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Research Article
In vitro Evaluation and *in vivo* Digestibility of Physically, Chemically and Biologically Treated Jatropha Meal

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ABSTRACT

Background and Objective: Protein considered the second main nutrient in diet formulation for all types of animals after energy. Present study aimed to evaluate the effect of different treatments on the nutritive value of Jatropha meal (JM) by *in vitro* and *in vivo* trials as protein source in sheep diet. **Materials and Methods:** Chemical composition, *in vitro* digestibility, gas production and phorbol esters (PE) were recorded for physically, chemically and biologically treated Jatropha meal. *In vivo* digestibility was measured by using 24 Barki rams randomly assigned into 4 nutritional groups (6 animals/treatment) as follow: 1) control ration and in 2, 3 and 4 groups cotton seed meal replaced with 30, 45 and 60% heated Jatropha meal (HJM). **Results:** The various treatments raised DM (Dry matter), CP (Crude protein), NFE (Nitrogen free extract) and ash, whereas reduced OM (Organic matter), CF (Crude fiber) and EE (Ether extract) content in JM, the results of *in vitro* dry matter disappearance (IVDMD) have a significant height ($p<0.01$) for physical followed by the chemical and biological treatments. Otherwise high significant results ($p<0.01$) for gas production for different treatments was observed. The different treatments decreased the concentration of PE in JM than untreated. **Conclusion:** It can be concluded that all treatments especially heat enhanced chemical composition, IVDMD of JM and gas production. Feeding values were better with the ratio 30 and 45%.

Key words: *Jatropha carcus* meal, gas production, digestibility, nitrogen balance, *in vitro* dry matter, organic matter, crude protein, crude fiber, ether extract

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Protein plays an important role in many aspects of male properties which include the increasing of body weight, sexual maturity[1]. Deficiency of protein sources used for ruminant's nutrition and costly importation of soybean meal is considered a problem in Egypt which caused in a need to look for unusual protein sources that help to decrease the shortage and to help for solving the problem. This has promoted to search for alternate sources of by-products as livestock feed. The effective use of by-products will broaden the feed base as well as bridge the gap between the supply and demand to a great extent. Further, if these by-products are produced locally, they will be economical and they can also partially/fully replace the traditional feed ingredients leading to better productivity because of their better nutritive value in terms of protein and energy. In Egypt, *Jatropha curcas* was planted in many regions (Luxor, Suez, Giza and Ismailia). The hectare of Jatropha plant surrender to 5 tons seeds given about 1.85 tons of oil within the year[2].

Jatropha curcas seed is rich in nutrients, high in protein (60%) and rich in oil (50 and 55%) content therefore, it can be used for biodiesel production[3]. Also because of its medical properties it can be used in both human and animal nutrition as vegetable feed additive for nutritive composition, antimicrobial and anti-inflammatory actions in the leaf and stem bark[4,5]. After the whole oil extraction of de hulled seeds of Jatropha, the major product stayed is the Jatropha kernel meal, Jatropha meal includes high crude protein content beginning with 40% to over 75% CP (DM basis) thus far it could be considerable feed supplement for farm animals producers[6,7]. Well oil extracted Jatropha meal contains (not as much of 2% oil) and starch amount ranges from 7-12% DM. Also, some minerals as phosphorus, calcium, potassium and magnesium are presented in a good amount in Jatropha kernel meal[8].

Despite its potential, the major problems with using Jatropha cake its high content of phorbol esters which inhibits using it in animal nutrition without methods for detoxification and some anti-nutritional factors which consider inhibitor activities like trypsin, saponins, phytate and lectins. *Jatropha* species contains phorbol esters in the seeds, stems, leaves, flowers, roots and all parts of the plant[9].

The highest concentration (from 2-6 g kg^{-1} DM) is presented in the seed kernel. There is 1-3 g kg^{-1} phorbol esters in non-detoxified kernel meal and the oil content is 2-7 g kg^{-1} (DM basis)[10]. The major methods used for reducing the anti-nutritional factors of Jatropha residues are physical treatment with heat, moist heat, chemical treatment with sodium hydroxide and methanol and hydrothermal dealing out based on the solubility of phorbol ester in short-chained organic solvents and considering it unstable in alkaline conditions[11], additionally biological treatment with many kinds of fungi were used[12,13,14].

About the *in vitro* studies, well-detoxified Jatropha meal can be used as a good quality source of protein in ruminant's nutrition. Though rich in protein and superior amino acid profile, presence of anti-nutritional factors limits its use as an animal feed. However, it can be processed using chemical as well as physical methods to reduce the incriminating factors and may be useful as feed supplements[15]. This work aimed the detoxification of Jatropha meal using raw material preparation and different methods for detoxification also to evaluate the effects of some physical, chemical and biological treatments to reach a detoxified meal and use it with different ratios in sheep rations.

MATERIALS AND METHODS

This study was carried out at the Nubaria Experimental Station, Abdelmonem Riad Village, Nubaria Governorate and in the Laboratories of Animal Production Department, National Research Center (NRC), Dokki, Giza, Egypt.

