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FERMENTATION VALUE OF SOME AGRO-INDUSTRIAL CO-PRODUCTS BASED ON GAS PRODUCTION KINETICS WITH DUAL RATE CONSTANTS

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ABSTRACT

Ten agro-industrial co-products (AICP) *viz.*, wheat (WB) and deoiled rice (DORB), gram chuni of green gram (GGC) and hyacinth bean (HBC), husk of Bengal gram (BGH), pigeon pea (PPH), hyacinth bean (HBH) and soy (SH) and ground shells of groundnuts (GSL) and almond (ASL) were evaluated for chemical composition and fermentation value (FV) aiding in the selection of feedstuffs in dairy rations. Total carbohydrates in AICPs consisted of 55 to 80% of NDF and 20 to 25% of NFC. CP in DORB was 49% bound to ADF but in other AICPs it was < 2.5%. Although macronutrient composition of DORB was better than WB, FV of DORB was 50% lesser than WB. Among husk, IVGP was higher in the order of HBH> SH> PPH> BGH which was more related to hemicellulose content than other cell wall constituents. Although fermentation rate of GSL and ASL were above 10% like WB, their extent of fermentation was 75 to 85% lesser than WB. Brans fermentation rate was ≥10% h^{-1} while gram chuni were fermented at a rate of 5 to 9% h^{-1}. Husk fermentation rate was slow (3 to 6% h^{-1}). CO_2 to methane ratio, extent and rate of fermentation were critical kinetic attributes to differentiate FV of AICPs. Net gas production of AICPs was greater with higher hemicellulose. Studies conclude that NGP or hemicelluloses of brans, chunis, husks or shells could be criteria for their selection to incorporate in dairy cattle ration.

Key words: Brans, by-products, dairy, husks, nutrition, shells

INTRODUCTION

Agro-industrial co-products (AICP) are in greater demand in dairy cattle rations (DCR) owing to advantage in anaerobic digestion of fibre, availability, production benefits and cost. Conventionally, different types of AICPs are incorporated in DCR based on chemical composition but their fermentation characteristics are ignored. Integrating chemical composition with fermentation characteristics is important because anaerobic fermentation in rumen is first step in the digestion of fibrous feedstuffs in ruminant animals. Therefore, integrating fermentation and chemical composition of feedstuffs is essential in feeding management of dairy animals (Makkar, 2004). Such approach is useful in ingesting higher dry matter (DM) by high milk yielding cows in excess to its rumen fill by taking advantage of variation in fermentability and fermentation kinetics of feedstuffs. Evaluation of fermentation value of feeds under anaerobic conditions is possible by *in vitro* gas production (IVGP) technique. Earlier studies reported the fermentation value of cereals (Singh and Srinivas, 2012), edible oilseed meals (Mohanavel and Srinivas, 2016a) and feed additives (Lokesha *et al.*, 2017) using IVGP as tool. AICPs are not only important ingredients in compounded DCR besides cereals and oilseed meals (OSM), but are also different kind of feedstuffs being rich in cell wall constituents. The objective of the present study was to find the fermentation value of some popularly used AICPs in DCR that was distinctive to their chemical composition.

MATERIALS AND METHODS

Chemical analysis

Popularly used AICPs in DCR such as bran (milling product consist of outer skin of seeds and part of pericarp, testa and germ) of wheat (*Triticum aestivum*) and deoiled rice (*Oryza sativa*), gram chuni (mix of seed coat and endosperm fraction of seed) of green gram (*Vigna radiata*) and hyacinth bean (*Lablab purpureus*), husk (outer shell or coat of the seed) of Bengal gram (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), hyacinth bean and soy (*Glycine max*) and ground shells of groundnuts (*Arachis hypogaea*) and almond (*Prunus dulcis*) were analyzed for proximate (AOAC, 2012) and cell wall composition (Van Soest *et al.,* 1991; AOAC, 2012). Organic matter (OM), total carbohydrates, non-fibrous carbohydrates (NFC) and hemicelluloses were derived mathematically.

