



Effect of supplementing canola quality rapeseed mustard cake on the metabolic profile and quality of milk in crossbred cows

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ABSTRACT

This study examined the comparative effect of incorporation of improved canola quality and high glucosinolates rapeseed mustard oil cake (RMC) in the supplement of crossbred cows on metabolic profile, thyroid hormones, milk yield and quality of milk. Lactating crossbred cows (18) were randomly divided into three groups of six each in a completely randomized design and allocated in three dietary supplements, viz. SBM, RMC and CM containing soybean meal (SBM), rapeseed mustard cake (RMC) and canola meal (CM), respectively as a major protein source for a period of 90 days. Daily intake of total dry matter and green fodder was comparable irrespective of dietary treatments. The supplement intake (kg/d) was significantly lower in the cows that were given RMC supplement compared to those given either SBM or CM supplement. Incorporation of CM in the supplement significantly increased daily and total milk yield (kg) as compared to RMC group. The milk thiocyanate level was significantly higher in RMC followed by CM and SBM groups. Blood-biochemical profile except significantly higher lactate dehydrogenase in RMC group remained comparable at different periods. Serum thiocyanate was significantly higher in RMC followed by CM and SBM. Serum T₃ and T₄ hormone levels were significantly lower in RMC fed group as compared to CM and SBM groups. It can be concluded that feeding of canola quality RMC (GSC5/6) may be used as complete replacement of soybean meal without any adverse effect on metabolic profile, thyroid hormones, milk quality and yield in crossbred cows.

Key words: Canola meal, Crossbred cows, Milk thiocyanate, Milk yield, Rapeseed mustard cake

The rapeseed mustard oil cake (RMC) can be readily used as a protein supplement in ruminant's diet due to its easy availability and low price compared to other oil cakes, having a high crude protein ranging from 34–38%, with a good balance of essential amino acid. However, Rapeseed–mustard cultivars prevalent in India have high erucic acid (43–57%) and high level of glucosinolates (GLS; 150–240 µmole/g) in seed meal. These are two nutritionally toxic undesirable factors present in RMC that limit its potential use as a protein supplement in ruminants. Although single or double low (00) varieties (low erucic acid and low GLS) of rapeseed have been developed in Canada, Europe, and Australia, these varieties could not be introduced in India due to their poor production performance under Indian agronomic conditions (Tripathi and Misra 2007). On the other hand, India is forced to reduce the erucic acid up to 2% and GLS content in seed meal up to 30 µmol/g to meet international accepted norms under WTO agreement. Canola meal may be used as an alternative to conventional protein supplements without any adverse effect (Paula *et al.* 2017) on health and production performance of

animals. Keeping this in view, it was proposed to investigate the effects of feeding canola meal and high glucosinolates RMC on metabolic profile, thyroid hormones, milk yield and quality of milk in crossbred cows.

MATERIALS AND METHODS

Experimental design and feeding: The experiment was carried out at the Livestock Production and Management Farm of the ICAR-Indian Veterinary Research Institute, Izatnagar. Eighteen crossbred (*Bos taurus* × *Bos indicus*) milch cows in their third and middle stage of lactation with a mean body weight of 401±19.7 kg and average milk yield of 7.6±0.4 kg/d were randomly divided into three treatments of six each in a completely randomized design. The cows were randomly allocated into three dietary treatments, viz. SBM, RMC and CM containing soybean meal, rapeseed mustard cake and canola meal, respectively as a major protein source. All the cows had a common managerial exposure prior to the experiment. The amount of supplements was adjusted at fortnight intervals as per the milk yield of each cow.

