

Effects of Different Treatment Methods on the Composition of Nutrients and Phorbol Ester Levels of *Jatropha* (*Jatropha curcas* L.) Seed Meal Produced in Ethiopia

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Abstract: Seed meal of *Jatropha* that is obtained after extracting the oil has high nutritive value in terms of crude protein that can be used as animal feed. However, *Jatropha* seed meal contains several anti-nutritive compounds that can be probably toxic to animals. The aim of the present study was therefore to evaluate the effects of different treatment methods on the composition of nutrients, anti-nutritional factors and metabolizable energy content of *Jatropha* seed meal. The seed meal was collected from a biodiesel industry at Bati, Oromia Zone of Amhara Region, Ethiopia and treated using sodium hydroxide, backing yeast and heat and compared with the control (untreated) *Jatropha* seed meal. The nutrient composition (Ash, Crude Protein, Ether Extract, Crude Fiber, Neutral Detergent Fiber, Acid Detergent Fiber and Acid Detergent Lignin), Phorbol ester, metabolizable energy and non-fiber carbohydrate contents were estimated using the standard formula. The result indicates that crude protein content varied from 26.97% in sodium hydroxide to 38.83% in the control treatments on a dry matter basis. The ash content of sodium hydroxide treated and the control were significantly higher ($p < 0.05$) than yeast and heat-treated treatments. The ether extract content of sodium hydroxide-treated was significantly lowest ($p < 0.05$) when compared to the other treatments. The crude fiber contents of sodium hydroxide (30.05) and yeast-treated *Jatropha* seed meal (33.67) were higher ($p < 0.05$) than heat-treated (28.54) and the control (24.3) treatments. The highest neutral detergent fiber and crude fiber contents were observed from yeast and sodium hydroxide treatments whereas the lowest from the control. The metabolizable energy contents of the control was higher ($P < 0.05$) than all other treated *Jatropha* seed meal. Sodium hydroxide and the control treatments had higher ($p < 0.05$) non-fiber carbohydrate content than the yeast treatment. The lowest (360.35) phorbol ester content was recorded from yeast-treated *Jatropha* seed meal followed by sodium hydroxide (872.15) treated while the highest (2285.9 $\mu\text{g TPA eq./g}$) was from untreated control. Treating the *Jatropha* seed meal with yeast improved the feed values, reduced the phorbol ester content and increased the crude protein and ether extract than the other treatments, which can be recommended as possible treatment option for JSM. Feeding trial of yeast-treated JSM is also recommended for further research.

Keywords: Biological treatment, Chemical treatment, Nutrient composition, Physical treatment



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1. Introduction

The productivity of poultry in the tropics has been limited by scarcity and high prices of the conventional protein and energy sources (Aberra *et al.*, 2011). The high cost of protein sources of poultry feed, their restricted availability and the unpredictability of their markets increase the need for utilization of other inexpensive sources of plant proteins (Yue and Zhou, 2009). Over the years there have been several studies such as Adejimi *et al.* (2011), Prasad *et al.* (2012), Abbas (2013) and

Aberra *et al.* (2013), where the non-conventional protein sources like *Jatropha* Seed Meal, *Moringa oleifera* leaf meal and Cocoa pod husks have been used in poultry ration as replacement of the conventional protein sources. Among the different non-conventional protein sources *Jatropha* seed meal has a great potential to complement and substitute soybean meal as a protein source which can be included in livestock diets (Makkar *et al.*, 2012).

Jatropha (*Jatropha curcas* L.), commonly known as physic nut, belongs to the Euphorbiaceae family

(Heller, 1996, Barros *et al.*, 2015) and is cultivated primarily for bio-fuel production. According to the Ministry of Water and Energy (MoWE, 2012), the production of jatropha has gained high attention in Ethiopia as a potential alternative and renewable source of energy due to rising oil prices globally. Jatropha produces a large quantity of seed (Heller, 1996) with a high oil content of 40 to 60% (Makkar *et al.*, 1997).

