1]

Parameters	Sodium butyrate (%)				SEM[2]	P-values	
	Control	0.05	0.10	0.20		Linear	Quadratic
Dry matter	71.23^{b}	72.11^{a}	72.18^{a}	72.43^{a}	0.137	<0.001	0.769
Gross energy	72.82^{b}	73.88^{a}	73.91^{a}	73.98^{a}	0.150	<0.001	0.253
Crude protein	51.70^{b}	53.64^{a}	53.82^{a}	53.98^{a}	0.277	<0.001	0.308
Total ash	46.28^{b}	47.95^{a}	47.98^{a}	48.05^{a}	0.272	0.006	0.384
Calcium	44.93^{b}	46.97^{a}	47.39^{a}	47.75^{a}	0.334	<0.001	0.743
Available phosphorus	34.33^{b}	36.49^{a}	37.74^{a}	38.13^{a}	0.465	<0.001	0.536

[1]The nutrients retention (%) by marker (chromic oxide) method was calculated as; Nutrient retention (%) = 100- [100 x (% Cr in feed/ % Cr in excreta) x (% nutrient in excreta/ % nutrient in feed)]
[2]Standard error of means
^{ab} Values with different superscripts in the same row differ significantly (P<0.05).

Nutrient retention

Dietary supplementation of increasing levels of sodium butyrate had greater (linear, P<0.05; Table 3) retention of CP, GE, ash, Ca and P. Moreover, retention of all nutrients were greater (P<0.05) in hens fed diet supplemented with different levels of sodium butyrate than that of hens fed the control diet. However, the digestibility of all nutrients remained comparable (P>0.05) among hens fed 0.05, 0.10 or 0.20 % sodium butyrate.

Excreta microflora

Hens fed diets supplemented with increasing levels of sodium butyrate had increased (linear, P<0.05) total anaerobic bacteria and *lactobacillus spp.* population (Table 4). Moreover, hens fed 0.2 % sodium butyrate supplemented diet had higher (P<0.05) total anaerobic bacteria and *Lactobacillus spp.* populations than that of hens fed the control diet. The total anaerobic bacteria and *Lactobacillus spp.* populations in hens fed 0.05 and 0.10 % sodium butyrate supplemented diets remained comparable (P>0.05) with hens fed the control and 0.2% sodium butyrate supplemented diets. The excreta *Clostridium* spp. and coliforms were tended to decrease (linear, P<0.10) with increase in dietary levels of sodium butyrate.

Table 4. Effects of sodium butyrate supplementation on bacterial load in the excreta (log_{10} CFU/g) of laying hens (d 56).

Parameters	Sodium butyrate (%)				SEM[1]	P-values	
	Control	0.05	0.10	0.20		Linear	Quadratic
Total anaerobic bacteria	8.45[b]	8.52[ab]	8.71[ab]	8.87[a]	0.064	0.021	0.160
Bifidobacterium spp.	7.79	7.80	8.15	8.23	0.095	0.103	0.280
Lactobacillus spp.	7.63[b]	8.05[ab]	8.11[ab]	8.24[a]	0.096	0.019	0.984
Clostridium spp.	6.60	6.40	6.35	6.30	0.062	0.089	0.973
Coliforms	6.77	6.66	6.52	6.47	0.064	0.099	0.610

[1] Standard error of means.

[ab] Values with different superscripts in the same row differ significantly (P<0.05).

Serum metabolites

Dietary supplementation of sodium butyrate had no effects on serum metabolite of older laying hens (P>0.05; Table 5).

Table 5. Effects of sodium butyrate supplementation on serum metabolite in laying hens (d 56)

Parameters	Sodium butyrate (%)				SEM[1]	P-values	
	Control	0.05	0.10	0.20		Linear	Quadratic
GOT (mg/dl)	157.00	156.50	155.25	151.75	2.688	0.608	0.665
GPT (mg/dl)	4.75	4.25	3.50	3.75	0.559	0.492	0.975
BUN (mg/dl)	0.73	0.60	0.55	0.58	0.077	0.456	0.874
Ca (mg/dl)	30.15	28.38	27.55	29.63	1.193	0.688	0.596
GLU (mg/dl)	214.00	218.75	221.00	218.50	1.334	0.116	0.489
TCHO (mg/dl)	97.00	94.00	95.50	96.00	1.734	0.785	0.652

[1]Standard error of means.
GOT: glutamic oxaloacetic transaminase; GPT: glutamic pyruvate transaminase; BUN: blood urea nitrogen; Ca: calcium; GLU: glucose; TCHO: total cholesterol

DISCUSSION

The organic acid family, named as butyric acid has been attracted by many researchers due to its superior effectiveness when employed as feed additive in animal and poultry diet. It have been reported that, butyrate have positive effects on villi growth (Andoh *et al.,* 1999), epithelial growth and metabolism (Topping and Clifton, 2001; Langhout and Sus, 2005) and have anti-inflammatory effects, serve as the energy source for the normal colonic epithelium, enhance appetite (Galfi and Bokori, 1990; Fernandez-Banares *et al.,* 1999; Brons *et al.,* 2002; Kanauchi *et al.,* 2003) and stimulate the growth of the duodenal mucosa (Hu and Guo, 2007). In the present study, we aimed to investigate the effect of salts of organic acid (Sodium butyrate) on feed intake, egg production, egg quality, serum metabolites and excreta microflora.

