GLUCOSINOLATES IN, MUSTERD AND RAPESEED OIL CAKES AND THEIR INFLUENCE ON THYROXINE SECRETION RATE AND GROWTH OF CROSSBRED CATTLE

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
IN PARTIAL FULFIMENT OF THE REQUIREMENT
FOR THE DEGREE OF

DOCTOR OF PHYLOSOPHY

IN

ANIMAL NUTRITION

BY

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DIVITION OF DAIRY CATTLE NUTRITION
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(I. C. A. R.)
KARNAL-132 001 (HARYANA), INDIA

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To my parents

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SENIOR SCIENTIST

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DATED MAY 31 , 1991.

C E R T I F I C A T E

This is to certify that the thesis entitled **"GLUCOSINOLATES IN MUSTARD AND RAPESEED OIL CAKES AND THEIR INFLUENCE ON THYROXINE SECRETION RATE AND GROWTH OF CROSSBRED CATTLE"** submitted by Mr. **AMRISH KUMAR TYAGI** in partial fulfilment of the requirement for the Award of the **DEGREE OF DOCTOR OF PHILOSOPHY** in **ANIMAL NUTRITION** of the National Dairy Research Institute (Deemed University), Karnal (Haryana) India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

(K.K.SINGHAL)
MAJOR ADVISOR & CHAIRMAN (GUIDE)

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LIST OF ABBREVIATIONS

ADF	:	Acid Detergent Fibre
ARC	:	Agriculture Research Council
BARC	:	Bhabha Atomic Research Centre
B.wt.	:	Body weight
CD	:	Critical Difference
CO_2	:	Carbon dioxide
CF	:	Crude Fibre
CH_4	:	Methane
Cm	:	Centimeter(s)
CP	:	Crude protein
CPM	:	Count per minute
DCP	:	Digestible crude protein
DM	:	Dry matter
EE	:	Ether extract
ffa	:	Free fatty acids
Fig	:	Figure
G	:	Girth
g	:	gram(s)
GNC	:	Groundnut cake
h	:	hour
H	:	Height
Hb	:	Haemoglobin
H_2SO_4	:	Sulphuric acid
HGRSM	:	High glucosinolate rapeseed meal
I/V	:	Intra-venous
K	:	Thyroxine disappearance rate
Kg	:	Kilogram
$KgW^{0.75}$	:	Metabolic body weight
L	:	litre(s)/Length
LGRSM	:	Low glucosinolate rapeseed meal
M	:	Molar
MC	:	Mustard cake
ME	:	Metabolizable energy
Meq	:	milliequivalent
Mcal	:	Mega calories

....contd.

Mg	:	Milligram
ml	:	millilitre
mm	:	millimeter
Min	:	Minute
N	:	Nitrogen
NDF	:	Neutral detergent fibre
NFE	:	Nitrogen free extract
ng	:	nanogram
NRC	:	National Research Council
OM	:	Organic matter
PBI	:	Protein bound iodine
PT	:	Plasma thyroxine
%	:	Percent
R^2	:	Coefficient of variation
RH	:	Relative humidity
RIA	:	Radio-immunoassay
rpm	:	Revolutions per minute
RSC	:	Rapeseed cake
RSM	:	Rapeseed meal
SBM	:	Soyabean meal
SE	:	Standard error
SRL	:	Trichloro acetic acid
TDN	:	Total digestible nutrients
TDS	:	Thyroxine distribution space
T_3	:	Tri-iodothyronine
T_4	:	Thyroxine
Temp.	:	Temperature
TH	:	Thyroidial hormones
TRH	:	Thyrotropin stimulating hormone
TSH	:	Thyroid stimulating hormone
TSR	:	Thyroxine secretion rate
TVFA	:	Total volatile fatty acids
uCi	:	Micro-curie
ug	:	Microgram
ul	:	Microlitre

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CHAPTER - I

INTRODUCTION

1. INTRODUCTION

The total oil seeds production in India is expected to be 19 million tonnes in 1990-91, the highest ever achieved in the past. As a matter of fact, the buoyancy in the oilseed sector started after establishing the Technology Mission in 1986-87 which gave the major thrust for oilseed production. India is the leading producer of Cruciferous oilseeds (**Brassica juncea, B.campestris, B.napus**) and about 90 percent of mustard/rape seed production is concentrated in six states namely, U.P., Rajasthan, Haryana, M.P., Gujarat and Punjab. The production of mustard seed in the country has taken a quantum jump during the **rabi** season of 1990-91 with the production estimate of 5.5 to 6.0 million tonnes, about 35 to 45 percent more than the previous year's production. The production of rape seed alone stood at 5.5 million tonnes during the same period. The combined production of mustard and rape seed surpassed the production of groundnut which used to be on top of the total oilseeds production in the country. Mustard (**B.campestris**) commonly known as **sarson** is a **rabi** crop and grown either alone or in combination with wheat, while rape seed (**B.campestris Var.toria**), known as toria, is a short duration catch crop taken between **rabi** and **kharif** seasons. The colour of mustard varies from pale yellow to dark brown while rape seed is generally dark brown in colour, smaller in size and more pungent than mustard seed.

Mustard and rape seed are grown primarily for their high oil content (about 40%) which contains high contents of erucic acid and other polyunsaturated fatty acids. The oilcakes of these oilseeds are utilized for livestock feeding but their use is restricted due to the presence of glucosinolates (previously known as thioglucosides). The glucosinolates check palatability and create goitrogenicity in animals (Hill, 1979). The problems of erucic acid in oil and glucosinolates in oil cakes attracted the attention of scientists world over. In some of the European countries scientists have evolved varieties of mustard and rape seed containing low erucic acid and glucosinolates such as Tower, Span, Bronowski, etc. In India efforts have been mainly directed towards higher oil yield and suitability of varieties under different agro climatic conditions. However,

possibility of changes in glucosinolate content with the variety cannot be ruled out.

Glucosinolates, present in the plants of Cruciferae family, are hydrolysed under the influence of an endogenous enzyme known as myrosinase, into a range of products. This enzyme is also produced by rumen microorganisms. The hydrolysing products essentially include glucose and the acid sulphate ions. The organic aglycone may undergo various re-arrangements, producing isothiocyanates, thiocyanates or nitriles. One of the main glucosinolates in rape seed meal has been the trivial name progoitrin which gets converted into the goitrin and causes goitrogenicity. Few products are volatile and strongly pungent, being responsible for the pungent or biting taste of mustard, rape seed etc. The ingestion of large amounts of glucosinolates may reduce feed intake, enlarge thyroid gland and reduce levels of circulating thyroid hormones which adversely affect the production performance of livestock.

In India mustard and rape seed oilcakes are utilized for feeding the ruminants since antiquity. Certain myths are associated with these oilcakes such as mustard oilcake is better than rape seed oilcake and in summer season mustard oilcake is superior to all other oilcakes as it gives cooling effect to the animals. Farmers feed water-soaked mustard/rape seed oilcake alongwith wheat straw and other ingredients by making **saani** particularly in summer season but such feeding system is subsequently discontinued with the onset of monsoon. Therefore animals are exposed to mustard cake supplemented ration only for few months. Farmers also have a belief that the mixing of common salt reduces the pungency of rape seed oilcakes and the milk of lactating animals fed on mustard/rape seed oilcakes exhibits longer shelf-life. There is no scientific data to substantiate these observations of the farmers. Contrary to the age old practice of mustard/rape seed oilcake feeding without any adverse impact on ruminants, scientific reports exhibit the few evidences of goitrogenic fraction(s) of these oilcakes. The contraditions of traditional and time tested practices and scientific reports prompted us to resolve the issues.

Oil cakes are proteinaceous materials having high dry matter (90-95%) and low lipid contents (1-10%). These are commonly used in the production of compounded feedstuffs which inevitably entails storage of both the raw materials and finished feeds. Expeller pressed oilcakes are also stored in the solvent extraction plants for quite some time during

which oilcakes are exposed to the changes in ambient temperature and relative humidity. Temperature and humidity not only influence the rate at which chemical changes take place but also the growth of insect pests and fungi e.g. **Aspergillus flavus** causing the aflatoxins production particularly in the groundnut oilcake. Moreover, high losses of oil content of groundnut cake during storage period of 8 to 11 months was reported (Sabale **et al.**, 1975) due to lipolysis, oxidation and polymerization. Kehar **et al.** (1956) did not find such a heavy loss of oil even in **ghani** pressed oilcakes which were supposed to contain higher oil content than expeller pressed or solvent extracted oil cakes. Defromont and Delahaye (1961) recommended that the moisture level of oilcakes must not exceed 10 percent and these should be stored in sacks rather than in bulk preferably in dark places for their safe storage. However, effect of storage on glucosinolates and highly unsaturated long chain fatty acids of mustard and rape seed oilcakes, which are important in the overall utilization of these cakes for livestock feeding, have not been reported so far.

Keeping the above mentioned aspects in view, present investigation has been initiated with the following objectives:

(i) To analyse the chemical composition of mustard and rape-seed oilcakes and the Indian varieties of these oilseeds with special reference to their glucosinolate content.

(ii) To study the effect of glucosinolate and oil contents of mustard oilcake on rumen fermentation in **in vitro** system.

(iii) To investigate the influence of glucosinolates of mustard and rape seed oilcakes on the growth, nutrient utilization and thyroxine secretion rate in crossbred calves.

(iv) To study the effect of storage on the chemical composition of mustard, rape seed and groundnut oilcakes.

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CHAPTER - II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Production of mustard and rape seed in 1990-91 has surpassed the previous records due to the new technology and strategy applied for oilseed production to achieve self-sufficiency. India has one of the largest areas in the world under oilseed crops and also the biggest importer of edible oils, a paradox which is hard to explain. It was with this background that the Technology Mission on oilseeds was set in 1986 in order to harness the best of production, processing and management technologies to accelerate self reliance. Besides India, Canada, USA and few European countries are the chief producers of rape seed.

Mustard/rape seed oilcake is consumed in the country for feeding ruminants because neither it is exported nor included in the ration of poultry. Mustard/rape seed oilcakes are traditionally used for the feeding of cattle and buffaloes and farmers prefer these over other oilcakes especially in the summer season. It is a common experience that these oilcakes contain pungent smell due to their glucosinolate contents which are goitrogenic and affect the animal's performance, however, no such report is available in the country where these are being traditionally used for livestock feeding.

2.1 CHEMICAL COMPOSITION

The knowledge of the chemical composition of mustard cake/rape-seed meal is rather incomplete, yet it is useful to examine this area to obtain a better understanding of the various factors that affect the nutritional value of the meal.

Rape seed contains 16.5 to 18.7 percent hulls of its weight (Appelqvist and Ohlson, 1972) equivalent to about 30 percent of oil free rape seed meal. Hulls contain about 12 to 24 percent lignin and 12 to 16 percent CP (on dry matter basis) and digested poorly. Commercial rape seed meal (RSM) contains 8-12 percent crude fibre, most of which is derived from hulls. The crude protein varies from 36 to 42 percent and ether extract from 1 to 12 percent depending upon the method of oil extraction, i.e.,

ghani, expeller or solvent extraction. Dhindsa and Gupta (1974) compared the chemical composition of the 30 strains of **raya** (**B.juncea** coss.) and reported that the oil content in the seeds varied from 28.45 to 41.45 percent whereas, the variation in CP content in the defatted oilcakes was from 39.38 to 46.08 percent. Though proximate composition of mustard and rape seed oilcakes seems to be similar, however, differences in their DM and N degradabilities due to the variations in the varieties of oilseeds and their processing conditions cannot be ruled out. Fiems **et al.** (1985) reported higher protein solubility of RSM than the soybean meal inspite of difference in their glucosinolate content. Negi **et al.** (1989) reported highest effective degradability of mustard cake than other oilcakes. Kumar and Walli (1989) reported lower effective DM degradability of RSC (**toria** cake) than groundnut cake, however, their effective CP degradabilities were in reverse order. They reported the RDP of rape seed and groundnut cakes as 14.65 and 37.08 percent, respectively and corresponding values of UDP as 19.36 and 8.78 percent which showed the higher UDP content of RSC than groundnut cake.

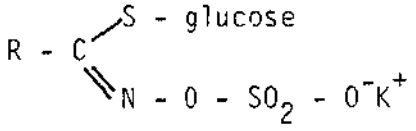
The protein value of RSM has been reported to be higher than the majority of other vegetable protein supplements due to its lysine and sulphur amino acids. Digna Ballester **et al.** (1970) estimated the biological quality of 12 samples of RSM and reported that all samples showed a high protein content and their amino acid pattern with a score of 80 with adequate level of available lysine. Bell and Jeffers (1976) compared the amino acid composition of oilcakes and reported that RSM and soybean meal (SBM) were comparable and RSM contained more sulphur amino acids and slightly lower lysine. Bourdon and Aumaitre (1990) reported similar results and concluded that amino acid content of RSM was influenced by the glucosinolate content of the meal. Singhal (1986) also reported the higher levels of sulphur amino acids and lysine in mustard and rape seed (toria) oilcakes than in groundnut cake. Besides amino acids, Singhal and Mudgal (1984) reported higher macro (Ca, P, Na, K, Mg) and micro minerals (Cu, Zn, Co, Mn) in mustard and rape seed oilcakes than in groundnut cake, however, differences between mustard and rape seed oilcakes were not high. Fenwick (1982) reported that bioavailability of minerals from RSM is lower than SBM.

2.2 FATTY ACID COMPOSITION OF MUSTARD/RAPESEED OIL

In India, expeller pressed mustard cake is generally used for livestock feeding which contains about 8-10 percent oil. The mustard oil contains highest percentage of poly unsaturated fatty acids among all the commercial oils/fat sources available for ruminants through oilcakes (Gohl, 1975). Eskin and Frenkel (1977) also reported the higher content of unsaturated fatty acids in rape seed oil than in soybean oil. They reported that rape seed oil contains Oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) as 56.1, 24.3 and 12.3 percent, respectively and the corresponding values for soybean oil were 46.6, 38.2 and 43.2 percent. Fatty acid composition of rape seed oil depends upon the variety and erucic acid (22:1) content of oil varied in the range of 2 to 25 percent depending upon the variety of rape seed and its complete removal was not possible (Ackman, 1977).

2.3 Glucosinolates

Presence of glucosinolates (formerly known as thioglucosides) in mustard/rape seed meal represent the single most important factor limiting its potential as a protein supplement. Glucosinolates are thioethers containing an organic aglycone:

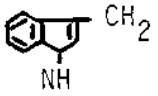
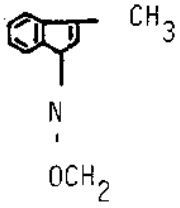


Where, R is an alkyl group. These are hydrolyzed by glucosinolases to B-D-glucose, HSO_4 and derivatives of the aglycone including isothiocyanates, nitriles, thiocyanates or similar structure. There are 50 identified glucosinolates of different chemical structure and VanEtten **et al.** (1969) have reviewed the natural glucosinolates found in foods and feeds. They occur widely in cultivated plants particularly in Cruciferae family. Most of the glucosinolates containing cruciferous that are important in animal nutrition, are in the genus Brassica.

The nature and amount of glucosinolates in rape seed meal (RSM) have been reviewed by Appelqvist and Ohlson (1972), Robbelin and Thies (1980), Larsen (1981) and Fenwick **et al.** (1983). Six glucosinolate

components are of significance in RSM (Table 2.1).

Table 2.1 Major glucosinolates of rape seed meal

Glucosinolates	Semi-systemic nature	R	Molecular weight of glucosinolates
Progoitrin	3-OH-3-butenyl	CH_2=CH-CHOH-CH_3	428
Gluconapin	3-butenyl	CH_2=CH$(CH_2)_2$	412
Glucobrassicanapin	4-butenyl	CH_2=CH$(CH_2)_3$	426
Napoleiferin	2-OH-4-pentenyl	CH_2=CH-CH_2-CH(OH)-CH_2	442
Glucobrassicin	3-indolyl-methyl		487
Neoglucobrassicin	1-methyl-3-indyl methyl		517

The glucosinolates are hydrolysed by an enzyme system myrosinase (glucosinolase or thioglucosidase) found in the plant and is released when plant material is crushed (masticated). The enzyme is also produced by rumen microorganisms. Upon hydrolysis at neutral pH, glucose and sulphate ions are split off and free (aglycone) goitrogenic compounds evolve such as oxazolidinethione (goitrin cyclizing from 2-hydroxy-3 butenyl isothiocyanate), various isothiocyanates and thiocyanates. Progoitrin, one of the main glucosinolates in rape seed meal is converted to goitrin (Fig 2.1) which has goitrogenic activity. Under some hydrolysis conditions such as low pH, nitriles are produced (Larsen, 1981) which may be more toxic than the usual products although the thyroid is not the primary organ

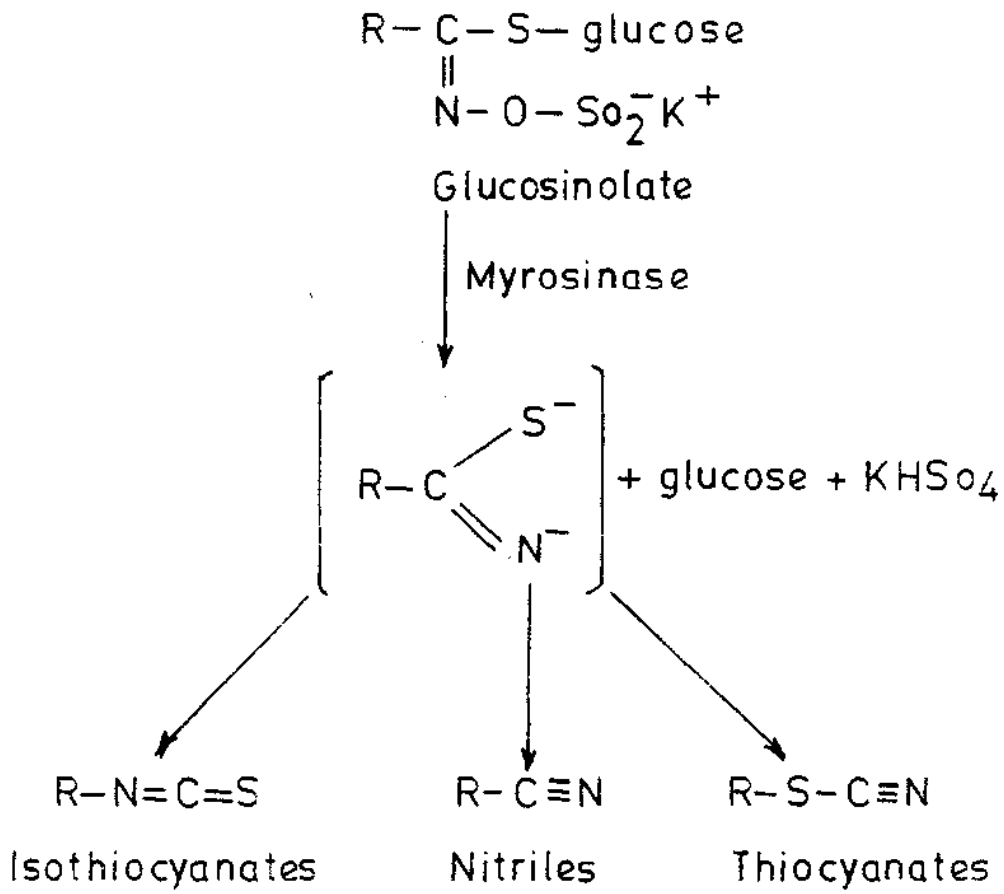


FIG. 2.1 HYDROLYSIS OF GLUCOSINOLATE IN DIFFERENT PRODUCTS

affected (VanEtten **et al.**, 1969). The glucosinolates present in RSM are biologically inactive (Appelqvist and Ohlson, 1972), however, the products of their hydrolysis during processing of seed and after ingestion of RSM are goitrogenic.

Seed coats or hulls of rape seed contain relatively small amount of glucosinolates (Josefsson, 1970). Evaluation of glucosinolate contents and of changes associated with plant breeding and seed processing are complicated by differences in assay methods and methods of expression of glucosinolate concentration. Some authors have expressed concentrations as mg/g, µmol/g or percent of oil free meal or seed. Some reported as total glucosinolates, some as specific glucosinolates and some as cleavage products (aglycones). Efforts have been made to present the total glucosinolates as percent of oil free meal of various cultivars of mustard/rape seed and other glucosinolates containing oil meals of different origin (Table 2.2).

Table 2.2 Glucosinolates content of different varieties of rape seed meal and other oil meals from various countries (% on DM basis)

Oilseed meal and its variety	Place	glucosinolate content/ range	Reference
(1)	(2)	(3)	(4)
Crambe	U.S.A.	11.1	McGhee **et al.**(1964)
Crambe abyssinica	U.S.A.	9-11	VanEtten **et al.**(1965)
B.napus	Sweden	6.85	Appelqvist and
B.campestris	-do-	3.77	Josefsson (1967)
Crambe abyssinica	-do-	4.07	-do-
White mustard (**Sinapis alba**)			
Cultivars Sv Seco	Sweden	7.91-10.12	Josefsson (1970)
Sv Trico	-do-	8.27-9.30	-do-
Sv 0405	-do-	8.37-8.94	-do-

....contd.

(1)	(2)	(3)	(4)
B.campestris Var dichotoma	U.S.A.	6.54	VanEtten **et al.**
B.campestris Sarson	U.S.A.	6.99	(1974)
B.campestris Toria	U.S.A.	6.84	-do-
B.juncea	U.S.A.	7.01	-do-
B.napus	U.S.A.	5.48	-do-
Rapeseed Tower	Canada	0.87	Papas **et al.**(1979)
Rapeseed Turret	Canada	1.54	-do-
Rapeseed Target	Canada	4.49	-do-
B.napus Target	Canada	4.51	Campbell **et al.**
B.napus Turret	Canada	1.55	(1980)
B.napus Tower	Canada	0.44	-do-
B.campestris Candle	Canada	0.50	-do-
Rape seed Tower	Canada	4.77	Laarveld **et al.**
Rape seed Midas	Canada	3.26	(1981a)
Rape seed meal	Europe	4-8	Fenwick (1982)
	Canada	3-5	-do-
B.campestris Torch	Canada	3.98	Bell (1984)
B.campestris Candle	Canada	1.18	-do-
B.napus Midas	Canada	6.58	-do-
B.napus Regent	Canada	1.10	-do-
B.napus Diamant	Europe	6.68	-do-
B.napus Erglu	Europe	0.65	-do-
Rape seed meal (HG)	Belgium	1.29	Fiems **et al.**(1985)
Rape seed meal (LG)	Belgium	0.10	-do-
Rape seed meal	France	5.76	Fenwick **et al.**(1986)
Rape seed meal	U.K.	3.68	Smith and Dacombe (1987)
B.napus	Britain	13.87	Stedman and Hill
B.campestris Canola	Canada	3.43	(1987)

....contd.

(1)	(2)	(3)	(4)
B.campestris Loras	Britain	0.76	Stedman and Hill
B.campestris Tower	Canada	1.44	(1987)
Rape seed meal (Solvent)	France	5.35-8.85	Bourdon and
Rape seed meal(Dehulled)	France	6.46	Aumaitre (1990)

The variation in glucosinolate content of RSM as evident from table 2.2 may be attributed to variety of seeds as well as the factors affecting rate and degree of myrosinase inactivation during cooking stage, amount of thermal decomposition in the desolventizing stage and the method of estimation as these factors may affect the amounts and hydrolysis of glucosinolates. Campbell **et al.** (1980) showed that some hydrolysis of glucosinolates may occur during commercial processing, even though it appears desirable to minimize such hydrolysis for the production of best quality edible oil and meal.

Intact glucosinolates are highly water soluble and can be extracted easily (VanEtten **et al.**, 1969), however, enzymic conversion of allylglucosinolate of mustard (**B.juncea**) into volatile allylisothiocyanate is the most promising technique to overcome the problem of glucosinolates (Mustakas **et al.**, 1965). The same technique is traditionally used in India for the utilization of mustard and rape seed meals as these are soaked into the water for few hours before feeding especially in the summer season and during this period allylisothiocyanate produced as a result of enzymatic reaction might have volatilized and glucosinolate content in the soaked meal reduced considerably.

Keeping in view the limitation of high glucosinolate content of rapeseed (**B.napus**, Cv Midas, Span, Torch) meal in livestock ration certain low glucosinolate and/or low erucic acid cultivars of rape seed such as Regent, Tower, Candle and Bronowski, 1780, and few others have been developed in Canada and Europe. In India efforts have been directed to develop the cultivars of Brassica species to get the higher yield of oil. The oil seeds of these cultivars are found to contain variable levels of oil, protein and allylthiocyanate (Dhindsa and Gupta, 1974), however, report about glucosinolate content of Indian rape seed and mustard of their oil meals is not available.

Dhindsa **et al.** (1975) reported the allylisothiocyanate content of eleven Brassica species and the values ranged from 0.317 to 0.545 percent with an average value of 0.388 percent in the whole seeds. Besides glucosinolates, rape seed meal contains sinapine 1 percent which is an ester of sinapic acid and choline and responsible for its bitter taste (Appelqvist and Ohlson, 1972) and palatability problem. Tannins (20-30 g/kg) are mainly found in seed coat of these oil seeds.

2.4 EFFECT OF GLUCOSINOLATES ON PALATABILITY

The palatability or acceptability of concentrate mixtures based on RSM as a protein supplement depends mainly on the level of its inclusion, the type of and level of glucosinolates and the process of which the RSM had been subjected. The acceptability is of great importance for high producing animals such as fast growing young animals and high yielding cows, particularly during peak lactation.

The pungent smell of hydrolysis products affects the palatability of rations supplemented with rape seed/mustard oilcakes. The diets containing low glucosinolate varieties of rape seed meal were consumed more readily than those containing high glucosinolate variety in pigs. To overcome the palatability problem of glucosinolates, additions of molasses or other flavouring agent showed only marginal improvement (Ingalls and Sharma, 1975). The inclusion of high glucosinolate rape seed meal at 15-20 percent of the concentrate for dairy cattle did not have any adverse affect on milk composition and milk production (Laarveld **et al.**, 1981a).

Stake **et al.** (1973) reported significantly lower daily grain mixture intake when RSM and SBM were compared in the diet of calves from birth to 14 weeks and as a result of this difference in feed intake, daily weight gain and feed efficiency ratios were influenced. Papas **et al.** (1979) reported the lower feed intake when high glucosinolate type RSM (HG-RSM) was included in the diet of calves at 25 percent level, than those fed on low glucosinolate RSM (LG-RSM) at similar level. Wheeler **et al.** (1980) used LG-RSM (Tower) as a sole protein supplement in the diet of weaning calves and reported no adverse affect on daily feed intake upto the age of 12 weeks. From experiments with growing bulls (> 100 kg live wt), it was concluded that HG-RSM can be included in grain mixtures upto

a level of 12 percent without negatively affecting feed intake and production traits (Olsson, 1978). As an upper limits of HG-RSM for large animals, they recommended 0.2 kg and for smaller animals 0.3 kg of HG-RSM/100 kg live weight. Wernli **et al.** (1973) concluded that RSM upto the levels of 16 percent may be successfully fed to growing calves at an age of 60-140 days.

The palatability of concentrate mixtures containing RSM depends on the age of the animals. Only limited amounts of RSM, at least of HG-type, are recommended for animals below 100 kg live weight. Beyond this weight substantial amounts of RSM can be used without impairing consumption. Canola Council of Canada has recommended the level of inclusion of high glucosinolate rape seed meal (HG-RSM) or low glucosinolate rape seed meal (LG-RSM) in calves ration as 200 g/kg concentrate mixture where as recommended level for HG-RSM and LG-RSM for dairy cows as 100 and 250g/kg concentrate mixture, respectively (Fenwick, 1982).

2.5 GOITROGENICITY OF RAPE SEED/MUSTARD OILCAKES

Glucosinolates **per se** apparently are non-toxic, however, several microbial species inhabiting the gastrointestinal tract were shown to have appropriate enzyme system for achieving glucosinolate hydrolysis. However, more pronounced glucosinolate breakdown and greater goitrogenicity seems to result from the addition of myrosinase to the diet (Bell, 1965) than has been found to occur in pigs or mice as a result of microbial actions in the gastrointestinal tract. Eggum **et al.** (1986) reported fast turnover and metabolism of glucosinolates in animals.

The first evidence that RSM contained goitrogenic properties apparently was that of Kennedy and Purves (1941) with rats. Subsequently, swine and poultry showed similar effects (Bell and Belzile, 1965), but until Iwarsson **et al.** (1973) and Geavy and Beranger (1975) reported evidence of goitre in growing bulls, it was assumed that ruminants might not be affected by rape seed goitrogens.

Most, if not all, of the glucosinolates found in rape seed may yield goitrogenic products after hydrolysis, although the modes of action may vary. Now it is clear from various reports that oxazolidinethione, various isothiocyanates, thiocyanates and certain nitriles are variously

capable of depressing iodine uptake, iodification, T_3:T_4 (Tri-iodothyronine:thyroxine) ratio and thyroid histology (Lo and Hill, 1971; Bell **et al.**, 1972; Nishie and Daxenbichler, 1980). Combination of glucosinolate hydrolytic products may show more pronounced effects on thyroid size and function than equivalent amounts of individual compounds administered singly (Langer, 1966).

2.5.1 Influence on thyroidal hormones

Thyroid gland has the unique capability to accumulate iodine and combine it into hormones-thyroxine (T_4) and 3-3', 5-triiodothyronine (T_3). The regulation of thyroidal hormones (TH) secretion by the thyroid gland is achieved by the interaction of their groups of hormones. Thyrotropin releasing hormone (TRH) stimulates the release of thyrotropin stimulating hormone (TSH) which in turn activates iodine uptake and releases thyroid hormones T_4 and T_3. Though in fact every cell of the body is a target of these hormones, yet a generalization can be made that the TH among other functions regulate growth and oxidative metabolism.

The hydrolytic products of glucosinolates mainly 5-vinyloxazolidine-2-thione (OZT) causes the thyroid enlargement. Papas **et al.** (1979) reported that T_4 levels in SBM, LG-RSM (Tower) and HG-RSM (Turret) fed cows were 4.2, 3.4 and 3.3 µg/100 ml plasma and the variation among groups was significant, however, effect on plasma T_3 levels was non-significant. In contrast to this, in an earlier experiment they reported that RSM had no goitrogenic effect (Papas **et al.**, 1978). They concluded from their experiments that T_4 levels in mature cattle are not accurate indicators of the changes in the size and histology of thyroid gland. They specifically observed that calves with enlarged thyroids maintained normal levels of T_4 and T_3 probably due to compensatory production of hormones by an enlarged thyroid. From the histology of calf thyroid they suggested that increased size of thyroid following the feeding of RSM was probably caused by intact glucosinolates and their metabolic products which are still unknwon. However, critical appraisal of their data indicated that goitrogenicity was possibly due to the combination of low iodine intake and the high glucosinolate content of RSM. Sharma **et al.** (1977) reported significantly higher serum thyroxine (T_4) level in cows fed SBM than those fed on RSM, however, RSM feeding to the level of 25 percent in concentrate mixture did not affect the milk production, adversely.

