



Effects of Fermented Rapeseed Meal on Growth Performance and Serum Parameters in Ducks

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ABSTRACT : A trial was performed to study the effects of feeding a diet containing solid-state fermentation rapeseed meal (FRSM) replaced soybean meal (SBM) on growth performance and serum biochemistry parameters of ducks and then to determine the appropriate proportion of soybean meal replacement. The 75% rapeseed meal and 25% blood meal were mixed and inoculated with the *Lactobacillus plantarum* and *Bacillus subtilis*. Over the 21-day fermentation, isothiocyanates were reduced from 72.7 to 14.1 mmol/kg. A total of 1,280 fifteen-day-old Cherry Valley ducks were randomly allocated into 4 dietary treatments, 4 replicate groups of 80 ducks each for a 30-day feeding trial. In four treatment groups, fermentation rapeseed meal replaced soybean meal at 0, 33, 67 or 100%, respectively. Results showed that feed intake of ducks fed 100% FRSM was greater ($p < 0.05$) than SBM and partial FRSM in both the finishing period (31-45 d) and entire feeding period (15-45 d). Daily gain increased gradually in the three treatment groups with augmenting FRSM over in the whole study period. In the growing period (15-30 d), compared with the SBM group, phosphorus and calcium content in serum from the FRSM group was improved ($p < 0.05$). Total protein concentration was lower in ducks fed 100% FRSM than SBM and 33% FRSM ($p < 0.05$). Concentrations of IgM were dramatically higher for animals fed 100% FRSM than in the SBM, 33% FRSM and 67% FRSM groups. In the finishing trail stage (31-45 d), only serum IgG content in 100% FRSM group was improved ($p < 0.05$). Therefore, rapeseed meal fermented with *Lactobacillus plantarum* and *Bacillus subtilis* is a promising alternative protein source and fermented rapeseed meal can completely replace soybean meal in duck diet and potentially reduce the cost of duck production. (**Key Words :** Duck, Growth Performance, Serum Parameter, Fermentation Rapeseed Meal, Soybean Meal)

INTRODUCTION

Soybean meal is commonly used as an effective plant-derived protein in the animal feed industry. However, 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 (Laudadio and Tufarelli, 2010). In China, rapeseed meal is the preferred alternative (Chiang et al., 2010).

Rapeseed meal is the byproduct remaining after rapeseed is processed and the oil is removed. It contains 34-38% crude protein and is a good source of protein for animal feeding. However, the presence of toxic glucosinolates limits its utilization, especially for young

animals (Elangovan et al., 2001; Tripathi and Mishra, 2006). Glucosinolates are hydrolysed by a myrosinase enzyme present in the rapeseed to release a range of products (Cheng et al., 2004). The most common products are isothiocyanates which cause reduced feed intake, impaired growth and induce goiter (Svetina et al., 2003; McNeill et al., 2004).

Solid state fermentation (SSF) has been reported to be an effective way to reduce anti-nutritional factors in rapeseed meal, such as glucosinolate (Al-Asheh and Duvnjak, 1995; Ebune et al., 1995), while producing a certain amount of rapeseed peptides. Furthermore, these fermented rapeseeds are highly digestible and nutritious, contributing important nutrients including rapeseed peptide, calcium, phosphate, and vitamins B (Marczak et al., 2003; Megias et al., 2006). It is not known whether these rapeseed peptides can improve the immune function of animals. Isothiocyanates can cause goiter and then the swelling of the thyroid may lead to the abnormal secretion of T3 and T4. Recent investigations have shown the use of fermented rapeseed meal can improve pig performance and influence

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Received December 22, 2010; Accepted February 28, 2011

serum biochemical parameters (Canibe and Jensen, 2003; Svetina et al., 2003). However, the data regarding the effects of fermented rapeseed meal on performance in ducks is limited. Therefore, the current experiment was conducted to evaluate the growth performance and detect serum biochemistry parameters in ducks fed a FRSM-based diet.

MATERIALS AND METHODS

Preparation of solid-state fermented rapeseed meal (FRSM)

The *Lactobacillus plantarum* and *Bacillus subtilis* used were obtained from the National Research Center for Industrial Microbiology, as CICC 20764 and CICC 10265. Dried rapeseed meal and blood meal were sieved through 40 mesh sieves. The 75% rapeseed meal and 25% blood meal (swine) were mixed and inoculated with CICC 20764 and CICC 10265 of 6% (V/W), respectively. The ratio of meals and water was 1:1.2. The mixture was added to a plastic bag, sealed and fermented for 3-weeks under anaerobic conditions. Fresh fermented samples were dried at 50-60°C for 3 days.

