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Effect of feeding rapeseed-mustard cake during pregnancy on the performance of ewes

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ABSTRACT

This study examined the effect of feeding high glucosinolate rapeseed-mustard cake (RMC) on the performance of ewes during pregnancy. Sixteen Muzaffarnagari ewes (28.9± 1.1 kg) were teased twice daily for oestrus detection and bred by single ram during standing oestrus. Ewes returning to heat again in the subsequent cycle were re-bred. Confirmed pregnant ewes (12) were divided randomly into 2 equal groups and allocated to 1 of the dietary treatment GNC or RMC, concentrate mixture containing groundnut cake (GNC) or RMC as major protein source was fed to respective animal group. The glucosinolates (Gls) content in RMC supplement was 30.75µmol/g. All the ewes were offered wheat straw *ad lib.* as basal roughage along with required amount of respective concentrate mixtures to meet their nutrients requirement for maintenance and pregnancy. Two metabolism trials were conducted, during early and late gestation. Supplementation of RMC to wheat straw based diet reduced the total DMI by gravid ewes but digestibility coefficient of various nutrients was comparable between both treatments. The digestible nutrient intake and nutritive value of diets during late pregnancy was reduced in RMC group. Net retention of N (g/d), per cent of intake or per cent of absorbed N was significantly lower in RMC fed gravid ewes during early and late gestation. All the gravid ewes gained weight throughout gestation period, however, gross gain after 21 weeks and net gain was lower in RMC fed animals. Blood-biochemical attributes (haemoglobin, PCV, glucose, serum proteins, urea, calcium and phosphorus, AST and ALT levels) did not differ significantly between RMC and GNC fed animals. Serum T_3 (triiodothyronine) did not differ significantly irrespective of dietary treatments, while, concentration of T_4 (tetra iodothyronine) hormone was significantly lower in RMC fed animals. It may be concluded that the feeding of RMC (41% in concentrate mixture) significantly reduced the palatability of concentrate, gross gain after 21 weeks of pregnancy, net gain and thyroxine concentration in blood of gravid ewes.

Key words: Ewes pregnancy, Glucosinolates, Performance, Rapeseed-mustard cake

Rapeseed-mustard cake (RMC) is one of the cheapest protein supplement traditionally used for feeding ruminants in India (Saha and Singhal 1993). RMC is a major by-product following the oil extraction from rapeseed-mustard seeds and it contains 34 to 38% crude protein (Bell 1984). Rapeseed-mustard cultivars prevalent in India have high erucic acid and high level of glucosinolates in seed meal (TERI 2003), which are nutritionally undesirable. However, the major limiting factor affecting efficient utilization of RMC in the animal diets is glucosinolates (Gls), a natural thioglucosides found in high amount (63–120 µmol/g DM) in Indian varieties (*Brassica juncea*) of RMC (Tyagi *et al*. 1997). Though, the Gls present in RMC are biologically inactive; they are hydrolyzed by endogenous enzyme, myrosinase (thioglucosidase, E.C 3.2.3.1) to yield active metabolites, viz. thiocyanate (SCN-), isothiocyanate, oxazolidone–2-thiones and nitrile compounds (Appelqvist and Ohlson 1972). The use of RMC as protein supplement to ruminants' diet is constrained due to its higher level of glucosinolates that have variable adverse effect on palatability, intake, nutrient utilization, thyroid hormone status and overall performance of the animals (Hill 1991, Tripathi and Mishra 2007). There is dearth of systematic studies exploring information regarding the effect of RMC on the reproductive performance of ewes to accurately decide their safe dietary level. Therefore, this experiment was undertaken to assess the effect of feeding rapeseed-mustard cake during pregnancy on the performance of ewes.

