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Nutrient composition, glucosinolate and vinyl-oxazolidine-thione profiling of Indian rapeseed (*Brassica juncea* Coss.) meal

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Presence of antinutritional factor(s) in any feedstuff impedes effective nutrient utilization by animals, besides causing specific adverse effects. This study includes eighteen different batches of Indian rapeseed (*Brassica juncea* Coss.) meal (RSM) sampled over a period of nine months for their evaluation in terms of nutrient composition, glucosinolate (GL) and vinyl-oxazolidine-thione (VOT) concentrations. Results showed that RSM contained a mean value (on dry basis) of crude protein (36.7%), ether extract (1.1%), ash (7.6%), crude fibre (10%), neutral detergent fibre (25%), acid detergent fibre (16.5%), hemicellulose (8.6%), total carbohydrates (54.7%), non-fibrous carbohydrates (29.7%), total digestible nutrients (77.9%) and metabolizable energy (11.7 MJ/kg). Furthermore, antinutritional factors like GL (μmol/g) and VOT (mg/g) are presented to be in the range of 36.2–72.2 (mean: 52) and 0–3.2 (mean: 1.0) respectively. Based on the species-wise tolerance limit for GL, the optimum inclusion level of RSM recommended for ruminants, pigs, rabbits, rats, poultry and fish is 15.6%, 1.5%, 13.5%, 1%, 10.4% and 6.9% respectively. Results of the present study are expected to maximize precision ration balancing for enhanced nutrient utilization whilst also curtailing any possible adverse effects of GL and VOT on production performance of animals.

Keywords: Antinutrients, glucosinolates, NIRS, protein feeding, rapeseed meal.

RAPESEED or Indian mustard (*Brassica juncea* Coss.) is a popular oilseed crop and the meal/cake obtained after oil extraction is a customarily used protein source for livestock feeding. Its continuous availability with high quality in conjunction with its advantages as value for money and poor human-edible potential have made rapeseed meal (RSM) an attractive and well established component in many formulated compound animal feeds. Nutritionally, RSM is characterized by high crude protein (CP: 35–40%) with much (~65%) of rumen degradable fraction[1,2], whilst its amino acid composition features a relatively rich methionine content[3,4]. On the other hand, RSM also

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presents sulphur containing plant secondary metabolites notably glucosinolates (GL; sinigrin, gluconapin, progoitrins, sinapine and glucobrassicanapine) and erucic acid that imparts pungent odour and bitter taste[1,3–5], thereby partly affecting dry matter (DM) intake. Besides this, enzymatic hydrolysis of GL either during processing or in the gut by myrosinase (thioglucosidase) releases goitrogenic compounds such as thiocyanates, isothiocyanates, nitriles and vinyl-oxazolidine-thione (VOT), which dictate the quality of RSM for animal feeding[6]. Nutritional valorization of RSM by means of water washing[7], heat treatment[8], solid-state fermentation[5], and supplementation of basal diet with iodine, copper, etc.[6] have been attempted to minimize the adversity of GL to livestock. Whereas, 'double zero' rapeseeds, often called canola (Canadian oil low in acid) meal containing <2% erucic acid and <30 μmol/g GL could safely constitute much higher proportion in animal diets[3,4] than conventional RSM. Furthermore, RSM of Indian origin is known to contain high GL than the temperate counterparts[6]. Nonetheless, literature documenting the concentration of GL and VOT of commercially traded RSM in India is largely limited hitherto, and at the same time, compelling evidence clues that there exists a considerable amount of variation in composition, energy value, amino acid profile and intestinal digestibility of RSM, for e.g., in China[9]. Consequently, it would also be pertinent to know the ideal inclusion level of RSM on the basis of species-wise GL tolerance to assist precision diet formulation under Indian context. In this endeavour, the present study was planned to screen GL and VOT concentrations in commercially traded RSM in India as well as to set an optimum dietary recommendations for diverse livestock species. Given the widespread popularity and usage of RSM in commercial formulations, understanding its quality becomes imperative for compound feed manufacturers in India.

The samples ($n = 18$) of RSM received at the R&D Centre (Animal Nutrition) of Indo Gulf Fertilisers (Jagdishpur, Amethi, UP, India) from May 2016 to January 2017 were included in the present study. Briefly, samples were sourced from the commercial feed manufacturing units located primarily at Varanasi and Kanpur, UP. The sampling procedure included drawing small proportion of samples from several bags (minimum 10) using sampling trier and composited into a single sample of approximately 500 g for subsequent laboratory evaluation. This procedure was repeated for every lot altogether constituting 18 samples in the aforementioned period of nine months.