Chemical analysis

All analyses were performed in duplicate. Fermented rapeseed meal was sampled and analyzed for dry matter by drying at 105°C for 5 h, ash by incineration at 550°C. Protein (N \times 6.25) was determined according to the method of Kjeldahl. Total isothiocyanates were measured according to Choi et al. (2004). Briefly, a 10 g sample was mixed with myrosinase (Sigma) to release isothiocyanates. Then the analysis of isothiocyanates was 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).

Experimental design

A total of 1,280 fifteen-day-old Cherry Valley ducks of an equal ratio of males and females were randomly allocated into the four dietary treatments (Table 1) of 4 replicates with 80 birds per group. The ducks were allowed free access to water and feed. In the four treatment groups, FRSM replaced soybean meal (SBM) at 0, 33, 67 or 100%, respectively. The experimental diets were FRSM supplied 0, 33, 67 and 100% SBM, respectively. This experiment was conducted in 2 phases consisting of a starter phase from d 15 to 30 and a finisher phase from d 31 to 45. At the end of each period (d 30 and 45), all birds and feed were weighed by pen. Daily weight gain, feed intake and feed conversion were determined from these data. The basal diet was supplemented with minerals and vitamins to meet the requirements for ducks (NRC, 1994). This experiment

started from 2009 November 3 to December 2 at Sun Poultry Co., Ltd. in Anhui, China. The birds' experimental area was simple shed and the control group diet (100% SBM) was prepared by Taiyang Poultry.

Blood sampling

Blood collection was carried out on the experimental ducks at two time intervals. One set (n = 8) was slaughtered at the age of 30 days and other set (n = 8) was slaughtered at 45 days of age. Blood samples were collected from the wing vein of the ducks at the age of 30 days and 45 days, the samples were centrifuged at 1,600 \times g for 15 min and collected the serum, the resulting serum was kept at -20°C until used.

Plasma samples were evaluated for: serum total protein (TP), urea nitrogen (UN), total phosphate (P), calcium (Ca), and immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), triiodothyronine (T3), thyroxine (T4).

Assay procedures of immunoglobulin-immunoturbidimetry

Table 2 summarizes assay arrangements and includes instrument (chemistry analyzer, RANDOX 204, England) settings. Concentrations of serum immunoglobulin-immunoturbidimetry were measured according to Feng et al. (2007). The first five tubes in a batch were standards, followed by unknown samples and controls. The data processor presented the standard curve as absorbance changes and as a slope equation with a correlation coefficient followed by the respective calculated results for each unknown sample. Alternatively, results can be calculated by graphical means, with use of the absorbance change during 200 s.

T3 and T4 levels

Serum was analyzed for T3 and T4 concentrations by RIA kits described previously (Davis et al., 2000). Samples were assayed in a single assay to avoid interassay variation. Intrassay coefficient of variation assessed by precision pool tubes was 1.9%. To test parallelism, stock solutions of T3 and T4 were serially diluted to each standard concentration. These were added to 1mL of pooled duck plasma samples; the logit by plot of percentage bound versus concentration was compared to each standard curve. The slopes and standard errors of the standard curves and spiked, pooled serum curves were similar.

Statistical analyses

Data were subjected to analysis of variance using the GLM procedure of SAS (SAS Institute, 1996) to test for significant differences among least square means and SEM. Pen was considered as the experimental unit for the performance data while individual bird was used as the

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

Ingredients (% as-fed)	SBM ^a		33% FRSM ^b		67% FRSM ^b		100% FRSM ^b	
	15-30 d	31-45 d	15-30 d	31-45 d	15-30 d	31-45 d	15-30 d	31-45 d
Corn	54.00	56.00	54.40	58.50	54.70	58.70	55.10	56.00
Wheat middling	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
rice bran	8.00	5.90	8.00	5.90	8.00	5.90	8.00	5.90
Soybean meal (43% CP)	10.50	5.50	7.00	3.67	3.50	1.84	0	0
Fermented rapeseed meal	0	0	3.15	1.65	6.30	3.30	9.45	4.95
Rapeseed meal	6	8	6	8	6	8	6	8
Cottonseed meal (39% CP)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Corn gluten meal	2.00	2.50	2.00	2.50	2.00	2.50	2.00	2.50
Fish meal (60% CP)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.20	0.35	0.20	0.35	0.20	0.35	0.20	0.35
Soybean oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Na ₂ SO ₄	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Limestone	1.20	1.40	1.20	1.40	1.20	1.40	1.20	1.40
CaHPO ₄	0.90	0.85	0.90	0.85	0.90	0.85	0.90	0.85
Chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Bentonite	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00
Premix ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zeolite	2.00	3.00	2.00	3.00	2.00	3.00	2.00	3.00
Nutrient content ²								
Crude protein	17.00	16.00	17.00	16.00	17.00	16.00	17.00	16.00
ME (MJ/kg)	11.72	11.80	11.72	11.80	11.72	11.80	11.72	11.80
Calcium	0.70	0.72	0.70	0.72	0.70	0.72	0.70	0.72
Total phosphorus	0.59	0.61	0.63	0.62	0.63	0.61	0.63	0.62
Ether extract	6.72	6.70	6.72	6.70	6.72	6.70	6.72	6.70
Ash	6.10	6.13	6.10	6.13	6.10	6.13	6.10	6.13
Lysine	0.84	0.83	0.83	0.80	0.80	0.79	0.80	0.78
Methionine+cystine	0.62	0.62	0.65	0.63	0.64	0.62	0.66	0.63

