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Effects of Feeding Solid-state Fermented Rapeseed Meal on Performance, Nutrient Digestibility, Intestinal Ecology and Intestinal Morphology of Broiler Chickens

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ABSTRACT : This trial was conducted to determine the effects of feeding a diet containing solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. A mixed liquid culture, containing approximately 5 log cfu/ml *Lactobacillus fermentum*, *Enterococcus faecium*, *Saccharomyces cerevisae* and *Bacillus subtilis* was prepared in a 1:1:1:1 ratio. A basal substrate (BS) containing 75% rapeseed, 24% wheat bran and 1% brown sugar was mixed with the liquid culture in a ratio of 10:3. Over the 30-day fermentation, isothiocyanates were reduced from 119.6 to 14.7 mmol/kg. A total of 168, day-old male Arbor Acres broiler chicks were assigned to one of three dietary treatments including a corn-soybean meal based control diet as well as two experimental diets in which the control diet was supplemented with 10% of the BS containing unfermented rapeseed meal or 10% of the BS containing rapeseed meal subjected to solid state fermentation. There were 8 pens per treatment and 7 birds per pen. From days 19-21 and days 40-42, uncontaminated excreta were collected from each pen for digestibility determinations. In addition, digesta from the colon and ceca were collected to determine the number of lactobacilli, enterobacteria and total aerobes. The middle sections of the duodenum, jejunum, and ileum were collected for intestinal morphology. Over the entire experimental period (d 1-42), the weight gain and feed conversion of birds fed fermented rapeseed meal were superior ($p<0.05$) to that of birds fed non-fermented rapeseed meal and did not differ from the soybean control. On day 42, birds fed fermented rapeseed meal had higher ($p<0.05$) total tract apparent digestibility coefficients for dry matter, energy, and calcium than birds fed non-fermented rapeseed meal. Colon and ceca digesta from broilers fed the fermented feed had higher ($p<0.05$) lactobacilli counts than birds fed the control and non-fermented rapeseed meal diets on day 21 and 42. Fermentation also improved ($p<0.05$) villus height and the villus height:crypt depth ratio in the ileum and jejunum on day 21 and 42. The results indicate that solid-state fermentation of rapeseed meal enhanced performance and improved the intestinal morphology of broilers and may allow greater quantities of rapeseed meal to be fed to broilers potentially reducing the cost of broiler production. (**Key Words :** Broiler, Intestinal Morphology, Nutrient Digestibility, Microbial Characteristics, Rapeseed Meal, Solid-state Fermentation)

INTRODUCTION

Soybean meal is the most commonly used source of supplementary protein for poultry and it is generally a consistent, high quality product (Britzman, 2006). Unfortunately, the cost of using soybean meal can be prohibitive and many poultry producers are looking for alternative sources of supplementary protein which may be available at a lower cost. In China, one such alternative is rapeseed meal.

Rapeseed meal is a coarse powdery material, produced from rapeseed cake after a series of preparatory physical processes followed by multi-stage extraction of its oil under hygienically controlled conditions. The meal is a good source of protein for animal feeding but the presence of toxic glucosinolates limit its utilization (Bourdon and Aumaitre, 1990; Elangovan et al., 2001; Tripathi and Mishra, 2006). Glucosinolates are hydrolysed by a myrosinase enzyme present in the rapeseed to release a range of breakdown products (Mithen et al., 2000; Cheng et al., 2004). The most common products are isothiocyanates which cause reduced feed intake, impaired growth and high

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mortality rates (Clandinin et al., 1966; Campbell and Smith, 1979; McNeill et al., 2004).

