THYROID STATUS AND GROWTH PERFORMANCE OF GROWING KIDS FED RAW OR WATER SOAKED RAPESEED-MUSTARD CAKE BASED DIET

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ABSTRACT

The study was undertaken to investigate the thyroid status and growth performance of young growing goats (kids) fed with raw or water soaked rapeseed-mustard cake (RMC) replaced for ground-nut cake in the diet. Eighteen male Barbari kids were randomly assigned to 3 dietary treatments of 6 each; GNC, RMC-dry and RMC-sani containing ground-nut cake, raw RMC and overnight water soaked (1:3 w/v ratio) RMC, respectively. The respective concentrate mixture and ad lib. wheat straw were offered for an experimental period of 180 days with monitoring of blood biochemical profile at 45 days intervals. All the kids were slaughtered as per the standard procedure; thyroid glands were collected, weighed and studied for histological changes if any. Total glucosinolates (GLS) content of RMC-dry concentrate mixture was 64.28 µmol/g DM while it was reduced by 31.96 % to 43.74 µmol/g DM in RMC-sani and nil in GNC concentrate mixture. The DM intake and weight gain of kids were significantly ($P<0.01$) lower in RMC treatments as compared to GNC. The levels of Hb, serum Proteins and Glucose were significantly lower and of Total Cholesterol and serum SCN^- were significantly ($P<0.01$) higher in RMC treatments as compared to their analogous values in GNC. Among RMC treatments, serum SCN^- was significantly ($P<0.01$) reduced in RMC-sani. Serum T_3 was similar among the treatments. Serum T_4 was significantly ($P<0.01$) lower by kids of RMC-dry as compared to GNC while it was comparable in RMC-sani with other treatments. Although no significant ($P>0.05$) difference was observed in weight of thyroid among the treatments, appreciable histological changes were noticed in the thyroid glands under RMC treatments relative to those of GNC; however, among RMC treatments, intensity of thyroid histological changes was comparatively low in RMC-sani. Despite reduction in the substantial amount of GLS content by water soaking of RMC, comparatively high levels of GLS content of raw or water soaked RMC based diets induced significant but mild hypothyroidic changes and poor growth performance of kids.

Key words: Thyroid status, Growth performance, Goats, Rapeseed-mustard cake, Glucosinolates

Introduction

Rapeseed-mustard cake, a cheap protein source (34 - 38 % CP) utilized for livestock feeding in India; but its dietary utilization is restricted by presence of large amount of glucosinolates (GLS) in Indian varieties of RMC (Agnihotri and Nutan Kaushick, 2000; Banday and Verma, 2003). The GLS are hydrolyzed by an endogenous enzyme 'Myrosinase' which is also produced by rumen microorganisms (Nugon-Baudon et al., 1990) into organic aglycones including isothiocyanate, thiocyanate (SCN^-), 5-venyl-oxazolidine-2-thione (Goitrin) and nitrile compounds. These biologically active compounds exert negative effects on the production performance and health status of animals fed RMC based diet which mainly related to drastic thyroid disturbance induced by them (Ahlin et al., 1994). The anti-thyroidal properties of GLS derivatives expressed by reduced production of thyroid hormones either by inhibition of Iodine uptake of thyroid or blocking the incorporation of Iodine into thyroxine precursors. In addition, the low circulatory levels of Triiodothyronine (T_3) and Thyroxine (T_4) stimulate excessive secretion of hypothalamic thyroid-stimulating hormone, which induces an increased thyroid follicle activity, resulting in a hypertrophy of the thyroid tissue (Mawson et al., 1994). However, studies concerning the set of problems encountered with the use of RMC in the diets for ruminants are still scarce although it is well established that its effect vary among species, and depend mainly upon the age of the animal and level of GLS in the diet (Vincent et al., 1988; Derycke et al., 1999). Among ruminant species, young caprines are more susceptible to thyroid disorders (Pachauri, 1997).