Laarveld and Christensen (1976) reported that feeding of HG-RSM caused hypo-thyroidism in lactating cows than those fed on LG-RSM or SBM containing complete diets. However, milk production was similar in all groups. It was concluded that RSM irrespective of their glucosinolate content was equivalent to SBM as a source protein supplement. Claypool **et al.** (1985) reported that canola, cottonseed or soybean meals feeding to calves did not affect their feed intake, plasma T_3 and T_4, significantly.

Thyroxine and protein bound iodine (PBI) tended to decrease and blood thiocyanate levels increased with increasing levels of dietary RSM in pig ration (Ochetim **et al.**, 1980), however, Paliwal **et al.** (1976) did not find any adverse effect on blood PBI of Haryana calves and Murrah buffaloes by feeding them mustard cake as an exclusive source of dietary protein.

Levels of plasma T_3 and T_4 are relatively insensitive index while evaluating the goitrogenic effects. A more sensitive index of thyroid functions is the thyrotropin releasing hormone (TRH) test (Ormston **et al.**, 1971). Using this test Laarveld **et al.** (1981b) demonstrated that feeding of HG-RSM at the level of 13.2 and 18.9 percent of concentrate mixture had goitrogenic effect to increase the pituitary sensitivity to TRH and to decrease the ability of the thyroid gland to secrete T_4. They also reported that T_3:T_4 ratios were not influenced by the type and level of RSM. Ahlin **et al.** (1987) also observed the significantly higher TSH levels in a TRH test in RSM fed cows than those fed on control diets, however, differences for milk yield among groups were non-significant.

2.5.2 Thyroxine secretion rate

Goitrogens have been shown to influence the thyroid activity through a direct effect on the pituitary TSH (Florsheim **et al.**, 1966) or on the thyroid gland (Topel and Merkel, 1966; Florsheim **et al.**, 1966). The best method to determine thyroid activity is through the determination of rate of thyroxine secretion. In fact, no effort has been made for determining the thyroxine secretion rate (TSR) following the feeding of RSM or mustard cake. However, feeding of leucaena seed, known for its goitrogenic content, i.e., mimosine, reduced the plasma T_4 levels and TSR in goats (Chakraborty, 1985).

2.5.3 Blood parameters

Papas **et al.** (1979) reported that feeding of HG-RSM (target) reduced certain blood parameters namely packed cell volume, haemoglobin, and erythrocyte counts. Other workers also reported similar observations following the feeding of high levels of RSM to dairy cows (Laarveld and Christensen, 1976), however, low dietary level of RSM did not appear to affect the blood parameters (Iwarsson **et al.**, 1973).

It is evident from above reports that thyroid function is affected by the feeding of RSM without much effect on the production performance of animals and the extent of effect depends upon the dietary levels of RSM and its glucosinolate content.

2.5.4 Effect of glucosinolates on rumen fermentation

The carbohydrate and protein moieties of protein supplements degrade in the rumen as a result of microbial fermentation into the organic acids, amino acids, ammonia carbon-dioxide and methane. Laarveld and Christensen (1976) used three complete diets containing SBM, HG-RSM and LG-RSM as protein supplements and taking into consideration of TVFA and its fractions did not find any adverse effect of glucosinolates on rumen fermentation and protein utilization.

Hunt **et al.** (1990) conducted **in vitro** and **in situ** trials to determine the effect of glucosinolate of rape seed forage of HG- and LG-varieties. **In vitro** results indicated that glucosinolates contained in HG-forage did not interrupt fermentation and the HG- was in fact more degradable than LG- as indicated by total VFA concentration. They concluded from their results that glucosinolates had no detrimental effect on digestion.

Huntaven **et al.** (1986) gave a basal diet with 50 percent grass silage and 50 percent rolled barley to the bulls in control group. In experimental group 30 percent barley was replaced with crushed rape seed. They reported lowered TVFA and ammonia-N concentration in rumen liquor of experimental group than that of control group. Rapeseed incorporation also increased the proportion of propionic acid and decreased the proportion of butyric acid in rumen liquor.

Naumenko **et al.** (1987) reported that replacement of sunflower meal with 10 or 15 percent RSM in diet of bulls did not adversely affect total VFA's or their molar proportions in rumen fluid.

2.6 EFFECT OF UNSATURATED FATTY ACIDS OF MUSTARD OIL ON RUMEN FERMENTATION AND METHANE PRODUCTION

Methane production during the process of ruminal fermentation limits the overall energetic efficiency of metabolism in the ruminant system. It has been reported that about 6 to 10 percent of dietary energy is wasted in the form of methane. Various methods such as changing the nature of the animal diet, use of methane inhibiting chemicals and manipulation of rumen ecosystem have been directed towards decreasing the methane production and thereby increasing the overall energetic efficiency of metabolism of nutrients.

Part of the methane, produced by microorganisms in the digestive tract of ruminants, arises from the reduction of carbon dioxide. This reduction accompanies the oxidation of formic acid in the rumen (Carrol and Hungate, 1955). Since the CO_2 produced is identical with the CO_2 pool of the rumen (Williams **et al.**, 1963), it is possible that hydrogen acceptors other than CO_2 added to the rumen might reduce methane production. They reported that intraruminal infusion of linolenic acid reduced the methane production and the reduction in methane production was considerably greater than that expected even assuming that all the three double bonds of the linolenic acid had been hydrogenated.

Jenkins (1987) conducted 4 **in vitro** trials to determine how ruminal fermentation is affected by source of fat, level of fat and combination of fatty acids. They reported that increasing level of fat caused no change in VFA levels except to decrease butyric acid from 12.1 to 9.9 percent of total VFA. They also reported that increasing the level of unsaturation of fatty acids decreased acetic and butyric acids, while increasing propionic acid. Czerkawski (1973) showed that incorporation of large amounts of linseed oil in the diet decreased the concentration of VFA in the rumen and acetic and butyric acids were mainly affected.

Henderson (1973) and Palmqvist **et al.** (1986) showed a detrimental effect of polyunsaturated fatty acids on rumen fermentation however, Chalupa **et al.** (1984) showed that long chain fatty acids do not interfere with ruminal fermentation.

Ferguson **et al.** (1990) showed that addition of fatty acids substrate (C16:0, 0.47%; C18:0, 36% and C18:1, 14%) in an **in vitro** rumen fermentor at different levels had no effect on total VFA production; however, acetate:propionate ratio reduced at higher level (15-20%) of substrate. Czerkawski **et al.** (1975) showed that concentration of VFA was reduced on high fat diet but the capacity of rumen contents to produce VFA (**in vitro**) increased.

Czerkawski **et al.** (1966a) reported lowered methane production by infusion of unsaturated fatty acids in the rumen. They showed that oleic acid (18:1) having 1.00 double bond per mole depress methane production by 1.70 moles per mole fatty acid. Similarly, linoleic (18:2) and linolenic acid (18:3) with a mean unsaturation of 1.72 and 2.40 double bonds per mole, respectively, depressed methane production to an extent of 1.79 and 2.05 moles per mole fatty acids, respectively. Methane production reduced by 13.8, 14.2 and 16.4 Kcal CH_4/100 kcal oleic, linoleic and linolenic acid infusion in the rumen sheep, respectively. The depression of CH_4 production tended to increase with increase in the unsaturation of fatty acids and the unsaturated fatty acids were found to be hydrogenated in the rumen. The introduction of double bond into an acid was calculated to reduce methane production by 0.24±0.09 moles per mole double bond. These results showed that the double bond of fatty acids complete with CO_2 for hydrogen. Czerkawski **et al.** (1966b) also reported the minor cellulose digestion following the infusion of fatty acids. Similar results were obtained in earlier experiment (Czerkawski **et al.**, 1966a) possibly due to the effect by inhibition of growth of gram positive methanogenic bacteria while allowing growth of cellulolytic bacteria, as Nieman (1954) reported that only gram-positive organisms are susceptible to the action of fatty acid in minute amounts.

The effect of fat supplementation on microbial protein synthesis indicated contradictory responses. Czerkawski **et al.** (1975) showed increased number of bacteria and decreased number of protozoa in the rumen of animals receiving the high fat diet.

Knight **et al.** (1978) found that the microbial protein synthesis efficiency increased markedly with the addition of linseed or coconut oils in the diet of sheep, however, no change was reported when cod liver oil was used (Sutton **et al.**, 1970).

It can be observed from this review that glucosinolates of mustard or rape seed does not affect the rumen fermentation, however, incorporation of mustard/rape seed oil, which contains high levels of unsaturated fatty acids (Eskin and Frankel, 1977) may affect the rumen fermentation by reducing the methane production.

2.7 PERFORMANCE OF GROWING ANIMALS

Rape seed and mustard oilcakes are important protein supplements for the feeding of all categories of ruminants, however, their palatability is influenced by their glucosinolates as discussed earlier. In a classical growth trial, Kehar **et al.** (1956) used differently processed mustard cake as a sole source of protein supplement in a 50 weeks duration experiment and reported an average growth rate in solvent, expeller and **ghani** extracted mustard cake fed calves as 390, 370 and 376 g/day and the variation among groups was not significant. Mukherjee and Kehar (1949) reported protein quality of mustard cake (Sarson) was superior than that of groundnut cake when these were fed along with straws. Kehar (1948) used different oilcakes along with paddy straw in the ration of growing calves and reported higher retention of absorbed nitrogen from mustard cake than from groundnut cake. From the data of Lander (1940,1941) there appear to be little difference between **toria** and sarson cakes as regards absorption of N, Ca and P. Thomke (1981) reviewed the effect of rape seed meal (RSM) feeding on the performance of ruminants.

Stake **et al.** (1973) reported inferior growth performance of young calves on feeding RSM (20% of concentrate mixture) as compared to soybean meal (SBM) control. Similarly, Olsson (1978) reported depressed growth in the first seven weeks of age by using 6 and 13 percent HG-RSM calf starter Vs SBM control. However, when calves were more than 80 kg body weight 13 percent level of RSM resulted in equal performance. However, Wood and Stone (1970) fed calves RSM upto 50 percent of the total DM intake without any adverse effect.

Schingoethe **et al.** (1974) showed that low glucosinolate rape seed meal (LG-RSM) can be fed to young calves with faster growth and superior feed efficiency than those fed on HG-RSM and they concluded that LG-RSM can provide 100 percent supplemental protein in the calf starter.

Bush **et al.** (1978) compared the performance of two groups of growing steers by feeding them corn silage, barley and Tower or Candle RSM and reported that growth, feed consumption and feed efficiency of steers in both the groups were not significantly different.

Ingalls and Seale (1971) fed heifers a 13.7 percent RSM diet from birth till first lactation and reported no significant effect on body weight gains, feed intakes, feed efficiency, reproduction or milk production.

Papas **et al.** (1979) compared the growth of calves fed on SBM, HG-RSM (Target) and LG-RSM(Tower) and reported the weight gain as 0.74, 0.52 and 0.75 kg/day, respectively. The results indicated reduced growth rate and feed intake in HG-RSM fed group than those fed on SBM or LG-RSM, however, RSM diets increased ($P \leq 0.01$) the weight of thyroid irrespective of their glucosinolate content as compared to control group. Iwarsson **et al.** (1973) also reported the increased weight of thyroid gland following the feeding of RSM in bulls, however, RSM fed bulls showed significantly higher weight gains, feed efficiency ratios and fat deposition as compared to those fed on SBM control. They suspected a slightly altered hormone secretion rate which might have lowered the basal metabolic rate.

Ahmed and Malik (1982) replaced cottonseed meal with RSM on protein equivalent basis in a year long growth study on Sahiwal calves and reported that RSM can be incorporated upto 70 percent of cottonseed meal without adversely affecting the growth rate and feed utilization.

Fiems **et al.** (1985) included 10 and 20 percent of RSM in the calf starter and compared the performance of calves with those fed on SBM supplemented calf starter. The growth rate averaged 0.80, 0.76 and 0.77 kg/day in SBM, 10 percent RSM and 20 percent RSM containing calf starter fed groups and the variation among groups was non-significant inspite of the lower protein quality and high glucosinolate content in RSM than in SBM.

Schwarz and Kirchgessuer (1989) reported that replacement of SBM with LG-RSM in the diet of 5 month old calves did not affect the feed intake, growth and carcass characteristics during a 336 days feeding experiment. Similarly, Claypool **et al.** (1985) reported that canola, cottonseed or soybean meals feeding to calves did not affect the feed intake, growth and blood triiodothyronine (T_3) and Thyroxine (T_4) significantly.

It is evident from this review that RSM feeding affect the growth of calves but their age and body weight are the main criterion. Only part of the protein requirement of young calves (less than 80 kg body weight) may be given through RSM preferably low glucosinolate type, however, calves over 100 kg body weight can be fed on RSM as a sole protein supplement under practical conditions.

2.8 DIGESTIBILITY OF RAPE SEED AND MUSTARD OILCAKES

The range of digestibility coefficients, i.e., CP, EE, CF, NFE of rape seed and **toria** (expeller) were 84-85, 91-93, 38-44 and 61-74 (Sen **et al.**, 1978). Sauer **et al.** (1982) also reported the protein digestibility of 81 percent in both HG- and LG-RSM. An earlier value of 73 percent CP digestibility (May and Bell, 1971) for HG-RSM may reflect changes in processing conditions in the crushing plants in addition to cultivar differences. The amount of hulls, present in these oilcakes, have significant effect on the nutrient digestibility. Removal of hulls resulted in improved digestibility (Sarwar **et al.**, 1981).

Jarl (1951) reported that RSM organic matter was 75.9 percent digestible and crude protein digestibility was 82.7 percent in lactating cows. Assuming digestibility of gross energy and organic matter to be similar, the DE/kg dry matter was assessed as 3,350 kcal. It was comparable with 3,213 kcal/kg, reported by Wood and Stone (1970) for 100 kg calves, and 3,300 kcal/kg for 150 kg calves (Stake **et al.**, 1973).

Stake **et al.** (1973) found no difference in digestibility of nutrients when commercial RSM, LG-RSM and soybean meal were compared as protein supplements for calves, however, Schingoethe **et al.** (1974) reported the highest apparent nutrients digestibility for SBM and lowest for RSM supplemented complete diets. Sharma **et al.** (1980) replaced 50 percent of a basal diet with SBM and RSM (two types, Tower and Candle) and reported that feeding of RSM reduced the digestibilities of DM, CP and ADF significantly, however, energy digestibility and nitrogen balances were similar in all the three groups. When the nutrient digestibility of three protein supplements were calculated by difference method, non-significant differences were observed in apparent digestibilities of DM, ADF and energy. Crude protein digestibility was significantly ($P<0.05$) higher for SBM than candle RSM and similar to Tower RSM.

Paliwal **et al.** (1976)) replaced 0, 33, 66 and 100 percent crude protein of groundnut cake with mustard cake in the ration of Haryana cattle and Murrah buffaloes and animals were given wheat straw **ad lib** as a source of roughage. They did not observe any significant variation for the digestibility of proximate principles among the treatments and species. Srivastava **et al.** (1962) also did not find any statistical difference in the digestibility coefficients of proximate principles of groundnut cake and RSM supplemented diets fed to Haryana calves.

Bush **et al.** (1978) compared the RSM of two varieties namely Candle and Tower (both low erucic acid, low glucosinolate) for their nutrient digestibility in sheep. The digestibility coefficients of diets containing Candle RSM with respect to dry matter, nitrogen, organic matter, energy, ADF and NDF were significantly higher than those of Tower RSM. This difference was attributed to the variation in the fibre content of both the meals.

Ingalls and Sharma (1975) did not find any variation in the dry matter intake and nutrient digestibility of rations containing a commercial RSM, LG-RSM and SBM. When LG-RSM (Bronowski) was used at 0, 10, 17 and 24 percent in the concentrate mixture for dairy cows, the DM intake, digestibility of different nutrients and nitrogen balances were similar in all the groups. They also reported that pelleting and adding either molasses or flavouring agent in concentrate mixture containing 19 percent RSM did not improve the dry matter consumption.

Laarveld and Christensen (1976) compared the nutrient digestibility of complete feeds containing LG-RSM(1788), HG-RSM (Span) and SBM as sources of protein in the ration of lactating cows and did not observe any variation in the digestibilities of nurients between the two varieties of RSM, however, dry matter and crude fibre digestibilities were lower ($P/0.05$) in SBM feed than those of RSM feeds. The difference in the nutrient digestibility or glucosinolate contents of the feeds did not alter VFA concentration in rumen fluid or milk production.

On comparing the RSM and SBM as exclusive sources of protein in the rations of lactating cows, Waldern (1973) reported that animal consumed RSM and SBM to the tune of 11.8 and 10.6 percent of total daily dry matter intake. Though the digestibility of crude protein was lower ($P/0.05$) in RSM group but nitrogen retention and efficiency of nitrogen utilization were comparable in both the groups.

Guglya **et al.** (1988) reported a reduced digestibility of crude fibre of RSM supplemented ration than that of sun-flower meal supplemented diet.

From the literature about the use of RSM in the diet of growing calves, it can be inferred that glucosinolates and its hydrolysing products were considered the major problems, however, in calves having higher body weight and in lactating cows glucosinolates did not affect the nutrient digestibility.

2.9 BODY COMPOSITION

Knowledge of body water, fat, protein and ash contents of living animals would aid the interpretation of data obtained in many biological experiments. Mitchell (1944) concluded that weight gain of growing animals varied in their water, fat and protein content and therefore, increment in body weight did not necessarily reflect equivalent nutritive effects of different feeds. Maynard and Loosle (1962) had clearly pointed out that it was important to assess the specific effects in terms of protein deposited at cellular level while comparing the efficiency of different protein sources during growth.

Geavy and Beranger (1975) reported significantly lower empty live weight and carcass weight of RSM fed bulls than those fed on groundnut cake (GNC), however, variation for carcass composition between groups was non-significant. At slaughter the thyroid weights of GNC fed animals were lighter than those fed on RSM.

Senger (1979) studied the effect of feeding of protected protein of groundnut cake on body composition of kids and did not find significant variations in the body water, body fat, body protein percentage of untreated, formaldehyde treated or tannic acid treated groundnut cake.

Manget Ram (1989) compared the body composition of three groups of crossbred calves fed on two types of urea-molasses-mineral block and a conventional concentrate mixture along with wheat straw **ad lib** and did not report significant variation in their body composition on live weight basis.

Bourdon and Aumaitre (1990) studied the effect of feeding high glucosinolate containing rape seed meal (HG-RSM) on carcass quality of pigs and reported a linear increase in the liver and thyroid weights with

increasing content of glucosinolate of rape seed meal, however, carcass measurements did not indicate any difference in carcass quality.

2.10 INFLUENCE OF STORAGE ON THE QUALITY OF OILCAKES

Oilseed cakes/meals are normally packed in gunny bags and stored in godowns for varying periods during which climatic conditions also vary. It is well known fact that the quality of stored feedstuffs is affected due to the natural action of enzymes of feed, chemical reactions such as oxidation, hydrolysis and growth of microorganisms. Various factors which influence the quality of stored products are moisture content, ambient temperature, relative humidity, initial quality of product, and type and duration of storage.

The presence of excessive moisture in the oilcakes leads to deterioration on storage and an increase in the free fatty acid (ffa) value. Lakshminarayana **et al.** (1973) reported that the oil value of oilcake decreased during the storage of one year with an increase in ffa content. They have recommended that undecorticated oilcakes should not be stored more than 90 days to avoid the oil loss and increased ffa value. The moisture content of stored product is also conducive for the fungal growth.

Safe storage time depends on a combination of moisture content of feed and ambient temperature (Saul and Lind, 1958) especially for the grains. The requirement for safe storage of feed ingredients made from grains are more exacting than those for whole grains. For example, the safe storage time for soybean meal at a given moisture content is considerably less than the safe storage time for whole soybean at the same moisture content. Animal feeds including grains and oilcakes are hygroscopic and come to a specific moisture content when exposed to air of a given temperature and relative humidity. Relative humidity above 70 percent is conducive to mould growth when temperature are favourable. Lowe and Apelt (1987) reported that oilseeds or oil meals can be safely stored at room temperature with relative humidity (RH) of, upto about 70 percent, however, at higher humidity conditions, the fat of meal deteriorates.

The initial quality of the products affect their quality during storage. In case of oilcakes, their oil content is influenced by the method of oil extraction namely **ghani**, expeller, and solvent extractions.

Kehar **et al.** (1956) studied the effect of storage of sesame, mustard, groundnut and linseed oilcakes processed by all the three methods of oil extraction by storing them in gunny bags in godowns upto 2 years. They reported no adverse affect of storage on the quality of solvent extracted oilcakes except sesame cake, where a decrease in ether extract content accompanied by minor changes in total carbohydrates was reported for the first four months. Same trend was noticed by **ghani** and expeller processed oilcakes. The periods upto which changes occurred in groundnut, sesame, mustard and linseed oilcakes were respectively 5, 12, 12 and 12 months for **ghani** pressed oilcakes and 4, 11, 6 and 12 months for expeller pressed oilcakes. During long term storage of feeds, oxidation of unsaturated fats take place accompanied by off flavours, an increase in total acidity and peroxide numbers with the formation of aldehydes and breakdown of vitamin A and E. However, presence of erucic acid in mustard oil checks its oxidation inspite of its high unsaturated fatty acid content because erucic acid inhibits the activity of lipoxygenases (Ory and St.Angelo, 1975). Moreover, tissue enzymes of oilseeds, e.g., lipases and lipoxygenases are arrested to large extent due to involvement of heat during the oil extraction (Sunde, 1973). The problem of development of rancidity in **ghani** or expeller pressed oilcakes was more than those obtained by solvent extraction method, obviously due to the difference in their oil content (Defromont and Delahaye, 1961). In their another experiment with the storage of groundnut cake (0.86% oil) they reported that storage in sacks in dark conditions was better than that in bulk and light conditions. They recommended that moisture level of the cakes must not exceed 10 percent during storage.

While studying the effect of storage of groundnut cake, Sabale **et al.** (1975) observed that 60 percent of original oil present in oilcake was lost during storage and the recoverable oil was darker and having higher free fatty acids value. Ramakrishna **et al.** (1973) reported that during the storage of niger seed cake for 180 days oil and total protein contents did not change, however, ffa value had risen from 1.4 to 5.5.

Blaha (1980) stored groundnut and soybean meals in bags at 20°C and RH 70-80 percent as well as at 30°C and RH 80 percent and reported non-significant changes in their proximate composition. However, slight decrease in fat content and increase in acid number of fat were observed. In addition to this, groundnut cake was found positive for aflatoxin.

Though storage of oilcakes at high humidity and temperature can accelerate the development of rancidity, however, greater importance is given to the toxin producing moulds such as **Aspergillus flavus** and its strains which flourish at higher humidity and temperature conditions during storage and produce aflatoxins namely aflatoxin B_1, B_2, G_1 and G_2. Aflatoxins production depends upon the moisture of substrate (Christensen, 1957), temperature (Semeniuk, 1954), nature of substrate (Mayne **et al.**, 1966), available oxygen (Shih and Marth, 1974) and relative humidity of atmosphere (Scott, 1957). Optimum temperature for aflatoxin production by **A.flavus** has been shown to be 25°C for aflatoxin B_1 and 30°C for aflatoxin G_1 (Rabie, 1965). Groundnut is highly susceptible to the contamination by aflatoxin and it has been observed that damage to the shell and splitting of kernels which can be caused by insects, drought or poor harvesting practices may contribute to the high level of aflatoxin. Various reports revealed that the feedstuffs including groundnut, linseed, cottonseed, safflower, sesame, sunflower, and other oilcakes available in different parts of India were containing varying levels of aflatoxins (Patel **et al.**, 1981; Singh **et al.**, 1984; Reddy **et al.**, 1984; Salunkhe **et al.**, 1987) but none of the report has indicated about the presence of aflatoxins in mustard and rape seed oilcakes.

As regards the glucosinolates and isothiocyanates in mustard/rape seed oilcake, Velisek **et al.** (1983) reported that these are lost partially during the storage of oil cake at ambient temperature for several months.

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CHAPTER - III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Different experiments were conducted to study the effect of glucosinolates of mustard and rape seed oilcakes on growth and thyroxine secretion rate in crossbred calves. The procedure involved and various analytical techniques followed under different experiments have been detailed phase wise as follows.

PHASE-I

3.1 CHEMICAL COMPOSITION OF OILCAKES AND INDIAN VARIETIES OF MUSTARD/RAPE SEED

3.1.1 Chemical composition of oilcakes

Groundnut cake (GNC), procured in bulk from market by the central stores of NDRI was taken for this study. Mustard cake (MC) and rape seed cake (RSC) were extracted in local oil mill after ascertaining the oilseeds.

3.1.1.1 Proximate composition:-

Proximate composition of GNC, MC and RSC was determined by the standard procedures according to AOAC (1984).

3.1.1.2 Total glucosinolates:-

Total glucosinolates content of oilcakes was determined according to procedure of McGhee et al. (1964) which is based upon the reaction of silver with thioglucosides. The details of procedure are given as follows:

Oilcakes were ground and 10g of each were added to the 200 ml of deionized boiling water and kept boiling for 5 min to inactivate the

enzyme (myrosinase). The enzyme inactivation step did not allow to prolong beyond 5 min to avoid the breakdown of glucosinolates. Thereafter, the contents were filtered on a Buchner funnel. The residue was washed with 50 ml hot water three times and filtered in the same manner. The volume of total filterate was made upto 500 ml. An aliquot of 25 ml was taken in a beaker to which 10 ml solution of 0.1N $AgNO_3$ and 25 ml ethanol (95%) were added. The contents were refluxed on a waterbath for 45 min, cooled to room temperature, volume was made to 100 ml with distilled water and centrifuged. An aliquote of 25 ml of supernatent was taken in 125 ml flask containing 2 ml of 6N nitric acid and 6 ml of 8 percent (w/v) ferric ammonium sulphate solution. The homogenous mixture was titrated to pale salmon colour against a solution of 0.01M potassium thiocyanate. A blank was also run with each determination. The percent glucosinolate was calculated as follows:

$$= \frac{\text{(blank titration)} \times 4 \times 0.01 \times \text{mol. wt.glucosinolate} \times \text{total volume}}{1000 \times 2 \times \text{Sample wt} \times 25} \times 100$$

3.1.1.3 Free fatty acid value:-

The oil content obtained during the ether extract determination of oilcakes was used to estimate the free fatty acids (ffa) value according to the procedure of Rao **et al.** (1972) as follows:

The known quantity of oil content present in the flask was dissolved into 25 ml of neutral ethyl alcohol and 3 drops of phenolphthalein indicator was added. The mixture was titrated against 0.1N NaOH.

$$\text{ffa content as oleic acid} = \frac{V \times N \times 28.8}{W}$$

Where,

V is the volume of 0.1N NaOH used
N is the normality of NaOH
W is the weight of oil

3.1.1.4 Amino acid composition:-

Amino acid composition of oilcakes namely groundnut, mustard and rape seed was determined as follows:

(i) Preparation of protein hydrolysate:-

Defatted oilcake sample (100 mg) was taken in test tube in duplicate and after addition of 5 ml of 6N HCl, it was sealed under the constant supply of nitrogen. The sealed tubes were kept at 110°C for 24h for complete hydrolysis of protein (Roach and Gehrke, 1970). At the time of analysis, seal was broken and the contents were filtered through Whatman filter paper No.1. The residue was washed thoroughly and volume was made to 10 ml in a volumetric flask.

(ii) Derivatization:-

An aliquot of 5 µl of protein hydrolysate was taken and mixed with 10 µl of solution of ethanol, water, triethylamine. After drying the sample under vacuum, 20 µl of derivatizing reagent (ethanol:triethylamine: water:phenylisothiocyarate::7:1:1:1) was added and mixed gently. After keeping the mixture for 20 min at room temperature (20°-25°C) the same was dried again using the vacuum pump. Thereafter, sample was diluted to 200 µl and used for the estimation of amino acids on HPLC.

(iii) Analysis:-

An aliquot (5 µl) of derivatized sample was injected into HPLC System (Waters model 510) fitted with absorbance detector model 440 adjusted at 254 nm and a PICO-TAG™ column (15 cm x 3.9 mm) having high efficiency stationary phase suitable for reverse phase separation of amino acids (Waters, USA). The Waters PICO-TAG eluents A and B were prepared according to the procedure given in operation manual of HPLC and flow rates were adjusted with the help of automatic gradient controller (Waters model 680). The derivatized standard mixture of amino acids (Pierce Chemical Company, USA) and unknown samples were injected under identical conditions. The responses of standard and unknown samples were recorded (Waters data model 745) and peaks were identified and their areas were compared to estimate the quantity of individual amino acid.

3.1.2 Chemical composition of Indian varieties of mustard/rape seed

Seven varieties of mustard/rape seed namely Raya RH 781, Raya RH 2859, Raya RC 781, Toria Shymgarh, Toria TH 83, Toria Sangram and Toria Kranti were procured from All India Coordinated Research Project on

Oilseeds, Haryana Agricultural University, Hissar.

In addition of these, the oilseeds of 5 more varieties, viz. Toria TH 109, B 054, B.Bold, **B.Carinata** and **B.napus** were procured from Indian Agricultural Research Institute, New Delhi. The oilseeds were crushed and analysed for proximate composition (AOAC, 1984), cell wall constituents (Goering and VanSoest, 1970) and total glucosinolates (McGhee **et al.**, 1964).

PHASE-II

3.2 EFFECT OF GLUCOSINOLATES AND MUSTARD OIL ON RUMEN FERMENTATION

Two separate experiments were carried out to study the effect of glucosinolates and mustard oil on rumen fermentation using one stage **in vitro** technique (Tilly and Terry, 1963) and following parameters were taken into consideration:

(i) Total volatile fatty acid(TVFA) concentration

(ii) Individual VFA proportions

(iii) Total gas production

(iv) Methane and CO_2 production

(v) Microbial protein synthesis

A crossbred male adult cattle, fitted with permanent rumen fistula, was taken as a donor animal which was fed on concentrate mixture (maize 50 parts, GN cake 28 parts, wheat bran 20 parts and common salt and mineral mixture 2 parts) and wheat straw **ad lib** as a source of roughage. Rumen liquor sample (about 1 litre) was drawn from various sites in rumen with the help of hard polythene tube prior to feeding and watering of animal into a prewarmed thermos flask. Rumen liquor was strained through 4 layers of muslin cloth and the strained rumen liquor (SRL) was stored in stoppered bottle, flushed with CO_2 gas and maintained at $39\pm1^{o}C$ in a thermostatically controlled waterbath.

3.2.1 Effect of glucosinolates on rumen fermentation

Glucosinolate content of 20 g mustard cake (containing 4.5% glucosinolate) was extracted quantitatively by boiling it with 200 ml water for 5 min followed by filtration through Whatman paper No.541.

