Effects of feeding a fermented product on egg production, faecal microflora and faecal pH in laying hens

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ABSTRACT

This study was conducted to investigate the effects of feeding fermented product (FP) to layers on laying performance, faecal microflora and pH. A total of 96, 13-week-old Babcock B380 pullets were used in the study. They were randomly assigned to four numerically equal groups with eight replicates per treatment, three birds per replicate. All the birds were caged individually. The treatments consisted of Diet 1 (Control), without FP, Diet 2 (FP3) contained 3% (w/w) FP (30g FP/kg diet), Diet 3 (FP6) contained 6% (w/w) FP (60 g FP/kg diet) and Diet 4 (FP9) contained 9% (w/w) FP (90 g FP/kg diet). The study was conducted for 14 weeks with 2 weeks of acclimatization. Fresh faecal samples were collected fortnightly started from 15 weeks of age. The feed intake, egg production, feed conversion ratio (feed intake, g/egg mass, g), egg mass (egg x egg production/100) and egg weight were not affected by the addition of FP at any level in the diets. However, the egg weight for FP6 was significantly higher than FP9. The faeces samples were cultured for lactic acid bacteria (LAB) and *Enterobacteriaceae*. The results obtained from this study showed that FP reduced (P<0.05) the faecal *Enterobacteriaceae* and pH value. Higher (P<0.05) counts of faecal LAB in layers fed with FP were observed. In conclusions, these results indicate that FP could be included up to 6% in the diets of layers contributed to heavier egg weight and shifting the microflora composition of the layers' gastrointestinal tract toward a beneficial balance.

KEY WORDS: fermented product, layer, egg, lactic acid bacteria, *Enterobacteriaceae*

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INTRODUCTION

Utilization of feed additive is a common practice to improve the productivity of animals. Addition of antibiotic growth promoter is the most common approach. However, the extensive use of antibiotics as growth enhancer may lead to the development of antimicrobial resistance against pathogenic bacteria species (Mikkelsen and Jensen, 2000). This may hamper the effectiveness of treatment in infected humans especially in the event of human-animal infections. Furthermore, the EU countries have already imposed ban on the use of antibiotic in the dietary of animals as growth enhancer. Recently, fermented feed was suggested as alternatives to the antibiotic growth promotants (Mikkelsen and Jensen, 2000; Demecková et al., 2002; van Winsen et al., 2002; Loh et al., 2003a,b). Therefore, it has become an important and rapidly expanding segment of the animal feed market. This is one of the avenues to minimize or even eliminate the need for antibiotics in feeds. Feeding fermented liquid and fermented fruits to animals resulted in the reduction of coliform numbers in the digestive tract (Mikkelsen and Jensen, 2000; van Winsen et al., 2001, 2002; Demecková et al., 2002; Canibe and Jensen, 2003; Højberg et al., 2003; Loh et al., 2003a,b; Heres et al., 2004). In addition, feeding fermented feed results in the increase of feed intake post-weaning and thus increase post-weaning growth rate in piglets (Mikkelsen and Jensen, 2000). It also has been reported that feeding fermented solid product (Loh et al., 2003a,b) has a similar effect as feeding fermented liquid feed (Mikkelsen and Jensen, 2000; Demecková et al., 2002; van Winsen et al., 2002; Canibe and Jensen, 2003; Højberg et al., 2003; Heres et al., 2004). However, there is no information on the effects of feeding fermented solid products to layers on their laying performance, faecal microflora counts and pH in faeces. Thus, the objectives of this experiment were to study the effects of different levels of FP inclusions in the diets of layers on the laying performance, population of faecal microflora and its pH.