All the experimental animals were penned individually in ventilated sheds with free access to fresh water and allowed exercise outdoors in an adjacent dry paddock daily. All the animals were adapted to their respective diets for 15

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days. During the experiment, the animals were offered a weighed quantity of respective test concentrate mixture as two equal meals in the morning at 7:00 AM and in the afternoon at 4:30 PM during the time of milking. The cows were offered green fodder [2:1 ratio of berseem (*Trifolium alexandrinum*) and oat (*Avena sativa*)] *ad lib.* along with required amount of iso-nitrogenous SBM, RMC and CM supplements to meet their nutrient requirements for maintenance and milk production as per Kearl (1982) for a period of 90 days. Daily dry matter (DM) intake and fortnightly body weights of all the cows were recorded before feeding in the morning. Daily milk yield of individual cows were recorded while milk sample from each cow was drawn once a week and stored at 4°C after adding 2–3 drops of potassium dichromate as a preservative until further analysis.

Chemical analyses: Feed samples offered, refusals, faeces voided and urine excreted were processed and analyzed for OM, CP, ether extract, NDF and ADF (AOAC 2000). The glucosinolates content of RMC and canola meal was analysed using HPLC following the desulphoglucosinolates method (Sang and Truscott 1984). Milk samples were analysed for total protein and fat content. Thiocyanate (SCN⁻) concentration of milk and serum samples was estimated by method of Bowler *et al.* (1944) as described for serum samples. Haemoglobin (Hb) and packed cell volume (PCV) were estimated in whole blood immediately after blood collection by cyanomethaemoglobin method (Dacie and Lewis 1969) and Wintrobe's tube, respectively. Serum glucose concentration was determined colorimetrically (Barham and Trinder 1972). The serum protein and albumin content were measured as per Vatzidis (1977) and Doumas *et al.* (1971), respectively. Serum urea was estimated by the method of Hallett and Cook (1971). Total cholesterol and activity of serum enzymes, viz. alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated by using diagnostic kits. Serum T₃ and T₄ hormones were estimated using diagnostic ELISA kits.

Statistical analyses: The data obtained were analyzed by using one way ANOVA (Snedecor and Cochran 1989) and post hoc tests through statistical package SPSS 20.0. The periodic alterations in metabolic profile and milk yield were analyzed by using repeated measures design using GLM procedure. Significance of treatments with respect to different characters was declared at ≤ 0.05 unless otherwise stated.

RESULTS AND DISCUSSION

Chemical composition of feeds: The supplements were iso-nitrogenous and comparable in terms of OM, total ash, NDF and ADF. The EE content of SBM was significantly ($P < 0.05$) lower as compared to RMC and CM supplements. The higher EE level in RMC and CM may be attributed to higher EE content of expeller pressed rapeseed mustard cake and canola meal used in this experiment (Liu *et al.* 1994, Palanivel 2008). The GLS content were 149.5 and 16.62

Table 1. Chemical composition of feed (% DM basis)

Attribute	Supplement			Green oats	Berseem
	SBM*	RMC**	CM [#]		
Organic matter	90.77	91.64	90.37	90.93	88.01
Crude protein	18.14	18.45	18.53	8.93	16.96
Ether extract	1.40	4.14	3.08	2.93	2.10
Total ash	9.23	8.36	9.63	9.07	12.00
NDF	52.47	48.00	49.14	67.09	54.13
ADF	11.97	13.95	13.75	39.96	38.79
Glucosinolates ($\mu\text{mol/g DM}$)	0.00	38.12	3.91	0.00	0.00

*SBM (kg/100 kg): Maize, 25.0; deoiled SBM, 17.0; wheat bran, 55.0; mineral mixture, 2.0; salt, 1.0. **RMC (kg/100 kg): Maize, 25.0; RMC, 25.0; wheat bran, 46.5; mineral mixture, 2.0; salt, 1.0. [#]CM (kg/100 kg): Maize, 25.0; canola meal, 23.5; wheat bran, 48.5; mineral mixture, 2.0 and salt, 1.0.

$\mu\text{mol/g DM}$ in RMC and CM, respectively. However, GLS content in RMC and CM concentrates were 38.1 and 3.9 $\mu\text{mol/g DM}$, respectively.