Filterate was added in glass bottles (500 ml) in varied

quantity, i.e., 0, 2, 4, 6 and 10 ml representing 0 (T_1), 9 (T_2), 18 (T_3), 27 (T_4) and 45 (T_5) mg glucosinolate, respectively in duplicate maintained at 39±1°C. Each bottle contained SRL (50 ml), Hungate **buffer** (50 ml), casein (100 mg) and starch (100 mg). Prewarmed distilled water was added to make the similar volume of contents in each bottle. After flushing the CO_2 gas, bottles were stoppered. Simultaneously, 0 hr control bottles were also kept in duplicate at room temperature after stopping their microbial activity by adding few drops of concentrated sulphuric acid.

The glass bottles (Borosil) were having the narrow ground neck in which a glass stopper having narrower neck was fixed with the help of standard joint. The neck of glass stopper was closed with a tight silicone lubricated rubber stopper (Beckton Dickinson USA). This arrangement was found suitable for collecting the gas quantitatively.

Hungate **buffer**:- The buffer solution used in this experiment was prepared by mixing two buffer solutions A and B as described by Prins (1987).

Solution A:- (% W/v) KH_2PO_4 0.3; NaCl. 0.6;$(NH_4)_2SO_4$ 0.3; $MgSO_4$ 7 H_2O 0.06; $CaCl_2$ $2H_2O$ 0.06. The Mg and Ca salts were dissolved separately in a minimum quantity of deionized water and added at the last moment to the other dissolved ingredients after about all the water has been added.

Solution B:-(% W/v) K_2HPO_4 0.3%

To make up the final buffer solution 1 part of solution A was mixed with 1 part of solution B and 2 parts of deionized water. The final buffer was prepared just before starting the experiment. The mixture was heated to boiling for a few minutes and then allowed to cool under the continuous flow of CO_2 on the surface of fluid to exclude the air. Sodium bicarbonate was added to reach a 0.5% (W/v) final concentration. Warm fluid (40°C) having pH about 6.7 was used.

3.2.1.1 Total gas production and its fractionation in methane and CO_2:-

All the bottles were incubated at 39±1°C in a constant temperature waterbath fitted with a shaker. Bottles were kept nearly submerged during the incubation in order to keep both the incubation fluid and the gas phase at similar temperature (39°C). After a period of 24 h the total gas present in each bottle was measured by puncturing the rubber

stopper by a fine needle as per the procedure of Prins (1987). The needle was attached to one of the two upper arm of a U shaped manometer (Fig 3.1). Total gas production was indicated by level of displacement of water.

After measuring the total gas, the gas sample was taken from each bottle with the help of a gas tight Hamilton syringe and the same was analysed for methane and CO_2 using gas chromatograph (Nucon, Series 5700) adjusted to the following conditions:

Gas chromatograph was fitted with a stainless steel column (2m x 2mm) which was filled with PORAPACK as the stationary phase. The flow of carrier gas (Hydrogen) was adjusted to 40 ml/min. The temperature of injection port, oven and detector (TCD) was adjusted to 40°C. The known amount of standard mixture of CO_2 and methane (EDT Research, London), containing 27.4 percent methane and 2.2 percent CO_2, was injected into the column with the help of gas tight Hamilton syringe. Peaks were identified and the peak area of each peak of standard mixture was compared with those of unknown samples to calculate the gases quantitatively.

3.2.1.2 Estimation of total volatile fatty acids (TVFA):-

After the analysis of gas samples, the contents of each bottle wereanalysed as per Barnett and Reid (1957).

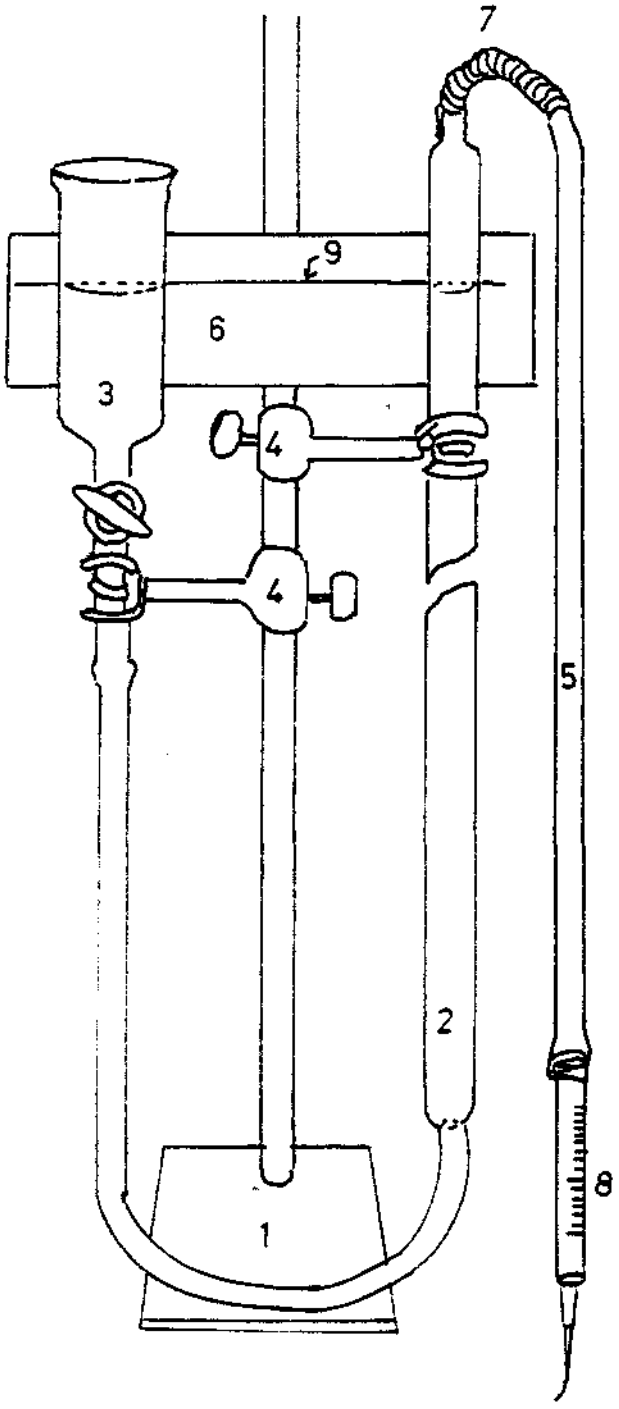
One ml sample was taken into the Markham's apparatus alongwith 1 ml of oxalic acid-potassium oxalate buffer (equal quantity of 5% oxalic acid and 10% potassium oxalate solutions). The distillate of about 80 ml was collected into a conical flask after closing the inlet and passing the steam into the mixture. The distillate was titrated against 0.01 NaOH using phenolphthalein as an indicator. Simultaneously, a blank sample was also carried out.

TVFA(meq/100 ml incubation mixture)= Vol of 0.01N NaOH used x 100

3.2.1.3 Estimation of individual VFA:-

Molar proportions of individual volatile fatty acids were estimated in the contents of each bottle as per the method of Erwin **et al.** (1961).

An aliquot of 4 ml from each bottle was taken and 1 ml of m-phosphoric acid (25% m phosphoric acid in 1N H_2SO_4) was added to it.



1. STAND
2. BURETTE
3. FUNNEL
4. CLAMPS
5. 1.5 M FLEXIBLE TUBING
6. GRAPH PAPER
7. IRON WIRE
8. PLASTIC SYRINGE W/O PLUNGER AND WITH NEEDLE ATTACHED
9. LINE FOR WATER LEVEL READINGS

FIG. 3.1 WATER MANOMETER FOR MEASURING GAS PRODUCTION IN *in vitro* RUMEN INCUBATIONS

After keeping the samples over night, the same were centrifuged at 10,000 rpm for 15 min in a refrigerated centrifuge and the supernatent was stored at sub-zero temperature for the analysis of individual volatile fatty acids.

The fractionation of VFA's was done by using gas chromatograph (Nucon, 5000) fitted with Flame ionization detector (FID) and a stainless steel column (2m x 2mm i.d.) filled with chromosorb 101 as a stationary phase. Flow rates of carrier gas (Nitrogen) through the column, fuel gas (IOLAR grade hydrogen) and air through the FID were 40, 25 and 240 ml/min, respectively. The temperatures of injection port, oven and detector were 200^{o}, 186^{o} and 240^{o}C, respectively. Known quantities of each standard VFA and their mixture were injected for the identification and quantitative estimation of each VFA. Molar percentage of each VFA was also calculated taking their peak areas into consideration.

3.2.1.4 Estimation of microbial protein synthesis:-

Microbial protein synthesis was determined according to the procedure of Lowry **et al.** (1951).

An aliquot of 50 ml from the contents of each bottle was taken into a conical flask and after adding 10 ml of 25% TCA, the flasks were kept over night for the complete precipitation of proteins (Shultz and Shultz, 1970). The contents of flask were centrifuged at 5000 rpm for 15 min and the supernatent was discarded. The pellet was washed again with 25% TCA solution followed by ethyl alcohol and centrifugation each time. Finally, pellet was dissolved in 0.1N NaOH and volume was made to 100 ml in volumetric flask. Aliquots in duplicate from each flask were used to estimate the microbial protein as per Lowry **et al.** (1951) using the spectrophotometer (Bausch and Lomb Spectronic-20) at 420 nm.

Data for different parameters were analysed statistically as per Snedecor and Cochran (1968).

3.2.2 Effect of mustard oil on rumen fermentation

Effect of mustard oil on rumen fermentation was studied in **in vitro** system discussed above.

Different levels of mustard oil, i.e., 0, 0.02, 0.04, 0.06 and 0.08 ml representing 0, 17, 34, 54, 68 mg oil were taken in incubation bottles in duplicate along with SRL (50 ml), Hungate **buffer** (50 ml),

defatted mustard cake (1g) and starch (100 mg) in each bottle. These bottles were incubated as per the above procedure at 39±1°C for 24 h. Total gas production and its fractionation in methane and CO_2, TVFA, VFA fractions and microbial protein synthesis were estimated as per the above described procedures. Experiment was repeated three times.

Data were analysed for each parameter according to the method of Snedecor and Cochran (1968).

PHASE-III

3.3 COMPARATIVE EFFECT OF GROUNDNUT,MUSTARD AND RAPE SEED OILCAKES ON GROWTH, NUTRIENT UTILIZATION AND THYROXINE SECFETION RATE IN CROSSBRED MALE CALVES

3.3.1 Selection and distribution of animals

Eighteen crossbred male calves of similar age and body weight were selected from the cattle herd of National Dairy Research Institute, Karnal and randomly distributed into 3 groups of 6 each. The details of their age, breed and body weight are given as follows. These calves were kept in stalls and fed individually. Deworming was done and calves were conditioned to concentrate and wheat straw based diet before starting the experimental feeding.

Details of distribution of experimental calves in different treatment groups

Animal No.	Date of birth	Body wt of animal at the time of selection
(1)	(2)	(3)
	GNC (Control) group	
KS 3923	8.10.88	117.00
KS 3925	19.10.88	88.00
KS 3939	25.12.88	71.00
KS 3938	25.12.88	52.00
KF 5004	27.12.88	68.00
KF 5011	31.12.88	43.00

....contd.

(1)	(2)	(3)
	MUSTARD cake (MC) group	
KS 3929	26.10.88	93.00
KS 3933	17.11.88	79.00
KF 5012	1.12.88	50.00
KF 5000	15.12.88	78.00
KF 5006	28.12.88	64.00
KF 5010	30.12.88	54.00
	RAPESEED cake (RSC) group	
KS 3931	9.11.88	80.00
KS 4995	25.11.88	67.00
KF 4996	1.12.88	80.00
KS 3935	12.12.88	52.00
KF 5005	27.12.88	61.00
KF 5007	29.12.88	58.00

3.3.2 Feeding treatments

First group (control) was fed on concentrate mixture containing GN cake (GNC) as a source of protein. In the concentrate mixture of groups 2 and 3 the protein supplied by GNC in control ration was replaced completely by expeller pressed mustard (MC) and rape seed cake (RSC), respectively. The composition of all the three rations is given in table 3.1. Initially, all the animals were given the control ration alongwith wheat straw **ad lib** as a source of roughage and green non-leguminous fodder (1 kg daily on fresh basis) to supply the vitamin A. Experimental rations 2 and 3 were introduced gradually to the respective groups and complete replacement was made within one week. The nutrient requirements of calves (NRC, 1978) were fulfilled by the concentrate mixture. The concentrate mixture was offered two times a day daily in the morning at 9.30 A.M. and in the afternoon at 3.30 P.M. along with wheat straw. Water was offered free choice two times a day. Dry matter intake through concentrate mixture and roughage were recorded once a week. The body weights of individual calf were recorded consecutively for two days in a week prior to their feeding and amount of concentrate mixture was adjusted accordingly.

Table 3.1 Composition of treatment rations

Ingredients	I	II	III
Maize grain	50.00	40.84	40.84
Groundnut cake	27.79	-	-
Mustard cake	-	39.16	-
Rape seed cake	-	-	39.16
Wheat bran	20.00	17.00	17.00
Mineral mixture	2.00	2.00	2.00
Common salt	1.00	1.00	1.00
Calculated CP	20.00	20.00	20.00
Calculated TDN	71.43	70.22	70.00

3.3.3 Weighing and body measurements

All the animals were housed in a well ventilated byre with concrete floor, with arrangements for the individual feeding. Healthy surrounding and proper cleanliness were maintained throughout the experimental period. During this period, the manger, floor and wall were cleaned regularly so as to keep the calves away from any infection. Measures were taken to control the ticks. Body weights of each calf were recorded consecutively for two days in a week. The weights were recorded in the morning before offering the feed and water and quantity of concentrate mixture was adjusted accordingly. The body weight formed the basis of determining the growth rate of animals. Following three body measurements were taken at monthly interval by using standard methods:

i) Body length from shoulder joint to tubercoxae

ii) Height at withers

iii) Heart girth

Experimental feeding continued for 35 weeks and during this period blood samples were collected from 5 animals in each group at monthly intervals for the measurement of haemoglobin, T_3 & thyroxine (T_4).

Haemoglobin was estimated immediately after collection of blood and for estimation of T_3 & T_4 blood plasma samples were preserved in a deep freeze.

3.3.4 Metabolism trial and balance studies

A metabolism trial of seven days duration was conducted on all the 18 calves after 35 weeks of feeding the experimental rations. A proper record of feed, fodder and water consumed by each calf was maintained during the metabolism trial.

3.3.4.1 Collection of faeces and urine:-

The calves were kept in the metabolic shed, specially constructed for the metabolism trial. The amount of faeces and urine voided by the experimental animals during 24 h was recorded each day in the morning for the entire period of seven days. Composite samples of urine and faeces were taken separately in a clean dried glass bottle fitted with stopper and brought to the laboratory each day for aliquoting.

3.3.4.2 Aliquoting of faeces and urine:-

Faeces samples (100g) were taken in shallow aluminium trays and dried daily at 100°C in an oven and pooled for 7 days for individual animal. An aliquot of 1/200 of the total faeces voided by each calf was preserved with 5 ml of 25 percent H_2SO_4 in pre-weighed plastic bottle. At the end of seven days collection period, the contents were weighed, mixed thoroughly and 50 g sample was taken in a Kjeldahl flask for digestion and estimation of nitrogen.

The urine collected in 24 h in containers was measured volumetrically daily.

An aliquot of 1/200 of total urine voided by individual animals was taken daily for nitrogen determination in glass bottle containing 30 ml of 25 percent H_2SO_4. After 7 days the volume was made to 500 ml with distilled water and an aliquot was drawn for the estimation of nitrogen.

3.3.4.3 Analysis of samples:-

All the samples were grounded individually in a micro-willey mill through 1mm seive, labelled and stored for further analysis. Samples of feeds, left overs and faeces were analysed for proximate principles

(AOAC, 1984) and cell wall contituents as per Goering and VanSoest (1970). Nitrogen in urine was analysed as per AOAC (1984).

3.3.4.4 Statistical analysis:-

Simple and multiple regression equations for predicting live weight on various correlated factors in the crossbred calves in different treatment groups were calculated according to the method of Snedecor and Cochran (1968).

Similarly, data on the efficiency of nutrient utilization and growth under different treatment groups were subjected to analysis of variance and critical difference test.

3.3.5 Collection of blood

Blood samples were collected from all the animals before starting the experimental feeding and thereafter at monthly intervals till the experiment was terminated by puncturing the jugular vein. To prevent coagulation, heparin was added as an anti-coagulant. Of this 1.0 ml blood was immediately processed for haemoglobin measurements. Remaining blood was centrifuged at 5000 rpm for 5 min in refrigerated centrifuge for separating the blood plasma and stored in deep freeze for the Tri-iodothyronine (T_3) and thyroxine (T_4) estimation.

3.3.6 Body composition

To determine the body composition of growing calves, antipyrine dilution technique given by Brodie **et al.** (1949) and modified by Wellington **et al.** (1956) was followed. The details of the procedure are as follow:

3.3.6.1 Experimental animals:-

After conducting the metabolism trial towards the end of growth studies as described earlier, four animals from each group were weighed for two consecutive days and average of these two weights was considered for this purpose. Animals were deprived of feed and water for 16-18 h prior to the administration of antipyrine.

3.3.6.2 Estimation of total body water:-

Thirty percent solution of antipyrine (2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one; Phenazone procured from Sigma Chemical Company, USA) was prepared in double glass distilled water. Twenty ml of 30 percent solution of antipyrine was injected quantitatively into the jugular vein of each animal. Blood samples were drawn at 180, 210, 240 and 270 min after injecting the antipyrine for its analysis in blood plasma. Just before injection, blood sample from each animal was also taken which served as blank during the determination of antipyrine in the plasma.

The blood was collected in test tubes having heparin as anticoagulant. The tubes were centrifuged at 3500 rpm for plasma separation.

The plasma concentration at zero hour was calculated by plotting the plasma antipyrine concentration at 180, 210, 240 and 270 min and extrapolating the straight line back to zero hour. Weighed quantity of plasma was dried by keeping it in an oven for over night and from this, plasma water content was determined. Correction was made to obtain actual antipyrine concentration. Correction was also made in the blank plasma sample by subtracting it from the concentration of antipyrine in subsequent plasma samples.

From antipyrine level in plasma water at zero hour, the total amount of body water was calculated by the formula (Soberman, 1950).

$$\text{Body water(litres)} = \frac{\text{Amount of antipyrine injected (mg)}}{\text{Plasma water level of antipyrine(mg/litre)}}$$

3.3.6.3 Determination of antipyrine in plasma samples:-

Two ml of plasma was taken in a 50 ml Erlanmeyer flask to which 4 ml of distilled water and 2 ml of zinc reagent (100g of $ZnSO_4.7H_2O$ and 40 ml of 6N H_2SO_4 were dissolved in water and diluted to one litre) were added. Now 2 ml of 0.7N NaOH was added drop by drop with continuous swirling of the flask, and shaken for an additional half a minute. Supernatant fluid was filtered using Whatman filter paper No.42.

Four ml of the clear supernatant was transferred to a quartz cuvette and 5 ml of distilled water was added. Now, 5 ml of 0.2 percent sodium nitrite solution was added to both the unknown and zero hour samples and the O.D was recorded at 350 nm after 25 min.

3.3.6.4 Preparation of standard curve:-

For standard curve 1 ml of 10 percent antipyrine solution was diluted to 100 ml with 0.07 N H_2SO_4 so that concentration of antipyrine at 10 µg/ml was obtained. Of this, 1.0, 2.0, 3.0, 4.0 and 5.0 ml were taken into five test tubes and 4.0, 3.0, 2.0 and 1.0 ml of 0.07 N H_2SO_4 was added in 1st, 2nd, 3rd and 4th test tubes, respectively so that each tube had an equal volume of 5 ml solution. The blank consisted of 5 ml of 0.07N H_2SO_4.

Optical density (OD) was recorded for each dilution at 350 nm in Hitachi spectrophotometer model 100-20 (Hitachi Ltd., Japan) before and after the addition of 0.5 ml of 0.2 percent sodium nitrite solution as described earlier.

Corrected OD were plotted against its concentration on a graph paper and a straight line was obtained.

Determination of empty body weight (kg), body fat (%), body protein (%) and body ash (%) are detailed as under:

3.3.6.5 Empty body weight:-

Empty body weight was determined by using the equation given by Bensadoum **et al.** (1963).

$$\text{Empty body wt} = 0.943X - 4.076$$

Where, X = Live body weight (kg)

3.3.6.6 Body fat:-

Body fat was determined by using the following equation (Reid **et al.**, 1963):

$$Y = 355.88 + 0.356X - 202.91 \log X$$

Where, Y = Fat in empty body (%)

X = Water in empty body (%)

3.3.6.7 Body protein:-

Body protein was determined by using the following equation (Reid

et al., 1963):

$$P = 0.2903X - 0.477$$

Where, P = Total protein (%)

X = Empty body water (%)

3.3.6.8 Body ash:-

$$A = 100-[\text{Body water}(\%) + \text{Body fat}(\%) + \text{Body protein}(\%)]$$

Where, A = Ash (%)

3.3.6.9 Statistical analysis:-

Statistical analysis of data on body composition was carried out as per Snedecor and Cochran (1968).

3.3.7 Tri-iodothyronine (T_3) and thyroxine status of crossbred calves during experimental period

3.3.7.1 Tri-iodothyronine(T_3):-

Plasma samples were thawed at the time of estimation of T_3 and T_4 concentrations.

The procedure specified by BARC for RIA of triiodothyronine was followed.

50 µl plasma and 300 µl assay buffer (pH 8.6) as used in T_4 estimation were taken in duplicate into 10 x 75 mm tubes. To these tubes 0.1 ml tracer and 0.1 ml antiserum were added. Contents of the tubes after gentle mixing were incubated for 45 min at 37°C. After incubation, 1.0 ml PEG solution was added to each tube. The tubes were gently mixed and centrifuged at 2000 Xg for 20 min.

Supernatent was discarded without disturbing the precipitates and radio-activity was counted in gamma scintillation counter. Standard tubes with a series of standards (0.015, 0.03, 0.06, 0.12 and 2.4 ng/ml) were also run alongwith unknown samples which consisted of 0.2 ml of assay buffer 0.1 ml of T_3 standards and 50 µl of T_3 free serum. Further processing was identical to the unkrown samples described above.

Besides, three sets of tubes were also processed in an identical fashion to find out non-specific binding, maximum binding of the tracer by the antibody and recovery. For measurement of total counts, 0.1 ml tracer was added in duplicate tubes and counted directly in gamma counter. Corrections were made for background counts. Zero binding was calculated as:

$$\%B = \frac{\text{Corrected av. counts of zero standard}}{\text{Corrected total counts}} \times 100$$

$$\%B/Bo = \frac{\text{Corected av. counts of std/sample}}{\text{Corrected av. counts of zero std.}} \times 100$$

% B/Bo on a logit scale was plotted against concentration of T_3 (ng/ml) on a log scale of a logit-log graph paper. Sample values were estimated from the standard curve. T_3 concentration of the samples was expressed as ng/ml.

3.3.7.2 **Thyroxine** (T_4):-

The procedure specified for radioimmunoassay (RIA) for thyroxine estimation in human serum by Bhabha Atomic Research Centre (BARC), Bombay was followed for estimating thyroxine level in bovine blood plasma. An aliquot of 0.1 ml of thawed plasma was diluted to 1 ml with assay buffer (pH 8.6), containing 0.1 percent gelatin in 0.14M Tris-Hydroxymethyl Amino Methane, in a marked test tube for each sample and 0.1 ml of this was pipetted in duplicate into 10 x 75 mm tubes, to which 0.1 ml assay buffer, 0.1 ml labelled thyroxine and 0.1 ml antiserum were added in succession followed by gentle mixing. The tubes were then incubated at 37°C for 30 min.

One ml of polyethylene glycol (PEG) was added to all the tubes, vortexed thoroughly, centrifuged at 2000 Xg for 20 min at 4°C. The supernatant was properly discarded and the tubes were wiped out with absorbant paper to the possible extent without touching or disturbing the precipitate. Simultaneously, standard tubes with a series of T_4 concentration (2.5, 5.0, 10.0 and 20.0 ng/ml in assay buffer) were also run along with unknown samples in an identical manner. In addition to

unknown and standard tubes, 3 sets of tubes were also run as follows:

- Blank tubes in duplicate, containing 0.3 ml assay buffer, 0.1 ml tracer to observe non-specific binding (NSB)
- Two tubes containing 0.1 ml assay buffer, 0.1 ml of tracer, 0.1 ml T_4 free serum and 0.1 ml antiserum to obtain maximum binding of the tracer by the antibody
- Recovery tubes with known amount of hormone in plamsa

Total counts were also obtained by taking 0.1 ml of tracer in duplicate tubes.

The radioactivity of all the tubes was measured in a Gamma counter and corrections were made for background counts. T_4 concentrations (ng/ml) in samples were estimated as per the calculations shown for T_3.

3.3.7.3 Haemoglobin(Hb):-

Blood haemoglobin was estimated as per Cohen and Smith method (1919) described by Oser (1968). Fresh blood measuring 0.05 ml was added to 10 ml of N/10 HCl to form acid haematin and optical density was measured at 520 nm. Known haemoglobin standard of 0.075g/100 ml was prepared as per the method of Wong (1928).

3.3.7.4 Estimation of thiocyanate content in blood plasma:-

Thiocyanate content in blood plasma of two animals from each group was estimated as per the method of Bowler (1944). The details of procedure are given as follows:

One ml of plasma was added into the test tube containing 2.5 ml of 20 per cent trichloro acetic acid and 6.5 ml distilled water. Contents were mixed by inversion several times, allowed to stand for about 10 min and filtered through a Whatman filter paper No.40. An aliquot of 5 ml of the filtrate was treated with 5 ml of ferric nitrate-reagent [80g $Fe(NO_3)_2 \cdot 9H_2O$ dissolved in 250 ml 2N HNO_3, volume made to 500 ml with distilled water] in the absence of day light. Contents were mixed gently and optical density was recorded at 460 nm within 15 min on a spectrophotometer. Standard curve was drawn using the different concentration of 0.01M solution of potassium thiocyanate.

3.3.7.5 Statistical analysis:-

Statistical analysis of data was carried out as per the method of Snedecor and Cochrar (1968).

3.3.8 Estimation of thyroxine secretion rate(TSR)

The thyroxine secretion rate (TSR) was estimated by the method of Yousef and Johnson (1967) in which TSR is determined as the product of plasma thyroxine, thyroxine disappearance rate (K) and thyroxine distribution space (TDS). The outline of whole procedure is presented in Fig 3.2.

After completing the growth trial and body composition determination experiment, 2 animals from each group (GNC, MC and RSC) were selected for estimation of thyroxine secretion rate (TSR). ^{125}I labelled L-thyroxine (75 μCi) was injected in each animal through jugular vein. Thereafter, blood samples of all the six animals were collected from the jugular vein in heparinized tubes using sterilized sharp needles. To estimate the thyroxine secretion rate (TSR) the blood samples were collected at 24, 48, 72 and 96 h intervals after injecting labelled L-thyroxine in the calves.

Plasma samples were obtained after centrifuging the blood samples under refrigerated conditions at 5000 rpm for 30 min. 2 ml plasma of each animal was subjected to gamma-counter (Iso Data model 500, USA) to measure the counts.

3.3.8.1 Determination of K(Disappearance rate of labelled thyroxine):-

Seventy five μCi of ^{125}I labelled L-thyroxine dissolved in 50 percent propylene glycol was injected in the jugular vein of each animal. The labelled material had 0.007 μg/ml thyroxine content.

Simultaneously, 0.1 ml of ^{125}I labelled L-thyroxine was diluted to 5 ml with 50 percent propylene glycol solution. This was used as a standard to express the counts as a percentage of dose administered. After injecting ^{125}I-T_4 blood samples were drawn at 24, 48, 72 and 96 h. Plasma was separated from these samples and the radioactivity of the 2ml plasma and standards were measured in a gamma counter. The results of the counts were plotted on a semilogarithmic coordinate system (ordinate logarithmic) with hours after injection of ^{125}I-T_4 as the abscissa and the activity as

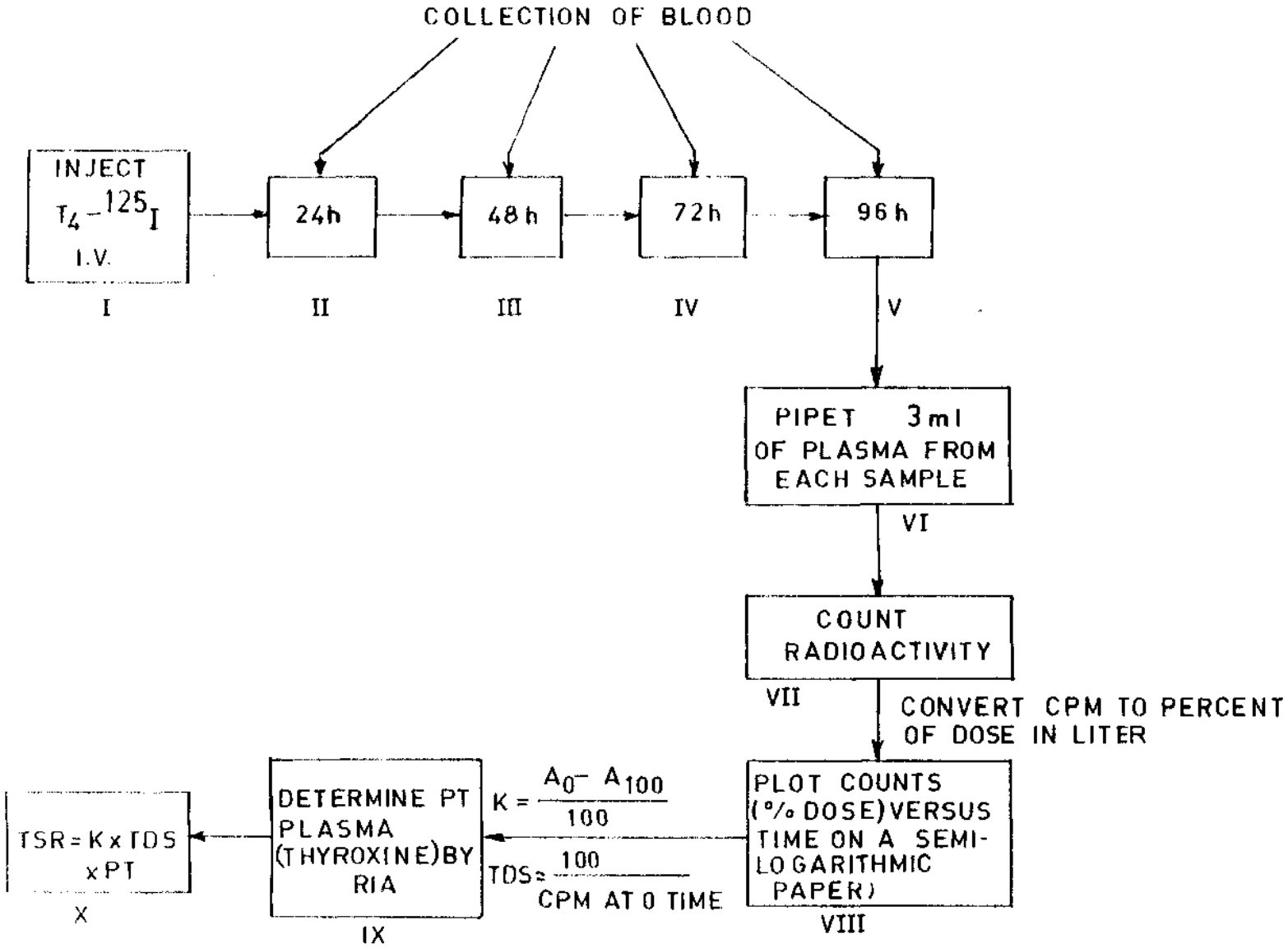


FIG 3.2 SCHEMATIC PRESENTATION SHOWING THE METHOD OF ESTIMATION OF THYROXINE SECRETION RATE

a percentage of the dose administered per litre of plasma as the ordinate. The rectilinear part of the curve was extrapolated to time zero and 100 h post-injection. The ^{125}I -T_4 disappearance rate (K) was calculated from the linear curve using the equation.

$$K = \frac{Ao - A_{100}}{100}$$

Where, Ao is the natural logarithm of ^{125}I activity at zero time and A_{100} is the natural logarithm of ^{125}I activity at 100 h post-injection.