The risk associated with high GLS content of RMC has been reduced significantly by the breeding of new oilseed rape cultivars with lower content of GLS. In addition, as the adverse nutritional effects associated with feeding of RMC have been attributed to the hydrolysis products from GLS, some technological treatments of RMC has been recommended either to inactivate the endogenous enzyme or to reduce the GLS content. It has been reported that water soaking of RMC is a cheap and conventional practice resulted in substantial reduction (36 %) of GLS content by enzymatic hydrolysis into volatile derivatives (Tyagi et al., 1997). The present study was therefore, taken to investigate the thyroid status and growth performance of growing kids fed with raw or water soaked RMC based diet.

Materials and Methods

Experimental animals and feeding

Eighteen male Barbari kids of similar age (~6 months) and body weight (11.10 ± 0.77 kg) were distributed into 3 dietary treatments of 6 each; GNC, kids fed concentrate mixture containing groundnut cake as a protein source; RMC-dry, kids fed concentrate mixture containing RMC replaced for groundnut cake; RMC-sani, kids fed overnight water soaked (1:3 w/v ratio) RMC concentrate mixture as mixed ration (*sani*) with known amount of part of daily allowance of wheat straw. Wheat straw in *ad libitum* and green non-legume fodder (200 g/day) were offered to kids after concentrate feeding in the morning hours. Daily DM intake through concentrate and roughage, and fortnight body weight of individual kids were recorded; quantity of concentrate mixture was adjusted accordingly as per Kearl (1982) feeding standards. The experimental feeding was continued for 180 days. Total GLS content (µmol/g DM) of raw and overnight water soaked RMC was analysed by HPLC (Sang and Truscott, 1984) and their levels in respective concentrate mixtures were calculated.

Blood sampling and analysis

Blood samples (10 ml) were collected from all the kids before starting the experimental feeding and thereafter at 45 days intervals till the end of experiment by jugular puncture before feeding in the morning hours. 2 ml of the sample was added with EDTA for the analysis of haematological parameters (Hb and PCV) and the remaining sample was centrifuged at 4 °C, and separated serum was stored at -20ºC for further analysis. Serum samples were analysed for Glucose, Total Cholesterol, Urea and serum Proteins, serum enzymes (LDH and SGOT) levels using commercial diagnostic kits manufactured by Span Diagnostics Ltd., Surat, India. Serum T_3 and T_4 concentrations were determined using the radioimmunoassay (RIA) kits procured from Board of Radiation and Isotope Technology, Mumbai (India). Serum SCN^- concentration was determined as per procedure of Bowler (1944) based on the colorimetric estimation.

Histology of thyroid gland

The thyroid glands were collected at the time of slaughter of all the kids after the end of experimental feeing, weighed and representative tissue pieces of 2 mm thickness was fixed in 10 % buffered formalin solution for 24 hours. The formalin fixed tissues were washed with

distilled water for 4 to 5 hours, dehydrated in ascending grades of alcohol, cleaned with benzene and embedded with multiparaffin (60ºC) to prepare paraffin block. Sections of 4 - 5 µ thickness were cut and stained by Hematoxylin and Eosin stain.

Results and Discussion

Dietary composition and feed intake

The concentrate mixtures were isonitrogenous (23% CP) and comparable with respect to other parameters related to their proximate principles. The GLS content of RMC concentrate mixture was 64.28 µmol/g DM, as RMC used contained 149.50 µmol/g DM of GLS. The analysed value of total GLS content in RMC was very high and quite comparable with the values reported earlier (Tyagi et al., 1997; Banday and Verma, 2003; TERI, 2003). The GLS content of water soaked RMC concentrate mixture was 42.14 µmol/g DM as its level in the water soaked RMC get reduced by 31.96 % to 101.72 µmol/g DM. The result was corroborating with Singhal and Senani (1991) who reported that water soaking (12 hours) of mustard oilcake reduced its GLS content by 30.7 %.

