Effects of enzyme addition on the nutritive value of broiler diets containing high proportions of hulled or dehulled Chinese double-low rapeseed meals

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Abstract

This study was conducted to investigate the effect of fibre-degrading enzymes on the nutritive value of broiler diets containing high proportions of hulled or dehulled Chinese double-low rapeseed meals (DLRM). Two two-phase basal diets (phase 1, 4 ~ 21 d of age; phase 2, 22 ~ 42 d of age) were formulated with either hulled (22.5% and 23.5% of diet for phase 1 and 2, respectively) or dehulled (20% and 21.5% of diet for phase 1 and 2, respectively) Chinese DLRM as the major protein source to meet NRC (1994) nutrient requirements. The two basal diets, respectively, plus enzymes A (xylanase + β-glucanase), B (xylanase) and C (xylanase + cellulase) created another six diets. The eight grower diets with triplicate each were used to predict responses of diets to exogenous enzymes by the in vitro two-stage incubation method. Subsequently, a two-phase performance trial was conducted with 288 four-d-old chickens assigned to eight diets with 6 replicate floor pens of 6 birds each. Overall, the digestibility of DM or NDF didn't differ ($P > 0.05$) due to meal types; enzymes B and C addition either to hulled or dehulled DLRM diets both resulted in increased ($P < 0.05$) CP and NDF digestibility compared with their respective controls. Birds fed dehulled DLRM diets had a higher ($P < 0.05$) growth rate, feed efficiency and lower ($P < 0.05$) feed intake than those feed hulled DLRM diets. Enzyme C addition to dehulled DLRM diets resulted in improved ($P < 0.05$) growth rate and feed efficiency during phase 1. Enzymes A and B addition elicited a positive response in feed intake and weight gain ($P < 0.05$), respectively. However, feed efficiency was affected ($P > 0.05$) by neither of the two enzymes. It would appear feasible that using appropriate fibre-degrading enzymes to improve feeding values of broiler diets containing Chinese DLRM. Responses of broilers to fibre-degrading enzymes could be highlighted by hull removal of fed DLRM.

Key words: Broilers, Chinese double-low rapeseed meal, digestibility, performance

Introduction

Double-low rapeseed meal (DLRM), more commonly known as canola meal is considered as a good protein source in poultry diets. Unfortunately, the relatively low digestible energy resulting from the high fibre content (Bell, 1993) still limits its dietary inclusion level. Mechanical dehulling was considered an alternative to reduce fibre content and, consequently, enhance the nutritive value. However, the reduction in dietary fibre following removal of hulls was mainly reflected by a decrease in insoluble fibre, lignin in particular, but total non-starch polysaccharides (NSP) still accounts for some 17.8-21.4%, as near as making no difference from that present in hulled DLRM (16-22%) (Campbell et al., 1995). At the same time, hull removal may cause an increased level of soluble fibre and worse viscosity problem for that a majority of soluble fibre is present in cotyledon of DLRM (Peng, 2001).In addition, analysis in our laboratory indicated that neutral detergent fibre (NDF) content of dehulled Chinese DLRM is 19.5% (as feed), approximately 50% higher than that of soybean meal (13.3%, NRC 1994), whereas NDF negatively affected energy and protein digestibility (Schullze et al., 1994). In short, the potentially increased soluble fibre and the remained high content of fibres such as NDF or NSP still limit the feeding value of DLRM although hull removal decreased fibre content in some degree.

Using fibre-degrading enzymes to breakdown NSP is considered to be a promising way that reducing the anti-nutritional effect of these fibre components in animal feeds. For DLRM, a total of about 15% carbohydrates including starch, free sugars and soluble NSP are encapsulated by cell walls and their actual contribution to digestible energy is modest (Bell, 1993). In this regard, adding an effective enzyme targeting cell wall fibres may improve the nutritive value of DLRM. In addition, the potential viscosity problem caused by relatively increased soluble fibre seems to make the enzyme addition to dehulled DLRM diet become more necessary (Tang et al., 2006).

In the present study, the first consideration was to comparatively evaluate responses of broilers to diets containing different types of DLRM (hulled versus dehulled). Another objective was to investigate the feasibility of using fibre-degrading enzymes to improve the nutritive value of broiler diets containing high proportions of hulled or dehulled Chinese DLRM as estimated from in vitro nutrient digestibility and broiler performance.

