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ENHANCEMENT OF NUTRITIVE VALUE OF
MUSTARD OIL MEAL (MOM)

presented by

Nanda Prakash Joshi

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PhD degree in Animal Science

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ENHANCEMENT OF NUTRITIVE VALUE OF MUSTARD OIL MEAL (MOM)

By

Nanda Prakash Joshi

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1986

ABSTRACT

ENHANCEMENT OF NUTRITIVE VALUE OF MUSTARDOIL MEAL (MOM)

By

Nanda Prakash Joshi

Mustard oil meal (MOM) was chemically characterized. On the average, it contained 38% crude protein (dry matter basis (dmb)) and 12.5 % ether extract (dmb). Gross energy content was 4.65 Kcal/g and metabolizable energy content was 3.29 Kcal/g. Glucosinolate content was 4.75 % (Fat Free (FF) dmb), erucic acid content 37.05 % (in residual oil) and tannic acid content was 2.86 % (FF dmb).

Autoclaving of MOM improved chick growth significantly over dry heat treated MOM at 121 C for 30 min or hot water treated MOM (100 C for 30 min). There was a progressive decline in weight gain upon increasing the dietary levels of MOM, irrespective of the treatments they were subjected to.

Complete defatting of MOM with acetone and autoclaved MOM supported similar gains.

Defatting of MOM with hexane improved chick growth significantly over raw MOM. There was further improvement upon acetone washing of defatted MOM. However, it was not as high as the growth response to canola meal.

There was 17 % decrease in total lysine content upon

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Nanda Prakash Joshi

autoclaving of MOM which indicates that the growth improvement upon feeding of autoclaved MOM was independent of lysine destruction.

A drum heating system was developed that was used to heat treat the MOM. MOM was treated at 85 and 100 C , with 12.5 - 37.5% moisture levels and heated for 15 - 60 min. Heat treated MOM also analyzed for total glucosinolates, erucic acid, total lysine, reactive lysine and metabolizable energy.

Availability of lysine in heat treated MOM was determined using a slope ratio assay (SRA) technique and dye binding assay (DBA) technique. On the average, lysine availability in heat treated MOM was significantly better than in raw. However, within the treatments there was no significant differences. SRA and DBA were well correlated ($r = 0.81$).

Supplementation of arginine and lysine in a MOM basal diet significantly improved chick growth over MOM basal diet. Thyroid size of the chicks fed arginine and lysine in the diet significantly reduced and was not significantly different from the controls.

DEDICATED TO MY PARENTS

Lt. Mrs. Bhakti M. Joshi and Mr. Ananda B. Joshi

ACKNOWLEDGEMENTS

The Author is deeply indebted to his major advisor, professor Dr. Robert J. Deans for his constant guidance, encouragement and friendship throughout this study.

Deep appreciation is expressed to professors Dr. Duane E. Ullrey, Dr. Robert J. Brunner and Dr. Darrell Fienup for their valuable suggestions and healthy criticism in preparing this manuscript and participating in this project as the author's graduate committee members.

Professors, Dr. Elwin R. Miller is thanked for valuable suggestions in designing feeding trials, Dr. Werner G. Bergen is thanked for amino acid analysis, Dr. Mel yokoyama for GC analysis.

Special thanks are given to Professor John L. Gill for his guidance and suggestions in designing experiments and statistical analyses.

The author sincerely expresses his gratitude to faculty and staff in the department for their friendship and willingness to help when needed.

The author also wishes to thank the organizations involved in supporting this project, namely: USAID through the IAAS/MUCI project, Tribhuwan University (TU), MUCIA and Michigan State University.

Gratitude is expressed to Professor Ram Chandra B. Singh, the then vice chancellor of TU for kindly granting study leave. The author appreciates the support given by Netra B. Basnyat, registrar at TU for this project.

Faculty and staff at IAAS are appreciated for moral support and encouragement.

Chairman Dr. Maynard Hogberg is sincerely thanked for providing financial support to complete this project.

Dr. Pao K. Ku is thanked for his help with the laboratory work. Fellow graduate student Douglas K. Hall is thanked for much help rendered during the author's stay.

Dr. Wayne Stockland , director of Supersweet Research Farms, Courtland, MN is thanked for amino acid analysis and also for the opportunity provided the author to work with amino acid analyzer at their facilities.

Learning Corporation is thanked for the opportunity to use their microcomputer.

Gratitude is expressed to Professor Emeritus Alfred M. Lucas who allowed the author to be his office mate and gave inspiration and fellowship throughout the stay.

My special thanks to my family members and relatives in Nepal for their encouragement and support. My sincere thanks goes to my wife, Mani, whose patience and emotional support helped throughout this study. My appreciation is also expressed to my daughter, Poonam, whose presence gave us joy and made life a little easier in graduate school.

NPJ

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KEY TO ABBREVIATIONS

RS = Rapeseed

MS = Mustard seed

MOM = Mustard oil meal

MOC = Mustard oil cake

RSM = Rapeseed meal

GNC = Ground nut cake

SBM = Soybean meal

CGM = Corn gluten meal

SSM = Sesame seed meal

CM = Canola meal

WM = Wheat middlings

DM = Dry matter

CP = Crude protein

EE = Ether extract

ME = Metabolizable energy

GE = Gross energy

TME = True metabolizable energy

OZT = Oxazolidinethione

ITC = Isothiocyanates

min = minutes

C = celsius

std.= Standard

Ref.= Reference

wt = weight

F/G= Feed to gain ratio

RL = Reactive lysine

TL = Total lysine

AL = Available lysine

ORGANIZATION OF THE MANUSCRIPT

For simplicity and clarity the main body of this manuscript is presented in the following fashion:

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Each experiment has an introduction, materials and methods and results and discussion section. Experiments in part I describe the chemical characteristics of mustard oil meal (MOM) , effects of different types of heat treatments, effects of defatting and effects of MOM levels in the diets of growing chicks.

In part II, effects of temperature, moisture and duration of heat treatment on the nutritive value of MOM are examined. Availability of lysine and effects of supplementation of amino acids to MOM basal diets are also studied.

INTRODUCTION

Mustard oil meal (MOM) is a byproduct of mustard seed (Brassica spp.) oil extraction. In Nepal, mustard is the major oilseed crop followed by ground nut, linseed and soybean. Mustard seed occupies a distinct first position in terms of acreage and total production. Sarson (Brassica campestris cv. sarson), toria (Brassica campestris cv. toria) and rai (Brassica juncea) are the three major Brassica oilseed crops grown. In trade, no distinction is made among these three oilseeds. Mostly mixtures of these seeds are traded in the market. Almost all oil extraction is done by the expeller process. In the 1984-85 fiscal year, 71,322 mt of mustard seed were produced, which is approximately 85% of the total production of all oilseeds (83, 920 mt). Mustard oil cake production was 44,226 mt (Nepal, 1985).

MOM is a rich source of protein (33-40%) and has relatively well balanced amino acid composition. Although protein quality appears to be better than that of ground nut cake (GNC), use in diets for animals has been limited due to toxicity problems. MOM would be a promising substitute for the relatively expensive GNC and soybean meal in poultry and swine rations in Nepal, if this toxicity could be reduced.

Toxicity problems associated with MOM are due to its high content of glucosinolates, erucic acid in the residual oil, phenolic compounds, high fiber content and possibly others yet to be identified. An antithiamine factor and a tripsin inhibitor have also been identified. Detoxification of MOM is necessary for its efficient utilization in livestock rations.

Nutritive value of MOM depends upon several factors including varieties, growing conditions and oil extraction procedure used. During expeller extraction of oil, some nutrient loss may occur. However, there is incomplete extraction of oil, as 9 - 15% of residual oil is left in the meal. Mustard oil is rich in erucic acid (32 - 42%), as a result, substantial amounts of erucic acid are present in MOM, contributing to its toxicity problem.

Several detoxification techniques have been used by researchers with varying degrees of success. Techniques thus far are only partially successful in the destruction of all antinutrient components in MOM. Heat treatment of some form is the most common method of detoxification. Aqueous extraction and treatment with chemicals like ammonia and ferrous sulfate have also been used to detoxify MOM. Most of these techniques induce loss of some nutrients along with the antinutritive factors. Ideally it would be desirable to eliminate all antinutritive factors without nutrient destruction for its fullest utilization. Unfortunately, some nutrient loss has to be tolerated to improve its nutritive

value.

A more desirable long term solution is to develop mustard seed varieties devoid of antinutritive characteristics. Several rapeseed types that are low in erucic acid and glucosinolates have evolved from extensive plant breeding research in Canada and European countries. In Canada, double-low rapeseed (low in erucic acid and low in glucosinolates) is marketed under the trade name of 'Canola'. However, rapeseed types completely devoid of glucosinolates are not available.

Mustard oil is the major edible oil in Nepal. It's distinct pungent smell is familiar to every Nepalese household, and it's value in preserving pickles is considered important particularly. Any proposed change in the characteristics of mustard oil needs careful evaluation.

Although substantial amounts of MOM are available in Nepal, limited information is available on the chemical characteristics of MOM and its nutritive value to livestock, restricting the use of this otherwise potential protein supplement. In this study, chemical characteristics of MOM from commercial sources in Nepal were established. It's nutritive value to the growing chick was also investigated.

Several heating techniques were tested in an attempt to improve it's nutritive value. An attempt was also made to partition the effects due to the oil fraction and other components such as glucosinolates and tannic acids. The study has been approached in two phases. In part 1, effects

of wet and dry heat treatments, defatting of MOM and maximum levels of inclusion in chick rations has been investigated. In part 2, a practical type of heating system was used. After evaluation of heat-treated MOM by in vitro and in vivo procedures, selected treatments were further evaluated for available lysine. Supplementation of a corn-MOM basal diet with amino acids was also investigated.

Following are the major objectives of this study:

1. To determine the following chemical characteristics of MOM

- a) Proximate analysis
- b) Mineral analysis
- c) Amino acid analysis
- c) Antinutrients
 - i. Total glucosinolates
 - ii. Allyl-isothiocyanate
 - iii. Erucic acid
- IV. Tannins

2. To determine metabolizable energy of MOM
3. To determine maximum levels of MOM that can be incorporated in chick rations.
4. To determine the effects of different types of heat treatment on the nutritive value of MOM
5. To determine the effect of defatting of MOM on its nutritive value

6. To partition the effects of the residual oil fraction from the other components in MOM
7. To determine the effects of heat treatments on the availability of lysine
8. To determine the effect of amino acid supplementation to corn-MOM basal diet

LITERATURE REVIEW

Nomenclature of Brassica oilseed crops

Mustard is a member of the genus Brassica of the family cruciferae. There are over 150 species of genus Brassica and most of them are cultivated as oilseed crops or vegetable crops. Mustard is chiefly grown in China, India, Canada, USA, USSR, Nepal, Pakistan, United Kingdom and other European countries (Wies, 1983). In the tropics and subtropics, it is grown as a winter crop. Mustard seed have been used as a spice, medicine and as a source of edible oil since ancient times.

There are two types of mustard, viz. white and black. There are seeds that look like black mustard seeds called rapeseeds. Due to their close resemblance they are often available as mixtures of these seeds in the market (Allikutty, 1976). In commerce, no distinction is made between black mustard seeds and rapeseeds (Shankarnarayana, et al., 1972). The English name mustard corresponds to rai and rapeseeds to sarson and toria, however they are commonly referred to as mustard. Mustard is the most important oilseed crop in Nepal followed by peanut, linseed and soybean. Common names and some of the types of mustard seed and rapeseed are given in Table 1.

TABLE 1. NOMENCLATURE OF MUSTARD AND RAPESEEDS

	Seed	Botanical name	Common name
1. Mustard	White	<u>Brassica alba</u> or <u>Sinapis alba</u>	Yellow mustard
	Black	<u>Brassica nigra</u>	Table mustard
		(L) Koch	
		<u>Brassica juncea</u> Coss	Brown mustard (Rai)
2. Rape		<u>Brassica campestris</u> var. Yellow sarson	Yellow sarson
		<u>Brassica campestris</u> var. Brown sarson	Brown sarson
		<u>Brassica campestris</u> var. Toria	Toria

Source: Shankarnarayana, et al. (1972)

Importance of Brassica oilseed crops

Rapeseed is one of the five most widely produced oilseeds in the world. Its biggest producers are India, China, Canada, France, Pakistan and East and West Germany (Becker and Tiernan, 1976; Weis, 1983). Canada is the major exporter of rapeseed, rapeseed oil and rapeseed meal. World production and distribution of major oilseed crops and their meals are given in Tables 2 and 3, respectively.

TABLE 2. MAJOR RAPESEED PRODUCING COUNTRIES AND PRODUCTION

	(in thousand tons)		
	1970	1975	1980
World	7,000	8,500	12,000
Canada	1,600	1,900	2,500
China	800	1,400	2,500
India	1,600	1,800	2,000
France	582	475	450
Poland	560	720	230
UK	9	61	250

Source (Weis, 1983)

TABLE 3. ESTIMATED WORLD PRODUCTION OF MAJOR OIL MEALS

	(in million tons)		
	1970	1975	1980
Coconut	1.3	1.6	1.8
Cotton seed	8.0	9.0	10.0
Groundnut	4.0	4.0	4.0
Linseed	1.8	1.3	1.4
Palm kernel	0.5	0.5	0.6
Rapeseed*	3.0	4.2	6.0
Sesame	0.7	0.8	1.0
Sunflower	2.0	3.5	5.0
Soybean	32.0	44.0	60.0
Total	53.5	69.0	90.0

Source: Weis (1983); * Includes mustard oil meal

Area and production of oilseeds in Nepal from the fiscal years 1975-76 through 1984-85 are presented in Table 4.

TABLE 4. AREA AND PRODUCTION OF MUSTARD SEEDS IN NEPAL

Fiscal year	Area (ha) 000'	Oilseed (mt) 000'	Mustard (mt) 000'	Mustard oil cake (mt) 000'
1975-76	1,13	68.5	58.0	36.0
1976-77	1,08	61.0	52.0	32.0
1977-78	1,33	78.5	67.0	41.0
1978-79	1,44	92.5	79.0	49.0
1979-80	1,18	63.0	53.0	33.0
1980-81	1,22	77.0	66.0	41.0
1981-82	1,14	79.0	67.0	42.0
1982-83	1,10	70.0	59.0	37.0
1983-84	1,10	73.0	62.0	39.0
1984-85	1,28	84.0	71.0	44.0

Source: Agricultural Statistics Division, Food and Agricultural Marketing Services (Nepal, 1985)

Uses of mustard and rapeseeds and their products:

Mustard seed is used as a spice in curry, pickles and sauces. It believed to act against the action of molds and yeasts. The major commercial products of mustard seeds are



mayonnaise, mustard paste and mustard flour. Brassica nigra is a source of condiments , although it has been largely replaced by Brassica juncea (Vaughan, 1977). Mustard seed oil is the primary vegetable oil used in cooking and also in preserving pickles in Nepal.

1. Oils: Rapeseed and mustard seeds yield 24 to 40 percent of fatty oils depending upon variety, growing conditions and season. These oils are rich in erucic acid (22 C:1). The oils from cruciferous seeds are composed of about 95-97% triacylglyceroles (or triglycerides) and only 0.3-0.5% free fatty acids (Appelqvist, 1971). The fatty acid composition of mustard oil is given in Table 5.

TABLE 5. FATTY ACID COMPOSITION (%) OF MUSTARD SEED OIL

Fatty acids	<u>B. campestris</u>		<u>B. juncea</u>
	Toria	Sarson	Rai
C 16	2.65	1.67	4.56
C 16:1	0.25	0.27	0.18
C 17	trace	trace	trace
C 17:1	" "	" "	" "
C 18	1.43	1.22	1.37
C 18:1	11.77	12.33	10.74
C 20	7.82	6.96	8.41
C 20:1	0.85	1.64	2.25
C 22	1.22	2.14	---
C 22:1	45.70	48.71	32.22-44.72
C 24	0.51	0.81	0.99
C 24:1	1.96	1.53	2.92

Source: Dutta and Ghosh (1970)

2. Meals: Sixty to sixty-two percent of the oilseed is made into oil cake. Residual meals are rich source of protein (33- 40 %). These meals are excellent sources of

protein for livestock. However, due to the toxic problems associated with these meals, the full potential has not yet been exploited. Expeller-processed MOM contains approximately 10-15% residual oil (Panda and Shrivastava, 1978).

Composition of MOM and RSM

Appelqvist (1971) observed that the existence of crop types with the group names rapeseed and mustard seed is one reason for the greater compositional variation in commercial samples of these meals. Composition of MOM and RSM varies with species and within a species among the varieties. Protein and oil content of several species of the genus *Brassica* are given in Table 6. Superior protein quality of *B. alba* compared to *B. juncea* and *B. campestris* has been reported (Pathak et al., 1973). Prasad (1978) observed that MOM from *B. juncea* was little lower in protein compared to *B. campestris* varieties. Double-low cultivars of rapeseeds yield excellent quality protein (Eggum, 1981).

