Short Communication

Effects of short chain fatty acid (SCFA) supplementation on performance and egg characteristics of old breeder hens

E. Sengor[#], M. Yardimci, S. Cetingul, I. Bayram, H. Sahin and I. Dogan
Faculty of Veterinary Medicine, Department of Animal Husbandry and Nutrition, Afyon Kocatepe University, 03200 Afyonkarahisar-Turkey
Afyon Kocatepe University, Faculty of Veterinary Medicine, Dept. of Animal Husbandry, Afyonkarahisar–Turkey

Abstract

A study was conducted to determine the effect of supplementing the diet of breeder hens with a short-chain fatty acid (SCFA) premix, containing 509 g fatty acid salts/kg of which 285 g were calcium butyrate, on their eggshell characteristics and the hatching percentage of the eggs. One thousand six hundred 66-week old White Bovans laying breeder hens were used in this experiment. They were housed in eight identical pens, each containing 200 birds, and four pens were used per treatment. The SCFA premix was included at 1000 mg/kg in the treatment diet, and fed for a period of nine weeks. Responses were compared with an unsupplemented treatment. Supplementation started when the hens were 66 weeks old. From day 75 eggs were collected for the next seven weeks and the occurrence of cracked, dirty and misshapen eggs was recorded, and the hatching percentage of the eggs was determined. Eggshell strength was lower in eggs from the control (1.76 ± 0.05) than from the treatment group (2.07 ± 0.03). The percentage of eggs produced by the control group (68.6 ± 0.08) was significantly lower than that by the supplemented group (71.5 ± 0.15). Percentage of dirty, cracked and misshapen eggs, and the hatchability percentage of the control group (1.15 ± 0.03, 3.44 ± 0.05, 6.27 ± 0.03 and 88.93 ± 0.06, respectively) were also significantly lower than in the group receiving SCFA (0.47 ± 0.03, 2.21 ± 0.03, 3.81 ± 0.03 and 93.36 ± 0.05, respectively). It was concluded that dietary supplementation of SCFA to layer breeder hens from 66 weeks of age onwards improved eggshell strength, reduced the percentage of dirty, cracked and misshapen eggs and increased the hatching percentage of the eggs. The positive responses were suggested to be largely due to the butyrate in the SCFA.

Keywords: Butyrate, SCFA, eggshell quality, hatching characteristics
[#] Corresponding author. E-mail: erolsengor@yahoo.com, sengor@aku.edu.tr

Eggshell quality is one of the main criteria for purchasing eggs by retail consumers. It is well known that eggshell strength deteriorates with the increase in age of a hen. This is a factor contributing to a reduced profitability for the producer. Eggshell quality is also important in parent stock breeding. It affects the hatchability, regulates the gaseous exchange during incubation and affects the number of hatching eggs to be set (Barnett *et al.*, 2004)

Egg size increases with age, resulting in thinner eggshells. On the other hand, increasing the calcium level in the feed reduces feed consumption, leading to deficiencies of other nutrients. Phosphorus together with calcium in the correct ratio, vitamin D and manganese are essential for eggshell formation. Therefore, good shell quality cannot be maintained solely by increasing the calcium level in the diet (Keshavarz, 1998). Rama *et al*. (2006) reported that over a period of 42 days feed intake of broilers was reduced ($P < 0.01$) from 3423 g to 3228, 3225 and 3043 g by increasing the calcium concentration of the diet from 6 to 7, 8 or 9 g/kg, respectively.

As a hen's age increases, mucosal cells in the intestinal wall weaken, and villi on the inner wall surface of the duodenum shorten and absorption of the nutrients required for eggshell formation becomes impaired, leading to weakness in the eggshell (Belyavin *et al*., 1987). Brons *et al.* (2002) credited butyrate derived from the fermentation of non-starch polysaccharides with improved gastrointestinal health in humans and reduced incidence of colon cancer. Butyric acid therefore appears to be a stimulant of villi growth. Butyrates are known to have some vital functions in supporting the mucosal membranes of the intestinal wall

(Andoh *et al*., 1999). Andoh *et al*. (1999) reported that of the abundant SCFAs, butyrate has been shown to have the most significant effects on colonic epithelial cells, both *in vivo* and *in vitro*. Butyrate serves as the primary energy source for the normal colonic epithelium and can stimulate the growth of the colonic mucosa. However, in tumour cell lines it inhibits growth and induces apoptosis. In some clinical studies the effectiveness of butyrate enemas in the treatment of active ulcerative colitis has been reported. The precise molecular mechanisms underlying this response have not been identified, but it is believed to be based partially on the inhibitory actions of butyrate on the production of pro-inflammatory mediators in the intestinal epithelium.