Jatropha meal preparation: *Jatropha curcas* seeds, cultivated in Luxor City (Egypt), were milled and the oil was extracted by hexane as solvent according to Hawash *et al.*[16] in the Oil's Unit, NRC to obtained *J. curcas* meal, the seed meal after extraction was sun dried to 90% DM and stored in a plastic bag in the laboratory and used for chemical analysis and detoxification studies (Table 1).

Jatropha meal was subjected for various treatments: physical, chemical and biological treatments to reduce its toxin content.

Table 1: Chemical composition of Jatropha meal (on DM basis)

	Chemical composition (%)						
Treatment	DM	OM	CP	CF	EE	Ash	NFE
Jatropha meal	91.70	91.48	22.00	42.00	1.83	8.52	25.65

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract

Treatments of Jatropha meal

Physical treatments: This stage was included two treatments:

Dry heat treatment: Jatropha meal was heated to 150°C for 60 min in an oven and then the seed meal was air dried and stored in a plastic bag in the laboratory for using.

Wet heat treatment: Jatropha meal was mixed with water to 66% moisture according to Makkar *et al.*[17] the mixture was made into a paste, covered with aluminum foil and heated to 150°C for 1 h in an oven, then the seed meal was air dried and stored in a plastic bag in the laboratory for using.

Chemical treatments

Chemical treatment processing: The JM was processed chemically according to Anandan *et al.*[18] with 5 g kg^{-1} DM sodium hydroxide (NaOH) or calcium hydroxide [$Ca(OH)_2$], 5 g kg^{-1} DM sodium chloride (NaCl) and 30 g kg^{-1} DM urea. JM was also subjected for soaking (overnight) and roasting (100°C for 30 min).

Biological treatment: *Trichoderma reesei* f-418, *Aspergillus oryzae* f-923 and *Aspergillus fumigates* f-993 were obtained from the Genetic and Cytology Department, National Research Center, Dokki, Giza, Egypt, were maintained on potato dextrose agar medium (PDA), grown at 28°C for 72 h, then stored at 4°C and re-cultivated every 2 months. The microorganisms were maintained on agar medium composed of (g L^{-1}) yeast extract 3 g, malt extract 30 g, peptone 5 g, sucrose 20 g and agar 20 g.

This study included two parts of experiments

The first part (laboratory trials): The first part was laboratory trials which were carried out to study the effect of using physical, chemical and biological treatments of JM, amino acid assay and determination the concentration of phorbol esters.

Treatments were designed as follow:

T1 : Jatropha meal untreated
T2 : Jatropha meal treated with heat (150°C for 1 h)
T3 : Jatropha meal with 66% moisture + heat (150°C for 1 h)
T4 : Jatropha meal treated with NaOH (5 g kg^{-1})
T5 : Jatropha meal treated with $Ca(OH)_2$ (5 g kg^{-1})
T6 : Jatropha meal treated with NaCl (5 g kg^{-1})
T7 : Jatropha meal treated with urea (30 g kg^{-1})
T8 : Jatropha meal treated with *Trichoderma reesei* f-418
T9 : Jatropha meal treated with *Aspergillus oryzae* f-923
T10 : Jatropha meal treated with *Aspergillus fumigates* f-993

Chemical analysis

Proximate composition: The proximate chemical analysis of untreated and treated Jatropha was determined according to AOAC[19] to determine dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash. Nitrogen free extract (NFE) was calculated by difference.

Amino acid analysis: Amino acid content was determined as described by Spackman *et al.*[20] and Moore *et al.*[21]. The analysis was performed in Central Service Unit, National Research Center, Egypt, using LC3000 amino acid analyzer (Eppendorf-Biotronik, Germany).

The second part (*in vitro* and *in vivo* trials): The second part was carried out to evaluate *in vitro* dry matter and organic matter disappearance, gas production for JM with different physical, chemical and biological treatments under study and determination of phorbol esters for some treatments. The treatments gave the best results were *in vivo* evaluated by digestibility and N-balance trials with rams.

***In vitro* valuation:** The *in vitro* dry matter and organic matter disappearance (IVDMD and IVOMD) were determined according to the method described by Tilley and Terry[22]. The *in vitro* gas production (GP) assay was carried out as described by Theodorou *et al.*[23] and adapted to the semi-automatic system of Mauricio *et al.*[24] using a pressure transducer in 120 mL serum bottles incubated at 39°C for 24 h. Ground samples (0.3 g as-fed) were incubated in 120 mL serum bottles along with 15 mL mixed rumen fluid and 30 mL of incubation MB9 medium. The composition of MB9 was NaCl (2.8 g), $CaCl_2$ (0.1 g), $MgSO_4.7H_2O$ (0.1 g), the pH was adjusted to 6.8 and CO_2 was flushed for 30 min[25]. This was done on triplicate samples, rumen liquor was collected from cannulated Barki sheep using a stomach tube. The rams were fed on clover hay to cover its requirement during 3 weeks before collecting the rumen liquor.

Determination of phorbol esters: Phorbol esters were determined according to Makkar and Becker[26]. The samples were extracted with methanol and an aliquot was loaded on a high-performance liquid chromatography (HPLC). The results were expressed as equivalent to phorbol-12-myristate 13-acetate as the standard which was detected at 29.8 min.

