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ORIGINAL ARTICLE

Ricin activity of raw and processed castor (*Ricinus communis*) bean meal as evaluated by haemagglutination test

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Abstract

Among various agro-forest based industrial byproducts, castor bean meal is hitherto wasted, though available in appreciable quantities in various parts of India and that could be utilized as a source of protein for livestock feeding. Even though castor bean meal is rich in protein (35-40%), it is seldom used as animal feed because of presence of incriminating factors such as ricin, ricinine, allergen and chlorogenic acid. An attempt was, therefore, made to reduce ricin content, the main culprit out of all the incriminating factors, which possess haemagglutinating and proteolytic activities by different processing methods such as physical and chemical methods. Physical methods comprised of soaking (12, 24 and 48 h), boiling (30 min), pressure cooking and roasting (50 and 100°C for 20, 30 and 40 min), while the chemical methods included sodium chloride (1, 2, 3% w/w), sodium hydroxide (0.25, 0.50, 1% w/w), calcium hydroxide (0.25, 0.50, 1% w/w) and urea ammoniation (1, 2, 3% w/w). Among various methods, treatment with 2% sodium chloride (common salt), 0.25% calcium hydroxide (lime) and 24 h water soaking were found promising for detoxification based on the reduction of haemagglutination of chicken RBC.

Keywords: Castor bean meal, ricin, detoxification, haemagglutination test

Introduction

Due to unavailability of optimum concentrate feeds to the desired level and its cost had further led to aggravate of existing shortage of 7 million tones of digestible crude protein and 50 million tones of total digestible nutrients (Jain and Singh, 1990). Hence, to fulfill the gap of demand and supply, nutritionists recommend the alternate feed resources for economic livestock production such as agro-forest based industrial byproducts (Pathak, 2003) and are in constant search of alternate feed resources. Unconventional oil cakes and agro-industrial by products are major protein and energy source to fulfill the deficit of protein and energy feeds. In most of the countries including India different oil cakes are used as main feed ingredients in formulations of rations for the ruminant animals (Sudake *et al.,* 2013). Castor bean meal, which is hitherto wasted, is one such byproduct available in various parts of India that could be utilized as a source of protein for livestock feeding (Lade *et al.*, 2013).

India, after Brazil, is the second largest producer of castor bean in the world and its production was 59 lakh tonne in India (FAO, 2002). But castor bean meal cannot be used as such as protein supplement as it contains antinutritional factors such as ricin, ricinine, allergen and chlorgenic acid and needs to be detoxified before being fed to livestock (Lade *et al.*, 2013). About 38.8 mg% of ricin in present (Anandan *et al.*, 2005) which is major toxic constituent in CBM.

Various processing methods such as water soaking, steam cooking, roasting and use of various chemicals alone or in combination to improve nutritive value of CBM were found partially successful (Anandan *et al.*, 2005). This CBM can be converted into wholesome protein substitute in livestock feeding after denaturation of ricin by various processing methods. Hence, efforts were required for effective detoxification and inclusion of such detoxified cake in the diets to see the performance of animals. Out of various antinutritional factors present in castor bean

meal, ricin the main culprit is known to possess proteolytic and haemagglutinating property of red blood cells. Hence an attempt was, therefore, made to detect toxin present in variously detoxified castor bean meal by comparative qualitative test using plate agglutination technique (Rao *et al.*, 1986).

Materials and Methods

Laboratory level processing of castor bean meal (CBM): In order to find out most promising methods of detoxification, laboratory level testing of variously processed castor bean meal was undertaken for the selection of easily adaptable method, keeping in view its application under field conditions. The various physical and chemical treatments employed during the preliminary laboratory level testing are as follows:

Physical treatments

Boiling: The ground CBM was boiled for 30 minutes with excess of water (1:6 w/v) which was then decanted, sun dried and ground for further testing.

Pressure cooking: The ground CBM was made into a thick paste with sufficient amount of water and pressure – cooked till the contents were converted into soft and puffy (approximately for 20 minutes) material. The cooked meal was then sundried and ground.

Water soaking: Powdered CBM was steeped in 3 parts of water for 12, 24 and 48 hour with intermittent stirring. Then the supernatant was decanted and the residual cake was sundried and ground.

Roasting: The CBM was roasted at 50°C and 100°C for 20, 30 and 40 min., respectively.

Chemical treatments

The chemical treatments, except urea ammoniation, were done as per the methods by Ambekar and Dole (1957) with slight modifications.