It was demonstrated that colonic fermentation of *Plantago ovata* seeds yields butyrate, which serves as a nutrient for intestinal epithelial cells. The presence of butyrate in inflammatory bowel disease (IBD) in humans reduced the inflammation by inhibiting the production of cytokines and the activation of the transcription factor nuclear factor – kappa B (NF-κB) (Fernandez-Banares *et al*., 1999). In addition, butyrate protects the intestinal mucosa against injury and promotes mucosal healing (Scheppach *et al*., 1992).

Administration of germinated barley foodstuff (GBF) augmented SCFA production which increased caecal butyrate levels and improved colonic epithelial cell proliferation in humans (Kanauchi *et al*., 2003). Furthermore, the flavonoid, bacalein, isolated from the roots of *Scutellaria baicalensis* Georgi (Labiatae), reduced and ameliorated the inflammatory symptoms of the dextran sulphate sodium (DSS)-induced colitis. Its effect was reported to be similar to that of sulphasalazine, the reference drug used for the treatment of ulcerative colitis in mice (Hong *et al*., 2002). Furthermore, the phytochemical tryptanthrin, isolated from the indigo plant, *Polygonum tinctorium*, may have therapeutic effects on colitis in mice (Micallef *et al*., 2002).

Butyrate shows some strong anti-inflammatory action both *in vivo* and *in vitro*. On the other hand, SCFA, especially butyrate, are used for improvement in cell propagation in the digestive tract and as an energy source for enterocyte cells of the intestinal epithelium (Wachtershauser & Stein, 2000). The SCFA have also been shown to stimulate the digestive enzymes in pigs. Galfi & Bokori (1990) found that the group receiving sodium n-butyrate, i.e. SCFA, had a better appetite, grew more rapidly, had a brighter skin and smoother coat. Ulcerative lesions were lower in the test group. The size and the number of the microvilli in the caecum and in the ileum were favourable in the test group indicating that the increased length of microvilli means an enlarged absorptive surface in the gut and increases the absorption of the nutrients. This leads to overall improvement in intestinal digestion, better feed utilization and consequently, much higher body mass gain (Galfi & Bokori, 1990).

Leeson *et al*. (2005) reported that 0.2% butyric acid supplementation of the diet of broiler birds increased breast meat yield ($P < 0.01$) compared to a non-supplemented control. Duodenal villi crypt depth was decreased by bacitracin supplementation compared to non-supplementation and 0.2% butyric acid supplemented birds ($P < 0.05$). These researchers indicated that unlike antibiotics, butyrate assisted in maintaining intestinal villi structure, compared with the negative effect of antibiotics, and that prior treatment reduced the devastating effects of coccidial challenges.

Sakata (1987) showed that intestinal infusion of butyrate into fistulated rats increased the proliferation of crypt cells in both the small and large intestines, while Sharma *et al*. (1995) suggested that the effect on crypt cell growth may reflect changes in the gut microflora, which is known to be a major modulator of epithelial cell activity. However, not much research has been conducted on increasing the shell strength of eggs of breeder hens by using the SCFA as a feed supplement. Schwartzer (2006) reported that percentage of microscopic hair cracks reduced significantly by including 250 g sodium butyrate/ton in the diets of layers older than 40 weeks. Microscopic hair cracks were reduced to 11.9% for the butyrate treatment from 16.2% in the control. It was also shown by Schwartzer, (2006) that the broken egg percentage reduced by 31%, 27%, 12% and 10% respectively at 28, 31, 35 and 42 weeks of age by including sodium butyrate in layer breeder diets.

This investigation was carried out to determine the effect of a SCFA premix high in butyrate on eggshell strength and hatching characteristics of eggs laid by old breeder hens.

One thousand six hundred White Bovans layer breeder hens with an average production level of 81.8% were used. Eight identical pens, each containing 200 birds, were assigned to the experiment, four for the SCFA treatment and four for the non-supplemented control. Stocking density was six females and one male per square metre. Treatments started when the hens were 66 weeks old. The diet contained 11.89 MJ

Table 1 The composition of the short chain fatty acid premix, Provimax

Ingredients	g/kg
Calcium butyrate	285
Calcium lactate	190
Calcium propionate	17
Fumaric acid	17
Sepiolite (as carrier)	245
Maize cob (as carrier)	246
Total	1000

ME/kg and 175 g crude protein/kg. A commercially available premix of SCFA (Table 1) containing mainly butyrate (Provimax marketed by Provimi Inc.) was included in the treatment diet at a rate of 1000 mg/kg diet. Diet formulation and the result of calculated analyses of the diets are shown in Table 2. Nine weeks later (at 75 weeks of age) 30 eggs were randomly collected from each pen to determine the eggshell strength. This was done using an instrument (DRIng. Georg Wazau, Plus tech., Germany) developed by Rauch (1965), and measured as kg/cm^2. The effect of SCFA on the egg production percentage and percentage of dirty eggs, cracked eggs and misshapen eggs, and hatching percentage of the eggs were recorded over the following seven weeks when the hens were between 75 to 81 weeks old.