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MATERIALS AND METHODS

Experimental animals: Muzaffarnagari ewes (16), weighing 28.9± 1.1 kg and 1.5 years of age, were teased twice daily for oestrus detection and were bred by single ram during standing oestrus. Ewes returning to heat again in the subsequent cycle were re-bred. The confirmation of pregnancy was done by palpitation method. Twelve confirmed ewes were divided into 2 equal groups and randomly allocated to GNC and RMC treatments, containing GNC and RMC as major protein source, respectively.

Experimental procedure: All the ewes were offered a basal diet of wheat straw *ad lib.* along with required amount of respective concentrate mixtures to meet their nutrients requirement for maintenance during early pregnancy (up to 60 days post-mating). However, from 60 days of pregnancy to term they were given pregnancy allowance (Kearl 1982) in the form of extra concentrate mixture. A small quantity of (200g) green fodder was provided to meet vitamin A requirement of ewes. Two metabolism trials of 9 days each (3 days acclimatization to metabolic cages followed by 6 days collection) were conducted, during early and late gestation. All the gravid ewes were provided water free choice and weighed at fortnightly intervals in the morning before offering the feed to find out the live weight changes during the study from mating to 24 h post lambing. The pregnancy product was estimated by the weight difference between live weight at 21 weeks of gestation and 24 h post lambing. The records of daily feed offered, residue left, faeces and urine voided were maintained for 6 days collection. Faeces and urine were collected in acidified containers and proportionate samples were preserved with 20% (v/v) sulphuric acid. Samples of feed, residue and faeces were dried at 70°C for 24 h or till content weight in a forced-draught oven for dry matter determination. Dried samples of feed, refusals and faeces were pooled for 6 days collection and preserved in airtight polythene bags for further analysis. Blood samples were collected through jugular vein puncture at 0, 45, 90 and 135 days intervals, serum was separated and preserved at –20°C until analysed. Another 2 ml blood sample was collected in tubes containing ethylene diamine tetra-acetate at 1 mg/ml blood, for haematological studies.

Chemical analyses: The glucosinolates content of RMC was analysed by the palladium complex method (Hassan *et al*. 1988). Samples of feeds, residues and faeces were milled to pass through a 1mm sieve and then analyzed as per AOAC (1995) to determine DM by the oven drying method, organic matter by muffle furnace incineration, crude protein by kjeldahl method, ether extract ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined following the methods of Van Soest *et al*. (1991). NDF was assayed with sodium sulphite, in the NDF reagent without alpha-amylase and the results were expressed with residual ash. Haemoglobin and packed cell volume (PCV) were estimated in whole blood immediately after blood collection by acid haematin method (Benjamin 1985) and Wintrobe's tube, respectively. Serum glucose concentration was determined colorimetrically (Hultmann 1959). The serum protein and albumin content were measured as per Wotton (1964) and Doumas *et al*. (1971), respectively. Serum urea was estimated by improved diacetyl monoxime method (Rahmatulla and Boyde 1980). Calcium (Ca) and phosphorus (P) in serum samples were analysed by Ferguson *et al*. (1940) and Thomas (1998), respectively. Activity of serum enzymes alanine aminotransferases (AST) and aspartate aminotransferases (ALT) were estimated as per standard methods described by Reitman and Frankel (1957). Serum concentration of triiodothyronine (T_3) and thyroxine (T_4) was determined by radioimmunoassay. Samples and calibrators were incubated with ^{125}I-labelled T_3 and T_4 tracers in antibody-coated tubes. After incubation, the liquid content of tubes was aspirated, and the bound radioactivity was determined in a gamma counter.

Statistical analyses: Results pertaining to nutrient digestibility, N-balance, and nutrient intake during both trial and blood biochemical parameters were analyzed applying repeated measures (Snedecor and Cochran 1994), for collection periods and 0 day values were used as covariate. The pregnancy variables were subjected to student's t-test to compare the difference between the treatment means. Significance was declared at $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of concentrate mixtures and major feed components is given in Table 1. The concentrate mixtures were iso-nitrogenous and comparable with respect to other related parameters (OM, NDF and ADF) except for EE, which was higher in RMC. The chemical composition of concentrate mixtures and wheat straw offered as basal feed was comparable with the earlier reports for Indian feeds and fodders (Patra *et al*. 2002, Rastogi *et al*. 2003, Dey *et al*. 2008, Dubey *et al*. 2012). Glucosinolates content in RMC supplement was 30.75 µmol/g.

