Running title: Organic acids in layer production
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The effect of supplementary formic and lactic acid mixtures on egg production, feed conversion ratio and egg quality of layers

(Kesan penambahan campuran asid formik dan lactik terhadap pengeluaran telur, kadar penukaran makanan dan kualiti telur ayam penelur)

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Key words: organic acids, egg production, feed conversion, egg quality, laying hens

Abstract

Two experiments with Lohmann brown layers (30 and 43 weeks old, respectively) were conducted to evaluate the effects of varying organic acid mixtures (formic and lactic acid; OA) on egg production, feed conversion and egg quality. Each experiment was of 6 weeks duration. Treatments were the same in both studies and consisted of the following five dietary treatments: A: control diet, B: control diet supplemented with 0.15% organic acid mixture (OA), C: control diet supplemented with 0.30% OA, D: control diet supplemented with 0.45% OA and E: control diet supplemented with 0.60% OA. In period 1 and 2, supplementation with OA mixture had no significant effect on feed intake, egg weight and Haugh reading for all treatments. Combined analysis of data for both periods showed that supplementation with OA mixture at the 0.6% level significantly improved egg production, mean and total egg weight and improved feed efficiency compared to the control. There were no improvements in Haugh reading and egg shell thickness with OA supplementation. There is a trend towards higher egg production, better FCR and more efficient use of ME and CP for egg production with OA inclusion especially at levels of 0.60%.

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Introduction

The digestibility, improve animal growth performance and efficiency of feed conversion from antibiotic supplementation in farm animal diets is due mainly to its impact on the gastrointestinal microflora or antimicrobial effect. Historically, it has been theorized that antibiotic supplementation is a means of establishing food animals as reservoirs for antibiotic resistant pathogens isolated from humans (Neu 1992).

There are concerns associated with increased antibiotic resistance in bacterial pathogens making treatment of clinical diseases more difficult. This has resulted in a heightened awareness regarding exposure of foodborne pathogens to therapeutic and growth promotion levels of antibiotics during preharvest phases of food animal production (Threlfall et al. 2000). On the other hand, organic acids (Cherrington et al. 1991) have a long history of being utilized as antimicrobial food additives and preservatives for preventing food deterioration and extending the shelf life of perishable food ingredients.

Although the use of antibiotic growth promotants (AGP) in broiler production is common, its use is not allowed during egg production due to residue deposition in the egg. As the layer bird ages, there is a decrease in digestive capacity due to the impaired quality of intestinal mucosal cells, decreased length of intestinal villi, lower egg production, increased feed conversion ratio and lower egg shell calcification. As there is very little information on organic acids in layer feeding, the objective of this study was to evaluate the effect of an organic acid mixture on egg production, feed conversion ratio (FCR) and egg quality.

Materials and methods

Two experiments were conducted using Lohmann brown layers housed individually in 450 mm x 260 mm cages. The first study (period 1) with 30-week-old-hens was for a duration of 6 weeks. A different group of birds was used in the second trial (period 2) with 43-week-old hens for the 6 weeks feeding duration.

A total of 150 Lohmann Brown hens were used and the layers (5 pullets/replicate and 6 replicates/treatment) kept under open housing were subjected to the following dietary treatments: Group A (Control), a typical corn-soybean meal based diet; Group B, control diet with 0.15% (Organic acid mixture, OA); Group C, control diet with 0.30% OA; Group D, control diet with 0.45% OA and Group E, control diet with 0.60% OA over the 6 weeks laying period. The experimental diet and minimum calculated nutrients in the typical corn-soybean meal diets are shown in *Table 1*.

The mineral premix contained the following minerals per kilogramme: iron 80 g, manganese 100 g, copper 15 g, zinc 80 g, iodine 1.0 g, selenium 0.2 g, cobalt 0.25 g, potassium 4 g, magnesium 0.6 g and sodium 1.5 g. The vitamin premix contained the following vitamins per kilogramme: vitamin A, 50 million IU; vitamin D3, 10 million IU; vitamin E, 75 g; vitamin K3 MNB, 20 g; vitamin B1, 10 g;vitamin B2, 30 g; vitamin B6. 20 g; vitamin B12, 0.10g; calcium D-pantothenate, 60 g; niacin, 200 g; folic acid, 5 g and biotin, 235 mg.