Seed meal of Jatropha is obtained after extracting the oil from the seeds. The meal has high nutritive value in terms of crude protein. The protein content of detoxified Jatropha kernel meal was estimated to be 665g/kg dry matter and was higher than soybean (*Glycine max*) meal, which is 471 g/kg dry matter (Kumar *et al.*, 2010). However, Jatropha seed meal contains several anti-nutritive compounds such as lectin, trypsin inhibitor (anti-trypsin), saponin, phytate, and phorbol esters (Makkar *et al.*, 1997). Of all the compounds, phorbol esters are considered as the main toxic compound in animal feeds (Makkar and Becker, 1998). The concentration of minerals and anti-nutritional substances in jatropha seed and kernel meal may differ with the soil and agro-climatic conditions where jatropha is grown (Chikpah and Demuyakar, 2012). The biological effects of these compounds include tumors promotion, a wide range of negative biochemical and cellular effects, alteration of cell morphology, induction of platelet aggregation and also serve as lymphocyte mitogens (Azzaz *et al.*, 2011).

These anti-nutritional factors therefore need to be inactivated or removed using different methods at a different level of application to make a comparison for the possible use of treated JSM as a feed (Makkar *et al.*, 1997). The removal of phorbol esters would transform JSM into a highly nutritious and high-value feed ingredient for monogastrics, fishes and ruminants (Haas and Mittelbach, 2000). In this regard, various researches have been conducted to evaluate the effects of different treatment methods on the nutritional values of Jatropha seeds.

A study conducted by Annongu *et al.* (2010) on the nutritional value and effects of physically (boiled, soaked, roasted) and bio-chemically (fermented and soaked in ethanol) treated Jatropha seed meal on the

cockerel diet found non-significant difference in feed intake and body weight gain compared to the control group. Studies by Aderibigbe *et al.* (1997) also indicated that Jatropha seed meal could be used as animal feed through adequate detoxifications using physical or chemical processes.

According to Martinez-Herrera *et al.* (2006), jatropha seed meal could be detoxified and the residual protein-rich seed cake remaining after extraction of the oil could form a protein-rich ingredient in feeds for poultry, pigs, cattle and even fish. Different reports indicated that the nutritive values and phorbol ester contents of jatropha seed meal and jatropha kernel meal are varied based on agro-climatic condition and treatment methods used to remove or reduce the toxic substances. Martinez-Herrera *et al.* (2006) reported high concentrations of Phorbol esters (3.85mg/g) of jatropha kernel collected in Coatzacoalcos region of Mexico. According to the authors, treating Jatropha seed meal collected from Coatzacoalcos with ethanol 90%, and ethanol 90% +NaHCO₃ at 1210C for 25 min decreased the phorbol ester content by 97.9% and 95.8%, respectively. Areghore *et al.* (2003) also reported that jatropha seeds treated with heat and washed four times with 92% methanol reduced Phorbol esters content to 0.09 mg/g and contained 68% crude protein which was higher than value of soybean (47.7%). Phorbol ester is heat tolerant and withstands roasting temperature as high as 160°C for 30 minutes (Chang *et al.*, 2014). Hence heat treatment alone may not be effective to remove phorbol ester from the seed and seed meal. Thus the combination of chemical (sodium hydroxide) and heat, and yeast and heat treatment methods may be successful in the reduction of toxicity of jatropha seed meal.

On the other hand, no information are available in the use of baking yeast as biological inoculate for treating jatropha seed meal to reduce the anti-nutritional factor and other toxic substances in Ethiopia. However, studies conducted by Celik *et al.* (2003) showed that yeast (*Saccharomyces cerevisiae* Meyen ex Hansen) additives can be beneficial in reducing the toxic effects of Aflatoxin. Therefore the present study was initiated to assess the efficacy of different methods in detoxification of phorbol ester in Jatropha seed meal produced in Ethiopia and evaluate

the nutritional composition of major nutrients and different carbohydrate fractions.

