Effect of feeding water soaked rapeseed-mustard cake (Brassica juncea) based diet on the performance of lactating cows

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ABSTRACT
The study was carried out to ascertain the effect of feeding water soaked rapeseed-mustard cake (RMC) based diet on the performance of lactating cows. Eighteen Holstein Friesian crossbred lactating cows were randomly allocated to 3 dietary treatments of 6 each viz. Control, RMC-dry and RMC-sani, respectively for an experimental period of 90 days. The glucosinolates (GLS) content of raw RMC was 149.50 µmol/g DM and it gets reduced by 31.96 per cent to 101.72 µmol/g DM in overnight water soaked (1:3 w/v ratio) RMC. The daily intake of concentrate and total DM by lactating cows did not differ significantly (P>0.05) irrespective of the dietary treatments. The digestibility coefficient (%) of DM, OM, CP, EE, NDF and ADF; nutrient density (%) and intake (g/kgW^{0.75}) of composite diets in terms of DCP and TDN did not differ significantly (P>0.05) irrespective of dietary treatments. Milk yield and its basic quality parameters including milk fat, milk protein, total solids and SNF content were comparable among the dietary treatments. Though thiocyanate (CNS) concentration of blood serum (8.65 to 12.65 µg/ml) and milk (24.77 to 39.84 µg/ml) was significantly (P<0.01) increased in RMC fed groups as compared to control, it was significantly (P<0.01) reduced in RMC-sani as compared to RMC-dry. The serum T3 (2.07 - 2.15 nmol/L) and T4 (70.62 - 73.67 nmol/L) concentrations were comparable among the dietary treatments. Alongwith substantial reduction in the GLS content of water soaked RMC, no adverse effect could be noticed by feeding raw or water soaked RMC based diet on the performance of lactating cows and CNS excretion in the body fluids get reduced by dose dependent intake of dietary GLS.

Key words: Lactating cows, Milk quality, Rapeseed-mustard cake, Water soaking.

INTRODUCTION
Rapeseed-mustard cake (RMC), a major by-product in oil extraction from rapeseed-mustard traditionally used as cheap protein supplement for feeding ruminants in India (Sahoo et al. 2006). However, efficient dietary utilization of RMC is limited due to presence of an anti-nutritional factor, Glucosinolates (GLS), a natural thioglycosides found in higher level (150 - 240 µmol/g DM) in the Indian variety (Brassica juncea) of RMC (Banday and Verma, 2003; TERI, 2003). On ingestion, these intact thioglycosides are enzymatically hydrolyzed into active metabolites including thiocyanate (CNS) those having variable adverse effect on palatability of the RMC based diet, thyroid activity and performance of the animals (Hill, 1991; Das and Singhal, 2005). In addition, there has also been great concern regarding use of RMC in animal feeding from the viewpoint of quality of animal products. Feeding of RMC based diet to lactating animals may result in transfer of CNS to the milk, causing goitrogenic changes in young ones or in humans consuming that milk due to higher levels of milk CNS binds the circulatory iodine and blocks its secretion in the milk (Tripathi and Mishra, 2007).

In order to alleviate toxic effects of GLS, various physical and chemical processing methods have been tried successfully by reducing the GLS content of RMC. Among these methods, water soaking is a conventional practice used to improve the palatability of RMC by substantial reduction (36 percent) of its GLS content (Tyagi et al. 1997). Crushing and soaking of RMC in water brings the endogenous ‘Myrosinase’ enzyme in contact with GLS resulting in substantial hydrolysis of GLS to volatile metabolites viz. isothiocyanate, CNS, nitriles and other degradation products. Therefore, the present study was planned to assess the relative effects of feeding raw or water soaked RMC based diet on the performance of lactating cows and CNS excretion in the body fluids.