The birds were randomly assigned and given the feed and water *ad libitum.* The OA (Fisher Chemicals) was a mixture of formic acid and lactic acid (1:4). Feed intake data for each replicate were collected weekly over the two experimental periods and pooled to give mean feed intake for each replicate for the period.

Eggs were collected and counted daily. All eggs produced on day 7, 14, 21, 28, 35 and 42 were weighed and pooled to give mean egg weight for each replicate for the period. At the 6th week, two egg samples from each replicate were taken for measurement of albumen height and egg shell thickness. The micrometers were AMES (Massachusetts, USA) albumen height micrometer and AMES thickness measurement gauge.

Haugh unit reading (Haugh, 1937) were derived from the equation:

$HU = 100 \log \{H-[\sqrt{G} (30W^{0.37} - 100) + 1.9\}$.

Data were subjected to analysis of variances using SAS Inst. (2000).

Results

Period 1: Feed intake and layer performance

Supplementation with OA mixture (*Table 2)* had no significant effect ($p > 0.05$) on feed intake, egg production, egg weight, FCR or Haugh reading for all treatments. Egg shell thickness was significantly higher in treatment E compared to treatment A and B.

Period 2: Feed intake and layer performance

Supplementation with OA mixture (*Table 2*) had no significant effect ($p > 0.05$) on feed intake, mean egg weight, Haugh reading and egg shell thickness for all treatments. However, egg production and total egg weight was significantly higher in treatment E compared to treatment A, B and C. While FCR was significantly lower ($p < 0.05$) in treatment E compared to other treatments.

Combined analysis of Period 1 and 2

Supplementation with OA mixture (*Table 3*) significantly lowered ($p < 0.05$) feed intake for treatment D and E compared to treatment A, B and C. Egg production and total egg weight was significantly higher ($p<0.05$) in treatment E compared to the other treatments.

Mean egg weight was significantly higher ($p < 0.05$) in treatment E compared to the control. FCR was significantly lower ($p < 0.05$) in treatment E compared to the other treatments. FCR of treatment D was also significantly lower ($p < 0.05$) than the control.

There were no significantly differences in Haugh readings between treatments. Egg shell thickness was significantly lower ($p < 0.05$) in treatment E compared to treatment B and C but all treatments were not significantly different to the control.

Comparison of feed intake and layer performance between period 1 and 2

A comparison of feed intake, FCR, egg production, Haugh reading and egg shell thickness of laying hens (*Table 4*) in period 1 and 2 showed that layers in period 1 performed significantly ($p < 0.05$) better than layers in period 2.

ME and protein intake and requirements for egg production

Supplementation with OA mixture (*Table 5*) had no significant effect ($p > 0.05$) on daily bird intake of ME and crude protein intake for all treatments in period 1. However, intake of ME and CP per kilogramme egg production was significantly lower ($p < 0.05$) in treatment E compared to the control.

In period 2, supplementation with OA mixture (Table 5) also had no significant effect ($p > 0.05$) on daily bird intake of ME and crude protein intake for all treatments. However, intake of ME and CP per kilogramme egg production was significantly lower (p <0.05) in treatment E compared to treatment A and B.

Discussion

Although there were no significant differences in feed intake, egg production, egg weight, FCR or Haugh reading between treatments, it can be observed that there is a trend towards better egg production and lower feed consumption with increasing OA supplementation in period 1. By comparison, supplementation with OA mixture also had no significant effect on feed intake, mean egg weight and Haugh reading for all treatments in period 2. However, egg production, total egg weight and FCR was significantly better in treatment E compared to the other treatments.