TABLE 6. OIL AND PROTEIN CONTENT OF BRASSICA OILSEEDS

Brassica oilseeds	Percent(dry basis)		
	Oil (%)	Protein(N*6.25)	
		Whole seed	Defatted meals
<u>Brassica napus</u>	44.7	23.8	43.1
<u>Brassica campestris</u>	42.7	23.8	41.5
<u>Brassica juncea</u>	37.2	27.5	43.8
<u>Brassica hirta</u>	28.1	31.2	43.4
<u>Brassica nigra</u>	32.4	31.2	46.2

Source : Miller, et al. (1962).

Gross chemical composition:

Extensive reviews of chemical composition and properties of proteins and glucosinolates in RSM and MOM are available in the literature (Appelqvist, 1971; Shankarnarayana et al., 1972; Bell, 1984). Marquard et al. (1979) observed that various types of mustard (B. juncea, B. nigra and Sinapis alba) grown in different ecological locations differed greatly in yield, content and composition of fats and protein. Proximate analysis of mustard oil meal is given in Table 7. Meals contain approximately 25% hull which lowers the nutritive value of canola (Mitaru et al., 1983).

TABLE 7. PROXIMATE ANALYSIS OF MOM

Component	<u>B. comp.</u>	<u>B. jun.</u>
Dry matter	91.9	91.80
Crude protein	35.8	33.20
Crude fiber	8.11	9.79
Ether extract	14.43	10.98
Total ash	7.59	9.03
Calcium	0.79	0.75
Phosphorus	1.91	1.79

Source : Prasad (1978)

Udaysekharrao and Srinivasrao (1980) found the crude protein and fat content of mustard seeds (B. juncea) to be 22.3% and 32%, respectively, which was similar to the results obtained by Miller et al. (1962). Zombade et al. (1980) found crude protein to be 33.9% in MOM. Bell and Jeffers (1976) found the protein content of RSM to be 35.9%.

Minerals and their availability:

MOM is a relatively rich source of calcium. Although it is also rich in phosphorous, availability is affected by phytic acid and high fiber content. Retention of all minerals are adversely affected by phytic acid and crude fiber (Nwokolo and Bragg, 1977). RSM has been found to bind more zinc compared to SBM (83.6% vs. 50.3). Removal of soluble polyphenols significantly decreased the zinc binding capacity of RSM (Seth et al., 1975a). Rapeseed protein concentrates contain 5.3-7.5% phytic acid assuming 28% phosphorous in phytic acid (Erdman, 1979). Shah et al. (1976) observed that adverse effect of Brassica protein concentrates containing high levels of phytic acid on zinc metabolism of young rats can be overcome by zinc supplementation.

Metabolizable energy:

Lodhi et al. (1969a) found that the available carbohydrate in RSM was only 15% compared to 23.6% for SBM using chemical and biological assays. This seemed to affect the ME value of MOM. Muztar and Slinger (1982) using a rooster assay determined the TME of 3 varieties of canola meals and found that TME ranged from 2.31 - 2.54 cal/g. However, their samples contained only 1.96 to 2.99 % ether extract.

Low ME values in RSM are due to its low available carbohydrate (11%) (Lodhi et al., 1969b; Zombade et al., 1980; Blair and Reichart, 1984), lower protein content , low

nitrogen absorbability (Rao and Clandinin, 1972) and high fiber content (Vose, 1974; Sarwar et al, 1981). Differences in ME values of RSM for different stages of growth of chickens have been reported (Lodhi et al, 1969). Expeller processed MOC (13% ether extract) gave ME value of 3017 cal/kg in white leghorn layers (Shrivastava et al., 1976b). The ME value of RSM was not found to be affected by progoitrin (Rao and Clandinin, 1970). Significant improvement in the ME value of RSM was achieved upon removal of tannins (Yapar and Clandinin, 1972); however, removal of gum had no effect on TME values for chickens (Sibbald, 1977). Levels of inclusion of RSM, age of chicks and the duration of feeding had no effect on the ME values in chicks and roosters (Bayley et al., 1974). ME value ranged from 1340 to 2000 kcal/ kg. ME value for canola meal were found to be 1900 kcal/kg for young chicks and 2000 kcal/kg for adult birds (Clandinin and Robblee, 1981). Lodhi et al. (1970a) found ME for RSM to be 1880 kcal/kg for 4 week old chicks and 1800 kcal/kg for layers.

Amino acid profile

Varietal differences in amino acid composition of rapeseed, turnip rape and soybean have been reported (Soluski and Sarwar, 1973). The amino acid compositions of selected Brassica species are given in Table 8.

TABLE 8. AMINO ACID PROFILE OF SELECTED BRASSICA SPECIES

Amino acids	<u>B. comp.</u>	<u>B. hirta</u>	<u>B. juncea</u>	<u>B. napus</u>	<u>B. nigra</u>
Lysine	6.048*	5.792	5.360	5.824	4.384
Methionine	1.920	1.552	1.664	1.776	1.504
Cystine	2.432	1.984	2.544	2.432	2.368
Isoleucine	3.792	3.312	3.776	3.648	3.760
Leucine	6.464	6.592	6.320	6.320	6.256
Phenylalanin	3.776	3.728	3.840	3.536	3.744
Tyrosine	2.736	3.296	2.672	2.624	2.640
Threonine	3.984	2.736	4.016	3.840	3.712
Valine	4.912	4.800	4.736	4.816	4.608
Histidine	2.720	2.624	2.576	2.576	2.528
Arginine	5.920	5.392	6.416	5.616	7.552

Source: Miller et al. (1962). * gm amino acid/16 gm N.

Protein quality in RSM and MOM

Protein quality of a feedstuff depends on many factors including the amino acid balance and availability (Wolzak et al., 1981). It is essential to consider amino acid balance as well as the quantity of the limiting amino acid proportionality patterns to evaluate dietary proteins. Kies (1981) concluded that bioavailability is secondary to amino acid proportionality patterns in determining the quality of a food protein. The optimum ratio between protein level and the most limiting amino acid is important (Fisher et al., 1960). Anwar and Clandinin (1971) observed that nitrogen solubility in saline or acidic solutions can be used as a measure of relative protein availability in different RSM samples. Solubility of nitrogen of RSM was significantly greater in 6N HCl than in 0.5% NaCl solution (Seth and Clandinin, 1975). Lodhi (1970) observed that nitrogen



absorbability was 79.9% for RSM and 85.4% for SBM. Goh et al. (1980) suggested that the solubility of RSM protein in 0.2% KOH is not a good measure of protein quality. They suggested that dye binding capacity of protein was significantly correlated to TPE values.

Haq et al. (1982) observed that free fatty acids increase rapidly when stored under room temperature, therefore expeller processed oil cakes should be hexane extracted at 40 to 70 C to preserve protein quality.

Amino acid availability:

Weight gain is a function of the absolute intake of the first limiting amino acid (Netke et al., 1969). As most feed grains are known to have lysine as a first limiting amino acid, it is essential to estimate availability values for lysine. RSM is low in lysine and also moderately low in arginine (Klain et al., 1956). Lodhi et al. (1974) observed that amino acid balance of MOM was better than GNC. Nwokolo et al. (1976) observed that all essential amino acids except methionine (78.4%) showed high availability, although significantly lower than in SBM. Availability of lysine was 94.4%. Vaidya et al. (1975) found available lysine in MOM to be 3.51 g/16 gm nitrogen. Zombade et al. (1980) found lysine content to be 3.97 g/16 g N in 14 samples of MOM analyzed. Udaysekharrao and Srinivasrao (1980) observed that Brassica juncea was low in tryptophan (0.5%). Glucosinolates in RSM have no effect on amino acid



availability (Salo, 1982; Rao and Clandinin, 1978).

Available lysine in a double low cultivar (tower rapeseed) for pig was 79.9% compared to 91.4% for SBM (Pearson and Bowland, 1976). Leslie and Summers (1975) found that arginine is the limiting amino acid in RSM for maximum chick growth, and the addition of arginine and methionine improved chick growth.

Purified diets require more arginine compared to practical corn-soy type diets (Snyder et al., 1956). Leslie et al. (1976) found that in RSM the ratio of arginine to lysine was about 1.1:1. It was also suggested that arginine is less available from RSM due to the fact that some of the arginine is utilized in the metabolic process involved in tannic acid excretion. Interaction of arginine, methionine and tannic acid has been suggested. It was demonstrated that arginine deficiency could be alleviated by narrowing the protein energy ratio in an arginine deficient diet for chickens (Scott and Forbes, 1958).

Procedures to determine available lysine

The traditional method of determining protein quality in feedstuffs has been digestibility determination. Biological assays are needed to establish protein bioavailability. However, they are expensive and time consuming. It also requires considerable quantity of samples which are not always available. Therefore, chemical methods have been developed to evaluate protein quality (Wolzak et al., 1981). A mathematical model useful in predicting available lysine due

to heat processing was developed (Wolf et al, 1983).

Chick growth assay:

A chick growth assay technique for amino acids was developed for the cases in which standard material cannot be fed free from the test material (Hill et al., 1966). Amino acid imbalances can be created using semipurified basal diets (casein - corn starch) and amino acid mixtures (low in one or several of the limiting amino acids) or protein supplements like gelatin (Harper, 1959). Dietary lysine level had no effect on feed intake and relative amino acid balance was without any effect on animal performance (Cieslak and Benevenga, 1984). The need of correction for increased protein levels, to measure available lysine in protein supplements has been stressed (Grau, 1948). However, Gupta (1958) did not observe an appreciable effect on lysine availability by the changes in the levels of test protein. Netke and Scott (1970) used a purified basal diet using crystalline amino acid mixtures deficient only in lysine to study the lysine availability in test ingredients. Dean and Scott (1965) developed a purified amino acid reference diet with and without added casein that gave similar growth as practical corn-soy diet.

Carpenter et al. (1963) used a sesame seed meal basal diet to assay available lysine using chick growth assay. Chick growth assay was more reliable than FDNB (Flouro-di nitro-benzene) method.

Plasma amino acid level:

It was demonstrated that the plasma level of the first limiting amino acid remains at a very low level in the blood irrespective of the severity of the deficiency (Zimmerman and Scott, 1965). It was also shown that arginine accumulates in plasma when the dietary level of lysine and valine is low. It was suggested that the plasma level of amino acids may be a good criterion to estimate availability of dietary amino acids.

Rapid assay using roosters:

Sibbald (1976) developed a method of determining true metabolizable energy (TME) in feedstuffs using adult rooster. In this method adult roosters are fasted for a given period of time (24 - 36 h) and force fed a known quantity of test ingredient and excreta is collected for 24 to 36 h. A pair of roosters are used for endogenous excretion. Feed intake, excreta collected from the roosters on test diets and from fasted roosters (endogenous excretion) are used to calculate TME. This method is simple and rapid. The same roosters can be used repeatedly. The TME method has been used for determining amino acid availability in feedstuffs (Likuski, 1978; Muztar et al., 1980). Farrell (1978) developed a rapid bioassay using adult roosters similar to Sibbalds' TME method to determine metabolizable energy. Campbell et al. (1981) observed negative nitrogen balance in TME determination using roosters. Available lysine value for RSM was 83 to 94% similar to SBM (Muztar et

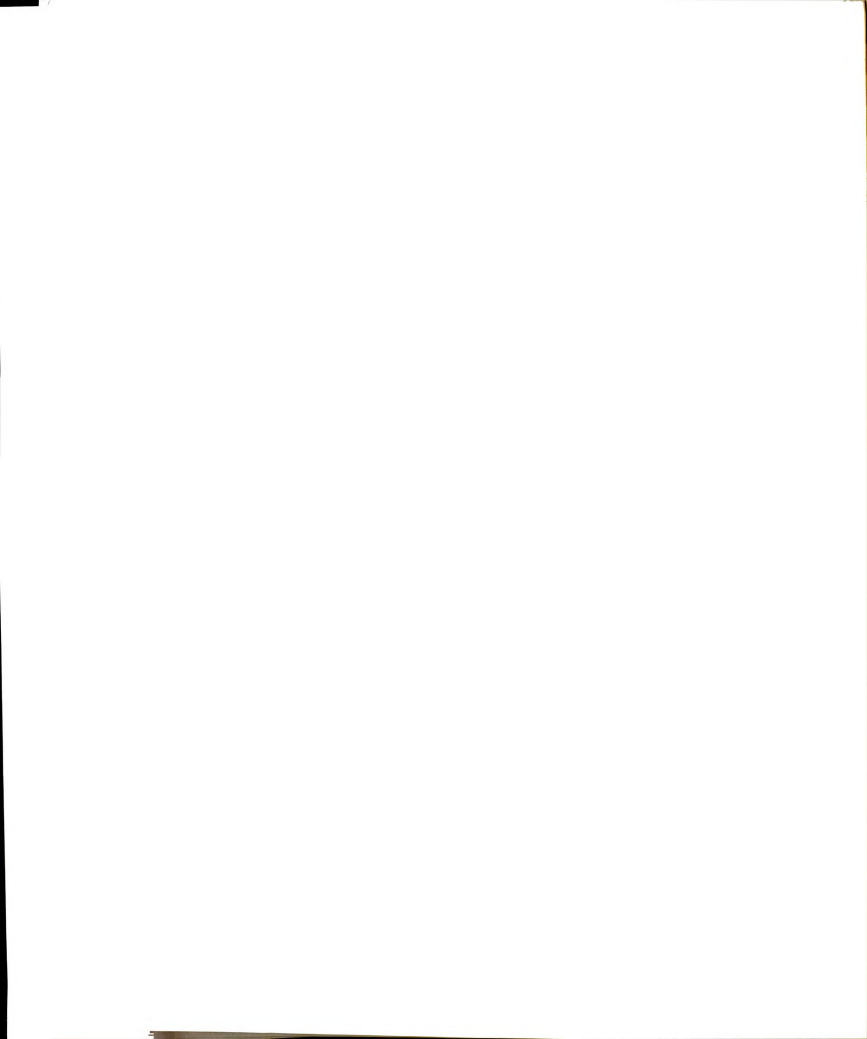
al., 1980). Heat damage to SBM protein was detected by rapid assay using adult roosters (Wallis and Balanave, 1984).

Chemical assays:

The TNBS(2,4,6-trinitribenzenesulfonic acid) method was comparable to FDNB method in the determination of available lysine (Kakade and Liener, 1969). Good correlation between the values obtained by the FDNB method and the growth assay procedures for available lysine in raw or unheated feedstuffs were observed. However, the FDNB method failed to take into account the rate of digestion and digestible peptides in heat treated proteins. Boctor and Harper (1968) found good correlation between growth assay and FDNB method for determining available lysine.

Dye binding assay:

A dye binding assay using acid orange 12 before and after treatment with propionic anhydride was rapid, inexpensive and highly reproducible (Addo and Hill, 1982). Goh and Clandinin (1978) found good correlation between dye binding capacity of protein and Kjeldahl's protein values. Goh et al. (1979a) found highly negative correlations between the dye binding capacity of the protein of RSM and the duration of autoclaving. Hurrell and Carpenter (1979) found that the dye binding technique is sensitive enough to detect the range of first 15% damage in feedstuffs. Goh et al. (1979b) modified the procedure developed by Goh and Clandinin (1978) and recommended it's use in conjunction



with Kjeldahl's nitrogen analysis to detect inferior RSM which are severely heat damaged. Goh et al. (1979c) found high correlations between dye binding capacity of RSM and total protein efficiency values of the meals.

Anti-nutritive components

Bell (1984) reviewed nutrients and toxicants of RSM.

Glucosinolates

Glucosinolates are a class of secondary compounds present mostly in cruciferous plants. General formula for glucosinolates was revised by Ettlinger and Lundeen (1956) and then confirmed by them in 1957. Glucosinolates originate from amino acids and are derivatives of thiohydroxamic acids. Excellent review on its biosynthesis and catabolism was done by Underhill (1980). Comprehensive reviews on glucosinolates are available in the literature (Tookey, 1980; Larsen, 1981; Kjaer, 1981) and specific reviews on analysis are also available (Rodman, 1978; Olsen and Sorensen, 1981).

Glucosinolates are stored in the parenchymal cells of the cotyledons of seeds. They are present in all parts of the plants, however seeds have highest concentration. More than 80 glucosinolates have been identified (Rodman, 1978). All the 300 species of 1500 crucifers analyzed contain 1 to 7 glucosinolates. These differ from one another through a structure of the organic radical or aglucon, the frequency of their presence and the properties of their hydrolysis

products (Maheshwari et al., 1981; Olsen and Sorensen, 1981). The majority of cultivated crucifers which contain glucosinolates are the source of food and condiments.

So far 10 of the 80 identified glucosinolates have been found in rapeseeds. Nomenclature and the presence of glucosinolates are given in Table 9.