Considerable interest has recently been shown in the use of fermented feeds to improve pig performance and influence the bacterial ecology of their gastrointestinal tract (Canibe and Jensen, 2003; Canibe et al., 2008). However, this technology has not been widely applied with poultry. Since fermentation has been shown to reduce the glucosinolate content of rapeseed meal (Rozan et al., 1996; Adarsh and Amandeep, 2001; Vig et al., 2001), the use of solid-state fermentation may improve the nutritive value of rapeseed meal when it is fed to poultry. Therefore, the present study was conducted to investigate the chemical and microbial characteristics of solid-state fermented rapeseed meal during fermentation and to determine the effects of fermentation on performance, nutrient digestibility, intestinal ecology and intestinal morphology when fed to broiler chickens.

MATERIALS AND METHODS

Preparation of solid-state fermented rapeseed meal

A mixed liquid culture containing approximately 5 log cfu/ml *Lactobacillus fermentum* (CGMCC No. 0843), *Enterococcus faecium* (CGMCC No. 1.2025), *Saccharomyces cerevisae* (CGMCC No. 2.1793) and *Bacillus subtilis* (MA 193), was prepared in a 1:1:1:1 ratio. A basal substrate containing 75% rapeseed meal, 24% wheat bran and 1% brown sugar was mixed with the liquid culture in a 10:3 ratio. The mixture was packaged and sealed in a newly-developed multi-layer polythene bag (25 kg capacity) equipped with a one-way valve to allow the release of any carbon dioxide produced during fermentation (Rou Duoduo Biotechnology Co., Beijing, China). This technology has been patented by the State Intellectual Property Office of the Peoples Republic of China under patent number PCT/CN20061001134.

During the initial stage of fermentation, *Saccharomyces cerevisae* yeast rapidly consumed the oxygen in the bag and produced large amounts of carbon dioxide. The gas pressure from carbon dioxide was discharged through the valve but the valve did not allow external air into the bag. This process created an anaerobic and acidic environment which provided both lactobacilli and *Bacillus* with optimum conditions in which to grow and reproduce. The fermentation was held at 30±3°C for 30 days. Samples of the solid-state fermented feed were collected on d 0, 1, 3, 7, 12, 20, and 30 for lactobacilli counts and chemical analysis. A total of 21 bags were prepared and 3 bags were opened on each day. Bags were discarded following sampling.

Lactobacilli counts and chemical analysis of solid-state fermented rapeseed meal

Ten-fold dilutions of the solid-state fermented rapeseed meal were prepared in sterile physiological saline for microbial enumeration. Lactobacilli were enumerated on MRS agar plates (Oxoid Ltd., Basingstoke, Hampshire, UK) following anaerobic incubation at 37°C for 24 h.

The pH value of the solid-state fermented feed was determined by weighing 20 g of feed into a 250 ml beaker and adding 200 ml of de-ionized water. pH was measured with a pocket-sized pH meter (Hanna Instruments, Woonsocket, RI). Total isothiocyanates were measured according to Choi et al. (2004). Briefly, a 10 g sample was mixed with myrosinase (Sigma, St. Louis, MO) to release isothiocyanates. Analysis of isothiocyanates was then performed on a HP 6890 gas chromatograph equipped with a HP 5873 mass-selective detector and a HP 6890 series auto-injector (Hewlett Packard, Wilmington, DE).

Feeding trial

The Animal Welfare Committee of China Agricultural University approved the animal care protocol for this experiment. A total of 168, day-old, male Arbor Acres broiler chicks were purchased from the Arbor Acres Poultry Breeding Company (Beijing, China). The experimental birds were assigned to one of three dietary treatments including a corn-soybean meal based control diet as well as two experimental diets in which the control diet was supplemented with 10% of the basal substrate containing unfermented rapeseed meal or 10% of the basal substrate containing rapeseed meal subjected to solid state fermentation. The fermented rapeseed diet was never prepared more than two days before feeding to prevent the high moisture feed from going moldy.