Digestibility and nitrogen balance trials: Twenty-four Barki rams with an average live body weight 32.9 kg and 180 days age were randomly assigned into 4 nutritional treatments (6 animals/treatment) to receive one of the following rations:

R1: Control ration [CFM_1 (0% heated Jatropha meal (HJM)+ peanut vines hay (PVH)], R2: [CFM_2 (30% HJM replacement of cotton seed meal)+PVH], R3: [CFM_3 (45% HJM replacement of cotton seed meal) +PVH], R4: [CFM_4 (60% HJM replacement of cotton seed meal)+PVH].

Formulation of the experimental concentrate feed mixtures is presented in Table 2.

The four rations consisted of CFM and peanut vines hay (PVH) used in 3:1 ratio. Chemical composition of different experimental rations is also used. Rations were offered to rams *ad libitum*, while, drinking water was freely available all daytime. Animals were individually confined to wooden metabolic crates.

Digestibility and N-balance trials were carried out to determine nutrients digestibility, nutritive values and N-balance for the four experimental rations. Digestibility trials consisted of 21 days, where 14 days were considered as a preliminary period to allow animals a suitable adaptation followed by 7 days for total collection of feces and urine.

Composite samples from collected feces and urine of each animal were taken for chemical analysis. The experimental diets were offered once a day at 9:30 am, samples of rations offered and residuals if any, were weighed daily during the collection period for further chemical analysis.

Statistical analysis: Data concerning *in vitro* DM and OM disappearance and *in vivo* nutrients digestibility trials were statistically analyzed according to SAS[27]. A one-way classification analysis followed by Duncan's multiple-range test[28] for testing the significance between means was used.

Table 2: Formulation of the experimental concentrate feed mixtures (on DM basis)

Ingredients	CFM_1	CFM_2	CFM_3	CFM_4
Yellow corn	58	58	58	58
Wheat bran	11	11	11	11
Soybean meal	07	07	07	07
Cotton seed meal (CSM)	20	14	11	08
Jatropha seed meal (JM)	00	06	09	12
Limestone	2.2	2.2	2.2	2.2
Vitamin and mineral premix[a]	0.1	0.1	0.1	0.1
Common salt	01	01	01	01
Sodium bicarbonate	0.5	0.5	0.5	0.5
Toxin binder	0.2	0.2	0.2	0.2
Total	100	100	100	100

CFM_1: For control ration, CFM_2: 30% Jatropha of CFM, CFM_3: 45% Jatropha meal replacement of CSM, CFM_4: 60% Jatropha meal replacement of CSM, [a]each 3 kg of vitamins and minerals premix contained Vitamin A: 4000000 IU, Vitamin D3: 1000000 IU, Vitamin E: 4000 mg kg^{-1}, Mg: 27000 mg kg^{-1}, S: 250 mg kg^{-1} , Mn: 9858 mg kg^{-1}, Se: 134 mg kg^{-1}, Zn: 20700 mg kg^{-1}, Cu: 1000 mg kg^{-1}, I: 600 mg kg^{-1}, Co: 800 mg kg^{-1}

RESULTS

Effect of the different treatments on chemical composition: The results of chemical composition of untreated and different treated JM in Table 3 showed that DM content was higher with treated comparing with untreated JM. The highest DM value was detected in JM treated with *A. fumigates* (96.7%). In contrast, the lowest value was observed in JM treated with *T. reesei* (91.4%).

On the opposite, physical treatments gave higher values as compared with the chemical and biological treatments. The highest OM value was recorded with untreated JM (91.48%). On the contrast the lowest value (85.3%) was recorded in T6 and T7 as a chemical treatment, while physical, biological and other chemical treatments indicated intermediate values (87.7 and 88.5%). The results obtained also showed that CP content was increased after different treatments compared with untreated JM (22.0%).

Generally, all treatments decreased CF content, using urea as chemical treatment was the finest treatment that led to reduce CF content (28.4%) then NaCl (30.9%) as a chemical treatment after that heat treatment (31.77%) as a physical one.

As shown in Table 3 results explained that EE values were decreased with all treatments. Ether extract content was higher with untreated JM compared with different biological treatments which led to decrease EE contents.

Heat treatment increased contents of NFE of JM by 7% in comparing with untreated JM. Also, most treatments recorded higher NFE values in comparison with the untreated JM.

Amino acids concentration: The major amino acid in JSM shown in Table 4 was glutamic acid (4.72%). Also, cystine (2.77%), arginine (2.52%), aspartic acid (2.25%), leucine (1.89%), alanine (1.40%) and phenylalanine (1.36%). On the other hand, the major amino acid in CSM was glutamic acid (18.1%), followed by 10.3, 8.9, 5.9, 5.2, 4.4, 4.4 and 4.3% for arginine, aspartic acid, leucine, phenylalanine, valine, serine and lysine, respectively.

Effect of physical, chemical and biological treatments on *in vitro* DM and OM disappearance: *In vitro* DM (IVDMD) recorded the better values with heat treatment as a physical treatment which improved IVDMD from 54.43.0-69.87% for untreated and treated JM, respectively, followed by *T. reesei* (64.93%) as a biological treatment and $Ca(OH)_2$ (64.88%) as a chemical treatment that shown in Table 5.

