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Variation of Phorbol Ester Contents in *Jatropha curcas* from Different Provinces in Thailand and the Application of its Seed Cake for Starter Broiler Diets

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Abstract: *Jatropha curcas* cultivated in different provinces in Thailand can be classified into toxic and non-toxic genotypes depending upon the phorbol esters content. Seed parts of each sample provided different concentrations of phorbol esters. *J. curcas* seed cake, a main by-product from biodiesel production, had a high protein level. The detoxified seed cake after ethanol extraction was formulated to be the starter broiler diet 1. The proteins isolated from the seed cake and from the detoxified seed cake were applied to diets 2 and 3 respectively. The phorbol ester contents of each diet were reduced by approximately 50% or higher after an extrusion process. The extrusion was therefore an effective method to remove phorbol esters.

Key words: *Jatropha curcas* · Phorbol esters · Starter broiler · Diets · Extrusion

INTRODUCTION

Jatropha curcas is a drought-resistant plant which belongs to the family *Euphorbiaceae*. It has been widely cultivated in Central and South America, south-east Asia, India and Africa [1]. Since it is a multipurpose tree, it has been promoted for planting in Thailand. It can be grown in low to high rainfall areas either in farms as a commercial crop or as a hedge to protect fields and prevent erosion. Its seeds contain a high amount of oil approximately 60-66% [2] which is a good source of biodiesel after esterification. Its seed cake, which is a by-product from biodiesel production, contains high content of protein with a well-balanced amino acid composition according to the FAO/WHO reference pattern, except for lysine [3].

J. curcas seed parts, however, contain several anti-nutritional factors: trypsin inhinitor, phytic acid, lectin and saponin. Additionally, phorbol esters found in *J. curcas* seed parts have been identified as main toxic compounds for animals and humans [4, 5]. Makkar *et al.* [6,7] reported that *J. curcas* species without phorbol esters have been classified to be non-toxic genotypes. Makkar and Becker [8] found that the seed cake after removing heat-stable toxic agent by 92% methanol was not toxic to rats. The objective of this study was to investigate the phorbol ester contents in different provinces of *J. curcas* and to apply *J. curcas* samples as protein sources in the starter broiler feed diets.

MATERIALS AND METHODS

Materials: Toxic and non-toxic genotypes of *J. curcas* seeds used in this study were obtained from different provinces in Thailand. The toxic genotypes were harvested from 4 provinces: Chiang Mai, Phare, Phitsanulok and Satun. Three non-toxic genotypes: A, B and A x B provided by Ladda Company Limited were harvested from Nakornpathom. *J. curcas* seed cake was obtained by a screw press of *J. curcas* seeds. The samples were stored in polyester plastic containers at -20°C before used.

Determination of Phorbol Ester Contents: Phorbol esters were extracted by the method of Saetae and Suntornsuk [9] and analyzed by HPLC according to the modified method of Hass and Mittelbach [10]. Four peaks of phorbol esters appeared between 4.5 and 5.8 min. The peaks were integrated and the results were expressed as equivalent to phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical) used as an external standard.

Preparation of Detoxified Seed Cake: The phorbol esters were removed from *J. curcas* seed cake by ethanol extraction following the method of Saetae and Suntornsuk [9]. Five grams of sample were extracted by 15 ml of 90% (v/v) ethanol with a shaking speed of 150 rev/min. This method was repeated four times in each sample.

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The seed cake passed this process was referred as the detoxified seed cake.

Preparation of Protein Isolates: *J. curcas* seed cake and its detoxified seed cake were suspended in distilled water (1:10). The pH of suspension was adjusted to 12.0 with 1 N NaOH and stirred at 50°C for 3 h for protein extraction. Then, the suspension was centrifuged at 1,837 × g (Rotanta 46R, Hettich, Germany) for 30 min. The supernatant was collected and adjusted to pH 4.0 for protein precipitation. The precipitated proteins were collected by centrifugation and washed twice by distilled water and then vacuum dried.

Starter Broiler Feed Formulations: Detoxified *J. curcas* seed cake, proteins isolated from the seed cake and from the detoxified seed cake were applied to be ingredients of starter broiler feed. The experimental diets for broilers consisted of 3 diets. All diets based on the starter broiler requirements. The control diet was adapted from the report of the Department of Livestock Development, Thailand. The diets 1 contained 10% of the detoxified seed cake while diets 2 and 3 contained 10% of protein isolated from the seed cake and 10% of protein isolated from the detoxified ones respectively. The starter broiler requires the optimum energy level of 3,000-3,200 kcal/kg ME per day. All diets were formulated to be isonitrogenous (23% crude protein) according to National Research Council [11] requirement of broiler chick. Calculated analysis of the experimental diets was done according to feed stuff analysis outline by the method of AOAC [12]. Chemical compositions of the feed ingredients were determined according to AOAC [12]. Carbohydrate contents were determined by difference.

