SHORT COMMUNICATION

Effect of solid state fermentation on nutrient content and ileal amino acids digestibility of canola meal in broiler chickens

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Abstract

The aim of the current study was to investigate the potential of Lactobacillus salivarius solid state fermentation for reduction of glucosinolate content in canola meal (CM) as well as the improvement of its nutrient digestibility for broiler chickens. Canola meal was treated with the L. salivarius in solid state fermentation for 30 days. Nutrients ileal digestibility was tested using 42-day-old broilers fed by either CM or fermented CM (FCM) as the sole source of energy and protein. The results showed that fermentation of CM using L. salivarius reduced glucosinolate content of CM by 38%. The digestibility coefficient was improved significantly for crude protein, Met, Cys, Arg, Asp, Glu, and Ser in FCM compared to CM. However, apparent metabolisable energy content of CM was not affected by fermentation. It appears that fermentation treatment of CM using L. salivarius may improve the overall nutritive value of CM for broiler chickens, reducing its total glucosinolate and crude fibre content by 38 and 16%, respectively.

Introduction

Canola is a particular type of genetically altered rapeseed with <2% erucic acid and <30 µm/g glucosinolate in air-dried oil-free meal (Bell, 1993). Canola meal (CM) is a by-product of canola seed-crushing after oil extraction process and contains high protein (40%) with a well-balanced amino acid (AA) composition (Newkirk, 2009). However, the high fibre and glucosinolate content limits the use of CM in chicken diets. The indigestible carbohydrate content of CM is high (Kocher et al., 2000). Bell (1993) reported that CM has an average 2.5% α-galacto-oligosaccharides and 18% non-starch polysaccharides (NSP) of which 1.5% is soluble. The soluble NSP tends to increase the digesta viscosity and reduce nitrogen digestion and absorption (Annison, 1991), subsequently resulting in poor growth performance. Moreover, glucosinolates in CM also reduce growth performance by decreasing voluntary feed intake, interfering with the synthesis of thyroid hormones, damaging liver and kidney, and causing anemia (Tripathi and Mishra, 2007). Inclusion of CM at 20% in diets of poultry has been reported to reduce apparent metabolisable energy (AME) (Mushtaq et al., 2007) as well as nutrients digestibility (Slominski and Campbell, 1990).

Solid state fermentation is regarded as one of the possible approaches to diminish the antinutritional factors and enhance the bioavailability of nutrients. Lactic acid fermentation may modify cereal carbohydrate composition as a result of microbial metabolism (Marklinder et al., 1996; Al-Asheh and Duvnjak, 1995). Skrede et al. (2003) indicated that fermentation decreased crude fibre (CF) content of wheat and barley and increased total carbohydrates and starch digestibility. Pal and Walia (2001) reported that solid state fermentation of rapeseed meal using Rhizopus oligosporus reduced its glucosinolate content by 45%. This reduction may occur due to utilisation of glucose and sulphur moieties of these compounds by the fungus. Fermentation by lactic acid bacteria (LAB) also reported to reduce cyanogenic toxicants in jojoba seed meal and trypsin inhibitor in black bean and soybean meal (Granito et al., 2002; Gao et al., 2013). A pilot study in our laboratory (unpublished) showed that fermentation of CM by Lactobacillus salivarius reduced CF and glucosinolate content. Yang et al. (2006) also indicated that fermentation of food waste using L. salivarius enhance breakdown of fibre. However, to the best of our knowledge, the effect of L. salivarius fermentation on nutrient digestibility of CM in broiler chickens is lacking. Hence, the present study investigated the potential of L. salivarius solid state fermentation for reduction of glucosinolate content in CM as well as improvement of its nutrient digestibility for broiler chickens.