Table 3: Effect of treatments on chemical composition of Jatropha meal (JM)

		Chemical composition (%) (DM basis)						
Treatment No.	Treatments	DM	OM	CP	CF	EE	Ash	NFE
T1	Untreated Jatropha	91.70^{f}	91.48^{a}	22.00^{c}	42.00^{a}	1.83^{a}	8.52^{d}	25.65^{ef}
Physical treatment								
T2	Heat	94.00^{c}	87.80^{bc}	22.00^{f}	31.77f	0.98^{cd}	12.20^{bc}	33.05^{a}
T3	Heat+Moisture	96.00^{b}	88.50^{b}	22.10^{ef}	36.70^{d}	0.57^{e}	11.50^{c}	29.13^{c}
Chemical treatment								
T4	NaOH (5 g kg^{-1} JM)	93.20^{d}	87.70^{c}	22.30^{e}	35.90^{de}	0.99^{cd}	12.30^{b}	28.51^{d}
T5	CaOH (5 g kg^{-1} JM)	92.70^{de}	88.33^{bc}	22.20^{ef}	35.30^{e}	1.25^{b}	11.67^{bc}	29.58^{b}
T6	NaCl (5 g kg^{-1} JM)	92.50^{e}	85.30^{d}	24.40^{c}	30.90^{g}	0.86^{d}	14.70^{a}	29.14^{c}
T7	Urea (30 g kg^{-1} JM)	93.20^{d}	85.30^{d}	30.40^{a}	28.40^{h}	1.20^{bc}	14.70^{a}	25.30^{ef}
Biological treatment								
T8	*T. reesei*	91.40^{f}	88.10^{bc}	24.30^{c}	40.10^{b}	0.25^{f}	11.90^{bc}	23.45^{g}
T9	*A. oryzae*	93.27^{d}	88.30^{bc}	23.60^{d}	37.90^{c}	0.38^{ef}	11.70^{bc}	26.42^{e}
T10	*A. fumigates*	96.70^{a}	88.30^{bc}	25.00^{b}	38.40^{c}	0.39^{ef}	11.70^{bc}	24.51^{f}
	±SE	0.30	0.31	0.58	0.75	0.08	0.31	0.54
	Significant	**	**	**	**	**	**	**

Means in the same columns with various superscripts are different at p<0.05, * Significant at p<0.05, ** Significant at p<0.01, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract

Table 4: Amino acids concentration (g/100 g sample) in Jatropha and cotton seed meal

Amino acid	Jatropha seed meal	Cotton seed meal	Soybean meal*
Essential amino acid			
Threonine	0.85	3.4	2.35
Valine	Not detected	4.4	2.86
Methionine	0.32	1.8	0.76
Isoleucine	0.97	3.2	2.88
Leucine	1.89	5.9	4.82
Phenylalanine	1.36	5.2	3.02
Histidine	0.84	3.2	1.56
Lysine	0.99	4.3	3.80
Arginine	2.52	10.3	4.45
Non-essential amino acid			
Aspartic acid	2.25	8.9	7.06
Serine	1.04	4.4	3.54
Glutamic acid	4.72	18.1	10.56
Glycine	0.78	4.1	2.50
Alanine	1.40	4.0	2.64
Cystine	2.77	1.9	1.06
Tyrosine	0.67	2.9	2.11

* Makkar and Becker[8] and Kumar *et al*.[42]

Table 5: Effect of physical, chemical and biological treatments on *in vitro* disappearance

Treatment No.	Treatments	IVDMD	IVOMD	IVDCPI (g/day)	IVDOMI (g/day)
T1	Untreated Jatropha	54.43^{bc}	70.48	279.76^{cd}	2854.98^{b}
Physical treatment					
T2	Heat	69.87^{a}	74.50	493.72^{ab}	3385.59^{a}
T3	Heat+Moisture	64.19^{ab}	65.32	276.31^{cd}	1670.23^{cd}
Chemical treatment					
T4	NaOH (5 g kg^{-1} JM)	63.51^{ab}	76.10	558.17^{a}	3444.85^{a}
T5	Ca $(OH)_2$(5 g kg^{-1} JM)	64.88^{ab}	69.30	323.62^{bc}	2561.38^{b}
T6	NaCl (5 g kg^{-1} JM)	43.20^{cd}	65.68	273.24^{cd}	1577.51^{cd}
T7	Urea (30 g kg^{-1} JM)	54.16^{bc}	66.58	285.50^{cd}	1948.39^{c}
Biological treatment					
T8	*T. reesei*	64.93^{ab}	66.92	305.23^{c}	2005.06^{bc}
T9	*A. oryzae*	43.19^{cd}	61.23	193.46^{d}	1054.66^{d}
T10	*A. fumigates*	36.16^{d}	66.61	381.08^{b}	2139.01^{bc}
	±SE	2.26	1.17	35.52	201.28
	Significant	**	NS	*	*

Means in the same row with various superscripts are different at p<0.05,* Significant at p<0.05, ** Significant at p<0.01, NS: Non-significant, IVDMD: *In vitro* dry matter disappearance, IVOMD: *In vitro* organic matter disappearance, IVDCPI: *In vitro* digestible crude protein intake, IVDOMI: *In vitro* digestible organic matter intake

Numerically insignificant higher values of IVOMD were recorded with JM treated NaOH (76.10%) as a chemical treatment and heat (74.50%) as a physical treatment. On the contrast, the other treatments decreased IVOMD compared with untreated JM.