MATERIALS AND METHODS
Experimental animals and diets: Eighteen multiparous Holstein Friesian crossbred (Bos taurus x Bos indicus) lactating cows (average milk yield of 9.41 ± 0.27 kg/day) at their second parity and mid stage of lactation with a mean live weight of 313.72 ± 15.85 kg were selected. The cows were randomly divided into three dietary treatment groups consisting 6 cows each viz. Control, RMC-dry and RMC-sani.
While groundnut cake is free from

Table 1: Ingredient composition (percent on as fed basis) of concentrate mixtures fed to lactating cows

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>RMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>25.0</td>
<td>-</td>
</tr>
<tr>
<td>Rapeseed-mustard cake</td>
<td>-</td>
<td>31.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>47.0</td>
<td>41.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Salt</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

sani in a completely randomized design. The animals were housed individually in a well-ventilated, roofed and cement-floored barn with provision made for individual feeding. All the experimental animals were offered a basal diet of green oats ad libitum along with required amount of respective iso-nitrogenous (21 per cent CP) concentrate mixture (Table 1) and wheat straw to meet their nutrients requirement for maintenance and milk production as per the Kearl (1982) feeding standards. The cows of Control and RMC-dry were individually offered with two equal portions of daily allowance of respective concentrate mixtures at the time of milking hours. For the cows of RMC-sani, RMC concentrate mixture has been soaked overnight in water at the ratio of 1:3 w/v ratio, mixed with part of daily allowance of wheat straw and fed as a mixed ration (Sani) in divided portions at the time of milking hours. The ration schedule was adjusted every fortnight after recording the daily milk yield and fortnight body weight changes to meet the nutrient requirements for maintenance and milk production. A digestion trial of 6 days duration was conducted after 60 days of experimental feeding during which samples of feed offered, residue left and faeces voided were collected and the representative samples were analysed for proximate components (AOAC, 1995) and fibre fractions (Van Soest et al. 1991).

Blood and milk samplings: Blood samples (10 ml) was collected from each cows by jugular venipuncture before feeding in the morning hours at 45 days interval starting from 0 to 90 days. The sample was centrifuged at 3000 rpm for 20 min to harvest serum. Serum samples were stored at -20°C until further analysis. Animals were hand milked twice daily (8.00 AM and 4.00 PM) in hygienic manner and yield gets recorded individually. Milk samples were drawn from each animal during both the times of middle stage of milking in a day at fortnight intervals starting from 0 to 90 days. After thorough mixing, a sample of 100 - 150 ml was taken by means of a dipper and transferred to a sample bottle with rounded corners (to avoid lodging of the milk solids) up to 3/4th level, and then bottle was corked tightly by a rubber stopper. The sample bottles were labeled properly and dispatched to laboratory in an ice box. Fat content of the milk samples was analysed immediately after collection and remaining samples were stored at 4°C after adding 2 - 3 drops of potassium dichromate as a preservative, until further analysis.

Laboratory analyses: The GLS content of raw and water soaked RMC was analysed using the HPLC method (Sang and Truscott, 1984). Serum Triiodothyronine (T3) and Thyroxine (T4) concentrations were determined using the radioimmunoassay kits (Cornelius, 1980). The milk samples were analysed for fat, titrable acidity and total solids as per ISI (1961) and protein content as per AOAC (1995). SNF content of milk was calculated by subtracting fat content from total solids content of milk accordingly. CNS concentration of milk and blood serum samples was estimated as per the procedure of Bowler (1944).

Statistical analysis: The data pertaining to various parameters were subjected to one way analysis of variance in a completely randomized design as per the methods of Snedecor and Cochran (1989) with the help of SPSS 11.0 software package. If F-values were significant, the means of different treatments were subjected to Duncan’s multiple range tests as described by Duncan (1955).