The birds were housed in wire-floored pens (90×60×40 cm) in an environmentally controlled room with continuous light. There were 8 pens per treatment and 7 birds per pen. Feed and water were available *ad libitum*. The room temperature was maintained at 35°C for the first 3 d and was reduced 3°C weekly until reaching 24°C. A temperature of 24°C was maintained until the end of the 42 d experiment. All birds were vaccinated against Newcastle disease on d 7 and 28 and against inactivated Infectious Bursa Disease vaccine on d 14 and 21.

This experiment was conducted in 2 phases consisting of a starter phase from d 1 to 21 and a finisher phase from d 22 to 42. At the end of each period (d 21 and 42), all birds and feed were weighed by pen. Daily weight gain, feed intake and feed conversion were determined from these data.

Digestibility determination

Chromic oxide (0.2%) was added to all diets as a digestibility marker and was fed throughout the experiment.

During days 19-21 and 40-42, uncontaminated excreta (free from feathers and feed) were collected from each pen. The excreta samples from the three collections from each cage were pooled within pen, weighed, and dried at 60°C for 48 h. The dried excreta samples were ground to pass through a 40 mesh screen, mixed thoroughly, and then homogenized using a laboratory grinder (1.0-mm screen) prior to analysis. Digestibility coefficients for dry matter, energy, calcium and phosphorus as well as nitrogen retention were determined using the equations for the indicator method described by Sauer and de Lange (1992) as follows:

$$\text{Apparent total tract digestibility (\%)} = 1-((Cr_2O_{3D}/Cr_2O_{3E})\times(N_E/N_D))\times 100\%$$

where Cr_2O_{3D} is the % chromic oxide in the assay diet; N_E is the % nutrient in the excreta; Cr_2O_{3E} is the % chromic oxide in the excreta and N_D is the % nutrient in the assay diet.

Collection of intestinal tissues and digesta

On d 21 and 42, one bird from each pen was randomly selected and euthanized for sampling. The digesta from the colon and ceca were collected separately and immediately immersed in liquid nitrogen and stored at -80°C until needed for bacteriological analysis. The middle sections of the duodenum (10 cm away from the pyloric junction), jejunum (proximal half of the remaining small intestine), and ileum (15 cm before the ileocecal junction) were aseptically isolated, flushed with 0.9% physiological saline solution, fixed with 4% formaldehyde-phosphate buffer, and kept at 4°C until microscopic assessment of mucosal morphology.

Chemical analysis

Diet and excreta dry matter (AOAC, 934.01), crude protein (AOAC, 954.01), calcium (AOAC, 927.02), phosphorus (AOAC, 965.17), ether extract (AOAC, 920.39), and ash (AOAC, 942.05) content were analyzed according

Table 1. Ingredient composition and nutrient content of experimental diets containing solid-state fermented rapeseed meal

Items	Starter phase (d 1 to 21)			Finisher phase (d 22 to 42)		
	Control	Unfermented rapeseed meal	Fermented rapeseed meal	Control	Unfermented rapeseed meal	Fermented rapeseed meal
Ingredients (% as-fed)						
Corn	55.89	50.87	50.87	59.76	54.73	54.73
Soybean meal (43% CP)	33.35	26.20	26.20	30.18	23.03	23.03
Fermented rapeseed meal	0.00	0.00	10.00	0.00	0.00	10.00
Unfermented rapeseed meal	0.00	10.00	0.00	0.00	10.00	0.00
Fish meal (64% CP)	4.50	4.50	4.50	3.00	3.00	3.00
Soybean oil	1.86	3.40	3.40	2.82	4.34	4.34
Limestone	1.27	0.88	0.88	1.20	0.85	0.85
Dicalcium phosphate	1.23	1.94	1.94	1.15	1.85	1.85
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.35	0.35	0.35
Chromic oxide	0.20	0.20	0.20	0.20	0.20	0.20
L-lysine·HCl (78%)	0.01	0.27	0.27	0.04	0.30	0.30
DL-methionine (98%)	0.19	0.24	0.24	0.10	0.15	0.15
Nutrient content[2]						
ME (Mcal/kg)	2.90	2.90	2.90	3.00	3.00	3.00
Crude protein	22.71	22.44	22.76	20.83	20.67	20.72
Calcium	1.02	1.03	1.00	0.92	0.88	0.93
Total phosphorus	0.79	0.85	0.82	0.70	0.72	0.76
Ether extract	4.89	6.31	6.21	5.92	7.21	7.15
Ash	7.21	7.85	7.93	5.72	6.12	6.07
Lysine	1.28	1.28	1.28	1.15	1.15	1.15
Methionine	0.58	0.58	0.58	0.45	0.45	0.45