In vitro gas production (IVGP)

IVGP was measured using 100 mL glass syringes (Menke and Steingass, 1988). Rumen liquor was collected from 3 cows fitted with flexible rumen cannula (large cannula #1C, M/s Bar Diamond, USA) and fed finger millet straw (*Elucina coracona*) and concentrate mixture (M/s Karnataka Milk Federation, Bangalore) having 89.7% dry matter (DM), 15.75% crude protein (CP), 12.75% total ash, 1.48% extractable ether (EE), 43.01% neutral detergent fibre (NDF), 26.1% acid detergent fibre (ADF) and 8.98%, acid detergent lignin (ADL) and 2.4% acid insoluble ash, in the ratio of 70:30. Rumen liquor (RL) was drawn before offering feed at 8:00 h (with 6 h gap from the last offering of drinking water), strained (SRL) through double layer muslin cloth and immediately used as incubation media. Oven dried samples of AICPs were taken as substrate, weighed and ground by lab mill (M/s Jaico Laboratory, India) using 1 mm sieve. Glass syringes were filled with 10 mL SRL, 200 mg substrate and 20 mL buffer. Piston was lubricated with petroleum jelly and fixed into the cylinder of syringe while removing any excess air. Syringes were kept in BOD incubator at 39°C on a slow moving rotor (6 rpm). Gas production in the syringes was measured at $^{1}/_{2}$, 1, 2, 4, 8, 12, 18, 24 36 and 48 h of fermentation intervals (FI). IVGP values at different intervals were adjusted to blank. Blank corrected IVGP values were fitted to generalize Mitscherlich model with two differential rate constants (France *et al.,* 1993); $G = A\,[1\text{-}e^{-c\,(t-L)\,-\,d\,(\sqrt{t}\,-\,\sqrt{L})}]$ wherein G denotes cumulative gas production at time 't', A is asymptotic gas production, L (h) is lag time and t½ is half-time (h) of A while c (h^{-1}) is rate constant of A at t½ and d (h^{-1}) is rate constant of A at first quartile. The fractional fermentation rate (FR) at t½ was calculated as $\mu½\ (h^{-1}) = c + (d/2\sqrt{t½})$. Metabolizable energy (ME) was calculated, as per Menke and Steingass (1988), from NGP based on following empirical formula:

$$ME = 0.157 \times NGP + 0.0084 \times CP + 0.022 \times EE - 0.0081 \times TA + 1.06\ (n = 200;\ R^2 = 0.94)$$

where NGP was net gas production & CP, EE and TA were crude protein, ether extract and total ash, respectively.

Statistical analysis

IVGP at different time intervals from different AICPs were tested using the ANOVA model included the repeat measure. Variance among different chemical parameters was analyzed in a completely randomized block design by using the model: $Y_{ij} = \mu + T_i + B_j + e_{ij}$. wherein Y_{ij} was any observation for which $X_1 = i$ and $X_2 = j$ (X_1 was primary factor and X_2 was block factors), μ was general mean and e_{ij} was random error component. Significance between estimated marginal means of treatments was tested using Duncan's multiple range test. Significant differences between means was differentiated with alpha superscripts. Statistical analyses were carried using statistical package for social science (SPSS, v14.0).

RESULTS AND DISCUSSION

Husk (outer shell or coat of seed) of Bengal gram (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), hyacinth bean and soy (*Glycine max*) and ground shells of groundnuts (*Arachis hypogaea*) and almond (*Prunus dulcis*) were analyzed for proximate (AOAC, 2012) and cell wall composition (Van Soest *et al.,* 1991; AOAC, 2012). Organic matter (OM), total carbohydrates, non-fibrous carbohydrates (NFC) and hemicelluloses were derived mathematically.

AICPs are important feed ingredients in DCR besides grains and oilseed meal (OSM) to improve the diet and milk quality. CP content in wheat bran (WB) and deoiled rice bran (DORB) was 14 and 15%, respectively, but DORB contained 49% total protein as indigestible protein fraction (Mohanavel and Srinivas, 2016a) bound to ADF in comparison to its proportion of just 1.4% in WB. Gram gram husk (GGH) contained some polished dust of grain and had 16% CP which was comparable to hyacinth bean chuni (HBC) [Table 1]. Soybean husk (SH) had 13% CP while hyacinth gram husk (HBH) and pigeon pea husk (PPH) had 7 and 9% CP. Bengal gram husk (BGH) had only 4% CP which is equivalent to CP content in groundnut shell (GSL) but, even less than almond shell (ASL). EE was highest (7.5%) in ASL. Gross energy (GE) in AICPs was 4 M cal kg^{-1}. Total carbohydrates in AICPs consisted of 55 to 80% NDF and 20 to 25% non-fibrous carbohydrates (NFC) and divergent to grains. NFC in AICPs was comparable to OSM. ADF bound CP was less than 2% in any AICPs, except DORB (7.43%). Lignin content in GSL and ASL was 35 and 39%, respectively while in other AICPs the content was as low as 2.55% in WB with a mean of 15.44%. Among brans and husks, only PPH had 23% lignin (Table 2).