Table 1: Table showing the feed intake and growth performance of kids under different dietary treatments.

	Treatments			
Attributes	**GNC**	**RMC-dry**	**RMC-sani**	**SEM**
Body Wt (kg)				
Initial	11.29	11.24	11.10	0.77
Final*	19.41^{a}	17.84^{b}	17.27^{b}	0.75
Total gain**	8.13^{a}	6.59^{b}	6.18^{b}	0.39
ADG (g/day)**	45.15^{a}	36.61^{b}	34.34^{b}	2.18
DM intake (g/day)				
Concentrate**	192.46^{a}	166.17^{b}	159.04^{b}	4.07
Wheat straw	136.98	142.66	141.66	1.60
Total DM*	329.44^{a}	308.83^{b}	300.69^{b}	9.00
GLS intake (µmol/day)	0.00^{c}	10681.41^{a}	6956.42^{b}	1039.95

$^{a\,b\,c}$ Mean values bearing different superscripts within a row differ significantly; ** (P<0.01) * (P<0.05)

The DM intake and weight gain of kids (Table 1) were significantly (P<0.05) lower in RMC treatments as compared to GNC which might be attributed to considerably high GLS content (>100 µmol/g DM) of both raw and water soaked RMC to exert deleterious effect on feed intake which in turn growth performance of kids (Hill, 1991). Average GLS intake (µmol/d) by kids was significantly (P<0.01) lower in RMC-sani as compared to that of RMC-dry and it was nil in GNC.

Blood biochemical profile

Parameters of hematology and blood chemistry (Table 2) have been used to assess the metabolic status of the animals.

Hematology

The Hb concentration (9.50 - 10.30 g/dl) was significantly (P<0.01) lower in RMC treatments as compared to GNC which could be attributed to GLS interference on normal copper metabolism which in turn reduced availability of copper for Hb synthesis (Ludke and Schone, 1988) and counter hypothyroidism (Pitt-Rivers and Trotter, 1964; Kaneko et al., 1997). Nevertheless, the levels of Hb and PCV in all the kids were within the reported physiological range (Hb, 8.0 - 12.0 g/dl; PCV, 22.0 - 38.0 %) for goats (Rodostits et al., 2003).

Table 2: Table showing hematological parameters, blood biochemical parameters and thyroid weight of kids under different dietary treatments.

Attributes	Treatments			SEM
	GNC	RMC-dry	RMC-sani	
Hematological parameters				
Hb (g/dl)**	10.30[a]	9.50[b]	9.56[b]	0.20
PCV (%)	35.14	33.97	34.06	0.68
Serum biochemical parameters				
T_3 (nmol/L)	1.21	1.19	1.15	0.06

T_4 (nmol/L)**	75.88[a]	64.66[b]	69.74[ab]	3.76
Serum SCN^- (µg/ml)**	6.54[c]	14.32[a]	11.11[b]	0.63
Glucose (mg/dl)**	57.23[a]	45.69[b]	44.31[b]	1.33
Total cholesterol (mg/dl)**	72.28[b]	90.14[a]	86.28[a]	2.51
Urea (mg/dl)	40.75	42.61	40.99	0.84
Total protein (g/dl)*	7.20[a]	6.90[b]	6.84[b]	0.13
Albumin (g/dl)*	4.20[a]	4.00[b]	3.93[b]	0.07
Globulin (g/dl)	3.00	2.89	2.89	0.09
LDH (IU/L)	320.53	331.36	324.82	4.63
SGOT (IU/L)	98.79	104.92	99.46	2.28
Thyroid gland (g/kg PSW)	0.070	0.072	0.073	0.002

Mean values bearing different superscripts in a row within periods differ significantly. ** (P<0.01), *(P<0.05)