Material and methods

*Basal diets and treatments:*Two two-phase basal diets for broilers (phase 1, 4 to 21 d of age; phase 2, 22 to 42 d of age) were formulated with either hulled or dehulled Chinese DLRM as the major protein source to meet NRC (1994) nutrient requirements. Basal diet 1 contained 22.5% (phase 1) and 23.5% (phase 2) hulled Chinese DLRM, and basal diet 2 contained

20% (phase 1) and 21.5% (phase 2) dehulled Chinese DLRM. The two basal diets, respectively, plus enzymes A (xylanase + β-glucanase), B (xylanase) and C (xylanase + cellulase) created another six diets. Three enzymes were all in powder form, and were directly added to the complete diet. All diets were in mash form. The diet formulations were presented in Table 1.

Table 1. Basal diet formulation (as fed basis)

Ingredients (g/kg)	Grower phase (4 ~ 21 d)		Finisher phase (22 ~ 42 d)	
	Basal diet 1	Basal diet 2	Basal diet 1	Basal diet 2
Corn	517.4	562.3	564	609
Soybean meal	190	185	125	120
Hulled Chinese double-low rapeseed meal	225	0	235	0
Dehulled Chinese double-low rapeseed meal	0	200	0	215
Methionine	1.3	1.4	1.2	1.3
Lysine	2.5	1.5	2.8	1.6
Salt	3.8	3.8	3.6	3.6
Soybean oil	25	10	35	15
Limestone	10	12	9	11
Dicalcium phosphate	20.0	19.0	19.4	18.5
Premix*	5	5	5	5
Nutrients as calculation				
ME (MJ/kg)	121.3	121.3	125.5	125.5
Crude protein	210	210	190	190
Salt	3.7	3.7	3.5	3.5
Calcium	10	10	9.5	9.5
Total phosphorus	7.8	7.8	7.5	7.5
Available phosphorus	4.7	4.7	4.5	4.5
Digestible lysine	10	10	8.9	8.9
Digestible Methionine+Cystine	7.2	7.2	6.7	6.7

*Provided per kg of diet: Vitamin A, 13500 IU; Vitamin D3, 3000 IU; Vitamin E, 22.5 mg; menadione, 3.0 mg; thiamine, 3.0 mg; riboflavin, 7.5 mg; niacin, 30 mg; d-panthothenic acid, 15 mg; Vitamin B6, 3.0 mg; Vitamin B12, 23 μg; d-biotin, 120 μg; folic acid, 1.5 mg. copper, 11 mg; iron, 100 mg; manganese, 110 mg; zinc, 100 mg; iodine, 0.8 mg; selenium, 0.3 mg. Arsanilic Acid, 90mg; Zinc Bacitracin, 50 mg.

In vitro two-stage enzyme incubation trial: The eight diets for phase 1 were incubated in triplicate with the *in vitro* two-stage enzyme incubation and dialysis procedure as described in detail by Peng (2000). The residues from the dialysis tubes were then frozen, freeze-dried and analyzed for DM and CP using the technique outlined by AOAC (1990). NDF content in diets and residue was determined by the method of Goering and Van Soest (1970). Each sample was analyzed in duplicate and the *in vitro* digestible DM, CP and NDF were calculated by subtracting the amount of DM, CP and NDF remaining in the residue from the present in the original diet. The digestibility coefficients were calculated from the following equation (taken CP as an example): CP digestibility coefficient = digestible CP (g/kg diet)/total dietary CP (g/kg diet)

Table 2. Effects of enzyme addition on in vitro digestibility of DM, CP and NDF. Values are means ± SD (n = 3)

Meal	Enzyme	DM (%)	CP (%)	NDF (%)
Hulled meal	Control	42.82 ± 1.90^{c}	53.50 ± 0.09^{e}	11.11 ± 1.34^{e}
Hulled meal	Enzyme A	43.81 ± 0.16^{bc}	56.38 ± 1.00^{d}	16.21 ± 0.72^{bcd}
Hulled meal	Enzyme B	45.34 ± 0.43^{ab}	58.70 ± 0.27^{ab}	20.12 ± 2.40^{b}
Hulled meal	Enzyme C	45.86 ± 0.94^{ab}	56.63 ± 1.22^{cd}	25.39 ± 2.14^{a}
Dehulled meal	Control	44.01 ± 2.14^{bc}	57.16 ± 1.02^{bcd}	13.63 ± 1.91^{de}
Dehulled meal	Enzyme A	45.84 ± 1.19^{ab}	58.45 ± 0.91^{abc}	14.67 ± 1.80^{cde}
Dehulled meal	Enzyme B	44.63 ± 0.71^{abc}	60.38 ± 0.12^{a}	19.33 ± 2.81^{bc}
Dehulled meal	Enzyme C	46.69 ± 0.87^{a}	59.64 ± 1.06^{a}	19.54 ± 2.20^{bc}
			—probability of greater *F* value in ANOVA—	
Source of variance				
Meals		NS	***	NS
Enzyme		*	***	***
Meal × Enzyme		NS	NS	0.10