It can be inferred from this two separate studies that differences between period 1 and 2 might be due to the OA mixture being ineffective against layers of a lower age (period 1) compared to older layers (period 2). As the layer bird ages, there is a decrease in digestive capacity due to the impaired quality of intestinal mucosal cells (Dibner and Buttin 2002), lower egg production, increased feed conversion ratio and lower egg shell calcification (Roberts 2004). Thus, OA supplementation is more appropriate for older layers. There was also a significant trend towards a thicker egg shell with increasing OA supplementation in period 1, but these differences were not seen in period 2.

There are some reports of improved production with organic acids used for poultry (Patten and Waldroup 1988; Izat et al. 1990; Skinner et al. 1991). Early study by Vogt and Matthes (1981) reported that fumaric acid improved feed efficiency by 3.5-4% in broilers and layers but rate of lay was not affected.

Combined analysis of the data from the two periods showed that supplementation with OA mixture at the 0.6% level significantly improved egg production, mean and total egg weight and enhancing feed efficiency compared to the control. The FCR and total egg production improved by 1.87% and 1.41% respectively in treatment E compared to the control and thus OA supplementation is effective in enhancing production over both periods.

Combined analysis of the data from the two periods showed no improvements in Haugh reading and egg shell thickness with OA supplementation implying that it has no effect on these egg characteristics. Internal egg qualities relates to the functional characteristics of an egg and Haugh reading is the standard method for determination of interior egg quality (Eisen et al. 1962) and rate of quality loss as measured by Haugh reading is a nonlinear function. However, Silversides and Scott (2001) reported that the Haugh equation might be wrong as the statistical association between albumen pH and egg weight was very low. They have suggested that if albumen quality is being used as a measure of freshness, then the albumen height is biased by the strain and age of hen, whereas the albumen pH is not and should be used.

Intake of calculated ME and CP per kilogramme egg production was significantly lower (p <0.05) in treatment E compared to the control for period 1 and 2 and this was due to more efficient and higher egg production at OA supplementation of 0.6%. Thus with higher levels of OA supplementation, less energy and protein was required per unit egg production and this improvement in feed efficiency has been reported by Vogt and Matthes (1981).

In addition, Garcia et al. (2007) also reported that formic acid increased ileal digestion of nutrients and had a positive effect on the intestine mucosa. The ME intake and CP intake values for production of per kilogramme eggs reported here were lower than values reported by Wong and Engku Azahan (2004). This might be due to differences in bird ages and the feed formulations.

Organic acids have been reported (Dibner and Buttin 2002) to improve protein and energy digestibilities by reducing microbial competition with the host for nutrients and endogenous nitrogen losses. It has also been reported to lower the incidence of subclinical infections and secretion of immune mediators, and by reducing production of ammonia and other growth-depressing microbial metabolites.

The internal quality of eggs and quality of egg shells are influenced by many factors. These include bird age, bird strain, nutrition, disease, management practices, water quality, housing conditions, temperature or stress (Roberts 2004). As the layers in period 1 were younger, it was expected that laying performance, feed efficiency and egg quality as measured by Haugh reading and egg shell thickness would be significantly better than the layers in period 2, and this was observed in this study.

Generally layers reach peak laying performance at 30-38 weeks while internal egg quality reach a peak at 20 weeks (Anon. 2006). From this study, with the exception of bird age, all the other factors mentioned by Roberts (2004) were similar between period 1 and 2. Although researchers have attempted to improve egg production and egg quality through nutrient supplementation, egg internal quality inevitably declines with age.

Williams (1992) reviewed factors that affect albumen height and Haugh reading and observed that a few nutritional factors may be implicated, but, overall, nutrition is relatively unimportant. The major influences on albumen height and Haugh reading are the strain and age of the hen laying the egg and storage time and conditions.