Extrusion Process: The feed ingredients of each diet were mixed thoroughly and pelleted by a co-rotating twin screw extruder (length/diameter ratio 25) (APV Baker, MPF 19:25, APV Baker, Peterborough, UK). The extruder was operated at 300 rpm. The liquid in the extruder barrel was set at 20 %. The temperature at the outlet die was 160°C. A die with 4.2 mm Ø was used and a die face cutter was operated to cut extrudates to 1.0 cm. The extrudates were referred to the feed diets.

RESULTS AND DISCUSSION

Phorbol Ester Contents of *J. Curcas* Seed Parts: The phorbol ester contents of *J. curcas* cultivated in different provinces in Thailand are shown in Table 1. The results showed that the phorbol ester concentrations varied from province to province of cultivation. Similar observations were found in the report of Makkar *et al.* [5]. *J. curcas* seeds used in this study, therefore, can be classified into 2 genotypes: toxic and non-toxic genotypes depending upon the phorbol ester concentrations. The higher the phorbol esters, the lower the acceptance of *J. curcas* seeds to be human food or animal feed. The phorbol esters were detected in *J. curcas* seed parts from Chiang Mai, Phrae, Phitsanulok and Satun provinces which were classified to be toxic genotypes. Fig. 1 illustrated the HPLC chromatogram of phorbol esters with four major peaks at retention times of 4.5-5.8 min. The phorbol ester peaks appear in this chromatogram were similar to those reported by Hass and Mittelbach [10] and Saetae and Suntornsuk [9]. Most contents of phorbol esters were found in the seed kernel compared to the seed shell. While the seed oil and kernel oil showed higher levels of phorbol esters than the seed cake and kernel

Table 1: Phorbol ester contents of *J. curcas* seed parts in different provenances of Thailand (on dry matter basis)

	Phorbol esters[a] (mg/g)[b]						
Provenances	Whole seed	Seed kernel	Seed shell	Whole seed cake	Whole seed oil	Seed kernel cake	Seed kernel oil
Chiang Mai	0.21 ± 0.00	0.15 ± 0.00	0.04 ± 0.00	0.07 ± 0.00	0.13 ± 0.00	0.05 ± 0.00	0.09 ± 0.00
Phrae	0.47 ± 0.01	0.38 ± 0.01	0.08 ± 0.00	0.20 ± 0.01	0.26 ± 0.01	0.14 ± 0.00	0.23 ± 0.01
Phitsanulok	0.31 ± 0.01	0.24 ± 0.01	0.06 ± 0.00	0.13 ± 0.01	0.16 ± 0.00	0.10 ± 0.00	0.14 ± 0.01
Satun	0.45 ± 0.01	0.35 ± 0.01	0.08 ± 0.00	0.19 ± 0.00	0.24 ± 0.01	0.13 ± 0.00	0.20 ± 0.01
Nakornpathom							
Genotype A	ND	ND	ND	ND	ND	ND	ND
Genotype B	ND	ND	ND	ND	ND	ND	ND
Genotype A x B	ND	ND	ND	ND	ND	ND	ND