[a-c]Values within a column with no common superscripts differ significantly ($P < 0.05$)
[***]$P < 0.001$, [*] $P < 0.05$

Feeding Trial: Single sex (male) Avian broiler chickens were raised from hatch to 4 d of age in brooders on commercial starter crumbles. At d 4, a total of 288 four-d-old chickens were used in the performance trial, and chickens were randomized among 48 floor pens with each 2 m^2 pen containing 6 chickens, which means six replicates (pens) per dietary treatment. Clean wood shavings were used as litter. Chickens had free access to feed and water. Lightening program, temperature and relative

humidity were according to conventional conditions. Temperature and relative humidity were recorded daily. Feed intake per pen was recorded daily throughout the experiment, and body weight data was recorded at d 21 and 42 of age. The total experiment conducted in two phases (growing phase, 4 ~ 21 d of age; finishing phase, 22 ~ 42 d of age) lasted 39 d.

Statistical Analysis: The study was conducted in a randomized complete block design. SAS (1989) was used to perform the statistical analysis used in this study. Data were analyzed according to the GLM procedure for ANOVA to determine the significance of the main effects (DLRM and enzyme addition) and interactions with the mean value of a pen as the experimental unit, and Duncan's multiple range test was used to separate means when significant effects ($P < 0.05$) were detected by multifactorial analysis of variance.

Results and discussion

Effects of meal type (hulled vs. dehulled) and enzyme addition on the in vitro digestibility

The in vitro digestibility of DM, CP and NDF following enzyme supplementation was shown in Table 2. Overall, the digestibility of DM or NDF was not significantly ($P > 0.05$) different due to meal types, whereas dehulled DLRM diets had a significantly ($P < 0.001$) higher CP digestibility than hulled DLRM diets, regardless of enzyme addition or not. Among the three enzymes, enzymes B and C addition either to hulled or dehulled DLRM diets both resulted in significantly ($P < 0.05$) increased CP and NDF digestibility compared with their respective controls. In contrast, a significant enhancement in CP and NDF digestibility was observed in hulled DLRM diets rather than in dehulled DLRM diets following enzyme A supplementation. Remarkably, with the inclusion of enzyme C, NDF digestibility was improved by 1.3-fold in hulled DLRM diet compared with 0.4-fold in dehulled DLRM diet. This could be explained by the difference in fibre components between hulled and dehulled DLRM. It was reported that the major NSP components found in DLRM were pectic polysaccharides, which include rhamnogalacturonan with associated side chains consisting of arabinose, galactose, and xylanase residues (Bacic et al., 1988). Further study revealed that the non-cellulase polysaccharides in DLRM consisted of arabinose (33%), xylose (13%), mannose (3%), rhamnose (2%), fucose (2%), uronic acids (30%), galactose (13%) and glucose (5%) (Slominski and Campbell, 1990). The high content of arabinose and xylose in DLRM indicated the presence of considerable amount of arabinoxylans (Slominski and Campbell, 1990), which with other polysaccharides including cellulase, xylans, and xyloglucans, are predominantly found in the hull fraction (Meng and Slominski, 2005). In this regard, more amounts of substrates for fibre-degrading enzymes such as cellulase and xylanase would be available in hulled DLRM diet than in dehulled DLRM, which may highlight the responses of DLRM-containing diets to fibre-degrading enzymes in terms of fibre degradation and, consequently, a higher improvement of NDF digestibility was observed for hulled DLRM diets compared with that for dehulled DLRM diets following the same enzyme addition. The varied NDF digestibility of hulled DLRM diets with the inclusion of different enzymes may be a result of the difference in enzyme components and sources. Xylanase, for example, even derived from the same source organism, can vary widely in their catalytic activities on various xylan substrates (Bedford and Schulze, 1998; Faulds et al., 2003; Frederix et al., 2003).