Intake and nutrient utilization: Supplementation of RMC during early and late gestation to wheat-straw based diet significantly ($P>0.05$) reduced the total DMI (g/kgW$^{0.75}$) in gravid ewes (Table 2). During early phase of gestation concentrate intake was comparable between RMC and GNC treatments but wheat straw intake was reduced significantly ($P<0.05$) with RMC supplementation. While, during late gestation concentrate intake was significantly ($P<0.05$) reduced in RMC fed group and wheat straw intake remained comparable between GNC and RMC treatments. It has been observed from earlier reports that the effects of supplementation of RMC in the rations of ruminants may be variable probably due to variations in the glucosinolates content of various cultivars and their dietary levels. Glucosinolates cause pungent odour due to the formation of volatile and pungent compounds after their hydrolysis, and a bitter taste (Hill 1991, Papas *et al*. 1979, Palanivel *et al.*

Table 1. Ingredient and chemical composition of feeds

Attributes	Dietary treatments		Wheat straw
	GNC	RMC	
Ingredients composition (%)			
Maize	30	30	-
Groundnut cake	29	-	-
Mustard oil cake	-	41	
Wheat bran	39	27	-
Mineral premix*	1	1	-
Salt (NaCl)	1	1	-
Chemical composition (% DM)			
Organic matter	92.35	92.09	92.27
Crude protein	21.29	21.98	3.27
Ether extract	2.53	4.51	0.98
Neutral detergent fibre	29.29	30.41	79.75
Acid detergent fiber	11.90	11.90	55.64
Glucosinolates**, (μmol/g DM)	0.0	30.75	0.0

*Contained (g/kg): calcium 215, phosphorus 95, sodium chloride 285, potassium iodine 2.5, iron 5.0, copper 0.8, cobalt 1.0, manganese 1.0 and sulphur 1.0.**MOC Gls, 75 μmol/g DM.

2010) that may contribute to lower feed intake when high glucosinolate containing RMC is included in the ration of ruminants (Hill 1991).

The digestibility coefficient of DM, OM, CP, NDF and ADF did not differ significantly (P>0.05) between RMC and GNC treatments during early and late gestation. The treatment and period interaction was also nonsignificant. The results indicated that difference in the level of glucosinolate intake did not adversely affect nutrient digestibility, and in agreement with earlier reports (Tripathi *et al*. 1999a, Kumar *et al*. 2002, Ravichandiran *et al*. 2008, Tayo 2010). The nutrient intake (g/kgW$^{0.75}$) and density (%) of composite diets in terms of DCP and TDN was comparable during early gestation, however, intake and concentration of nutrients by gravid ewes was significantly (P<0.05) reduced in RMC fed animals.

N-intake (g/d) was comparable between RMC and GNC group of gravid ewes during early gestation, however, during late gestation N-intake was significantly (P<0.05) lower in RMC fed group. Similarly, faeces N-excretion (g/d) was significantly (P<0.05) lower in RMC fed animals during late gestation. Urinary excretion of N (g/d) did not differ significantly (P<0.05) between RMC and GNC treatments during entire gestation period. Net retention of N (g/d), percent of intake or percent of absorbed N (an indication of apparent biological value) was significantly (P<0.05) lower in RMC fed gravid ewes during early and late gestation. Present results are in conformity with the report of Tripathi *et al.* (1999b), who reported low N retention (as % of N intake) in ewes fed water soaked MOC compared to GNC.