^a SBM = Soybean meal. ^b FRSM = Fermented rapeseed meal.

¹ The premix provided the following (per kilogram of diet): vitamin A, 1,800 IU; vitamin D, 1,220 IU; vitamin E, 1,800 mg; vitamin K, 150 mg; vitamin B₁, 100 mg; vitamin B₂, 500 mg; Pantothenic, 800 mg; niacin, 3,500 mg; vitamin B₆, 120 mg; folic acid, 80 mg; vitamin B₁₂, 1 mg; Cu, 800 mg; Fe, 6,000 mg; Ze, 6,000 mg; Mn, 8,000 mg; I, 70 mg; Se, 40 mg.

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

experimental unit for serum parameters.

RESULTS

The chemical composition of fermentation rapeseed meal

Characteristics of solid-state fermented rapeseed meal

Table 2. Assay settings for immunoturbidimetry of immunoglobulin

Sample serum	IgG 5 µl, IgA 15 µl, IgM 20 µl
Antiserum	300 µl
Temperature	37°C
Wavelength	340 nm
Light setting	Operate
Measure interval	120 s

are shown in Table 3. The dry matter content of the solid-state fermented rapeseed meal dropped from 88.1% to 93.4 while crude protein content was increased from 37.1% to 58.4%. Crude fat raised 1.4% and calcium and phosphorus showed little change. Over the 21 day fermentation,

Table 3. Characteristics of solid-state fermented rapeseed meal during fermentation

Diet ^a	RSM ^b	FRSM ^c
Dry matter (%)	93.4	88.1
Crude protein (%)	37.1	58.4
Isothiocyanates (mmol/kg)	72.7	14.1
Crude fat(g/kg)	14.8	16.2
Calcium (g/kg)	8.2	8.3
Phosphorus(g/kg)	8.6	8.5

^a On a dry matter basis. ^b RSM = Rapeseed meal.

^c FRSM = Fermented rapeseed meal.

Table 4. Effect of fermented rapeseed meal on growth performance of ducks¹

Criteria	SBM ²	33% FRSM ³	67% FRSM ³	100% FRSM ³	SEM ⁴
15-30 day-old					
Daily weight gain (g)	79.8	82.3	82.2	81.8	0.8
Daily intake (g)	164.5	169.0	168.5	169.4	1.02
Feed conversion ratio	2.06	2.05	2.05	2.07	0.01
31-45 day-old					
Daily weight gain (g)	78.1	75.9	76.3	80.1	0.6
Daily intake (g)	273.9 ^a	270.5 ^a	271.9 ^a	284.7 ^b	1.03
Feed conversion ratio	3.51	3.56	3.56	3.55	0.02
15-45 day-old					
Daily weight gain (g)	78.9	79.1	79.3	81.3	0.5
Daily intake (g)	210.1 ^a	212.2 ^a	214.4 ^a	224.9 ^b	0.85
Feed conversion ratio	2.66	2.68	2.70	2.77	0.01

¹ Values are presented as means; n = 4 for DG, DFI and FC per treatment. Means within rows with different letters differ significantly (p<0.05).

² SBM = Soybean meal. ³ FRSM = Fermented rapeseed meal. ⁴ SEM = Standard error of the mean.

Means within rows with different letters differ significantly (p<0.05).

isothiocyanates were reduced dramatically from 72.7 to 14.1 mmol/kg.