Table 3. Feed intake of broilers fed Chinese double-low rapeseed meal diets with and without enzyme addition. Values are means ± SD (n = 6)

Meal	Enzyme	Feed intake (g/d)		
		Grower phase (4 ~ 21 d)	Finisher phase (22 ~ 42 d)	Overall phase (4 ~ 42 d)
Hulled meal	Control	43.5 ± 1.0[a]	131.8 ± 5.0[a]	91.1 ± 2.9[a]
Hulled meal	Enzyme A	42.7 ± 1.3[ab]	127.6 ± 4.7[ab]	88.4 ± 3.0[ab]
Hulled meal	Enzyme B	43.4 ± 2.0[a]	131.8 ± 6.1[a]	91.0 ± 3.9[a]
Hulled meal	Enzyme C	43.4 ± 0.7[a]	132.3 ± 6.1[a]	91.3 ± 3.1[a]
Dehulled meal	Control	42.3 ± 1.4[ab]	122.6 ± 6.6[b]	85.5 ± 3.9[b]
Dehulled meal	Enzyme A	43.2 ± 1.3[ab]	130.0 ± 3.4[a]	90.0 ± 1.9[a]
Dehulled meal	Enzyme B	43.3 ± 0.6[a]	126.8 ± 6.6[ab]	88.3 ± 3.7[ab]
Dehulled meal	Enzyme C	41.8 ± 1.6[b]	127.9 ± 4.8[ab]	88.2 ± 3.3[ab]
		—probability of greater *F* value in ANOVA—		
Source of variance				
Meals		NS	*	*
Enzyme		NS	NS	NS
Meal × Enzyme		NS	0.09	0.08

[a-b]Values within a column with no common superscripts differ significantly ($P < 0.05$)
*$P < 0.05$

Effects of Meal Type and enzyme addition on Broiler Performance

To comparatively evaluate responses of broilers to diets containing different types of DLRM (hulled versus dehulled), we incorporated the two basal diets on calculated equal-energy (ME) and equal-protein basis with hulled or dehulled DLRM as the major protein source. Feed intake, growth rates and feed conversion ratio measured over the grower phase (4 ~ 21 d), finisher phase (22 ~ 42 d) and overall phase (4 ~ 42 d) were shown in Tables 3, 4 and 5, respectively. Interestingly, no difference in feed intake was observed between the two DLRM diets during the growing phase. In contrast, during the finishing and overall phase birds fed dehulled DLRM diets had a significantly lower feed intake than those fed hulled DLRM

diets. Consequently, during these two phases, birds fed dehulled DLRM diets had a significantly higher feed efficiency (gain:feed) than those fed hulled DLRM diets. Considering that dietary available energy was normally the determinant factor that affects the feed intake of birds, the relatively lower feed consumption for dehulled DLRM diets may suggest that the available energy of dehulled DLRM be underestimated when the diets were formulated.

Table 4. Average daily gain of broilers fed Chinese double-low rapeseed meal diets with and without enzyme addition. Values are means ± SD (n = 6)

Meal	Enzyme	Average daily gain (g/d)		
		Grower phase (4 ~ 21 d)	Finisher phase (22 ~ 42 d)	Overall phase (4 ~ 42 d)
Hulled meal	Control	33.4 ± 0.8^{b}	64.7 ± 4.3^{ab}	50.2 ± 2.5^{abc}
Hulled meal	Enzyme A	31.7 ± 1.1^{c}	62.7 ± 2.7^{b}	48.4 ± 1.8^{c}
Hulled meal	Enzyme B	33.6 ± 1.2^{b}	65.3 ± 2.5^{ab}	50.7 ± 1.1^{abc}
Hulled meal	Enzyme C	34.0 ± 1.2^{ab}	63.8 ± 4.8^{ab}	50.1 ± 2.7^{bc}
Dehulled meal	Control	33.3 ± 2.5^{b}	65.4 ± 4.8^{ab}	50.6 ± 2.5^{abc}
Dehulled meal	Enzyme A	34.8 ± 1.6^{ab}	66.9 ± 2.6^{a}	52.1 ± 1.3^{ab}
Dehulled meal	Enzyme B	35.7 ± 0.6^{a}	61.7 ± 2.4^{b}	49.7 ± 1.6^{c}
Dehulled meal	Enzyme C	35.4 ± 1.2^{a}	67.1 ± 3.3^{a}	52.5 ± 1.9^{a}
		—probability of greater *F* value in ANOVA—		
Source of variance				
Meals		***	NS	*
Enzyme		**	NS	NS
Meal × Enzyme		*	*	*

[a-c]Values within a column with no common superscripts differ significantly (P < 0.05)
[***]P < 0.001, [**]P < 0.01, [*]P < 0.05

Table 5. Feed:gain of broilers fed Chinese double-low rapeseed meal diets with and without enzyme addition. Values are means ± SD (n = 6)