Blood-biochemical parameters: Haemoglobin, serum glucose, serum urea, serum protein, albumin, globulin, A: G

Table 2. Effect of feeding high glucosinolates RMC on intake and nutrient utilization by gravid ewes

Attributes	Early -gestation			Late-gestation			Significance		
	GNC	MC	Pooled SE	GNC	MC	Pooled SE	Treat (T)	Period (P)	T × P
kg W$^{0.75}$	13.46	12.14	0.28	14.38	13.18	0.44	*	NS	NS
Dry matter intake									
g/d	859.5^{b}	653.2^{a}	53.68	912.6^{b}	673^{a}	54.27	*	NS	NS
% BW	2.7^{b}	2.4^{a}	0.09	2.6^{b}	2.1^{a}	0.11	*	NS	NS
g/kg W$^{0.75}$	63.2^{b}	52.2^{a}	2.86	63.9^{b}	50.4^{a}	3.04	*	*	NS
Intake, g/kg W$^{0.75}$									
Concentrate	23.1	23.6	2.08	32.2^{b}	20.7^{a}	2.20	*	*	NS
Straw	40.1^{b}	29.9^{a}	2.75	31.7	29.6	2.73	*	*	NS
Digestibility of nutrients (%)									
DM	49.2	51.8	1.17	53.2	49.9	1.18	NS	NS	NS
OM	51.7	54.23	1.12	55.6	52.5	1.11	NS	NS	NS
CP	53.9	55.0	1.49	61.0	55.2	2.24	NS	NS	NS
NDF	38.6	38.5	1.11	36.3	38.0	1.20	NS	NS	NS
ADF	34.6	34.0	1.28	34.1	36.3	1.09	NS	NS	NS
Nutrient density (%)									
DCP	5.7	6.5	0.73	7.7^{b}	6.1^{a}	0.46	*	*	NS
TDN	49.8	52.1	1.07	54.0^{b}	50.9^{a}	1.00	*	*	NS
Plane of nutrition (g/kg W$^{0.75}$)									
DCP	3.6	3.4	0.28	4.83^{b}	3.0^{a}	0.11	*	*	NS
DOM	30.2	26.7	1.38	32.9^{b}	24.6^{a}	1.87	*	*	NS
TDN	31.7	27.9	1.47	34.5^{b}	25.8^{a}	1.96	*	*	NS

[abc] Means with different superscripts in a row (period) differ significantly (P< 0.05); DCP, digestible crude protein; DOM, digestible organic matter; TDN, total digestible nutrients.

Table 3. Effect of feeding high glucosinolates RMC on nitrogen (N) utilization by gravid ewes

Attributes	Early -gestation			Late-gestation			Significance		
	GNC	MC	Pooled SE	GNC	MC	Pooled SE	Treat (T)	Period (P)	T X P
N intake, g/d	14.3	12.2	1.11	18.2^{b}	11.7^{a}	1.24	*	*	NS
N-excretion, g/d									
Faeces	6.43	5.43	0.42	7.0^{b}	5.2^{a}	0.44	*	*	NS
Urine	3.5	4.4	0.43	5.4^{b}	4.2^{a}	0.41	*	*	NS
Total	9.9	9.8	0.66	12.6	9.5	0.78	NS	NS	NS
N-balance									
g/d	4.3^{b}	2.3^{a}	0.66	5.8^{b}	2.2^{a}	0.78	*	NS	NS
% of N intake	29.0^{b}	18.3^{a}	2.55	30.6^{b}	19.2^{a}	2.61	*	NS	NS
% of N absorbed	53.0^{b}	32.6^{a}	5.10	48.4^{b}	34.6^{a}	3.05	*	NS	NS

^{abc} Means with different superscripts in a row (period) differ significantly (P< 0.05).