Mean ± standard deviation of triplicate analyses, ND: Not detected

[a] Equivalent to phorbol 12-myristate, 13 acetate [b] mg/g whole seed

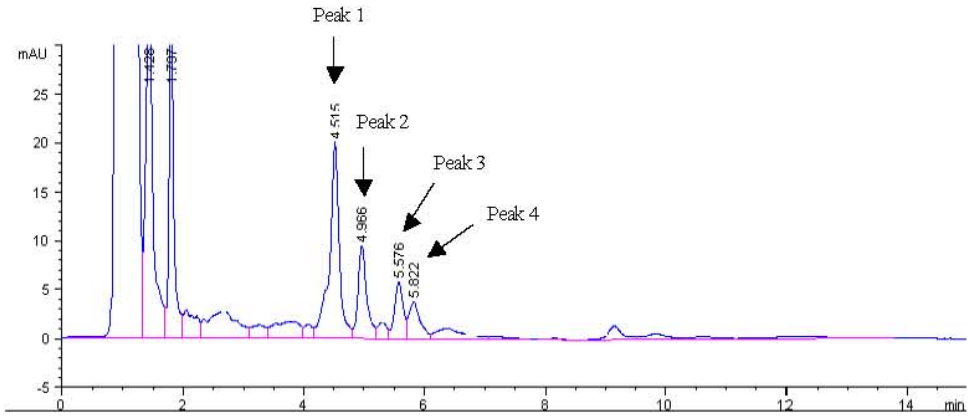

Fig. 1: HPLC chromatogram of the extract from toxic *J. curcas* seed

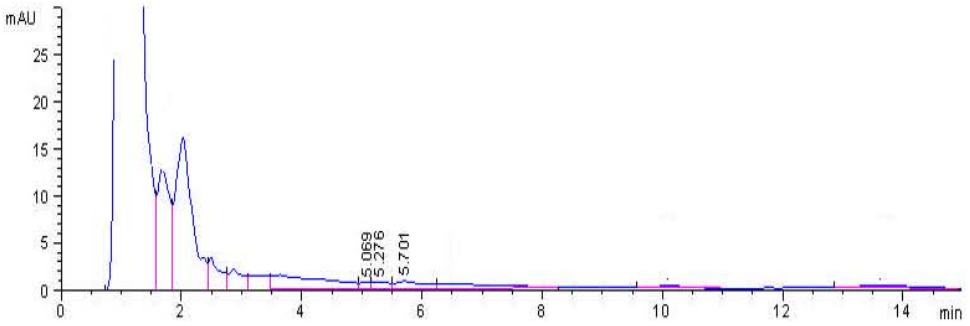

Fig. 2: HPLC chromatogram of the extract from non-toxic *J. curcas* seed

cake respectively. Three genotypes of *J. curcas* seeds obtained from Nakornpathom province were classified as non-toxic genotypes since the phorbol esters were not detected. This was confirmed by the HPLC chromatogram without the phorbol ester peaks found (Fig. 2). Martínez-Herrera *et al.* [3] reported that the non-toxic seed kernels after roasting were consumed by local people in Mexico.

Starter Broiler Feed Diets from *J. Curcas* Samples: *J. curcas* seed cake is a main by-product obtained from biodiesel production. One kg of *J. curcas* seed provides approximately 1 liter of oil and 3 kgs of seed cake. The seed cake therefore should be promoted to be a high value product, for example, animal feed. Since the majority of *J. curcas* cultivated in Thailand is a toxic genotype, the seed cake from this genotype of *J. curcas* should be studied. The detoxified *J. curcas* seed cake and the proteins isolated from seed cake and from its detoxified seed cake were formulated to be starter broiler feeds.The feed ingredients of each diet were mixed and pelleted by an extruder. The experimental diets for broilers are presented in Table 2. Table 2 showed that the protein content and metabolizable energy of each diet were maintained in ranges of approximately 23-24% (w/w) and 3,200-3,300 Kcal/kg respectively. Increasing proportions

Table 2: Percentage of ingredients in the starter broiler diets

	Diet			
Ingredient (%)	Control	1	2	3
Degermed maize flour	45.2	43.2	60.2	60.2
Dehulled full fat soybean meal	45.0	37.0	20.0	20.0
Detoxified *J. curcas* seed cake	-	10.0	-	-
Protein isolated from seed cake	-	-	10.0	-
Protein isolated from detoxified seed cake	-	-	-	10.0
Meat and bone meal	8.0	8.0	8.0	8.0
Dicalcium phosphate (P18)	1.0	1.0	1.0	1.0
DL - Methionine	0.2	0.2	0.2	0.2
Salt	0.35	0.35	0.35	0.35
Vitamin/mineral premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Protein[a] (%)	23.9	23.3	24.6	24.5
Metabolizable energy (ME)[a] (Kcal/kg)	3,322	3,212	3,228	3,228

[a] The data were obtained from calculations
-: Not added

Table 3: Phorbol ester contents of the starter broiler diets before and after extrusion process

	Phorbol esters[a] (mg/g)[b]	
Diets	Before extrusion	After extrusion
Control	ND	ND
1	0.02 ± 0.00	0.01 ± 0.00
2	0.03 ± 0.00	0.01 ± 0.00
3	ND	ND

Mean ± standard deviation of triplicate analyses; ND: Not detected
[a] Equivalent to phorbol 12-myristate,13 acetate; [b] mg/g whole seed

of *J. curcas* materials led to decreasing proportions of soybean meal which is an expensive protein source.

Phorbol Ester Contents of Starter Broiler Feed Diets: The phorbol ester contents of each diet are shown in Table 3. The phorbol esters were found in very low levels in the diets 1 and 2. They were decreased after the extrusion process. The percent reduction of phorbol esters in diets 1 and 2 were approximately 50%. The phorbol esters were not found in diet 3 neither before nor after the process as well as in the control diet. This result related to the report of Devappa and Swamylingappa [13] which claimed that in protein isolation steps, for example, steam injection, washing and removal of oil in the whey might reduce the content of phorbol esters to an undetectable level. The phorbol ester contents detected in all diets were lower than rat tolerable level (0.09 mg/g) [14]. Although phorbol esters are heat-stable, they can be partly removed by the extrusion process. Therefore, the extrusion can be an alternative method to remove the phorbol ester contents from the feed diets.

CONCLUSION

Phorbol esters are the main toxic agents in *J. curcas*. Thus, toxic and non-toxic genotypes of *J. curcas* can be classified by the phorbol ester concentrations. Since *J. curcas* seed cake is a low value by-product from biodiesel, it was applied as a protein source in the starter broiler diets. The starter broiler diets formulated in this study maintained the optimum energy levels and also protein contents according to the starter broilers requirement. The phorbol esters were removed by extrusion since their contents were markedly decreased. Therefore, extrusion could be an effective process to eliminate the phorbol esters.

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