The growth performance of ducks

The performance of the ducks is presented in Table 4. Performance did not differ among the treatment groups during the starter period while differences were noted during the finishing period and the entire feeding. Over the

finishing period and the entire 30 day growth trial, the feed intake of ducks fed 100% FRSM was significantly higher (p<0.05) than SBM and partial FRSM. Daily weight gain and feed conversion did not differ statistically between the treatments groups whole study period.

Changes of serum biochemistry parameters

Serum biochemistry parameters are shown in Table 5. In

Table 5. Effect of fermented rapeseed meal on serum parameters in ducks¹

	SBM ²	33% FRSM ³	67% FRSM ³	100% FRSM ³	SEM ⁴
30 days					
Urea nitrogen (mmol/L)	0.28	0.31	0.29	0.27	0.01
Total phosphorus (mmol/L)	2.59	2.80 ^b	2.83 ^b	2.91 ^b	0.22
Ca (mmol/L)	2.52 ^a	2.64 ^b	2.65 ^b	2.73 ^c	0.02
Total protein (g/L)	39.71 ^a	39.88 ^a	37.78 ^{ab}	37.03 ^b	0.81
IgG (g/L)	0.21	0.20	0.21	0.20	0.01
IgM (g/L)	0.50 ^b	0.50 ^b	0.52 ^b	0.65 ^a	0.03
IgA (g/L)	0.24	0.24	0.25	0.23	0.02
T3 (mmol/L)	1.49	1.56	1.73	1.47	0.01
T4 (mmol/L)	17.87	17.61	16.61	15.93	0.81
45 days					
Urea nitrogen (mmol/L)	0.21	0.25	0.22	0.21	0.01
Total phosphorus (mmol/L)	2.50	2.54	2.52	2.51	0.04
Ca (mmol/L)	2.74	2.83	2.75	2.82	0.03
Total protein (g/L)	41.50	40.68	38.96	40.39	0.84
IgG (g/L)	0.20 ^b	0.22 ^{ab}	0.21 ^{ab}	0.35 ^a	0.01
IgM (g/L)	0.55	0.57	0.58	0.57	0.02
IgA (g/L)	0.22	0.21	0.22	0.21	0.01
T3 (mmol/L)	1.35	1.33	1.38	1.31	0.02
T4 (mmol/L)	17.62	16.12	16.09	17.92	0.72

Values with different letters in the same row differ significantly (p<0.05).

¹ Values are mean for eight ducks. Means within rows with different letters differ significantly.

² SBM = Soybean meal. ³ FRSM = Fermented rapeseed meal. ⁴ SEM = Standard error of the mean.

the starter phase, the content of serum total phosphorus and calcium was higher ($p < 0.05$) for birds fed FRSM than SBM and serum calcium concentration was highest in Group 100% FRSM. Total protein concentration for ducks fed 100% FRSM was lower than SBM and 33% FRSM ($p < 0.05$) and decreased gradually with an increasing amount of FRSM. The concentrations of IgM was dramatically higher for ducks fed 100% FRSM than SBM, 33% FRSM and 67% FRSM, but did not differ from birds fed SBM and partial FRSM groups.

In the finishing stage (31-45 d), all blood biochemistry parameters in Group SBM and Group FRSM did not differ statistically, except for IgG. The highest concentration of IgG was observed in groups fed 100% FRSM, and the lowest content was observed in groups fed SBM, whereas no change in IgG content occurred between Group 33% FRSM and Group 67% FRSM.

DISCUSSION

Fermentation changed the physical and nutritional characteristics of rapeseed meal (Table 3). Crude protein and crude fat were increased after fermentation. The increase in protein may be due in part to the decreased carbohydrate content after fermentation. The loss of dry matter at the expense of fermentable sugars during fermentation with bacteria, fungi or yeast, which are optimal sources of proteins, could be a possible reason for such an increase in crude protein (Rozan et al., 1996). Vig and Walia (2001) demonstrated that fermentation can increase nitrogen and protein content of rapeseed meal. The crude fat concentration increased slightly (1.4%) and this increase is most likely a reflection of the decline in dry matter content rather than an actual increase in fat content. The most significant effect of the solid-state fermentation was the dramatic reduction in the isothiocyanate content in the rapeseed meal which declined by about 80%. Chiang et al. (2010) reported that isothiocyanates were reduced dramatically from 119.6 to 14.7 mmol/kg in a 30 day fermentation. Vig and Walia (2001) reported that glucosinolates decreased after fermentation with the reduction being 13.7%, 25.1%, 33% and 43.1% after 2, 5, 8 and 10 days of fermentation, respectively. Reduction of glucosinolates during fermentation may be due to utilization of glucose and sulphur moieties of these compounds by microbial enzymes (Tripathi and Mishra, 2006). This explanation is supported by Verbiscar et al. (1981), who reported that after 21 days of *Lactobacillus* fermentation at 26°C, total toxicants were lowered by 95%-98%.