In the present study, dietary supplementation of sodium butyrate had no effects on feed intake and feed conversion efficiency of laying hens. Present results are in good agreements with data reported by Yesilbag and Colpan (2006), who observed no effects of organic acids on feed intake and feed conversion efficiency of laying hens. In contrast to report by Yesilbag and Colpan (2006) and the present results, some of the previous studies with supplementation of organic acids reported improved performance and feed efficiency of laying hens (Patten and Waldroup, 1988; Vugt, *et al.,* 2001; Denli *et al.,* 2003; Langhout and Sus, 2005; Soltan, 2008). These variations in results might be due to stage of laying and doses of the organic acid supplemented. In the present study we used laying hen in their late laying cycle (65 week old).

With increase in age of laying hens intestinal epithelial cells become weaker and the villi on the inner wall surface of the lumen shorten which results in to impaired nutrients absorption, eggs shell abnormality and weaken the egg shell strength (Belyavin *et al.,* 1987). In addition, it have been reported that shell specific gravity was gradually decreased with increase in age of laying hens (Castillo *et al.,* 2004; Narvaez-Solarte *et al.,* 2006; Swiatkiewicz and Koreleski, 2008). The consequence of this problem may deal with the deficiency of absorbed calcium that required for building up the egg. Etches (1987) confirmed that Dietary Ca is essential for eggshell synthesis, and its needs are affected by the stage of eggshell formation. In poultry, the absorption of minerals, especially calcium for eggshell formation is taking place along the entire gastrointestinal tract (Sugiyama *et al.,* 2007). Previous studies investigated the role of organic acids in activation of hydrochloric acid and digestive enzymes in gastrointestinal tract (Eidelsburger, 1997), to stimulate the digestibility of mineral such as Ca, P, Mg and Zn (Kirchgessner and Roth, 1988), to enhance availability of dietary energy in bird (Pirgolzliev *et al.,* 2008).

Some of the previous studies reported that dietary supplementation of high level of organic acids and salts of organic acids have positive effect on laying rate but have no effects with lower level of supplementation (Gama *et al.,* 2000; Yesilbag and Colpan, 2006). Sohail and Roland (2000) and Safaa *et al.,* (2008) reported linear increase in calcium level and specific gravity of the egg shell when birds fed diet supplemented with organic acids. In the present study, sodium butyrate had no effect on egg production and egg weight of laying hens. In contrast to the present results, Van *et al,.* (2001) and Isaias *et al.,* (2009) reported increased egg weight when hens fed diet containing high doses (500 ppm) of sodium butyrate.

In the present study, layers fed diet supplemented with sodium butyrate had linear improvement in egg shell strength and reducing in broken egg percentage. Improvement in egg shell strength and reducing in broken egg percentage might be due to ability of sodium butyrate to improvement of mineral utilization by laying hen (Kirchgessner and Roth, 1988).

In the present study, dietary supplementation of increasing levels of sodium butyrate linearly increased the total anaerobic bacteria and *Lactobacillus* spp. count; whereas the *Clostridium* spp. and coliforms tended to decrease with increase in dietary sodium butyrate. In general, beneficial intestinal bacteria or microflora provide positive effects to the intestinal physiology by maintaining the homeostasis in intestinal via activate tool like receptors under normal condition and protects susceptibility to colonic wound, stimulate the epithelial cell development and forming and activating immunity and play role as a stimulant in nutrient uptake and metabolism (Hooper and Gordon, 2001). Butyrate has been found to have direct effect on mucin secretion in poultry, which support antibacterial activity on *E. coli*, *Salmonella* spp. and *Clostridium* spp. (Van *et al.,* 2006). Therefore,

organic acids have been applied ~~to~~ against pathogenic microorganisms viz. *E. coli*, *Salmonella* spp. and *Clostridium* spp. (Roth and Kirchgessner, 1998; Van *et al.,* 2006). In the present study laying hens fed diet supplemented with sodium butyrate increased populations of *Lactobacillus* spp., which might have effect on improving the physiology of the intestinal tract and nutrient absorption.

In conclusion, the results obtained in present study indicates that dietary supplementation of sodium butyrate had beneficial effects on egg shell strength, nutrient retention and on beneficial gut bacteria while detrimental to the pathogenic bacteria of the gut of laying hens. Therefore, sodium butyrate can be used as feed additive for laying hen during late laying cycle.