MATERIAL AND METHODS

Experimental birds and diets

This experiment was carried out on 96 13-week old Babcock B380 pullets with an average body weight of $1.19 \, kg \pm 0.01$ (BW ± SE). All the birds were kept individually in wire cages ($36 \times 30 \times 35$ cm) in an open layer house. They were randomly allotted to four dietary treatments, with eight replicates per treatment, three birds per replicate. The dietary treatments comprised of basal diet without the FP as control, and experimental diets containing 3% FP (w/w) (FP3), (w/w) (FP6) and 9% (w/w) (FP9). The contents of the FP used in this study consisted of, %:

(w/w) lime 9, (v/w) molasses 1, (w/w) rice bran 53.5, (w/w) fish (*Rastrelliger kanagurta*) 35, (v/w) vinegar 1 and (w/w) starter cultures 0.5. The starter cultures comprised of *Lactobacillus plantarum* isolated from 'tempeh', a local fermented food as described by Foo et al. (2003). The fermentation process was conducted according Loh et al. (2003a,b). The experimental feeds were provided in mash form. The diet compositions of pullet and layer are shown in Tables 1 and 2, respectively. The diets were formulated as isonitrogenous and isoenergetic. The dry matters of the pullet and layer diets were ranged from 88.20 to 88.36 % and the *Enterobacteriaceae* count was 0.83 ± 0.16 ($\log_{10}$ cfu/g). The birds were adapted to the respective diets for 2 weeks and the experimental period was conducted for another 14 weeks. Individual body weight was measured at the beginning and end of the experiment. Water and feed were supplied *ad libitum*. The birds were raised under natural light circumstances. The feed was offered early in the morning (08.00) and in the evening (17.00). Feed intake was measured weekly for each treatment groups. Feed conversion ratio (FCR) was then calculated based on the formula: FCR = feed intake (g)/egg mass (g).

Table 1. Compositions of pullet diets, from 13-18 weeks of age

Item	Control	FP3	FP6	FP9
Ingredients, %				
maize	60.15	58.65	56.81	54.97
palm oil	2.50	2.20	1.94	1.70
soyabean meal (44%)	31.30	30.10	29.20	28.28
L-lysine	0.20	0.20	0.20	0.20
DL-methionine	0.20	0.20	0.20	0.20
MDCP	3.00	3.00	3.00	3.00
limestone	1.10	1.10	1.10	1.10
salt	0.40	0.40	0.40	0.40
vitamin premix[1]	0.08	0.08	0.08	0.08
mineral premix[2]	0.07	0.07	0.07	0.07
choline chloride	1.00	1.00	1.00	1.00
FP	-	3.00	6.00	9.00
Calculated values				
ME, MJ/kg	12.23	12.23	12.23	12.23
crude protein, %	18.89	18.81	18.84	18.86
lysine, %	0.98	0.98	0.98	0.98
methionine, %	0.42	0.43	0.43	0.44

[1] the vitamin-premix per kg of diet, IU: vit. A 50.0, vit. D_3 8.0, g: vit. E 200, vit. B_2 20.0, vit. B_6 20.0, vit. B_{12} 1.0, vit. K_3 10.0, biotin 0.8, panthothenic acid 80.0, folic acid 10.0, niacin 130.0, anticaking agent 20.0 and antioxidant 0.2

[2] the mineral premix provides the following amounts per kg of diet, g: Mn 20.0, Fe 80.0, Zn 80.0, Cu 10.0, Co 0.2, I 0.3 and Se 0.3

FP - fermented product

Table 2. Compositions of layer diets, from 19 weeks to 29 weeks of age

Item	Control	FP3	FP6	FP9
Ingredient, %				
maize	60.60	58.55	57.00	54.30
palm oil	1.55	1.35	1.05	0.97
soyabean meal (44%)	25.50	24.75	23.60	23.38
L-lysine	0.06	0.06	0.06	0.06
DL-methionine	0.16	0.16	0.16	0.16
MDCP	2.50	2.50	2.50	2.50
limestone	8.00	8.00	8.00	8.00
salt	0.50	0.50	0.50	0.50
vitamin premix[1]	0.07	0.07	0.07	0.07
mineral premix[2]	0.06	0.06	0.06	0.06
choline chloride	1.00	1.00	1.00	1.00
FP	-	3.00	6.00	9.00
Calculated values				
ME, MJ/kg	11.42	11.42	11.41	11.41
crude protein, %	16.22	16.29	16.23	16.49
lysine, %	0.88	0.89	0.89	0.90
methionine, %	0.42	0.43	0.43	0.44

[1] the vitamin-premix per kg of diet, IU: vit. A 50.0, vit. D_3 8.0, g: vit. E 200, vit. B_2 20.0, vit. B_6 20.0, vit. B_{12} 1.0, vit. K_3 10.0, biotin 0.8, panthothenic acid 80.0, folic acid 10.0, niacin 130.0, anticaking agent 20.0 and antioxidant 0.2

[2] the mineral premix provides the following amounts per kg of diet, g: Mn 20.0, Fe 80.0, Zn 80.0, Cu 10.0, Co 0.2, I 0.3 and Se 0.3

FP - fermented product

Samples collection and microflora counts

During the laying period (from 21 to 29 weeks of age), the eggs were collected daily. The number of egg produced including the normal and abnormal eggs from all the treatments was recorded and only normal eggs were weighed. Egg mass was calculated based on the formula: egg mass = (egg weight × egg production)/100.