The various quality parameters like CP, ether extract (EE), ash, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) as well as GL and VOT were estimated by near-infrared reflectance spectroscopy (NIRS™, DS 2500 F, FOSS, Hillerød, Denmark enabled with Trouw Nutrition Mosaic Network). Measurement of unground sample (150 g) was done using large sample cup at 25°C. Before every measurement, instrument diagnostics for hardware was done. Also, performance tests were run and subsequently confirmed by calibration check cell. In addition, the following calculations were made[10–12]

Hemicellulose (%) = NDF (%) – ADF (%)

[10]Total carbohydrates (CHO, %) = 100 – ash (%) – CP (%) – EE (%)

[10]Non-fibrous CHO (NFC, %) = CHO (%) – NDF (%)

Nitrogen free extractives (NFE, %) = CHO (%) – CF (%)

[11]Total digestible nutrients (TDN, %) = 0.9918 × CP (%) + 1.272 × EE (%) + 0.0318 × CF (%) + 0.8904 × NFE (%)

[12]Metabolizable energy (ME, MJ/kg) = TDN (%) × 0.15.

Descriptive statistical tools of MS-Office Excel software were applied, and the data on each parameter was averaged and presented with standard error (SE).

Table 1 illustrates detailed chemical composition, GL and VOT profiles in 18 samples of RSM. The range of CP (34.8–39.2%), EE (0.1–1.8%) and CF (9.2–10.6%) observed across samples is in close concordance with BIS[13] specifications for high fat RSM, the corresponding values being 35%, 8% and 9% for CP, EE and CF respectively. Also, the ash content (7.6%) observed lies within the maximum limit of 8% (ref. 13). Indeed, the results indicate that the RSM traded in Indian market complies with specifications, thus assuring quality, as analysed over a period of nine months. This broadly corroborates with many previously published results for RSM[3–5,8,14–16]. The ME content calculated using prediction equation[11] in the present study (11.7 MJ/kg) is slightly higher than 10.4 MJ/kg reported previously with *in vitro* Hohenheim gas test[14], whereas it is lower than 13.6 MJ/kg (ref. 16), as obtained by employing the NRC[10] prediction model. The concentration of GL (μmol/g) is in the range of 36.6–72.2 (mean: 52), while that of VOT (mg/g) is in the range of 0–3.2 (mean: 1.0). In this aspect, the literature data assembled by Tripathi and Mishra[6] found a wide range of GL content of 42.6 to 186 μmol/g in Indian RSM. Similarly, Tayo *et al.*[3] reported 150–240 μmol/g of GL in Indian rapeseed-mustard varieties, albeit the present findings concur closely to 55 μmol/g as reported by Tyagi[7] for Indian mustard cake. The level of VOT in the 18 market samples of RSM averaged to be 1 mg/g, which is quite low for regular RSM, as double zero meals (canola) generally contain such small levels[6]. This might be due, in part, to the differences in estimation procedures and/or relatively small sample size considered in the present study.

Table 1. Chemical composition (%), metabolizable energy (MJ/kg), glucosinolate (μmol/g) and VOT (mg/g) contents in Indian rapeseed meal

Sample no.	CP	EE	Ash	CF	NDF	ADF	HC	CHO	NFC	TDN	ME	GL	VOT
1	36.7	1.7	7.7	9.5	26.7	16.2	10.5	53.9	27.2	78.4	11.8	61.4	0.5
2	37.8	0.1	8.3	9.3	31.1	16.9	14.2	53.9	22.8	77.5	11.6	71.6	0.4
3	36.2	1.5	7.4	10.2	25.4	16.0	9.4	54.9	29.5	77.9	11.7	44.1	0
4	36.7	1.2	7.7	10.4	28.0	16.6	11.4	54.4	26.4	77.4	11.6	45.7	0.5
5	36.0	1.4	7.3	10.6	23.9	16.4	7.5	55.3	31.4	77.6	11.6	49.3	0.1
6	36.0	1.1	7.6	10.0	23.4	16.7	6.7	55.3	31.9	77.7	11.7	57.2	1.3
7	36.2	1.0	7.3	9.4	22.3	16.4	5.9	55.5	33.2	78.5	11.8	70.8	1.9
8	35.9	1.2	7.2	9.7	23.9	16.4	7.5	55.7	31.8	78.4	11.8	65.3	1.2
9	35.5	1.0	7.4	10.3	25.1	16.4	8.7	56.1	31.0	77.6	11.6	43.7	1.6
10	34.8	1.0	7.1	10.0	26.8	16.6	10.2	57.1	30.3	78.0	11.7	37.9	0.6
11	35.6	1.1	7.4	10.1	25.4	16.7	8.7	55.9	30.5	77.8	11.7	43.5	1.6
12	37.2	0.1	8.1	9.2	24.0	17.0	7.0	54.7	30.7	77.7	11.7	72.2	1.3
13	35.7	1.3	7.2	10.2	23.8	16.3	7.5	55.8	32.0	78.0	11.7	43.2	1.8
14	36.7	1.3	7.4	10.3	23.8	16.9	6.9	54.6	30.8	77.8	11.7	45.1	1.1
15	35.9	0.1	8.4	9.6	25.4	17.1	8.3	55.7	30.3	76.9	11.5	69.9	3.2
16	38.9	1.8	7.4	10.3	23.3	15.5	7.8	51.9	28.6	78.2	11.7	37.3	0.2
17	39.2	1.7	7.2	10.1	23.2	15.8	7.4	51.9	28.7	78.6	11.8	36.2	0
18	38.9	1.5	7.9	10.0	25.0	16.4	8.6	51.7	26.7	77.9	11.7	41.4	0.1
Mean	36.7	1.1	7.6	10.0	25.0	16.5	8.6	54.7	29.7	77.9	11.7	52.0	1.0
SE	0.30	0.53	0.09	0.10	0.50	0.10	0.47	0.36	0.60	0.10	0.01	3.08	0.20