NGP on WB increased at rapid rate at 8 h of FI. IVGP was 2.7 times greater than DORB (Table 3). Such distinction between two types of brans was related to hemicellulose content in cell walls rather than macronutrients. Proximate analysis of DORB appeared better than WB but vice-versa was the case for NGP. This indicated that fermentation of DORB was about 50% lesser than WB. Among husk, NGP was higher in the order of HBH> SH> PPH> BGH which was also related to difference in hemicelluloses than other constituents of cell content and cell walls. Fermentation of husks was accelerated from 4 to 8 h after incubation. NGP on shells of groundnut and almond was only 20% of brans or chunis or husks that was attributed to higher NDF content (80%) and lesser available CP (4%) and NFC (6.6%). Proximate and cell wall composition only provide the quantitative information on chemical entity while IVGP was reflection of fermentability of feedstuff where web like network of different chemical entities need to be hydrolyzed to their simple forms. IVGP thus, represents microbial degradation as well as feed characteristics (Srinivas and Krishnamoorthy, 2005).

Table 1: **Proximate chemical composition of agro-industrial co-products (% on dry matter basis)**

Feedstuffs	DM	OM	CP	EE	TCHO	TA	AIA	GE (M cal kg^{-1})
Wheat bran	92.48^{a}	96.45^{de}	14.00^{de}	4.28^{c}	78.17^{c}	3.56^{bc}	0.45^{ab}	4.07^{e}
DORB	95.87^{d}	88.06^{a}	15.25^{ef}	2.09^{b}	70.71^{a}	11.94^{f}	2.58^{f}	3.63^{a}
Green gram chuni	93.68^{bc}	94.60^{c}	16.07^{ef}	4.37^{c}	74.16^{b}	5.4^{d}	0.89^{cd}	4.00^{d}
Hyacinth bean chuni	94.03^{c}	93.85^{b}	16.43^{f}	2.19^{b}	75.23^{b}	6.15^{e}	1.15^{e}	3.86^{b}
Bengal gram husk	95.58^{d}	96.11^{d}	4.34^{a}	0.49^{a}	91.28^{g}	3.89^{c}	0.80^{cd}	3.88^{b}
Hyacinth bean husk	94.10^{c}	96.65^{de}	6.95^{bc}	1.76^{b}	87.95^{f}	3.35^{bc}	0.52^{b}	3.95^{c}
Pigeon pea husk	92.72^{ab}	96.54^{de}	8.51^{c}	0.08^{a}	87.96^{f}	3.46^{bc}	0.44^{ab}	3.88^{b}
Soybean husk	98.71^{e}	94.97^{c}	12.57^{d}	1.64^{b}	80.77^{d}	5.03^{d}	0.55^{bc}	3.88^{b}
Groundnut shell	98.30^{e}	96.89^{e}	4.70^{ab}	3.88^{c}	88.32^{f}	3.11^{b}	0.35^{ab}	4.07^{f}
Almond shell	92.08^{a}	98.03^{e}	6.23^{abc}	7.50^{d}	84.30^{e}	1.97^{a}	0.23^{a}	4.30^{g}
SEM	0.38**	0.18**	0.74**	0.31**	0.85**	0.18**	0.09**	0.02**

All the values are average of three replications. Values bearing different alphabetic superscripts in a column differ significantly; **P < 0.01

Table 2: **Cell wall constituents of agro-industrial co-products (% on dry matter basis)**