Dry matter intake: Inclusion of RMC significantly ($P < 0.05$) reduced the concentrate intake (kg/d) in milch cows relative to their counterparts in SBM and CM groups. GLS cause pungent odour due to the formation of volatile and pungent compounds after their hydrolysis, and a bitter taste that may contribute to lower feed intake when high GLS containing RMC is incorporated in the ration of ruminants (Hill 1991, Papas *et al.* 1979). Similar to present findings, Sharma *et al.* (2007) and Ravichandiran *et al.* (2008) also reported decreased concentrate intake of high GLS rapeseed meal relative to SBM in crossbred calves and heifers. In the present study, palatability of RMC concentrate was relatively poor (14% lower) and cows spent more time to eat the RMC supplement, which might be due higher GLS intake in RMC (1662 vs 193 $\mu\text{mole g/kg W}^{0.75}$) as compared to CM. Intake of green and total DM (kg/d) was parallel ($P > 0.05$) among the treatment groups.

Milk yield and quality: Incorporation of canola meal (CM) in the supplement of crossbred cows significantly ($P < 0.05$) increased daily and total milk yield (kg), fat and protein yield (g/d) as compared to RMC group, however, cows given SBM supplement had an intermediary position between RMC and CM. The daily milk yield by crossbred cows is depicted in Fig. 1. The results of present study were in accordance with the earlier workers, who compared the effects of feeding canola quality meal either with soybean meal or cottonseed meal and reported higher average milk yield (4% FCM) in canola ration than the control (Sharma *et al.* 1977, MacLean and Laarveld 1991). However, Hill (1991) reported decline in milk yield in cows fed RMC with higher GLS. The lower milk yield in RMC group may be credited to comparatively lower intake of concentrate moiety; however, higher daily yield of fat and protein in CM group may be attributed to higher milk yield in CM than RMC.

Titrate acidity value of raw milk was comparable

Table 2. Intake and milk yield and quality of milk in lactating cows

Attribute	Supplement			SEM	P value
	SBM	RMC	CM		
<i>Body weight (kg)</i>					
Initial	399.67	406.83	396.5	19.66	0.981
Final	434.17	441.5	436.00	19.55	0.970
<i>Intake</i>					
Total dry matter (kg/d)	10.73	10.35	11.12	0.23	0.411
Dry matter (% BW)	2.67	2.57	2.81	0.11	0.698
Concentrate (kg/d)	4.46 ^b	3.96 ^a	4.49 ^b	0.10	0.040
Green fodder (kg/d)	6.27	6.39	6.62	0.19	0.780
<i>Lactation performance</i>					
Total milk yield (kg)	607.08 ^{ab}	557.39 ^a	629.83 ^b	13.43	0.030
Milk yield (kg/d)	6.75 ^{ab}	6.19 ^a	6.99 ^b	0.15	0.030
Fat yield (g/d)	301.03 ^{ab}	276.68 ^a	312.31 ^b	6.62	0.030
Protein yield (g/d)	234.63 ^{ab}	214.55 ^a	243.22 ^b	5.08	0.021
Titration acidity	0.14	0.14	0.14	0.006	0.381
Organoleptic evaluation score*	87.74	87.12	87.73	0.28	0.652

*Based on score of 0–100. ^{ab}Mean values bearing different superscripts within a row differ significantly.

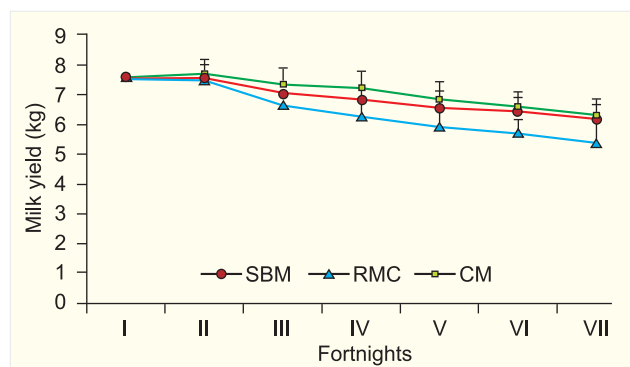


Fig. 1. Effect of feeding canola meal on milk yield by crossbred cows.