3.3.8.2 Determination of thyroxine distribution space(TDS):-

The rectilinear curve, used for the estimation of "K", was extrapolated to time 'zero' and the concentration of ^{125}I-T_4 administered was obtained by assuming that the distribution of ^{125}I-T_4 was uniform in this space. Its volume was calculated by the following equation:

TDS = 100/radioactivity per litre as a percent of dose at zero time

3.3.8.3 Determination of PT:-

It was estimated by standard radioimmunoassay technique.

3.3.8.4 Determination of thyroxine secretion rate (TSR):-

The TSR per day was calculated as the product of TDS x K/day x PT (mg/litre). The values were expressed as mg/100 kg body wt/day.

3.3.8.5 Statistical analysis:-

Data was analysed according to the method of Snedecor and Cochran (1968).

PHASE-IV

3.4 EFFECT OF STORAGE ON THE QUALITY OF OILCAKES

Expeller processed groundnut, mustard and rape seed oilcakes, each packed in gunny bags (about 80 kg) were kept in the godown where other feed ingredients were also stored. The storage of these oilcakes was done

after checking the initial quality in the month of April. Thereafter, samples were drawn at monthly intervals from each bag upto a period of one year to study the influence of storage on their quality. Relative humidity (RH) and temperature were recorded at weekly intervals in the godown. Following parameters were selected to check the quality of oilcakes.

i. Proximate principles

ii. Total glucosinolates content

iii. Free fatty acid value

iv. Total aflatoxins

3.4.1 Proximate principles

Proximate principles were determined according to the methods of AOAC (1984).

3.4.2 Total glucosinolates

Total glucosinolates content of oilcakes was estimated according to the procedure of McGhee **et al.** (1964), as described under 3.1.1.2.

3.4.3 Free fatty acids value

The ffa value of the oil content of oilcakes was estimated as per the procedure of Rao **et al.** (1972) as described under 3.1.1.3.

3.4.4 Estimation of aflatoxins

Samples of GN cake, mustard cake and rape seed cake were collected at the time of their storage in April, 1989 and then after one year of their storage, i.e., in April 1990, and quantitative estimation for their aflatoxins was performed after their extraction, purification and derivatization by high performance liquid chromatographic (HPLC) technique. The detailed procedure is as follows:

3.4.4.1 Extraction:-

Aflatoxins were extracted from the oilcakes as per the method of AOAC (1984). Fifty gram sample was blended with 250 ml methanol:water

(55:45), 100 ml hexane and 2 g sodium chloride for one min at high speed and filtered through Whatman filter paper No.0. An aliquot (25 ml) of aqueous methanol phase of filtrate was taken into a separating funnel and equal amount of chloroform was added. After vigorous shaking for one min, chloroform phase was drained into a glass beaker and chloroform was evaporated at low temperature under the flow of nitrogen gas till it is condensed to about 2 ml.

3.4.4.2 Purification:-

Extracted material was purified as per the procedure of Walter et al., (1980) by passing the condensed solution through a column filled with silica gel and having a layer of anhydrous sodium sulphate on top of it. Sample was gently transferred to the top layer and eluted with the help of chloroform. The eluent collected in a beaker was again condensed to about 2 ml by evaporating the chloroform at low temperature under the nitrogen gas flow. The condensed mixture was transferred into 2 dram vial and dried it completely.

3.4.4.3 Derivatization:-

The purified residue was dissolved in 5 ml chloroform and two aliquots of 1 ml each were taken in separate vials and chloroform was evaporated as per the usual procedure. Thereafter, 2 ml solution of benzene and acetonitrile (98:2) was added into each vial and filtered through micropore filter paper. This filtrate was dried again as described earlier and the dried residue was derivatized by adding trifluroacetic acid (TFA) as per the procedure of Roberta (1978). Excess TFA was evaporated completely and 400 µl dissolving solution (Water:methanol:acetic acid 6:2:2) was added. This final solution was used for quantitative estimation of aflatoxins.

Standard aflatoxins B_1, B_2, G_1 and G_2 (Sigma Chemical Company, USA) were individually derivatized and their mixture was used as an external standard.

Aflatoxins estimation was done using reverse phase HPLC (Waters model 510 Millipore Corpn., Massachusetts) fitted with fluoroscence detector and data module (Waters model 745) to increase selectivity and

sensitivity. The stainless steel column (10 cm x 8 mm) was filled with NOVAPAK 18TM as the stationary phase. The pH of mobile phase (Water:methanol:acetic acid 6:2:2) was adjusted to 4 and rate of flow was 1.2 ml/min. A 10 μl derivatized individual aflatoxin and mixture of aflatoxins standard and samples were injected. The responses of individual aflatoxin, mixture of aflatoxins and samples were recorded on data module. Peaks for individual aflatoxins were identified and peak areas of unknown and external standard were compared to estimate the each component of aflatoxins in oilcake samples.

3.4.5 Statistical analysis

The data on chemical composition were examined for differences among the oilcakes and periods using analysis of variance technique prescribed by Snedecor and Cochran (1968).

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CHAPTER - IV

RESULTS

4. RESULTS

Results obtained from the various experiments conducted during the course of study are presented in this chapter.

4.1 CHEMICAL COMPOSITION OF OILCAKES AND INDIAN VARIETIES OF MUSTARD/RAPE SEED

4.1.1 Chemical composition of oilcakes

Groundnut cake (GNC), Mustard cake (MC) and Rape seed cake (RSC) were analysed for their proximate principles, total glucosinolates and amino acid profile and the results on percent dry matter basis are recorded in tables 4.1 and 4.2.

4.1.1.1 Proximate composition and glucosinolate contents:-

Crude protein percentage in GNC (47.71) was higher than MC (36.27) and RSC (36.33) oilcakes. Crude fibre content was lower in GNC than in MC and RSC and the respective values were 7.80, 9.18 and 9.22 percent. Ether extract percentage was highest in RSC (10.63) followed by MSC (8.94) and GNC (7.27). MC and RSC were got extracted at local expellar oil mill whereas GNC was procured from the market which was reflected in their free fatty acids (ffa) values. The average ffa value for GNC, MC and RSC was 45.24, 22.72 and 22.16, respectively. Glucosinolates were found absent in GNC, however, total glucosinolates in MC and RSC were 4.40 and 3.54 percent, respectively (Table 4.1).

4.1.1.2 Amino acid profile:-

Amino acid profile of oilcakes (Table 4.2) showed that GNC contained higher level of total amino acids than MC and RSC and the latter two oilcakes were similar in this respect. Essential as well as non-essential amino acids except sulphur containing amino acids were higher in GNC than in MC and RSC.

Table 4.1 Chemical composition of ground, mustard and rapeseed cakes

Parameters	GNC (% DM basis)	MC (% DM basis)	RSC (% DM basis)
Organic matter	92.13	91.13	92.34
Crude protein	47.71	36.27	36.33
Crude fibre	7.80	9.18	9.22
Ether extract	7.27	8.94	10.63
NFE	29.35	37.54	36.16
Glucosinolates	0.00	4.40	3.54
FFA	45.24	20.72	22.16

Table 4.2 Amino acid profile of groundnut, mustard and rapeseed oilcake (% of total amino acids)

Amino acids	Groundnut cake	Mustard cake	Rapeseed cake
Aspartic acid	0.71	0.43	0.96
Glutamic acid	0.90	0.57	1.06
Serine	4.88	6.21	5.58
Glycine	2.92	4.62	3.77
Histidine	1.13	1.95	0.96
Arginine	3.53	1.02	2.58
Threonine	1.29	1.97	1.04
Alanine	10.62	7.94	6.52
Proline	35.76	38.04	39.22
Tyrosine	12.30	15.05	14.05
Valine	0.37	32.88	0.28
Methionine	0.75	1.14	0.96
Cystine	1.20	1.69	1.64
Isoleucine	1.96	0.83	0.55
Leucine	0.78	0.83	0.72
Phenylalanine	21.05	16.35	19.04
Lysine	0.97	0.90	1.15

4.1.2 Glucosinolate content, proximate composition and fibre fractions of Indian varieties of mustard/rape seed

Seven varieties/strains of mustard/rape seed, developed at All India Coordinated Research Project on Oilseeds, HAU, Hissar, were analysed for their glucosinolate contents, proximate composition and cell wall constituents. These seven varieties were Raya RC 781, Raya RH 2859, Raya RH 781, Toria Shyamgarh, Toria TH 83, Toria Sangram, and Toria Kranti. Besides these varieties, 5 more varieties viz. Toria TH 109, B-054, B.Bold, **B.carinata** and **B.napus** were collected from Indian Agricultural Research Institute, New Delhi, and analysed for their glucosinolate and oil contents.

4.1.2.1 Glucosinolate content

Glucosinolate content in mustard/rape seed varieties namely Raya RC 781, Raya RH 2859, Raya RH 781, Toria Shyamgarh, Toria TH 83, Toria Sangram, Toria Kranti; Toria TH 109, B-054, B.Bold, **B.carinata** and **B.napus** was 1.61, 2.24, 3.27, 2.58, 2.90, 4.92, 4.01, 3.21, 1.60, 4.34, 3.57 and 1.77 percent on DM basis of oilseed. These figures showed that glucosinolate content was present in all the varieties, however, it varied in the range of 1.60 to 4.92 percent. The total glucosinolates in the oil free meal of these varieties has also been presented in table 4.3 and it was evident that highest glucosinolate content was in the variety of rape seed known as Toria Sangram which was having fairly high level of oil content. Few other strains of rape seed (toria), viz., Toria Shymgarh, Toria Sangram, and Toria Kranti had almost similar oil content, but their glucosinolate contents had large variations. The results showed that despite the similar oil content Toria Shymgarh had about 40 percent lower glucosinolate content than Toria Sangram. Similar trend was observed with other varieties like Raya RC 781, B-054 and **B.napus.** Present results did not reveal any relationship between oil and glucosinolate contents of different varieties/strains of mustard and rape seed.

4.1.2.2 Oil content:-

The data on oil percentage in different varieties of mustard/rape seed are presented in table 4.3. The oil content, varying in the range

Table 4.3 Total glucosinolates, proximate composition and fibre fraction of cell wall of different varieties of mustard/rapeseed (% on DM basis)

Varieties	Oil(%)	Total glucosinolate (%)	Oil free meal basis %				NDF	ADF	Cellulose	Hemicellulose
			Total glucosinolate	CP	TCHO	Mineral matter				
Raya RC 781	35.28	1.61	2.54	40.80	52.51	6.69	25.23	19.38	10.69	5.85
Raya RH 2859	32.89	2.24	3.55	42.09	52.47	5.44	27.44	22.26	12.63	5.18
Raya RH 781	34.16	3.27	5.16	42.81	50.61	6.58	27.31	23.19	13.45	4.12
Toria Shymgarh	38.85	2.58	4.33	46.23	49.07	4.70	23.54	18.63	10.35	4.51
Toria TH 83	35.38	2.90	4.53	43.74	51.16	5.10	26.34	21.83	10.88	4.51
Toria Sangram	38.36	4.92	7.31	42.76	50.55	6.69	25.27	20.27	11.61	4.69
Toria Kranti	38.96	4.01	6.56	35.46	58.86	5.68	35.54	29.36	17.08	6.18
Toria TH 109	37.86	3.21	5.16	-	-	-	-	-	-	-
B-054	37.10	1.60	2.54	-	-	-	-	-	-	-
B.Bold	36.85	4.34	6.87	-	-	-	-	-	-	-
B.carinata	36.44	3.57	5.61	-	-	-	-	-	-	-
B.napus	35.38	1.77	2.73	-	-	-	-	-	-	-

- Not estimated

of 32.89 to 38.96 percent on DM basis, showed a large variation among the varieties. The highest oil content was observed in Toria Kranti.

4.1.2.3 Crude protein:-

Crude protein content in oil free meal of different varieties of mustard/rape seed are presented in table 4.3. The crude protein in seven varieties, namely, Raya RC 781, Raya RH 2859, Raya RH 781, Toria Shymgarh, Toria TH 83, Toria Sangram and Toria Kranti was 40.80, 42.09, 42.81, 46.23, 43.74, 42.76 and 35.46 percent, respectively. The crude protein in these seven varieties varied in the range of 35.46 and 46.23 percent and the highest crude protein content was observed in Toria Shymgarh and lowest in Toria Kranti.

4.1.2.4 Total carbohydrates and mineral matter:-

The total carbohydrates in seven different varieties of mustard/rape seed namely, Raya RC 781, Raya RH 2859, Raya RH 781, Toria Shymgarh, Toria TH 83 , Toria Sangram and Toria Kranti was 52.51, 52.47, 50.61, 49.07, 51.16, 50.55 and 58.86 percent, respectively. The oil free meal of Toria Kranti contained highest level of total carbohydrates whereas other varieties were similar in this respect. The mineral matter in the above mentioned seven varieties varied in the range of 4.70 to 6.69 percent in fat free meal of mustard/rape seed.

4.1.2.5 Cell wall constituents:-

The cell wall constituents of different varieties of rape seed (toria) and mustard are given in table 4.3. The NDF fraction varied in the range of 23.54 to 35.54 percent and it was found highest in Toria Kranti than in other varieties. The ADF and cellulose followed the same trend. Hemicellulose varied in the range of 4.12 to 6.18 percent and highest value was observed in Toria Kranti. The cell wall constituents showed a large variation in the fibre composition of different varieties and the higher fractions of NDF and ADF in oilseeds of Toria Kranti indicated its higher proportion of hulls.

4.2 EFFECT OF GLUCOSINOLATES AND MUSTARD OIL ON RUMEN FERMENTATION

4.2.1 Effect of glucosinolates on rumen fermentation

Total glucosinolates obtained from mustard cake by hot water extraction were added at different levels, i.e., 0 (Control T_1), 9 (T_2), 18 (T_3), 27 (T_4) and 45 (T_5) mg added in the **in vitro** system to study their effect on rumen fermentation. The results are presented in table 4.4.

4.2.1.1 Total volatile fatty acids (TVFA):-

TVFA production in treatments T_1, T_2, T_3, T_4 and T_5 was 85.00±0.36, 85.13±0.47, 85.67±0.23, 87.50±0.34 and 87.50±0.29 meq/litre respectively and the variations among treatments were not significant (Table 4.4). The TVFA increased non-significantly with increasing levels of glucosinolates due to the higher availability of N which accompanied the glucosinolates in the form of soluble-N of oilcake.

4.2.1.2 Individual volatile fatty acids (VFA):-

The molar proportions of acetate, propionate and butyrate varied significantly ($P/0.05$) among the treatments. The acetate to propionate ratios were 1.90, 1.90, 1.90, 1.85 and 1.84 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. These results indicated that varying levels of glucosinolates altered the microbial fermentation of substrate.

4.2..1.3 Total gas production:-

The total gas production in treatments T_1, T_2, T_3, T_4 and T_5 was 12.15±0.50, 14.16±0.31, 18.70±0.82, 19.50±0.90 and 22.74±0.52 ml in **in vitro** system, respectively. These results further revealed that the glucosinolates did not affect the microbial activity. The increase in the production of total gas was due to the higher microbial activity induced by the presence of soluble nitrogen and increased availability of glucose as a result of glucosinolates hydrolysis. The total gas production from 9, 18, 27 and 45 mg glucosinolates was obtained by subtracting the control (T_1) values from those obtained in treatments T_2, T_3, T_4 and T_5 values. The figures in respective treatments were 2.01, 6.55, 7.35, 10.59 ml. The

Table 4.4 Effect of different levels of glucosinolates on rumen fermentation and gas production (**in vitro**)

Treatments	TVFA meq/l	Molar proportions			A:P	Total gas (ml)	Methane %	CO_2 %	CH_4 ml	CO_2 ml	Microbial protein ,g/100ml SRL
		Acetate	Propio-nate	Buty-rate							
T_1	85.00 ±0.36	53.33^a ±0.01	27.95^a ±0.02	18.72^a ±0.01	1.90 ±0.01	12.15^a ±0.50	27.45^a ±0.97	72.55^a ±0.97	3.36^a ±0.14	8.67 ±0.16	122.0^a ±2.0
T_2	85.13 ±0.47	53.31^a ±0.01	27.99^a ±0.02	18.70^a ±0.01	1.90 ±0.02	14.16^b ±0.31	31.19^b ±1.18	68.80^b ±1.18	4.19^b ±0.09	9.97 ±0.10	188.0^b ±2.0
T_3	85.67 ±0.23	53.42^a ±0.02	28.04^a ±0.01	18.54^a ±0.03	1.90^c ±0.02	18.70^c ±0.82	32.09^b ±0.92	67.91^b ±0.92	5.79^c ±0.26	12.91 ±0.30	203.0^c ±4.6
T_4	87.50 ±0.34	53.34^a ±0.03	28.17^a ±0.01	19.48^a ±0.03	1.85 ±0.01	19.50^c ±0.90	33.34^b ±0.95	66.64^b ±0.95	6.15^c ±0.36	13.35 ±0.38	217.0^d ±4.6
T_5	87.50 ±0.29	53.05^b ±0.01	28.86^b ±0.03	18.09^b ±0.03	1.84 ±0.13	22.74^d ±0.52	34.44^b ±1.37	65.56^b ±1.37	7.83^d ±0.30	14.91 ±0.31	232.0^e ±2.0

^{abcde}values with different superscripts in a column differ significantly ($P/0.05$)

proportion of methane was 27.45 percent in treatment T_1 where there were no glucosinolates. It increased progressively in all the other treatments with increasing level of glucosinolates. Increase in the levels of glucosinolates increased the total gas as well as methane and CO_2. Production of total gas, methane and CO_2 varied significantly ($P/0.05$) among the treatments (Table 4.6).

4.2.1.4 Microbial protein synthesis:-

Microbial protein synthesis in treatments T_1, T_2, T_3, T_4 and T_5 was 122.00±2.00, 188.00±2.00, 203.00±4.63, 217.00±4.63 and 232.00±2.00 mg/100 ml incubation mixture, respectively. These results indicated that the microbial protein synthesis increased with the increasing levels of glucosinolate extract. The contribution of 9, 18, 27 and 45 mg glucosinolates extract was also calculated by subtracting the control value from the respective values obtained in other treatments. The figures were 66.0, 81.0, 95.0 and 110.0 mg/100 ml SRL, respectively in treatments T_2, T_3, T_4 and T_5. This increasing trend of microbial protein synthesis with increasing level of glucosinolates indicated that glucosinolates as such or its hydrolysis products were not harmful for the rumen microflora. Further, it also showed that the soluble-N content present in the glucosinolate extract was utilized efficiently by microbes probably due to the availability of sulphur which was available as a result of hydrolysis of glucosinolates. Variations among treatments for microbial protein synthesis were found significant ($P/0.05$) as indicated by the table 4.6.

4.2.2 Effect of mustard oil on rumen fermentation

Effect of mustard oil addition at 0 (T_1), 17 (T_2), 34 (T_3), 51 (T_4) and 68 (T_5) mg levels along with one gram mustard cake (solvent extracted) was investigated on **in vitro** rumen fermentation and the results are presented in table 4.5.

4.2.2.1 Total volatile fatty acids(TVFA):-

The TVFA concentration in treatments T_1, T_2, T_3, T_4 and T_5 flasks was 84.66, 85.27, 85.54, 86.08 and 86.22 meq/litre respectively. These results indicated that the level of addition of oil, i.e., substrate in

Table 4.5 Effect of different levels of mustard oil on rumen fermentation and gas production (**in vitro**)

Treatment	TVFA meq/l	Molar proportions			A/P ratio	Total gas (ml)	CH_4 (%)	Total CH_4 (ml)	CO_2 %	CH_4/g oil(ml)	Microbial protein mg/100ml SRL
		Acetate	Propio-nate	Buty-rate							
T_1	84.66 ±0.33	53.33[a] ±0.01	27.95[a] ±0.11	18.72 ±0.08	1.90 ±0.01	12.00[a] ±0.25	24.16 ±1.82	2.89[a]	75.89[a] ±1.82	-	133.0[a] ±11.0
T_2	85.27 ±0.21	53.16[a] ±0.49	27.95[a] ±1.04	18.89 ±0.90	1.90 ±0.02	17.87[b] ±0.72	23,66 ±2.43	4.22[b]	76.34 ±2.43	78.32	177.0[b] ±11.0
T_3	85.54 ±0.21	52.42[b] ±0.42	29.16[b] ±1.01	18.42 ±1.02	1.79 ±0.02	21.50[b] ±0.81	21.34 ±3.30	4.58[b]	78.66 ±3.30	75.88	210.0[c] ±5.6
T_4	86.08 ±0.30	52.04[b] ±0.51	29.61[b] ±1.05	18.35 ±0.81	1.75 ±0.02	22.50[c] ±1.01	20.96 ±3.02	4.71[b]	79.04[b] ±3.02	53.52	215.0[c] ±4.6
T_5	86.22 ±0.21	51.56[b] ±0.83	30.11[b] ±1.00	18.33 ±1.03	1.71 ±0.03	22.90[c] ±0.71	19.88 ±2.85	4.55[b]	80.12 ±2.85	39.70	215.0[c] ±3.67

[abc]values with different superscripts in a column differ significantly (P/0.05)

Table 4.6 Analysis of variance for different parameters of **in vitro** rumen fermentation studies

Source of variations	d.f.	Mean sum of squares								
		TVFA	Acetate	Propionate	Butyrate	Total gas	Methane	CO_2	Total methane	Microbial protein
					Effect of glucosinolates on rumen fermentation					
Replicates	5	22.82	0.003	0.013	0.30	2.58	6.57	8.81	0.42	54.85
Treatments	4	87.11	1.16	4.68	5.99	110.92	17.47	26.56	13.34	10648.00
Error	20	36.81	0.007	0.007	0.30	2.41	5.03	6.72	0.36	38.72
					Effect of mustard oil on rumen fermentation					
Replicates	5	37.62	3.87	10.67	8.81	12.84	145.43	152.85	2.52	309.76
Treatments	4	37.13	4.199	7.06	0.90	163.43	6.14	195.40	12.12	14705.53
Error	20	29.02	1.049	9.15	5.15	4.32	19.79	8.52	0.46	204.89

in vitro system did not influence the TVFA concentration significantly (Table 4.6).

4.2.2.2 Individual volatile fatty acids(VFA):-

The molar proportions of individual VFAs changed during the course of fermentation. The proportions of acetate decreased with the increasing levels of oil content. Simultaneously, proportions of propionate increased with the increasing level of oil. The proportions of butyrate, however, were not influenced by the addition of oil. The butyrate proportion was same in the control (T_1) and in the rest of the treatments (T_2-T_5). The acetate-propionate ratios in treatments T_1, T_2, T_3, T_4 and T_5 were 1.90±0.01, 1.90±0.02, 1.79±0.02, 1.75±0.02 and 1.71±0.03, respectively. These results indicated that with the increasing level of oil the ratios of acetate to propionate narrowed down during **in vitro** fermentation.

4.2.2.3 Production of gases:-

The total gas production as a result of addition of unsaturated fatty acids containing oil, i.e., mustard oil was obtained by subtracting the control (T_1) value from those obtained in treatment T_2, T_3, T_4 and T_5. It indicated that the total gas produced from 17, 34, 51 and 68 mg oil was 5.87, 9.50, 10.50 and 10.90 ml, respectively during 24 h fermentation. These results showed that the oil content served as the source of energy after its lipolysis during the course of fermentation. Gas production increased with increased level of oil content. The proportion of methane in total gas reduced with simultaneous increase in CO_2 proportion at all the levels of oil ($P<0.05$). Total methane production from oil content in T_2, T_3, T_4 and T_5 was calculated as 1.34, 2.58, 2.73 and 2.70 ml while CO_2 was 4.54, 6.93, 7.78 and 8.21 ml, respectively. Methane production per gram of oil in corresponding treatments was calculated as 78.82, 75.88, 53.52 and 39.70 ml, establishing a decreasing trend in methane production on increasing the level of unsaturated triglycerides, i.e., mustard oil.

4.2.2.4 Microbial protein synthesis:-

Microbial protein synthesis in treatment T_1 was 133.0 mg/100 ml incubation mixture which indicated that there was substantial utilization

of mustard cake by the microbes. Further, microbial protein synthesis increased in treatments T_2 and T_3, thereafter, the increase was very small. The microbial protein synthesis in treatments T_2, T_3, T_4 and T_5 was 177.01 ±11.00, 210.01±5.6, 215.00±4.6 and 215.0±3.6 mg/100 ml incubation mixture respectively. The corresponding values obtained as a result of energy supplementation were 44.0, 77.0, 82.0 and 82.0 mg/100 ml. These figures showed that the efficiency of microbial protein synthesis increased with the increase in the energy availability only upto treatment T_3. Thereafter the variations among T_3, T_4 and T_5 were not significant.

4.3 EFFECT OF GROUNDNUT, MUSTARD AND RAPE SEED OILCAKES ON GROWTH, NUTRIENT UTILIZATION AND THYROXINE SECRETION RATE

4.3.1 Chemical composition of treatment rations

The data on proximate composition and cell wall constituents of different treatment concentrate mixtures, wheat straw and green fodder (berseem) are given in table 4.7.

The concentrate mixtures given to groups GNC, MC, RSC were containing 21.84, 22.15 and 22.56 percent crude protein, respectively. The crude protein of wheat straw and green fodder was 3.88 and 18.38 percent on dry matter basis. All the three concentrate mixtures were isonitrogenous. The ether extract in GNC, MC and RSC rations was 3.50, 5.01 and 5.69 percent, respectively. The cell wall constituents, i.e., NDF and ADF values in corresponding groups were 34.35, 15.15; 31.15, 14.31 and 30.51, 13.75. The wheat straw contained 80.83 percent NDF and 65.67 percent ADF.

The glucosinolate content of concentrate mixture of GNC, MC and RSC groups was 0, 1.72 and 1.40 percent on DM basis, respectively. Glucosinolates were not detected either in wheat straw nor in green berseem fodder.

4.3.2 Feed:gain ratio and growth rate

The data on total DM consumed through roughage as well as through concentrate mixture and growth performance of calves in different groups during the total experimental period of 35 weeks are presented in Table 4.8. The weekly body weight gain by the calves in different groups has been depicted in Fig 4.1.