TABLE 9. NOMENCLATURE AND THE PRESENCE OF GLUCOSINOLATES
BRASSICA SEEDS

Nomenclature of aglucones	Trivial names	Presence
3-propyl-(allyl)	sinigrin	<u>B. jun.</u> , <u>B. nigra</u> , <u>C. abyss.</u> , <u>B. hirta</u>
3-butenyl	gluconapin	<u>B. camp.</u> , <u>B. nap.</u> , <u>C. abyss.</u>
4-pentenyl	glucobrassic- anapin	<u>B. camp.</u> , <u>B. nap.</u>
4-methylsulfinylbutyl	glucoallyssin	<u>B. camp.</u>
6-methylsulfinylpropyl	glucoiberin	<u>B. nap.</u>
5-methylsulfinylpentyl	glucoraphanin	<u>B. camp.</u>
2-phenylethyl	gluconasturtin	<u>B. nap.</u> , <u>C. abyss.</u> <u>B. camp.</u>
(R)-2-hydroxy-3butenyl	progoitrin	<u>B. camp.</u> , <u>B. nap.</u>
(S)-2-hydroxy-3-butenyl	epi-progoitrin	<u>C. abyss.</u>
2-hydroxy-4-pentenyl	napoleiferin	<u>B. camp.</u> , <u>B. nap.</u>
p-hydroxybenzyl	sinalbin	<u>B. hirta</u> , <u>B. nap.</u> <u>Sinapis alba</u>
3-indolyl-methyl	glucobrassicin	<u>B. nap.</u>

Source : Meith, et al. (1983a)

In B. compestris , gluconapin and glucobrassicinapin constitute 35-45% of each and progoitrin constitutes about 20%, where as in B. napus, the major glucosinolate is progoitrin (60-70%) and gluconapin and glucobrassicinapin constitute about 15-20% each. Traces of other glucosinolates may be present. B. juncea, B. nigra and B. hirta as well as Crambe abyssinica contain exclusively allylglucosinolate (sinigrin). The proportion of glucosinolates in species and

varieties of Brassica differs considerably and is conditioned by environmental factors and changes in the course of vegetation periods. Varietal differences in goitrogenic properties of RSM were observed (Clandinin et al., 1959). In general B. napus contain on average, more glucosinolates (4-8%) than B. campestris (3-6%). Summer varieties of B. napus have lesser glucosinolates compared to the varieties grown in winter.

Hemingway and Vaughan (1960) found that mustard seed samples from Nepal contained only allyl isothiocyanate and was identified as B. juncea. Kirk et al. (1964) and Jensen et al. (1953b) confirmed that B. juncea contained only allylglucosinolate (sinigrin). B. juncea contain quantitatively higher amount of single glucosinolate, however B. campestris and B. napus contain several types of glucosinolates (Rahaman and Quddus, 1979). Gland et al. (1981) analyzed 700 genotypes of Brassica and observed that B. napus did not contain sinigrin, where as B. campestris contain sinigrin. Glucosinolate contents of some cultivars of Brassica are presented in Table 10. In Brassica napus the main constituent was found to be 3-butenylisothiocyanate (Jensen et al., 1953a).



TABLE 10. GLUCOSINOLATE CONTENTS OF RAPESEED MEALS^a

Brassica species and cultivars	Total glucosinolates (mg/gm)
<u>B. napus</u>	
Target*	9.50
Tower *	1.29
<u>B. campestris</u>	
dichotoma(pusa kalyan)	9.70
T-g, Toria**	17.80
T-42, Yellow sarson**	6.06
<u>B. juncea</u>	
T-63, Brown mustard**	15.10

Source: Srivastava et al. (1976); a In dry defatted meal;

* Canadian cultivars; ** Indian cultivars.

Enzyme:(Myrosinase)

Myrosinase (Thioglucoside glucohydrolase, E.C.3.2.3.1) is a glycoprotein with two peptide chains with a molecular weight of approximately 130,000 daltons (Tookey, 1973).

Myrosinase in Brassica seeds is stored in a special cell called idioblasts. Bjorkman and Johonson (1972) identified 3 isoenzymes of myrosinase in Sinapis alba. It is now widely believed that myrosinase is a single enzyme system. Myrosinase from Crambe abyssinica and B. napus were stimulated by ascorbic acid (Tookey, 1973; Srivastava and Hill, 1974; West et al., 1977) whereas from Sinapis alba, it was not responsive to ascorbic acid (Schwimmer, 1961; Srivastava and Hill , 1974). The enzyme can be extracted along with protein fractions with sodium chloride followed by (NH₄)₂SO₄ fractionation (Rao et al., 1978).



Aglucons:

Aglucones are formed due to enzymatic hydrolysis (Olsen and Sorensen, 1980; Leeson, 1984), when myrosinase from idioblasts and glucosinolates from parenchymal cells come in contact at proper moisture and temperature. Bell et al. (1971) reported that oriental mustard (B. juncea) contains only allyl isothiocyanate and yellow sarson (B. campestris) contains butenyl isothiocyanate. Autolysis of most rapeseeds produce nitriles and small amounts of goitrin. However, upon incubation of heat treated seed meals with myrosinase produced only small amounts of nitriles (Paik, et al., 1980).

The detailed description of enzymatic and non-enzymatic hydrolysis is given in Figure 1 (page 27). In the gastrointestinal tract of laying hens, different products of hydrolysis of goitrin results under different conditions (Smith and Campbell, 1976). Astwood et al. (1949) determined substances in RSM having equal potency of thiouracil for antithyroid activity in man as L-5-vinyl-2-thiooxazolidone.

Major hydrolysis products of B. juncea and B. nigra plants were allyl and 2-phenyl isothiocyanates (Cole, 1976). It was also observed that autolysis of glucosinolates in several crucifers produced 1-cyano-2,3-epithiopropene and 1-cyano-3,4-epithiopentenes, in addition to the 1-cyano-3,4-epithiobutane (Cole, 1975). In B. nigra and B. juncea traces of 1-cyano-epithioalkanes were also found.

Kameoka and Hashimoto (1980) analyzed steam volatile oils from B. juncea, B. hirta and B. compestris and identified total of fifty five compounds. However the major compounds were allylisothiocyanate, dimethyl trisulfide, 3-butenyl isothiocyanate, 3-phenyl propionitrile and benzyl isothiocyanate. Matsumoto et al. (1975) found that B. napus type rapeseed meal contains more oxazolidinethione (OZT) and isothiocyanate (ITC) compared to B. compestris type RSM. Daxenbichler et al. (1979) identified organic nitrile, 1-cyano-2-hydroxy-3-butane in cabbage by autolysis. Mixed samples of B. compestris and B. juncea oil seed cakes contained 1.11 to 2.61 mg of OZT/g of meal and 3.18 to 4.85 mg of ITC/g of meal (Vaidya et al., 1975a). Matsumoto et al. (1975) suggested that antithyroid activity of OZT was much stronger than ITC.

Glucosinolate determination:

A good review on advances in glucosinolate analysis is available in the literature (Olsen and Sorensen, 1981). Most of the earlier methods used in quantifying glucosinolates in RSM and MOM have been based on the measurement of aglucons produced by enzymatic or non-enzymatic hydrolysis of the glucosinolates.

McGhee et al. (1965) used a sulfur ion balance method based on the principle described by Shaw (1959) to quantify total glucosinolates in mustard seeds. This procedure is simple and a way to measure total thioglucoside with good



accuracy.

Spectrophotometric methods have been used to quantify thioglucosides by measuring oxazolidinethione (Wetter, 1957; Wetter and Youngs, 1976) and allylisoithiocyanate (Devani et al., 1976; Devani et al., 1978; Mukhopadhyay and Bhattacharya, 1983). Titrimetric method has been used by Croft (1971) to measure total thioglucoside in RSM. Argentimetric methods have been used in measuring allylisoithiocyanate to estimate the total thioglucosides (Wetter, 1955; Vangheesdale and Bichot, 1970).

Enzymatically released glucose as a measure of glucosinolates in many crucifers have been used (VanEtten et al., 1974; VanEtten et al., 1976; VanEtten and Daxenbichler, 1977; Daxenbichler et al., 1979; Carlson et al. 1981).

More recently chromatographic techniques have been used to quantitatively identify glucosinolates (Youngs and Wetter, 1967; Daxenbichler et al., 1970; Underhill and Kirkland, 1971; VanEtten et al., 1976; Thies, 1976).

Newer methods using HPLC and GLC in combination, and GLC-MS are available to measure intact glucosinolates (Olsen and Sorensen, 1981). While these methods are efficient for isolation, separation and quantification of glucosinolates they require more sophisticated equipment. Choice of the method depends upon the purpose of analysis and degrees of accuracy desired, in addition to the accessibility.



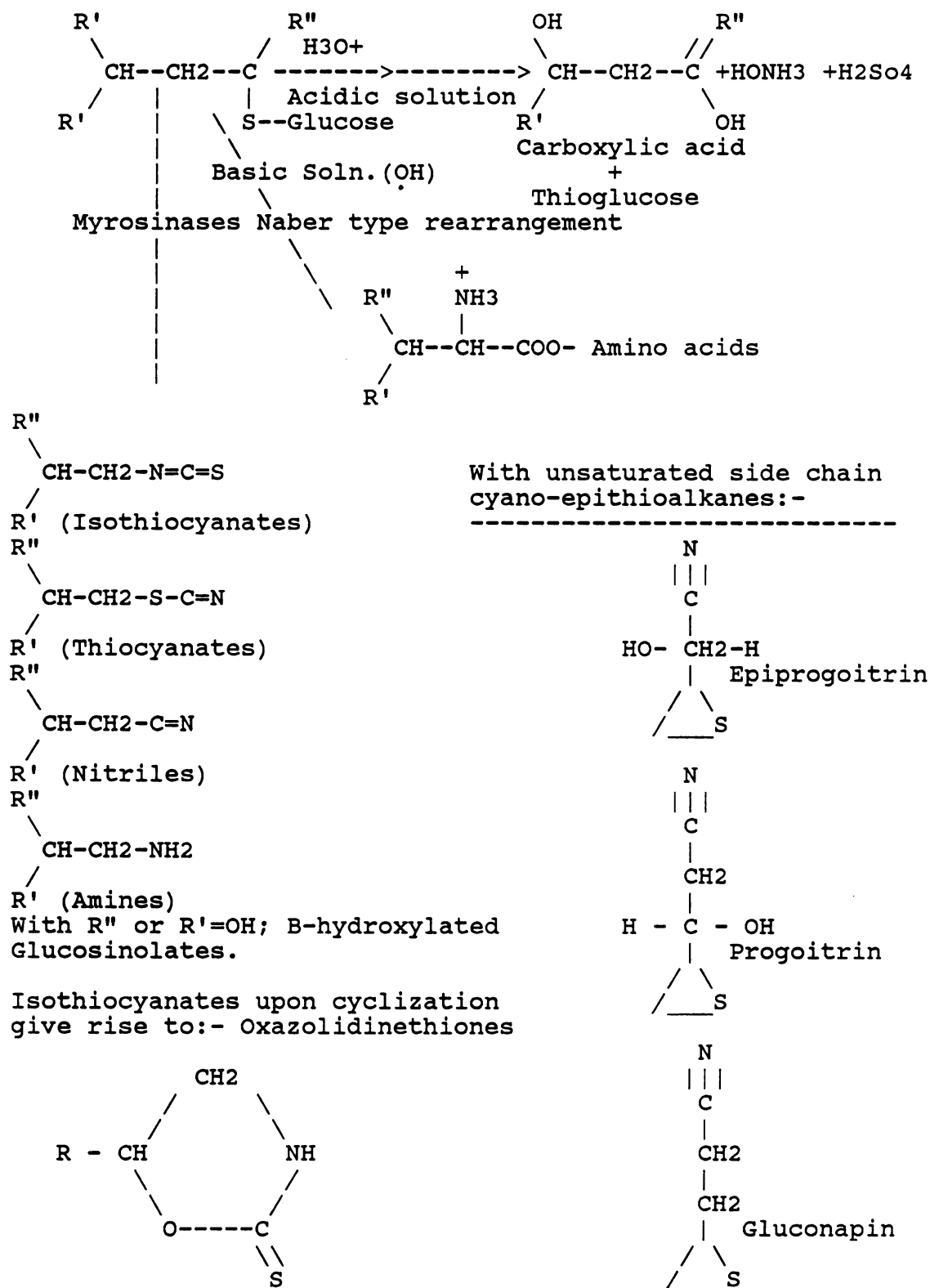


Figure 2. Enzymatic and non-enzymatic degradation of Glucosinolates; Adapted from Olsen and Sorensen(1980)

Nutritional Implications:

The goitrogenic effect of the OZT is due to the fact that it inhibits the synthesis of thyroid hormones which can not be overcome by iodine supplementation (Gmelin and Virtanen, 1960). Glucosinolates per se are not toxic but the hydrolysis products due to the action of enzyme myrosinase yield toxic allyl isothiocyanates, goitrins and organic nitriles (Leeson, 1984).

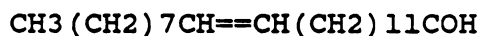
In general, effects of glucosinolates on broiler chicks are reduced growth rate, reduced feed intake, and enlarged thyroids and reduced egg production, liver haemorrhage, enlarged thyroids, reduced egg iodine content and egg tainting in layers (Leeson, 1984). Toxicity is closely related to the residual amount of glucosinolates (Elnokrashy et al., 1975). Myrosinase inactivated dehusked RSM was found to contain low fiber and high quality of protein (Agren and Ekmund, 1977). Bille et al. (1983) suggested that myrosinase aggravates the toxic and antinutritional effects of some glucosinolates. Akiba and Matsumoto (1979) observed that there is very little contribution of microorganisms in the enlargement of the thyroid in chicks.

Toxicity in RSM may be attributed to the production of nitriles during hydrolysis of the raw meals (Lo and Hill, 1972). Josefsson and Munck (1968) found that growth was depressed in mice only when levels of potential isothiocyanates and oxazolidinethiones exceeded 1 mg/g in the

diets. Brak and Henkel (1978) found good correlation between content of nitriles in RSM and repression of feed intake. Weight gain of rats and chicks were inversely proportional to the levels of nitrile rich RSM (Shrivastava, et al., 1975).

Allyl isothiocyanate contents of the meals from B. juncea, B. compestris and B. alba were found to be 0.45%, 0.26% and 0.22% respectively (Pathak, 1973). Lodhi et al. (1980) suggested the presence of goitrogenic compounds that are not reversed by iodine supplementation. Lodhi et al. (1970b) found that up to 0.054 to 0.069% OZT in the diet had no effect on ME for 4 or 6 week old chicks, however at 0.09% level, ME value slightly decreased.

Erucic acid



Erucic acid is an isomer of docosanoic acid and is a characteristic acid of mustard and rapeseed oils. Fogerty et al. (1978) confirmed that rapeseed oil contains only the cis form of 13-docosanoic acid (erucic acid). Oleic acid is a precursor of eicosenoic and erucic acids, in their biosynthesis (Downey and Craig, 1964; Rahaman and Quddus, 1979).

Conventional varieties of rapeseed and mustard seed yield oils containing 40-50% erucic acid (C22:1) and 8-10% eicosenoic acid (C20:1) (Dutta and Ghosh, 1970; Spencer, 1975; Srivastava et al., 1976b; Prasad and Rao, 1977). The amount of residual oil left in the meals depends upon the

method of oil extraction used. A higher percentage of oil in the meal may mean higher percentage of erucic acid in the meal, however varietal differences are to be taken into account. Usually 10-15% residual oil is left in the meals by expeller process (Panda and Shrivastava, 1978), which amounts to 4 to 6% of erucic acid. Ruckemann (1978) developed a rapid method of determining erucic acid in oils similar to the method prescribed in AOAC (1985).

Nutritional implications:

Nutritional properties of rapeseed oil have been extensively reviewed by Slinger (1977) and effect of dietary erucic acid by Prasad and Rao (1977). Feeding of rapeseed oil with high erucic acid as a major source of dietary energy resulted in reduced growth and undesirable histopathological changes in the cardiac and skeletal muscles in rats (Abdellatif and Vles, 1970; Bhatnagar and Yamashiro, 1979) and ducklings (Abdellatif and Vles, 1970; Abdellatif et al., 1972). The main deleterious factor in rapeseed oil is erucic acid (Chand and Sadgopan, 1980). Erucic acid in mustard and rapeseed oils may inhibit mitochondrial oxidation of other fatty acids causing accumulation of triglycerides and cholesterol esters. Lipidosis is the acute effect of mustard oil feeding in rats(Ray et al., 1979). Incorporation of erucic acid in the test diets at the levels provided by 20 and 30% expeller processed MOM adversely affected the performance of chicks in as much the

same way and almost to a similar extent as the MOM diets (Prasad et al., 1978a). Similar results were obtained by Prasad and Rao (1977). It was suggested that the primary factor limiting the utilization of MOC in poultry is the presence of erucic acid (Prasad, et al., 1978a). Prasad and Rao (1982) suggested that the erucic acid may be an important deleterious agent adversely affecting chick performance. They observed improvement in chick performance equivalent to GNC fed chicks upon hexane extraction and reconstitution of mustard oil cake. Lall and Slinger (1973) found that when *B. napus* or *B. compestris* meals were fed to the layers, erucic acid content in the egg yolk was 0.6%, whereas low erucic acid rapeseed oil produced eggs containing only 0.2% erucic acid in the egg yolk.

Mustard seed of Czech origin was found to contain significantly lower amounts of erucic acid compared to its fatty acids of glycerides (Pokorny and Zeman, 1971). Appreciable variation in erucic acid content among the varieties of mustard seeds from India was reported by Kharchenko (1969).

