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EFFECT OF AMMONIA AND UREA TREATMENT ON THE *IN VIVO* DIGESTIBILITY OF MUSTARD SEED HULLS

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Mustard seed hulls (MSH) were treated with 1-5% (w/w) aqueous ammonia or urea and 20% added water for 45 days at ambient temperature confined system. Under these conditions, the maximum reduction in lignin contents (27.0 and 20.0%) and maximum increase in nitrogen contents (49.2 and 65.0%) was observed when MSH was treated with 5% aq. ammonia and 5% urea respectively. Maximum increase in *in vivo* digestibility of dry matter, nitrogen crude fibre, minerals and organic matter was observed when MSH were treated with 2% ammonia or 4% urea. The improvement in digestibility indicated that ammonia or urea treated mustrard seed hulls has a great potential as feed ingredient.

Key words: *In vivo* digestibility, Nutritive feed, lignin.

Introduction

Intrest in the use of agro-industrial wastes i.e. straws, crop residues, oil seed hulls as feed for ruminants has increased in recent years since other feeds such as grains, oilseed cakes and high quality forages have become more expensive. Many attempts have been made to improve the feed value of low quality roughages and agro-industrial wastes by subjecting these to different physico-chemical and biochemical treatments [1-6]. Mustard seed hulls which contain in average 17.2% protein, 16.4% oil and 18.7% crude fiber [7] can be an excellent source of energy for the ruminants but the cellulose and lignin reduce its digestibility. It could be used as ruminant feed after improving its digestibility by treatment with ammonia or urea becuase the ruminants can convert these non-protein nitrogen compounds into useful microbial protein which partially meet their protein requirements [8,9].

The present work was undertaken to study the effect of ammonia and urea treatment on the *in vivo* digestibility of the mustard seed hulls.

Material and Methods

Mustard seeds (*Brassica juncea*) variety RL-18 were procured from Ayub Agricutural Research Institute, Faisalabad.

The mustard seed were cleaned and ground in hand mill. The kernel and hull fractions of seeds separated by air-classification as reported elsewhere [9]. 500 Gram seeds were steamed at 1 kg/sq. cm. bucket for 15 mins. The wet seeds were spread on trays 1'x2.5', dried in an oven at 80±2° for an hour and passed through a hand mill to crack the hulls. The crushed seeds were allowed to fall from a hole in the centre of a drum (size, length: 90 cm, dia. 50 cm, open on both sides) and were subjected to air draught at right angle. The hulls fraction being lighter was blown away at distance and the Kernels fraction settled in the farm of a heap due to the difference in their particle size and rate of fall. The hulls were ground to 60 mesh size and mixed with 1-5% urea or aqueous ammonia (w/w basis). The substrate (200 g) with 20% added water was incubated at ambient temperature (ranging from 16 to 40°) for 45 days in 500 ml size canning jars. The ammoniated or urea treated mustard seed hulls were dried in a vacuum oven at 60° to moisture level below 20%.

Moisture, nitrogen, crude protein, crude fibre, fat, minerals and lignin contents of untreated hulls powder were determined according to A.O.A.C. Methods [10].

Digestibility trial. In vivo digestibility trials were carried out in nylon bags on a rumen fistulated cow. A dry Sahiwal cow weighing 350 kg was obtained from M/s. Packages Dairy Farm Ltd., Pakistan and fistulated. The cow was given necessary treatment for one month and kept at maintenance ration. After one month, the samples of untreated and treated mustard seed hulls were infused in the rumen as per standard technique [11] in six replicated first washed with distilled water, then with alcohol and finally with distilled water until washings gave no colour for soluble materials. The washed samples were dried at 105 ± 2° to constant weight. The results of *in vivo* digestibility were analysed statistically and standard deviation of each sample was also calculated according to Snedecore methods [12].

Results and Discusssion

The oil from mustard and rape seeds is extracted by pressing or refluxing with solvent. The hulls are not removed because a fair amount of oil is left unextracted in hulls. Dehulling of seeds is of paramount importance for preparation of protein rich mustard meal or protien isolates for incorporation into poultry feed or foodstuffs [9,14]. The hull fraction of seeds obtained after dehulling is used as roughage