Fig 4.1:AV. BODY Wt. GAIN IN THREE

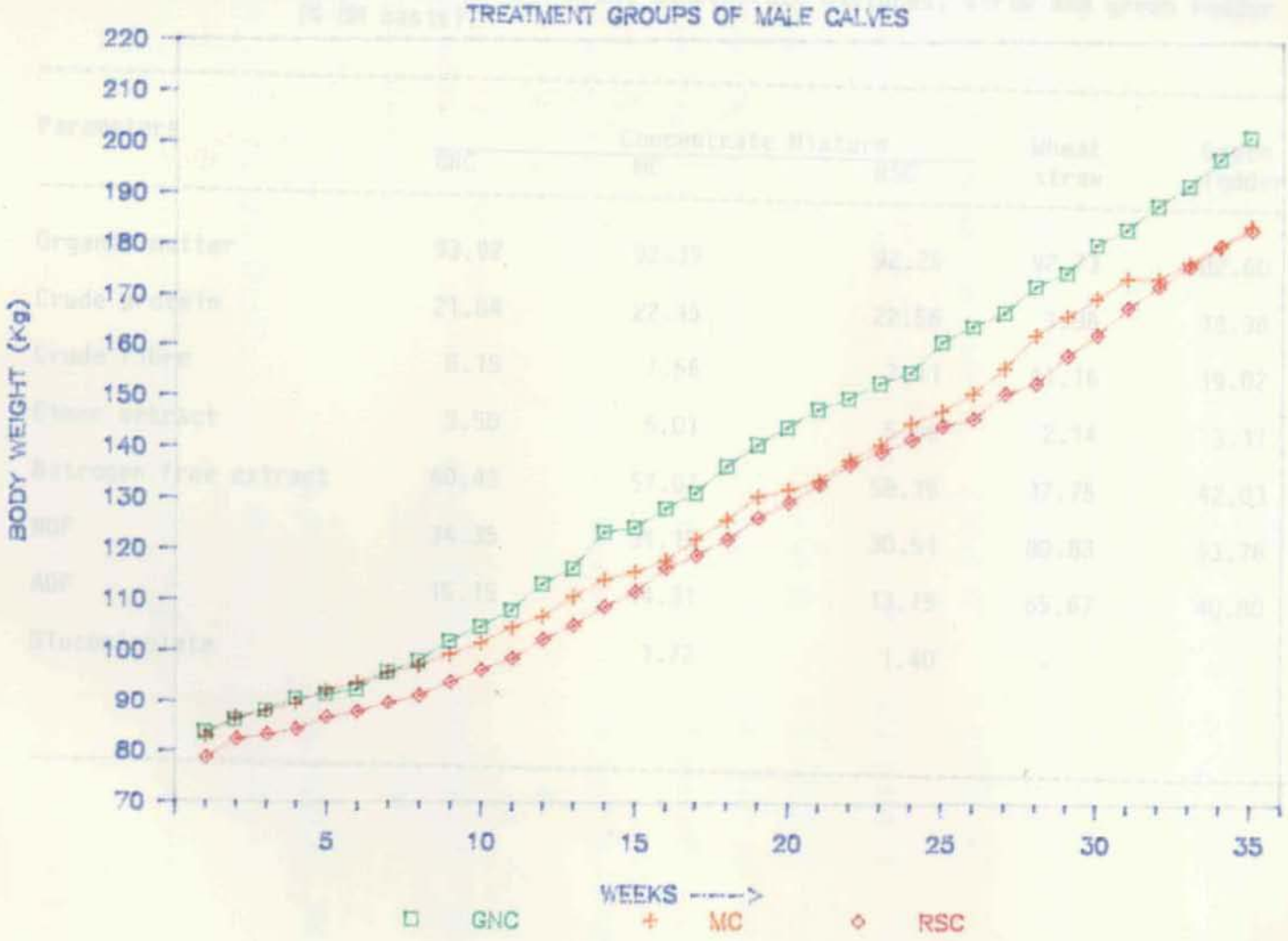


Table 4.7 Composition of different concentrate mixtures, straw and green fodder (% DM basis)

Parameters	Concentrate Mixture			Wheat straw	Green fodder
	GNC	MC	RSC		
Organic matter	93.92	92.39	92.25	92.73	82.60
Crude protein	21.84	22.15	22.56	3.88	18.38
Crude fibre	8.15	7.56	7.41	41.16	19.02
Ether extract	3.50	5.01	5.69	2.14	3.17
Nitrogen free extract	60.43	57.07	58.19	37.75	42.03
NDF	34.35	31.15	30.51	80.83	53.76
ADF	15.15	14.31	13.75	65.67	40.80
Glucosinolate	-	1.72	1.40	-	-

Table 4.8 Average growth rate and feed conversion efficiency in different treatment groups (whole experimental period of 35 weeks)

Parameters	Groups		
	GNC	MC	RSC
Initial body wt (kg)	83.93±9.47	83.14±6.30	78.78±7.63
Final body wt (kg)	201.68±14.19	184.31±9.67	183.45±10.97
Wt gain in 35 weeks (kg)	117.93±3.12	101.17±4.43	104.67±6.69
Daily body wt gain(g)	480.6	412.9	427.2
Av.growth rate/week(b value)	3.32±0.17	3.07±0.14	3.22±0.24
Total DMI through concentrate mix(kg)	541.95±38.69	523.39±24.72	510.33±33.32
Total DMI through roughage (kg)	506.90±9.88	512.58±14.35	502.33±17.61
Total DMI (Kg)	1048.19±48.00	1036.13±37.99	1012.67±50.50
Feed intake/kg gain (kg)	8.89±0.41	10.24±0.45	9.67±0.59
Total CP intake in 35 weeks (kg)	147.96±9.44	144.11±6.24	140.95±8.33
CP intake/kg gain (kg)	1.25	1.42	1.34
Total TDN intake in 35 weeks (kg)	601.81±38.05	590.25±27.47	577.41±32.62
TDN intake/kg gain (kg)	5.11	5.83	5.51
Total ME intake in 35 weeks (Mcal)	2178.56±137.77	2136.71±99.46	2090.25±118.10
ME intake/kg gain (Mcal)	18.50	21.11	19.95

The total DM consumption in GNC, MC and RSC groups was 1048.19±48.00, 1036.13±37.99 and 1012.67±50.50 kg, respectively. The average roughage:concentrate ratio was 1:1.06 in GNC group, 1:1.02 in MC group and 1:1.01 in RSC group. The average gain in body weight during whole experimental period in GNC, MC and RSC groups was 117.93±3.12, 101.17±4.43 and 104.67±6.69 kg, respectively. The average daily gain in body weight was 480.6 g in GNC group, 412.9 g in MC group, and 427.2 g in RSC group. The average growth rate per week (b value) in GNC, MC and RSC groups was 3.32±0.17, 3.07±0.14 and 3.22±0.24, respectively (Table 4.8). The feed:gain ratios in corresponding groups were 8.89±0.41, 10.24±0.45 and 9.67±0.59.

The total feed consumption and the total body weight gain in control (GNC) group were higher than those in MC and RSC groups. However, there was no statistical variation among groups and among calves within the group for the total feed consumption, total weight gain and growth rate (Table 4.12). Total CP consumption during the experimental period of 35 weeks in the GNC, MC and RSC groups was 147.96±9.44, 144.11±6.24 and 140.95±8.33 kg, respectively. The CP value per kg body wt gain in corresponding groups was estimated as 1.25, 1.42 and 1.34 kg. The CP consumption per kg body weight gain in MC and RSC groups was higher by 13.6 and 7.2 percent over that of GNC group. Average TDN and ME consumption during the whole experimental period were 601.81±38.05, 590.25±27.47 and 577.41±32.62 kg and 2178.56±137.77, 2136.71±99.46 and 2090.25±118.10 Mcal in GNC, MC and RSC groups, respectively. The corresponding values for TDN and ME intake per kg body wt gain were 5.11, 5.83 and 5.51 kg and 18.50, 21.11 and 19.95 Mcal (Table 4.8). The total CP and energy consumed during the whole experimental period were higher in GNC group, however, its CP and energy consumption per kg body weight gain were lower than those of other two groups. The variations among the groups were not significant (Table 4.8).

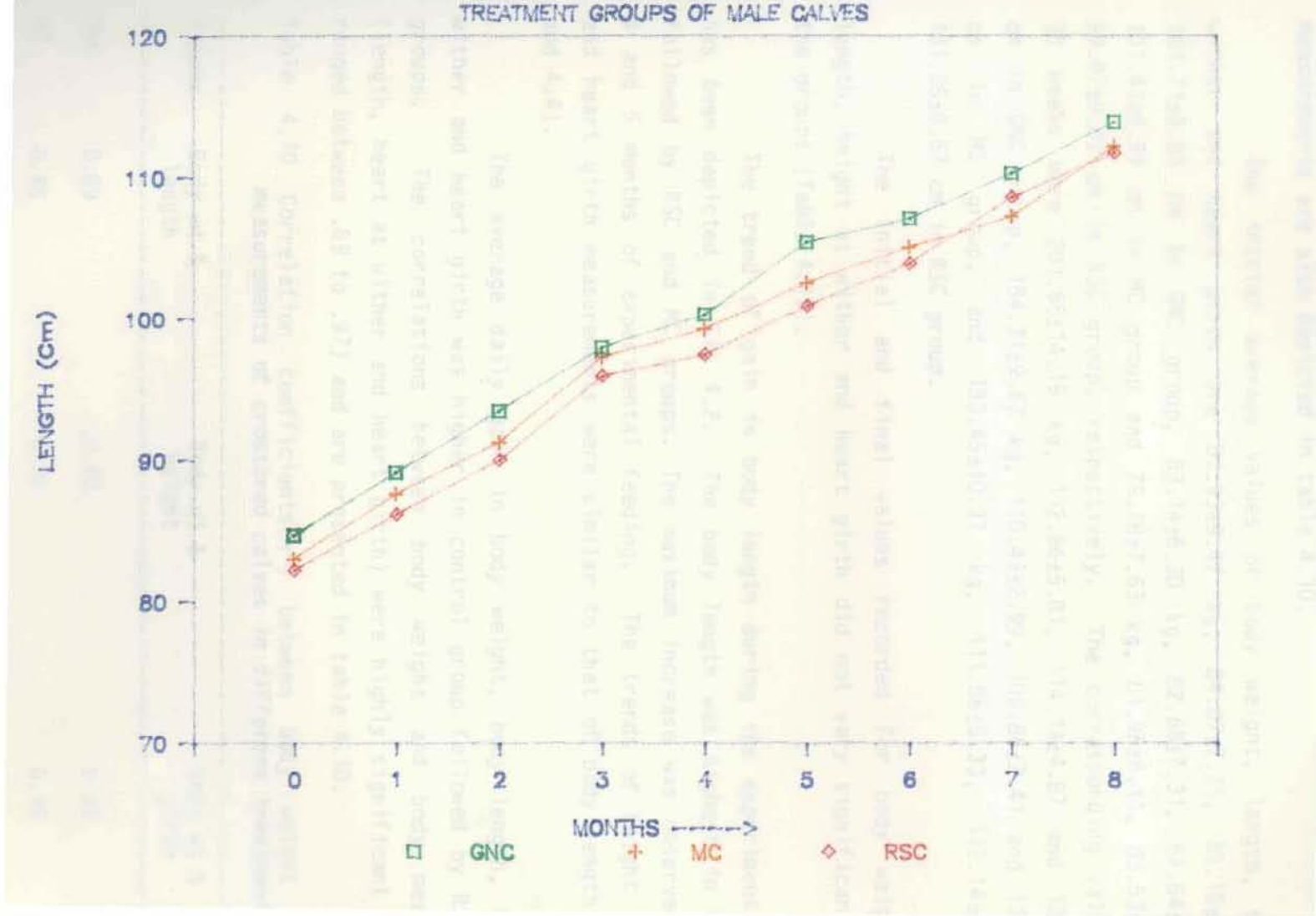
4.3.3 Body measurements

The data on body measurements, i.e., length, height at wither and heart girth at the time of starting and after 35 weeks of experimental feeding are presented in table 4.9. The data on length, height at wither and heart girth recorded at monthly intervals are presented in Figs 4.2,

Table 4.9 Effect of different treatment rations on body measurement of crossbred calves

Parameters	GNC	MC	RSC
No. of animal	6	6	6
Initial body wt (kg)	83.93±9.47	83.14±6.30	78.78±7.63
Final body wt (kg)	201.68±14.19	184.31±9.67	183.45±10.97
Daily weight gain (g)	480.6	412.9	427.2
Initial body length (cm)	84.07±7.45	82.68±7.31	81.96±6.11
Final body length (cm)	112.86±5.81	110.43±2.99	111.86±5.33
Body length gain (mm)	117.50	113.26	122.04
Initial height at wither (cm)	85.18±6.55	84.64±2.57	83.53±5.10
Final height at wither (cm)	114.14±4.87	109.86±2.41	112.14±6.51
Daily increase in height at wither (mm)	118.20	102.93	116.77
Initial heart girth (cm)	101.71±9.83	101.43±6.99	99.07±9.02
Final heart girth (cm)	134.86±7.92	131.86±5.67	131.85±8.57
Daily increase in heart girth (mm)	135.30	124.20	133.79

Fig 4.2 : AVERAGE BODY LENGTH IN THREE



4.3 and 4.4, respectively. The correlation between body weight and body measurements are also depicted in table 4.10.

The initial average values of body weight, length, height at wither and heart girth are 83.93±9.47 kg, 84.07±7.45, 85.18±6.55 and 101.71±9.83 cm in GNC group, 83.14±6.30 kg, 82.68±7.31, 84.64±2.57 and 101.43±6.99 cm in MC group and 78.78±7.63 kg, 81.96±6.11, 83.53±5.10 and 99.07±9.02 cm in RSC group, respectively. The corresponding values after 35 weeks were 201.68±14.19 kg, 112.86±5.81, 114.14±4.87 and 134.86±7.92 cm in GNC group, 184.31±9.67 kg, 110.43±2.99, 109.86±2.41 and 131.86±5.67 cm in MC group, and 183.45±10.97 kg, 111.86±5.33, 112.14±6.51 and 131.85±8.57 cm in RSC group.

The initial and final values recorded for body weight, body length, height at wither and heart girth did not vary significantly among the groups (Table 4.12).

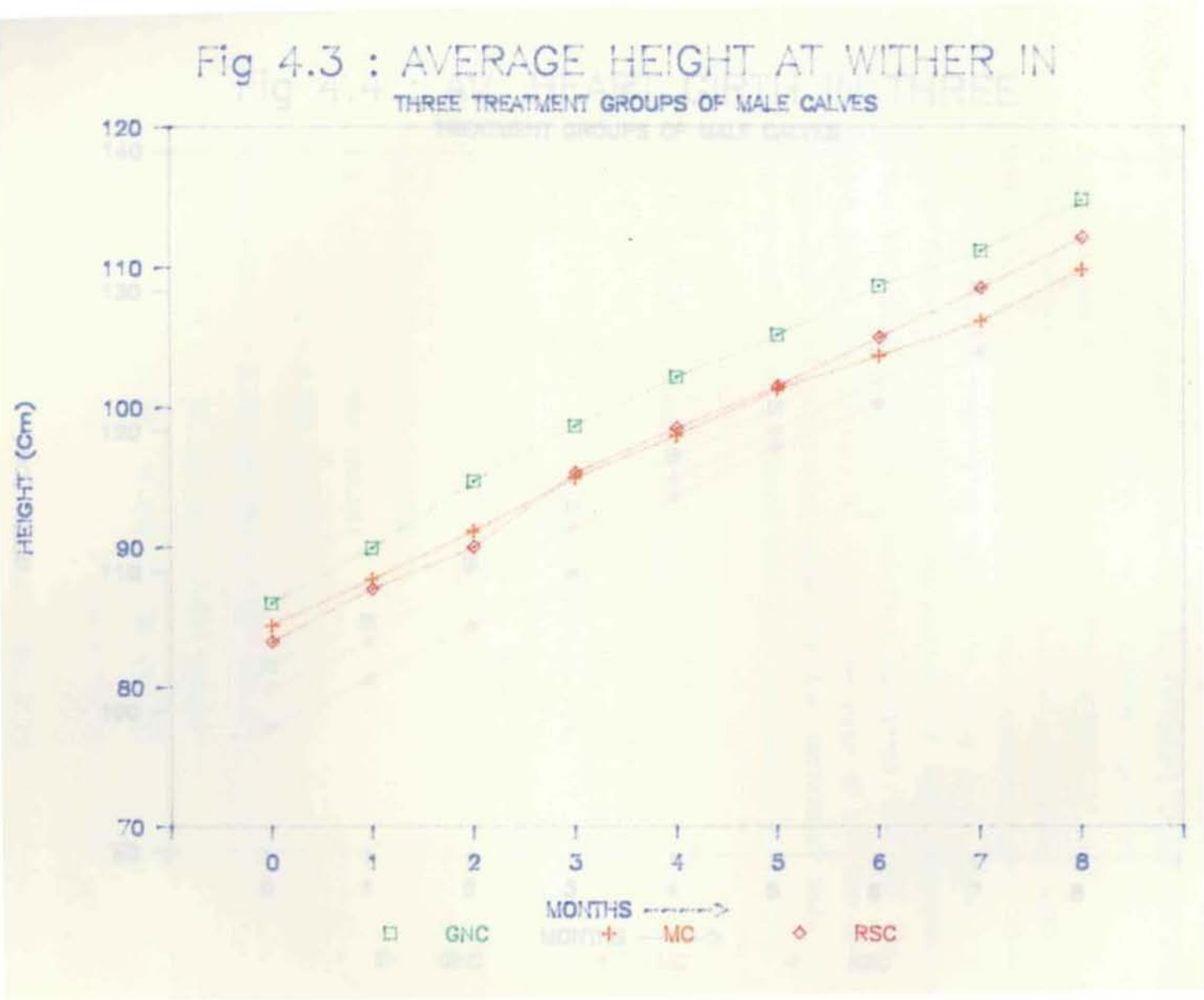
The trend of gain in body length during the experimental period has been depicted in Fig 4.2. The body length was highest in GNC group followed by RSC and MC groups. The maximum increase was observed between 4 and 5 months of experimental feeding. The trends of height at wither and heart girth measurements were similar to that of body length (Fig 4.3 and 4.4).

The average daily gain in body weight, body length, height at wither and heart girth was higher in control group followed by RSC and MC groups. The correlations between body weight and body measurements (length, heart at wither and heart girth) were highly significant (R^2 value ranged between .89 to .97) and are presented in table 4.10.

Table 4.10 Correlation coefficients(r) between body weight and body measurements of crossbred calves in different treatment groups

Groups	Body wt & length	Body wt & height	Body wt & girth
GNC	0.89	0.90	0.92
MC	0.95	0.96	0.95
RSC	0.97	0.96	0.96

Fig 4.3 : AVERAGE HEIGHT AT WITHER IN



4.3.4 Relationship between body weight and measurements

In order to relate the feed input to body weight gains, certain body measurements, which can easily be recorded, were recorded and used for developing the prediction equations in different treatment groups. The results of prediction equations in different groups of crossbred calves are detailed in table 4.11. The prediction equations in crossbred calves for 4-13 months period revealed that either of the three measurements (girth, height or length) alone could be accurately used for predicting body weights in all the three groups, however, girth measurement alone gave the optimum prediction in all the three group because R^2 values were higher than 90.0 percent. Combinations of body measurements were also tried for developing the prediction equations for all the three groups which showed satisfactory R^2 value. The data of all the three groups were pooled and final prediction equations for individual body measurements as well as combined measurements were also developed (Table 4.11).

Table 4.11 Prediction equation of body weight with body measurements (Length, height and girth)

	R^2
Groundnut cake group	
B.W. = -224.00 + 3.62L (±0.12)	0.82
B.W. = -222.26 + 3.55H (±0.08)	0.75
B.W. = -227.10 + 3.08G (±0.10)	0.92
B.W. = -236.60 + 2.69L (±0.25) + 1.03H (±0.25)	0.83
B.W. = -236.73 + 1.36L (±0.26) + 2.01G (±0.22)	0.87
B.W. = -231.49 + 0.38H (±0.26) + 2.79G (±0.21)	0.85
B.W. = -236.31 + 1.37L (±0.28) + 0.04 H (±0.26) + 2.03G (±0.25)	0.87
Mustard cake group	
B.W. = -215.49 + 3.50L (±0.11)	0.78
B.W. = -227.99±3.61H (±0.16)	0.65

...contd.

...contd.Table 4.11

	R^2
B.W. = -224.05±3.02G (±0.08)	0.91
B.W. = -204.96 + 4.01L (±0.33) + 0.61H (±0.37)	0.78
B.W. = -241.78 + 1.43L (±0.19) + 1.96G (±0.16)	0.86
B.W. = -252.78 + 0.98H (±0.18) + 2.43G (±0.13)	0.84
B.W. = -238.72 + 1.59L (±0.34) + 0.17H (±0.30) + 1.96G (±0.16)	0.85
Rapeseed cake group	
B.W. = -203.76 + 3.37L (±0.10)	0.93
B.W. = -228.85 + 3.56H (±0.085)	0.91
B.W. = -210.25 + 2.91G (±0.04)	0.97
B.W. = -221.62 + 2.18L (±0.25) + 1.34H (±0.27)	0.93
B.W. = -214.02 + 0.47L (±0.18) + 2.54G (±0.13)	0.97
B.W. = -207.56 + 0.17H (±0.18) + 3.04G (±0.13)	0.97
B.W. = -206.99 + 0.73L (±0.18) + 0.59H (±0.20)+ 2.77G (±0.15)	0.97
Pooled	
B.W. = -215.98 + 3.51L (±0.05)	0.82
B.W. = -226.33 + 3.58H (±0.07)	0.75
B.W. = -221.29 + 3.10G (±0.04)	0.87
B.W. = -226.51 + 2.84L (±0.15) + 0.77H (±0.16)	0.83
B.W. = -231.24 + 1.14L (±0.12) + 2.13G (±0.10)	0.89
B.W. = -230.75 + 0.54H (±0.12) + 2.62G (±0.95)	0.88
B.W. = -230.04 + 1.20L (±0.15) + 0.10H (±0.14) + 2.15G (±0.10)	0.89

Table 4.12 Analysis of variance for different parameters during the whole experimental period of 35 weeks

Source of variation	d.f.	B.wt	Total DMI	DMI Conc.mix	DMI roughage	Feed:gain	Growth rate/week
Replicates	5	446.36	3292625.7	5538.8	1101836.8	2.00	0.49
Treatments	2	146.17	3387472.5	1807.4	1086046.2	2.34	0.10
Error	10	119.95	3190580.0	7014.4	1073326.3	2.77	0.18

Source of variation	d.f.	Initial b.measurement			Final b.measurement		
		Length	Height	Girth	Length	Height	Girth
Replicates	5	6.04	34.63	10.32	14.60	72.93	20.04
Treatments	2	8.03	21.53	4.91	10.42	17.76	32.19
Error	10	50.38	80.59	32.63	26.81	47.65	26.02

4.3.5 Intake of dry matter and organic matter

The data of dry matter (DM) and organic matter (OM) in different groups during the metabolism trial period are given in Table 4.13.

The average daily DM intake in GNC, MC and RSC groups was 4.52, 4.38 and 4.30 kg and the DM intake per 100 kg body wt was estimated as 2.23±0.05, 2.29±0.11 and 2.45±0.21 kg, respectively. The corresponding values for DM intake per kg $W^{0.75}$ were 84.29±1.21, 84.50±3.58 and 84.14±3.86 g. However, the variation among groups was not significant (Table 4.15). The organic matter and digestible organic matter (DOM) intake in GNC, MC and RSC groups were 4.21, 4.20 and 3.85 kg, and 2.75, 2.62 and 2.33 kg, respectively. The corresponding values for DOM intake/kg $W^{0.75}$ were 52.87, 50.55 and 45.59 g. The variations for organic matter intake observed among the groups and among the animals within the groups were not significant (Table 4.15).

4.3.6 Digestibility of nutrients

Digestibility coefficients for the proximate principles and cell wall constituents in different treatment groups are presented in table 4.13.

The average digestibility coefficients for dry matter and organic matter in GNC, MC and RSC groups were 61.79±1.16, 59.59±2.00 and 59.29±1.08; 65.28±1.18, 62.46±2.49 and 60.62±1.74 percent, respectively. The digestibility coefficient of crude protein was 68.34±1.35 in GNC group, 71.82±1.63 in MC group and 69.66±1.03 in RSC group. The values for the percent digestibilities of crude fibre, ether extract and NFE in GNC, MC and RSC groups were 52.90±1.56, 51.75±2.62 and 52.11±2.51; 78.09±2.11, 79.45±0.79 and 78.78±1.43; 68.86±2.15, 60.97±2.95 and 62.75±2.88. The average digestibility coefficients of ADF and NDF were 50.43±2.17 and 43.39±1.72 in GNC group, 48.67±2.40 and 42.36±3.02 in MC group and 48.86±1.71 and 44.91±1.57 in RSC group, respectively, and the variations among groups were not significant (Table 4.15).

4.3.7 Nitrogen metabolism

The data for nitrogen intake, its excretion through faeces and urine and nitrogen balances in different groups are presented in Table 4.14.

Table 4.13 Average DM intake and digestibility of nutrients in different groups (During metabolism trial)

Parameters	Groups		
	GNC	MC	RSC
Av.body weight (kg)	202.52±13.56	193.25±10.07	189.67±13.28
DMI through concentrate mix (kg)	2.54	2.54	2.33
DMI through roughage (kg)	1.98	1.84	1.97
Total DM intake (kg)	4.52±0.21	4.38±0.12	4.30±0.29
DMI(kg)/100 kg B.wt	2.23±0.05	2.29±0.11	2.45±0.21
DMI(g)/kgW$^{0.75}$	84.29±1.21	84.50±3.58	84.14±3.86
Digestible Org.Matter(DOM) Intake (kg)	2.75±0.14	2.62±0.16	2.33±0.14
DOM intake/kgW$^{0.75}$(g)	52.87±0.55	50.55±2.63	45.59±2.79
Digestibility coefficients			
Dry matter	61.79±1.16	59.59±2.00	59.29±1.08
Organic matter	65.28±1.18	62.46±2.49	60.62±1.74
Crude protein	68.34±1.35	71.82±1.63	69.66±1.03
Crude fibre	52.90±1.56	51.75±2.62	52.11±2.51
Ether extract	78.09±2.11	79.45±0.79	78.78±1.43
NFE	68.86±2.15	60.97±2.95	62.75±2.88
ADF	50.43±2.17	48.67±2.40	48.86±1.71
NDF	43.39±1.72	42.36±3.02	44.91±1.57

Table 4.14 Average nitrogen intake, excretion and balance in different treatment groups

Parameters	Groups		
	GNC	MC	RSC
N-intake through conc.mixture	87.14±5.48	90.39±3.93	85.12±4.63
N-intake through straw	10.16±1.27	8.90±2.17	7.58±1.89
N-intake through fodder	3.28	3.28	3.28
Total N intake (g)	100.58±5.90	102.57±3.92	95.98±6.49
N intake(g)/kgW$^{0.75}$	1.94±0.02	1.98±0.02	1.88±0.02
N intake/kg DOM (g)	36.57	39.14	41.19
Faecal-N excretion (g)	31.62±1.60	28.79±1.57	28.82±1.23
Urinary-N excretion (g)	44.75±3.29	47.42±1.25	41.25±2.30
Total excretion (g)	76.37±4.55	76.21±2.13	70.07±2.73
N-balance (g)	24.25±2.83	25.86±4.16	25.91±5.75
N balance/kgW$^{0.75}$ (g)	0.46	0.50	0.51
N digested (g)	68.96±4.94	73.78±3.79	67.16±5.37
N-digestibility/kg W$^{0.75}$ (g)	1.39±0.06	1.38±0.60	1.32±0.09
Faecal-N as % N intake	31.65±1.34	28.06±1.63	30.34±1.03
Urinary-N as % N intake	44.49±1.54	46.61±2.30	44.03±3.90
% retention of total-N intake	23.96±2.09	24.74±3.18	25.62±4.73
% retention to digested-N	33.05±2.26	34.67±4.41	36.40±6.45

Table 4.15 Analysis of variance for DM intake and digestibility of nutrients

Source of variation	d.f.	Mean sum of squares						
		DMI	CP intake	TDN intake	DM Dig.	OM Dig.	CP Dig.	CF Dig.
Replicates	5	42.45	13977.39	0.30	15.99	29.74	21.59	71.43
Treatments	2	7.04	2672.98	0.23	22.64	32.92	18.51	90.23
Error	10	22.21	3871.08	0.07	11.71	11.75	5.91	36.66

Source of variation	d.f.	Mean sum of squares					
		EE Dig.	NFE Dig.	ADF Dig.	NDF Dig.	N intake	N balance
Replicates	5	21.31	41.60	31.78	17.21	356.67	218.31
Treatments	2	5.29	102.64	9.89	5.60	68.50	7.76
Error	10	10.74	44.19	27.81	31.76	98.94	72.63

Daily nitrogen intake in GNC, MC and RSC groups was 100.58±5.90, 102.57±3.92 and 95.98±6.49 g while the nitrogen intake per kg $W^{0.75}$ was 1.94, 1.98 and 1.88 g, respectively. Concentrate mixture supplied the major portion of nitrogen in all the groups. The nitrogen intake (as percent of total intake) through concentrate mixture in GNC, MC and RSC groups was 87.14, 90.39 and 85.12, respectively. The corresponding values for the faecal and urinary excretion were 31.62±1.60, 28.79±1.57 and 28.82±1.23; 44.75±3.29, 47.42±1.25 and 41.25±2.30 g per day. The nitrogen balance in GNC, MC and RSC groups was found to be 24.25±2.83, 25.86±4.16 and 25.91±5.75 g per day, respectively. Nitrogen efficiency was found to be 23.96±2.09 percent in GNC group, 24.74±3.18 percent in MC group and 25.62±4.73 percent in RSC group. Average N intake/kg digestible organic matter intake was 36.57 g in GNC group, 39.14 g in MC group and 41.19 g in RSC group and these values indicated that the animals were getting sufficient supply of fermentable-N.

Statistical analysis of data (Table 4.15) indicated that the variations among groups for nitrogen intake and for nitrogen balance were not significant. However, the variation observed among animals within groups for nitrogen balance was significant ($P/0.05$).

4.3.8 Plane of nutrition

The plane of nutrition of the calves under different groups during the trial period have been presented in table 4.16 and have been compared with the values given by NRC (1978) feeding standard.

The average daily CP consumption in GNC, MC and RSC groups was 628.45±37.04, 641.08±24.44 and 599.88±40.60g, while DCP consumption was 430±30.82, 464±26.50 and 419±37.04g, respectively. The daily TDN intake in respective groups was observed to be 2.83±0.16, 2.72±0.13 and 2.49±0.17 kg. Average metabolizable energy (ME) intake was 10.21±0.59 Mcal in GNC group, 9.84±0.49 Mcal in MC group and 9.01±0.64 Mcal in RSC group. In general, the CP intake in all the groups was higher than the values given by NRC (1978). However, TDN intake was lower by 8.70, 9.03 and 15.02 percent as compared to the values given by NRC (1978) in GNC, MC and RSC groups, respectively. As far as the ME intake is concerned, it was 89.09, 88.88 and 83.70 percent of the requirement suggested in NRC (1978).

Table 4.16 Plane of nutrition of experimental calves and its comparison with NRC (1978) values (During metabolism trial period)

Parameters	Groups		
	GNC	MC	RSC
Average Body wt (kg)	202.52±13.34	193.25±10.07	189.67±13.29
Average metabolic body weight ($W^{0.75}$ kg)	53.68	51.76	50.99
CP consumed (g/day)	628.45±37.04	641.08±24.49	599.88±40.60
CP consumed/$kgW^{0.75}$ (g)/day	11.70	12.38	11.76
NRC requirement for CP(g/day)	602.00	581.68	570.90
CP consumed as % of NRC value	104.39	110.21	105.07
DCP consumed (g/day)	430 ± 30.82	464 ± 26.50	419 ± 37.04
DCP consumed/$kgW^{0.75}$(g/day)	8.01± 1.17	8.96± 0.98	8.22± 1.31
TDN consumed (kg/day)	2.83 ± 0.16	2.72 ± 0.13	2.49 ± 1.31
TDN consumed/$KgW^{0.75}$ (g/day)	52.71±0.87	52.55±0.76	48.83±1.07
NRC requirement for TDN (kg)	3.10	2.99	2.93
TDN consumption as % of NRC requirement	91.29	90.97	84.98
*ME consumption(Mcal/day)	10.21±0.59	9.84±0.49	9.01±0.64
ME consumption/$kgW^{0.75}$ (Mcal)	0.19	0.19	0.18
NRC requirement for ME (Mcal)	11.46	11.07	10.86
ME consumption as % of NRC requirement	89.09	88.88	83.70

*1 kg TDN = 3.62 Mcal ME (Kearl, 1982)

From the statistical analysis of data (Table 4.15), it was evident that the intake of CP and TDN did not vary significantly among the groups. But the variations among animals within the groups were found to be significant ($P/0.05$).

4.3.9 Body composition

The response of glucosinolates offered through the MC and RSM supplemented rations on body composition of crossbred male growing calves was compared with those fed on control ration. The body composition estimated in terms of percentage of water, fat, protein and ash are presented in table 4.17.

The average content of body water (percent) in MC and RSM groups was not significantly different from that of the control (GNC) group. Body water was found to be 67.74±0.60, 67.88±0.20 and 69.10±0.25 percent (live weight basis) in GNC, MC and RSM groups, respectively. However, body water on empty body weight basis in these groups was 62.52±0.56, 62.49±0.023 and 63.6±0.23 percent (Table 4.17). Fat percentage also did not vary among the groups and the corresponding values on empty body weight basis were 13.70±0.58, 13.73±0.25 and 12.51±0.24 percent. The protein percent in these groups was observed to be 17.73±0.19, 17.65±0.07 and 18.00±0.06 while the ash was 6.04±0.17, 6.12±0.06 and 5.82±0.06 percent respectively. Analysis of variance of the data (Table 4.18) showed that the differences among the groups were not significant for all the parameters measured for body composition.