Materials and methods

Fermentation procedure

Twenty kg of CM (Brassica napus) were fermented for 30 days using L. salivarius. The L. salivarius was an isolate from Malaysian fermented soybean (tempel) (GenBank accession number: KF303794). The meal was inoculated with freeze dried L. salivarius at 0.1% based on dry matter (DM) content. The inoculation was first prepared by suspending the appropriate weight of L. salivarius powder in water to increase the moisture content of CM to 70% (Rodriguez-Leon et al., 2008). The inoculant suspension was sprayed over the CM and mixed thoroughly. The colony forming units (cfu) of L. salivarius was 10^7/g CM. The treated CM was placed in plastic barrel, sealed and incubated for 30 days at room temperature (28 to 32°C). Control CM was prepared at the same time without inoculant addition. At the end of the fermentation period, samples of the untreated and inoculated CM were dried at 50°C for 3 days and ground for chemical analyses. The pH value and cfu were determined at the initial and final stage of fermentation using a portable pH meter (Hanna Instruments, Woonsocket, RI, USA).

Birds and experimental procedure

All experimental procedures were conducted in accordance with Universiti Putra Malaysia...
Research Policy on animal care. A total of 50 day-old male broiler chickens (Cobb 500) were obtained from a local hatchery and raised in groups of 5 in 10 battery cages with wire floors in a conventional open-sided house (maximum 34°C and minimum 24°C). Chicks were fed commercial broiler starter in crumble form [2900 kcal ME/kg; 21% crude protein (CP)] and grower in pelleted form [3050 kcal ME/kg; 19% CP] from day 1 to 21, and day 22 onwards, respectively. Water was available at all times.

On day 42, 36 chickens were chosen for digestibility study according to their body weight (2200±50 g). This selection was to eliminate misleading factor of body weight. Groups of three birds were then assigned randomly to 12 cages. All birds were allowed a 4-day adaptation period where they were fed a corn-soybean based diet with 30% CM (basal diet, Table 1) (Soleimani et al., 2010). Two experimental diets containing either CM or fermented CM (FCM) as the sole source of energy and protein were prepared (Table 1) (Ahmed et al., 2014; Jia et al., 2012). Both diets contained titanium dioxide (0.5%) as an indigestible marker. Following the adaptation period, the birds were fasted for 24 h and then allowed to consume the experimental diets (either CM or FCM base, Table 1). Birds were slaughtered 4 h after the beginning of feeding by halal neck cut for collection of ileal content. The 4 h sampling time has been shown to be optimal for ileal digesta sampling in broiler chickens (Kadim and Moughan, 1997). Upon complete immobilisation, the body cavity was opened, and the ileum (from Meckel’s diverticulum to a point 40 mm proximal to the ileocecal junction) was removed. The ileum was then divided into two parts, and the contents of the lower half of the ileum were collected by gentle flushing with distilled water from a syringe into a plastic container. Digesta samples of birds within a cage were pooled, freeze-dried and stored at -20°C.

Sample analyses

The samples were finely ground using a coffee grinder (Panasonic, Osaka, Japan) and DM, CP, ether extract (EE), CF and ash were determined according to AOAC methods 925.09, 988.05, 920.39, 978.10 and 942.05, respectively (AOAC, 1990). Gross energy (GE) was measured using an adiabatic oxygen bomb calorimeter (C 2000 basic; IKA, Staufen, Germany). Total glucosinolate was determined based on alkaline degradation and subsequent reaction of released 1-thioglucose with ferricyanide (Jezek et al., 1999; Gallaher et al., 2012). Amino acid concentrations in the diet and ileal digesta were determined by high-performance liquid chromatography according to the procedures described by Strydom and Cohen (1994). Pre-column derivatisation was done with ACCQ reagent (6-aminooquinyl-N-hydroxysuc-cinimidyl carbamate; Waters Corporation, Milford, MA, USA). Cys and Met were analysed as cysteic acid and methionine sulfone by oxidation with performic acid for 16 h at 0°C and neutralisation with hydrobromic acid before hydrolysis. Trp contents were determined following alkaline hydrolysis of the sample with 4.3 M LiOH·H2O for 16 h at 120°C and neutralisation with 6 M HCl. Quantification of the other AA was done by hydrolysing the sample in 5 M LiOH·H2O for 22 h at 110°C. Titanium dioxide was determined according to procedures described by Short et al. (1996).