REFERENCES

Andoh, A., Bamba, T. and Sasaki, M. 1999. Physiological and anti-inflammatory roles of dietary fiber and butyrate in intestinal functions. *J. Parenter. Enteral. Nutr*., **23** (5): 70-73.

AOAC, 2007. Official Methods of Analysis of the Association of Official Analytical Chemists International, 18th ed. Gaithersburg, MD, USA.

Belyavin, C.G., Boorman, K.N. and Volynchook, J. 1987. Egg quality in individual birds. In: Egg Quality-Current Problems and Recent Advances. *Poult. Sci. Symp.* Series 20. Eds. Wells, R.G. and Belyavin, C.G., Butterworths, Borough Green, Sevenoaks, Kent TN 15 8PH, England. **87**: 105-122

Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C. M. and Baker, D.H. 2000. The effects of citric acid on phytate phosphorus utilization in young chicks and pigs. *J. Anim. Sci.*, **78**: 682–689.

Brons, F., Kettlitz, B. and Arrigoni, E. 2002. Resistant starch and the butyrate revolution. *Trends Food Sci. Technol.*, **13**: 251-261.

Castillo, C., Cuca, M., Pro A., Gonzalez, M. and Morales, E. 2004. Biological and economic optimum level of calcium in White Leghorn laying hens. *Poult. Sci*., **83**: 868–872.

Choi, J.Y., Shinde, P.L., Kwon, I.K., Song, Y H. and Chae, B.J. 2009. Effect of wood vinegar on the performance, nutrient digestibility and intestinal microflora in weanling pigs. *Asian-Aust. J. Anim. Sci.*, **22**: 267-274.

Denli, M., Okan. F. and Celik, K. 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pakistan J. Nutri.*, 2: 89-91.

Eidelsburger, U. 1997. Optimierung der Futterqualität ist nur ein Teilaspekt. In: Schweinewelt, Nr. Januar, p. 18-21.

Etches, R.J. 1987. Calcium logistics in the laying hen. *J. Nutr.*, **117**: 619-628.

Fenton, T.W. and M. Fenton, 1979. An improved method for chromic oxide determination in feed and feces. *Can. J. Anim. Sci.*, **59**: 631-634.

Fernandez-Banares, F., Hinojosa, J., Sanchez-Lombrana, J.L., Navarro, E., Martinez-Salmeron, J.F., Garcia-Puges, A., Gonzalez-Huix, F., Riera, J., Gonzalez-Lara, V., Dominguez-Abascal, F., Gine, J.J., Moles, J., Gomollon, F. and Gassull, M.A., 1999. Randomized clinical trial of Plantago ovata seeds (dietary fiber) as compared with mesalamine in maintaining remission in ulcerative colitis. Spanish Group for the Study of Crohn's Disease and Ulcerative Colitis (GETECCU). *Am. J. Gastroenterol*., **94**: 427-433.

Galfi, P. and Bokori, J. 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. *Acta Vet. Hung*., **38**: 3-17

Gama, N.M.S.Q., Oliveira, M.B.C., Santin, E. and Berchieri, Jr. A. 2000. Supplementation with organic acids in diet of laying hens. *Ciencia Rural*, **30**: 499–502.

Hooper, L.V. and Gordon, J.I. 2001. Commensal host-bacterial relationships in the gut. *Science,* **292**: 1115-8.

Hu, Z. and Guo, Y. 2007. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. *Anim. Feed Sci. Technol.*, **132**: 240-249

Isaias, S.H., Elizabeth, P.H., Ezequiel, S.R., Benjamin, F.M., Jorge, H.E., Jose, L.L.V. and Ernesto, A.G. 2009. Effect of sodium butyrate on diets for laying hens on the productive performance, egg quality and intestinal vill. *Vet. Mex*., **40**(4): 397-403

Kanauchi, O., Serizawa, I., Araki, Y., Suzuki, A., Andoh, A., Fujiyama, Y., Mitsuyama, K., Takaki, K., Toyonaga, A., Sata, M. and Bamba, T. 2003. Germinated barley foodstuff, a prebiotic product, ameliorates inflammation of colitis through modulation of the enteric environment. *J. Gastroenterol*., **38**: 134-141.

Kirchgessner, M. and Roth, F.X. 1988. Ergotrope Effekte durch organische Säuren in der Ferkelaufzucht und Schweinemast. *Übersichten zur Tierernährung*, **16**: 93-108.

Koreleski, J. and Swiatkiewicz, S. 2004. Calcium from limestone meal and grit in laying hen's diet– effect on performance, eggshell and bone quality. *J. Anim. Feed Sci*., **13**: 635-645.