Table 4. Effect of feeding high glucosinolates RMC on blood-biochemical parameters by gravid ewes

Parameters	Treatments		Pooled SE	Periods*				Pooled SE
	GNC	RMC		0	45	90	135	
Hb (g/dl)	10.3	10.0	0.21	9.6^{a}	11.6^{c}	8.9^{a}	10.6^{b}	0.42
Serum glucose (mg/dl)	46.8	48.6	2.02	47.4	41.2	46.5	45.5	1.43
Serum urea (mg/dl)	16.9	17.0	1.01	17.5	16.7	16.8	16.9	1.56
Albumin (g dl)	3.4	3.5	0.04	3.9^{c}	2.8^{a}	3.5^{b}	3.5^{b}	0.06
Globulin (g/dl)	3.3	3.2	0.09	2.9^{a}	3.8^{c}	3.1^{a}	3.3^{bc}	0.13
Total protein (g/dl)	6.6	6.7	0.10	6.8	6.6	6.6	6.8	0.18
A:G	1.1	1.2	0.04	1.4^{c}	0.76^{a}	1.2^{b}	1.1^{b}	0.05
Ca (mg/dl)	12.0	12.2	0.09	11.8	12.4	12.2	11.9	0.12
P (mg/dl)	5.8	5.9	0.09	5.8	6.0	6.2	5.9	0.12
AST (U/ml)	117	106	4.43	89^{a}	73^{a}	134^{b}	149^{b}	6.26
ALT(U/ml)	29.3	32.5	2.47	26.3	33.1	32.5	32.0	3.49
T_3 hormone (nm/L)	1.46	1.29	0.051	1.34	1.55	1.37	1.23	0.071
T_4 hormone (nm/L)	76.16^{b}	62.5^{a}	1.83	82.0	78.20	57.83	59.35	1.87

*Values average of both groups; ^{abc} Means with different superscripts in a row (period) differ significantly (P< 0.05).

ratio, serum calcium and phosphorus, AST and ALT levels of gravid ewes did not differ significantly (P>0.05) between RMC and GNC fed groups. Blood-biochemical attributes were remained within the normal range of variations suggested under physiological stages for sheep (Kaneko 1997). Treatment and period interaction was significant (P<0.05). Levels of Hb, albumin, globulin, A: G ratio and AST differed (P<0.05) among periods, which are in general likely due to normal metabolic changes in the animals. Present results are in concurrence with the report of Ravichandiran *et al*. (2008), who reported comparable values of various target blood-biochemical parameters except decreased level of serum urea between high and low glucosinolates RMC fed calves. However, serum urea level was comparable between both the groups in present study because the extent of degradation of high glucosinolates RMC and GNC was also comparable.

Serum T_3 and T_4 hormones: T_3 (triiodothyronine) did not differ significantly irrespective of dietary treatments, however, concentration of T_4 (tetra iodothyronine) hormone was significantly (P<0.01) lower in RMC as compared to GNC. The decrease in T_4 hormone in RMC treatment indicated that feeding of RMC is likely to alter thyroid functional activity in gravid ewes. The goitrogenic activity of RMC is attributed to its degradation product, thiocyanate that inhibits trapping of iodide, and subsequently their accumulation in the follicular cells of thyroid as well as release of hormones, mainly T_4 from thyroid (Schone *et al*. 1990, Tripathi *et al*. 2001a, Ravichandiran *et al*. 2008, Tayo 2010).

Serum thiocyanate: Serum thiocyanate (SCN^-) level in gravid ewes ranged from 1.72 to 24.20 µg/ml. Mean serum SCN^- level increased significantly (P<0.01) in RMC fed group as compared to GNC (Fig.1). Similarly, SCN^-levels remained higher (P<0.01) from II to IV periods in RMC than GNC group. The increase (P<0.01) in serum SCN^- concentration in RMC, which may be due to higher consumption of Gls. The Gls degradation in rumen by

Table 5. Effect of feeding high glucosinolates RMC on pregnancy variables by gravid ewes