Meal	Enzyme	Feed:gain ratio		
		Grower phase (4 ~ 21 d)	Finisher phase (22 ~ 42 d)	Overall phase (4 ~ 42 d)
Hulled meal	Control	1.31 ± 0.04^{ab}	2.05 ± 0.18^{ab}	1.82 ± 0.12^{a}
Hulled meal	Enzyme A	1.35 ± 0.03^{a}	2.04 ± 0.07^{ab}	1.83 ± 0.06^{a}
Hulled meal	Enzyme B	1.29 ± 0.05^{ab}	2.02 ± 0.10^{abc}	1.80 ± 0.07^{a}
Hulled meal	Enzyme C	1.28 ± 0.05^{b}	2.08 ± 0.13^{a}	1.83 ± 0.09^{a}
Dehulled meal	Control	1.27 ± 0.09^{bc}	1.88 ± 0.20^{c}	1.69 ± 0.13^{b}
Dehulled meal	Enzyme A	1.24 ± 0.06^{bc}	1.94 ± 0.07^{abc}	1.73 ± 0.05^{ab}
Dehulled meal	Enzyme B	1.22 ± 0.03^{cd}	2.06 ± 0.14^{a}	1.78 ± 0.09^{ab}
Dehulled meal	Enzyme C	1.18 ± 0.05^{d}	1.91 ± 0.06^{bc}	1.68 ± 0.04^{b}
		—probability of greater *F* value in ANOVA—		
Source of variance				
Meals		***	*	***
Enzyme		*	NS	NS
Meal × Enzyme		NS	NS	NS

[a-d]Values within a column with no common superscripts differ significantly (P < 0.05)
[***]P < 0.001; [**]P < 0.01; [*]P < 0.05

As for the growth rate, the results of two-way ANOVA analysis showed that birds fed dehulled DLRM had a higher (P < 0.05) growth rate than those fed hulled DLRM during the grower and overall phase, respectively. This seemed contradictory to Campbell et al. (1995). However, it needs to note that a significant interaction between enzymes and meal types occurred during these two phases. In detail, enzyme supplement in dehulled DLRM diets resulted in significantly improved weight gain, but not in hulled DLRM diets. Furthermore, during any experimental phase, there were no differences in growth rate between the two controls containing hulled and dehulled DLRM, respectively. Therefore, it would appear that the observed difference was resulted from enzyme addition, not from the change in meal type. In contrast, Kracht et al. (1999) compared the influence of graded rapeseed meal levels (7%, 14%, 21%) from hulled and dehulled rapeseed on growth performance and found that the in the average of the three levels the weight gain of broilers fed dehulled rapeseed meal diets rose about 53 g (=3.5%) compared with that fed hulled rapeseed meal diets although at a substitution level of 21% the growth decreased. The inconsistency may be associated with the difference in diet formulation between these studies. Remarkably, to show the effect of dehulling, the energy content of the diets was not equalized in the study by Kracht et al. (1999), whereas soybean oil were used as an energy supplement to achieve equalized energy for the two types of DLRM diets in the present study. Therefore, the equalized energy may in part mask and, consequently, result in underestimate of the improved feeding values of dehulled DLRM in comparison to hulled DLRM.

Overall, enzyme C had a higher efficacy than enzymes A and B. For example, enzyme C addition to birds aged 4 to 21 d increased weight gain by 5.9% (35.4 g/d vs. 33.3 g/d, $P < 0.05$), and decreased feed:gain ratio by 7.1% (1.18 vs. 1.27, $P < 0.05$) compared with the control without enzyme addition. Also, birds fed enzyme C-supplemented diet had the highest feed efficiency over the total experimental phase. In contrast, adding enzyme B to dehulled DLRM diets enhanced the growth rate of birds aged 4 to 21 d, but the feed efficiency was not improved. Similarly, enzyme A had a positive effect on feed intake, but its adding value was discounted by the resulted high feed:gain ratio. Remarkably, in the current study, improved growth performance by enzyme supplementation was observed in dehulled DLRM diets but not in hulled DLRM diets, although enzyme addition to either of the two types of diets both resulted in increased nutrient digestibility in vitro. As discussed previously, the difference in responses of the two types of diets to similar enzyme addition may be associated with the difference in fibre components and their anti-nutritional effects. Furthermore, water-soluble NSP seemed to be more susceptible to enzyme action especially under a short digesta transit time in the gastrointestinal tract (Danicke et al., 1999; Meng and Slominski, 2005). It would appear that dehulled DLRM may produce more complex anti-nutritional effect than hulled DLRM when incorporated into broiler diets and, consequently, hull removal of DLRM may highlight the responses of broilers fed DLRM diets to exogenous enzymes as evidenced from the current study.

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