Sodium chloride: The ground CBM was steeped in 1, 2 and 3 % (w/w) solution of sodium chloride (sea salt) with water at the ratio 1:1.2 (w/v) each for 6, 12 and 24 h. The soaked meal was spread in aluminum trays and dried in hot air oven at 40°C. The ground sample was used for further testing.

Sodium hydroxide: The ground CBM was steeped in 0.25, 0.50 and 1.00 % sodium hydroxide (w/w) solution with water at the ratio of 1:1.2 (w/v) each for 12, 24 and 48 h. The soaked material was spread in aluminum trays and dried in hot air oven at 40°C. The ground sample was used for further testing.

Calcium hydroxide: The ground CBM was steeped in 0.25, 0.50 and 1.00 % calcium hydroxide (lime) (w/w) solution with water at the ratio of 1:1.2 (w/v) each for 12, 24 and 48 h. The residue was oven dried at 40°C, ground and stored for further testing.

Urea ammoniation: The ground CBM was ensiled in water (1:1.2w/v) containing fertilizer grade urea @ 1, 2 and 3% (w/w) in air tight containers each for 12, 24 and 48 h, respectively. They were oven dried at 40°C and ground.

Preparation of crude castor bean meal extracts (CCBME)

Exactly, 25 g of CBM was stirred mechanically in 150 ml of normal saline (0.9% NaCl). The contents were then filtered through muslin cloth and the filtrate so obtained was centrifuged at 2000 rpm for 5 min. An aliquot of clear supernatant (CCBME) of both processed and raw meal was further utilized to test the haemagglutinating activity of the ricin by comparative qualitative test using plate agglutination technique (Rao *et al.*, 1986a).

Haemagglutinating activity in raw and treated castor bean meal

About 5 ml of blood was collected from chicken in equal volume of sterile Alsever's solution (Dextrose, 20.50 g; soldium citrate, 8.00 g; citric acid, 0.55 g; sodium chloride, 4.20 g and distilled water 100 ml) and centrifuged at 1000 rpm for 10 minutes to sediment red blood cells (RBC). The supernatant (Plasma and Alsever's solution) was discarded carefully without disturbing RBC's settled at bottom. The RBC's were then washed three times with phosphate buffer saline (PBS) through centrifugation and diluted further with PBS to a final suspension of 0.5%. The modified microhaemagglutiantion test was carried out in Laxbro plate (Olsnes *et al.*, 1974; Aregheore *et al.*, 1998) and for each experimental diet average values of five observations were presented.

Results and Discussion

Incorporation of processed castor bean meal in the concentrate mixture replacing SBM protein of control concentrate mixture did not influence the percent (%) chemical constituents in terms of OM, CP, TCHO, total ash, calcium and phosphorus but the percentage of EE and CF were found higher in raw and processed castor bean meal incorporated concentrate mixtures (Table 1 and 2).

Table 1: Ingredient (% on as fed basis) of concentrate mixtures

Ingredients	Diets				
	D1	D2	D3	D4	D5
Maize	35	35	35	35	35
SBM	20	-	-	-	-
Raw CBM	-	34	-	-	-
Salt treated CBM	-	-	34	-	-
Lime treated CBM	-	-	-	34	-
Water soaked CBM	-	-	-	-	34
Wheat bran	42.0	28.0	28.7	28.1	28.0
Mineral mixture	2	2	2	1.9	2
Salt	1	1	0.3	1	1

Where, D1, (Control) soybean meal; D2, Raw castor bean meal; D3, NaCl treated castor bean meal; D4, $Ca(OH)_2$ treated castor bean meal; D5, Water soaked castor bean meal.

Table 2: Chemical composition (% on DM basis) of concentrate mixtures[#] and oat straw

Particulars	Diets*					
Nutrients	D1	D2	D3	D4	D5	Oats straw
OM	90.57	90.11	91.45	92.01	90.57	91.4
CP	20.87	19.0	19.10	19.00	19.05	2.71
EE	2.30	4.11	3.31	3.60	3.74	0.80
CF	8.57	14.31	14.27	15.05	16.07	37.83
NFE	58.83	52.69	54.77	54.36	51.71	50.06
Total ash	9.43	9.89	8.55	7.99	9.43	8.60
TCHO	66.40	67.00	69.04	69.41	67.78	87.89
Calcium	0.34	0.25	0.36	0.27	0.31	0.13
Phosphorus	0.96	1.05	1.04	1.03	1.00	0.17

[#]Vitablend $(AD)_3$ @ 6 g/kg concentrate mixture was added containing vitamin A - 50,000 IU and D_3 5000 IU per gram.