irrespective of treatments and remained within the normal range. Generally, titration acidity is a rapid test to access the quality of raw milk and progressive increase in titration acidity in milk is associated with increased storage time. The organoleptic tests of milk from cows did not reveal any significant score difference irrespective of dietary treatments. Earlier observations showed that the undesirable oxidized flavour of milk was decreased in cows given RMC in comparison to those given soybean meal or linseed meal (Astrup 1983). CM being low GLS content did not impart any off-flavors on sensory test of milk.

Milk thiocyanate (SCN): The mean thiocyanate level of milk was significantly ($P < 0.01$) higher in RMC followed by CM and SBM groups. Though the level of milk thiocyanate was increased significantly ($P < 0.01$) in RMC and CM groups with the advancement of feeding trail but the effect was more prominent in RMC as compared to CM group (Fig. 2). The level of milk SCN⁻ (12.55 to 15.01 µg/

ml) in RMC fed milch cows was comparable to the values reported by Papas *et al.* (1979) in crossbred cows fed with RMC cake (26%). Thiocyanate, one of the end products of GLS hydrolysis in GI tract of cows fed with RMC, get absorbed into the blood and transferred to the milk and its concentration in milk was directly related to dietary GLS (Papas *et al.* 1979, Vincent and Hill 1988).

Metabolic profile: The levels of Hb, PCV, serum glucose, total proteins, albumin and globulin and serum

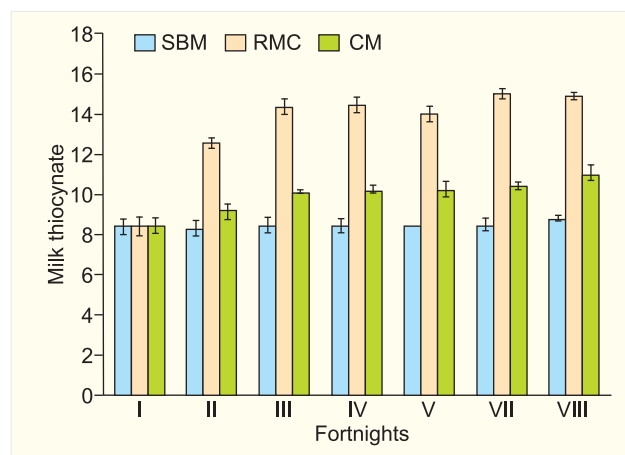


Fig. 2. Effect of feeding canola meal on milk thiocyanate level (µg/ml) in cows.

urea, cholesterol remained comparable under different treatments (Table 3) and within the normal physiological range for cattle (Kaneko *et al.* 1997, Radostits *et al.* 2003). The activity of serum enzymes, viz. ALT, AST and ALP (IU/ml) in cows did not differ ($P > 0.05$) irrespective of dietary treatments, however, LDH level was higher ($P < 0.05$) in RMC group than CM and SBM. Though the LDH activity in this study were within the normal physiological range of 123–445 IU/l reported in ruminants (Radostits *et al.* 2003, More *et al.* 2006). It indicates that the levels and duration of exposure to dietary GLS from CM did not have adverse