Variables	Dietary treatments		Pooled SE
	GNC	RMC	
Live weight (kg) of ewes			
At mating	28.75	28.5	1.25
21 weeks of gestation	36.18	34.98	1.13
Gross gain (after 21 weeks of pregnancy) weeks pregnancy)	7.43[b]	5.90[a]	0.518
Pregnancy product	6.02	6.8	0.451
Net gain	1.45[b]	–0.83[a]	0.421
24 h post lambing	30.17	28.25	0.568
Birth weight of lambs	2.97	2.65	0.150
Gestation length (days)	147.83	154.16	3.06
Survivability (%)	100.0	100.0	-

[ab] Means with different superscripts in a row differ significantly.

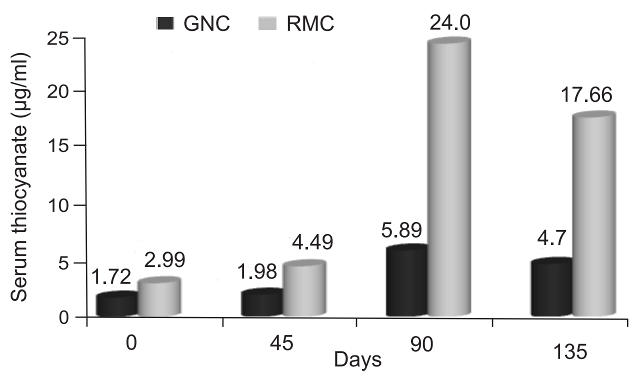

Fig. 1. Effect of feeding RMC on serum thiocyanate level by gravid ewes.

myrosinase enzyme liberates SCN^-, which is absorbed into the circulation and detected in blood. Tripathi *et al*. (2001b) reported higher SCN^- in blood of mustard meal fed lambs (26 µg/ ml). There are 2 sources of serum SCN^- from Gls. First, SCN^- is a product of Gls degradation by microbial thioglucosidase in the intestine (28.78%); second, nitriles from Gls degradation release CN-, which is detoxified by thiosulphate S transferases forming SCN^- (Oginsky *et al*. 1965, Schone *et al.* 1997).

Pregnancy variables: The live weight (kg) of ewes at mating, 21 weeks of gestation, pregnancy product, live weight at 24 h post lambing did not differ significantly ($P>0.05$) between RMC and GNC fed animals (Table 5). Gravid ewes under both treatments had positive weight gain throughout gestation period. However, gross gain (after 21 weeks of pregnancy) and net gain was significantly ($P<0.01$) lower in RMC as compared to GNC treatment. The concentrate intake was lower by 35.7% in RMC fed animals to that in GNC fed animals due high Gls content of RMC. The metabolites of Gls reduced protein synthesis in liver (Kozlowski *et al*. 1990), thyroid hormone status, and growth of young animals (Cox-Ganser *et al*. 1994, Ravichandiran et al. 2008). Body loss was reported in sheep consuming diets containing Gls 1.2 to 2.2 µmol/g DM during lactation (Mandiki *et al*. 2002). Present results are also in conformity with the earlier reports indicating poor growth performance with lower ADG in lambs on RMC diets (Kossaibatti and Bryant 1994, Tripathi *et al*. 1998, Tripathi and Mishra 2008). The birth weight of lambs and gestation length (days) was comparable between RMC and GNC treatments. All the gravid ewes under both treatments gave birth to the single lamb with 100% survivability of lambs.

On the basis of results, it may be concluded that feeding of rapeseed-mustard cake from traditional genotypes (Gls, 75 mmol/g DM) appear to have no adverse effect on nutrient utilisation in gravid ewes. However, feeding of RMC significantly reduced the palatability of concentrate, gross gain after 21 weeks of pregnancy, net gain and thyroxine concentration in blood of gravid ewes. More studies are required to assess the reproductive performance of animals on feeding RMC in relation to glucosionolate content and to work out correction strategies.

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