Phenolic compounds

The presence of 'phenolic fractions' is a characteristic feature of all plant tissues. They all possess an aromatic ring bearing a hydroxyl substituent. The major phenolic constituents of Brassica and Sinapis oilseeds is sinapine, a choline ester of sinapic acid (Kozłowska et

al., 1983). Smaller amounts of p-coumaric acid, ferrulic acid, p-hydroxybenzoic acid and caffeic acid are also present in rapeseed (Durkee and Thievierge, 1975). Free phenolic acid was not found in mustard and rapeseed meals. Chlorogenic acid in *B. compestris* has also been identified. Structures of some of the phenolic acids are given in Figure 2. Ismail and Eskin (1979) measured sinapine in rapeseed protein concentrates using colorimeter requiring titanium tetrachloride.

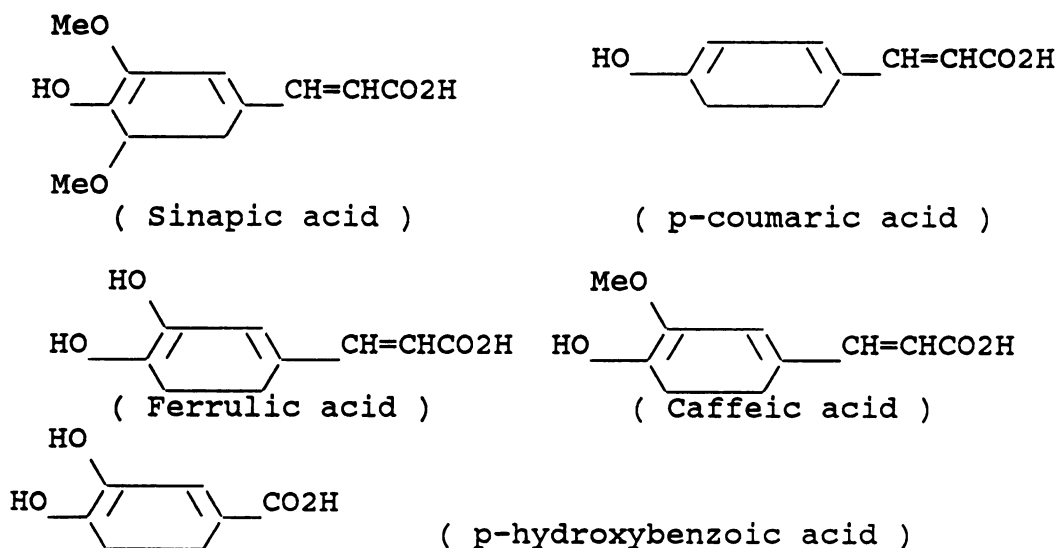


Figure 3. Some of the phenolic acids present in RS and MS

Tannin is an important polyphenol present in rapeseed and mustard. There are two types of tannin viz. condensed tannins (derivatives of flavanols; (C₆-C₃-C₆)_n) and hydrolyzable tannins (esters of a sugar, usually a glucose with one or more trihydroxybenzene carboxy acid) (Gross, 1981). Hydrolyzable tannins are mixtures of gallic acid, m-gallic acid, trigallic acid and galloylated glucose. The chemistry

of tannin is most complex and non uniform (Harborne, 1980). Vaidya et al. (1975a) found that MOM contained 2.05 to 3.74% of tannic acids.

Nutritional implications

Phenolic acids in the seeds cause deterioration in taste, odor and color of protein concentrates. Oxidized phenolic compounds bind with essential amino acids such as lysine and methionine forming complexes which are unassimilable in the gastrointestinal tract of animals and men.

Sinapine level more than 1% in the diet was found to adversely affect palatability (Josefsson and Uppstrom, 1976). Sinapic acid contributes to the bitter taste of rapeseed flour (Rutkowski and Kozlowska, 1979). Tainted eggs (fishy odor in eggs) laid by some strains of layers laying brown shelled eggs have been linked to feeding of RSM in the diets (Hobson-Frohock et al., 1977; Pearson et al., 1979). Fishy odor is produced by trimethylamine (TMA). Feeding of RSM in the diets of tainters resulted in tainted eggs and deletion of the meal from the diets halted the production of tainted eggs (Overfield and Elson, 1975). Hobson-Frohock et al. (1975) devised a method to select the tainters from nontainters to study the effect of RSM feeding on egg taints. Goh et al. (1983) found that when constant amount of sinapine is fed to the layers, OZT not the total glucosinolate tend to increase %age of fishy eggs.

Hepatic TMA oxidase is the enzyme that oxidizes TMA in its excretory form. Hepatic TMA oxidase activity was found to be lowered by feeding RSM to the hens (Pearson et al., 1979a). It was demonstrated that OZT in RSM is an important factor in producing tainted eggs in sensitive hens, along with thyroid enlargement (Pearson et al., 1979b). Pearson et al. (1980a) demonstrated that even the feeding of low glucosinolate RSM (cv. tower) could depress TMA oxidation in sensitive hens and result in tainted eggs. Pearson et al. (1980b) ruled out that sinapine is involved in the depression of TMA oxidation, when RSM is fed as a sole source of TMA in the diet. It was observed that unabsorbed choline in the intestine is associated with TMA production by intestinal bacteria (March and McMillan, 1980). Lee et al. (1982) could not confirm that the tainters are less efficient at excreting TMA oxide. It was concluded that progoitrin is the major constituent of B. compestris meals for the potentials of tainting (Pearson , 1983). Addition of antimicrobial drugs to laying rations containing RSM is unlikely to reduce TMA content of egg produced by the tainters (Goh, et al., 1982a).

Growth was inhibited more by hydrolyzable tannins than by condensed tannins (Joslyn and Glick, 1969). Gallic acid and catechin depressed growth, however ellagic acid was without any effect. None of these compounds effected fecal nitrogen excretion (Glick and Joslyn, 1970a). Toxicity of tannin decreased with increase in age and weight of rats



(Glick and Joslyn, 1970b). When 5% tannic acid was in the diet, supplementation with choline or methionine did not improve growth whereas supplementation of casein improved growth indicating that effect of tannins may be reduced at higher protein levels. Prasad and Rao (1980) did not find any adverse effect of tannin components, on chick growth and feed efficiency singly or in combinations, at the levels present in 20 and 30% of MOM in the diets. As low as 0.5% dietary tannic acid caused a depression in growth of chickens and 70% mortality occurred in 7 to 11 days at the 5% dietary level (Vohra et al., 1966). They also found that ME was also reduced along with reduced nitrogen retention. Cholesterol level was also increased.

Egg production and health of hens were not adversely affected by sinapine content in the diet (Overfield and Elson, 1975) nor was growth depressed in the hens fed RSM (Clandinin, 1961a). Prasad and Rao (1982a) found that tannic component had little or no effect on the utilization of MOM in chicks.

Other toxic factors

An anti thiamine factor was identified as 3,5-dimethoxy-4-hydroxy cinnamic acid (methyl sinapate) by Bhattacharya and Chaudhary (1974). Zemen et al. (1964) observed the evidence of the presence of an unknown component presumably an unsaturated hydroxy acid in RSM.

Detoxification of MOM and RSM:

Extensive review on physiochemical, microbiological and enzymatical treatments of brassica seeds on antinutritional components is available in the literature (Meith et al., 1983b). The techniques thus far developed can be broadly classified under the following categories (Maheshwari et al., 1981):

1. Inactivation of the enzyme myrosinase

The intact glucosinolates are not removed during oil extraction but toxicity prevails only if they are hydrolyzed in the digestive tract. Myrosinase inactivation starts at temperatures above 70 °C if the moisture content is 6 to 10%.

2. Hydrolysis and removal of hydrolytic products

Volatile isothiocyanates can be steam stripped however, oxazolidinethione and other non volatile products of autolysis (enzyme hydrolysis) remain in the meal. Autolysis starts at 45 to 50 °C in the presence of high moisture content.

3. Removal of glucosinolates and their hydrolytic products:

Glucosinolates are highly soluble in water. Diffusion extraction from whole seeds prior to hydrolysis by myrosinase is effective in detoxification of RSM (Rutkowski and Kozłowska, 1979). Dry heat treatment at 80 to 90 °C for 15 to 30 minutes inactivates myrosinase and leaves intact glucosinolates in the meal. After inactivation of

myrosinase, treatment with 0.01 N NaOH at 60 C removes most of the glucosinolates (Soluski, 1972). Five to six 1-hr diffusion extraction of intact seeds of rapeseed also removes most of the glucosinolates. Inactivation of myrosinase by boiling water was effective. However, there is nutrient loss whenever there is aqueous leaching.

4. Plant breeding techniques to breed out glucosinolates:

In Canada and European countries newer varieties of rapeseed have been developed with low levels of glucosinolates and low levels of erucic acid. They are called double low cultivars. Varieties of rapeseed that are low in erucic acid (<1%) are available however varieties having zero glucosinolates are not yet available.

Effect of processing:

Processing techniques influence the bioavailability of protein, some positively and some negatively (Kies, 1981). Solvent processed meals supported 15% higher weight gain and 10% more efficient feed to gain ratio in rats (Goering et al., 1960). Prepress solvent processed RSM is nutritionally superior to expeller processed meal which may be due to the fact that lysine damage is spared in solvent processing (Clandinin, 1967).

The nutritive properties of rapeseed meals are considerably improved if the glucosinolates are removed by autolysis followed by solvent extraction (Mukherjee et al., 1976). When 70% acetone extraction and autolysis were

compared, both gave satisfactory results, however, acetone washing was more effective (Mukherjee, et al., 1979).

Excessive heat treatment during the oil extraction adversely affected nutritional value of resulting meals (Clandinin et al., 1959). Yule and McBride (1978) observed that solvent processed meal was nutritionally superior to expeller processed RSM. Cooking temperature of rapeseed should be such that at least 6% residual oil is left in the resultant meals to get good quality meals (Clandinin and Tajcner, 1961b). Removal of glucosinolates prior to oil extraction gives good quality meals (Mustakas et al., 1962). There was a 30.69% reduction in lysine and 22.92% reduction in arginine by open pan roasting of mustard seeds prior to oil extraction (Capper et al., 1982). Effect of open pan roasting on essential amino acid composition of mustard seeds prior to oil extraction is given in table 11. Eapan et al. (1968) found that wet heating of RS prior to oil extraction produced better quality of both oil and meal. Dry heating was unsatisfactory in inactivating myrosinase and oil color. Lower temperature cooking (98.8 C for 30 min) gave higher lysine content and higher fat content in the resultant meals (Clandinin and Tajcner, 1961b).

Two hour heat treatment of RS at 90 C caused no change in glucosinolate whereas a 12.38% reduction occurred when heated at 150 C for 2 h (Akiba and Matsumoto, 1979b). No adverse effect of treating mustard seeds at 125 C for 30

min prior to oil extraction was observed, on metabolizable energy value for the growing chicks (Vaidya et al., 1975b).

TABLE 11. AMINO ACID COMPOSITION OF ROASTED AND UNROASTED MUSTARD SEED CAKE*

Amino acids	Unroasted	Roasted
Arginine	2.40	1.85
Lysine	2.15	1.49
Histidine	1.01	0.85
Isoleucine	1.74	1.49
Leucine	2.60	2.31
Phenylalanine	1.51	1.35
Methionine + Cystine	1.01	0.82
Threonine	1.86	1.49
Tryptophan	0.46	0.50
Valine	1.98	1.81

Source: Capper, et al. (1982); * DMB

Heat treatments:

Jansen et al. (1975) suggested that it is the effectiveness of heat penetration that is important in the inactivation of antigrowth factors in soybeans rather than the moisture levels.

Higher temperature of oil extraction is responsible for the impaired digestibility in pigs fed expeller processed RSM (Salo, 1982). Mild heat treatment are more effective in detoxification than severe treatment (Garcha et al., 1976). Heat treatment of the seeds prior to the oil extraction did not show any variation in the tannin content and OZT, however ITC content was lowered (Vaidya et al., 1979).



Wet heat treatment:

Many researchers have shown that various types of heat treatments improve the nutritive quality of plant proteins (Olsen et al., 1975; Bayley and Summers, 1975; Srihara and Alexander, 1983; Srihara and Alexander, 1984). Thermal processing affects protein quality by rendering the basic amino acids especially lysine unavailable (Wolzak et al., 1981). Increasing moisture content of RSM from 12 to 40% before autoclaving improved chick growth (Hijikuro and Takemasa, 1979a). Goitrin content was almost eliminated and the destruction of lysine and arginine were also low, when RSM was autoclaved at 120 C for 60 min at 30% moisture level (Hijikuro and Takemasa, 1979b). Myrosinase activity of rapeseed was completely destroyed by the heat treatment at 90 C for 15 minutes at 8% moisture level (Appelqvist and Josefsson, 1967).

Progressive decline in available lysine value in RSM was reported upon autoclaving at 120 C from 15 to 120 minutes (Rayner and Fox, 1976). Steam heating of RSM reduced nutritive value, However, there was lesser destruction of nutritive value when compared to ammoniation (Lipinska et al., 1978).

Steam heating of RSM (cultivar start) supported growth similar to SBM diets (Bock et al., 1981). Chickens fed CM desolventized at 100 C (after oil extraction) supported faster growth rate compared to chickens fed CM desolventized at 25 (Shires et al., 1982). Steam volatilization of

ITC after enzyme hydrolysis gave satisfactory RSM (Goering et al., 1960).

Steaming at 100 C for 30 minutes ensured no loss in available lysine and good quality RSM (Rauchberger et al., 1979). Wet heating of the rapessedes prior to oil extraction produce better quality of the meals and oils, whereas dry heat treatment was unsatisfactory in inactivating myrosinase (Eapan et al., 1968). Autolysis of RSM at 37 C for 1-hr followed by steaming at atmospheric pressure for 1-hr improved the feeding value (Srivastava and Hill, 1976).

Autoclaving of RSM at 15 psi improved chick growth independent of lysine destruction and total lysine was reduced by 24-36%, whereas dry heating at 115-120 C showed only little damage in lysine (Gray, et al., 1957).

Dry heat treatment:

Heat treatment of RSM at 150 C for twohours reduced physiologically active goitrin from 20.32 mg/100 gm of meal (in unheated RSM) to 5 mg/100 gm of meal (in heated meal). At 90 C no glucosinolate reduction occurred (Akiba and Matsumoto, 1979a). Results of thermal detoxification are given in Table 12. Severe over heating of protein supplemental feeds result in seriously depressed availability of all amino acids , however lysine is more sensitive to heat (Meade , 1972).

Prolonged heating of MOM at higher temperatures and lower moisture contents were detrimental to the nutritive



value (Mustakas et al., 1965). Optimum nutritive value of low glucosinolate RSM was obtained by heat treatment of the seeds with moisture content of 8% at 100 to 110 C for 15 to 60 minutes (Josefsson, 1975). Heat treatment of RSM at 100 C was more effective than at 90 C for chick growth, however, at 10 to 15% moisture level most of the lysine was destroyed and at higher moisture (30 - 40%) methionine was also destroyed (Josefsson and Munck, 1972).

Hot water treatment:

Hot water treatment of RSM removed a goitrogenic factor from the meals (Nakaya and Tagannis, 1965). Feeding hot water treated RSM up to the 30% level had no adverse effect on chick growth and no adverse effect on iodine content in the thyroids (Nakaya, 1965).

Microwave treatment:

Microwave treatment of dehulled RSM for 1.5 minutes with 10 or 13% added moisture in the meals reduced some glucosinolates, however, goitrogenicity of the meal was not different from untreated RSM (Maheshawri et al., 1980). Optimum moisture contents for the microwave treatment for the inactivation of myrosinase was found to be 14 to 16% (Medeiros et al., 1978). Marked improvement in the nutritive value of whole rapeseed by microwave treatment occurred at 329-399 C for 5-15 seconds (Woodly et al., 1972).

Water treatment:

Two extractions of RSM in water did not improve PER in rats nor was there any change in liver histology, however thyroid histology was changed (Ballester et al., 1977). Double water extraction with water was found to be more effective in reducing OZT and ITC (Ballester et al., 1970b). Diffusion extraction of glucosinolates was found to be effective in reducing glucosinolates (Bock et al., 1979). After aqueous ethanol extraction of rapeseed, hot water washing of RSM three times reduced the glucosinolate content of the meal by 95% of raw untreated meal (Ekmund et al., 1971a). Protein efficiency ratio of such meal was found to be 2.9 compared to 2.1 for the meal washed only once (Ekmund et al., 1971b). Jones described a water extraction procedure that removes 90% glucosinolates and the resultant meal was also rich in amino acids. Mustakas et al. (1976) described a procedure to remove glucosinolate by water washing. Kozłowska et al. (1972a) found that aqueous extraction of glucosinolates from RSM resulted in loss of soluble nitrogen and other nutrients. At 20% MOM dietary level, chick growth improved upon MOM treatment with hot water, whereas cold water treatment of MOM depressed growth. At 30% level, however, both the treatments depressed growth significantly (Prasad and Rao, 1978).