A total of 3 g of faecal samples were collected from each replicate of each experimental group at 15, 17, 19, 21, 23, 25, 27 and 29 weeks of age during the experimental period. Fresh faecal samples were collected immediately after defecation. The samples were then placed in different sterile universal bottles in order to determine the faecal pH, numbers of LAB and *Enterobacteriaceae*. The pH of the faeces was directly measured with a pH meter. The faecal (10% w/v) was suspended in sterile peptone water and incubated for an h before further 10-fold dilutions (v/v) were made with peptone water for total LAB and *Enterobacteriaceae* counts. Total LAB counts were spread plated on MRS-agar (Merck®) and incubated at 30°C for 48 h, whereas *Enterobacteriaceae* were plated on EMB-agar (Merck®) and incubated at 37°C for 24 h as described by

Loh et al. (2003a,b). Numbers of colony forming units (CFU) are expressed as $\log_{10}$ CFU per g.

Statistical analysis

The data were analysed using the one-way analysis of variance (ANOVA) in accordance to a completely randomised design model (SAS®, 1991). Significant differences between treatments means were compared using the Duncan's multiple range test at P<0.05.

RESULTS

The effect of feeding fermented product (FP) on growth and laying performance in layers from 15 to 29 weeks of age is presented in Table 3. No significant difference (P>0.05) results were found for initial and final body

Table 3. Effects of feeding fermented product on growth and laying performance in layers from 15 to 29 weeks of age

Item	Treatments				Signi-ficance
	control	FP3	FP6	FP9	
Initial body weight, kg/bird	1.20 ± 0.01	1.19 ± 0.01	1.19 ± 0.01	1.17 ± 0.01	ns
Final body weight, kg/bird	1.67 ± 0.02	1.66 ± 0.02	1.68 ± 0.02	1.64 ± 0.02	ns
Average daily feed intake, g/day/bird	91.03 ± 1.82	92.64 ± 1.02	89.25 ± 1.32	91.26 ± 1.07	ns
Average egg production, at 29 weeks of age, %	85.71 ± 4.38	88.69 ± 2.58	82.14 ± 3.36	91.07 ± 2.24	ns
Average feed conversion ratio, g feed intake/g egg mass	1.96 ± 0.07	2.05 ± 0.09	2.10 ± 0.06	2.10 ± 0.07	ns

the results are presented as mean values ± SE
values with different superscripts within rows differ significantly at P<0.05
[ns] no significant difference
* P<0.05