The Statistical software package, SPSS (1998), was used (Afyon Kocatepe University) to analyze the data. The data for eggshell strength and egg production in the control and treatment groups were not presenting normal distribution curves. Therefore, the Mann-Whitney U-test was applied for determining the significance of differences (Z). The data for egg characteristics (dirty eggs, crack eggs, misshapen eggs and hatching eggs) were normally distributed, and the Student's t-test was applied for determining the significance of differences.

Table 2 Feed ingredients of the diet and calculated chemical analysis (as fed basis)

Ingredients	Control %	Treatment %	Calculated analyses mg/kg	
Maize	46.30	46.22	Dry matter	886
Wheat	19.92	19.90	Crude protein	175
Soyabean meal	9.46	9.46	ME (MJ/kg)	11.89
Full fat soya	4.98	4.98	Calcium	36.5
Maize gluten meal	4.98	4.98	Av. phosphorus	4.3
Limestone	8.47	8.47	Lysine	7.9
DiCaP	1.15	1.15	Methionine	4.2
Fish meal	3.49	3.49	Meth+Cyst	7.5
NaCl	0.35	0.35	Sodium	1.9
Vitamin premix	0.25	0.25		
Mineral premix	0.10	0.10		
Dl-methionine	0.05	0.05		
Colin chloride	0.05	0.05		
D-biotin	0.05	0.05		
Salmonella inhibitor	0.20	0.20		
Mycotoxin binder	0.20	0.20		
Provimax	0	0.10		

The eggshell strength of the eggs from the control group was lower ($P < 0.05$) than that of the treatment group (Table 3). It is generally accepted that the eggshell becomes thinner as a hen ages, with 75 weeks of age considered to be an old age for breeder hens. Belyavin *et al*. (1987) suggested that since the mucosal cells in the intestinal wall weaken and villi on the inner wall surface of duodenum shorten with age, the nutrient absorption would become less efficient, leading to weak shells in eggs laid by older birds. The improved shell strength of eggs from birds receiving SCFA could be attributed to regenerated mucosal cells in the intestinal wall leading to the better absorption of nutrients.

Table 3 Eggshell strength of the eggs from breeder hens at 75 weeks of age after receiving a supplemental short chain fatty acid mixture from 66 weeks of age

	n	Eggshell strength (kg/cm^2) (Mean ± s.e.).	Z
Control	120	1.76 ± 0.05	-4.801
Treatment	120	2.07 ± 0.03	

s.e. - Standard error; $P < 0.05$

The control group produced fewer ($P < 0.05$) eggs (68.6%) compared to the SCFA supplemented group (71.5%) (Table 4). It is suggested that due to SCFA and in particular to butyrate supplementation, the better absorption of nutrients through the gut wall as a result of healed villi was manifested as an increase in egg production.

Table 4 Egg production percentage of old breeder hens between 75 and 82 weeks of age when receiving a supplemental short chain fatty acid mixture from 66 weeks of age

	Control (mean ± s.e.)	Treatment (mean ± s.e.)	Z
Egg Production (%)	68.63 ± 0.08	71.47 ± 0.15	-8.53

$P < 0.05$ s.e. - standard error

Table 5 Egg characteristics of old breeder hens between 75 and 82 weeks of age when receiving a supplemental short chain fatty acid mixture from 66 weeks of age

Egg Characteristics	Control (mean ± s.e.)	Treatment (mean ± s.e.)	t
Dirty egg (%)	1.15 ± 0.03	0.47 ± 0.03	16.44
Crack egg (%)	3.44 ± 0.05	2.21 ± 0.03	22.39
Misshapen egg (%)	6.27 ± 0.03	3.81 ± 0.03	53.10
Hatching egg (%)	88.93 ± 0.06	93.36 ± 0.05	-60.02

$P < 0.05$; s.e. - standard error; t - student t-test

The percentage of dirty eggs, cracked eggs and misshapen eggs as well as the hatching percentage (Table 5) in the control group were lower ($P < 0.05$) than in the treatment group. In the control group 6727 eggs were recorded of which 78 (1.15%) were dirty, while in the SCFA treatment 7003 eggs were collected of which 33 (0.47%) were dirty. Incidence of dirty eggs is affected mainly by the incidence of cracked eggs, the availability of sufficient nest boxes and the frequency of egg collection. Since frequency of egg collection and number of nest boxes were similar in both groups, cracked eggs would be the only possible cause of the dirty eggs. Weak eggshells tend to crack easily, and nest box liners become soiled by cracked egg contents, causing newly laid eggs to be dirty. The percentage of cracked eggs and consequently the percentage of dirty eggs are strongly influenced by eggshell weakness. Since the control group did not receive additional butyrate, the shell of the eggs should be weaker, as expected (Belyavin *et al*., 1987).