Table 3. Metabolic profile of lactating cows

Attribute	Supplement			SEM
	SBM	RMC	CM	
Haemoglobin (g/dl)	10.29	10.26	10.22	0.89
Packed cell volume (%)	41.61	41.13	41.55	0.19
Glucose (mg/dl)	46.49	45.41	45.87	0.99
Urea (mg/dl)	48.51	48.78	48.39	0.18
Cholesterol (mg/dl)	163.08	164.01	163.32	0.30
Total protein (g/dl)	7.18	7.01	7.18	0.07
Albumin (g/dl)	4.06	3.90	4.09	0.10
Globulin (g/dl)	3.25	3.29	3.25	0.10
A:G ratio (g/dl)	1.27	1.22	1.31	0.07
AST (IU/l)	91.69	92.15	91.75	0.17
ALT (IU/l)	35.13	35.77	35.22	0.24
LDH (IU/l)	552.83 ^a	560.76 ^b	553.25 ^a	0.96

^{ab}Mean values bearing different superscripts within a row differ significantly ($P < 0.05$).

effect on physiological functions of liver, heart or skeletal muscles. However, higher level of LDH in RMC fed group indicates slight adverse effect on physiological functions of target organs, which may be attributed to higher GLS intake by milch cows.

Serum thiocyanate (SCN): Mean serum thiocyanate was significantly ($P < 0.01$) higher in RMC followed by CM and SBM groups. Though thiocyanate level after 90 day feeding was increased significantly ($P < 0.01$) in RMC and CM groups but effect was more pronounced in RMC as compared to CM, which may be due to higher consumption of GLS in RMC group. The level of serum thiocyanate remained unaffected in SBM group. The thiocyanate level is depicted in Fig. 3. The GLS degradation in rumen by myrosinase enzyme liberates SCN^- , which is absorbed into the circulation and detected in blood. Palanivel (2008) reported higher SCN^- in blood of RMC fed milch cows ($14.9 \mu\text{g/ml SCN}^-$). The lower serum SCN^- level of CM as compared to that of RMC may be a dose dependent response of dietary GLS, which was in agreement with the reports from previous experiments with different animal species (Schone *et al.* 1990, Tripathi *et al.* 2001).

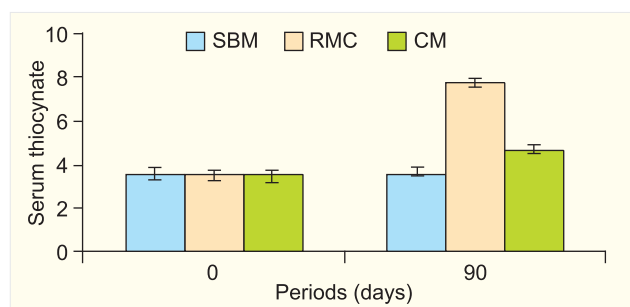


Fig. 3. Effect of feeding canola meal on serum thiocyanate level ($\mu\text{g/ml}$).

Serum T_3 and T_4 hormones: The mean serum levels of T_3 and T_4 were significantly ($P < 0.05$) lower in RMC fed group as compared to their counterparts in CM and SBM groups (Fig. 4). The periods and treatment interaction was also significant ($P < 0.05$). The levels of T_3 and T_4 decreased significantly ($P < 0.05$) at 90 d in RMC as compared to CM and SBM groups. The levels of T_3 and T_4 were within the reported range of 0.7–2.2 and 54.05–110.68 nmol/l, respectively (Kaneko *et al.* 1997, Radostits *et al.* 2003). Similarly, the values of present study were comparable to reports of Papas *et al.* (1979) for milch cows fed with Tower or 1821 RSM. The levels of T_3 and T_4 were 2.35 to 2.53 and 60.49 to 63.06 nmol/l, respectively. Generally, goitrogenicity caused by dietary inclusion of RMC in adult ruminants vary with level of RMC inclusion, GLS and iodine content of the diet, fermentation process and age of the process in adult ruminants described by French workers has been shown to lower the goitrogenicity of rapeseed products (Geavy and Beranger 1975). Similarly, adult animals are more efficient in detoxification of GLS breakdown products not only by hepatic pathway but also as a result of rumen activity as compared to young ruminants