[1] The premix provided the following (per kilogram of compound feed): zinc, 60 mg; iron, 95 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.35 mg; selenium, 0.3 mg; vitamin A, 10,000 IU; vitamin D_3, 2,750 IU; vitamin E, 30 IU; vitamin K_3, 2 mg; vitamin B_{12}, 12 μg; riboflavin, 6 mg; nicotinic acid, 40 mg; pantothenic acid, 12 mg; pyridoxine, 3 mg; biotin, 0.2 mg; choline chloride, 800 mg.

[2] Crude protein, calcium, phosphorus, ether extract, and ash are analyzed values. The metabolizable energy and amino acid content were calculated.

to AOAC (1990). Gross energy was measured by an automatic adiabatic oxygen bomb calorimeter (Model 1281, Parr, Moline, IL, USA). Chromic oxide was determined following the method of Mueller (1956).

Intestinal ecology

For bacterial enumeration, about 1 g of digesta from the colon or ceca was diluted 10-fold with sterile physiological saline resulting in dilutions ranging from 10^{-1} to 10^{-8}. Lactobacilli were enumerated on MRS agar (Oxoid Ltd., Basingstoke, Hampshire, UK) following anaerobic incubation at 37°C for 24 h. Enterobacteria were quantified on McConkey agar (Beijing Haidian Microbiological Culture Factory, Beijing, China) and total aerobes were cultured on Nutrient agar (Beijing Haidian Microbiological Culture Factory, Beijing, China) following aerobic incubation at 37°C for 24 h. Each dilution was determined in triplicate. The microbial enumerations are expressed as $\log_{10}$ colony forming units per gram. The concentration of lactobacilli, enterobacteria and total aerobes were determined by a visual count of colonies using the best replicates from dilutions that resulted in 30 to 300 colonies.

Small intestinal morphology

Villus height and crypt depth were measured according to Li et al. (1990). The intestinal samples from the middle sections of the duodenum, jejunum, and ileum were prepared using conventional paraffin embedding techniques. Briefly, all small intestinal segments were immediately fixed in 10% neutral-buffered formalin, and then embedded in paraffin. Villus height and crypt depth were measured at 40×magnification using an Olympus microscope (CK 40, Olympus Optical Company, Shenzhen, China). Measurements of villus height and crypt depth were taken only from sections where the plane of the section ran vertically from the top of villus to the base of an adjacent crypt. Values presented are means from 7 samples of villi measured from the tip to the crypt mouth and 7 associated crypts measured from the crypt mouth to the base (Xu et al., 2003).

Statistical analysis

Data were subjected to analysis of variance using the GLM procedure of SAS (SAS Institute, 1996). Pen was considered as the experimental unit for the performance and digestibility data while individual bird was used as the experimental unit for microbial counts and measures of intestinal morphology. Differences among treatments were separated by Duncan's multiple range test. Results were expressed as least square means and SEM. Probability values less than 0.05 were considered statistically significant.

RESULTS

Solid-state fermented rapeseed meal

The dry matter content of the solid-state fermented rapeseed meal dropped from 67.1% to 65.0% while crude protein content was increased from 33.8% to 36.6% after the 30 d fermentation period (Table 2). pH dropped from 5.94 at the start of fermentation to 5.42 after 30 days. Over the 30 day fermentation, isothiocyanates were reduced dramatically from 119.6 to 14.7 mmol/kg. The lactobacilli count increased rapidly during the first three days of fermentation and slowly declined thereafter. These counts were maintained at about 6.5 log cfu/g for the remainder of the fermentation period (Table 2).