4.3.10 Tri-iodothyronine(T_3)concentration

The data of average tri-iodothyronine (T_3) concentration in the blood plasma of calves of GNC, MC and RSC groups estimated at the monthly intervals by radioimmunoassay technique are presented in table 4.19.

Initially, the average plasma T_3 concentration in GNC, MC and RSC groups was 0.37±0.03, 0.29±0.02 and 0.30±0.01 ng/ml, respectively and the variation among groups was not significant as the calves were fed on similar diet, i.e., normal concentrate mixture and wheat straw **ad lib.** The average T_3 values in GNC group were 0.41±0.09, 0.37±0.01, 0.40±0.12, 0.38±0.13, 0.25±0.03, 0.37±0.00, 0.27±0.03 & 0.66±0.06 ng/ml after 1, 2, 3, 4, 5, 6, 7 and 8 months of experimental feeding respectively. The

Table 4.17 Data on body composition of crossbred calves in different treatment groups (% empty body weight basis)

Groups	Water	Fat	Protein	Ash
GNC	62.52 ± 0.56	13.70 ± 0.58	17.73 ± 0.19	6.04 ± 0.17
MC	62.49 ± 0.23	13.73 ± 0.25	17.65 ± 0.07	6.12 ± 0.06
RSC	63.66 ± 0.23	12.51 ± 0.24	18.00 ± 0.16	5.82 ± 0.06

Table 4.18 Analysis of variance for body composition

Source of	d.f.	Mean sum of squares			
		Water	fat	Protein	Ash
Replicates	4	0.584	0.606	0.033	0.045
Treatments	2	2.240	2.410	0.125	0.129
Error	8	0.790	0.850	0.084	0.076

Table 4.19 Plasma Tri-iodothyronine (T_3) and Thyroxine (T_4) concentrations (ng/ml) in different treatment groups at various monthly intervals during the growth trial

Month	Period of experimental feeding (months)	GROUPS					
		GNC		MC		RSC	
		T_3	T_4	T_3	T_4	T_3	T_4
April	0	0.37±0.03	29.61±12.66	0.29±0.00	24.95±4.79	0.30±0.01	28.31±4.45
May	1	0.41±0.09	39.50±7.56	0.30±0.16	36.62±11.42	0.49±0.09	25.67±4.90
June	2	0.37±0.01	51.85±12.33	0.19±0.01	43.48±8.96	0.40±0.11	20.95±4.55
July	3	0.40±0.12	39.05±4.26	0.10±0.07	49.50±16.26	0.56±0.20	45.14±12.72
August	4	0.38±0.13	45.89±7.32	0.20±0.03	43.75±8.27	0.41±0.41	31.04±6.00
September	5	0.25±0.03	40.98±5.63	0.40±0.09	33.74±1.89	0.40±0.10	40.50±9.39
October	6	0.37±0.00	56.16±6.48	0.41±0.10	48.68±13.27	0.21±0.03	50.75±6.89
November	7	0.27±0.03	74.22±10.17	0.44±0.16	67.38±15.57	0.28±0.01	55.23±12.41
December	8	0.66±0.06	87.78±9.63	0.37±0.04	75.91±15.13	0.54±0.23	65.02±6.75
Average		0.39±0.03	51.44±6.04	0.30±0.03	47.11±5.30	0.39±0.04	40.29±4.97

corresponding values in MC group were 0.30±0.16, 0.19±0.01, 0.10±0.07, 0.20±0.03, 0.40±0.09, 0.41±0.10, 0.44±0.16 amd 0.37±0.04 ng/ml and in RSC group the values were 0.49±0.09, 0.40±0.11, 0.56±0.20, 0.41±0.41, 0.40±0.10, 0.21±0.03, 0.28±0.01 and 0.54±0.23 ng/ml. The variations among months of experimental feeding were significant ($P/0.05$) in all the three treatment groups (Table 4.21).

The overall average value of T_3 concentration in GNC, MC and RSC was 0.39±0.03, 0.30±0.03 and 0.39±0.04 ng/ml, respectively. The variations among groups were found to be significant ($P/0.05$). Plasma T_3 concentration in MC group was significantly lower ($P/0.05$) than in GNC and RSC groups, however, variations between latter two groups were non-significant (Table 4.21).

4.3.11 Thyroxine(T_4)concentration

The data of average thyroxine (T_4) concentration in the plasma of calves fed on GNC, MC and RSC supplemented diets at different stages of growth are given in table 4.19.

Initially, the average plasma T_4 concentration in GNC, MC and RSC groups was 29.61±12.66, 24.95±4.79 and 28.31±4.45 ng/ml, respectively and the variations among groups were not significant. The average plasma T_4 concentrations were increased with increasing the age of calves in all the groups, however, levels in general were lower in MC and RSC groups as compared to GNC group. The overall average value of plasma thyroxine (T_4) in GNC, MC and RSC groups was 51.44±6.04, 47.11±5.30 and 40.29±4.97 ng/ml, respectively.

The average T_4 values of GNC group after 1, 2, 3, 4, 5, 6, 7 and 8 months of experimental feeding were 39.50±7.56, 51.85±12.33, 39.05±4.26, 45.09±7.32, 40.98±5.63, 56.16±6.48, 74.22±0.17 and 85.78±9.63 ng/ml, respectively and the corresponding values in MC group were 36.62±11.42, 43.48±8.96, 49.50±16.26, 43.75±8.27, 33.74±1.84, 46.68±13.27, 67.38±15.57 and 75.81±15.13, and in RSC group were 25.67±4.90, 20.95±4.55, 45.14±12.72, 31.04±6.00, 40.50±9.39, 50.75±6.89, 55.23±12.41 and 65.02±6.75. Statistical analysis of data (Table 4.21) revealed that there was no significant variation among groups, however, variations among different periods were significant ($P/0.05$).

4.3.12 Haemoglobin

Haemoglobin (Hb) content in the blood of calves fed on different treatment rations was estimated at monthly intervals upto 8 months of experimental feeding and data have been presented in table 4.20.

The average initial Hb content in GNC, MC and RSC groups was 11.73±0.19, 11.60±0.18 and 11.67±0.25 percent, respectively. The overall average Hb content in corresponding groups was 11.60±0.08, 11.55±0.08 and 11.55±0.02 percent. Statistifal analysis of data indicated that there were non-significant variations among the treatments and periods (Table 4.21).

4.3.13 Thiocyanate content in blood plasma

The data on thiocyanate content in blood plasma of the two animals from each group at monthly intervals are given in table 4.20.

Thiocyanates were not detected in the plasma of GNC group throughout the experimental period of eight months. Thiocyanates were also not detected in the MC and RSC groups in the samples drawn before starting their experimental diets. The trend of thiocyanate content was similar in MC and RSC groups. However, overall average thiocyanate content was higher in MC group (8.90±0.11 µg/ml) than in RSC group (7.92±1.68 µg/ml).

4.3.14 Thyroxine secretion rate (TSR)

The data on thyroxine secretion rate in experimental animals of different groups as determined by thyroxine turn over method are presented in table 4.22.

The thyroxine disappearance rate (K) was 2.10 and 2.09 x 10^3 in animal No.5004 and 4994 of control group (GNC), 1.96 and 1.85 x 10^3 in animal No.5000 and 3933 of MC group and 1.89 and 2.22 x 10^3 in animal No.3935 and 4995 of RSC group, respectively. The average K values in GNC, MC and RSC groups were 2.09 x 10^3, 1.90 x 10^3 and 2.05 x 10^3, respectively (Fig 4.5).

The thyroxine distribution space (TDS) was 39.44 and 39.77 litre in animal No.5004 and 4994 of GNC group, 41.01 and 42.00 litre in animal No.5000 and 3933 of MC group and 41.54 and 38.84 litre in animal No.3935

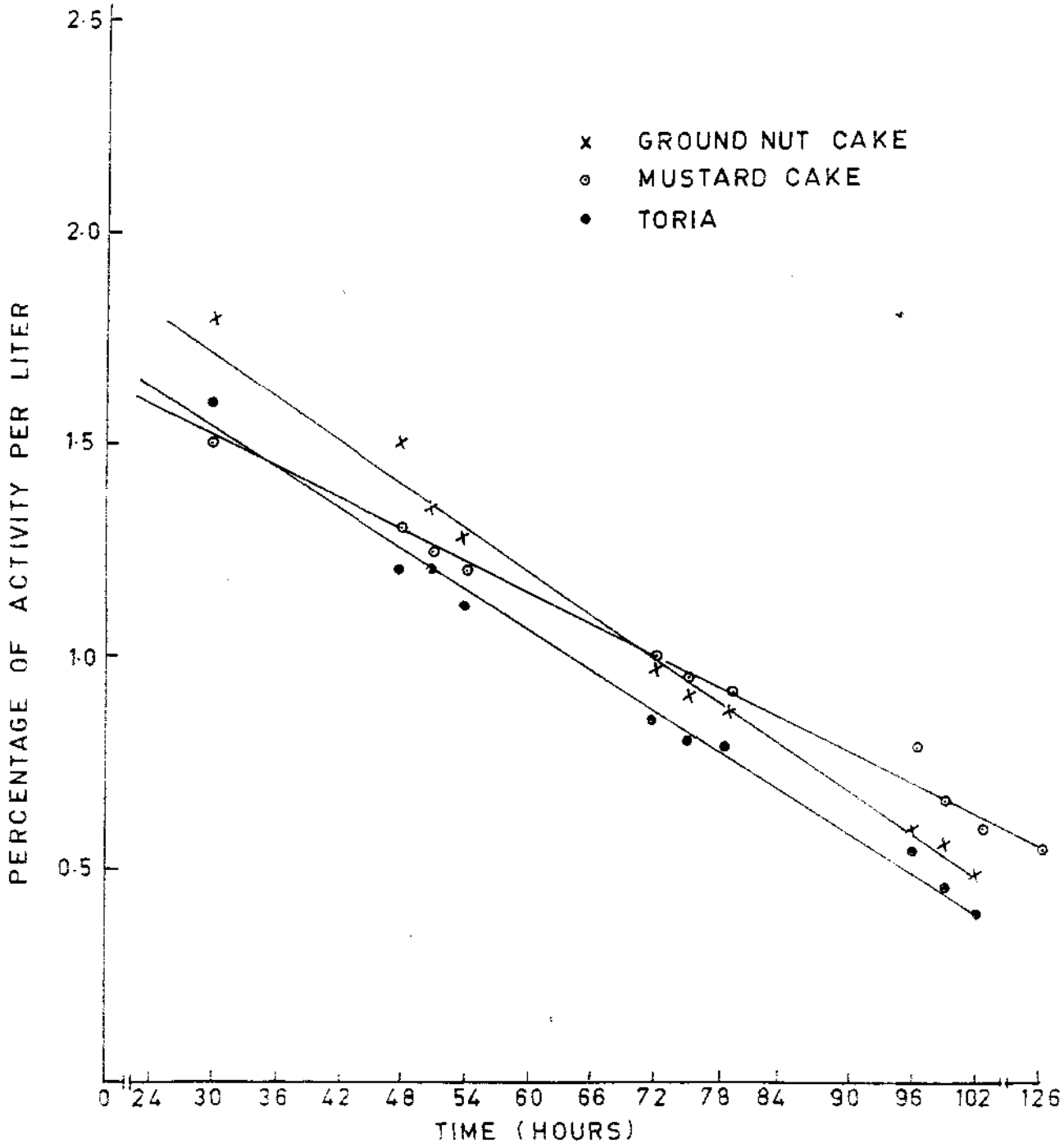


FIG. 4.6 ESTIMATION OF THYROXINE DISAPPEARANCE RATE IN THREE DIFFERENT TREATMENT GROUPS OF MALE CROSS BRED CALVES

Table 4.20 Blood haemoglobin and plasma thiocyanate contents at monthly intervals in different treatment groups of crossbred calves

Month	Period of exp. feeding	Haemoglobin(%)			Thiocyanate(ug/ml)		
		GNC	MC	RSC	GNC	MC	RSC
April	0	11.73 ±0.19	11.60 ±0.18	11.67 ±0.25	0.00	0.00	0.00
May	1	11.47 ±0.23	11.60 ±0.18	11.67 ±0.16	0.00	7.14	0.00
June	2	11.45 ±0.20	11.60 ±0.27	11.48 ±0.11	0.00	5.94	6.32
July	3	11.67 ±0.16	11.60 ±0.11	11.48 ±0.11	0.00	6.34	7.12
August	4	11.60 ±0.11	11.29 ±0.26	11.48 ±0.11	0.00	7.12	4.76
September	5	11.92 ±0.14	11.92 ±0.19	11.47 ±0.18	0.00	7.52	7.12
October	6	11.99 ±0.12	11.60 ±0.18	11.48 ±0.16	0.00	14.68	15.46
November	7	11.92 ±0.14	11.67 ±0.12	11.60 ±0.18	0.00	12.68	9.92
December	8	11.60 ±0.18	11.67 ±0.18	11.16 ±0.18	0.00	9.92	12.68
Average		11.60 ±0.08	11.55 ±0.08	11.55 ±0.02	0.00 ±0.00	8.90 ±1.12	7.92 ±1.68

Table 4.21 Analysis of variance for tri-iodothyronine (T_3), thyroxine (T_4) and haemoglobin (Hb)

Source of variation	d.f.	Mean sum of squares		
		T_3	T_4	Hb
Replicates	4	0.037	1090.31	0.46
Periods	8	0.035	3700.62	0.01
Treatments	2	0.127	1947.83	0.13
Error	120	0.012	442.50	0.31

Table 4.22 Estimation of Thyroxine secretion rate and related parameters in different groups

Animal	Body wt (kg)	K x 10^{-3}	TDS/Lit	TDS/Lit per 100 kg B.W.	PT ug/100 ml	Total TSR mg/Lit	TSR mg/Lit per 100 kg B.W. per day
			GNC				
5004	207.50	2.10	39.44	20.24	6.97	0.057	0.027
4994	192.25	2.09	39.77	20.68	8.57	0.071	0.037
Average	199.87	2.09	39.60	20.46	7.77	0.064	0.032
			MC				
5000	200.00	1.96	41.01	20.50	5.56	0.045	0.022
3933	201.00	1.85	42.00	20.89	7.72	0.060	0.029
Average	200.50	1.90	41.50	20.69	6.64	0.052	0.025
			RSC				
3935	183.00	1.89	41.54	20.69	8.36	0.066	0.036
4995	158.00	2.22	38.84	24.58	6.50	0.056	0.035
Average	170.50	2.05	40.19	22.63	7.43	0.061	0.035

and 4995 of RSC group, respectively. The average TDS value per animal of GNC, MC and RSC was 39.60, 41.50 and 40.19 litre. The average TDS value per 100 kg body weight in corresponding groups was 20.46, 20.69 and 22.63 litre. Statistical analysis of data indicated non-significant variations between the animals as well as among the treatment groups (Table 4.23).

Table 4.23 Analysis of variance for thyroxine disappearance space and thyroxine secretion rate

Source of	d.f.	Mean sum of squares			
		TDS	TDS/100 kg	TSR	TSR/100 kg
Treatments	2	1.89	2.84	.00007	.00005
Replicates	1	0.32	3.71	.00006	.00004
Error	2	1.92	2.01	.00009	.00001

Plasma thyroxine (PT) as determined by RIA technique was 6.97 and 8.57 µg/100 ml in animal No.5004 and 4994 of GNC group, 5.56 and 7.72 µg/100 ml in animal No.5000 and 3933 of MC group and 8.36 and 6.50 µg/100 ml in animal No.3935 and 4995 of RSC group, respectively. The average PT value in GNC, MC and RSC groups was 7.77, 6.64 and 7.43 µg/100 ml, respectively and the variations among treatment groups were not significant.

Thyroxine secretion rate (TSR) was calculated taking into account the thyroxine disappearance rate, TDS and PT. The TSR in animal No.5004 and 4994 of GNC group, 5000 and 3933 of MC group, and 3935 and 4995 of RSC group was 0.057, 0.071; 0.045, 0.060; and 0.066 and 0.056 mg/litre, respectively. The average TSR per animal of GNC, MC and RSC was 0.064, 0.052 and 0.061 mg/litre, respectively, and the average TSR per 100 kg body weight in corresponding groups was 0.032, 0.025 and 0.035 mg/litre. Statistical analysis of data did not reveal any significant variation among the different treatment groups (Table 4.23).

4.4 EFFECT OF STORAGE ON QUALITY OF OILCAKES

The effect of storage on the chemical composition (on % DM basis) of groundnut cake (GNC), mustard cake (MC) and rapeseed cake (RSC) was studied at monthly intervals for one year. The data obtained for quarterly intervals have been presented in table 4.24. The meteorological data has been shown in Fig 4.6.

4.4.1 Variations in temperature and relative humidity during the storage period

The changes in the temperature and relative humidity in the godown during the course of study havebeen presented in Fig 4.6. A maximum of 80-85 percent RH was observed for a brief spell during the last week of June and then again during the month of December but most of the times the RH was below 70 percent. Temperature in godown varied from 15^{O} to $42^{O}C$, the lowest being in January and the highest in June. During the remaining period, the temperature varied between 25^{O} to $30^{O}C$.

4.4.2 Proximate composition

4.4.2.1 Dry matter:-

The average initial DM content of GNC, MC and RSC was 97.75, 97.72 and 97.35 percent, respectively. The average DM in GNC, MC and RSC during the periods of May-July, Aug-Oct, Nov-Jan and Feb-April was 96.30, 96.27, 95.27 and 95.37; 96.24, 96.19, 95.43 and 95.30; and 96.20, 96.24, 95.38 and 95.20 percent, respectively (Table 4.24). The overall average DM content of GNC, MC and RSC was 96.39±1.10, 96.33±1.03 and 96.27±1.00 percent, respectively. The statistical analysis of data (Table 4.25) revealed that the variation among oilcakes was not significant. However, the decrease in DM percentage during storage period was significant ($P/0.05$).

4.4.2.2 Crude protein:-

The average initial CP content of GNC, MC and RSC was 47.71, 36.27 and 36.33 percent (on DM basis), respectively. Though, there was a decrease in CP content during the storage period, the extent of decrease

Fig 4.6 :RELATIVE HUMIDITY & TEMPRATURE

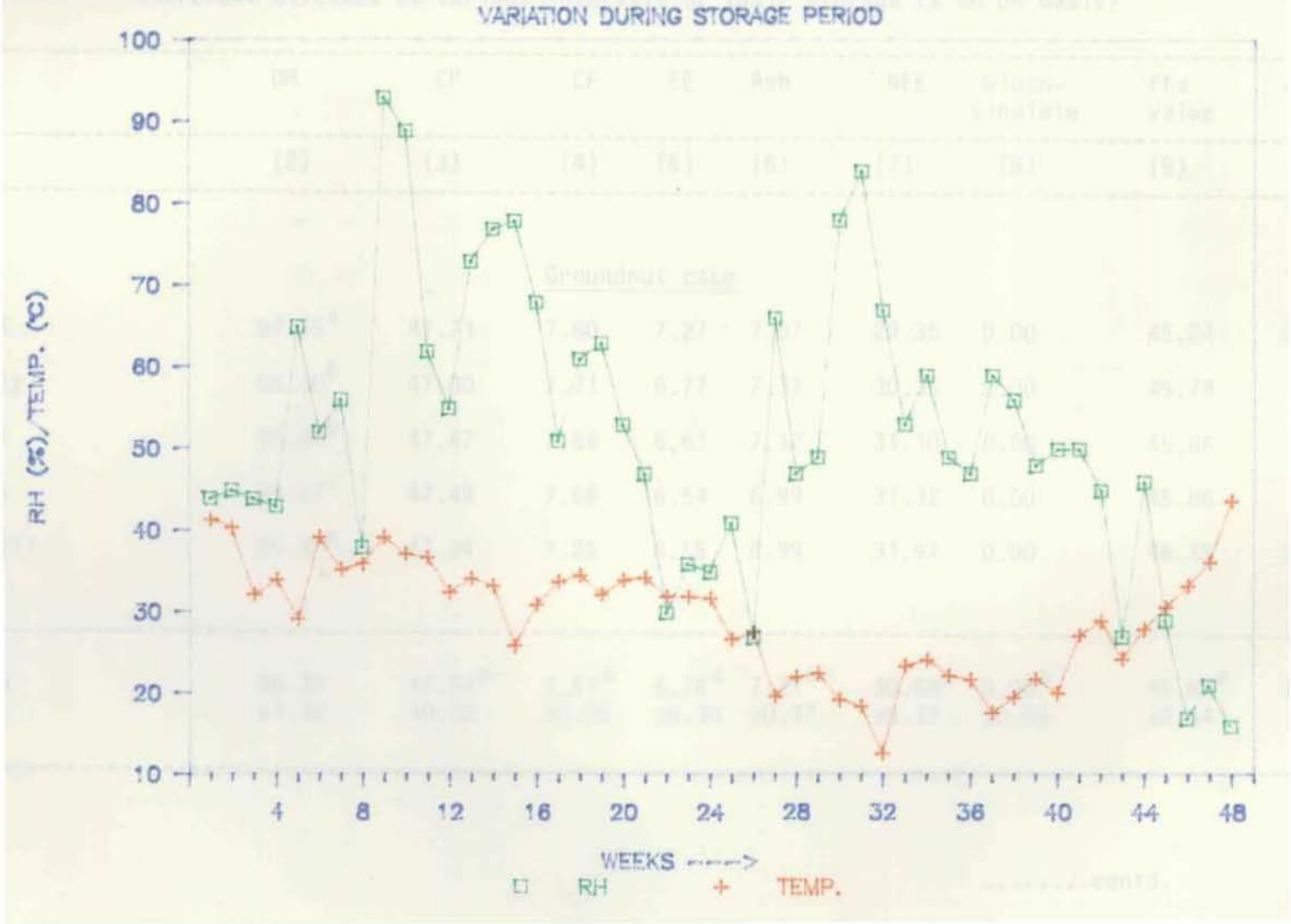


Table 4.24 Proximate composition, glucosinolates content, ffa value and aflatoxins content of different oilcakes at various intervals of their storage (% on DM basis)

Months	DM	CP	CF	EE	Ash	NFE	Gluco-sinolate	ffa value	Aflato-xins(ng/g)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
				Groundnut cake					
April	97.75[a]	47.71	7.80	7.27	7.87	29.35	0.00	45.24	21.50
May-July	96.30[b]	47.80	7.71	6.77	7.37	30.35	0.00	45.78	-
Aug-Oct	96.27[b]	47.47	7.64	6,67	7.12	31.10	0.00	45.86	-
Nov-Jan	95.27[c]	47.49	7.66	6.54	6.99	31.32	0.00	45.86	-
Feb-April	95.37[c]	47.24	7.25	6.55	6.99	31.97	0.00	46.15	20.83
Average	96.39 ±1.10	47.54[a] ±0.22	7.51[a] ±0.05	6.76[a] ±0.30	7.27[a] ±0.37	30.68[a] ±0.37	0.00[a] ±0.00	45.67[a] ±0.34	21.16

.....contd.

.....contd.Table 4.24

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
		Mustard cake							
April	97.72[a]	36.27	9.18	8.94	8.07	37.54	4.69	20.72	4.56
May-July	96.24[b]	35.98	9.45	8.92	7.98	37.87	4.37	20.61	-
Aug-Oct	96.19[b]	35.62	8.98	8.87	7.44	39.09	4.33	20.21	-
Nov-Jan	95.43[b]	35.87	8.46	8.80	7.85	39.02	4.33	21.97	-
Feb-April	95.30[b]	35.69	8.66	8.60	7.84	39.21	4.35	21.21	6.99
Average	96.33 ±1.03	35.88[b] ±0.25	8.95[b] ±0.39	8.83[b] ±0.13	7.84[b] ±0.24	38.54[b] ±1.12	4.40[b] ±0.07	20.95[b] ±0.26	5.77
		Rapeseed cake							
April	97.35[a]	36.33	9.22	10.63	7.66	36.16	3.56	22.16	6.18
May-July	96.20[b]	35.37	9.03	10.56	7.78	37.26	3.51	22.78	-
Aug-Oct	96.24[b]	35.22	8.94	10.26	7.56	38.02	3.42	22.77	-
Nov-Jan	95.38[c]	35.18	8.96	10.23	7.54	38.09	3.42	22.81	-
Feb-Apr	95.20[c]	34.12	8.66	10.18	7.46	39.58	3.48	22.80	4.89
Average	96.27 ±1.00	35.24[b] ±0.78	8.96[b] ±0.20	10.37[c] ±0.20	7.60[b] ±0.11	37.82[b] ±0.25	3.54[c] ±0.11	22.63[b] ±0.27	5.53

[abc]values with different superscripts in a column differ significantly ($P/0.05$)

Table 4.25 Analysis of variance for different parameters for quality of groundnut, mustard and rapeseed oilcakes during storage

Source of variation	d.f.	Mean sum of squares							
		DM	CP	CF	EE	Ash	NFE	FFA	Gluco-sinolates
Treatments	2	1.582	643.972	2.064	45.511	1.220	382.799	2443.986	4.096
Months	12	7.588	1.221	0.500	0.168	0.142	3.140	0.274	0.051
Error	24	0.999	1.393	0.309	0.104	0.091	1.613	0.161	0.092

was not significant. The CP content varied from 47.80 to 47.24 percent in GNC, 36.27 to 35.62 percent in MC and 36.33 to 34.12 percent in RSC during storage. Statistical analysis of data indicated that the variation in CP content during the storage for all the three oilcakes was not significant. However, CP content of GNC was significantly higher ($P/0.05$) (Table 4.25) than those of MC and RSC.

4.4.2.3 Crude fibre:-

The initial CF content of GNC, MC and RSC was 7.80, 9.18 and 9.22 percent, respectively. The CF content decreased during storage irrespective of oilcakes. The overall average CF content in GNC, MC and RSC was 7.61±0.05, 8.95±0.39 and 8.96±0.20 percent, respectively (Table 4.24). GNC was having lower ($P/0.05$) CF content than MC and RSC, however, the variations in CF content during the monthly intervals were not significant.

4.4.2.4 Ether extract:-

The initial EE content in GNC, MC and RSC was 7.27, 8.94 and 10.63 percent (on DM basis), respectively. The average percent values for GNC, MC and RSC were 6.77, 8.92 and 10.56 during May-July; 6.67, 8.87 and 10.26 during Aug-Oct; 6.54, 8.80 and 10.23 during Nov-Jan, and 6.55, 8.60 and 10.18 during Feb-April, respectively. The overall average EE content in GNC, MC and RSC was 6.76±0.30, 8.83±0.13 and 10.37±0.20 percent, respectively. Statistical analysis (Table 4.25) of data revealed that the EE content in RSC was significantly higher ($P/0.05$) followed by MC and GNC. However, the variations during the different periods of storage for all the three oilcakes were not significant.

4.4.2.5 Ash:-

The initial ash content in GNC, MC and RSC was 7.87, 8.07 and 7.66 percent on DM basis, with the overall average values of 7.27±0.37, 7.84±0.24 and 7.60±0.11 percent, respectively. The variations in the values obtained at monthly intervals for each oilcake were not significant. Ash content of MC and RSC was similar but significantly higher ($P/0.05$) than that of GNC.

4.4.2.6 Nitrogen free extract:-

The initial value of NFE in GNC, MC and RSC was 29.35, 37.54 and 36.16 percent, respectively. The NFE content registered an increasing trend during all the 4 quarters, irrespective of oilcake. The overall average NFE value of GNC, MC and RSC was 30.68±0.37, 38.54±1.12 and 37.82±0.25 percent, respectively. There were no significant variations during the different months of storage for each oilcake. The NFE content of MC and RSC was similar but found to be significantly higher ($P/0.05$) (Table 4.25) than that of GNC.

4.4.3 Free fatty acids (ffa)

The initial level of ffa was 45.24 in GNC, 20.72 in MC and 22.16 in RSC. The ffa content registered an increasing trend throughout the period of storage and it was observed to be increased from 45.24 to 46.15 in GNC, 20.72 to 21.21 in MC and 22.16 to 22.80 in RSC. The overall average value of 45.67±0.34, 20.95±0.26 and 22.63±0.27 were recorded for GNC, MC and RSC, respectively. The levels of ffa in three oilcakes were significantly different ($P/0.05$) (Table 4.25) and it was highest in GNC followed by RSC and MC. The variations in ffa content during different storage periods were not significant for all the three oilcakes.

4.4.4 Glucosinolates

The glucosinolate content was not detected in GNC while the initial values of total glucosinolates in MC and RSC were observed to be 4.69 and 3.56 percent on DM basis, respectively. Average percentage of glucosinolates in MC and RSC were 4.37 and 3.51 during May-July, 4.33 and 3.42 during Aug-Oct, 4.27 and 3.73 during Nov-Jan, and 4.35 and 3.48 during Feb-April. The overall average glucosinolate in MC and RSC was 4.40±0.07 and 3.54±0.11 percent. The results showed that the average glucosinolate content in MC was about 24 percent higher ($P/0.05$) than that in RSC. The variations in glucosinolate content in MC as well as in RSC during different periods of storage were not significant.

4.4.5 Aflatoxins

The concentration of total aflatoxins present in GNC, MC and RSC initially and at the end of the storage for one year under normal conditions have been presented in table 4.24.

Aflatoxin B_1 was the major constituent of total aflatoxins in GNC, whereas aflatoxin G_1 was the major one in MC and RSC. The initial concentration of total aflatoxins in GNC, MC and RSC was 21.5, 4.56 and 6.18 ng/g (fresh basis), respectively and the corresponding values after one year of storage were 20.83, 6.99 and 4.89 ng/g.

These results showed that the total aflatoxins content did not change during the entire storage of one year.

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CHAPTER - V

DISCUSSION

5. DISCUSSION

5.1 CHEMICAL COMPOSITION OF OILCAKES AND INDIAN VARIETIES OF MUSTARD/RAPESEED

5.1.1 Chemical composition of oilcakes

The CP content of GNC was higher than those of MC and RSC, however, latter two oilcakes were similar in their CP contents. Ether extract was highest in RSC (10.63%), followed by MC (8.94%) and GNC (7.20%). The differences in ether extract content of oilcakes may be attributed to the differences in their processing conditions during oil extraction. The CF contents of GNC, MC and RSC were 7.80, 9.18 and 9.22 percent, respectively and these values were higher than those reported by Sen **et al.** (1978) for these oilcakes. Appelqvist and Ohlson (1972) reported similar chemical composition of rapeseed meal.

Absence of glucosinolates in GNC was obvious as groundnut is not a member of Cruciferea family. However, MC contained higher glucosinolate content (4.40%) than RSC (3.54%). This difference was due to the difference in the varieties of the oilseeds. Large variations in the glucosinolate content of rapeseed meal obtained from different varieties of rapeseed of Canadian and European origin have been reported (Table 2.2). Variations in Indian varieties of mustard/rapeseed were also observed (Table 4.3). Papas **et al.** (1979) reported the glucosinolates in three Canadian varieties of rapeseed meal, i.e., Towar, Turret and Target as 0.87, 1.55 and 4.49 percent, respectively, whereas VanEtten **et al.** (1965) observed the glucosinolates as high as 9 to 11 percent in U.S. varieties of rapeseed meal. In comparison of U.S. varieties, the mustard and rapeseed oilcakes taken in present study were containing marginal glucosinolate content.