The results from the Table 5 showed that some treatments improved IVDCPI than untreated JM. Chemical treatment with NaOH was the best treatment which led to increase IVDCPI value (558.17 g) as a chemical treatment followed by heat (493.72 g) as a physical treatment then *A. fumigates* (381.08 g) as a biological one, on the other hand *A. Oryzae* as a biological treatment was the lowest treatment compared with untreated JM.

The results of IVDOMI showed increasing in treatments of NaOH (3444.85 g) and heat (3385.59 g), respectively. While the other treatments values were lower than untreated JM.

Effect of physical, chemical and biological treatments on *in vitro* gas production: Lowest value of gas production (GP) recorded with JM treated by *A. Oryzae* (25.80 mL) as a biological treatment then JM treated with wet heat (34.51 mL) as a physical treatment (Table 6).

On the other hand, heat treatment as a physical treatment was the highest (58.90 mL) in gas production followed by NaOH treatment (57.32 mL) compare with untreated JM. There were slight differences among treatments detected in gas production soluble fraction (GPSF) content.

Most treatments decreased GPSF, the lower value in GPSF found recorded to JM treated with NaCl (16.63 mL), while higher value found observed with JM treated with *A. fumigates* (45.01 mL), followed by heat treatment (37.75 mL) then NaOH treatment (33.13 mL). Gas production of non-soluble fraction (GPNSF) content was lower in different JM treatments except with heat treatment (46.34 mL) and NaOH (53.73 mL) which was higher than untreated JM. While the lowest value was recorded with JM treated with *A. fumigates* (18.61 mL) as a biological treatment.

Phorbol esters (PE) content in Jatropha meal after treatments: Data in Table 7 cleared that physical (heat), chemical ($Ca(OH)_2$) and biological (*A. fumigates*) treatments had an observed effect in decreasing phorbol esters (PE) content in Jatropha meal.

The best reduction value of PE values recorded with Jatropha meal treated with heat (0.040 mg g^{-1}) then the treatment with *A. fumigates* (0.047 mg g^{-1}) as a biological treatment then chemical treatment by $Ca(OH)_2$ (0.055 mg g^{-1}) in a comparison with untreated Jatropha meal.

Chemical composition of experimental rations: Results in Table 8 showed that the experimental rations were not different in DM and OM, but there was slight reduction in CP content for R4 (13.61%) compared with R1. On the contrast, increasing in CF and EE content from (12.52%) and (3.20%) for R1 to (14.14%), (3.24%) for R4, respectively.

Table 6: Effect of treatments on *in vitro* gas production

Treatment No.	Treatments	GP mL/200 mg DM	GPSF mL g^{-1} DM	GPNSF mL g^{-1} DM
T1	Untreated Jatropha	50.22^{abc}	31.15^{b}	39.41^{abc}
Physical treatment				
T2	Heat	58.90^{a}	37.75^{ab}	46.34^{ab}
T3	Heat+Moisture	34.51^{cd}	23.23^{bc}	31.09^{bcd}
Chemical treatment				
T4	NaOH (5g kg^{-1}) JM)	57.32^{ab}	33.13^{ab}	53.73^{a}
T5	CaOH(5 g kg^{-1} JM)	45.17^{abc}	29.17^{b}	38.69^{abc}
T6	NaCl (5 g kg^{-1} JM)	40.69^{bcd}	16.63^{c}	36.39^{abcd}
T7	Urea (30 g kg^{-1} JM)	37.22^{cd}	27.52^{bc}	30.84^{bcd}
Biological treatment				
T8	*T. reesei*	45.29^{abc}	29.17^{b}	30.53^{bcd}
T9	*A. Oryzae*	25.80^{d}	22.57^{bc}	20.88cd
T10	*A. fumigates*	47.84^{abc}	45.01^{a}	18.61^{d}
	±SE	2.24	2.57	2.39
	Significant	**	*	**

Means in the same row with various superscripts are different at p<0.05,* Significant at p<0.05,** Significant at p<0.01, GP: Gas production DM, GPSF: Gas production soluble fraction, GPNSF: Gas production non-soluble fraction

Table 7: Effect of treatments on phorbol esters in Jatropha meal

Treatments	Area	Concentration (mg g^{-1} sample)
Un-treated Jatropha	349	0.069
Jatropha treated with heat	204	0.040
Jatropha treated with $Ca(OH)_2$ 0.5 g kg^{-1}	280	0.055
Jatropha treated with *A. fumigates*	248	0.047

Table 8: Chemical analysis of tested feeds and experimental rations (%) (on DM basis)