Percentage of misshapen eggs in the control (6.27 ± 0.03) was higher ($P < 0.05$) than in the treatment group (3.81 ± 0.03). Minerals required for normal eggshell formation in hens must be released by their shell glands in the correct amounts and in the correct time for a good quality eggshell. This process requires that the absorption and metabolism of nutrients are adequate. The improvement could be due to the additional butyrate in the diet supporting mucosal membranes of the intestinal wall and improving the length of villi, thus improving the availability of the nutrients required for eggshell formation (Galfi & Bokori, 1990; Andoh *et al*., 1999; Wachtershauser & Stein, 2000). In the present study percentage of eggs hatching was higher ($P < 0.05$) in the treatment (93.36 ± 0.05) than in the control group (88.93 ± 0.06). Hatching eggs should not be cracked, be dirty or misshapen. Since these specifications were better in the supplemented than in the control group, the results were accepted as normal and possibly due to the effect of butyrate on hatching egg characteristics.

Results from this experiment suggested that the problem of eggshell weakness experienced in older birds could be overcome by the supplementation of butyrate in layer diets at a level of 285 mg/kg. Similar experiments could be carried out on parent stock after forced moulting at locations where forced moulting is practiced. It can be concluded that SCFA, in this case butyrate, supplemented at a level of 285 mg/kg to the diet of old layer parent stock would improve eggshell strength, reduce the percentage of dirty eggs, cracked eggs and misshapen eggs, and therefore increase the percentage of eggs hatching. Therefore, a potential benefit of SCFA supplementation for the poultry breeder industry would be an increased number of potentially hatchable eggs.

References

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

Barnett, D.M., Kumpula, B.L., Petryk, R.L., Robinson, N.A., Renema, R.A. & Robinson, F.E., 2004. Hatchability and early chick growth potential of broiler breeder eggs with hairline cracks. J. Appl. Poult. Res. 13, 65–70

Belyavin, C.G., Boorman, K.N. & 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. & Belyavin, C.G., Butterworths, Borough Green, Sevenoaks, Kent TN 15 8PH, England. 87, 105-122.

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

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. & 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. & Bokori, J., 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. Acta Vet. Hung. 38, 3-17.

Hong, T., Jin, G.B., Cho, S. & Cyong, J.C., 2002. Evaluation of the anti-inflammatory effect of baicalein on dextransulfate sodium-induced colitis in mice. Planta. Med. 68, 268-271.

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

Keshavarz, K., 1998. Further investigations on the effect of dietary manipulation of protein, phosphorus, and calcium for reducing their daily requirement for laying hens. Poult. Sci. 77, 1333-1346.

Leeson, S., Namkung, H., Antongiovanni, M. & Lee, E.H., 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84, 1418-1422.

Micallef, M.J., Iwaki, K., Ishihara, T., Ushio, S., Aga, M., Kunikata, T., Koya-Miyata, S., Kimoto, T., Ikeda, M. & Kurimoto, M., 2002. The natural plant product tryptanthrin ameliorates dextran sodium sulfate-induced colitis in mice. Int. Immunopharmacol. 2, 565-578.

Rama Rao, S.V., Raju, M.V.L.N., Reddy, M.R. & Pavani, P., 2006. Interaction between dietary calcium and non-phytate phosphorus levels on growth, bone mineralization and mineral excretion in commercial broilers. Anim. Feed Sci. Technol. 131, 135-150.

Rauch, W., 1965. The use of hen eggshell elasticity to estimate the shell strength. Arch Geflugelkd. 29, 467-477. (In German).

Sakata, T., 1987. Stimulatory effect of short-chain fatty aids on epithelial cell proliferation in the rat intestine: A possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Br. J. Nutr. 58, 95-103.

Scheppach, W., Sommer, H., Kirchner, T., Paganelli, G.M., Bartram, P., Christl, S., Richter, F., Dusel, G. & Kasper, H., 1992. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 103, 51-56.

Schwarzer, K., 2006. The use of sodium butyrate in combination with free organic acids in poultry. AVPA Scientific Meeting – Gold Coast 2006, Australia.

Sharma, R., Schumarcher, U., Ronaasen, V. & Coates, M., 1995. Rat intestinal mucosal responses to a microbial flora and different diets. Gut 36, 209-214.

SPSS, 1998. 10.0 Package program, User's Guide, SPSS Inc, Chicago, Illinois, USA.

Wachtershauser, A. & Stein, J., 2000. Rationale for the luminal provision of butyrate in intestinal diseases. Eur. J. Nutr. 39, 164-171.