*Concentrate mixtures contained: D1, (Control) soybean meal; D2, Raw castor bean meal; D3, NaCl treated castor bean meal; D4, $Ca(OH)_2$ treated castor bean meal; D5, Water soaked castor bean meal.

The EE content of the raw and processed CBM incorporated concentrate mixtures was higher than SBM included (2.30%), which might be due to high fat content of the expeller CBM utilized in preparing concentrate mixtures.

The Haemagglutination activity of crude extract of raw and variously processed castor bean meals was analyzed and average values for five observations were presented in Table 3. This analysis is based on the matrix formation and expressed (HA unit) as reciprocal of end point of dilution (Aregheore *et al.*, 1998; Katole *et al.*, 2010; Katole *et al.*, 2011; Katole *et al.*, 2013).

The laboratory level testing of raw and variously processed CBM for haemagglutinating activity revealed that the raw, roasted and 12 h water soaked CBM had the highest HA units clearly indicating the toxic effect. This also indicated that these methods (roasted and 12 h water soaked) are inefficient to detoxify CBM. Lectins are known to be heat labile and their activity can be decreased by heat treatment (Liener, 1994), however, in present study lectin activity was not reduced at 100°C roasting and was observed upto 1:8 dilution, beyond that lectin activity reduced. In case of raw CBM it was well expected in view of the presence of ricin, a toxicant well known for its agglutinating activity against the mammalian red blood cells (Bolley and Holmes, 1958). In the present study, pressure cooking and boiling were found effective in reducing ricin -

Table 3: Haemagglutinating activity of ricin of CBM on chicken RBC

Particulars	Serial Dilution							HA unit*
Processed CBM	1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Raw CBM	+	+	+	+	+	+	-	64
0.25% NaOH	+	+	-	-	-	-	-	4
0.5% NaOH	+	+	-	-	-	-	-	4
1% NaOH	+	+	-	-	-	-	-	4
1% NaCl	+	+	-	-	-	-	-	4
2% NaCl	+	-	-	-	-	-	-	0
3% NaCl	-	-	-	-	-	-	-	-
0.25% Ca(OH2)	-	-	-	-	-	-	-	-
0.5% Ca(OH2)	-	-	-	-	-	-	-	-
1% Ca(OH2)	-	-	-	-	-	-	-	-
1% Urea	-	-	-	-	-	-	-	0
2% Urea	-	-	-	-	-	-	-	0
3% Urea	-	-	-	-	-	-	-	0
Boiling	-	-	-	-	-	-	-	0
Pressure cooking	-	-	-	-	-	-	-	0
Roasting (20 min 50°C)	+	+	+	+	-	-	-	16
Roasting (30 min 50°C)	+	+	+	+	-	-	-	16
Roasting (40 min 50°C)	+	+	+	+	-	-	-	16
Roasting (20 min 100°C)	+	+	+	-	-	-	-	8
Roasting (30 min 100°C)	+	+	+	-	-	-	-	8
Roasting (40 min 100°C)	+	+	+	-	-	-	-	8
Water soaking (12 hrs)	+	+	+	+	-	-	-	16
Water soaking (24 hrs)	-	-	-	-	-	-	-	0
Water soaking (48 hrs)	-	-	-	-	-	-	-	0

*Expressed as reciprocal of end point of dilution (HA Unit)

content. Borchers (1949) and Rao *et al.* (1986a) inactivated heat labile toxins through autoclaving or cooking which proved better in reducing HA activity. Urea ammoniation did not yield satisfactory results in the present experiment on the contrary Rao *et al.* (1986a) found urea ammoniation to be more effective in reducing the ricin content.

Among various physical processing methods, water soaking for 24 h and chemical methods like NaCl (2% salt) and $Ca(OH)_2$ (0.25% lime) treatments were found to be effective in achieving nil HA activity.

Earlier reports also indicated that water soaking (Shrivastava, 1987) and NaCl (Kiran kumar, 1998; Agarwal, 2001) and $Ca(OH)_2$ (Wankhade, 2005) treatments were found to be effective in reducing the ricin content of the CBM on laboratory level testing which were similar to the present findings.

Conclusion

Thus, from the observations it is concluded that 24 h water soaking along with NaCl (2%) and $Ca(OH)_2$ (0.25%) treatments were found promising in reducing ricin content in castor bean meal basing on nil HA activity with chicken RBC. Further long term animal experimentations are warranted.

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