	Tested feeds		Experimental rations			
Items	JM	CSM	R1	R2	R3	R4
Dry matter (DM)	91.70	90.00	86.11	86.18	86.22	86.26
Organic matter (OM)	91.43	95.00	94.56	94.40	94.30	94.20
Crude protein (CP)	22.00	26.00	13.97	13.79	13.70	13.61
Crude fiber (CF)	42.00	24.00	12.52	13.33	13.73	14.14
Ether extract (EE)	1.85	1.50	3.20	3.22	3.23	3.24
Crude ash	8.57	5.00	5.44	5.60	5.70	5.80
Nitrogen free extract (NFE)	25.58	43.50	64.87	64.06	63.64	63.21

JM: Jatropha meal, CSM: Cotton seed meal, R1: Control ration [CFM_1 (0% heated Jatropha meal (HJM)+ peanut vines hay (PVH)], R2: [CFM_2 (30% HJM replacement of cotton seed meal)+PVH], R3: [CFM_3 (45% HJM replacement of cotton seed meal)+PVH], R4: [CFM_4 (60% HJM replacement of cotton seed meal)+PVH]

Table 9: Effect of the experimental rations on digestibility and nutritive values (%) of rams

	Experimental rations					
Items	Control (R1)	R2	R3	R4	±SE	Significant
Nutrients digestibility (%)						
DM	54.59^{a}	55.88^{a}	56.14^{a}	50.22^{b}	0.91	*
OM	63.92^{a}	60.98^{a}	62.78^{a}	54.93^{b}	1.15	**
CP	59.16^{a}	58.12^{a}	52.85^{b}	54.03^{b}	0.94	*
CF	53.55^{a}	52.89^{a}	52.32^{a}	49.11^{b}	0.62	*
EE	64.93^{c}	76.41^{a}	66.04^{bc}	75.27^{ab}	2.01	*
NFE	81.02^{a}	77.49^{b}	75.03^{c}	76.92^{b}	0.69	**
Nutritive values (%) on DM						
TDN	67.19^{a}	66.95^{a}	65.92^{a}	63.78^{b}	0.44	**
DCP	7.76^{a}	7.64^{a}	6.97^{b}	6.54^{b}	0.164	**

Means in the same row with various superscripts are different at p<0.05, SE: Standard error of the mean, * Significant at p<0.05, ** Significant at p<0.01, TDN: Total digestible nutrients, DCP: Digestible crude protein, R1: Control ration [CFM_1 (0% heated Jatropha meal (HJM)+PVH], R2: [CFM_2 (30% HJM replacement of cotton seed meal)+PVH], R3: [CFM_3 (45% HJM replacement of cotton seed meal)+PVH], R4: [CFM_4 (60% HJM replacement of cotton seed meal)+PVH], DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract

Effect of the experimental rations on digestion coefficients and nutritive value on rams: Digestibility coefficients of the experimental rations Table 9 showed significantly differences (p<0.05) for the different nutrients. The lowest value of dry matter digestibility recorded with R4 (50.22%). However, there were insignificant differences between R1, R2 and R3 compared with control (without JM).

On the other hand, as for CP ration one (R1) showed higher digestibility values (p<0.05) and with no significant difference with R2 and the lowest significant difference value noticed with R3. Also, crude fiber (CF) with R1 which recorded higher digestibility values (p<0.05) without significant differences with R2 and R3 and the lowest significant observed with R4 (49.11%).

Regarding ether extract, R2 scored higher EE digestibility value in comparison with other experimental rations. There were significant differences between R1 and other experimental rations in nitrogen free extract digestibility.

Also, there were insignificant differences between R2 and R4, while R3 indicated lower NFE digestibility value compared with different experimental rations. As for the nutritive value (Table 9), the control ration (R1) indicated highest (p<0.05) nutritive values in terms of total digestible nutrients (TDN) and digestible crude protein (DCP) compared the last experimental rations. While, the lowest values were recorded with R4 (60% JM).

Effect of different experimental rations on nitrogen balance of rams: Results of nitrogen utilization recorded significant differences among experimental rations in different nitrogen terms (Table 10). Highest total nitrogen intake (TNI) was observed with control ration (R1) and R2 while decreased in R3 and the lowest (p<0.05) TNI was observed with R4.

Nitrogen balance indicated that all experimental rations realized positive nitrogen balance. However, the control ration (R1) preserved more (p<0.05) N but with insignificant difference with R2 these two groups recorded higher N balance compared with the other groups R3 and R4 which were the lowest one.

Generally, dietary nitrogen utilization favored R1 (the control group) followed by R2 (30% Jatropha ration) as the most efficient group in utilization of the dietary N of the treated Jatropha rations and R3 then R4 had the lowest value in dietary nitrogen utilization.