Calculations

The AME and nutrient digestibility of diets were determined using the following formulas on DM basis:

\[
AME (\text{kcal/kg diet}) = \text{GE}_{\text{diet}} - \left[ \frac{\text{GE}_{\text{digesta}} \times (\text{marker}_{\text{diet}}/\text{marker}_{\text{digesta}})}{10} \right],
\]

where AME = (Apparent metabolisable energy), \(\text{GE}_{\text{diet}}\) = gross energy of diet, \(\text{GE}_{\text{digesta}}\) = gross energy of digesta, \(\text{marker}_{\text{diet}}\) = marker intake in diet, and \(\text{marker}_{\text{digesta}}\) = marker output in digesta.

Digestibility(%) = 100 - [(markerdiet/markerdigesta) \times (nutrientdiet/nutrientdigesta)] \\
(Driver et al., 2006);

The AA output and digestibility were calculated using the following equations:

\[
\text{AA output (mg/kg DM intake) } = \frac{\text{AA}_{\text{diet}} \times (\text{marker}_{\text{diet}}/\text{marker}_{\text{digesta}})}{100},
\]

Apparent AA digestibility (%) = \(\text{AA}_{\text{diet}}/\text{AA}_{\text{diet}}\times 100\) (Kadim and Moughan, 1997)

Statistical analysis

All statistical analyses were carried out with the Student’s t-test using TTEST procedure of SAS (SAS, 2002).

Results and discussion

The pH values determined at the initial and final stage of fermentation were 5.6 and 4.0, respectively, and cfu were \(2 \times 10^6\) and \(1 \times 10^7\), respectively. The final pH was within the range

| Table 1. Feed composition of experimental diets. |
|-----------------------------------------------|
|                                | Basal diet\(\text{a}\) | Assay diets |
|                                | CM   | FCM   |
|-------------------------------|------|-------|
| Ingredients, %                |      |       |
| Corn                          | 54.97| -      |
| Soybean meal (44%)            | 4.10 | -      |
| Palm oil                      | 5.00 | -      |
| Corn gluten                   | 1.00 | -      |
| Dicalcium phosphate           | 2.50 | 0.60  |
| Calcium carbonate             | 0.50 | 0.60  |
| Premix\(f\)                   | 1.00 | 1.00  |
| Salt                          | 0.30 | 0.30  |
| L-lysine                      | 0.30 | -      |
| Choline chloride              | 0.08 | -      |
| Sodium bicarbonate            | 0.05 | -      |
| DL-methionine                 | 0.20 | -      |
| CM                            | 30.00| 97.00 |
| Titanium dioxide              | 0.50 | 0.50  |
| Calculated analysis           |      |       |
| Metabolisable energy, MJ/kg   | 12.9 | 9.9   |
| CF, %                         | 20   | 39.9  |
| L-lysine, %                   | 1.0  | 1.6   |
| DL-methionine, %              | 0.6  | 0.8   |
| Methionine+cysteine, %        | 1.0  | 1.8   |
| Methionine, %                 | 0.8  | 1.7   |
| Threonine, %                  | 1.7  | 1.7   |

CM, canola meal; FCM, fermented canola meal; CP, crude protein. “The basal diet was fed to all birds for 4 d before introduction of the assay diets. Premix provided the following (per kilogram of diet): vitamin A, 2000 IU; vitamin D\(_3\), 400 IU; vitamin E, 1.8 mg; vitamin B\(_12\), 3.5 mg; riboflavin, 1.4 mg; pantothenic acid, 2 mg; nicotinic acid, 7 mg; pyridoxine, 0.25 mg; folic acid, 0.15 mg; menadione, 0.3 mg; thiamin, 0.15 mg; manganese oxide, 35 mg; ferrous sulfate, 35 mg; zinc oxide, 30 mg; copper sulfate, 60 mg; cobalt carbonate, 5 mg; potassium iodine, 0.6 mg; selenium vanadate, 0.09 mg.