CP, crude protein; EE, ether extract; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; HC, hemicellulose; CHO, total carbohydrates; NFC, non-fibrous carbohydrates; TDN, total digestible nutrients; ME, metabolizable energy; GL, glucosinolates; VOT, vinyl-oxazolidine-thione.

Table 2. Recommendations for optimum inclusion of Indian rapeseed meal (RSM) for various species of livestock and fish based on glucosinolate (GL) profile

Species	Tolerance limit (μmol/g diet)[a]	Optimum inclusion (%)[b]	Range (%)[c]
Ruminants[d]	1.5–4.2	15.6	13–20
Pigs	0.78	1.5	1.1–2.2
Rabbits	7.0	13.5	9.5–19.5
Rats	0.5	1.0	0.7–1.4
Poultry	5.4	10.4	7.5–15
Fish	3.6	6.9	5–10

[a]According to Tripathi and Mishra[6]; [b]Based on mean GL of 52 μmol/g; [c]Based on range of GL detected (36.2–72.2 μmol/g); [d]Assuming concentrate intake at 0.3 of total dry matter intake, and for all other animals complete diet was considered when RSM constituted the sole source of dietary GL.

The GL tolerance[6] was used to arrive at optimum level of RSM that can be included in the diet without exhibiting any deleterious effect on nutrient utilization and performance. It was observed that optimum level of RSM for ruminants, pigs, rabbits, rats, poultry and fish was 15.6%, 1.5%, 13.5%, 1%, 10.4% and 6.9% respectively (Table 2). In high yielding dairy cows (milk: 47 kg/day), Swanepoel *et al.*[17] incorporated low GL containing RSM (canola meal) up to 17% in the total mixed ration. Although the present results recommend a safe level of up to 20% for ruminants, earlier researchers in India have used almost double the level, for instance, in buffaloes up to 39% (ref. 18) and small ruminants up to 36% (ref. 19) with no discernable compromise in performance. This reiterates that ruminants can tolerate greater levels of GL in RSM when fed balanced diet adequate in energy, protein and micronutrients. Pigs are found to be more sensitive than other species for GL leading to the lowest level of RSM recommendation of only 2% amongst all species. Nevertheless, considering GL tolerance level of 2.5 μmol/g (ref. 1), RSM inclusion could be increased up to 7%. In the case of finishing pigs, Torres-Pitarch *et al.*[20] used RSM of low GL (isothiocyanate: 1469 mg/kg) up to 20% satisfactorily with small but significant ($P < 0.05$) compromise in the digestibility of organic matter and CF compared with 12% soybean meal (SBM)-based diets. On the contrary, Schöne *et al.*[21] replaced SBM by 7.5% RSM in pig diets. In line with our findings on rabbits, Gasmi-Boubaker *et al.*[22] found 14% (DM basis) as optimum level of RSM to substitute SBM in crossbred rabbit diet. In rodent species like rats, a controlled experiment involving standard GL supplementation revealed a level of 0.5 μmol/g GL as the upper limit[23], because higher levels resulted in negative curvilinear reduction ($P < 0.01$) in intake, growth and thyroid hormone status[24]. In regards to poultry species, Jayanthi *et al.*[15] advocated 15% RSM to be optimum for commercial broiler chickens without compromising zoo-technical, haematological aspects and carcass characteristics. Moreover, brown shelled laying hens, when fed diets with 12–24% RSM could result in undesirable fishy egg taints[25]. These data support our recommendation of maximum 15% RSM for poultry. Aquaculture nutrition heavily relies upon fish meal as quality conventional protein source, and vegetable proteins like SBM, RSM, etc. are also under continuous evaluation. Apart from GL, high fibre in RSM may also possibly limit its use in carnivorous fish. Similar to the present outcome of 5–10%, prior researchers established 5–12.5% RSM as ideal level for piscine diets[26,27].

It is apparent from the above discussion that the extent of inclusion of RSM is equivocal in the literature and the observed discrepancies might have occurred, due to differences in the processing techniques as well as genotypes of *Brassica* sp. (high GL versus low), across different studies[1,3].

The screening of market samples of Indian RSM over nine months revealed an average concentration of GL and VOT of 52 μmol/g and 1 mg/g respectively. Furthermore, fixing the recommended optimum inclusion of RSM based on the actual GL profile in the practical ration formulation software would be expected to aid precision diet formulation for various categories of livestock and aquaculture.

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