Feedstuffs	Cell content	NDF	ADF	Hemi-celluloses	Cellulose	ADL	NFC	NDF-N	ADF-N
Wheat bran	44.79^{f}	55.21^{a}	8.05^{a}	47.16^{e}	7.49^{a}	2.55^{a}	26.51^{cd}	2.49^{a}	0.19^{a}
DORB	38.16^{de}	61.84^{bc}	30.78^{c}	31.06^{e}	13.72^{b}	15.29^{e}	20.81^{bc}	12.80^{c}	7.43^{f}
Green gram chuni	45.36^{f}	54.64^{a}	30.92^{c}	23.72^{d}	26.46^{c}	5.56^{ab}	24.92^{bcd}	2.86^{a}	1.38^{bcd}
Hyacinth bean chuni	42.38^{ef}	57.62^{ab}	22.01^{b}	35.60^{e}	14.70^{b}	7.73^{bc}	23.77^{bcd}	3.02^{ab}	1.18^{bc}
Bengal gram husk	31.11^{bc}	71.22^{e}	69.47^{g}	1.75^{a}	55.92^{g}	10.57^{cd}	26.27^{cd}	3.31^{ab}	1.04^{ab}
Hyacinth bean husk	28.09^{b}	71.91^{e}	56.59^{e}	15.31^{c}	49.28^{f}	5.38^{ab}	19.38^{b}	3.07^{ab}	1.31^{bcd}
Pigeon pea husk	34.16^{cd}	65.84^{cd}	62.24^{f}	3.59^{ab}	39.60^{d}	22.55^{f}	25.57^{cd}	2.17^{a}	2.20^{de}
Soybean husk	37.95^{de}	62.05^{bc}	45.34^{d}	16.71^{c}	34.23^{c}	10.91^{d}	23.74^{cd}	4.46^{b}	2.07^{cde}
Groundnut shell	18.20^{a}	81.80^{a}	75.61^{h}	6.19^{ab}	40.60^{de}	35.28^{g}	9.62^{a}	2.62^{a}	1.63^{cde}
Almond shell	20.31^{a}	79.69^{f}	75.54^{h}	4.15^{ab}	43.25^{e}	38.55^{h}	6.58^{a}	3.46^{ab}	2.32^{e}
SEM	1.44**	1.44**	0.78**	1.91**	1.00**	1.01**	1.62**	0.44**	0.29**

The values are mean of three replications. The values bearing different alphabetic superscripts in a column differ significantly from each other; **P < 0.01

Cumulative IVGP (G) was higher in HBH. NGP on leguminous husks was more (Srinivas and Krishnamoorthy, 2005). t½ was more than 12 h in husks and even in shells. Among husks also t½ was much delayed in BGM and PPH significantly ($P< 0.01$) because of more lignin content. As per Srinivas and Swain (2009) the kinetic parameters t½ and c are related to the physical characteristics of feeds. Srinivas and Swain (2009) reported that initial rate of IVGP was higher in feedstuffs when carbohydrates to CP ratio were narrow i.e. 1:1 or 1:2. Fermentation in feedstuffs having carbohydrates to CP ratio of 7:1 may accelerate only after 12 h. Although AICPs are different than grains in type of carbohydrates, mean NGP of AICPs (269 mL g^{-1}) was comparable to grains (248 g mL^{-1}) but much higher than many edible oilseed (92 mL g^{-1}) meals (Singh and Srinivas, 2012; Mohanavel and Srinivas, 2016a). NDF complexity in shells was greater than husks because of lignification. Although limited part of shells was potentially fermentable, the solubility of fermentable part was rapid than husks. Similar was true for DORB. It was observed that in feedstuffs where there is more complexity

Table 3: ***In vitro*** **gas production (mL g^{-1} substrate) on agro-industrial co-products**