Broiler performance

The performance of the broilers is presented in Table 3. Performance did not differ among the treatment groups during the starter period while differences were noted during the finishing period. Over the entire 42 day growth trial, the weight gain of birds fed fermented rapeseed meal was superior ($p<0.05$) to that of birds fed unfermented rapeseed meal and did not differ from the control. Feed conversion was significantly ($p<0.05$) poorer for birds fed unfermented rapeseed meal compared with the control group while feed conversion for birds fed fermented rapeseed meal did not differ from the control.

Total tract apparent digestibility of nutrients

The results of the digestibility study are shown in Table 4. In the starter phase, the total tract apparent digestibility of dry matter and phosphorus was higher ($p<0.05$) for birds fed fermented rapeseed meal than unfermented rapeseed

Table 2. Characteristics of solid-state fermented rapeseed meal during fermentation

Time (d)	Dry matter (%)	pH	Crude protein (%)	Lactobacilli (log cfu/g)	Isothiocyanates (mmol/kg)
0	67.1	5.94	33.8	4.05	119.6
1	66.5	5.91	34.0	4.34	99.5
3	63.5	5.64	34.6	6.89	37.9
7	66.1	5.58	35.2	6.82	35.1
12	64.2	5.47	36.1	6.70	27.9
20	63.8	5.47	36.3	6.54	20.6
30	64.0	5.42	36.6	6.41	14.7

Table 3. Effects of solid-state fermented rapeseed meal on the performance of broilers (d 1-42)

Items[1]	Diet			SEM	p-value
	Control	Unfermented rapeseed meal	Fermented rapeseed meal		
Days 1 to 21					
Weight gain (g/d)	34.2	32.8	34.7	0.70	0.55
Feed intake (g/d)	45.1	46.1	46.6	0.51	0.19
Feed conversion	1.32	1.40	1.34	0.03	0.19
Days 22 to 42					
Weight gain (g/d)	73.8[a]	67.3[b]	72.5[a]	0.77	0.05
Feed intake (g/d)	120.6	115.9	121.2	1.03	0.06
Feed conversion	1.63[b]	1.72[a]	1.67[ab]	0.02	0.05
Days 1 to 42					
Weight gain (g/d)	53.9[a]	50.2[b]	53.6[a]	0.70	0.05
Feed intake (g/d)	82.6	80.6	84.1	0.59	0.09
Feed conversion	1.54[b]	1.62[a]	1.57[ab]	0.01	0.05

[a, b] Means in the same row with no common superscripts differ significantly ($p<0.05$). Data were means of 8 pens of birds.

Table 4. Effects of solid-state fermented rapeseed meal on total tract apparent nutrient digestibility (%) in broilers determined on day 21 and 42

Items	Diet			SEM	p-value
	Control	Unfermented rapeseed meal	Fermented rapeseed meal		
Day 21					
Dry matter	77.7[a]	72.9[b]	77.4[a]	0.83	0.05
Energy	79.9	77.6	82.7	0.64	0.12
Calcium	57.0	56.8	58.0	1.22	0.91
Phosphorus	58.2[ab]	49.4[b]	60.2[a]	1.93	0.05
Nitrogen retention	65.7	68.3	69.1	1.12	0.45
Day 42					
Dry matter	71.5[b]	68.1[b]	75.8[a]	1.02	0.05
Energy	79.3[ab]	76.2[b]	82.7[a]	1.01	0.05
Calcium	56.3[a]	43.3[b]	50.6[a]	1.63	0.05
Phosphorus	48.2	47.2	49.1	1.28	0.86
Nitrogen retention	58.0	54.5	57.9	1.77	0.69