Chiang et al. (2010) reported that the weight gain of broilers fed fermented rapeseed meal was superior ($p < 0.05$) to that of birds fed unfermented rapeseed meal. 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. In our experiment, only daily feed intake for ducks fed with 100% FRSM significantly increased ($p < 0.05$) during the finishing period (30-45 d) and entire feeding period (15-45 d). Compared to soybean meal, the protein digestion coefficient and the availability of amino acids are lower for rapeseed meal. Increased rapeseed feed intake compared to soybean feeds may compensate for this difference to some extent. In a broiler experiment reported by Newkirk et al. (1997) the difference between ileal protein digestibility of soybean meal and that of rapeseed meal was only half of the respective difference in the experiment. Palander et al. (2004) showed protein digestibility coefficients of rapeseed products were typically lower than those of soybean products in growing turkeys. In addition, because the rapeseed meal was fermented by *Lactobacillus* and glucosinolates decreased, its palatability was improved. This may be one of the reasons for the promoting effect of FRSM on feed intake. The feed conversion rates were generally low, which may be related to cold weather as there was heavy snow in the finishing phase and the experimental area consisted of simple sheds surrounded by plastic curtain insulation.

In the current study, compared to ducks fed SBM, a higher content of serum total phosphorus and calcium in ducks fed FRSM was observed in the first trail stage which may be associated with the increase of available phosphorus and calcium in fermented rapeseed meal. Although the total phosphorus and calcium content in fermented RSM remained the same as that of unfermented RSM, microbial phytases can be used to reduce phytic acid content in rapeseed, as a result, the bioavailability of phosphorus and calcium were improved. This explanation is supported by researchers, who reported that several microorganisms were tested for their ability to produce phytase and some of them were used in reduction of phytic acid content in rapeseed meal during solid state fermentation (Nair, La-flamme and Duvnjak, 1991; Al-Asheh and Duvnjak, 1995; Ebune et al., 1995). However, it is interesting that serum total protein concentration in the finishing stage of the trial had a significant decrease in our study, compared to the control, this results needs to be researched further.

The level of serum IgM and IgG may be associated with the increase of rapeseed peptide and bioactive peptides in fermented rapeseed meal. Wang et al. (2003) concluded that adding 3 g small peptides/kg basal diets fed to piglets increased the concentration of immunoglobulin. Fermentation affects the characteristics of proteins in rapeseed meal. In our experiment, rapeseed peptides content was increased from 0.8% to 4.6% over the 21 d fermentation (data not shown). Xue et al. (2009) isolated

and obtained bioactive peptides from rapeseed meal as well as the peptides might improve immune function.

The concentration of T3 and T4 was determined in order to obtain information on possible effects of isothiocyanates of the diets containing rapeseed products. Our results showed that no significant differences due to treatment ($p>0.05$) were noted in serum T3 level. However, there was a linear decrease ($p>0.05$) in serum T4 level in response to increasing FRSM in the first trail stage, but no change in the finishing trail stage. It may be that young ducks more sensitive to a small amount of isothiocyanates. The FRSM used in this trial only contained 14.1 μ moles isothiocyanates per g dry matter (DM). Therefore, this may be a major reason why T3 concentration was unchanged. Consumption of isothiocyanates is known to cause decreased levels of thyroid hormones (Bell, 1984). Lardy and Kerley (1994) noted similar T3 and T4 responses in growing beef steers fed rapeseed meal. The present results showed fermentation of rapeseed meal did not affect the function of thyroid in ducks.

In conclusion, fermentation significantly reduced the isothiocyanate content of the rapeseed meal. As a result, duck performance was similar to the performance obtained from ducks fed soybean meal. The results of the current experiment also indicated that fermented rapeseed meal could increase the level of IgG, IgM, total phosphorus, and calcium content and did not affect T3 content in serum. Therefore, rapeseed meal fermented with *Lactobacillus plantarum* and *Bacillus subtilis* is a promising alternative protein source and fermentation rapeseed meal could substitute for soybean meal and potentially reduce the cost of duck production.

ACKNOWLEDGMENTS

This study was supported by grants from National Scientific and Technical Personnel Services Company Action Projects under award number 2009GJC30021 and Anhui Agricultural Science and Technology Transformation Fund Programs under award number 09150306006.

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