2. Materials and Methods

2.1. Description of the study area

Jatropha seed meal was collected from a biodiesel industry situated at Bati district in Oromo Zone of Amhara Region, Ethiopia, 420 km Northeast of Addis Ababa located between 11°11'N latitude and 40°1'E longitude. The detoxification process and the chemical analysis were conducted in Nutrition Laboratory of the School of Animal and Range Sciences, College of Agriculture, Hawassa University, Hawassa, Ethiopia, while the phorbol ester analysis was carried out at the University of Hohenheim, Stuttgart in Germany.

2.2. Detoxification methods used

2.2.1. Physical (moist heat) treatment

Jatropha seed meal was made from the de-husked *J. curcas* seed and pressed using expeller machine made for the extraction of diesel oil by pressing mechanically in a biodiesel factory at Bati town. The meal was grouped as treated and untreated. The untreated jatropha seed meal was the meal directly from the biodiesel unit as a by-product without any further treatment (control) and the treated groups went through sodium hydroxide + autoclaving, yeast + autoclaving and autoclaving treatments. Accordingly, four treatments of JSM were used in the present study.

Physical treatment of jatropha seed meal was carried out using autoclaving described by Aregheore *et al.* (2003). Three replicates of jatropha seed meal of approximately 1000 g each were taken, covered with aluminum foil and placed in an autoclave at 121°C for 30 min. The autoclaved samples were removed and placed in a bucket, washed with distilled water four times and the water decanted. Then the samples were dried in an oven at 60°C for 48 hours and ground to pass through 1 mm sieve size and stored in sampling glass bottles with screw caps.

2.2.2. Chemical treatment

The chemical treatment of jatropha seed meal was carried out using sodium hydroxide + heat as described by Aregheore *et al.* (2003). A hammer mill with a sieve size of 4 mm was used to grind about 1000 g of jatropha seed meal. The grounded meal

was placed in three laboratory trays made of stainless steel to replicate and mixed with 5% sodium hydroxide solution to form a paste. The paste was heated in an autoclave at 121°C for 30 min. The autoclaved samples were removed and placed in a bucket, washed with distilled water four times and decanted the water. The samples were then dried in an oven at 60°C for 48 hours and ground to pass through 1 mm sieve size and stored in sampling glass bottles with screw caps for further chemical analysis.

2.2.3. Biological treatment

The biological treatment was conducted according to the method of Sumati *et al.* (2010) using baking yeast (*Saccharomyces cerevisiae*) purchased from the local super market. Approximately 2000 g of the JSM was grounded to pass through 4 mm sieve size and steamed at 121°C for 15 min. The jatropha seed meal samples were allowed to cool at room temperature. The cooled samples were then mixed with the baking, instant yeast (Jami-instant® commercial name) at 3g/kg jatropha seed meal up to about 60% moisture content level to the original weight of the sample. Then the sample was covered with plastic to make anaerobic condition and was incubated for 24 hours. After 24 hour of incubation the growth of yeast was terminated by oven drying the jatropha seed meal at 70°C for 24 hours. The sample was the allowed to cool at room temperature and grounded to pass through 1 mm sieve size and stored in sampling glass bottles with screw caps.

2.3. Determination of chemical composition of Jatropha Seed Meal

The dry matter, crude fat, and an ash content of the jatropha seed meal were determined using the standard methods described by AOAC (1990). Nitrogen (N) was determined by Kjeldhal procedure and crude protein was calculated using the formula $N \times 6.25$ (Sosulski and Imafidon 1990). The metabolizable energy content of JSM was estimated according to the equation proposed by Wiseman (1987) as indicated below.

$$ME = 3950 + 54.4EE - 88.7CF - 40.8 \quad [1]$$

Where,

ME = Metabolizable Energy in kilocalorie per kg dry matter

DM = Dry Matter,

EE = Ether extract
 CF = Crude Fiber
 Kcal = kilocalorie (Kcal kg⁻¹DM)
 Kg = kilogram