RESULTS AND DISCUSSION

Experimental diets: While groundnut cake is free from GLS, the GLS content of raw RMC is very high (149.50 µmol/g DM) and quite comparable with the values reported earlier (Banday and Verma, 2003; TERI, 2003). Overnight water soaking of RMC reduced its GLS content by 31.96 per cent to 101.72 µmol/g DM. The result was concurrent with the observation of Singhal and Senani (1991) who reported 30.7 per cent reduction in the GLS content of mustard oilcake by 12 hours water soaking as the result of enzymatic hydrolysis. Similarly, Tyagi et al. (1997) reported that simple water soaking (1:10 w/v ratio, 6 hours) of high GLS rapeseed meal (HG-RSM) followed by drying at 60°C reduced the GLS content by about 36 per cent (from 144.0 to 92.2 µmol/g DM). Generally GLS remain chemically stable within the cytoplasm until brought into contact with the endogenous enzyme Myrosinase following tissue disruption (Mithen, 2006). After physical damage of plant tissue, GLS are broken down by Myrosinase, releasing glucose and a complex variety of biologically active volatile metabolites (Holst and Williamson, 2004). However, water soaking of low GLS rapeseed meal (LG-RSM) reduced its GLS content (55.00 µmol/g DM) by 76% after 8 hours of soaking (Tyagi, 2002). The endogenous enzymatic hydrolysis of GLS therefore, varies with level and composition of GLS content of RMC, moisture level and soaking period. Myrosinase enzyme has maximum activity at 15 - 40 % moisture level during 6 - 8 hours of soaking period (Kim and Rhee, 1993; Tyagi, 2002). Gluconapine, Sinigrin, and Pentenyl-GLS detected in RMC in the ratio of 77:14:9 were enzymatically degraded at the rate of 82, 66 and 46 %, respectively (Tyagi, 2002).
Feed intake and digestibility of nutrients: The intake of concentrate component, green fodder and total DM by experimental cows was comparable (P>0.05) among dietary treatments (Table 2). Similarly, short term experiments with either HG-RSM or LG-RSM containing varying levels of GLS at 17 - 30 % of the concentrate mixture did not reveal any negative effect on feed intake in lactating cows (Emanuelson, 1994). The digestibility of DM, OM, CP, EE, NDF and ADF for various experimental diets did not differ significantly (P>0.05) irrespective of dietary treatments. The results of present study are in agreement with the earlier findings revealed no significant difference in the digestibility of nutrients by lactating ewes fed with raw or water soaked RMC based diet containing different levels of GLS (Tyagi et al. 1997; Tripathi et al. 1999).

Blood biochemical profile: The serum CNS concentration of RMC fed cows was significantly (P<0.01) higher as compared to Control (Table 3), and it was comparable to earlier reported value of 10.35 - 13.93 ìg/ml in crossbred cows fed with expeller or solvent extracted mustard cake based diet (Saha and Singhal, 1993). However, among RMC treatment groups, serum CNS concentration was significantly (P<0.01) lower in RMC-sani which might be attributed to significantly (P<0.05) low dietary GLS intake by lactating cows of RMC-sani. The dose dependent response for dietary GLS intake on serum CNS concentration in experimental cows is in close agreement with the results from previous experiments with lactating cows (Bibi and Bachman, 1997) and lactating ewes (Tripathi et al. 1999). Though serum CNS concentration was significantly (P<0.01) increased in RMC fed cows, the serum T3 and T4 concentrations were similar irrespective of the dietary treatments and within the suggested range of 0.7 - 2.2 nmol/L (T3) and 54.05 - 110.68 nmol/L (T4) by Rodostits et al. (2003). The levels of T3 and T4 were not affected by the treatments in this experiment, which is in accordance with earlier studies (Zech et al. 1995; Sustala et al. 2003). The fermentation process in adult ruminants has been shown to lower the goitrogenicity of RMC based diet (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 (Hill, 1991).