Feedstuff	Time interval of gas production (h)										Total gas (mL g^{-1})
	0.5	1	2	4	8	12	18	24	36	48	
Wheat bran	3.04^{bc}	8.10^{c}	17.98^{g}	54.24^{g}	121.73^{g}	40.56^{d}	36.28^{d}	25.27^{d}	38.14^{b}	4.58^{ab}	349.94^{e}
DORB	3.88^{bc}	4.41^{b}	11.37^{ef}	36.82^{e}	45.18^{e}	23.29^{c}	24.71^{c}	15.84^{c}	14.43^{a}	7.78^{b}	187.71^{c}
Green gram husk	1.12^{a}	2.77^{b}	0.81^{a}	5.55^{a}	27.07^{c}	25.33^{c}	29.35^{cd}	32.34^{e}	32.59^{b}	22.38^{c}	179.32^{c}
Bengal gram husk	0.83^{a}	0.29^{a}	3.63^{b}	6.73^{a}	12.40^{a}	19.14^{c}	34.97^{d}	58.79^{g}	78.03^{d}	81.25^{f}	296.06^{d}
Hyacinth bean husk	4.02^{c}	3.70^{b}	13.86^{f}	33.93^{e}	76.64^{f}	63.35^{f}	135.79^{g}	55.83^{g}	49.93^{c}	19.01^{c}	456.05^{h}
Pigeon pea husk	1.94^{ab}	2.73^{b}	11.38^{ef}	20.18^{d}	36.63^{d}	38.45^{d}	34.90^{d}	37.58^{e}	119.26^{e}	33.92^{e}	336.98^{e}
Soybean husk	0.46^{a}	9.02^{c}	9.89^{de}	44.97^{f}	31.98^{cd}	60.61^{f}	113.22^{f}	37.24^{e}	52.87^{c}	1.37^{a}	361.62^{f}
Hyacinth bean chuni	7.50^{d}	12.72^{d}	29.01^{h}	78.16^{h}	50.11^{e}	48.89^{e}	47.06^{e}	48.16^{f}	34.62^{b}	27.22^{d}	383.47^{g}
Groundnut shell	0.56^{a}	3.08^{b}	6.54^{c}	13.59^{c}	19.24^{b}	11.33^{b}	8.81^{b}	7.12^{b}	7.95^{a}	5.41^{ab}	83.63^{b}
Almond shell	3.39^{bc}	2.93^{b}	8.43^{cd}	10.19^{b}	8.52^{a}	2.93^{a}	1.74^{a}	0.44^{a}	11.08^{a}	4.60^{ab}	54.24^{a}
SEM	0.62**	0.78**	0.91**	1.18**	1.94**	2.25**	2.42**	1.94**	2.47**	1.55**	7.02**

Values bearing different alpha superscripts in a column differ significantly; **P < 0.01

Table 4: *In vitro* gas production kinetics on agro-industrial co-products

Feedstuffs	NGP (mL g^{-1})	Lag (h)	T½ (h)	k (10^{-2})	d (10^{-2})	ME# (M cal kg^{-1})	Fermentation rate (% h^{-1})	CO_2:CH_4
Wheat bran	347.33^{g}	0.17^{a}	8.54^{a}	8.16^{g}	16.32^{f}	2.84^{f}	10.96^{g}	72:28^{f}
Deoiled rice bran	185.85^{d}	0.18^{ab}	9.13^{ab}	7.65^{f}	15.29^{ef}	1.46^{c}	10.19^{f}	64:36^{d}
Green gram chuni	161.82^{c}	0.43^{d}	17.35^{d}	4.26^{c}	7.98^{b}	1.42^{c}	5.29^{c}	71:29^{c}
Hyacinth bean chuni	377.12^{h}	0.20^{ab}	10.18^{b}	6.85^{e}	13.70^{d}	3.01^{g}	9.01^{e}	70:30^{g}
Bengal gram husk	227.77^{e}	0.92^{e}	26.03^{f}	2.69^{a}	3.31^{a}	1.90^{d}	3.01^{a}	73:27^{e}
Hyacinth bean husk	432.46^{i}	0.28^{bc}	14.03^{c}	5.00^{d}	9.94^{c}	3.46^{h}	6.34^{d}	74:26^{g}
Pigeon pea husk	292.29^{f}	0.37^{cd}	21.48^{e}	3.26^{b}	7.61^{b}	2.40^{e}	4.09^{b}	74:26^{e}
Soybean husk	343.88^{g}	0.28^{bc}	13.91^{c}	5.00^{d}	10.01^{c}	2.77^{f}	6.34^{d}	68:32^{g}
Groundnut shell	82.59^{b}	0.19^{ab}	9.39^{ab}	7.52^{f}	15.06^{e}	0.84^{b}	10.01^{f}	66:34^{a}
Almond shell	53.71^{a}	0.19^{ab}	9.15^{ab}	7.66^{f}	15.32^{ef}	0.67^{a}	10.22^{f}	46:54^{a}
SEM	6.96**	0.03**	0.47**	2.07**	0.40**	0.05**	0.23**	2.91**