Serum SCN^-, T_3 and T_4 hormones

Serum SCN^- concentration was linearly (P<0.01) increased at different periods and it was significantly (P<0.01) higher in RMC treatments (11.11 - 14.32 µg/ml) as compared to GNC (6.54 µg/ml) due to GLS intake. Among RMC treatments, serum SCN^- concentration was significantly (P<0.01) lower in RMC-sani as compared to that of RMC-dry. Serum SCN^- concentration reflected the markedly different levels of total GLS intake by kids and was closely correlated with average daily GLS intake as expected (Spiegel et al., 1993; Schone et al., 1997; Tripathi et al., 2001). The SCN^- ions are major degradation products from indolyl GLS. When absorbed from digesta to the blood, the SCN^- ions are transferred to different vital organs (Emanuelson et al., 1993). It may competitively inhibit the transfer of Iodide to the thyroxine precursors in the thyroid gland; SCN^- ions and Iodide are thus considered to be important in relation to studies of the quality of RMC (Nicolas Mabon et al., 1999). The SCN^- also derived from isothiocyanate (Van Etten, 1969) and cyanate (CN^-) which released from nitrile compounds (Schone et al., 1997), and produced same effects.

Serum T_3 concentration was varied in a narrow range throughout the experimental period and did not differ significantly ($P>0.05$) among the treatments. However, serum T_4 concentration showed a sequential decline with advancement of feeding period and it was significantly ($P<0.01$) lower in RMC-dry as compared to GNC. The kids given RMC-sani diet exhibited an intermediate T_4 value as observed for GNC and RMC-dry treatments. This could be attributed to a possible mild hypothyroidic condition arising out of different concentrations of serum SCN^- between RMC treatments. Dietary SCN^- affect the iodination of T_3 to T_4 by blocking the iodide pumping mechanism of thyroid gland, resulting in serum T_4 concentration get reduced without change in serum T_3 concentration (Papas et al., 1979). Similar finding was observed in different species fed with raw RMC (Aherne and Lewis, 1978; Papas et al., 1979; Lardy and Kerley, 1994). However, as serum SCN^- concentration reduced in kids fed with water soaked RMC based diet, T_4 concentration was increased to comparable levels with other two treatments.

Serum metabolic and enzyme profiles

Serum glucose level was significantly ($P<0.01$) declined periodically and lower in RMC treatments (44.31 - 45.69 mg/dl) as compared to GNC (57.23 mg/dl) which might be attributed to anti-thyroidal properties of GLS. The thyroid gland, by helping with selective activity of the intestinal tract for sugar, increases blood sugar if it is overactive and decreases blood sugar if it is under active. Glucosinolates through their anti-thyroid activity could be hypothesized to decrease the normal metabolism and thereby reduce circulating glucose levels in the body (Ludke and Schone, 1988). The level of serum cholesterol was followed the reverse trend to serum glucose level in the kids among treatments. Serum cholesterol in RMC treatments showed an increase over the period than that of GNC, however within the physiological range of 70 - 130 mg/dl for goats (Kritchevsky, 1958) which attributable to long term feeding of RMC inducing, possibly a mild hypothyroidic condition. Depressed thyroxine levels can create increased serum cholesterol by preventing the formation of bile to reduce cholesterol level which also used as diagnostic accuracy (66%) for hypothyroidism especially in small animals (Kaneko et al., 1997).

The levels of total serum protein and albumin were significantly ($P<0.05$) lower in RMC treatments which could also be attributed to mild hypothyroidism (Walmsley and White, 1994; Rosenthal, 1997). Similar findings were reported in lactating ewes (Tripathi et al., 1999) and growing lambs (Tripathi et al., 2001) fed with mustard oilcake based diet. The

LDH and SGOT enzyme activities of all the kids did not differ significantly (P>0.05) among treatments and within the physiological range of 123 - 392 IU/L and 167 - 513 IU/L, respectively for goats (Kaneko et al., 1997). It indicates that the levels and duration of exposure to dietary GLS from RMC did not have adverse effect on physiological functions of liver, heart or skeletal muscles of kids.