weights, average daily fed intake, egg production and FCR between the treatment groups. Figure 1 shows the effects of feeding FP on egg mass of layers. There was no significant difference (P>0.05) in egg mass among the treatment groups throughout the experimental period. However, the egg produced by hens fed with FP had slightly lower mass as compare with the control hens during the laying period. The egg weight of layers after feeding with FP is shown in Figure 2. The egg weight was only found to be significant different (P<0.05) after 25 weeks of age. The egg weight of FP6 was significantly higher (P<0.05) than the FP9 from 26 to 29 weeks of age. However, the egg
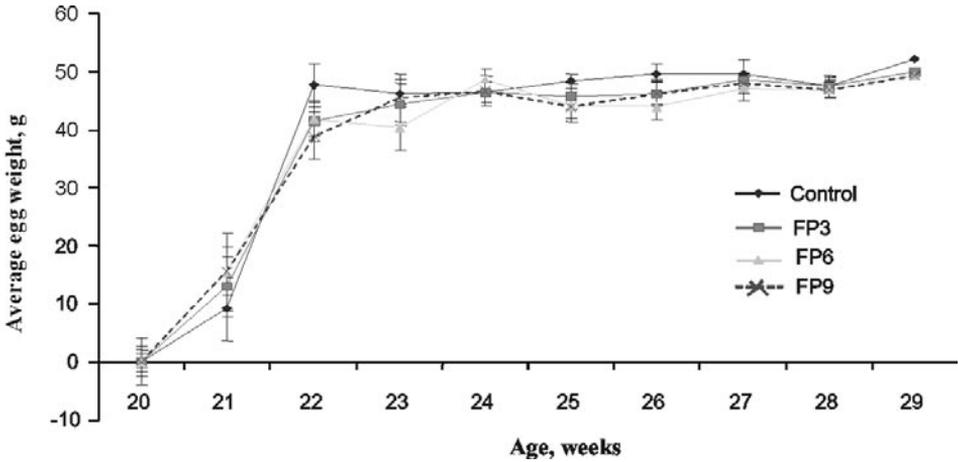
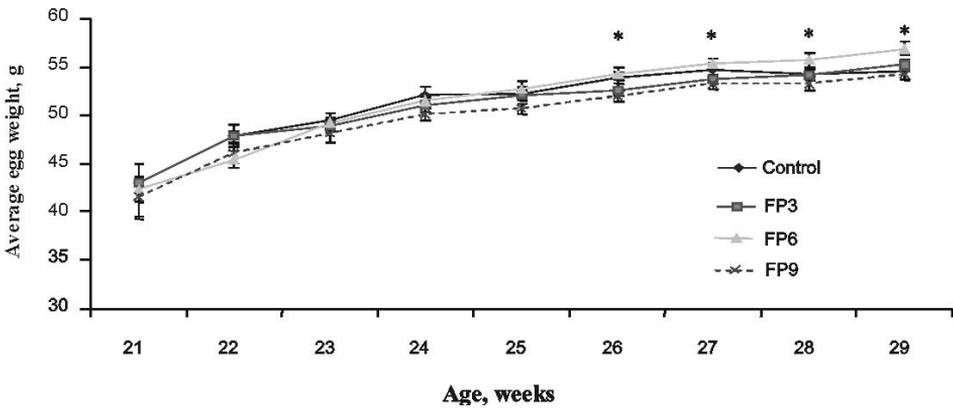Figure 1. Effects of feeding FP on egg mass of layers, mean ± SE, g

Figure 2. Effects of feeding FP on egg weight of layers, mean ± SE, g, * P<0.05

weights of FP3 and control were found to be significantly lower (P<0.05) compared to the FP6 at 26 and 29 weeks of age, respectively.

The faecal *Enterobacteriaceae* counts (Table 4) for all the treatment groups were similar (P>0.05) at the beginning of the experiment (15 weeks of age). However, the faecal *Enterobacteriaceae* counts for the FP6 and FP9 groups were significantly lower (P<0.05) compared to the control groups from 17 weeks of age onwards. Similarly, the FP3-fed group had significantly lower (P<0.05) faecal *Enterobacteriaceae* counts as compare with the control group except at 15 and 29 weeks of age.

Table 4. Effects of feeding fermented product on faecal *Enterobacteriaceae* counts ($\log_{10}$ cfu/g) in layers

Age, weeks	Treatments				Significance
	control	FP3	FP6	FP9	
15	7.23 ± 0.07	7.03 ± 0.09	6.96 ± 0.12	6.83 ± 0.06	ns
17	$7.90^a \pm 0.05$	$6.79^b \pm 0.09$	$7.45^b \pm 0.08$	$7.38^b \pm 0.12$	*
19	$6.35^a \pm 0.09$	$6.11^b \pm 0.06$	$6.55^b \pm 0.14$	$6.03^b \pm 0.12$	*
21	$6.83^a \pm 0.09$	$5.81^b \pm 0.07$	$5.52^c \pm 0.07$	$4.85^d \pm 0.05$	*
23	$6.25^a \pm 0.03$	$5.72^b \pm 0.11$	$5.56^b \pm 0.07$	$4.96^c \pm 0.11$	*
25	$6.49^a \pm 0.09$	$5.89^b \pm 0.12$	$5.34^c \pm 0.12$	$4.62^d \pm 0.08$	*
27	$6.32^a \pm 0.09$	$5.75^b \pm 0.09$	$5.00^c \pm 0.07$	$4.64^d \pm 0.09$	*
29	$5.92^a \pm 0.09$	$5.69^a \pm 0.13$	$5.24^b \pm 0.08$	$4.64^c \pm 0.08$	*

the results are presented as mean values ± SE
values with different superscripts within rows differ significantly at P<0.05
[ns] no significant difference
* P<0.05