Despite the higher unsaturated fatty acid contents of the oil of mustard as well as of rapeseed than that of groundnut (Gohl, 1975), higher free fatty acids (ffa) value of GNC than in RSC and MC was probably

due to difference in the timings of their oil extraction. GNC was already stored for some time before taking it for present experiment and ffa value of oil content of GNC might have already increased due to lipolysis during storage. MC and RSC were obtained just after the oil extraction, therefore, the ffa values of their oil contents were almost similar and lower than that of GNC. The minor difference in the ffa values of MC and RSC may be attributed to the varieties of the oilseeds (Ackman, 1977).

The total amino acids content was higher in GNC than in MC and RSC, obviously due to its higher protein content. All essential and non-essential amino acids except sulphur containing amino acids were also higher in GNC than in MC and RSC. Similar to the present observations, Bell and Jeffers (1976), Singhal (1986) and Bourdon and Aumaitre (1990) reported that rapeseed and mustard oilcakes were containing higher quantities of methionine and cystine than in soybean meal or groundnut cake. Present results also indicated that MC and RSC were having identical amino acid profile.

It can be concluded from these results that MC and RSC were having similar proximate composition and amino acid profile which was different than that of GNC. However, RSC was containing lower glucosinolate content than that in MC.

5.1.2 Glucosinolate content, proximate composition and fibre fractions of Indian varieties of mustard/rapeseed

The total glucosinolate content, proximate composition and fibre fractions of different Indian varieties of mustard/rapeseed (Brassica species) are presented in table 4.3.

Total glucosinolates of twelve different varieties of mustard/rapeseed varied in the range of 1.61 to 4.92 percent on dry matter basis of oilseeds. Since glucosinolates remains in the oilcake during the course of oil extraction, glucosinolate content was, therefore, expressed on oil free meal basis and the values varied in the range of 2.54 to 7.31 percent. Lowest glucosinolate content was recorded in Raya RC 781 whereas Toria Sangram showed the highest glucosinolate content. These results revealed that there was no relationship between oil and glucosinolate contents of mustard/rapeseed as Toria Shymgarh and Toria Sangram varieties were containing similar oil content but difference in their glucosinolate content was very wide. Similar pattern was observed with Raya RC 781 and

Toria TH 83. On the other hand, Raya RH 2859 was having lowest oil content but its glucosinolate content was at marginal level. Since total amount and kind of glucosinolates in mustard/rapeseed are largely under genetic control (Wetter and Craig, 1959; Yadava **et al.**, 1984) the variations in different varieties were obvious. Smith and Dacombe (1987) estimated the total glucosinolate content in British varieties of rapeseed and showed wider variation than that observed in Indian varieties taken in this experiment. Many Canadian and European workers (Papas **et al.**, 1979; Campbell **et al.**, 1980; Laarveld **et al.**, 1981a; Bell, 1984; Stedman and Hill, 1987) also reported the variations in glucosinolate content in different varieties of rapeseed meal (Table 2.2). VanEtten **et al.** (1965) reported higher glucosinolate content as in rapeseed meal of US origin than that recorded for Indian varieties. Similar to present observations Dhindsa and Gupta (1974) and Dhindsa **et al.** (1975) also reported large variations in allylthiocyanates, oil and protein contents of Brassica species.

Variety Toria Kranti was found to contain highest level of oil content besides the substantial quantity of glucosinolates. However, proximate composition of this variety indicated that its oil free meal contained lowest level of crude protein and highest level of total carbohydrates than all other varieties. This mmay be attributed to the comparatively higher proportion of hulls in the oilseed of this particular variety which was evident from its higher levels of neutral and acid detergent fibre contents (Table 4.3) because hulls contain about 80 percent of NDF and 67 percent of ADF (Bell and Shires, 1982). All other varieties were containing similar content of total carbohydrates which were reflected in their almost similar fibre fractions, i.e., NDF, ADF, cellulose and hemicellulose, indicating the similar hulls proportions in these varieties of mustard/rapeseed.

Since glucosinolate content of rapeseed/mustard oilcake is an important consideration for their use in livestock feeding and these are under genetic control in Brassica oilseeds, therefore, vigorous efforts should be made to reduce their glucosinolates without sacrificing the oil content by suitable plant breeding methods.

5.2 EFFECT OF GLUCOSINOLATES AND MUSTARD OIL ON RUMEN FERMENTATION

5.2.1 Effect of glucosinolates on rumen fermentation

The addition of different levels of glucosinolates, i.e., 0 (T_1), 9 (T_2), 18 (T_3), 27 (T_4) and 45 (T_5) mg on **in vitro** rumen fermentation did not have adverse affect on TVFA concentration (Table 4.4). However, molar proportion of propionate increased significantly ($P \leq 0.05$) in treatments T_4 and T_5 than in treatments T_1, T_2 and T_3 (Table 4.6). These results are in line with those of Naumenko **et al.** (1987) and Hunt **et al.** (1990) who reported that glucosinolates of RSM or rapeseed forage had no deterimental effect on digestion or rumen fermentation irrespective of their glucosinolate content. The increased percentage of propionate in treatments T_4 an T_5 may be attributed to the higher availability of glucose as a result of hydrolysis of glucosinolates during the fermentation. It may also be due to the higher availability of soluble nutrients which were present in the glucosinolate extract. It was also evident from these results that hydrolysis products of glucosinolates, e.g., isothiocyanates, thiocyanates, nitriles did not affect the rumen fermentation adversely.

Higher microbial activity in treatments T_2, T_3, T_4 and T_5 than in control (T_1) was manifested in the form of significantly higher ($P \leq 0.01$) production of total gas including methane and microbial protein synthesis (Table 4.6). The decreased CO_2 production with the increasing levels of glucosinolate extract (which also contained soluble nutrients of mustard cake) might probably be due to its conversion into methane by combining with hydrogen gas (which was also produced during rumen fermentation) with the release of some energy (Daniels **et al.**, 1984).

Protein synthesis was significantly higher ($P \leq 0.05$) with each level of glucosinolates extract as compared to that of control (T_1). It could be due to the presence of soluble-N in the extract as observed by Kumar and Walli (1989) who reported that the protein solubility of mustard cake was higher than that of other oilcakes. Besides this, the sulphate ions which were available as a result of hydrolysis of glucosinolates, might have facilitated the higher protein synthesis as demonstrated by Arora **et al.** (1974) using labelled sulphate compounds.

From this experiment it can be concluded that the glucosinolates or its hydrolytic products did not affect the rumen fermentation. Soluble

nutrients of mustard cake obtained alongwith glucosinolate extract and the hydrolysing products such as sulphate ions and glucose of glucosinolates also helped in the synthesis of microbial protein.

5.2.2 Effect of mustard oil on rumen fermentation

Response of addition of graded levels of mustard oil at 0 (T_1), 17 (T_2), 34 (T_3), 51 (T_4) and 68 (T_5) mg which constituted 0, 1.67, 3.28, 4.85 and 6.36 percent of mustard cake taken as substrate **in vitro** rumen fermentation was evaluated in terms of production of TVFA, individual VFAs, total gas production including methane and CO_2 and microbial protein synthesis, and the data are presented in table 4.5.

Increasing the level of oil in **in vitro** system did not influence the TVFA concentration and molar percentage of individual VFAs significantly (Table 4.6). However, there was a decreasing trend for acetate and increasing trend for propionate with the increasing level of oil. Czerkawski (1973) observed similar changes in the rumen fermentation irrespective of dietary level of linseed oil in sheep. However, he reported that there were some changes in fermentation pattern such as inhibition of methane and decreased concentration of acetate as observed in the present experiment. In an another experiment, Czerkawski **et al.** (1975) showed that the addition of fat did not affect the VFA production significantly.

Similar to the observations in the present study, Huntaven **et al.** (1986) reported that the proportions of propionate increased in the rumen due to the feeding of ground rapeseed, however, contrary to the present findings they reported decreased concentration of TVFA which was probably due to the other dietary components. Chalupa **et al.** (1984) emphasised the influence of nature of fatty acid on rumen fermentation and by using the different levels of fatty acids, they demonstrated that the fatty acids in the form of triglycerides induce small changes on rumen fermentation as compared to free fatty acids.

Jenkins (1987) reported that the increase in the level of fat did not influence the rumen fermentation but decreased the butyrate content.Fergusan **et al.** (1990) also reported that the mixture of palmitic, stearic and oleic acids (20% of substrate) did not influence the VFA production in **in vitro** fermentation.

Many reports (Jenkins and Palmquist, 1984; Jenkins, 1987) showed the detrimental effect of fats on rumen fermentation. The fermentative inhibition is restricted to the saturated fatty acids as they form more insoluble salts than the unsaturated ones during **in vitro** fermentation. Since unsaturated fat was taken in the present experiment, therefore, no detrimental effect was observed on rumen fermentation.

Total gas production increased significantly ($P \leq 0.05$) in treatments T_2 and T_3 than in treatment T_1 (Table 4.5). However, differences among T_3, T_4 and T_5 were non-significant (Table 4.6). This trend may be due to the limited utilization of glycerol component of triglycerides that resulted from lipolysis of added fat, because certain microorganisms such as **butyrivibrio** were reported to have a powerful lipase activity (Hobson and Summers, 1966) which catalyse the production of glycerol and free fatty acids from the dietary fat. The glycerol is glycogenic and enters the glycolytic pathways and yields 21 moles of ATP and CO_2 (McDonald **et al.**, 1987). This might have contributed to the production of higher amounts of total gas as well as CO_2 in treatments T_2 and T_3. It seems that the capacity of rumen microbes to lipolyse the available fat is limited since the gas production was not higher in treatments T_4 and T_5 inspite of having higher levels of oil.

The triglycerides of mustard oil are fairly rich in unsaturated fatty acids and the oleic acid ($C_{18:1}$) is the predominant one (Eskin and Frenkel, 1977). The double bonds of these fatty acids act as a 'sink' for hydrogen produced during rumen fermentation thereby reducing the loss of energy in the form of methane (Czerkawski, 1986). Singh and Hawke (1990) also reported the lipolysis and biohydrogenation of unsaturated fatty acids of hay in **in vitro** system.

The percentage of methane in total gas reduced with increasing levels of mustard oil. However, variations among treatments were non-significant (Table 4.6). It may be due to the presence of oleic acid in the mustard oil having only one double bond to act as a 'sink' for hydrogen. Methane production per gram of mustard oil reduced on increasing the level of mustard oil. Czerkawski (1973) also reported the same trend on addding the increasing level of linseed oil in the diet of sheep. The methane production per g oil only in treatments T_3, T_4 and T_5 reduced by 3.73, 32.09 and 49.63 percent, respectively, than that observed in treatment T_2 (Table 4.5). Czerkawski (1966) observed the rapid hydrogena-

tion of linolenic acid in the rumen and reported that there was a reduction in the production of methane by 17 kcal per 100 kcal of additional unsaturated fatty acids in the diet.

The decreasing trend in the production of CO_2 gas per g oil with increasing level of mustard oil indicated that the hydrogen produced during the rumen fermentation was consumed for the biohydrogenation of unsaturated fatty acids and not by the methanogens as evident from the lowered methane production without disturbing rumen fermentation. Czerkawski (1986) also reported that when methane production is inhibited, the intensity of fermentation of the substrates is not markedly affected and the available hydrogen is utilized by alternative means as observed in the present experiment.

The increased availability of energy from glycerol moiety of added triglycerides (mustard oil), through the glycolytic pathways in treatments T_2 and T_3, has further manifested in an increased ($P/0.05$) synthesis of microbial protein. Similar to other observations, variations in microbial protein synthesis among the treatments T_3, T_4 and T_5 were not significant (Table 4.6). These results further strengthened the contention that beneficial effect of additional fat can only be achieved at lower level of addition. Though Czerkawski **et al.** (1975) observed that the number of bacteria increased with simultaneous decrease in number of protozoa on increasing the level of fat in the diet but recently, Srivastava and Mani (1991) showed from their **in vivo** studies that the dietary fat increased the total-N concentration in the rumen liquor of animals as compared to those not received the fat.

Similar to present observations, Knight **et al.** (1978) reported that the microbial protein synthesis increased following the addition of unsaturated oils in the diet of sheep.

From the study it can be concluded that the oil content of mustard cake upto 3.28 percent improved ($P/0.05$) the rumen fermentation. The higher levels, however, neither had improving effect nor had the detrimental affect on rumen fermentation. The unsaturated fatty acids of mustard oil reduced the methane production during the **in vitro** rumen fermentation.

5.3 EFFECT OF GROUNDNUT, MUSTARD AND RAPESEED OILCAKES ON GROWTH, NUTRIENT UTILIZATION AND THYROXINE SECRETION RATE

5.3.1 Chemical composition of treatment rations

Concentrate mixtures given to GNC, MC and RSC groups were isonitrogenous. The concentrate mixture of RSC group contained highest ether extract content followed by those given to MC and GNC groups and the difference was due to variation in the ether extract of groundnut, mustard and rapeseed oilcakes, used in the present experiment as indicated in table 4.1. The oilcakes were having almost similar crude fibre content (Table 4.1) but the concentrate mixtures showed small variations in crude fibre, NDF and ADF fractions which may be attributed to the presence of other ingredients in varying quantity. The proximate composition of wheat straw and berseem fodder was similar to the composition reported by Sen **et al.** (1978).

MC ration was containing higher level of total glucosinolate content than that of RSC and GNC rations. It was attributed to the highest level of glucosinolate in mustard cake as indicated in table 4.1.

5.3.2 Influence of glucosinolates on feed intake and growth performance of crossbred calves

Three groups of young calves (4-5 months) were given GNC, MC and RSC as a sole source of protein in their ration. During first three weeks, calves in MC and RSC groups did not consume the full quantity of concentrate mixture given to them and instead they preferred the straw over concentrate mixture to fulfil their dry matter requirements. The DM intake in GNC group was highest followed by RSC and MC groups during the first 3 weeks of experimental feeding. It was due to the high proportion (39.16 percent) of mustard as well as rapeseed cake in the respective concentrate mixture which were pungent in nature because of their glucosinolate content. The lower DM intake in MC group than in RSC group may be attributed to the difference in their glucosinolate content, as mustard cake was containing higher glucosinolates than rapeseed cake. MC and RSC supplemented concentrate mixtures were containing 1.72 and 1.40 percent glucosinolate (on DM basis) respectively. The improved palatability of rations containing low glucosinolate meal is of prime importance in main-

taining their acceptability for fast growing young animals. On an average MC and RSC were given @ 585 g per day to each calf which was higher than the recommended level of 200 g rapeseed meal per day (Fenwick, 1982) in the ration of calves. The glucosinolates cause pungency due to the formation of volatile and pungent compounds after their hydrolysis. Papas **et al.** (1979) also reported the lower feed intake when high glucosinolate containing RSM was included in the ration of calves in comparison to those fed on low glucosinolate RSM. Stake **et al.** (1973) also found the poor palatability of RSM in comparison to soybean meal. Besides the pungent flavour of glucosinolates the bitter taste of sinapine (Appelqvist and Ohlson, 1972) may be the another possible reason for the poor palatabilities of MC and RSC rations. Comparatively lower intake of feed, particularly the concentrate mixture, in MC and RSC groups than in GNC group during first three weeks of experimental feeding resulted in lower body weight gain as evident from Fig 4.1. After the adaptation to experimental rations, animals started consuming their whole quota of concentrate mixture both in MC and RSC groups. However, the speed of consumption was highest in GNC group followed by RSC and MC groups. Calves in groups MC and RSC showed the tendency to consume the concentrate mixture when it was offered alongwith straw, whereas, calves in control group did not show such tendency. Feed intake increased linearly with the increase in body weight of calves in all the three groups throughout the experimental period. Total DM intake during the 35 weeks of experiment through concentrate mixture was 541.95, 523.39 and 510.33 kg, respectively, in GNC, MC and RSC groups, respectively. The corresponding values for roughage were 506.90, 512.58 and 502.33 kg (Table 4.8). The average DM intake per day in GNC, MC and RSC groups was 4.27, 4.23 and 4.13 kg, respectively. The variations among groups, however, were rot significant (Table 4.12). These results revealed that the pungent taste of mustard or rapeseed cakes may create the problem for palatability only for a few weeks of their introduction in the ration but in the long run animals get adapted for their taste and flavour.

Average body weight gain in GNC, MC and RSC groups was 480.6, 412.9 and 427.2 g per day, respectively. The corresponding regression coefficient for growth per week (b value) was 3.32±0.17, 3.07±0.14 and 3.22±0.24 (Table 4.8). The statistical analysis (Table 4.12) of data did not reveal significant variation among groups for weight gain per day as well as for growth rate per week. Growth performance of calves during the

35 weeks of experimental period (Fig 4.1) revealed that the level or source of glucosinolate did not affect the growth performance of calves. The similar growth performance in different groups may be attributed to the fact that the glucosinolate or its hydrolysis products did not affect the rumen fermentation as observed previously in **in vitro** experiment and as also reported by Naumenko **et al.** (1987) and Hunt **et al.** (1990). Moreover, the unsaturated fatty acid content of mustard and rapeseed cakes in MC and RSC groups might have reduced the energy losses by reducing the mechane production as observed in an earlier **in vitro** experiment.

Kehar **et al.** (1956) fed the differently processed mustard cake as well as linseed cake to the tune of 50 percent of the concentrate mixture along with wheat straw **ad lib** as a source of roughage in a 50 weeks long feeding experiment on Haryana calves and reported the similar growth pattern as observed in the present experiment. Inspite of feeding high levels of glucosinolate through mustard and rapeseed cakes, the growth in MC and RSC groups was similar to that fed on groundnut cake which was probably due to the higher levels of sulphur containing amino acids (Table 4.2) and rumen undegradable protein contents of mustard and rapeseed cakes than in groundnut cake (Kumar and Walli, 1989). Kehar **et al.** (1948), Mukherjee and Kehar (1949) concluded that the utilization of mustard cake was better than the groundnut cake when fed to animals alongwith straw.

Various workers reported that the rapeseed meal can replace completely or partially the soybean meal (Wood and Stone, 1970; Ingalls and Seale, 1971; Iwarsson **et al.**, 1973), linseed meal (Whiting, 1965), cottonseed meal (Schwarz and Kirchgessuer, 1989; Ahmad and Malik, 1982) in the ration of growing animals without affecting their performance. In contrast, many reports (Stake **et al.**, 1973; Geary and Beranger, 1975; Olsson, 1978; Papas **et al.**, 1979) showed the adverse effect of rapeseed meal feeding on growth of calves. Some reports provided the evidence that the production potential of ruminants was affected by the HG-RSM feeding while production by LG-RSM feeding was at par with conventional source of protein (Schingoethe **et al.**, 1974; Bush **et al.**, 1978; Papas **et al.**, 1979). A critical analysis of the data showed that the age and/or body weight of experimental animal and level of glucosinolates were the most important criterion to assess the effect of RSM on growth. The calves having more than 80 kg body weight performed well even on HG-RSM as an exclusive source of protein in their diets without any adverse effect on growth.

In the present experiment, the initial average body weight of calves was about 80 kg and they utilized the mustard and rapeseed cakes supplemented rations, inspite of their high levels of glucosinolate content, at par with groundnut cake ration. Similar growth rate in crossbred calves was reported by Tomar (1983) and Anon (1984).

5.3.3 Plane of nutrition

Average daily DM intake in GNC, MC and RSC groups was 4.27, 4.22 and 4.13 kg respectively. The values for CP, TDN and ME intake in corresponding groups were 603.9, 588.2 and 575.3 g; 2.45, 2.41 and 2.36 kg; and 8.89, 8.72 and 8.53 Mcal (Table 4.8). However, the variations among groups were not significant (Table 4.12). The values for CP intake were higher than the values reported by NRC (1978), but the intake of DM, TDN and ME were almost similar to the recommended values. The feed intake per kg body weight gain was highest in MC group (10.24 kg) followed by RSC (9.67 kg) and GNC (8.90 kg) groups and the variations among groups were not significant. Similar values of feed efficiency was reported by Sharma and Bakalkar (1989) in crossbred calves. CP intake per kg gain in body weight was 1.25, 1.42 and 1.34 kg in GNC, MC and RSC groups, respectively (Table 4.8). The corresponding values for TDN intake were 5.11, 5.83 and 5.51 kg. These values were similar to those reported by Tomar (1983) in crossbred calves fed on high protein diet. The roughage to concentrate ratios in GNC, MC and RSC groups were 1:1.06, 1:1.02 and 1:1.01, respectively. Similar roughage to concentrate ratio in all the three groups fed on isonitrogenous and isocaloric rations was responsible for similar nutrient intake and growth performance. Mallikarajunappa (1981) also reported a growth rate of 460 g/day in buffalo calves by keeping them on rations having equal roughage to concentrate ratios.

5.3.4 Body measurements

Besides the body weight, body measurements such as body length, height at wither and heart girth were recorded at the beginning of experiment and thereafter at monthly intervals in all the three groups (Fig 4.2, 4.3 and 4.4). There was no significant variation among the groups or among the animals within the group for the initial and final values of each body measurement. The daily increase in body length (L), height at

wither (H) and heart girth (G) was similar in all the three groups. Body wt of an animal seems to be function of muscular and skeletal growth. Therefore, correlations among body weights and body measurements were estimated in different treatment groups and presented in table 4.10.

The correlations of body weight with length, height at wither and heart girth of crossbred male calves in GNC group were 0.89, 0.90 and 0.92, respectively. The corresponding correlations in MC group were 0.95, 0.96 and 0.95 and in RSC group were 0.97, 0.96 and 0.96. All the correlation coefficients were very high and significant. Similar to these observations very high correlations of body weight with body length and heart girth in crossbred cattle have been reported in literature (Gill **et al.**, 1971; Parekh **et al.**, 1976; Tomar, 1983). The high correlations between body weight and body measurements suggested that there was no differential effect of dietary source of protein on overall growth of crossbred calves.

Relationship between body weight and body measurements among different groups were worked out using multiple linear regression equation to determine the effect of length, height and heart girth on the body weights of crossbred calves (Table 4.11). Each of the three independent variables (H, L, G) was fitted separately in the equation to measure the degree of relationship. Later on one more variable was introduced into the regression equation to measure the combined effect of two variables on body weight. Finally, third variable was introduced to assess the effect of all the three variables on the body weight. The introduction of height as an additional variable with length in the regression model of body weight did not improve the value of R^2 significantly in all the three treatment groups. Further, this additional variable was found to be not significant. However, the introduction of girth (G) as an additional variable improved the value of R^2 and it was found to have a significant effect on the body weight alongwith length (L). Even when the effect of all the three variables like length (L), height (H) and girth (G) was seen on the body weight, the effect of height was found to be not significant in all the groups except in RSC group. The non-significant effect of height (H) on the body weight may be due to its high correlation with other independent variables like length and girth. The variables included in the prediction equation explained more than 80 percent of the total variation. Tomar (1983) also found that the effect of length, height

and girth on body weight was significant and they were highly intercorrelated among themselves.

The individual coefficients of length, height and girth did not differ significantly in different treatment groups. The effect of girth on body weight was strong followed by length and height as revealed from the high R^2 values in all the three groups. The results are in agreement with those obtained in crossbred calves by Rao (1977) and Tomar (1983). The individual regression coefficients showed that for every one cm increase in length, height and girth, the average body weight is expected to increase by 3.62, 3.55 and 3.08 kg in GNC group, 3.50, 3.61 and 3.02 kg in MC group, and 3.37, 3.56 and 2.91 kg in RSC group, respectively. Various combinations of measurements, however, did not increase the R^2 values.

Since the differences in the body weight and in the body measurements of the three groups were not significant, therefore, the data of all the three groups were pooled which revealed that for every one cm increase in length, height and girth, the body weight of crossbred calves in the age group of 4-13 months will increase by 3.51, 3.58 and 3.01 kg, respectively. The pooled data also showed that the length and girth are the dependable measurements for predicting the body weight of crossbred calves in the age group of 4-13 months. Body measurements in various combinations did not improve the R^2 value of the equation. These results suggested that the measurement of girth or length could be accurately used for predicting their body weights of crossbred calves during the age of 4 to 13 months of age irrespective of their feed although the girth alone could give the highest accuracy. These findings are in concurrence with the observations of Parekh **et al.** (1976) and Tomar (1983).

5.3.5 Effect of mustard and rapeseed oilcakes on nutrient utilization

5.3.5.1 Nutrient intake:-

The data of nutrient intake during metabolism trial, conducted after 35 weeks of experimental feeding on all the three groups of animals, are presented in table 4.13.

The average DM intake in GNC, MC and RSC groups was 4.52±0.21, 4.38±0.12 and 4.30±0.29 kg, respectively. The concentrate mixture constituted 56.12, 57.99 and 54.18 percent of total DM intake in

corresponding groups. The oilcake consumption was 705 g in GNC group, 994 g in MC group and 912 g in RSC group. The glucosinolate intake was 0, 43.6 and 32.46 g in GNC, MC and RSC groups, respectively which showed that the glucosinolate intake in MC group was about 34 percent higher than in RSC group and about 44 percent higher than in GNC group. However, such large variations among groups in glucosinolate intake did not yield any adverse effect on DM intake calculated on percent body weight basis as well as on metabolic body size basis. This may be attributed to the adaptation of calves to their respective diets during the long period of experimental feeding. Bush **et al.** (1978) fed the steers with 680 g per day of two types of LG-RSM and did not observe any adverse effect on feed consumption and feed efficiency.

5.3.5.2 Nutrient digestibility:-

Average digestibilities of proximate principles and cell wall constituents in GNC, MC and RSC groups are presented in Table 4.13.

In general, the nutrient digestibilities were higher in GNC group than those in MC and RSC groups. However, variations among groups were not significant for all the nutrients (Table 4.15). Kehar **et al.** (1956) also reported similar digestibilities of crude protein, ether extract and total carbohydrates when differently processed mustard and linseed oilcakes constituted 50 percent of concentrate mixture in wheat straw based ration of growing Haryana calves. Similar to present results, Srivastava **et al.** (1962) and Paliwal **et al.** (1976) also observed that the variations in the digestibility of nutrients were not significant when mustard cake replaced the groundnut cake in the rations of Haryana cattle and buffaloes. Digestibility coefficients for ADF and NDF did not vary among the groups (Table 4.15), however, lower NDF digestibility in all the treatment groups may be attributed to the high level of concentrate feeding. Present results could also be corroborated by the findings of Stake **et al.** (1973), Ingalls and Sharma (1975) and Sharma **et al.** (1980), who replaced soybean meal of the ration either with different levels or with different varieties of rapeseed meals without any adverse affect on nutrient digestibility. Sharma **et al.** (1980) also showed that the ADF and NDF digestibilities of two varieties of rapeseed meal and soybean meal supplemented diets were similar as observed in the present experiment.

These results also showed that the difference in the level of glucosinolate intake did not yield any adverse effect on the nutrient

digestibility indicating the fact that the glucosinolate did not affect the rumen fermentation as observed in **in vitro** experiment (Table 4.4). Laarveld and Christensen (1976) also showed that the glucosinolate did not affect the nutrient digestibility in cows. However, contrary to the present observations, Bush **et al.** (1978) reported significant variations in the digestibilities of nutrients of two varieties of rapeseed meal. Similarly, Schingoethe **et al.** (1974) recorded lower digestibilities of nutrients of rapeseed meal containing diet than that of soybean meal containing diet.

It may be concluded from these observations that the intake and digestibilities of nutrients of groundnut, mustard and rapeseed oilcakes did not differ significantly. In addition, the glucosinolates either from mustard or rapeseed oilcakes did not affect the nutrient consumption and their digestibility adversely in crossbred calves.

5.3.5.3 Nitrogen metabolism:-

Data on nitrogen intake, its excretion and balance in GNC, MC and RSC groups are presented in table 4.14.

The data revealed that the concentrate mixture was the main source of nitrogen supply in each group as per the planning of experiment. Nitrogen supply through concentrate mixture was 87.14, 90.39, and 85.12 percent of total N intake in GNC, MC and RSC groups, respectively. The variations among groups were, however, not significant (Table 4.15). Nitrogen intake per kg $W^{0.75}$ was higher in MC group (1.98 g) followed by GNC group (1.94 g) and RSC group (1.88 g). Nitrogen supply in all the groups was as per the recommendations of NRC (1978) for growing male calves.

N intake per kg digestible organic matter (DOM) intake in GNC, MC and RSC groups was 36.57, 39.14 and 41.19 g, respectively and these values were higher than the value (30 g per kg DOM) suggested by ARC (1980) to fulfil the nitrogen requirements of animals.

Average digestible nitrogen intake as percent of total nitrogen intake in GNC, MC and RSC groups was 68.96, 73.78 and 67.16, respectively, which showed that the nitrogen from mustard and rapeseed cake was digested better than the nitrogen from groundnut cake probably due to the higher sulphur containing amino acids(Table 4.2) and higher availability of sulphur as a result of hydrolysis of glucosinolates. Similar to present

observations, Mukherjee and Kehar (1949) reported that the protein quality of colza cake (rapeseed oilcake) and mustard cake was superior to that of groundnut cake when these were fed alongwith straw based diet, irrespective of the level of CP of diets. Stake **et al.** (1973) and Waldern (1973) also reported the similar digestible nitrogen intake from rapeseed meal and other oil meals despite the differences in intake and retention of nitrogen from different protein supplements.

Similar and efficient nitrogen utilization in all the three treatment groups may be attributed to the amino acid contents of the experimental oilcakes, because the amino acids and available energy might have utilized for the efficient microbial protein synthesis as observed in **in vitro** experiment (Table 4.4). Nolan and Leng (1972) and Ganthorne and Nader (1976) also reported that the preformed amino acids were used to a large extent in microbial protein biosynthesis.

Urinary nitrogen excretion was similar in all the three groups but higher than the faecal nitrogen loss (Table 4.14). Present results revealed that 44.49, 46.61 and 44.03 percent of total nitrogen consumed was excreted through urine in GNC, MC and RSC groups, respectively, and the variations among groups were not significant (Table 4.15). This may be due to the almost similar potential degradabilities of nitrogen of the groundnut, mustard and rapeseed (**Toria**) oilcakes (Kumar and Walli, 1989).

Nitrogen balances were positive in all the three groups and these were found to be highest in RSC group (25.91 g) followed by MC group (25.86 g) and GNC group (24.25 g), and the nitrogen balances were corroborated by the body weight gains during the trial period. Similar pattern of nitrogen balances were reported by Mukherjee and Kehar (1949), however, contrary to their report, nitrogen balances did not vary significantly among the groups. Nitrogen retention as percent of total nitrogen consumption in GNC, MC and RSC groups was 23.96, 24.74 and 25.62, respectively and variations among groups were not significant. Sharma **et al.** (1980) also reported that the complete replacement of soybean meal with two varieties of rapeseed meal did not affect the nitrogen utilization efficiency in young bull calves. Similar to present observations, Bush **et al.** (1978) reported that the variations in the nitrogen retention as percent of total nitrogen intake in two groups fed on two types of rapeseed meal at equal level were not significant. These results get the support from the findings of **in vitro** experiment (Table 4.4) where nitrogen

utilization was not influenced by the different levels of glucosinolates.