TABLE 12. DETOXIFICATION OF MOM BY THERMAL TREATMENT

Treatments	Conditions of Treatment			Reduction in		
	Temp. [C]	Moisture [%]	Time [min]	Myr. %	ITC %	OZT %
1.Heating of	80	3.6	15	100		
defatted	90	3.6	15	92		
meal in a	80	6.4	15	34		
closed system	90	6.4	15	6		
in presence of	80	8.4	15	31		
water	90	8.4	5	4		
	80	7.4	15	40		
2.Heating of deftd.						
meal in open syst.	105	7.9	60	83		
in presence of	130	7.9	60	36		
water						
3.Thermal treatmt.	90		5	10	0.8	1.2
of deftd. meal	90		120	4	0.8	1.0
in presence of	95		5	10	0.9	1.1
8% moisture	95		120	0	0.7	0.9
	100		5	4	0.9	1.1
	100		120	0	0.6	0.8
	105		5	7	0.9	1.1
	105		120	1	0.5	0.8
4.Toasting or						
steaming of	105		30		0.58	0.39
deftd meal	135		30		0.34	0.13
	105		90		0.56	0.07
	135		90		0.27	tr.
	105*		30		0.37	0.33
	135*		90		0.13	0.23
5.Autoclave trt.						
of deftd. meal	121		20		6.2mg/	
(toria 29mg					100 g	
per 100 g)	121		30		2.3mg/	
					100 g	
6. Hot water trt.						
(sarson 20.4 mg	100		20		3.1 mg/	
per 100 g)					100 g	
	100		30		4.4 mg/	
					100 g	
7.Roasting of deftd.	105				0.27	0.51
meal (OZT=0.88;	115				0.24	0.46
ITC=0.61)	125				0.16	0.38
	135				0.04	0.31

Sources: 1 & 2, Applequist and Josefsson (1967); 3, Josefsson (1975); 4, Meith (1983); 5 & 6, Garch et al (1976) 7, Meith et al (1983). * Steaming



Chemical treatments:

MOM treated with ferrous sulfate reduced oxazolidinethione content by 88% and isothiocyanate by 74% (Daghir and Mian, 1976) and the ME value was also improved (Daghir and Charlabous, 1978). Bell et al. (1971) found that ferrous sulfate treated mustard had no allylisothiocyanate. Mild alkali or acid treatment of oilseed meals were more effective in detoxification than severe treatments (Garcha et al., 1976). Sodium hydroxide treatment eliminates myrosinase activities (Bhatti and Soluski, 1972). Autolysis or washing with 70% acetone were satisfactory in removing glucosinolates (Mukherjee et al. 1979). RSM treated with 2% soda ash supported significantly higher weight gain in pigs compared to pigs fed untreated RSM at 5%level, of inclusion in the diets (Newman, et al., 1973). Beneficial effect of treating mustard meal with 3.8% sodium carbonate in swine and mice diets were reported (Sarwar and Bell, 1980). They attributed this improvement to the removal of sinapine. Ammoniating RSM in the presence of steam in the solventization process decreased sinapine and oxazolidinethione contents in the resulting meals (Goh et al., 1982b). Ammoniation of mustard meal improved chick performance up to the 15% dietary level. However, at the 20% level there was a need to maintain lysine levels to improve performance (Blair, 1984). Ethanol treatment of RSM at 60 C completely destroyed myrosinase and more glucosinolates were removed (Josefsson and Munck, 1972). Kirk et al. (1971) observed

that salts of iron and copper and to some extent zinc, promoted destruction of epi-progoitrin in crambe seed meal. Ammoniation is believed to reduce glucosinolates in RSM (Keith and Bell, 1982).

Feeding values of MOM and RSM

In ruminants:

Increase in thiocyanate ions in milk from cows fed RSM was reported (Rutkowski, 1971; Iwarsson, 1973; Papas et al., 1979a). Thyroid weight was increased and 24 hr thyroid radio-iodine uptake as well as lowered serum protein bound iodine (PBI) occurred in rats fed milk from cows fed RSM in the diet (Iwarsson and Nilsson, 1973). Iodine concentration in milk of cows fed RSM was also observed (Iwarsson, 1973). Depression in appetite and off flavour in milk from cows fed RSM at the rate of 1.2 kg/day was reported (Eggum, 1981). RSM at 14% level in grain mixture fed ad libitum to lactating cows reduced feed intake without depressing milk production, however, at the 19% level in the grain mixture RSM significantly depressed milk production (Ingalls and Sharma, 1975). A case of toxicity in bull (Allikutty, 1976) and in a she buffalo (Shreemannarayana, 1976) have been reported due to over consumption of mustard seeds. Iwarsson et al. (1973) found enlarged thyroids in bulls fed 15% RSM which was significantly different from the control group.

Lindell (1981) observed no adverse effect of 10% RSM in lactating cows. Moss (1972) suggested that SBM can be replaced by RSM in the grain diets without any serious problems for dairy cows. Varietal differences in RSM were observed in calf rations (Schingoethe, et al., 1974). Feeding of 3% rapeseed gum had no adverse effect on apparent digestibility of nutrients, rate of gain, feed intake or feed efficiency of steers (Mathison, 1978). Wintering beef cattle can tolerate replacement of SBM by mustard meal in their diets, in lamb however, MOM feeding decreased wool growth (Peterson and Thomas, 1970).

Addition of 20 to 30 % RSM in potato silage resulted in high quality silage as bacteria and yeasts were inhibited (Czarnocka-Roczniakowa et al., 1972; Kozłowska et al., 1972a). It was also suggested that glucosinolates in RSM were substantially reduced by lactic acid in silage (Kozłowska et al., 1972b). Formaldehyde treatment of RSM was found to be beneficial on growth when fed to cross bred calves (Bedi and Jain, 1978). Palatability of RSM as a protein source in grain mixtures, depends on the levels of inclusion of RSM, the type and levels of glucosinolates, the process to which RSM has been subjected and also the age of the animals (Thomke, 1981). Ten percent high-glucosinolate RSM and up to 25% low-glucosinolate RSM could be incorporated in dairy rations without any adverse effect (Thomke, 1981).

In nonruminants:

Swine: Feed intake was inversely proportional to glucosinolate content of the diet in swine feeding (Lee and Hill, 1983). Regent rapeseed (full fat) were found to be a little better than candle seed for swine (Salo, 1980a). ME for regent was 4841 kcal/kg of DM and for candle it was 4473 kcal/kg. Replacement of SBM by erglu cultivar of RSM in pig rations did not affect growth (Peterson and Sculz, 1976). Pearson and Bowland (1976) concluded that double low cultivars of rapeseed yield RSM that can substantially replace SBM in the diets. Singam and Lowerence (1979) observed that tower and erglu RSM were less acceptable than SBM, when offered abruptly to the young growing pigs. Candle RSM was 30% better in nutritive value for pigs compared to span-torch variety of rapeseed (Salo, 1980b). Palatability in young pigs was found to be a problem when 20% RSM is incorporated in swine growing rations (Salo, 1982). Rundgren (1983) found that 10% low glucosinolate RSM can be incorporated in sow rations, 50% in growing finishing ration and only 5% in growing finishing rations. It was also observed that palatability is the main limiting factor in starter rations. Eggum (1981) observed that pigs were less affected by the glucosinolates in double low cultivars than poultry or ruminants.

Depressed serum thyroxin level and possibly thyroid dysfunction in pigs fed high and low glucosinolate RSM,

were observed however, it was much severe in the case of the group fed high - glucosinolate RSM (McKinnon and Bowland, 1979). No adverse effect of incorporation of 10% rapeseed gum in a 15% gum free RSM was incorporated in swine diets (McCuaig and Bell, 1981). Upto 15% RSM (cultivar candle) can be incorporated in pig rations without any adverse effect (Bell, et al., 1981). Bayley et al.(1969) suggested that prepressed solvent processed RSM may be fed at 11% level in a corn-soy diets supplemented with methionine and lysine in finishing swine without any adverse effect.

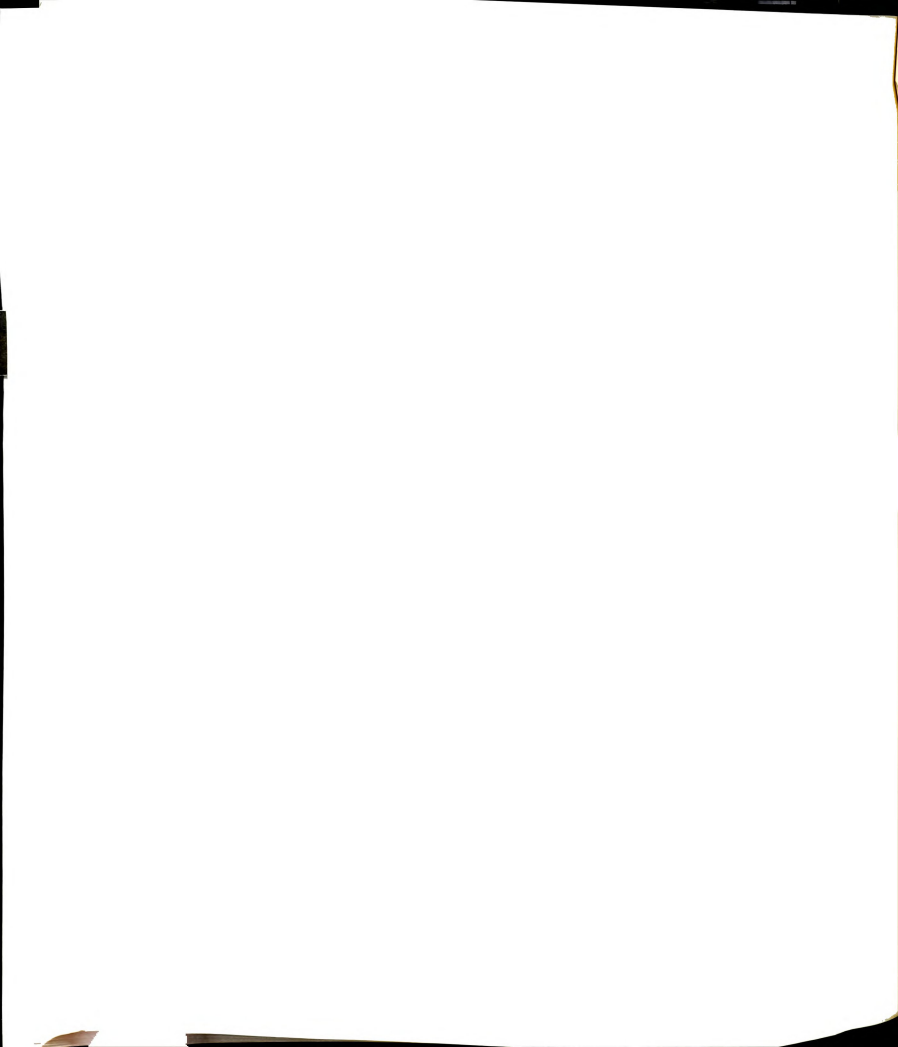
Ammoniation is generally believed to reduce glucosinolates, however no advantage was observed in feeding ammoniated RSM to pigs (Keith and Bell, 1982). Ammoniated canola was inferior to steam treated RSM (Keith and Bell, 1983).

Poultry:

Growing chicks:

There is lack of unanimity in the replacement value of RSM or MOM for SBM or GNC in poultry rations. Five or ten percent of MOM (sarson) heated or unheated, did not adversely affect chick growth, however at 15% level both the treatments lowered the gain significantly compared to GNC-reference diet (Vaidya and Panda, 1974).

MOM at 20% or more in chick starter diets adversely affected the performance of chicks and it was suggested that erucic acid may be the primary factor limiting the



utilization of MOM in poultry diets (Prasad et al., 1978a). Incorporation of MOM or RSM at the 10% level had no adverse effect on chick growth (Srivastava and Prasad, 1976; Marangos et al., 1974; Leeson et al., 1978; Matsumoto et al., 1979).

Replacement of GNC by MOM up to 80% level had no adverse effect on growth and feed efficiency of growing chicks (Benerjee et al., 1975; Benerjee et al., 1976). No adverse effect of replacing GNC by MOM in chick starter diets was reported (Prasad et al., 1974; Ballester et al., 1970; Mandal et al., 1981). Good quality of RSM protein was suggested by Ballester et al. (1970a). Replacement of upto 50% of SBM by RSM had no adverse effect on chick growth (Hijikuro and Takemasa, 1979c). Significant decrease in slaughter weight of broiler chicks fed 10% RSM was observed (Kozlowski et al., 1981). Up to 15% of high-glucosinolate RSM and up to 20% of low-glucosinolate RSM have been recommended (Clandinin and Robblee, 1981). Incorporation of 1.5% gum in the diet did not affect chick growth (Summers and Leeson, 1977; March and Soong, 1978). Replacement of 35% SBM by RSM in chick diets depressed growth by 25% (Kratzer, et al., 1954). Yule and McBride (1978) observed that even 10% (expeller processed) or 15% (solvent processed) RSM depressed growth and decreased feed efficieny in growing chicks. Replacement of GNC by expeller processed MOC at 50 or 100% in chick rations lowered feed

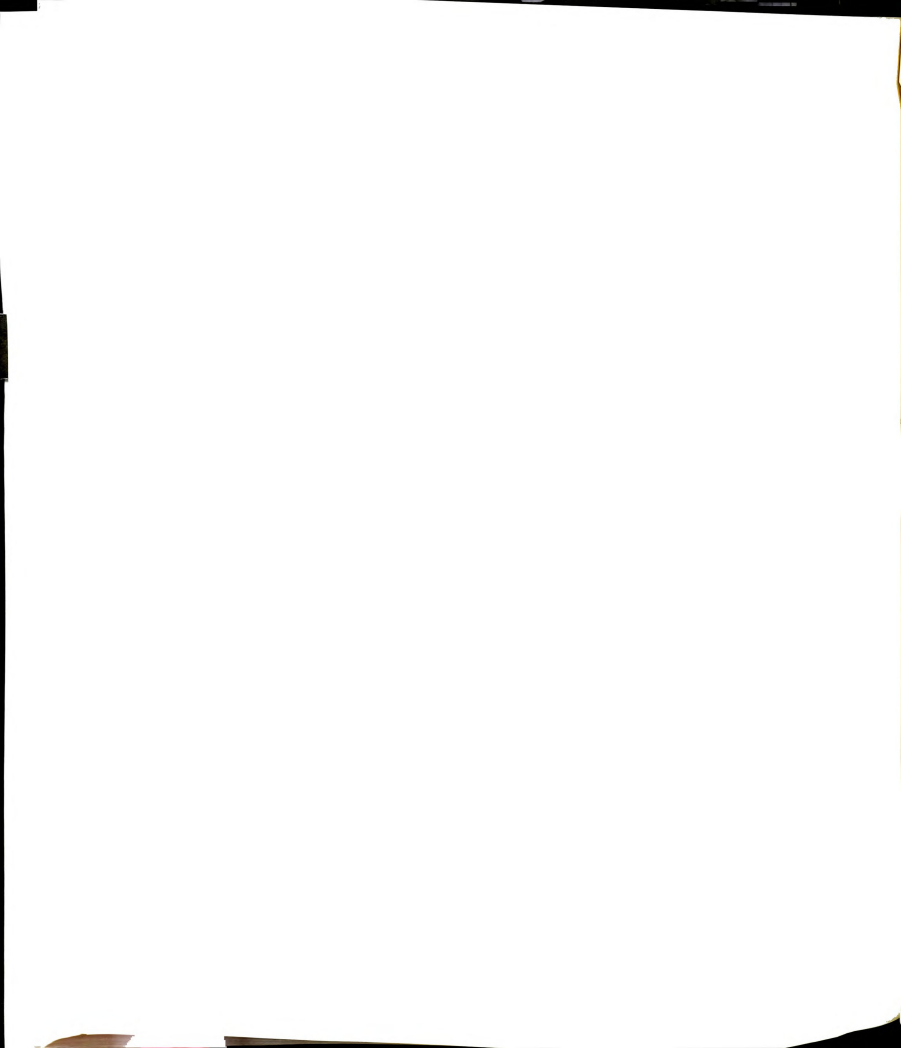
intake and chick growth (Sadgopan et al., 1983). Even inclusion of heat treated RSM at 20% level resulted in lower rate of gain compared to 10% heated RSM in broiler ration (Olomu et al., 1973). Disagreement in toxic effect of sinigrin in MOM was put forward by Panda and Pradhan (1966). They observed significantly better growth in chicks fed MOM diet as compared to chicks fed GNC.

Progressive decline in growth of chicks upon increasing levels of RSM in the diet was reported (Klain et al., 1956). Incorporation of 20% Midas variety of RSM in the diet decreased growth rate and it was lesser for heat treated meal (Paik, et al., 1981). Incorporation of 15% Tower rapeseed meal did not adversely affect the performance of broiler chicks (Summers and Leeson, 1978). Upto 5% canola seed hulls in broiler diets did not adversely affect growth and feed efficiency (Mitaru, et al., 1983).

Internal Lesions:

Haemorrhagic liver syndrome in white rock chicks fed diets supplemented with 50% RSM and 50% full fat rapeseed were reported (Yamashiro et al., 1977). Perotic hock joints in young turkeys, fed 25% RSM (cv. target) in the diet were reported (Moody et al., 1978). Perotic effect on chicks fed 30% RSM is attributed to factor(s) other than nitrogens (Holmes and Roberts, 1963).

Thyroid size for chicks fed Midas was 4 to 20 times greater than for controls, whereas Tower rapeseed



resulted in only a 2 to 3 times greater thyroid size.