[a, b] Means in the same row with no common superscripts differ significantly ($p<0.05$). Data were means of 8 birds per treatment.

meal. Digestibility coefficients for birds fed fermented rapeseed meal did not differ from birds fed the control. During the finisher phase, the total tract apparent digestibility of dry matter, energy and calcium were significantly higher ($p<0.05$) for birds fed fermented rapeseed meal than unfermented rapeseed meal. Digestibility coefficients for birds fed fermented rapeseed meal were similar to those of birds fed the control with the exception of dry matter digestibility which was higher ($p<0.05$) for birds fed fermented rapeseed meal than the control.

Intestinal ecology

Microbial counts are shown in Table 5. During both the starter and finisher phase, the total numbers of lactobacilli in the colon and ceca of birds fed fermented rapeseed meal were significantly higher ($p<0.05$) than those of birds fed unfermented rapeseed meal or the control. The counts of total aerobes or enterobacteria did not differ due to treatment during either the starter or finisher phase.

Small intestinal morphology

Dietary treatment had no effect on villus height, crypt depth, and villus height to crypt depth ratio in the duodenum during either the starter or finisher phase (Table 6). In contrast, fermented rapeseed meal increased ($p<0.05$) villus height and villus height to crypt depth ratio in the ileum and jejunum compared with unfermented rapeseed meal during the starter phase. In addition, villus height to crypt depth ratio in the jejunum was significantly higher for

Table 5. Microbial counts of lactobacilli, enterobacteria, and total aerobes in the colon and ceca digesta (log cfu/g) of broilers fed different dietary treatments

	Diet			SEM	p-value
	Control	Unfermented rapeseed meal	Fermented rapeseed meal		
Day 21					
Colon					
Lactobacilli	5.33[c]	6.38[b]	7.61[a]	0.31	0.05
Enterobacteria	4.10	4.78	4.05	0.16	0.11
Total aerobes	5.58	5.32	5.74	0.15	0.53
Ceca					
Lactobacilli	5.88[b]	6.53[b]	7.88[a]	0.30	0.05
Enterobacteria	4.38	5.27	4.86	0.19	0.18
Total aerobes	6.07	6.40	6.37	0.14	0.64
Day 42					
Colon					
Lactobacilli	6.27[b]	5.82[b]	7.93[a]	0.38	0.05
Enterobacteria	4.13	4.88	3.61	0.39	0.43
Total aerobes	6.03	5.68	6.17	0.15	0.45
Ceca					
Lactobacilli	6.78[b]	7.12[b]	8.66[a]	0.35	0.05
Enterobacteria	5.55	5.09	5.06	0.30	0.80
Total aerobes	6.77	6.74	5.96	0.22	0.25

[a-c] Means in the same row with no common superscripts differ significantly ($p<0.05$). Data were means of 8 birds per treatment.

birds fed fermented rapeseed meal than for birds fed the control.

During the finisher phase, birds fed either fermented rapeseed meal or the control diet had higher ($p<0.05$) villus height in the ileum and jejunum than birds fed unfermented rapeseed meal. These birds had a significantly higher ($p<0.05$) villus height to crypt depth ratio in the ileum as well. No differences were found between birds fed fermented rapeseed meal and the control during the finisher phase.