On the basis of these results it can be concluded that the nitrogen of mustard and rapeseed oilcakes were utilized as efficiently as that of groundnut cake when straw was offered **ad lib** as a source of roughage. Difference in the glucosinolate content of mustard and rapeseed oilcakes did not affect the nitrogen utilization when these were the sole source of nitrogen supply in the diet of crossbred calves.

5.3.5.4 Plane of nutrition:-

The data on plane of nutrition of experimental calves in different treatment groups during the metabolism trial period are given in table 4.16.

Average crude protein consumption in GNC, MC and RSC groups was 628.45, 641.08 and 599.88 g per day, respectively. These values were about 4.39, 10.21 and 5.07 percent higher than those recommended by NRC (1978) for growing bulls. Crude protein intake did not vary significantly among the groups (Table 4.15). DCP intake in GNC, MC and RSC groups was 7.5, 20.2 and 10.55 percent higher than the values recommended by Sen **et al.** (1978) for growing bull calves. These observations indicated that the calves were supplied with adequate levels of crude protein as well as DCP to fulfil the nutritional requirements. There was no significant variation among groups for TDN intake and it was 2.83, 2.72 and 2.49 kg in GNC, MC and RSC groups, respectively. The TDN intake in respective groups was 91.29, 90.97 and 84.98 percent of the values recommended by NRC (1978) as well as Sen **et al.** (1978). Lower TDN intake than the requirement may be attributed to the feeding of wheat straw as a source of roughage. Similarly, ME intake in GNC, MC and RSC groups was 89.09, 88.88 and 83.70 percent of the recommended allowance (NRC, 1978). These results revealed that the calves were offered 10 to 15 percent lower energy than the recommended allowances. However, it did not affect the nutrient utilization in crossbred calves.

5.3.6 Body composition

The data on body composition of crossbred calves in GNC, MC and RSC groups (Table 4.17) showed that the variations were not significant among the treatment groups (Table 4.18). As the plane of nutrition in all

the three groups in terms of nutrients intake and their digestibility did not differ significantly and the variations in body weight gain were also found to be not significant. In this study the GNC was replaced by MC and RSC but the CP and TDN intakes were similar in all the three groups. Mangat Ram (1989) also did not find any significant difference in the body composition of crossbred calves fed on two types of urea-molasses-mineral blocks and conventional concentrate mixture. However, the values for fat percentage observed in the present experiment were higher than those recorded by Mangat Ram (1989) which was due to the fact that he did not calculate the fat percentage on the basis of empty body weight basis.

Tomar (1983) also reported similar body composition of crossbred calves of similar age group. The body water was 68.86 percent while protein, fat and ash were 13.93, 17.02 and 5.86 percent, respectively. Similar body composition in the present experiment irrespective of dietary protein supplement indicated that the yield and quality of carcass from such animals will be similar as observed earlier by Bourdon and Aumaitre (1990) on feeding the RSM supplemented diet. Similar body composition in all the three experimental groups also indicated that the glucosinolates did not have any detrimental effect on the body composition of crossbred calves.

5.3.7 Tri-iodothyronine and Thyroxine concentration

Average tri-iodothyronine (T_3) and thyroxine (T_4) concentrations determined at monthly intervals during the entire period of experimental feeding in GNC, MC and RSC groups are presented in table 4.19.

The range of plasma tri-iodothyronine (T_3) was 0.25 to 0.66 ng/ml in GNC group, 0.10 to 0.44 ng/ml in MC and 0.21 to 0.61 ng/ml in RSC group. The overall average T_3 concentration in GNC, MC and RSC groups was 0.39, 0.30 and 0.39 ng/ml, respectively. Significantly lower ($P \leq 0.05$) T_3 concentration in MC group than in GNC and RSC groups may be attributed to the higher plasma thiocyanate content which arised as a result of hydrolysis of glucosinolates of mustard cake and have the capacity to reduce the iodine uptake. Similar to present observations, Khurana (1983) reported significant variations in plasma T_3 concentration during the prepubertal period of crossbred female calves, however, he reported higher T_3 values than those observed in present experiment.

The plasma thyroxine concentration (ng/ml) were in the range of 26.91 to 65.78 in GNC group, 24.95 to 75.91 in MC group, and 28.31 to 65.02 in RSC group. In general, thyroxine concentration increased with age in all the three treatment groups and variations among months were significant ($P<0.05$). The fluctuations observed during the course of experimental period may be attributed to the interaction of hormone level and climatic conditions. Khurana (1983) also reported the similar trend of T_4 concentration and its interaction with environmental changes in crossbred female calves during the age of 6 to 30 months. The overall average plasma T_4 concentration in groups GNC, MC and RSC was 51.44, 47.11 and 40.29 ng/ml, respectively, which showed a decreasing concentration in calves fed on mustard and rapeseed cakes due to their glucosinolate contents, however, variations among groups were not significant. These results depicted that feeding of glucosinolates to young calves either through mustard or rapeseed oilcake continuously for eight months did not affect the plasma thyroxine concentration significantly. Khurana (1983) reported higher average concentration of plasma T_4 (58.30 ng/ml) than those observed in present experiment which may be attributed to sex of calves as females have higher hormonal concentration than those of males.

Dietary glucosinolate intake in GNC,MC and RSC groups was 0, 43.60 and 32.46 g/day, respectively. Glucosinolate intake in MC and RSC groups decreased the plasma T_3 and T_4 concentrations during the experimental period probably due to their hydrolysis products, viz., 5-vinyloxazolidenethione (OZT), isothiocyanates and thiocyanates which might have affected the iodine uptake and caused thyroid enlargement and in turn reduced the tri-iodothyronine and thyroxine concentration. Plasma T_3 concentration decreased continuously upto the four months of experimental feeding, however, the T_3 concentration was increased in the subsequent period and was at par with those in GNC and RSC groups. The decrease in plasma T_4 concentration in MC and RSC groups was not significantly different from GNC group. The apparent decrease in plasma T_3 and T_4 concentration was sufficient to maintain the metabolic activity of the calves since the body weight gain (Table 4.8) and nutrient utilization (Table 4.13) were not affected in these groups. The present results revealed that the thyroid was not affected severely even after exposing the young calves to the high levels of glucosinolates for eight months. Hill (1979) also reported that despite the enlarged thyroid after the feeding of rapeseed oilcake, the thyroid hormone levels remained

unaffected and permitted normal metabolism in animals. Laarveld and Christensen (1976), Sharma **et al.** (1977), Papas **et al.** (1979) and Ahlin **et al.** (1987) also reported reduced plasma T_3 and T_4 concentrations in rapeseed oilcake fed cows than those fed on soybean meal without any adverse affect on milk production and concluded that increased size of thyroid following the feeding of rapeseed meal was probably due to intact glucosinolates and/or their unknown metabolic products, however, thyroid enlargement did not affect the production potential of animals. Similar assumption was supported by Papas **et al.** (1979) from the histology of thyroid of calves exposed to varying glucosinolate containing diets.

Similar to present observations, Paliwal **et al.** (1976) did not find any adverse effect of mustard oilcake feeding as an exclusive source of dietary protein on protein bound iodine (PBI) in blood plasma of Haryana cattle and Murrah buffaloes. Claypool **et al.** (1985) also reported that feeding of rapeseed, cotton seed or soybean meals did not affect the plasma T_3 and T_4 concentrations and growth of calves.

Dietary iodine was adequate and similar in all the three experimental rations as these were supplemented with equal quantity of mineral mixture. Moreover, supplementary iodine in the rapeseed meal containing diet did not alter the thyroid function when plasma T_4 concentration and milk yield were taken into consideration (Laarveld **et al.**, 1981c).

5.3.8 Haemoglobin

Data of average haemoglobin content in the blood of crossbred experimental calves in GNC, MC and RSC groups, determined at monthly intervals, are presented in table 4.20.

Average blood haemoglobin varied in the range of 11.45 to 11.92 percent in GNC group, 11.29 to 11.92 percent in MC group and 11.48 to 11.67 percent in RSC group with an average value of 11.60, 11.55 and 11.55 percent, respectively. The haemoglobin was similar to the normal values in all the treatment groups. Variations among the treatment groups as well as among the months were not siginificant (Table 4.21) for blood haemoglobin content. The observations indicated that haemoglobin was not influenced by the feeding of experimental diets as well as by the age of crossbred calves. Iwarsson (1973) also reported that feeding of rapeseed

meal did not affect the blood parameters including haemoglobin. Laarveld and Christensen (1976) and Papas **et al.** (1979) showed the reduced blood haemoglobin following the feeding of rapeseed meal, however, variations between control and experimental groups were not significant.

It can be concluded that feeding of mustard and rapeseed cakes did not affect the haemoglobin content of the crossbred calves.

5.3.9 Thiocyanate content in blood plasma

Average thiocyanate content in blood plasma of MC and RSC groups were 8.90±1.12 and 7.92±1.68 µg/ml, respectively (Table 4.20). Thiocyanate content in blood plasma of GNC group was not detected in the samples drawn from GNC group at any stage of experimental period. Higher plasma thiocyanate content in MC group than in RSC group may be attributed to the higher glucosinolate content of mustard cake than in rapeseed cake obviously due to the more release of thiocyanates as a result of hydrolysis of glucosinolates by the microbial species inhabiting in the gastrointestinal tract (Marangos and Hill, 1974). Barker (1936) reported that thiocyanate ions were possessing the antithyroid activity. Present results also showed that the concentrations of plasma tri-iodothyronine (T_3) and thyroxine (T_4) were depressed more pronouncely in MC group than in RSC group probably due to the presence of thiocyanates. Greer **et al.** (1964), Lo and Hill (1971), Bell **et al.** (1972) and Schulz and Lebzien (1988) also reported that various hydrolyzing products of glucosinolates including thiocyanates affect the thyroid function.

Laarveld **et al.** (1981c) also did not detect the thiocyanate in the milk of control group, however, feeding of rapeseed meal at 20 percent level of concentrate mixture increased the milk thiocyanate content, which was not influenced by the dietary levels of iodine.

These results indicated that feeding of mustard cake and rapeseed cake increased the plasma thiocyanate content and the increase was influenced by the dietary glucosinolates level.

5.3.10 Thyroxine secretion rate

The data on thyroxine secretion rate and related parameters, determined by isotope dilution technique in three treatment groups are presented in table 4.22.

Apparent changes in the volume of distribution of thyroxine or thyroxine distribution space (TDS) reflect an actual change in the space occupied by the circulatory thyroxine or it represents a change in relative concentration of the hormone in a particular compartment or organ in that space. Average value for TDS per 100 kg body weight in GNC, MC and RSC groups was 20.46, 20.69 and 22.63 litres, respectively, and the variations among groups were non-significant (Table 4.23) which indicated that actual changes in the space occupied by circulating thyroxine in all the groups, were similar irrespective of their dietary glucosinolate level. No report is available in literature about the changes in TDS after the feeding of rapeseed meal to the animals.

Animal's daily thyroxine secretion rate (TSR) in GNC, MC and RSC was 0.032, 0.025 and 0.035 mg /lt/ 100 kg body weight, respectively. Daily TSR was appeared to be lower in MC group than other groups probably due to the decreased T_3 and T_4 concentrations as a result of elevated thiocyanate content, however, variations among groups were non-significant. Khurana and Madan (1985) also reported similar values of TSR in crossbred calves of similar age group using same technique, however. Singh **et al.** (1969) reported higher values in crossbred calves than those observed in present experiment. Laarveld **et al.** (1981b) conducted the TRH test on cows fed on rapeseed meal containing diet. They did not find any difference in thyroid stimulating hormone following the injection of TRH before and after feeding the experimental diet with or without supplementary iodine and concluded that feeding of rapeseed meal did not affect the thyroid function.

It was evident from these results that glucosinolates of mustard and rapeseed oilcakes did not affect the thyroxine concentration or its secretion rate in crossbred calves significantly.

It can be concluded from these results that growth performance of crossbred calves fed on mustard or rapeseed cakes as exclusive dietary source of protein in wheat straw based ration was comparable with those fed on control ration. Glucosinolate content of mustard cake was higher than rapeseed cake, however, problem of palatability was encountered in both the groups and animals took about 3 weeks in complete adaptation. Feeding of mustard cake reduced the plasma tri-iodothyronine (T_3) concentration significantly ($P<0.05$) as compared to groundnut cake and rapeseed fed groups during the 35 weeks of experimental feeding.

due to the elevated level of plasma thiocyanate content which arised as a result of hydrolysis of glucosinolates. However, thyroxine (T_4) and thyroxine secretion rate did not vary significantly among the groups.

5.4 EFFECT OF STORAGE ON THE QUALITY OF OILCAKES

There was no change in colour, texture and odour of groundnut cake (GNC), mustard cake (MC) and rapeseed cake (RSC) during the entire period of storage.

5.4.1 Temperature and relative humidity

The temperature and relative humidity in the godown recorded wide variations, but during the major part of storage period, the temperature was in the range of 25-30°C and the relative humidity was below 70 percent (Fig 4.6) providing safe storage conditions for the oilcakes.

5.4.2 Proximate composition

DM content reduced ($P/0.05$) during the storage period in all the three oilcakes particularly during May to July. However variations in the DM contents of oilcakes were not significant (Table 4.25). These results showed that the temperature and relative humidity influenced the equilibrium moisture content of all the three oilcakes in a similar fashion.

The data on proximate composition, glucosinolate and aflatoxins contents in GNC, MC and RSC at different stages of storage period are given in table 4.24.

Proximate principles, i.e., crude protein, crude fibre, ether extract, ash and nitrogen free extract of GNC, MC and RSC, in general, did not change significantly during the storage. However, during the first four months of storage period crude protein, crude fibre and ether extract were observed to decrease slightly resulting in an increase in nitrogen free extract. The ether extract content was reduced by 9.9 percent in GNC, 3.8 percent in MC and 5.2 percent in RSC after the storage of 12 months. The changes in proximate composition may be attributed to the comparatively higher relative humidity during the first four months. Similar changes in the storage of expeller oilcakes were reported by Kehar **et al.** (1956). The influence of higher relative humidity on proximate

composition particularly the oil content of oilcake was elucidated by Sabale **et al.** (1975). Similar were the observations of Blaha (1980) and Lowe and Apelt (1987) who reported that the higher relative humidity caused a slight decrease in fat content of oilcakes. A slight decrease in oil content and in percentage of protein were also observed by Defromont and Delahaye (1961) during the storage of groundnut cake for 13 months.

5.4.3 Free fatty acids value

The initial ffa value of GNC was higher than that of MC and RSC (Table 4.24). It must be due to the fact that the GNC procured from the market might have already stored prior to its use for storage experiment. Since MC and RSC were freshly extracted in a local oil mill prior to storage, their ffa values were similar and lower ($P/0.05$) than that of GNC. The ffa value increased by 2.0 percent in GNC, 2.36 percent in MC and 2.88 percent in RSC after the storage for 12 months. However, variations among different months of storage for each oilcake were not significant. The increase in ffa value during the storage of oilcakes, especially of groundnnut cake was also reported by Defromont and Delahaya (1961), Sabale **et al.** (1975) and Francis and Wood (1980). However, our results indicated that the extent of increase in ffa was very less. It could be due to the better storage conditions provided for the oilcakes as recommended by Defromont and Delahaya (1961). He suggested that the storage of oilcakes in sacks and in ventilated godowns at 18 to 20°C at 60 to 70 percent relative humidity was less deleterious to their quality. Almost similar conditions were maintained in present experiment for the storage of oilcakes.

Inspite of having higher oil content and higher degree of unsaturated fatty acids, MC and RSC did not develop rancidity as measured by ffa value, during their storage. This may be attributed to the presence of erucic acid ($C_{22:1}$) in mustard and rapeseed oils which exerts inhibitory action on the activity of lipoxygenases (Downey and Klassen, 1976).

5.4.4 Glucosinolates

Glucosinolates were not observed in GNC while MC showed significantly higher ($P/0.05$) glucosinolates than RSC which was due to the difference in the variety of the oilseeds as observed in experiment 1 (Table 4.1) and also reported by Appelqvist and Ohlson (1972), Robbelin

and Thies (1980), Larsen (1981) and Fenwick **et al.** (1983). Glucosinolate contents of MC and RSC reduced by 7.24 and 2.24 percent, respectively after 12 months of the storage from their initial levels. However, variations in the glucosinolate content of MC and RSC during different months of storage were statistically not significant. The higher reduction of glucosinolates in MC during storage may be attributed to the higher level of glucosinolates and absorption of moisture during storage which might have facilitated the hydrolysis of glucosinolates by endogenous enzyme known as myrosinase. Velisek **et al.** (1983) also reported the reduction of glucosinolates of rapeseed meal during several months of storage at ambient temperature.

5.4.5 Aflatoxins

The concentrations of total aflatoxins content of GNC, MC and RSC at the initial stage of storage and after 12 months of storage are presented in table 4.24. The data indicated that the GNC contained higher concentration of aflatoxins than in MC and RSC at initial as well as at final stages of storage. However, total aflatoxins content of any of the three oilcakes was not affected during storage despite the variations in the ambient temperature and relative humidity. This may be attributed to the low moisture content (less than 6%) of oilcakes (Christensen, 1957), sufficient aeration (Shih and Marth, 1974) and lower relative humidity (lower than 70% on an average) in the godown (Scott, 1957). All types of aflatoxins, i.e., B_1, B_2, G_1 and G_2 were observed in initial and final samples of GNC as reported by Diener and Davis (1966). However, aflatoxin G_2 was not detected in MC and RSC. This was supported by the fact that the amount and relative proportions of different aflatoxins produced by the **A.flavus** depend upon the strain, medium, culture conditions and isolation procedure (Reddy **et al.**, 1972; Orth, 1973). Present values of aflatoxins of oilcakes were lower than those reported by Patel **et al.** (1981) and Sinha and Arora (1985).

It can be inferred from this experiment that the GNC, MC and RSC can be conveniently packed in gunny bags and stored in well aerated godown without affecting their quality. Minor changes in proximate composition of these oilcakes may take place during the early months of storage. However, production of aflatoxins did not take place even in the groundnut

cake which is considered to be most susceptible to the fungi, due to the low moisture content (less than 6 percent). It can also be concluded that glucosinolate content of mustard and rapeseed oilcakes decreased during storage, though the decrease was statistically not significant.

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CHAPTER - VI

SUMMARY

6. SUMMARY

Oil cakes, the vegetable protein supplements, are the vital components of balanced animal rations to exploit the production potential of ruminants. Mustard and rapeseed (**toria**) cakes are extensively used for feeding the cattle and buffaloes in our country and their use is expected to increase due to the enhanced production of **Brassica** oil seeds. However, the glucosinolate content of these oil cakes is reported to reduce feed intake, cause thyroid enlargement resulting in reduced levels of circulatory thyroid hormone in ruminants. Amount of glucosinolates vary from variety to variety of **Brassica** oil seeds. Glucosinolates themselves are not problematic to the animals but their hydrolysing products such as thiocyanates, isothiocyanates, nitriles, oxazolidinethione affect the iodine uptake and may cause the goitrogenicity and poor production performance of animals due to the malfunctioning of thyroid.

The oil cakes are generally stored for fairly long periods before their consumption. Since the oil cakes contain 7 to 12 percent oil besides proteins and carbohydrates, therefore, variations in temperature and relative humidity may cause some chemical changes as a result of oxidation and development of toxin producing fungi which may be detrimental to their quality.

These vital nutritional aspects about the use of mustard and rapeseed cakes in animal ration which need thorough investigations have not been studied in our country. The present study has, therefore, been undertaken to determine the variations in the chemical composition including glucosinolates in Indian varieties of **Brassica** oil seeds and the effect of storage on the quality of oil cakes. Effect of glucosinolates on rumen fermentation, growth performance and thyroxine secretion rate in crossbred calves were also studied.

The salient features of the investigation, planned and executed under four different experiments are presented as under:

6.1 CHEMICAL COMPOSITION OF OIL CAKES AND INDIAN VARIETIES OF MUSTARD/RAPESEED

6.1.1 The proximate composition and amino acid profile of mustard cake (MC), and rapeseed cake (RSC) were almost similar but different than that of groundnut cake (GNC). The total quantity of amino acids were higher in GNC than in MC and RSC, however, levels of methionine and cystine were higher in both MC and RSC than in GNC. Glucosinolate content was not detected in GNC while its level was higher in MC (4.40%) than in RSC (3.54%).

6.1.2 The chemical composition of twelve varieties of mustard and rapeseed revealed that the oil content varied in range of 32.89 to 38.96 percent, highest being in Toria Kranti. The glucosinolate content on the other hand varied from 1.60 to 4.92 percent on dry matter basis of the oil seeds, the highest being in Toria Sangram among the different varieties of mustard/rapeseed. The results indicated that there was no relationship between oil and glucosinolate contents of different varieties of **Brassica** oil seeds.

6.1.3 Proximate composition of seven varieties of mustard/rapeseed indicated that CP and total carbohydrates varied in the range of 35.46 to 46.23, and 49.07 to 58.86 percent on DM basis, respectively. The fibre fractions, i.e., ADF, NDF also showed large variations indicating the varying proportions of hulls in different varieties of **Brassica** oil seeds.

6.2 EFFECT OF GLUCOSINOLATES AND MUSTARD OIL ON RUMEN FERMENTATION

In the first experiment varying levels of glucosinolates, i.e., 0, 9, 18, 27 and 45 mg were taken **in vitro** system to study their effect on rumen fermentation. In another experiment varying levels of mustard oil, i.e., 0.17, 0.34, 0.51 and 0.68 mg, which constituted 1.67, 3.28, 4.85 and 6.36 percent of oil of substrate (solvent extracted mustard cake), respectively, were taken in

in vitro system to elucidate the effect of unsaturated fatty acids of mustard oil on rumen fermentation.

6.2.1 Varying levels of glucosinolates did not affect the rumen fermentation adversely as indicated by the concentrations of TVFA, molar proportions of individual VFA, total gas production, and its constituents CO_2 and methane. The significant variations in molar proportions of individual VFA, enhanced gas production and improved microbial protein synthesis by increasing the glucosinolate level were attributed to the utilization of sulphate ions and glucose, available as a result of hydrolysis of glucosinolates and also to the water soluble nutrients of mustard cake.

6.2.2 Addition of mustard oil at different levels did not influence the rumen fermentation adversely. On increasing the mustard oil the molar proportion of propionate increased ($P/0.05$) with a decrease in acetate proportion without affecting the proportion of butyrate and TVFA concentration. Production of total gas was also increased ($P/0,05$) by increasing the level of mustard oil upto 0.34 mg, however, proportion of methane in total gas was reduced. Microbial protein synthesis increased by the addition of mustard oil upto the level of 0.34 mg, thereafter the influence of enhanced levels of mustard oil on protein synthesis was not significant. The results showed that 3.28 percent oil content of mustard cake was optimum for rumen fermentation.

6.3 EFFECT OF GROUNDNUT, MUSTARD AND RAPESEED OIL CAKES ON GROWTH, NUTRIENT UTILIZATION AND THYROXINE SECRETION RATE

Eighteen male crossbred calves of similar age and body weight were distributed upto three groups of 6 each. First group (GNC) was fed with control diet containing groundnut cake as a protein supplement. In the ration of second (MC) and third (RSC) groups, the protein of groundnut cake of control ration was replaced by mustard and rapeseed cake, respectively. All the groups were fed their respective concentrate mixture to fulfil the nutritional requirements of calves as per NRC (1978) and wheat straw was given **ad lib** along with one kg fresh seasonally available green fodder to supply the vitamin A. Growth

performance, feed conversion efficiency and nutrient utilization were studied during 35 weeks duration feeding experiment. Circulatory levels of T_3 and T_4, haemoglobin and plasma thiocyanate content were estimated at monthly intervals. Finally body composition and thyroxine secretion rate were estimated in the calves of all the three groups.

6.3.1 The glucosinolate content of all the three iso-nitrogenous rations given to GNC, MC and RSC groups was 0, 1.72 and 1.40 percent on dry matter basis. Palatability of mustard and rapeseed containing rations was lower than that of control ration during the first three weeks of experimental feeding. Even after adaptation the consumption of concentrate mixture was slower in MC and RSC groups than in GNC group. The calves of MC and RSC groups preferred the mixture of concentrate and wheat straw over concentrate mixture alone.

6.3.2 The average daily gain in body weight in GNC, MC and RSC groups was 480.60±13.76, 412.90±19.50 and 427.20±29.49, g respectively and the feed intake per kg gain in body weight in corresponding groups was 8.90±0.41, 10.24±0.45 and 9.67±0.59 kg. The average growth rate per week (b value) was 3.32±0.17 kg in GNC group, 3.07±0.14 kg in MC group and 3.22±0.24 kg in RSC group. Though the growth rate and feed conversion efficiency were higher in GNC group than those in MC and RSC groups but variations among groups were not significant. Crude protein, TDN and ME intake per kg gain in body weight in GNC, MC and RSC groups were also not varied significantly among the groups.

6.3.3 The average daily gain in body weight, body length, height at wither and heart girth were higher in GNC group than in other two experimental groups, however, variations among groups were not significant. Prediction equations based on body measurements revealed that either of the three measurements (length, height at wither or heart girth) alone or their combinations can be used for predicting the body weight of crossbred calves in different treatment groups. However, heart girth measurement can only be used as a reliable measurement for predicting the body weight of crossbred calves in the age group of 4-13 months.

6.3.4 Average dry matter intake/100 kg body weight in GNC, MC and RSC groups was 2.23±0.05, 2.29±0.11 and 2.45±0.21 kg, respectively. The dry matter intake and digestibility coefficients of proximate principles and cell wall constituents did not vary significantly among the groups. The digestibility coefficient varied in the range of 59.29 to 61.79 for dry matter, 60.62 to 65.28 for organic matter, 68.34 to 71.82 for crude protein, 51.75 to 52.90 for crude fibre, 78.09 to 79.45 for ether extract, 60.97 to 68.86 for NFE, 48.67 to 50.43 for ADF and 42.36 to 44.91 for NDF.

6.3.5 Nitrogen intake in GNC, MC and RSC groups was 100.58±5.90, 102.57±3.92 and 95.98±6.49 g per day, respectively. Urinary nitrogen as percent of total nitrogen intake in corresponding groups was 44.49±1.54, 46.61±2.30 and 44.03±3.90. Nitrogen balances were positive in all the treatment groups and nitrogen retention as percent of total nitrogen intake was 23.96±2.09 in GNC group, 24.74±3.18 in MC group and 25.62±4.73 in RSC group. Nitrogen intake, its excretion and balances did not vary significantly among the groups.

6.3.6 The crude protein intake in GNC, MC and RSC groups was higher by 4.29, 10.21 and 5.07 percent than NRC (1978) feeding standard while TDN intake in corresponding group was lower by 8.71, 9.03 and 15.02 percent to the recommended values.

6.3.7 Body composition of the crossbred calves in GNC, MC and RSC groups revealed that the percentage of water, fat, protein and ash did not differ significantly among the groups.

6.3.8 Average plasma tri-iodothyronine (T_3) concentration in MC group (0.30 ng/ml) was significantly ($P<0.05$) lower than in GNC (0.39 ng/ml) and RSC group (0.39 µg/ml).

6.3.9 The average value of plasma thyroxine (T_4) in GNC, MC and RSC groups was 51.44, 47.11 and 40.29 ng/ml, respectively. Though the T_4 concentrations in MC and RSC groups were lower than in GNC group, however, variations among groups were not significant.

6.3.10 Blood haemoglobin content of crossbred calves did not vary significantly in all the three experimental groups throughout the feeding experiment.

6.3.11 Thiocyanate content was not detected in the plasma samples of GNC group at any stage of experimental feeding. However, thiocyanate content in plasma of MC and RSC groups increased with the progress of feeding period and average plasma thiocyanate content was higher in MC group (8.90±1.12 µg/ml) than in RSC group (7.92±1.68 µg/ml).

6.3.12 The average thyroxine secretion rate per 100 kg body weight in GNC, MC and RSC groups was found to be 0.032, 0.025 and 0.035 mg/litre, respectively and the differences among the different treatment groups were not significant.

6.4 EFFECT OF STORAGE ON QUALITY OF OIL CAKES

6.4.1 The temperature and relative humidity varied in the range of 15 to 42°C and 22 to 85 percent, respectively, during the storage period of 12 months. However, temperature above than 30°C and relative humidity above than 70 percent were recorded only for the brief period (June-Aug) during the initial stage of storage.

6.4.2 Dry matter content of groundnut, mustard and rapeseed cakes decreased significantly ($P/0.05$) during the first four months of storage. Proximate constituents such as crude protein, crude fibre and ether extract were decreased in all the three oil cakes with an increase in NFE during the storage period, however, variations among the samples drawn at monthly intervals of each cake were not significant.

6.4.3 Free fatty acids (ffa) value of groundnut, mustard and rapeseed cakes increased by 2.01, 2.36 and 2.88 percent after the storage of 12 months, however, variations among the samples drawn at monthly intervals for each cake were not significant.

6.4.4 Average glucosinolate content of mustard cake (4.40%) was significantly higher (P/0.05) than that of rapeseed cake (3.54%). It was decreased by 7.24 percent in mustard cake and 2.24 percent in rapeseed cake during the entire period of storage, however, effect of storage on glucosinolate content of mustard and rapeseed cakes was not significant.

6.4.5 Groundnut cake was containing higher aflatoxins content than mustard and rapeseed cakes, however, aflatoxins content of all the three cakes did not change during their storage period.

CONCLUSION

It can be concluded from these results that mustard and rapeseed oil cakes were similar in their chemical composition and amino acid profile, however, there was a difference in their glucosinolate content due to the difference in their variety as indicated by the large variations among different varieties of mustard/rapeseed. Glucosinolate did not affect the rumen fermentation adversely rather its hydrolysis products such as sulphate ions and glucose helped in enhancing the microbial protein synthesis. The oil content of mustard cake reduced the methane production due to hydrogenation of its unsaturated fatty acids. Mustard and rapeseed cakes can replace costlier groundnut cake without affecting the growth rate performance and feed conversion efficiency of crossbred calves in the age group of 4-13 months. However, long term feeding of mustard or rapeseed cake as an exclusive source of dietary protein may affect the thyroid function, however, such situation does not arise in field conditions because animals are exposed to mustard/rapeseed oil cake supplemented ration, only for 2 to 3 months mainly during the summer season and with the starting of monsoon the feeding of these oil cakes are generally discontinued due to the plentiful availability of green fodder. It was also evident from these results that storage of oil cakes in gunny bags in godown does not affect their feeding quality during a period of 2 months.

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