The crude fiber, neutral detergent fiber, acid detergent fiber and acid detergent lignin were analyzed according to the method of Van Soest et al. (1991) using ANKOM²²⁰ Fiber technology[®]. Non-fiber carbohydrate content was determined based on the formula below as described by NRC (2001).

$$\text{NFC} = 100 - (\text{NDF} + \text{CP} + \text{EE} + \text{ash}) \quad [2]$$

Where,

NFC = Non-fiber carbohydrate
 NDF = neutral detergent fiber,
 CP = Crude protein
 EE = Ether Extract

Nitrogen free extract was computed by the difference of organic matter and the sum of crude fiber, ether extract and crude protein. Hemicellulose was estimated as the difference between neutral detergent fiber and acid detergent fiber while cellulose estimated as the difference between acid detergent fiber and acid detergent lignin.

$$\text{NFE} = 100 - \% \text{Water} - \% \text{Ash} - \% \text{CP} - \% \text{EE} - \% \text{CF} \quad [3]$$

Where,

NFE = Nitrogen free extract
 CP = Crude Protein
 EE = Ether Extract
 CF = Crude fiber

2.4. Determination of the anti-nutritional components

Phorbol ester was extracted by the method described by Makkar et al. (1997) using HPLC-UV analysis method. All samples were analyzed in duplicate at the laboratory of University of Hohenheim, Stuttgart, Germany.

2.5. Statistical analysis

The data were analyzed using the General Linear Models (GLM) Procedure (SAS, 2002) and means were separated by the Duncan's Multiple Range Test (Duncan, 1955) at $p < 0.05$. The following model was used for the analysis of the collected data.

$$Y_{ij} = \mu + T_i + e_{ij} \quad [4]$$

Where,

Y_{ij} = Response of variables
 μ = Over all mean
 T_i = Treatment effect on nutrient compositions
 e_{ij} = Experimental error

3. Results

3.1. Chemical Composition of Jatropha Seed Meal

The chemical compositions of treated and untreated Jatropha seed meal are presented in Table 1. The crude protein content of the control (untreated) was higher ($p < 0.05$) than the sodium hydroxide and Yeast treated jatropha seed meal (Table 1). Sodium hydroxide treated and control (untreated) exhibited the highest ash content ($p < 0.05$). The ether extract content of jatropha seed meal treated with Sodium hydroxide was lowest ($p < 0.05$). However, there were no differences among Yeast and heat-treated and control treatments.

The highest ($p < 0.05$) neutral detergent fiber and crude fiber contents were recorded for yeast and Sodium hydroxide-treated jatropha seed meal while the lowest ($p < 0.05$) crude protein and neutral detergent fiber contents were recorded from the control. The neutral detergent fiber contents of Sodium hydroxide, and Yeast-treated jatropha seed meal were greater ($p < 0.05$) compared with heat-treated and control treatment. The lowest acid detergent lignin content was recorded from the control treatment compared to the other treatments, which were statistically similar.

The composition of different carbohydrate fractions of treated and untreated JSM is presented in Table 2. There were differences in non-fiber carbohydrate content, cellulose, hemi-cellulose and nitrogen free extract content across the different treatments. The findings indicated that the non-fiber carbohydrate content varied across the treatments with higher ($P < 0.05$) values recorded in Sodium hydroxide treated and control (untreated) treatments and lowest in Yeast treated jatropha seed meal. The study also showed that the hemicelluloses values varied across the treatments with higher ($P < 0.05$) values recorded in Sodium hydroxide and Yeast treated jatropha seed

meal while the control (untreated) had the least hemicelluloses. The nitrogen-free extract values were highest ($P<0.05$) in the Sodium hydroxide treated samples with no differences recorded across the other

treatments including the control (untreated). The metabolizable energy value was highest ($P<0.05$) in the control treatment while the lowest ($P<0.05$) in Sodium hydroxide-treated jatropha seed meal.