DISCUSSION

A mixed liquid culture of *Lactobacillus fermentum*, *Enterococcus faecium*, *Saccharomyces cerevisae* and *Bacillus subtilis* was used as the inoculum in the present study. It is typically difficult to co-incubate *Bacillus subtilis* MA139 (which requires an aerobic environment) with *Lactobacillus fermentum* (which are anaerobic bacteria) in the production of fermented feed (Koneman et al., 1983). However, in the present experiment, we used a newly-patented process (State Intellectual Property Office of the Peoples Republic of China, Patent Number PCT/CN20061001134) to facilitate the co-culture of the two bacteria. The key to the process is the use of *Saccharomyces cerevisae* to consume the oxygen inside the bag to enhance a better environment for anaerobes including lactobacilli. The specially developed fermenting bag (Rou Duoduo Biotechnology Co., Beijing, China), which is equipped with a one-way valve, functioned in regulating the concentration of carbon dioxide metabolized by the aerobic strains of bacteria. The *Bacillus subtilis* used has been shown to be useful in decreasing the counts of enterobacteria shed in the feces of piglets (Guo et al., 2006). The *Lactobacillus fermentum* and *Enterococcus faecium* used in the present experiment have been previously shown to prevent diarrhea in *E. coli* challenged piglets (Huang et al., 2004).

The counts of total lactobacilli (6.89 log cfu/g) obtained in the solid-state fermented rapeseed meal obtained in this study were much lower than those obtained with fermented liquid feed, which can reach as high as 9.0-9.4 log cfu/g (van Winsen et al., 2001; Canibe and Jensen, 2003; Canibe et al., 2006; Canibe et al., 2008). The lower numbers of lactobacilli may be caused by the lower water content of the solid-state fermented feed as the feed to water ratio used was just 10:3 while feed to water ratios of 1:2 to 1:2.75 have been reported with fermented liquid feed (van Winsen et al., 2001; Canibe and Jensen, 2003; Canibe et al., 2006; Canibe et al., 2008).

The decline in the dry matter content of the solid-state fermentation feed (3.1 percentage units) was most likely caused by the consumption of carbohydrate by yeast and aerobic bacteria. The absence of oxygen during the later stages of the fermentation would impair the growth and

Table 6. Effects of solid-state fermented feed on small intestinal morphology

Items	Diet			SEM	p-value
	Control	Unfermented rapeseed meal	Fermented rapeseed meal		
Day 21					
Villus height (μm)					
Duodenum	1,473	1,360	1,513	33.49	0.13
Jejunum	1,047^{ab}	958^{b}	1,133^{a}	27.08	0.05
Ileum	486^{a}	419^{b}	500^{a}	13.93	0.05
Crypt depth (μm)					
Duodenum	203	192	212	5.09	0.28
Jejunum	170	160	145	4.86	0.11
Ileum	139	155	125	5.38	0.08
Villus height/crypt depth ratio					
Duodenum	7.3	6.9	7.6	0.15	0.35
Jejunum	6.2^{b}	6.1^{b}	7.9^{a}	0.28	0.05
Ileum	3.5^{ab}	2.7^{b}	4.2^{a}	0.22	0.05
Day 42					
Villus height (μm)					
Duodenum	1,515	1,474	1,644	36.75	0.14
Jejunum	1,247^{ab}	1,158^{b}	1,371^{a}	33.91	0.05
Ileum	575^{ab}	537^{b}	613^{a}	13.08	0.05
Crypt depth (μm)					
Duodenum	119	107	130	8.20	0.54
Jejunum	125	114	114	3.47	0.37
Ileum	109	125	107	3.38	0.13
Villus height/crypt depth ratio					
Duodenum	14.1	15.0	13.2	1.01	0.81
Jejunum	10.1	10.3	12.2	0.41	0.07
Ileum	5.3^{a}	4.3^{b}	5.8^{a}	0.20	0.05

$^{a, b}$ Means in the same row with no common superscripts differ significantly ($p<0.05$). Data were means of 8 birds per treatment.

metabolism of yeast thus explaining the fact that the dry matter content reached a plateau after about 12 days of fermentation. The crude protein concentration increased slightly (2.8 percentage units) and this increase is most likely a reflection of the decline in dry matter content rather than an actual increase in protein content. The decrease in pH from 5.94 to 5.42 which occurred during fermentation was most likely the result of the increased concentration of organic acids produced by the increased numbers of lactobacilli (van Winsen et al., 2000; Canibe et al., 2006; Canibe et al., 2008).