The faecal LAB counts (Table 5) for all the treatment groups were similar (P>0.05) at the beginning of the experiment (15 weeks of age). The faecal LAB counts for the control and FP3 fed groups were not significantly different (P>0.05) at 17, 21, 25, 27 and 29 weeks of age. However, the faecal LAB counts for the FP6 and FP9 fed groups were significantly (P<0.05) higher than the control group.

Table 5. Effects of feeding fermented product on faecal LAB counts ($\log_{10}$ cfu/g) in layers

Age, weeks	Treatments				Significance
	control	FP3	FP6	FP9	
15	6.16 ± 0.11	6.06 ± 0.11	6.25 ± 0.04	6.06 ± 0.10	ns
17	$6.20^a \pm 0.06$	$6.24^a \pm 0.02$	$6.31^b \pm 0.07$	$6.60^c \pm 0.07$	*
19	$6.50^a \pm 0.05$	$6.74^b \pm 0.08$	$6.73^b \pm 0.03$	$7.05^c \pm 0.07$	*
21	$5.91^a \pm 0.10$	$6.17^b \pm 0.09$	$6.42^c \pm 0.07$	$6.56^c \pm 0.07$	*
23	$5.90^a \pm 0.09$	$6.13^b \pm 0.06$	$6.44^c \pm 0.05$	$6.71^d \pm 0.09$	*
25	$5.73^a \pm 0.09$	$5.75^a \pm 0.08$	$6.28^b \pm 0.05$	$6.53^c \pm 0.08$	*
27	$5.98^a \pm 0.06$	$6.05^a \pm 0.06$	$6.48^b \pm 0.08$	$6.65^b \pm 0.09$	*
29	$5.78^a \pm 0.08$	$5.98^a \pm 0.11$	$6.31^b \pm 0.07$	$6.60^c \pm 0.10$	*

the results are presented as mean values ± SE
values with different superscripts within a row differ significantly at P<0.05
[ns] no significant difference
* P<0.05

The faecal pH (Table 6) of birds at 15 weeks of age for all the experimental groups was not significant different (P>0.05). There was a trend faecal pH was lower in those birds fed with FP than the control. Significant difference (P<0.05) was observed in the faecal pH between FP9 and control groups after 15 weeks of age. Apparently, the faecal pH of the FP9 group remained the lowest (P<0.05) throughout the experimental period.

Table 6. Effects of feeding fermented product on faecal pH in layers

Age, weeks	Treatments				Significance
	control	FP3	FP6	FP9	
15	6.73 ± 0.07	6.85 ± 0.05	6.71 ± 0.02	6.73 ± 0.02	Ns
17	6.81[ab] ± 0.03	6.85[a] ± 0.05	6.71[b] ± 0.04	6.55[c] ± 0.04	*
19	6.90[a] ± 0.06	6.79[ab] ± 0.09	6.70[ab] ± 0.07	6.68[b] ± 0.04	*
21	7.00[a] ± 0.05	7.03[a] ± 0.09	6.85[ab] ± 0.06	6.72[b] ± 0.06	*
23	7.57[a] ± 0.05	7.52[a] ± 0.15	7.40[ab] ± 0.08	7.19[b] ± 0.03	*
25	7.09[a] ± 0.06	6.99[a] ± 0.06	6.92[a] ± 0.05	6.65[b] ± 0.11	*
27	7.17[a] ± 0.07	6.85[b] ± 0.08	6.95[b] ± 0.08	6.89[b] ± 0.06	*
29	7.06[a] ± 0.04	6.86[a] ± 0.07	6.88[a] ± 0.05	6.59[b] ± 0.12	*

the results are presented as mean values ± SE

values with different superscripts within rows differ significantly at P<0.05

[ns] no significant difference

* P<0.05

DISCUSSION

Supplementation of FP at 3, 6 and 9% in layer diet had no effect on body weights, fed intake egg production, FCR and egg mass. There was no mortality recorded during the experimental period. However, feeding higher levels of FP, i.e. 9% decreased the egg weight compared to control. Reduction in egg weight could be associated with dietary unsaturated fatty acids due to the present of fish in the FP (Law et al., 2007). Gonzalez-Esquerra and Leeson (2000) reported that consumption of unsaturated fatty acids causes a decrease in circulating triacylglycerols of birds and then limit the availability of lipid for yolk formation.