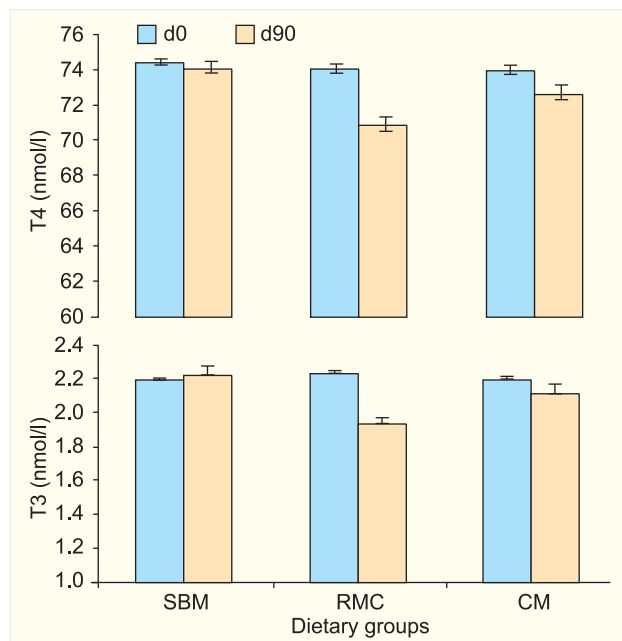


Fig. 4. Effect of feeding canola meal on serum T_3 and T_4 hormones in lactating cows.

(Hill 1991). In the present study inclusion of canola meal in the concentrate mixture at 23.5% level did not exert any adverse effect on T_3 and T_4 hormones in cows.

Based on the present results, it can be concluded that feeding of canola quality RMC such as GSC 5/6 may be used as a complete replacement of soybean meal without any adverse effect on metabolic profile, thyroid hormones, milk yield and quality of milk in crossbred cows. However, traditional genotype of RMC (GLS: $149.5 \mu\text{mol/g}$) significantly reduces the palatability of concentrate and lactation performance of cows.

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REFERENCES

- AOAC. 2000. *Official Methods of Analysis*. 18th ed. Association of Official Analytical Chemists, WA, US.
- Astrup H N. 1983. Improvement in milk taste obtained with rapeseed products. Proceedings of 6th International Rapeseed Congress & General Assembly, May 17–19, Paris, France. pp. 1622–1627.
- Barham D and Trinder P. 1972. An improved color reagent for the determination of blood glucose by the oxidase system. *Analysts* **97**: 142–45.
- Bowler R G. 1944. The determination of thiocyanate in blood serum. *Journal of Biochemistry* **38**: 385–88.
- Dacie Z and Lewis S. 1969. *Practical Hematology*. 4th edn. J and A Churchill, London, UK.
- Doumas V T, Watson W A and Biggs H G. 1971. Albumin standards and the measurement of serum albumin with Bromocresol green. *Clinica Chimica Acta* **31**: 87–96.
- Geavy Y and Beranger C A. 1975. Rapeseed meal in animal