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because of their low digestibility. The untreated mustard seed hulls (MSH) obtained after dehulling of seeds contained 1.2% nitrogen, 12% fat, 18% crude fibre, 18% ash and 8.5% lignin on dry matter basis. MSH were treated with 1-5% (w/w) aqueous ammonia or urea to improve their feed value. The effect of ammonia or urea treatment on the chemical composition of MSH is shown in Table 1 and 2. A gradual increase of 20.0-49.2% and 25.8-65.0% in nitrogen content was observed when MSH was treated with 1-5/ammonia or urea (w/w) respectively. The increase in the nitrogen content of ammonia or urea treated MSH was due to the formation of ammonium salts i.e. combinationation of ammonia with organic acid, in MSH during its fermentation in a confined system. These ammonium salts are metabolished to form amino acids and cellular proteins by microorganism in cattle rumen [13]. Thus increase in nitrogen level of MSH would help to improve its nutritional value when feed as feed materials to the cattle. The crude fibre contents of MSH showed maximum increase of 5% and 3.8% over the control after treatment with 5% ammonia or urea respectively. This increase appeared to be related with decrease in lignin content. Maximum reduction in lignin content (20%) was observed when MSH were treated with 5% ammonia or urea respectively. The decrease in lignin content appeared to be due to its partial chemical/biochemical decomposition into simple fragements. The increase in nitrogen and crude fibre contents and decrease in lignin contents is in accordance with the findings of Garrett *et al.* [2], and Blake [6] who observed similar changes after ammoniation of cereal straws and cannola meal. The increase in nitrogen and decrease in lignin contents may be viewed as an important development in enhancement of the nutritive value of MSH. Other constituents i.e. fat and ash showed non-significant change after different treatment.

Steaming of crop or wood residues is done to upgrade the carbohydrates availability of lignocellulose. The cleavage of acetyl group in this process provides an acidic medium conducive to hydrolytic action [14, 15]. Similarly, the subdivision of cellulosic materials into very small particles by grinding markedly increased their susceptibility to hydrolytic, enzymatic and microbiological attacks [1,4]. The treatment of crop residues with alkali agents e.g. NaOH, Ca $(OH)_2$, NH_4OH etc., have a swelling action on the lignocellulosic fibres and is thought o be the results of cleavage of ester bonds on Xylan Chains acting as cross links that restrain swelling [16]. Mustard seed hulls were ammoniated or urea treated to improve the digestibility.

TABLE 1. CHEMICAL COMPOSITION* OF MUSTARD SEED HULLS AFTER AMMONIATION.

Treatment	Nitrogen(%)	Increase(%)	Crude fibre(%)	Increase(%)	Lignin(%)	Decrease(%)
Mustard seeds hulls (as such**)	1.20	–	18.0	–	8.5	–
1 % Ammonia	1.44	20.0	18.2	1.1	7.6	10.6
2 % Ammonia	1.54	28.3	18.4	2.2	7.2	15.3
3 % Ammonia	1.58	31.6	18.6	3.3	7.0	17.6
4 % Ammonia	1.65	37.5	18.9	5.0	6.4	24.7
5 % Ammonia	1.79	49.2	19.0	5.5	6.2	27.0

* On dry matter basis – average of three replicates, ** Control.

TABLE 2. CHEMICAL COMPOSITION* OF MUSTARD SEED HULLS AFTER TREATMENT WITH UREA.

Treatment	Nitrogen(%)	Increase(%)	Crude fibre(%)	Increase(%)	Lignin(%)	Decrease(%)
Mustard seed hulls (as such)**	1.20	–	18.0	–	8.5	–
1 % Urea	1.51	25.8	18.1	0.6	8.1	4.7
2 % Urea	1.56	30.0	18.3	1.7	7.8	8.2
3 % Urea	1.83	52.5	18.4	2.2	7.5	11.7
4 % Urea	1.95	62.5	18.6	3.3	7.1	16.5
5 % Urea	1.98	65.0	18.7	3.8	6.8	20.0

* On dry matter basis – average of three replicates, ** Control.

TABLE 3. *IN VIVO* DIGESTIBILITY* OF MUSTARD SEED HULLS AFTER AMMONIATION.