A prerequisite for successful efforts to minimize the impact of antibiotic resistance in the animal industry is development and implementation of alternative antimicrobials and the feed supplements industry is clearly moving in this direction as can be observed by the many biological and herbal non-antibiotic growth promoters (AGP) products widely available. Many studies have documented the effects of organic acids on performance in young swine, particularly early-weaned piglets (Partanen 2001) and the author concludes that dietary acids have a beneficial effect, especially on weaned piglets, that is primarily associated with changes in the gastrointestinal microflora.

From this study, it can be inferred that there is a trend towards higher egg production, better FCR and more efficient use of ME and CP for egg production with OA inclusion especially at levels of 0.60%. Organic acids can have significant benefits in poultry production but additional research is still needed on its impact on replacing AGP.

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Abstrak

Dua kajian dengan ayam penelur baka Lohmann berwarna perang (pada umur 30 dan 43 minggu) dijalankan untuk menilai kesan beberapa aras campuran asid organik (asid formik dan asid lactik) terhadap pengeluaran telur, kecekapan penukaran makanan dan kualiti telur. Tempoh setiap kajian ialah 6 minggu. Lima perlakuan diet yang sama digunakan dalam kedua-dua kajian tersebut, iaitu: A: diet kawalan; B: diet kawalan yang ditambah dengan 0.15% campuran asid (OA); C: diet kawalan yang ditambah dengan 0.30% OA; D: diet kawalan yang ditambah dengan 0.45% OA; dan E: diet kawalan yang ditambah dengan 0.60% OA. Pada fasa pertama dan kedua, penambahan dengan campuran OA tidak ketara berbeza antara perlakuan bagi pengambilan makanan, berat telur dan bacaan Haugh. Analisis data dari fasa pertama dan kedua yang digabungkan menunjukkan penambahan dengan OA pada aras 0.6% meningkatkan pengeluaran telur, purata dan jumlah berat telur dan kecekapan penukaran makanan dengan ketara berbanding dengan kawalan. Penambahan dengan OA tidak meningkatkan bacaan Haugh dan ketebalan kulit telur antara perlakuan pada kedua-dua fasa. Didapati ada trend ke arah peningkatan pengeluaran telur, kecekapan penukaran makanan dan penggunaan ME dan CP dengan lebih cekap bagi pengeluaran telur apabila OA ditambah di dalam diet, khususnya pada aras 0.6%.

Table 1. Feed composition and calculated nutrients of experimental diet

Ingredient	Amount %	Nutrient	Minimum supplied	Units
Maize	51.35			
Palm oil	1.50	ME	11.21	MJ/kg
Soybean meal	22.00	Crude Protein	17.00	%
DCP	1.11	Crude Fat	3.00	%
Limestone	10.10	Fibre	3.00	%
NaCl	0.30	Linoleic acid	1.45	%
Lysine	0.05	Calcium	4.00	%
Methionine	0.10	P. Available	0.32	%
Mineral premix	0.10	Sodium	0.16	%
NaHCO3	0.15	Chloride	0.16	%
Rapeseed meal	5.00	Choline	1300.00	mg/kg
DDGS	2.00	Arginine	0.70	%
Choline	0.04	Cystine	0.25	%
Natuphos 5000	0.01	IsoLeucine	0.53	%
Vitamin premix	0.20	Lysine	0.82	%
Wheat pollard	6.00	Methionine	0.39	%
		Met+Cys	0.58	%
		Tryptophane	0.17	%
		Tyrosine	0.40	%
		Valine	0.53	%
		Vitamin E	20.0	mg/kg

Table 3. Effect of organic acids supplementation on feed intake, FCR, egg production, haugh reading and egg shell thickness in laying hens over combined periods

Treatment	A	B	C	D	E
Daily Feed Intake (g/bird/day)	108.08a	107.96a	108.08a	107.69b	107.60b
Tot feed intake per group (kg)	136.18a	136.03a	136.18a	135.69b	135.58b
Egg production (%)	89.48bc	89.36c	89.40bc	89.83b	90.39a
Mean egg weight (g)	62.10b	62.25ab	62.35a	62.23ab	62.33a
Total egg weight per group (kg)	69.97b	70.05b	70.16b	70.40b	70.96a
FCR	1.948a	1.944a	1.943a	1.929b	1.912c
Haugh unit	88.28a	88.18a	88.23a	88.29a	88.25a
Shell thickness (mm)	0.351ab	0.350b	0.350b	0.353ab	0.356a