Table 1: Chemical compositions of treated and untreated Jatropha Seed Meal

Nutrient content (% in DM)	NaOH-treated	Yeast-treated	Heat-treated	Control (untreated)	SEM \pm	P-value
Ash	10.00 ^a	8.19 ^b	8.37 ^b	9.37 ^a	0.25	0.0025
CP	26.97 ^c	33.39 ^b	34.93 ^{ab}	38.83 ^a	1.42	0.0022
EE	3.3 ^b	9.14 ^a	9.09 ^a	8.67 ^a	0.75	0.0001
NDF	48.38 ^a	46.52 ^a	39.64 ^b	34.27 ^c	1.72	0.0001
ADF	25.62 ^a	24.97 ^{ab}	22.87 ^{bc}	20.49 ^c	0.69	0.0074
ADL	13.59 ^a	11.75 ^a	10.90 ^a	7.71 ^b	0.74	0.0089
CF	33.05 ^a	33.67 ^a	28.54 ^b	24.30 ^c	1.16	0.0001

SEM = standard error of the mean; JSM = Jatropha seed meal; DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CF = crude fiber; NaOH = sodium hydroxide

Note: Means in the same row with similar letter/s are not significantly different at ($p<0.05$)

Table 2: Composition of carbohydrate fractions in treated and untreated Jatropha Seed Meal

Nutrient content (% in DM)	NaOH-treated	Yeast-treated	Heat-treated	Control (untreated)	SEM \pm
Non-fiber CHO	11.28 ^a	2.75 ^b	7.96 ^{ab}	8.86 ^a	1.79
Cellulose	12.03	13.21	11.96	12.77	1.29
Hemicellulose	22.75 ^a	21.55 ^a	16.76 ^b	13.77 ^c	0.79
Nitrogen-free extract	26.61 ^a	15.59 ^b	19.66 ^b	18.83 ^b	1.36
Metabolizable energy (Kcal kg ⁻¹ DM)	1202.73 ^d	1461.57 ^c	1914.48 ^b	2267.24 ^a	47.363

SEM = standard error of the mean; DM = dry matter

Note: Means in the same row with similar letter/s are not significantly different at ($p<0.05$)

3.2. Anti-nutritional components

The results presented in Table 3 indicate that the Phorbol esters content was lowest in yeast-treated jatropha seed meal. Conversely the highest ($P<0.05$) Phorbol esters content was recorded in untreated jatropha seed meal.

Table 3: Phorbol esters content Jatropha Seed Meal

Treatment	PE	SEM \pm	p-value
NaOH-treated	872.15 ^c	11.05	0.0001
Yeast-treated	360.35 ^d	22.25	0.0001
Heat-treated	1553.6 ^b	91.9	0.0001
Control (untreated)	2285.9 ^a	86.50	0.0001

PE = Phorbol esters ($\mu\text{gTPA eq./g}$); SEM = standard error

Note: Means in column followed by the same letter/s are statistically similar at $p<0.05$ probability level

4. Discussion

4.1. Nutritive values of JSM

The lower value of crude protein in sodium hydroxide, yeast, and heat-treated jatropha seed meal compared with the control could be due to denaturing effect of the heat during the process of autoclaving (Emiola *et al.*, 2003; 2007). The higher crude protein content of yeast treatment compared with sodium hydroxide could be as a result of the addition of microbial protein during fermentation (Belewu, 2008). The crude protein content of jatropha seed meal in the untreated control (38.83%) was higher than the values reported by Chikpah and Demuyakor (2012) where the crude protein content of jatropha seed meal collected from four different areas ranged between 27.33-29.61%. The variation in crude protein content with the previous study could be attributed to differences in agro-climatic conditions

in which the *Jatropha* grew harvesting season, age of the plant and methods of treatment used to remove or reduce the toxic substances (Martinez-Herrera *et al.*, 2006). However, the crude protein content of sodium hydroxide and yeast treated groups in this study is similar to results of some studies (Abdel-Shafy *et al.*, 2011) where de-hulled *Jatropha* seeds had values between 27-33%.