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of LAB fermentation in silage making (Filya et al., 2007), and the reduction in CF was as 
expected due to the diminishing nutrients for 
bacterial growth. Our results also showed that 
CM fermentation by L. salivarius increased CP 
and decreased CF and glucosinolate (P<0.05) 
(Table 2). However, there were no significant 
differences between CM and FCM. Enhancement of CP in 
FCM in the present study is in agreement with 
report of Rozan et al. (1996) and it may 
explained by the loss of DM at the expense of 
fermentable sugars during fermentation 
process with LAB. The positive effects of 
L. salivarius in CF reduction may be attributed to 
the presence of polysaccharidases as studies of 
Skrede et al. (2001, 2003) confirmed that 
fermentation by LAB successfully reduced levels 
of total and soluble dietary fibre and non- 
starch carbohydrates in wheat and barley 
whole meals.

Solid state fermentation has been previously 
used for detoxification and destruction of 
undesirable factors present in vegetable 
protein sources. Pal and Walia (2001) used 
Rhizopus oligosporus fermentation for 
rapeseed meal treatment and observed a reduction 
in CF as well as other antinutritional factors 
including glucosinolate, binoxazolidones and 
phytic acid by 25.5, 43.1, 34, and 42.4%, respec- 
tively. Although the observed glucosinolate 
level reduction in FCM in the current study 
(38%) was lower than the report of Pal and 
Walia (2001), it is still advantageous to use L. 
salivarius over R. oligosporus as the later may 
increase the accumulation of undesirable end 
products such as aflatoxins (Mienda et al., 
2011). Reduction in glucosinolates during 
fermentation may be due to the utilisation of glu- 
cose and sulphur moieties by microbial 
enzymes. This explanation is supported by 
observation of Verbiscar et al. (1981). The 
authors showed that after 21 days of jujuba 
meal fermentation by LAB at 26°C, total toxic- 
cants decreased by 55 to 98%. This was due to 
modification of cyano group by the nitrilase 
enzyme of LAB (Legras et al., 1990).

The results from the digestibility trial 
showed that the CP digestibility of FCM was 
higher (P<0.05) compared to CM, but not the 
AME (Table 3). Among the various AA, the 
enhancement of digestibility was observed for 
Met, Cys, Arg, Asp, Gln, and Ser. The observed 
protein quality might be due to the secretion of 
enzymes such as cellulase, phytase, and 
xylanase during the growth of microbes to 
convert the fibre materials for monosaccharide 
(Ugwuanyi et al., 2008). Some bacteria from 
the group of LAB, such as L. cellulosus, P. pen- 
tosaceu, L. fermentum, L. brevis and L. plan-
tarum, have been reported to have proteinase and aminopeptidase activities (Muğula et al., 2003). Pranoto et al. (2013) reported that, during fermentation, proteolysis could produce more peptides and AA and therefore increase digestible and soluble protein portion. In addition, some LAB such as L. plantarum have tannase activity (Duodu et al., 2003), breaking tannin complex with protein. Thereby, a combination of hydrolysis of protein matrix and releasing protein from complexes may attributed to LAB fermentation. The improvement in CP digestibility may also be attributed to the lower fibre content in FCM. This finding is supported by the reports of Siregar et al. (1982) and Brenes et al. (2002) that increase in dietary fibre or oligosaccharides reduced apparent protein digestibility in duck and chicken.

Conclusions

Solid state fermentation of CM using L. salivarius reduces CF (by 16%) and glucosinolate content (by 38%), while enhances the CP and some essential AA. This treatment may improve the overall nutritive value of CM for broiler chickens.