Histology of thyroid gland

Although no significant (P>0.05) difference was observed in weight of thyroid (g/kg PSW) among the treatments, the histological study of thyroid gland revealed that the effects of RMC feeding per se was indicative of mild thyroid perturbations due to long term feeding of RMC to the growing kids.

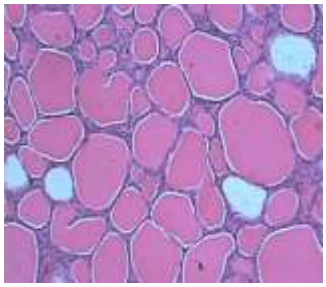

Figure 1: Image showing histological section of thyroid tissue of kid fed GNC diet

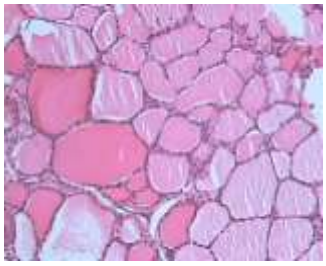

Figure 2: Image showing histological section of thyroid tissue of kid fed RMC-dry diet

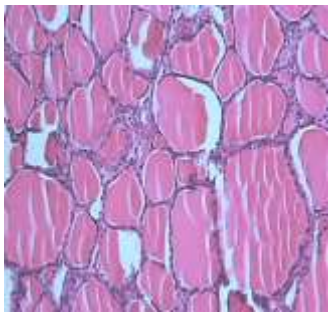

Figure 3: Image showing histological section of thyroid tissue of kid fed RMC-sani diet

The histological section of thyroid (Figure 1) from kids of GNC had shown unique features of thyroid viz. almost uniform size and shaped follicles lined by flattened epithelial cells and contained homogenous colloid with little interfollicular tissues. On the other hand, the histological sections of thyroid from kids under RMC treatments had shown hyperplasia and hypertrophy of the follicles indicated by variable in size and shape, and loss of colloid indicated by variable in its colour intensity. However, thyroid (Figure 2) from kids fed RMC-dry diet containing large amount of GLS showed more pronounced histopathological changes than those (Figure 3) from kids fed RMC-sani diet containing substantially reduced amount of GLS. Similar finding of dose dependent effect of GLS on thyroid histological changes was observed in calves fed with two varieties of rapeseed meal containing different levels of GLS (Papas et al., 1979).

The possibility of mild hypothyroidism due to GLS intake by ruminants may also vary according the age of the animal since pre-ruminant kids have a weak ability to detoxify GLS metabolites due to non-functional rumen and have enhanced demand of Iodine for the thyroxine precursors (Walinder, 1972; Papas et al., 1979; Derycke et al., 1999). Generally, Iodine absorbed in the body as Iodide ions get oxidized to organic Iodine by the action of thyroid peroxidase enzyme before incorporated in to thyroxine precursors. In the presence of GLS derivatives, the action of thyroid peroxidase shifted from oxidation of Iodide to that of GLS derivatives (Schone et al., 2001). This detoxification of GLS derivatives by thyroid peroxidase consumes excess organic Iodine and demand for Iodine taken by thyroglobulin resulted in compensatory hyperplasia and hypertrophy of thyroid epithelia (Taurog, 1985).

Conclusions

Despite reduction in the substantial amount of GLS content of RMC by overnight water soaking, high GLS content of both raw and water soaked RMC based diet induced significant but mild hypothyroidic changes and poor growth performance of growing kids; however, intensity of changes in thyroid activity was comparatively low in kids fed with water soaked RMC based diet. In future, it will be better to develop low GLS oilseed rape cultivars by breeding in addition to physical processing methods.

Abbreviations

RMC	rapeseed-mustard cake
GLS	glucosinolates
DM	dry matter

SCN^-	thiocyanate
T_3	triiodothyronine
T_4	thyroxine
CP	crude protein
ADG	average daily gain
PSW	Pre-slaughter weight

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