Langhout, P. and Sus, T. 2005. Volatile fatty acids improve performance and quality. *Int. Poult. Prod.* **13**(3): 17.

Liem, A., Pesti G.M. and Edwards, H M. 2008: The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. *Poult. Sci.,* **87**: 689-693.

Narvaez-Solarte, W., Rostagno, H.S., Soares, P.R., Uribe- Velasquez, L.F. and Silva, M.A. 2006. Nutritional requirement of calcium in white laying hens from 46 to 62 wk of age. *Int J. Poult. Sci.,* **5**: 181–184.

NRC. 1994. Nutrient Requirement of Poultry. 9th Edn. National Academy Press, Washington, DC.

Nys Y. 1999. Nutritional factors affecting eggshell quality. *Czech J. Anim. Sci.*, **44**: 135–143.

Nys Y. 2001. Recent developments in layer nutrition for optimising shell quality. In: Proceedings of 13th European Symposium of Poultry Nutrition. Blankenberge, Belgium, 45–52.

Omogbenigun, F.O., Nyachoti, C.M. and Slominski, B.A. 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. *J. Anim. Sci.,* **81**: 1806–1813.

Park, K.W., Rhee, A.R., Um, J.S. and Paik, I.K. 2009 Effect of dietary available phosphorus andorganic acids on the performance and egg quality of laying hens. *J. Applied Poult. Res.,* **18**: 598-607.

Partanen, K.H. and Mroz, Z. 1999. Organic acids for performance enhancement in pig diets. *Nutr. Res. Rev.*, **12**: 117–145.

Patten, J.D. and Waldroup, P.W. 1988. The use of organic acids in broiler diets. *Poult. Sci.,* **67**: 1182- 1187.

Pirgolzliev, V., Murfhy, T.C., Owens, B., George, J. and McCann, M.E. E. 2008. Fumaric and sorbic acis as additives in broiler feed. *Res. Vet. Sci.*, **84**: 387-394.

Roland, A. 1986. Eggshell quality IV. Oyster shell versus limestone and the importance of particle size or solubility of calcium source. *World Poult. Sci. J.,* **42**: 166-71.

Roth, F.X. and Kirchgessner, M. 1998. Organic acids as feed additives for young pigs: Nutritional and gastrointestinal effects. *J. Anim. Feed Sci.*, **7**: 25-33.

Safaa, H.M., Serrano, M.P., Valencia, D.G., Frikha, M., Jimenez-Moreno, E. and Mateos, G.G. 2008. Productive performance and egg quality of brown egg-laying hens in the late phase of production as influenced by level and source of calcium in the diet. *Poult. Sci.*, **87**: 2043–2051.

Sengor, E., Yardimci, M., Cetingul, S., Bayram, I., Sahin, H. and Dogan, I. 2007. Effects of short chain fatty acid (SCFA) supplementation on performance and egg characteristics of old breeder hens. *South African J. Anim. Sci.,* **37**: 158-163

Sohail, S.S. and Roland, D.A. Sr. 2000. Influence of phytase on calcium utilization in commercial layers. *J. Appl. Poult. Res*., **9**: 81–87.

Soltan, M.A. 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some blood serum parameters in laying hens. *International J. Poult. Sci.*, **7**(6): 613-621.

Sugiyama, T., Kikuchi, H., Hiyama, S., Nishizawa, K. and Kusuhara, S. 2007. Expression and localisation of calbindin D28k in all intestinal segments of the laying hen. *Br. Poult. Sci.,* **48**(2): 233-238.

Swiatkiewicz, S. and Koreleski, J. 2008. The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality. *Vet. Med.,* **53**: 555–563.

Topping, D.L. and Clifton, P.M. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev*., **81**(3): 1031–1064.

Van Immerseel, F., Russell, J., Flythe, M., Gantois, I., Timbermont, L. and Pasmans, F. 2006. The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. *Avian Pathol.,* **35**: 182-188.

Van Vugt Pna, Wijtten Pja, Perdok, H.B. and Langhout, D.J. 2001. Provimax improves the technical performance and eggshell quality of laying hens. 13th European Symposium Poultry Nutrition; 2001 September 30-04 October; Blankenberge, Belgium. Dundry Lodge, France Lynch, Stroud, Gloucestershire GL6 8LP, UK. The World's Poultry Science Association, 2001. 15-16.

Vogt, H., Mathes, S. and Harnisch, S. 1981. The effects of organic acids in the rations on the performance of broiler and laying hens. *Arch. Geft.,* **45**: 221-232.

Vugt, V. 2001. The effect of sodium butyrate on the technical performance in layers. 13th Eur. Symp. on Poultry Nutri., Bremen, 2003.

Yesilbag, D. and Colpan, I. 2006. Effects of organic acid supplemented diets on growth performance, egg production and quality and on serum parameters in laying hens. *Revue de Med. Vet.,* **157**: 280–284.