The most significant effect of the solid-state fermentation was the dramatic reduction in the isothiocyanate content in the rapeseed meal which declined by over 90%, and this result was comparable with that obtained with fermented cottonseed meal where the antinutritional factor gossypol was reduced by solid state fermentation (Zhang et al., 2007). The decomposition of isothiocyanates increased as the duration of the fermentation period increased and this result was similar to the study of Vig and Walia (2001). The reduction in isothiocyanates during fermentation may be due to utilization of glucose and the sulphur moieties of these compounds by microbial enzymes (Vig and Walia, 2001; Tripathi and Mishra, 2006).

Feeding rapeseed meal to broilers typically causes reduced feed intake, impaired growth, and high mortality rates compared with feeding soybean meal (Clandinin et al., 1966; Campbell and Smith, 1979; McNeill et al., 2004). Our results support this earlier work as birds fed the diet containing unfermented rapeseed meal had poorer weight gain and feed conversion than birds fed the soybean meal control. In contrast, the performance of birds fed fermented rapeseed meal was similar to that of the control likely reflecting the reduction in isothiocyanates in the fermented feed.

The improved nutrient digestibility (dry matter, energy and calcium) of the diets containing fermented rapeseed meal compared with unfermented rapeseed meal resulted in improved broiler performance. A beneficial effect of

fermentation on nutrient digestibility and energy utilization has been shown previously in mink and salmon (Skrede et al., 2001; Skrede et al., 2002).

Lactobacilli have the ability to inhibit the growth of putrefactive and pathogenic bacteria (Paton et al., 2006). In the current study, inclusion of fermented rapeseed meal successfully enhanced the growth of lactobacilli in the colon and ceca compared with either the control diet or the unfermented rapeseed meal diet. In agreement with our findings, van Winsen et al. (2001) and Canibe and Jensen (2003) indicated that supplementation with fermented feed increased the gastrointestinal lactobacilli population. All these data suggest that fermented feed may act in a similar manner to probiotics to improve gastrointestinal health (Paton et al., 2006). A balanced microbial population would support a healthy intestinal tract resulting in better control of intestinal pathogens.

The villus height to crypt depth ratio is a very useful measure to estimate the absorption capacity of the small intestine (Montagne et al., 2004). Maximum digestion and absorption is believed to occur as the villus height to crypt depth ratio increases in weaned pigs (Pluske et al., 1996). Changes in the intestinal morphology such as reduced villus height and deeper crypt may also indicate the presence of toxins (Xu et al., 2003). In the present study, increased villus height and increased villus height to crypt depth ratio in the ileum and jejunum were observed in broilers supplemented with fermented rapeseed meal compared with unfermented meal. The increased villus height and villus height to crypt depth ratio might be associated with the increased numbers of beneficial bacteria (lactobacilli) observed (Xu et al., 2003). The increased villus height to crypt depth ratio produced an intestinal structure more oriented to digestion, with improved absorptive and hydrolysis potential, as well as requiring fewer nutrients to be directed towards intestinal maintenance (Pluske et al., 1996). Thus, with fermented rapeseed, the intestinal structure of the ileum and jejunum is more favorable for the bird and may help to explain the improvement in weight gain and feed conversion observed during the finisher phase.

In conclusion, solid-state fermentation of rapeseed meal significantly reduced the isothiocyanate content of the rapeseed meal. As a result, broiler performance was significantly improved compared with birds fed unfermented rapeseed meal and was similar to the performance obtained with broilers fed soybean meal. Compared with unfermented rapeseed meal, solid-state fermentation improved nutrient digestibility, increased the numbers of lactobacilli in the colon and ceca and enhanced the small intestinal structure of the broilers. Solid-state fermentation of rapeseed meal may allow greater quantities of rapeseed meal to be fed to broilers potentially reducing the cost of broiler production.

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