- feeding comparison of oil meal of toasted or fermented rapeseed, soyabean, oil meal and groundnut oil meal for fattening bull. *Annales De Zootechnie* **4**: 209–15.
- Hallett C J and Cook J G H. 1971. Produced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. *Clinica Chimica Acta* **35**: 33–37.
- Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–3597.
- Hill R. 1991. Rapeseed meal in the diets of ruminants—A review. *Nutrition Abstract Review* **B61**: 139–55.
- Kaneko J J, Harvey J W and Bruss M L. 1997. *Clinical Biochemistry of Domestic Animals*. 5th edn. Academic Press, San Diego, CA, US.
- Kearl L C. 1982. Multipurpose cattle, pp 71–81. *Nutrient Requirements of Ruminants in Developing Countries*. (Ed) Kearl L C. International Feedstuffs Institute, Utah Agricultural Experimental Station, Utah State University, Logan, UT, US.
- Liu Y G, Steg A, Smits B and Taminga S. 1994. Crambe-meal: Removal of glucosinolates by heating with or without additive and water extraction. *Animal Feed Science and Technology* **48**: 273–87.
- McClean C and Laarveld B. 1991. Effect of somatotropin and protein supplement on thyroid function of dairy cattle. *Canadian Journal of Animal Science* **71**: 1053–61.
- More T. 2006. *Animal Clinical Biochemistry*. Kalyani Publishers, Ludhiana, India.
- Palanivel M. 2008. Effect of feeding high glucosinolates rapeseed–mustard cake on the performance of ruminants and quality assurance of meat and milk. PhD Thesis, IVRI, Izatnagar, Uttar Pradesh, India.
- Papas A, Ingalls J R and Campbell L D. 1979. Studies on the effects of rapeseed meal on thyroid status of cattle, glucosinolate and iodine content of milk and other parameters. *Journal of Nutrition* **109**: 1129–39.
- Paula E M, Monteiro H F, Silva L G, Benedeti P D B, Daniel J L P, Shenkoru T, Broderick G A and Faciola A P. 2017. Effects of replacing soybean meal with canola meal differing in rumen-undegradable protein content on ruminal fermentation and gas production kinetics using 2 *in vitro* systems. *Journal of Dairy Science* **100**(7): 5281–92.
- Radostits O M, Gay C C, Blood D C and Hinchcliff K W. 2003. *Veterinary Medicine—A Textbook of the Disease of Cattle, Sheep, Pig, Goats and Horse*. 9th edn. W B Saunders, St. Louis, MO, US.
- Ravichandiran S, Sharma K, Dutta N, Pattanaik A K, Chauhan J S and Agnihotri A. 2008. Comparative assessment of soybean meal with high and low glucosinolate rapeseed–mustard cake as protein supplement on performance of growing crossbred calves. *Journal of the Science of Food and Agriculture* **88**: 832–38.
- Sang J P and Truscott R J W. 1984. Liquid chromatographic determination of glucosinolates in rapeseed as desulphoglucosinolates. *Journal of the Association of Official Analytical Chemists* **67**: 829–33.
- Schone F, Ludke H, Winnerfeld K, Kirchner E, Grun M, Ludke H and Henning A. 1990. Copper and iodine in pig diets with HGRSM. Part III. Treatment of rapeseed meal with copper and the effect of iodine supplementation on trace element status and some related blood parameters. *Animal Feed Science and Technology* **57**: 653–62.
- Sharma H R, Ingalls J R and Mckirdy J A. 1977. Effects of feeding a high level of tower rapeseed meal in dairy rations on feed intake and milk production. *Canadian Journal of Animal Science* **57**: 653–62.
- Sharma K, Dutta N, Patra A K, Singh M, Pattanaik A K, Ravichandiran S, Chauhan J S, Agnihotri A and Kumar A. 2007. Effect of replacing soybean meal with Indian canola quality or high glucosinolate rapeseed–mustard meal on performance of growing crossbred calves. *Livestock Research for Rural Development*. Volume 19, Article #142.
- Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. East West Press Pvt. Ltd., New Delhi, India.
- Song M K and Kennelly J J. 1989. Effect of ammoniated barley silage on ruminal fermentation, nitrogen supply to the small intestine, ruminal and whole tract digestion, and milk production of Holstein cows. *Journal of Dairy Science* **72**: 2981–90.
- Tripathi M K and Mishra A S. 2007. Glucosinolates in Animal Nutrition: a review. *Animal Feed Science and Technology* **132**: 1–27.
- Tripathi M K, Tyagi A K and Karim S A. 2001. Performance of weaning lambs maintained on mustard and groundnut meal based diets. *Indian Journal of Animal Sciences* **39**: 261–67.
- Vatzidis H. 1977. An improved biuret reagent. *Clinical Chemistry* **23**: 908.
- Vincent I C and Hill R. 1988. Low glucosinolate rapeseed meal as a protein source for milk production. *Animal Production* **46**: 505.