References

Ahmed, A., Zulkiifli, I., Farjam, A.S., Abdullah, N., Liang, J.B., 2014. Extrusion enhances metabolizable energy and ileal amino acids digestibility of canola meal for broiler chickens. Ital. J. Anim. Sci. 13:3032.

Al-Asheh, S., Duvnjak, Z., 1995. Phytase production and decrease of phytic acid content in canola meal by aspergillus carbonarius in solid-state fermentation. World J. Microb. Biot. 11:228-231.

Annison, G., 1991. Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens. J. Agr. Food Chem. 39:1252-1256.

AOAC, 1990. Official methods of analysis. 15th ed., Association of Official Analytical Chemists, Washington, DC, USA.

Bell, J., 1993. Factors affecting the nutritional value of canola meal: a review. Can. J. Anim. Sci. 73:689-697.

Brenes, A., Marquardt, R., Guenter, W., Viveros, A., 2002. Effect of enzyme addition on the performance and gastrointestinal tract size of chicks fed lupin seed and their fractions. Poultry Sci. 81:670-678.

Driver, J.P., Atencio, A., Edwards, H.M., Pesti, G.M., 2006. Improvements in nitrogen-corrected apparent metabolizable energy of peanut meal in response to phytase supplementation. Poultry Sci. 85:96-99.

Duodu, K., Taylor, J., Belton, P., Hamaker, B., 2003. Factors affecting sorghum protein digestibility. J. Cereal Sci. 38:117-131.

Filya, I., Muck, R.E., Contreras-Govea, F.E., 2007. Inoculant effects on alfalfa silage: fermentation products and nutritive value. J. Dairy Sci. 90:5108-5114.

Gallaher, C.M., Gallaher, D.D., Peterson, S., 2012. Development and validation of a spectrophotometric method for quantification of total glucosinolates in cruciferous vegetables. J. Agr. Food chem. 60:1358-1362.

Gao, Y.-L., Wang, C.-S., Zhu, Q.-H., Qian, G.-Y., 2013. Optimization of solid-state fermentation with Lactobacillus brevis and Aspergillus oryzae for trypsin inhibitor degradation in soybean meal. J. Integr. Agric. 12:869-876.

Granito, M., Frias, J., Doblado, R., Guerra, M., Champ, M., Vidal-Valverde, C., 2002. Nutritional improvement of beans (Phaseolus vulgaris) by natural fermentation. Eur. Food Res. Technol. 214:226-231.

Jezek, J., Haggett, B.G.D., Atkinson, A., Rawson, D.M., 1999. Determination of glucosinolates using their alkaline degradation and reaction with ferricyanide. J. Agr. Food Chem. 47:4669-4674.

Jia, W., Mikulski, D., Rogiewicz, A., Zdunczyk, Z., Jankowski, J., Slominski, B.A., 2012. Low-fiber canola. part 2. nutritive value of the meal. J. Agr. Food Chem. 60:12231-12237.

Kadim, I., Moughan, P., 1997. Ileal amino acid digestibility assay for the growing meat chicken-effect of the imposition of a fasting period and the nature of the test diet. Brit. Poultry Sci. 38:285-290.

Kocher, A., Chot, M., Porter, M., Broz, J., 2000. The effects of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. Poultry Sci. 79:1767-1774.

Legras, J., Jory, M., Arnaud, A., Galzy, P., 1990. Detoxification of cassava pulp using brevibacterium sp. R312. Appl. Microbiol. Biot. 33:529-533.

Marklinder, I., Haglund, Å., Johansson, L., 1996. Influences of lactic acid bacteria on technological, nutritional, and sensory properties of barley sour dough bread. Food Qual. Prefer. 7:283-292.

Mienda, B.S., Idi, A., Umar, A., 2011. Microbiological features of solid state fermentation and its applications. An overview. Available from: http://researchin-biotechnology.com/article/ViewFile/60/57

Muğula, J., Nnko, S., Narhvs, J., Serhaug, T., 2003. Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. Int. J. Food Microbiol. 80:187-199.