Treatment	Digestibility (%) after 48 hrs.				
	Dry matter	Nitrogen	Crude fibre	Minerals	Organic nitrogen
Mustard seed hulls (as such)**	$35.9^{a} \pm 1.9$	$32.2^{a} \pm 1.8$	$25.6^{a} \pm 1.2$	$41.8^{a} \pm 1.5$	$35.1^{a} \pm 1.1$
1 % Ammonia	$43.2^{b} \pm 2.1$	$34.8^{a} \pm 2.0$	$29.4^{b} \pm 1.1$	$49.9^{b} \pm 1.3$	$41.7^{b} \pm 1.3$
2 % Ammonia	$44.3^{b} \pm 1.5$	$38.2^{b} \pm 2.4$	$33.2^{b} \pm 1.5$	$58.3^{b} \pm 1.8$	$42.1^{b} \pm 1.5$
3 % Ammonia	$41.0^{b} \pm 1.4$	$45.4^{b} \pm 1.6$	$31.5^{b} \pm 1.1$	$47.5^{b} \pm 1.5$	$39.5^{b} \pm 1.6$
4 % Ammonia	$38.6^{a} \pm 2.0$	$39.1^{b} \pm 1.4$	$25.9^{a} \pm 1.5$	$44.2^{a} \pm 1.8$	$37.9^{a} \pm 1.5$
	$36.1^{a} \pm 1.7$	$38.7^{b} \pm 2.2$	$15.0^{a} \pm 1.2$	$43.5^{a} \pm 2.0$	$35.6^{a} \pm 1.2$

*Average of six replicates with standard deviations; ** Control; ^{a,b} Means within a column not sharing a common superscript differ significantly ($P<0.05$).

TABLE4. *IN VIVO* DIGESTIBILITY* OF MUSTARD SEED HULLS AFTER TREATMENT WITH UREA.

Treatment	Digestibility (%) after 48 hrs				
	Dry matter	Nitrogen	Crude fibre	Minerals	Organic matter
Mustard seed hulls (as such)**	$35.9^{a} \pm 1.9$	$33.2^{a} \pm 1.8$	$25.6^{a} \pm 1.2$	$41.8^{a} \pm 1.5$	$35.1^{a} \pm 1.1$
1 % Urea	$40.8^{a} \pm 1.2$	$33.5^{a} \pm 1.5$	$27.2^{a} \pm 1.2$	$44.3^{a} \pm 2.0$	$39.0^{a} \pm 2.0$
2 % Urea	$42.5^{b} \pm 1.5$	$35.9^{a} \pm 1.2$	$28.8^{b} \pm 1.1$	$47.1^{b} \pm 1.5$	$41.1^{b} \pm 1.6$
3 % Urea	$43.8^{b} \pm 1.3$	$39.5^{b} \pm 1.4$	$30.0^{b} \pm 1.3$	$49.0^{b} \pm 1.5$	$42.5^{b} \pm 1.3$
4 % Urea	$45.5^{b} \pm 1.6$	$40.8^{b} \pm 1.5$	$31.5^{b} \pm 1.5$	$51.7^{b} \pm 1.7$	$44.8^{b} \pm 1.8$
5 % Urea	$42.9^{b} \pm 1.8$	$40.5^{b} \pm 1.2$	$30.7^{b} \pm 1.7$	$48.4^{b} \pm 2.1$	$42.1^{b} \pm 2.3$

*Average of six replicates with standard deviations; ** Control; ^{a,b} Means within a column not sharing a common superscript differ significantly ($P<0.05$).

In vivo digestibility of MSH treated with 1-5% (w/w) ammonia is given in Table 3. It is evident from the results that MSH treated with 2% ammonia showed maximum *in vivo* digestibility of dry matter (44.3%), crude fibre (33.2%), minerals (58.3%) and organic matter (42.1%). Maximum nitrogen digestibility (45.4%) was observed when MSH was treated with 3% ammonia. A gradual increase in ammonia contents 4 and 5% during treatment decreased the *in vivo* digestibility of different constituents of MSH. The decrease appeared to be due to increase in pH which inhibited the growth of the rumen microflora. The observations are inline with the findings of Gill [13] who reported increase in pH and ammonia poisoning in case of dry ammoniated roughage.

In vivo digestibility of MSH treated with different concentrations of urea (1-5% w/w) is presented in Table 4. Maxi-mum dry matter, nitrogen, crude fibre, minerals and organic matter *in vivo* digestibility was found to be 45.5, 40.8, 31.5, 51.7 and 44.8% respectively when treated with 4% urea. Further increase in urea to 5%, decreased the *in vivo* digestibility of treated MSH. Maximum increase in digestibility of 4% urea treated MSH seemed to be due to better growth condictions for ruminal microflora and hence better utilization of treated agricultural wastes as reported by Garrett *et al.* [2] Panjarthinam and Laximinarya [17] and Niazi *et al.* [5].

The maximum increase in the digestibility of MSH with 2% ammonia or 4% urea seemed to be due to breaking of bond between lignin and cellulose and better utilization of unbound residual ammonia as non-protein nitrogen source for protein synthesis in the rumen of animal as reported by Hartley *et al.* [18] and Shah and Firdous [19].

It is evident from the present investigations that mustard seed hulls can be converted into a nutritive feed after proper treatment with ammonia or urea.

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