Toxic effects of MOM may be confined to young poultry (Prasad, et al., 1973). Responses to RSM feeding may vary from one genotype of chicken to another (Proudfoot et al., 1983).

Palatability:

Summers et al. (1982) suggested that palatability could be the part of the problem of inferiority of canola seed compared to soybean meal diets. Palatability is the problem in young birds and they adapt to RSM feeding after about 3 weeks (Woodly et al., 1972).

Laying hens:

Egg production:

No adverse effect of replacing 100% of crude protein of GNC in layers ration by RSM (Brahmakshetriya et al., 1975). Shrivastava et al. (1975b) observed significant reduction in egg production even at 10% level of MOC inclusion in the diets. There was progressive decline in egg production by increasing levels of MOC.

RSM in layers reduced egg production (Summers, et al., 1971; Marngos and Hill, 1976). Complete replacement of GNC by MOC in layers diet reduced egg production, increased the numbers of days for the first egg laid and decreased the percent hatchability from the fertile eggs (Agarwala, 1984).

Incorporation of 1.5% gum did not affect the egg production in laying hens (Summers et al., 1978). Egg



production was depressed by feeding 12% RSM in the diet (Kubota, et al., 1972). RSM (B. napus) at 12% level in layer ration depressed egg production and reduced egg white (Marangos and Hill, 1976). Even 10% MOM reduced egg production significantly in layer rations (Shrivastava et al., 1976a).

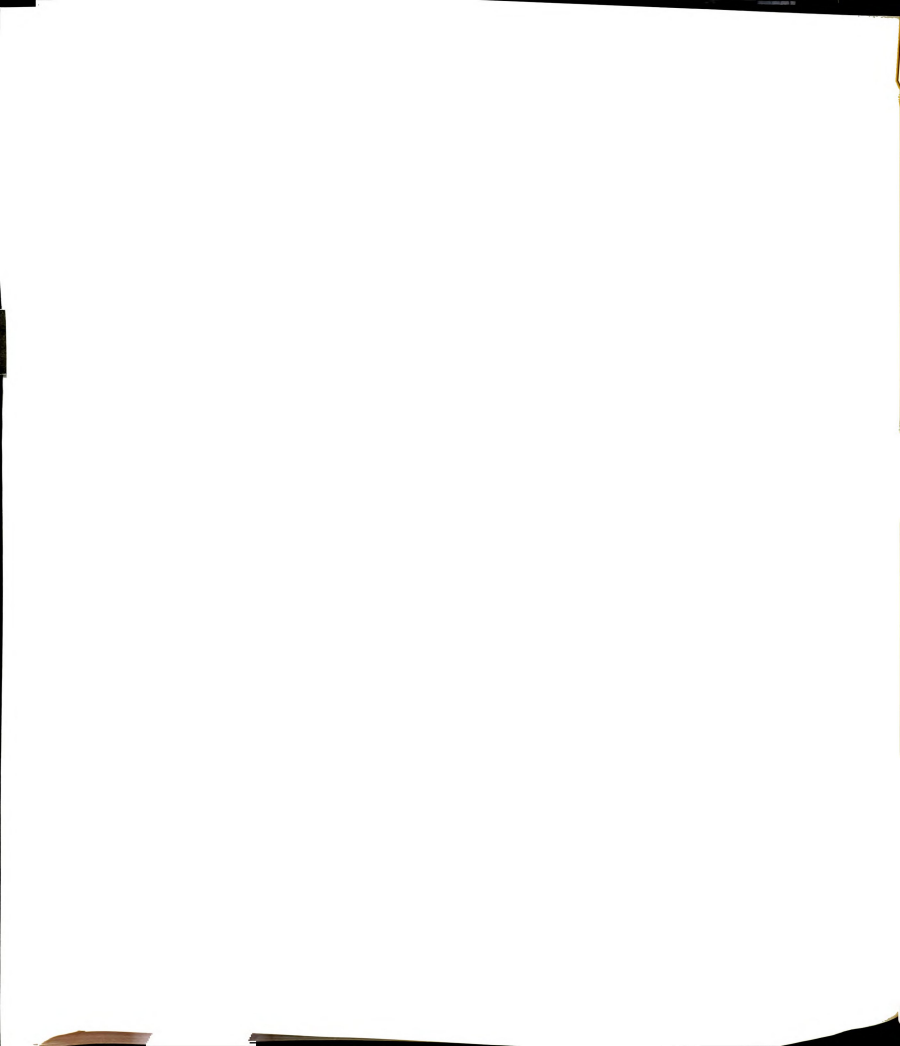
Significant reduction in egg production by replacement of GNC from GNC-based diet by 10 or 20% MOM. along with deterioration of internal quality of egg was also reported (Singh and Agarwala, 1975). RSM up to 17% in the diet of starting and growing pullets could be fed without any adverse effect in growth and subsequent egg production (March, et al., 1975). Five to 10% RSM (cv. candle) can be incorporated in layer diets without any adverse effect in egg production (Slinger, et al., 1978). Double low cultivars of rapeseeds (low in glucosinolates and low in erucic acid) yield excellent quality rapeseed proteins (Eggum, 1981).

No adverse effects of replacing GNC by MOC at 50 to 100 % level were observed in pullets whereas in layers egg production and feed efficieny was depressed (Sadgopan, et al., 1983).

Internal Lesions :

Haemorrhagic liver syndrome occurred in laying hens fed 15% RSM (cv. span) in the diet (Leeson et al., 1976).

Histological studies of thyroid gland revealed that thyroxine biosynthesis was inhibited by feeding RSM to



chickens (Summers et al., 1971, Summers et al., 1978) and it was suggested that growth depression and hyperthyroidism in birds fed RSM in the diet may not simply be due to reduction in serum thyroxine concentration (Summers et al., 1977). Hypertrophy of thyroids were reported when 12% RSM was incorporated in layer rations (Marangos and Hill, 1976). Intact glucosinolates in hen diets produced moderate increase in thyroid size, which further increased in size upon addition of myrosinase (Papas, et al., 1979b).

Palatability:

The presence of an appetite depressing factor has been suggested as there was a significant decrease in feed consumption upon increasing levels of MOM in the diets (Srivastava et al., 1975b). Palatability was not a problem in laying and breeding hens (O'neil, 1957). Layers adapted to a 20% level of RSM after adjusting to the initial phase of RSM feeding (Jackson, 1969). Protein supplement containing 10% RSM was satisfactory in turkey breeder diets (McGregor, 1964).

Justification for proposed study:

Feeding value:

There is no agreement amongst the researchers as to how much of MOM can be satisfactorily fed to livestock. In the past it was believed that the toxicity due to MOM was limited to the monogastric animals. However, recent literature reveals that there is a toxic effect in ruminants as well. In this study the model for studying the antinutritive characteris of MOM was growing chicks.

Some researchers suggested that MOM can replace 80 - 100% of GNC from a GNC based broiler rations. However, others have found that even 35 % replacement could lead to reduced growth. Some suggested that upto 10 % of MOM may be incorporated in chick starter rations, wheras others found severe growth depression upon feeding even 10 % MOM. These differences may possibly be attributed to the varietal differences, agroclimatic conditions and processing techniques.

Processing technique:

Expeller processed meal contains high amounts of residual oil (10 - 15 %), which may contain high amounts of erucic acid. Prepress solvent processed or solvent processed residual meal contains virtually no residual oil and thus devoid of erucic acid.

Temperature and moisture level at the time of processing

influences the quality of MOM. At proper temperature and moisture conditions, enzymatic hydrolysis of the glucosinolates yield toxic aglucons that enhance the toxicity problem of MOM. Heat treatment of mustard seeds prior to oil extraction has been found to be beneficial for oil extraction and also improves general feeding value. However, mustard nutrient loss due to heat treatment needs consideration. Open pan roasting of mustard seeds prior to oil extraction reduced lysine content by 36 %. and the availability of lysine was reduced from 94 % to 76 %.

Detoxification:

Inactivation of endogenous enzyme (myrosinase), removal of the glucosinolates or it's hydrolytic products reduce toxicity associated with the glucosinolates. Chemicals like ethanol, ammonia, acetone, sodium carbonate, sodium hydroxide and other similar compounds have been found to reduce toxicity in MOM, however, they are rather costly processes.

Diffusion extraction of the glucosinolates from the intact seeds prior to oil extraction has been found to be effective as the glucosinolates are highly water soluble.

Different types of heat treatment of seeds or meal have also been found to be effective in reducing toxicity in MOM. Overall nutritive value of MOM was found improved by wet heat treatment and dry heat treatment at high temperatures (121 C) has negative effective effect on nutritional quality

of MOM. However, effective practical systems of heat and moisture treatments have not been devised.

Most of the detoxification techniques described in the literature also induce some nutrient loss. The degree of loss in the nutrients vary from one technique to another.

Short of plant breeding techniques, compromise has to be made between the losses occurring due to detoxification and the gains due to improved nutritive value as a result of the treatments.

With this background, the proposed study is being undertaken to chemically characterize MOM, to partition the effects of antinutritive components, to determine the effects of various types of heating system on the nutritive value, the effects of temperature, moisture and heating period on lysine availability and the effects of amino acid supplementation on chick growth.

This study examines the following aspects of MOM feeding:

1. Partitioning the antinutrient components:

Limited information is available on the effect of various components of MOM. In this study we propose to partition the antinutritive effects due to erucic acid, the glucosinolates and tannic acids as these three make up the major antinutritive components.

2. Improvement in nutritive quality of MOM by heat treatment:

Effect of moisture, temperature and the duration of

heat treatment influence the nutritional quality of MOM. In the literature there is abundance of information on laboratory heating techniques such as autoclaving, static oven etc. There is lack of information on the nutritive value of MOM, when it is heated while in motion. There is also a derth of information on availability of lysine in MOM upon heat treatment at lower temperature and high moisture levels.

In this study it is proposed to test a practical type of heating system (rotating drum heating system) with flexibility in use of source of heat, to study the impact of lower temperature and high moisture level on the antinutrient composition and lysine availability in MOM.



EXPERIMENTAL PART I

Part I consists of the following experiments:

1. Experiment 1 : Chemical characterization of mustard oil meal (MOM)
2. Experiment 2 : Effects of wet heating (autoclaving) and dry heating (oven) and the levels of mustard oil meal (MOM) on chick growth
3. Experiment 3 : Chick growth assay Comparison of autoclaved and hot water treated mustard oil meal (MOM)
4. Experiment 4 : Effects of heating and water treatment of mustard oil meal (MOM) on amino acid composition
5. Experiment 5 : Effects of autoclaving defatted and undefatted mustard oil (MOM) on chick growth at different levels of intake
6. Experiment 6 : Partitioning the effects of erucic acid and other antinutritive components of mustard oil meal (MOM) using chick growth assay

PART I: EXPERIMENT 1: CHEMICAL CHARACTERIZATION OF MUSTARD

OIL MEAL (MOM)

INTRODUCTION

Information is limited on the chemical characteristics of Nepalese mustard oil meal (MOM) . Capper et al. (1982) analyzed MOM from Nepal produced from Brassica nigra. Hemingway and Vaughan (1960) analyzed several samples of mustard seeds (Brassica juncea) from Nepal and all of them contained only allylisothiocyanate.

It is essential to know the chemical characteristics of MOM to study its nutritive value. In this section chemical characteristics of MOM are described.

Materials and Methods:

During the period of these studies, three batches of mustard oil cake (MOC) were shipped from Nepal on three different occasions. Upon arrival the cake was ground and vacuum packed in plastic bags and stored in a freezer at below -40 C till needed for feeding trials. Samples were taken for laboratory analysis after grinding . Proximate analyses, mineral analyses and essential amino acid analyses are presented in Tables 13, 14 and 15, respectively.

TABLE 13. PROXIMATE ANALYSES (%) OF MOM*

Components	Batch 1	Batch 2	Batch 3
Dry matter	91.20	90.70	87.50
Crude protein	39.58	37.82	38.16
Ether extract	10.34	11.40	13.18
Crude fiber	6.36	6.95	7.43
Ash	12.61	7.72	8.00
NFE	22.31	26.81	20.73

* Dry matter basis (DMB)

TABLE 14. MINERAL ANALYSES OF MOM*

Elements	Batch 1	Batch 2	Batch 3
Calcium (%)	1.85	0.63	0.62
Phosphorus (%)	1.55	0.88	1.01
Cobalt (ppm)	<2	na	3.
Copper (ppm)	24	na	6.
Magnesium (%)	0.76	na	0.44
Manganese (ppm)	74	na	42.
Potassium (%)	1.21	na	1.22
Sodium (ppm)	66	na	40.
Zinc (ppm)	78	na	55.
Iron (ppm)	759	na	569.

na = not analyzed; All mineral analyses were done at Supersweet Research Farm, Courtland MN, through the courtesy of Multifood International Inc.

* As fed basis

TABLE 15. AMINO ACID ANALYSIS (%) OF MOM*

Amino acid	Batch 1	Batch 2	Batch 3
Lysine	1.69 ^a	2.31	2.94
Methionine	0.91	0.70	0.71
Cystine	0.46	0.54	0.67
Arginine	4.08	2.27	2.76
Histidine	1.05	1.05	1.26
Isoleucine	1.59	1.59	1.97
Leucine	2.76	2.75	3.37
Phenylalanine	1.68	1.50	1.84
Tyrosine	1.20	1.18	1.21
Threonine	2.10	1.76	2.11
Tryptophan	0.61	0.46	0.46
Valine	2.10	2.08	2.59

* Amino acid analysis were done at Supersweet research farm, Courtland MN, by the courtesy of Multifood International Inc.;^a DMB

Gross energy was determined by adiabatic bomb calorimeter (AOAC, 1980). Reactive lysine values were determined by a method modified in our laboratory (unpublished data, appendix A-I, page 136) that was similar to the procedure described by Hurrell and Carpenter (1979). Metabolizable energy was determined by a modified TME method of Sibbald (1977). The procedure for ME determination is given in appendix A-VII (page 144). Erucic acid was determined as 13-cis-docosenoic acid after methylation (AOAC, 1980). The procedure is given in appendix A-III (page 139). Total fat extraction for erucic acid determination was done by the method described by Folch et al. (1957). The procedure is given in appendix A-II (page 138).

Total glucosinolate was determined by the method described by McGhee et al. (1965). The procedure is given in

appendix A-IV (page 141).

Allylisothiocyanate was determined after steam volatilization by a GC method (AOAC, 1984). The procedure is given in appendix A-V (page 142). Tannin was determined as quercitannic acid by the method described in AOAC (1984). The procedure for the determination of tannin is given in appendix A-VI (page 143).

GE, ME, RL, erucic acid, total glucosinolates, allyl isothiocyanate and tannin content of raw MOM from batch 3 are presented in Table 16.

TABLE 16. NUTRITIVE AND ANTINUTRITIVE COMPONENTS OF MOM

Components	Amount
Gross Energy (cal/kg)	4653 ^a
Metabolizable energy (cal/kg)	3295 ^a
Reactive Lysine (g/kg CP)	46.16 ^a
Erucic acid (% in oil on DMB)	37.05
Total glucosinolates (%)	4.75 ^b
Allyl isothiocyanate (%)	tr
Tannins (as quercitannic acid) (%)	2.86 ^b

^a DMB; ^b Fat free DMB

Results and discussion:

There were no appreciable differences in protein concentrations in three batches of MOM (Table 13). There were slight differences in fat concentrations in MOM. Batch 1 was markedly higher in calcium and phosphorus content compared to the other two batches (Table 14).

MOM from batch 1 was lower in lysine compared to the other two batches. However, arginine was remarkably

higher in batch 1 than in batch 2 and 3. Methionine and tryptophan were also a little higher in batch 1 than in batch 2 and 3. Lysine content of samples from Nepal ranged 4.95 - 7.71 g/16 g N which is much higher than the values of 2.91 g/16 g N reported by Prasad (1978) for MOM from India. Values reported in this study are closer to the values reported by Miller et al. (1962)

The only known background about the MOM recieved, were that it belonged to the genus Brassica, and that it was expeller processed. Not knowing what it had gone through prior to oil extraction or how long and how it was stored, it was difficult to explain the discrepancy in lysine content. The lower lysine value in batch 1 cannot be explained by possible heat damage during oil extraction as the arginine value was rather high compared to the other batches.

The ME value for MOM using the TME method of Sibbald was higher than the value obtained by Shrivastava et al. (1976). They reported a value of 3017 kcal/kg as opposed to 3295 kcal/kg found in this study.

Reactive lysine in MOM was within the range published for RSM. The available lysine value was 77%. Capper et al. (1982) reported the availability of lysine in MOM to be 94%. However, they found a severe decrease in availability of lysine (to 74%) when mustard seeds were open pan roasted for 20 to 30 min prior to oil extraction.

Erucic acid in the residual oil was within the range

reported by Dutta and Ghosh (1970). Total glucosinolate content on a fat-free dry matter basis was within the range reported by Appelqvist (1971) for Brassica juncea(3 - 6%).

Tannin content of MOM was similar to the value reported by Vaidya et al. (1975a).