Mushtaq, T., Sarwar, M., Ahmad, G., Mirza, M., Nawaz, H., Mushtaq, M.H., Noreen, U., 2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broiler chickens. Poultry Sci. 86:2144-2151.

Newkirk, R., 2009. Canola meal: feed industry guide. Available from: http://www.canola-council.org/media/516716/canola_meal_feed_guide_english.pdf

Pal, V.A., Walia, A., 2001. Beneficial effects of Rhizopus oligosporus fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (Brassica napus) meal. Bioresource Technol. 78:309-312.

Pranoto, Y., Angrahinini, S., Efendi, Z., 2013. Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. Food Biosci. 2:46-52.

Rodriguez-Leon, J., Soccol, C., Pandey, A., Rodriguez, D., 2008. Factors affecting solid-state fermentation. In: A. Pandey, C. Soccol and C. Larroche (eds.) Current developments in solid-state fermentation. Springer, New York, USA, pp 26-47.

Rozan, P., Villaum, C., Bau, H., Schwertz, A., Nicolas, J., Mejean, L., 1996. Detoxication of rapeseed meal by Rhizopus oligosporus sp T3: a first step towards rapeseed protein concentrate. Int. J. Food Sci. Tech. 31:83-90.

SAS, 2002. SAS user’s guide: statistics. SAS Inst. Inc., Cary, NC, USA.

Scott, T.A., Silversides, F.G., Classen, H., Swift, M.L., Bedford, M.R., 1998. Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent digestible energy of wheat and barley. Poultry Sci. 77:456-463.

Short, F.J., Gorton, P., Wiseman, J., Boorman, K.N., 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215-231.

Siregar, A., Cumming, R., Farrell, D., 1982. The nutrition of meat-type ducks. 2. The effects of variation in the energy and protein contents of diets on biological performance.
and carcass characteristics. Aust. J. Agr. Res. 33:865-875.
Skrede, G., Herstad, O., Sahlstrøm, S., Holck, A., Slende, E., Skrede, A., 2003. Effects of lactic acid fermentation on wheat and barley carbohydrate composition and production performance in the chicken. Anim. Feed Sci. Tech. 105:135-148.
Skrede, G., Sahlstrøm, S., Skrede, A., Holck, A., Slende, E., 2001. Effect of lactic acid fermentation of wheat and barley whole meal flour on carbohydrate composition and digestibility in mink (Mustela vison). Anim. Feed Sci. Tech 90:199-212.
Slominski, B.A., Campbell, L.D., 1990. Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agr. 53:175-184.
Soleimani, A.F., Kasim, A., Alimon, A.R., Meimandipour, A., Zulkifli, I., 2010. Ileal endogenous amino acid flow of broiler chickens under high ambient temperature. J. Anim. Physiol. An. N. 94:641-647.
Strydom, D.J., Cohen, S.A., 1994. Comparison of amino acid analyses by phenylisothiocyanate and 6-aminoquinolyl-n-hydroxysuccinimidy carbamate precolumn derivatization. Anal. Biochem. 222:19-28.
Tripathi, M., Mishra, A., 2007. Glucosinolates in animal nutrition: a review. Anim. Feed Sci. Tech. 132:1-27.
Ugwuanyi, J.O., Harvey, L.M., McNeil, B., 2008. Protein enrichment of corn cob heteroxylan waste slurry by thermophilic aerobic digestion using Bacillus stearothermophilus. Bioresource Technol. 99:6974-6985.
Verbiscar, A.J., Banigan, T.F., Weber, C.W., Reid, B., Swingle, R.S., Trei, J.E., Nelson, E.A., 1981. Detoxification of jojoba meal by lactobacillii. J. Agr. Food Chem. 29:296-302.
Yang, S., Ji, K., Baik, Y., Kwak, W., McCaskey, T., 2006. Lactic acid fermentation of food waste for swine feed. Bioresource Technol. 97:1858-1864.