Table 10: Effect of the experimental rations on the nitrogen balance

Items	Experimental rations				±MSE	Significant
	R_1	R_2	R_3	R_4		
No. of animals	6	6	6	6		
Nitrogen intake (g/h/day)	15.58[a]	15.48[a]	9.47[b]	8.13[b]	1.10	**
Fecal nitrogen (g/h/day)	5.66[a]	5.80[a]	2.94[b]	2.51[b]	0.40	*
Urinary nitrogen (g/h/day)	2.02	1.85	1.11	1.14	0.06	NS
Total nitrogen excretion (g/h/day)	7.68[a]	7.65[a]	4.05[b]	3.65[b]	0.40	*
Nitrogen digested (g/h/day)	8.17[a]	7.56[a]	4.24[b]	3.62[c]	0.71	**
Nitrogen balance (g/h/day)	7.90[a]	7.83[a]	5.42[b]	4.48[c]	0.73	**
Nitrogen balance intake (%)	50.70[b]	50.58[b]	57.23[a]	55.10[a]	3.12	**
Nitrogen digested intake (%)	59.15[a]	58.14[a]	52.79[b]	47.30[c]	1.51	**

Means in the same row with various superscripts are different at p<0.05, * Significant at(p<0.05, ** Significant at p<0.01, NS: Non-significant, R1: Control ration [CFM_1 (0% heated Jatropha meal (HJM)+PVH], R2: [CFM_2 (30% HJM replacement of cotton seed meal)+PVH], R3: [CFM_3 (45% HJM replacement of cotton seed meal)+PVH], R4: [CFM_4 (60% HJM replacement of cotton seed meal)+PVH]

DISCUSSION

The protein content found in the untreated seed meal was less than that reported by Makkar *et al.*[29] (58-62%), Michael *et al.*[9] (37.68%), Martinez-Herrera *et al.*[30] (31.1-34.5%) and Xiao *et al.*[31] (59.6%), but according to Makkar *et al.*[17] the kernel has about 22.2-27.70 % of CP. Rakshit *et al.*[32] reported that the protein value (22.16%) in Jatropha seed cake gained from the full seeds pressed followed by soxhlet extraction (0.8% lipid)using hexane as solvent.

Treatment with chemical and biological may affected in the increments in CP content because of inserting urea in the chemical treatments and due to growing fungi throw the biological treatment (microbial protein). These results agreed with Martinez-Herrera *et al.*[30] and Xiao *et al.*[31]. Also, Ojediran *et al.*[33] said that increasing in CP as result of processing Jatropha than untreated. Material used in this work had the large amount of shells may cause decreasing in the protein content, which additionally add to raise fiber content in the samples.

The CF value found in this study ranged from 28.4- 42.0%. These results agreed with De Souza *et al.*[34] (36.68%). Decreasing in content of CF of the experimental treatments might be resulting in secreting the enzymes throw the biological treatment[35]. That result in agreement with those reported by Michael *et al.*[9]. Different biological treatments led to decrease ether extract (EE) content to the lowest value. These results were compatible with those showed by Guedes *et al.*[36] who suggested that the treatments reduced lipid content and raising the protein content on the opposite actions, which led to an increasing in the nutritional values of the treated Jatropha seed meal.

Generally, all treatments increased ash content compared with the untreated JM. These results agreed with Antyev *et al.*[37] who found that fat and ash had a negative correlation observed in the different treatments because defatting increase the concentration of minerals. Findings of present study agreed with Abo El-Fadel *et al.*[38] who found that treated JM with lactobacillus as a biological treatment decreased CF, increased CP and increased ash content. Meanwhile, other treatments (with heat) had quite similar for CF, decreased CP and all physical, chemical and biological treatments raised ash value compared with the untreated JM.

As general evidence, amino acids composition had higher concentration in cotton seed meal CSM than those in Jatropha seed meal JSM. These results agreed with those obtained by Abd El-Rahman *et al.*[39]. Makkar and Becker[8] found that the levels of essential amino acids in treated Jatropha meal except of lysine were higher than in soybean meal (SBMs). These results had higher concentrations than those obtained by Apiwatanapiwat *et al.*[40]. Michael *et al.*[9] reported that methionine and lysine values were good enough to improve the fish performance. Values reported in the present study were lower than those obtained by Antyev[41] and Kumar *et al.*[42].

Based on *in vitro* studies, soybean meal had higher *in vitro* organic matter (OM) digestibility and metabolizable energy using gas method than wet heated Jatropha kernel meal. Heat treatment for Jatropha meal used to protect protein from the ruminal digestion. However, Ruales and Nair[43] found that too much heat treatment may lead to over-protect protein, making its digestion in the abomasums and small intestine was unavailable[38] and may enhance the release of energy from the nutrients.

Results were agreed with those obtained by Makkar *et al.*[8] and Makkar *et al.*[10] who found that 24 h *in vitro* rumen protein degradation of Jatropha species and much lower than soybean meal (43.29% vs. 81). Another study reported that *in vitro* OM digestibility of soybean meal was higher than Jatropha meal (64 vs. 49-61%)[44] and the elimination methods

of anti-nutritional factors in Jatropha meal could increase it (from 51-61%)[45]. Heating humidity is numerous more efficient in inhibiting trypsin inhibitor activity[10].

Results in Table 7 were similar with the results obtained by Katole *et al.*[15] and Devappa and Swamylingappa[46] who reported that treatment Jatropha meal with NaCl and $Ca(OH)_2$ and methanol were indicated positive effect in decreasing 85.0%, 83.2% and 90% phorbol ester (PE), respectively.