Allylisothiocyanate in MOM could not be detected by the method described by AOAC (1984). A possible explanation for the failure to detect this compound is that the enzyme myrosinase may have been completely destroyed during the extraction of oil. Appelqvist and Josefsson (1967) reported that myrosinase was completely destroyed at 8% moisture level and at 90 C for 15 minutes. Moisture content in MOM analyzed for allylisothiocyanate was 12.5%.

PART I: EXPERIMENT 2: EFFECTS OF WET HEATING (AUTOCLAVING)
AND DRY HEATING (OVEN) AND THE LEVELS OF MUSTARD
OIL MEAL (MOM) ON CHICK GROWTH

INTRODUCTION

Wet heat treatment of RSM improves its nutritive value to chickens (Rauchberger et al., 1979; Bock et al., 1982). Improvement in quality of RSM upon wet heat treatment was observed by Eapan (1968). Chick growth was improved upon feeding RSM autoclaved at 15 psi (Gray et al., 1957), which was independent of lysine destruction. Autoclaving of RSM for 60 min at 120 C and 30% moisture level eliminated goitrin (Hijikuro and Takemasa, 1979b).

Limitation of use of mustard oil meal (MOM) and rapeseed meal (RSM) have been reported by several authors (Agarwala, 1964; Kubota et al., 1972; Srivastava and Prasad, 1976; Marangos et al., 1978; Matsumoto et al., 1979; Hijikuro and Tkemasa, 1979b; Kozlowska et al., 1981). It was attributed to the content of glucosinolates (El-nokrashy et al., 1975) in RSM and erucic acid in MOM (Prasad et al., 1978).

Replacement of GNC by MOM at the 80% level (Benergy, et al., 1974), at the 100% (Prasad et al., 1974; Mandal et al., 1981) and at the 100% level of RSM (Ballester et al., 1974) did not affect chick growth. March et al. (1975) did not find

any adverse effect of feeding RSM as the sole source of protein to layers. Hijikuro and Takemasa (1979c) replaced 50% SBM by RSM without any adverse effect in chick starter diets.

This experiment was designed to investigate the effect of dry and wet heat treatments on the nutritive value of MOM. Effect of incremental levels of MOM in chick growth was also investigated.

Materials And Methods:

MOM used in this trial was from batch 1.

Heat treatments:

1) Autoclaving: MOM was thinly spread in an aluminium pan and placed in an autoclave and steam heated for 30 min at 121 C. After the treatment, the pan was left overnight to equilibrate with the atmosphere.

2) Oven heating: MOM was thinly spread in an aluminium pan and placed in an oven (preheated to 121. C) and heated for 30 min. After the treatment, the pan was left overnight to equilibrate with the atmosphere.

Diet preparation:

Basal component: In all the diets, the basal component provides 70 % with the remaining 30% containing combinations of MOM and substitute component which simulates MOM. The composition of the basal component and substitute component

are presented in Table 17. Combinations of these components are given in Table 18. All the diets were equicaloric and equinitrogenous (ME: 2531 Kcal/kg; CP: 18.00%).

TABLE 17. COMPOSITION OF BASAL AND SUBSTITUTE COMPONENTS*

Ingredients**	Basal	Substitute
Corn starch	14.02	-----
Wheat middlings	45.44	11.50
Yellow corn	36.56	-----
Corn gluten meal	-----	80.50
Dical	0.76	1.57
Limestone	0.33	3.38
Coomon salt	0.71	-----
Mineral mix ¹	0.04	-----
Vitamin mix ²	0.43	-----
Lysine HCL (78.4%)	0.37	1.06
Methionine HCL(98%)	0.19	-----
Arginine (99.9%)	-----	2.00
Sand	1.15	-----

* As fed basis;** Detailed composition in appendix B-1,2 (page 145); 1 & 2 appendix B-3,4 (page 146)

TABLE 18. DIETARY COMBINATIONS

#	Diets	% MOM	% Substitute	% Basal
1	Raw MOM	30	0	70
2	Dry heated	30	0	70
3	Autoclaved	30	0	70
4	Raw MOM	20	10	70
5	Dry heated	20	10	70
6	Autoclaved	20	10	70
7	Raw MOM	10	20	70
8	Dry heated	10	20	70
9	Autoclaved	10	20	70
10	Control	0	30	70

Experimental procedure:

This experiment was designed as a 2 X 3 factorial plus control (Gill, 1978). Twelve day old meat-type chicks, previously raised on commercial chick starter rations in a



battery brooder were wing banded, weighed and ranked by weight in groups of 8 chicks. One chick from each weight group was randomly selected and assigned to one of the twenty pens. Each pen had eight chicks representing eight weight groups. Ten dietary treatments were randomly assigned to twenty pens (2 replicates/dietary treatment). Weight and feed consumptions were recorded daily for two weeks. Chicks were fed ad libitum and fresh water was available at all times.

Results and discussion:

Initial weight, two week weights of chicks on experimental diets, feed consumption and feed to gain ratios are given in Table 19.

TABLE 19. PERFORMANCE OF CHICKS IN EXPERIMENT 2

Diet #	Initial weight (g)	Final weight (g)	Feed consumption (g)	F/G (Ratio)
1	87.1	205.4	298.2	2.5
2	84.7	201.5	289.5	2.4
3	85.8	217.7	308.4	2.3
4	85.9	216.9	288.6	2.2
5	83.3	213.9	276.0	2.1
6	87.6	234.4	319.9	2.2
7	84.9	201.3*	243.8	2.1
8	86.9	206.3*	263.8	2.2
9	90.4	228.1*	296.1	2.1
10	85.9	204.6	251.2	2.1

* Excluded from statistical analysis; SE of treatment mean = ± 5.69

Weight gain was subjected to ANOVA (Appendix C-2, page 147). Autoclaving significantly improved growth com-

Weight gain was subjected to ANOVA (Appendix C-2, page 147). Autoclaving significantly improved growth compared to oven heat treated MOM ($P < .05$). Autoclaving of MOM supported higher growth rates compared to other treatments at all levels of incorporation. However, the differences were more prominent at lower levels of incorporation. In Figures 3, 4 and 5, weekly weights of chicks at 10, 20 and 30% in the diets are presented.

These results show that a progressive decline in growth occurred with increasing levels of MOM ($P < .05$). However, the decline was more severe for the raw and dry heated MOM diets.

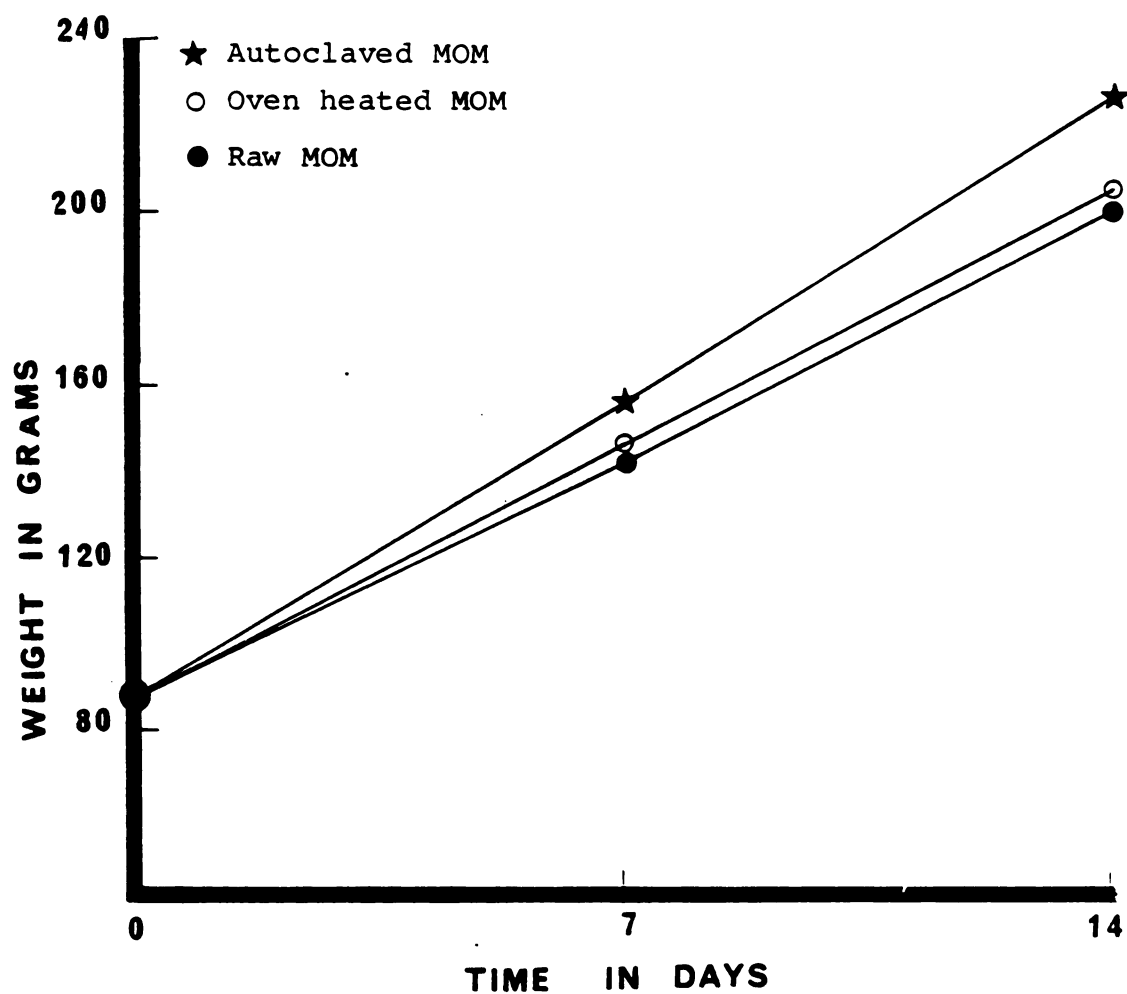


Figure 3. Weekly weight of chicks fed autoclaved, oven heated and raw MOM at 10% level in the diets

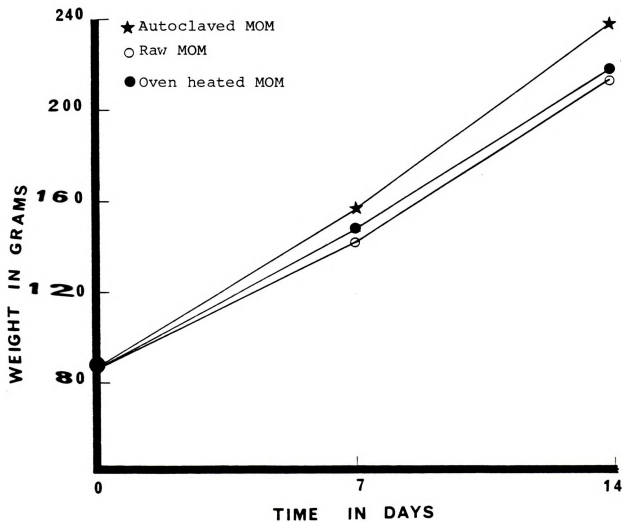


Figure 4. Weekly weight of chicks fed autoclaved, oven heated and raw MOM at 20% level in the diets

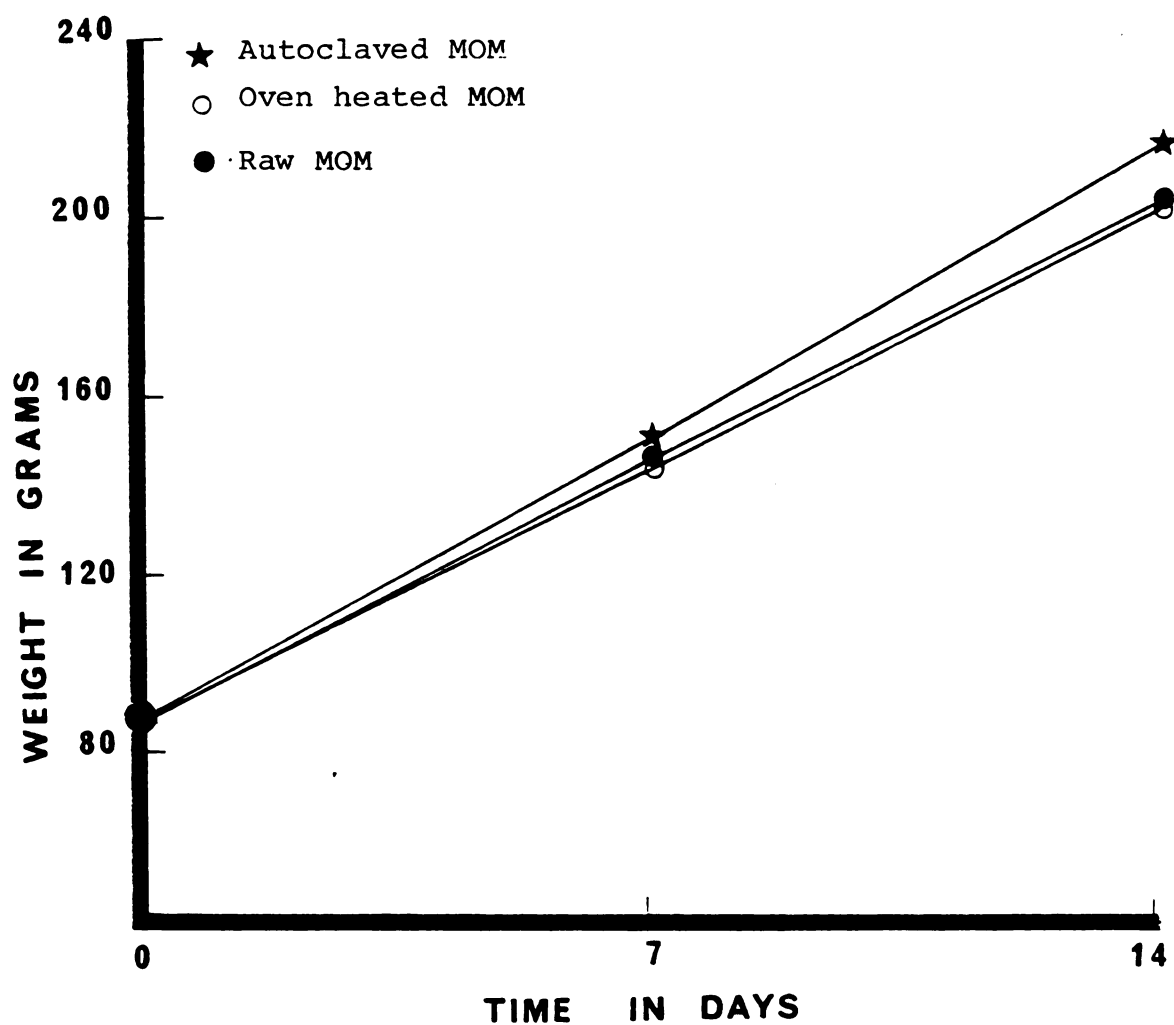


Figure 5. Weekly weight of chicks fed autoclaved, oven heated and raw MOM at 30% level in the diets

PART I: EXPERIMENT 3: CHICK GROWTH ASSAY TO COMPARE THE
NUTRITIVE VALUE OF AUTOCLAVED AND HOT WATER
TREATED MUSTARD OIL MEAL (MOM)

INTRODUCTION

Autoclaving has been shown to improve nutritive value of MOM by (Gray et al., 1957), and also in experiment 2, part I of this study. Autoclaving also destroys lysine, an important amino acid. Autoclaving is not practical except in laboratory conditions.

Hot water treatment of RSM was beneficial in improving its nutritive value (Prasad and Rao, 1978). Hot water extraction of RSM reduced toxicity in growing chicks (Nakaya, 1965a; Nakaya, 1965b; Nakaya and Tagannis, 1965; Lo and Hill, 1971). Olomu et al. (1974) observed improvement in the quality of the product upon autoclaving or hot water treatment of rapeseed.

It was observed in previous experiments that higher rates of incorporation of MOM without heat treatment impaired growth in growing chicks. Heating of MOM improved growth even at higher levels of incorporation.

It seems that wet heating has a beneficial effect on chick growth. This experiment was designed to compare the nutritive value of autoclaved MOM and hot water treated MOM

at two levels of dietary incorporation.

Materials and methods:

MOM from batch 2 was used in this study.

Hot water treatment:

Water in a large pan was brought to boiling and then MOM was added to the boiling water, with continued heating for 30 minutes. It was occasionally stirred. Thermocouples were placed at different depths to measure the product temperature during the process. After the heat treatment the slurry was spread on shallow pans and dried in forced draft oven for 18 hours at 45 C. The slurry formed a hardend crust, so it was ground in a Wiley mill. The meal was left in pans over night to equilibrate with the atmosphere.

Autoclave treatment: See experiment 2, part 1 (page 67).

Diet preparation:

A corn-soy basal diet was formulated and soybean meal was replaced by mustard oil meal at two levels of replacement. All the diets were equicaloric and equinitrogenous (ME: 3200 Kcal/kg; CP: 23.0%). Trace minerals and vitamins were added according to NRC (1977) recommendations. Composition of experimental diets are given in table 20.