Values bearing different letters are significantly different ($p < 0.05$)

Table 4. Comparison of feed intake, layer performance end egg quality between period 1 and 2

Treatment	Period 1	Period 2
Daily Feed Intake (g/bird/day)	105.47b	110.30a
Total Feed Intake per group (kg)	132.89b	138.98a
Egg production (%)	92.90a	86.49b
Mean egg weight (g)	61.24b	63.27a
Total egg weight per group (kg)	71.68a	68.93b
FCR	1.854b	2.016a
Haugh unit	90.69a	85.81b
Shell thickness (mm)	0.354a	0.349b

Values bearing different letters are significantly different ($p < 0.05$)

Table 2. Effect of organic acids supplementation on feed intake, FCR, egg production, haugh reading and egg shell thickness in laying hens for period 1 and 2

	Period 1 (30 weeks old laying hen)					Period 2 (43 weeks old laying hen)				
Treatment	A	B	C	D	E	A	B	C	D	E
Daily feed intake (g/bird/day)				105.65a						
	105.50a									
	105.65a									
	105.27a									
	105.27a									
	110.51a		110.43a		110.51a		110.11a	109.94a		
Total feed intake per group (kg)				133.12a						
	132.93a									
	133.12a									
	132.64a									
	132.64a									
	139.25a		139.14a		139.25a		138.74a	138.52a		
Egg production (%)	92.79a									
	92.70a									
	92.76a									
	93.03a									
	93.21a									
	86.16b	86.03b	86.04b	86.63b		87.57a				
Mean egg weight (g)	61.07a									
	61.24a									
	61.34a									
	61.22a									
	61.32a									
	63.13a	63.26a	63.36a	63.24a		63.35a				
Total egg weight per group (kg)				71.41a						
	71.53a									
	71.69a									
	71.76a									
	72.02a									
	68.53b	68.58b	68.64b	69.04ab		69.89a				
FCR	1.864a									
	1.858a									

	1.857a										
	1.848a										
	1.841a										
	2.032a	2.029a	2.029a	2.010a	1.982b						
Haugh unit	90.65a	90.62a	90.73a	90.68a	90.75a	85.91a	85.75a	85.73a	85.89a	85.75a	
Shell thickness (mm)	0.353b	0.353b	0.354ab		0.355ab	0.356a	0.348a	0.347a	0.347a	0.351a	0.355a

Statistical comparison between treatments made within period only

Values bearing different letters are significantly different ($p < 0.05$)

Table 5. Effect of organic acids supplementation on calculated ME and protein intake of layers in period 1 and 2

Parameter	Period 1 (30 weeks old laying hen)					Period 2 (43 weeks old laying hen)				
	Group A	Group B	Group C	Group D	Group E	Group A	Group B	Group C	Group D	Group E
ME intake (kJ/bird/day)	1184.34a	1182.66a	1184.34a	1180.04a	1180.04a	1238.85a	1237.88a	1238.85a	1234.36a	1232.40a
ME intake (MJ/kg egg)	19.39a	19.31ab	19.31ab	19.28ab	19.24b	19.63a	19.57ab	19.55abc	19.52bc	19.45c
CP intake (g/bird/day)	17.96a	17.94a	17.96a	17.89a	17.89a	18.78a	18.77a	18.80a	18.72a	18.69a
CP intake (g/kg egg)	294.08a	292.88ab	292.83ab	292.32ab	291.83b	297.62a	296.74ab	296.50abc	295.98bc	295.03c

Statistical comparison between treatments made within period only

Values bearing different letters are significantly different (p <0.05)