The ash content for untreated *jatropha* seed meal in the present study was lower (9.37%) than that of earlier reports, which ranged between 9.8 and 10.8% in defatted *jatropha* seed meal collected from four different regions in Mexico (Martinez-Herrera *et al.*, 2006). On the other hand, Ojediran *et al.* (2014) reported the ash content of defatted *jatropha* seed meal was about 6.13%. The higher value of ash content in sodium hydroxide treatment in the present study could be attributed to the addition of some elements like sodium from the chemical used for treatment.

The values of ether extract observed in the control (8.67) and yeast treated (9.14%) *jatropha* seed meal in the present study are slightly lower compared to untreated *J. curcass* kernel (9.35%) and fungus-treated (10.85%) as reported by Ojediran *et al.* (2014). On the other hand Abdel-Shafy *et al.* (2011) reported 4.38% ether extract from solvent-extracted *Jatropha* seed meal. The reason for the higher value of ether extract for untreated *jatropha* seed meal in the current study could be attributed to the mechanical expeller methods of extraction that might have not extracted the oil exhaustively. The mechanical presses have low extraction efficiencies, about 8-14% of the available oil remain in the press cake (Bamgboye and Adejumo, 2007).

The acid detergent fiber value (25.62%) in sodium hydroxide treated *jatropha* seed meal was lower than the value reported by Chivandi *et al.* (2004) in unshelled *Jatropha* seed (34.4%). The value of crude fiber content observed in sodium hydroxide treated was similar to the findings of Chikpah and Demuyakor (2012) for raw *Jatropha* seed meal (24.72%) but much higher than reported for defatted *Jatropha* seed meal (4.9 – 6.1%) from four different regions in Mexico (Martinez-Herrera *et al.*, 2006).

The nitrogen free extract content of treatment methods recorded in the present study was ranged from 18.83% to 26.61% which is generally lower than the findings of Abo El-Fadel *et al.* (2011) which ranged from 31.13% to 31.92%. However the values of nitrogen-free extract obtained from untreated and yeast-treated JSM in the current study were higher than those reported by Ojediran *et al.* (2014) for untreated JSM (11.09%) and by Belewu *et al.* (2010) for yeast-treated JSM (4.73%). The difference in crude fiber content with pervious study might be due to the difference in treatment methods, levels of application, agro-climate and harvesting season. The observed metabolizable energy value in this study was lower than the value reported for *Jatropha* kernel by Nessim *et al.* (2017) possibly because of the lower ether extract values.

4.2. Anti-nutritional components

The results in the present study indicated that the methods used to detoxify the *jatropha* seed meal influenced the value of phorbol ester contents. The higher phorbol ester value in the heat-treated (moist heat) *jatropha* seed meal could be attributed to the capacity of phorbol ester to withstand high temperature up to 160⁰C for 30 min as reported by Kumar and Sharma (2008), Belewu and Sam (2010) and Chang *et al.* (2014). The results revealed that the phorbol ester contents recorded in all treatments in the present study were lower than that of earlier findings reported by Ojediran *et al.* (2014) where the phorbol ester content of raw defatted *Jatropha* meal was 2.49mg/100g (2490 µgTPA eq./g). The lower phorbol ester in yeast treated *jatropha* seed meal in this study indicates that this treatments is more effective compared to other detoxifying methods evaluated.

5. Conclusion

The study revealed that sodium hydroxide treatment did not reduce the fiber content, neutral detergent fiber, acid detergent fiber, acid detergent lignin and crude fiber but increased the ash content. Except for the control (untreated), the crude protein and ether extract contents in sodium hydroxide-treated *jatropha* seed meal were lower than the values in other treatments. However, no differences between yeast and heat-treated *jatropha* seed meal in the crude protein and ether extract contents were observed. The

sodium hydroxide and yeast treatments reduced the phorbol ester content in the present study. Generally, yeast treatment increased crude protein, ether extract and decreased phorbol ester content of jatropha seed meal, which can be recommended for detoxification of jatropha seed meal so as to be used as animal feed. Further studies towards feeding trial of yeast treated JSM is also recommended.

Conflict of Interest

The authors declared that there is no conflict of interest

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