Makkar *et al.*[17] and Chivandi *et al.*[47] observed reduction in Jatropha PE by 95% and 87.7% with the extraction of the oil with 80% ethanol or 92% methanol processes or using double solvent extraction (hexane and ethanol system) and heat treatment for meal containing <1% oil. On the other hand, other studies found that heat treatment only is not efficient method in decreasing the PE level and were most effective with chemical treatments with 3% NaOH or $NaHCO_3$ in decreasing the PE content to 55% and curcin completely.

Abo El-Fadel *et al.*[38] observed that, results obtained were highly variable with *A. oryzae* recording the maximum detoxification of 45% followed by *P. ostreatus* and *B. allili* where in 35% detoxification was obtained. Also, Makkar *et al.*[17] reported that Jatropha cake has been considered detoxified when the concentration of PE is only 0.11 mg g^{-1} in the raw material.

Michael *et al.*[9] noticed that the methods of treatments used did not remove these anti-nutritional compounds completely, but could be reduce the level of these factors with smallest effect on the nutritional value.

Martinez-Herrera *et al.*[30], Belewu *et al.*[12], Rakshit *et al.*[32] and Abo El-Fadel *et al.*[38] reported that heat treatment reduced the concentration of some anti nutritional factors like trypsin inhibitor as well as lectin by about 75.54% and 83%, respectively and phytic acid in JM. while biological treatment has more positive effect on reducing the concentration by about 82% and 86.7%, respectively. These results were compatible with Ojediran *et al.*[33], Antyev *et al.*[37] and Makkar *et al.*[29].

Low CP digestibility recorded in the R3 and R4 treatment may be due to the low amount of feed intake from rations. These results agreed with Kumar *et al.*[42] who reported that observed changes could be attributed to several factors such as palatability, acceptance of diets, the presence of toxic and anti-nutritional compounds and protein and energy digestion in diets.

Katole *et al.*[48] found that the DM, OM, CP and nitrogen intake were less ($p<0.05$) among animals feeding of JM and reduced feed intake in sheep, the less intake of Jatropha-rations might be caused by the phorbol ester and/or curcin[15,49]. However, apparent digestibility of DM, OM and CP values were not different between the experimental groups. Likewise, feeding of treated Jatropha meal decreased feed intake and digestibility in goats[7] and in rat[32]. Also, Rakshit *et al.*[32] reported that the content of trypsin inhibitor and another anti-nutritional factor effect deficiently on (CP) digestibility.

Crude protein digestibility of concentrate feed mixture (CFM) containing untreated JM was less than CP digestibility of CFM containing treated JM as a result of rising content of trypsin inhibitors on untreated JM. In the meantime, the degradation of CP with the biological treatment was higher than heat treatment, which may cause by excessive protection by the treatment with heat.

On the contrary to this, Katole *et al.*[15] and Deshpande[50] observed the comparable digestibility of DM, OM and CP as compared to the control group in sheep. Low digestibility and absorption of nutrients may be the cause of low growth performance. Li *et al.*[51] found that when JM was used to replace soybean meal exceeding 30% (PE concentration of the diet at 5.50 mg kg^{-1}), it reduced energy, nitrogen utilization and enzyme activities, caused significant damage to the intestinal structure and reduced absorption of protein and energy of the digestive tract[52]. These results might due to the higher crude fiber content in JM.

More nitrogen intake may be lead to increase the nitrogen retention. The variability in dietary N retained may probably because of an increasing in utilization of the ammonia in the rumen of the animal. The nitrogen excretion was resulted high in the current study, which agrees with the recorded results with sheep[53]. The phorbol ester in the concentration of 0.13 mg g^{-1} and anti-nutritional factors and purgative properties of Jatropha meal JM could be the reason of less intake and high excretion of nitrogen between the animals[49,54].

Results of the present study agreed with those obtained by Da Silva *et al.*[55] who found that increasing levels of soy bean meal (SBM) substitution with TJC (Treated Jatropha Cake) resulted in decreasing in the nitrogen-intake, nitrogen absorbed and nitrogen balance linearly ($p<0.05$) also, the inclusion of TJC in the diets were not affected on the urinary urea nitrogen and the absorbed- nitrogen.

CONCLUSION

Generally, physical treatments (heat, wet heat), chemical treatments (NaOH, $Ca(OH)_2$, NaCl, urea) and biological treatments (*T. reesei*, *A. oryzae* and *A. fumigates*) could be used to inactivated successfully anti-nutritional factors (phorbol esters, total phenols, trypsin inhibitor activity, phytic

acid and saponins) in *Jatropha curcas* meal to be a protein source in ruminants' rations. Also, *in vitro* and *in vivo* digestibility trials referred to heat treated Jatropha meal could be used in sheep rations without adverse effects with the ratio of 30 and 45% from protein sources in diets.

SIGNIFICANCE STATEMENT

This study confirmed that the anti-nutritional factor in Jatropha meal could be decreased by heat, chemical and biological treatments up to the safe level for animals. Also, the results of the *in vitro* and *in vivo* evaluation were promised to use Jatropha meal as a new protein source in ruminants' diet.

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