Milk yield and quality: Feeding of raw or water soaked RMC based diet to experimental cows did not exert adverse impact on milk yield and its basic quality parameters (Table 4). The efficiency of milk production by cows was also comparable among the dietary treatments. Similarly, lactating cows fed with different varieties of RSM (1788-RSM, 940-
Table 4: Milk yield and its quality parameters of lactating cows under different dietary treatments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>RMC-dry</th>
<th>RMC-sani</th>
</tr>
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<tbody>
<tr>
<td><strong>Milk Yield</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole milk (kg/day)</td>
<td>9.48±0.18</td>
<td>9.45±0.19</td>
<td>10.03±0.25</td>
</tr>
<tr>
<td>4% FCM (kg/day)</td>
<td>10.85±0.14</td>
<td>10.82±0.12</td>
<td>10.96±0.12</td>
</tr>
<tr>
<td>ECM (kg/day)</td>
<td>11.68±0.12</td>
<td>11.62±0.11</td>
<td>11.73±0.09</td>
</tr>
<tr>
<td>Fat yield (g/day)</td>
<td>444.49±10.49</td>
<td>444.00±11.00</td>
<td>464.29±12.31</td>
</tr>
<tr>
<td>Protein yield (g/day)</td>
<td>326.98±6.20</td>
<td>322.10±6.90</td>
<td>340.90±8.54</td>
</tr>
<tr>
<td>EMP (kg DMI/kg 4% FCM)</td>
<td>1.05±0.02</td>
<td>1.07±0.03</td>
<td>1.06±0.02</td>
</tr>
<tr>
<td><strong>Milk Quality</strong></td>
<td></td>
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</tr>
<tr>
<td>Fat (%)</td>
<td>4.70±0.22</td>
<td>4.69±0.14</td>
<td>4.64±0.12</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.44±0.03</td>
<td>3.41±0.03</td>
<td>3.40±0.02</td>
</tr>
<tr>
<td>Total solids (%)</td>
<td>13.72±0.12</td>
<td>13.59±0.09</td>
<td>13.50±0.10</td>
</tr>
<tr>
<td>SNF (%)</td>
<td>9.04±0.10</td>
<td>8.92±0.09</td>
<td>8.94±0.10</td>
</tr>
<tr>
<td>CNS (µg/ml)</td>
<td>24.77±0.17</td>
<td>39.84±0.84</td>
<td>32.81±0.63</td>
</tr>
<tr>
<td>Titrable acidity*</td>
<td>2.03±0.02</td>
<td>2.01±0.02</td>
<td>2.00±0.01</td>
</tr>
</tbody>
</table>

*Mean values bearing different superscripts in a row differ significantly (P<0.01)

FCM - Fat corrected milk; ECM - Energy corrected milk; EMP - Efficiency of milk production

RSM and commercial varieties containing varying GLS levels at 25% of the concentrate mixture in place of soybean meal revealed no significant change in milk yield, fat, protein and SNF content of milk (Sharma et al.1977). However, lactating cows fed high levels of RSM based diet had shown lower milk yield with reduced milk fat, protein and SNF content of the milk which was associated with lower DM intake and nutrient utilization of animals (Waldern, 1973; Fisher and Walsh, 1976). Titrable acidity (TA) value of raw milk was also similar irrespective of dietary treatments. Generally, determination of TA is a preliminary step to access the quality of raw milk and progressive increase in TA value of milk is associated with increased storage time.

The mean milk CNS concentration (32.81 to 39.84 µg/ml) of RMC fed cows was significantly (P<0.01) higher as compared to Control, and it was comparable to the values reported in crossbred cows fed with mustard cake based diet (Tyagi and Singhal, 2002). However, among RMC fed groups, it was significantly (P<0.01) lower in RMC-Sani which might be attributed to significantly (P<0.05) low dietary GLS intake by lactating cows of RMC-sani. CNS, one of the end products of GLS hydrolysis in gastro intestinal tract of lactating cows fed with RMC based diet, get absorbed into the blood and transferred to the milk and its concentration in milk was directly related to dietary GLS intake (Papas et al., 1979; Laarveld et al., 1981; Vincent and Hill, 1988). CNS is a ubiquitous normal constituent of milk at low levels participating in the lactoperoxidase (LP) system, an unspecific antimicrobial in milk (Reiter, 1985).

CONCLUSION

The lactating cows fed water soaked RMC based diet have shown comparable performance with those fed raw RMC based diet, and CNS excretion in the body fluids get significantly lowered for those fed with water soaked RMC based diet by reducing substantial level of its GLS content as compared to those fed with raw RMC based diet.

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