Values bearing different alphabetic superscripts in a column differ significantly; **P < 0.01
Estimated from NGP using empirical formula of Ozkul *et al.* (2011)

in nutrients due to complex nature in cell walls, the fermentation of free form of nutrients in such feedstuffs was faster (Srinivas and Gupta, 1994). A typical example is cottonseed meal in which initial fermentation was fast but extent of fermentation was less (Mohanavel and Srinivas, 2016a). Earlier IVGP studies on grains or OSM a correlation with *in vivo* microbial protein and autocorrelation with milk production (Singh and Srinivas, 2016; Mohanavel and Srtinivas, 2016b).

The t½ ranged from 8.54 h in WB to 26.03 h in PPH. One rate constant as suggested by Krishnamoorthy *et al.* (1991) appears to be incomplete to characterize the rate of fermentation. Fermentation of some feedstuffs was rapid in early part of incubation but in some other feedstuffs it accelerated subsequently. This is possibly due to the variation in degradation of nutrient network in the feedstuffs. Henceforth, bifurcating the rate constants as suggested by Thornley and France (2005) into rate of fermentation before and after t½ would improve fermentation characterization of feedstuffs. Fermentation rate of AICPs ranged from 3 to 11% h^{-1} compared to 5 to 11% h^{-1} in edible OSM (Mohanavel and Srinivas, 2016a) and 10 to 12.5% in grains (Singh and Srinivas, 2012).

Although fermentation rate of GSL and ASL were above 10% like WB, their extent of fermentation was 75 to 85% lesser than WB. Hence, fermentation rate alone represents fermentable part of feedstuffs. It hardly explains physical complexity of nutrients. Therefore, when feedstuffs are judged for fermentation value based on IVGP kinetics, it is necessary to consider both extent and rate of fermentation. The ratio between CO_2 and methane was a cross-check to the fermentation pattern of feedstuff i.e. slow or fast fermenting! Methane proportion would be higher in slow fermenting feedstuffs like DORB, GSL and ASL. Thus, CO_2 to methane ratio also could be considered for characterizing the fermentation value of feedstuffs. In any case, the ratio between both the gases may not be helpful in their quantification based on net gas production in a closed syringe owing to properties of gases as explained by basic and combined gas laws *viz*., Avagrado's, Boyle's, Charles' and Gay-Lussac's gas laws. Fermentation rate of AICPs ranged from 3 to 11% h^{-1} as compared to 5 to 11% h^{-1} in OSM and 10 to 12.5% in grains (Singh and Srinivas, 2012; Mohanavel and Srinivas, 2016a). IVGP kinetics was not comparable between similar types of AICPs; such as bran or husk or chuni or outer shell although their physical entity and chemical composition were comparable. The difference in fermentation kinetics of similar type of AICPs was basis for differentiation of their fermentation value and formulating DCR. Heterogeneity in fermentation kinetics between grains and OSM was not as conspicuous as in AICPs. The extent of fermentation of WB, SH and HBC were comparable even though they are different types of AICPs. Compared to Menke and Steingass (1988), Ozkul *et al.* (2011) have suggested the inclusion of ADF besides CP, EE and NFE in empirical formula to estimate ME from NGP. Inclusion of ADF in ME estimation in formula resulted in ME estimate ranging from 2.26 to 2.60 as compared to 0.67 to 3.01 Mcal kg^{-1} DM without ADF. Unlike other concentrate feedstuffs AICPs are rich in NDF, hence ME estimation using NGP requires revisit. Among all the AICPs, the fermentation kinetics of WB was superior, but the extent of fermentation was 20% more in HBH. Brans fermentation rate was higher ≥10% h^{-1} while gram chuni were fermented at a rate of 5 to 9% h^{-1}. Husk fermentation rate was slow (3-6% h^{-1}).

Conclusion: The significant variation in fermentation kinetics between brans, chunis, husk and shells indicated that CO_2 to methane ratio and the extent and rate of fermentation were critical attributes to differentiate the fermentation value of AICPs. Net gas production of AICPs was greater with higher hemicelluloses, hence NGP or hemicelluloses% of brans, chunis, husks or shells could be good criteria of FV for their choice in DCR.

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