The FP used in this study was rich in LAB, a low pH and high concentration of lactic acid (Loh et al., 2003b). It has been shown that LAB has antagonistic effect on harmful bacteria either *in vitro* or *in vivo* studies (Lindgren and Dobrogosz, 1990; Forestier et al., 2001; Foo et al., 2003). Oral administration of FP (6 and 9%) rich in these beneficial bacteria has been shown to be able to reduce the faecal *Enterobacteriaceae* count. This was attributed to the reduction in faecal pH as a result of FP supplementation and increased proliferation of the faecal LAB in the present study. The faecal *Enterobacteriaceae* populations reflected the *Enterobacteriaceae*-LAB balance in the gastro-intestinal tract (GIT) of the host animal. Several *in vivo* studies reported that the numbers of *Enterobacteriaceae* in the GIT of the fermented feed fed groups were lower compared to the control group (van Winsen et al., 2001, 2002; Canibe and Jensen, 2003; Højberg et al., 2003; Loh et al., 2003a,b). This could be due to the fact that intestinal LAB are able to produce antimicrobial compounds such as bacteriocin and organic acids, which are bacteriostatic against pathogenic bacteria (Adam and Nicolaides, 1997; Holzapfel, 2002). The undissociated organic acids decrease the pH when it passes through

the cell wall of the bacteria (Immerseel et al., 2003). In the internal environment of the bacteria cell, the molecules will be dissociated and decreased the internal pH. This in turn stops the enzymatic processes and collapses the proton motive force. Growth is inhibited because the cell must spend more energy to maintain internal pH and this energy cannot be used for other metabolic processes. This probably resulted in less numbers of *Enterobacteriaceae* that could pass the crop and gizzard, which would reduce the probability of *Enterobacteriaceae* reaching the caeca and be excreted through the faeces (Heres et al., 2003a,b).

Laying hens fed diets supplemented with FP (6 and 9%) had higher faecal LAB counts as compare with the control group. However, 3% FP did not have any effect on faecal LAB count. These results indicate that the LAB count could be elevated as the inclusion level of FP more than 6%. The acidifying effect of the FP on the GIT provided a suitable environment for the growth of LAB (Loh et al., 2003a,b) as reflected in the LAB population in the faeces. The LAB competes against enteropathogens for nutrient, binding and receptor sites (Gallaher et al., 1996; Fujiwara et al., 1997; Kailasapathy and Chin, 2000). They have strong inhibitory effect in preventing the adherence, establishment and replication of pathogenic bacteria (Jin et al., 1998). The LAB are important components for a balanced microflora in the gastrointestinal tract.

Reduction of faecal pH was observed in layers fed with FP9. This further confirm earlier reports by Heres et al. (2003a,b, 2004) and Loh et al. (2003a,b). Feeding of acidified feed reduce the crop pH to pH 4.5 (Cox et al., 1972) and therefore lower the pH in GIT, resulting in a lower faecal pH. A number of factors may account for the beneficial effect of FP and these may act independently or synergistically. It was suggested that the FP reduced pH in the GIT thereby probably enhanced the activity of volatile fatty acids.

CONCLUSIONS

In conclusion, the results of feed intake, egg production, feed conversion ratio and egg mass were not affected by the level of fermented product (FP) added in the diet. However, the addition of 6 and 9% FP diets reduced the *Enterobacteriaceae* population in faeces and increased the faecal LAB population. Feeding of FP was able to reduce the level of faecal pH. Considering the results of egg weight, faecal microflora counts and pH, addition of FP up to 6% in the diet contributed better effects as compare with the rest of treatment groups. These results indicate that FP has the potential to be added in layers feed to promote better health performance without adversely affecting production parameters.

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