TABLE 20. COMPOSITION OF EXPERIMENTAL DIETS*

Ingredients**	Diets				
	1	2	3	4	5
SBM (44% CP	42.60	20.00	20.00	31.00	31.00
MOM (34.9% CP)	----	30.00	30.00	15.00	15.00
Yellow Corn	45.00	36.00	36.00	41.00	41.00
Corn oil	7.95	9.60	9.60	8.67	8.67
Dical	3.13	2.66	2.66	3.07	3.07
Limestone	0.29	----	----	----	----
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin Mix ¹	0.30	0.30	0.30	0.30	0.30
Mineral Mix ²	0.03	0.03	0.03	0.03	0.03
Methionine	0.20	0.08	0.08	0.10	0.10
Lysine	----	0.17	0.17	0.04	0.04
Sand	----	0.66	0.66	0.29	0.29

* As fed basis; ** Detailed composition in Appendix B-1,2 (page 145); 1 & 2 in appendices B-3 and B-4 (page 146)

Materials and methods:

The experiment was designed in a 2 X 2 factorial plus control (Gill, 1978). Fifty, 12 day old Barred Rock meat type chicks previously raised on commercial broiler starter ration were selected from a batch of 200 birds. Birds weighing 101 gm to 110 gm were selected and were randomly assigned to 10 pens (5 birds/pen). Five dietary treatments were randomly assigned to ten pens (2 pens/treatment). Birds were fed ad libitum and fresh water was available at all times. They were housed in a battery brooder. Weight gain and feed consumption were recorded at 3 day intervals.

Results and Discussion:

Weight gain, feed consumptions and feed to gain ratio are presented in Table 21. Final weight was subjected to ANOVA (appendix C-3; page 148).



TABLE 21. PERFORMANCE OF CHICKS IN EXPERIMENT 3

Diet #	Initial Wt. in grams	Final Wt. in grams*	Feed Consumption in grams	F/G ratio
1.	105.10	219.00	236.14	2.07
2.	104.30	203.00	234.64	2.37
3.	103.40	194.10	211.51	2.33
4.	103.80	207.80	277.01	2.18
5.	101.70	204.90	230.00	2.22

* 9 day weight gain; SE of treatment means = ± 2.606

Increasing levels of MOM in the diets resulted in lower rates of growth ($P < .05$). Autoclaving of the meal produced significantly higher growth than boiling water treatment ($P < .05$). However, the performance of the chicks on the standard reference diet was numerically much better than any treatment. Figure 6 shows weight at three day intervals. Irrespective of treatment there was decline in weight upon increasing the level of MOM from 15% to 30%. The difference between hot water and autoclave treated MOM was more distinct at the 30% level.

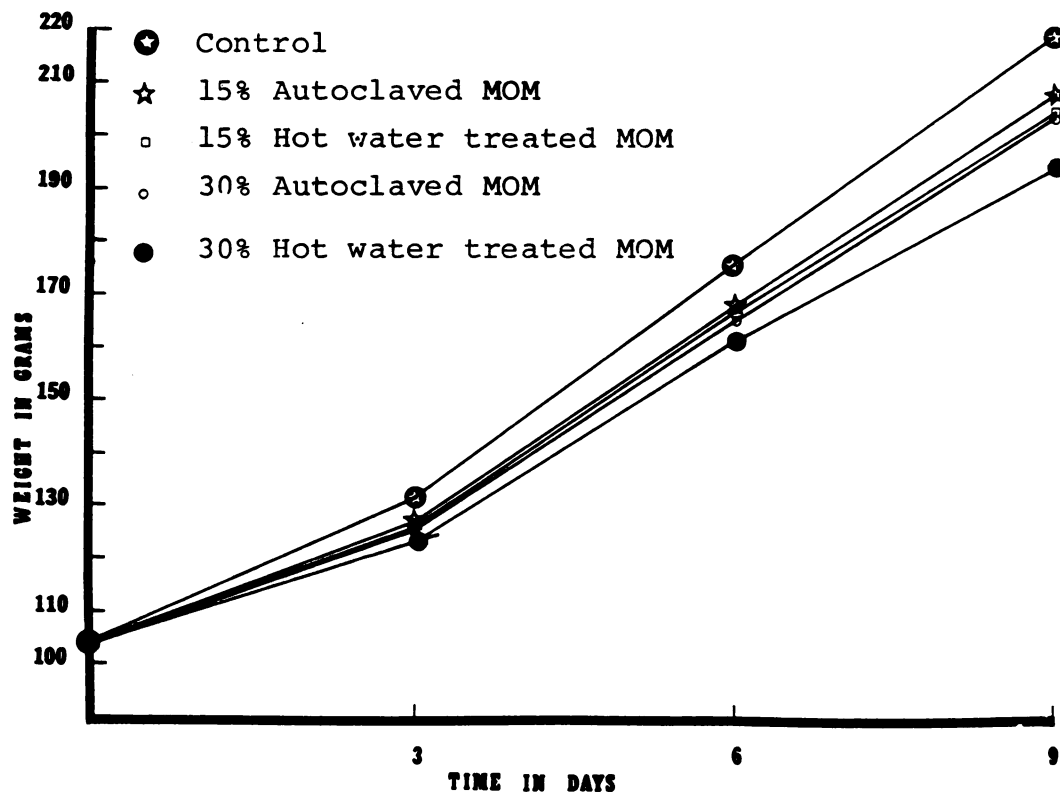


Figure 6. Weight gain of chicks fed autoclaved and hot water treated mustard oil meal (MOM) at two levels of intake

PART I: EXPERIMENT 4: EFFECTS OF HEATING AND WATER
TREATMENT ON AMINO ACID COMPOSITION OF MUSTARD OIL
MEAL (MOM)

INTRODUCTION

Processing techniques influence the bioavailability of protein, some positively and some negatively (Kies, 1981). Thermal processing affects protein quality, rendering basic amino acids, especially lysine, unavailable (Wolzak et al., 1981). Thermal treatments of many feed by-products have been found to be beneficial. However, nutrient losses do occur during such treatments. In experiments 1 and 2 of part one of this manuscript, autoclaving of MOM was beneficial in improving the feeding value of MOM. Capper et al. (1982) observed severe loss in lysine content of MOM due to the fact that mustard seed was subjected to heat treatment.

This experiment was conducted to see the effects of temperature and water treatments on the amino acid composition of MOM.

Materials and Methods:

Table 22 shows the treatment combinations used in this experiment. MOM used in this experiment was from batch 3.

TABLE 22. TEMPERATURE AND MOISTURE COMBINATIONS

Treatments #	Temperature C	Moisture %	Time min
1. Raw	-----	-----	-----
2. Autoclaved	121	12.6	30
3. Oven heated	121	12.6	30
4. Added moisture & Oven dried	121	50.0	30*
5. Soaked in cold water	4	500.0	720**

* Dried in force draft oven for 12 h at 50 C

** Dried in force draft oven for 18 h at 50 C

Preparation of samples:

Soaking: MOM was soaked in cold water for 12 hours (in a cold room). Five liters of water was used per kg of MOM. The supernatant portion was decanted and the residual was filtered using several layers of chese cloth. The resultant slurry was spread thin in a large aluminium pan and dried in a force draft oven at 50 C for 18 h. The hardened crust was ground in a Wiley mill and left over night to equilibrate with the atmosphere.

Addition of water: One hundred grams of distilled and deionized water was added to two hundred grams of MOM, forming a slurry. The slurry was then heated in an oven at 121 C for 30 minutes. While still in a slurry form, it was dried in a force draft oven till it formed a crust (10 h).

Autoclave treatment: See experiment 2, part I (page, 67).

Oven heat treatment: See experiment 2, part I (page, 67).

Results and Discussion

Amino acid profile of MOM before and after treatments are given in Table 23.

TABLE 23. EFFECT OF HEAT AND MOISTURE TREATMENT
ON THE AMINO ACID COMPOSITION OF MOM

Treatment #'s	1	2	3	4	5
Amino acids	(% on DMB)				
Lysine	2.98*	2.47	2.66	2.73	2.57
Arginine	2.76	2.49	2.70	2.77	2.09
Methionine	0.72	0.75	0.72	0.73	0.59
Cystien	0.68	0.64	0.69	0.66	0.46
Histidine	1.26	1.23	1.27	1.27	1.09
Isoleucine	1.97	1.96	1.94	1.93	1.73
Leucine	3.37	3.35	3.32	3.33	2.92
Phenylalanine	1.84	1.79	1.81	1.79	1.56
Tyrosine	1.21	1.06	1.12	1.35	0.97
Threonine	2.11	2.11	2.07	2.12	1.87
Valine	2.59	2.67	2.56	2.54	2.28
Tryptophane	----	0.41	0.46	----	----
Crude protein	33.5	35.0	36.0	36.0	30.6
Moisture	12.6	8.2	5.6	5.8	6.7

* Means of 3 determinations.

There was a decrease of 17% in lysine due to autoclave treatment, 10.7% decline due to oven heating of MOM, 8% decline with the addition of moisture (50%) and 13.7% following cold water washing of MOM. Arginine destruction due to autoclaving was 9%. Cold water washing of MOM reduced arginine content by 24.27%. There was a marked loss of crude protein (14%) due to cold water washing.

PART I: EXPERIMENT 5: EFFECT OF AUTOCLAVING DEFATTED AND
UNDEFATTED MUSTARD OIL MEAL (MOM) ON CHICK GROWTH
AT DIFFERENT LEVELS OF INTAKE

INTRODUCTION

In previous trials it was observed that autoclaving of mustard oil meal supported higher growth compared to dry heated meal, untreated meal and hot water treated meal. Garcha et al. (1976) reported that phenols were greatly reduced by defatting rapeseed meals. Yule and McBride (1978) observed that solvent processed RSM was better than expeller processed. It has been suggested that erucic acid may be the major factor in the antinutritive characteristic of MOM (Slinger, 1973; Prasad, 1977; Prasad et al., 1978; Chand and Sadgopan, 1980). Prasad and Rao (1982) observed improvement in chick performance upon hexane extraction of MOM.

This experiment was designed to investigate the effect of oil extraction from the meal and effect of autoclaving after oil extraction. Effect of addition of mustard oil to a standard reference diet has also been examined.

Treatment of Mustard oil meal:

Defatting of meal: Mustard oil meal was soaked in acetone overnight and decanted in an aluminium pan (previously

dried and weighed). Acetone was allowed to evaporate under the hood with blowing air fan till the smell of acetone was gone. The amount of fatty oil was calculated by the difference of the weight of aluminium pan before and after evaporation of acetone. Only 4.70% residual oil was left in the defatted meal. Defatted meal had a completely different color, possibly due to the fact that the color factor was in the oil fraction of the meal.

Autoclaving of meals:

Defatted and undefatted mustard oil meals were autoclaved as described in experiment 2, part I (page, 67).

Diet Preparation:

Dietary composition of the test diets are given in Table 24. All the diets were equicaloric and equinitrogenous (ME: 2930 Kcal/kg; CP: 18.00%).

TABLE 24. COMPOSITION OF EXPERIMENTAL DIETS

Ingredients	Corn-Soy diet Diet # 13	Corn-MOM diet 14
Yellow Corn	67.61	53.94
SBM	27.39	-----
MOM	-----	39.90
Corn Oil	0.55	2.39
Dical	3.19	2.89
Limestone	0.36	0.09
Mineral Mix	0.03	0.03
Vitmin Mix	0.30	0.30
Common salt	0.50	0.50
Methionine	0.13	0.06

Effect of addition of mustard oil to a standard reference diet was also investigated. Diets 15 and 16 were

constructed by adding 5.27% corn oil and mustard oil respectively to diet # 13. Mustard oil added was equivalent to the amount present in diet 14. Since mustard oil contains 37.01% erucic acid, the erucic acid content of the diet 14 and 16 is 1.90%.

TABLE 24. CONTD'

Ingredients	Diet numbers and type						
		Autoclaved MOM		Acetone	Autoclaved MOM		
	Diet #	4	5	6	10	11	12
		10 %	20 %	30 %	10 %	20 %	30 %
Yellow corn	65.85	62.85	61.08	64.60	61.60	61.08	
SBM	20.16	13.15	5.92	20.40	13.40	5.92	
MOM	9.52	19.03	28.55	8.89	17.78	26.68	
Corn Oil	-----	0.37	0.35	1.17	2.30	2.19	
Dical	3.11	3.03	2.95	3.12	3.03	2.95	
Limestone	0.30	0.23	0.17	0.29	0.23	0.18	
Salt	0.50	0.50	0.50	0.50	0.50	0.50	
Mineral Mix	0.03	0.03	0.03	0.03	0.03	0.03	
Vitamin Mlx	0.30	0.30	0.30	0.30	0.30	0.30	
Methionine	0.11	0.09	0.07	0.11	0.09	0.07	

TABLE 24. CONTD'

Ingredients	Diet numbers and type					
	Raw MOM			Acetone washed		
	1	2	3	7	8	9
	10 %	20 %	30 %	10 %	20 %	30 %
Yellow corn	64.60	61.60	58.57	64.60	60.34	58.57
SBM	20.40	13.40	6.43	20.40	13.66	6.43
MOM	10.00	20.00	30.00	9.33	18.66	27.99
Corn oil	0.38	0.76	1.15	0.46	1.32	3.05
Dical	3.12	3.03	2.96	3.12	3.04	2.97
Limestone	0.29	0.23	0.16	0.29	0.23	0.17
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral Mix	0.03	0.03	0.03	0.03	0.03	0.03
Vitamin Mix	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.11	0.07	0.07	0.09	0.09	0.07

Experimental

The experiment was designed as a 2 X 2 X 3 factorial (Gill, 1978). One positive control (diet #13) and One negative control (diet # 14) diets were also included. One hundred twenty eight day-old chicks were were wing banded and weighed, before randomly being assigned to thirty two pens (4 birds/replicate). Sixteen dietary treatments were randomly assigned to the pens (2 replicates/treatment). Chicks were fed ad libitum and fresh water was available at all times. Three days were considered as pretreatment period therefore the weight on 3rd day was considered as the initial weight. Chicks were housed in a battery brooder. Weight gain and feed consumptions were recorded at weekly intervals.

Results and Discussion

Weight gain, feed consumption and feed to gain ratio after two weeks on test diets are presented in Table 25 and 26. Two week weight gain was subjected to ANOVA (Appendix C-5; page 149). Figure 7 shows the effect of levels of treated and untreated MOM in the diets. Figure 8 shows the weekly weight of chicks fed diets supplemented with mustard oil or corn oil.

There was significant improvement due to autoclaving of MOM ($P < 0.009$). Mean response to the levels of MOM in the diets were found significant ($P < 0.05$) for the levels from

10% to 30%. Because of mild interaction between defatting and levels, effect of defatting was tested at 3 levels of dietary incorporation using Studentized t- test (Gill, 1978). At 10 and 20 % levels, defatted MOM supported significantly higher growth rate compared to undefatted MOM ($P < 0.05$). However at 30% level of MOM there was no significant difference in growth of chicks fed defatted or undefatted MOM.

Figures 9, 10 and 11 are presented to clearly indicate the differences in weight gain pattern at 10, 20 and 30% levels of MOM in the diets respectively.

TABLE 25.FINAL WEIGHT, FEED INTAKE AND FEED/GAIN RATIOS OF CHICKS IN EXPERIMENT 5

Description of diets	MOM %	Init. Wt. (g)	Weight gain (g)	Feed consumed (g)	F/G ratio
Raw MOM diet	10	46.2	136.0	179.2	2.0
Autoclaved MOM	"	44.0	155.9	190.1	1.7
Acetone washed	"	45.2	149.0	209.0	2.1
Acet. Wd. Auto. MOM	"	44.7	159.5	225.1	1.9
Raw MOM	20	45.2	124.7	189.1	2.4
Autoclaved MOM	"	38.0	134.9	210.9	2.2
Acetone washed MOM	"	43.4	143.5	208.0	1.9
Acet. Wd. Auto. MOM	"	43.8	146.9	215.6	2.2
Raw MOM	30	45.8	120.0	178.6	2.4
Autoclaved MOM	"	41.9	120.6	159.7	2.0
Acetone washed MOM	"	44.4	116.2	173.6	2.4
Acet. Wd. Auto. MOM	"	43.4	119.6	186.1	2.4

SE for treatmenets = +- 3.18

In Table 26 the effect of addition of mustard oil to the standard referance diet is presented. The results indicate

that there is detrimental effect of adding mustard oil in the diets. Significant weight reduction occurred due to mustard oil ($P < 0.05$). Weekly weight of chicks fed diets 13, 14, 15 and 16 are presented in figure 11. ANOVA for two week weight is presented in appendix C-6 (page 150).

TABLE 26. PERFORMANCE OF CHICKS FED MUSTARD OIL
ADDED DIETS IN EXPERIMENT 5

Diet Treatment #	Initial weight	Final weight	Feed consumption	Feed/gain ratio
13. Corn-soy std diet	40.4	157.5	191.2	1.6
14. Corn-MOM control	44.0	109.3	179.2	2.7
15. #1 +Corn oil	43.7	160.6	217.4	1.9
16. #1 +Mustard oil	44.7	140.6	198.4	2.1

SE of treatment means = ± 6.69

Diets 14 and 16 are equal in erucic acid content. However, the growth decline in chicks fed diet 16 was less than due to diet 14 ($P < 0.05$). It is reasonable to believe that there are still antinutritive factors in MOM, in addition to erucic acid.

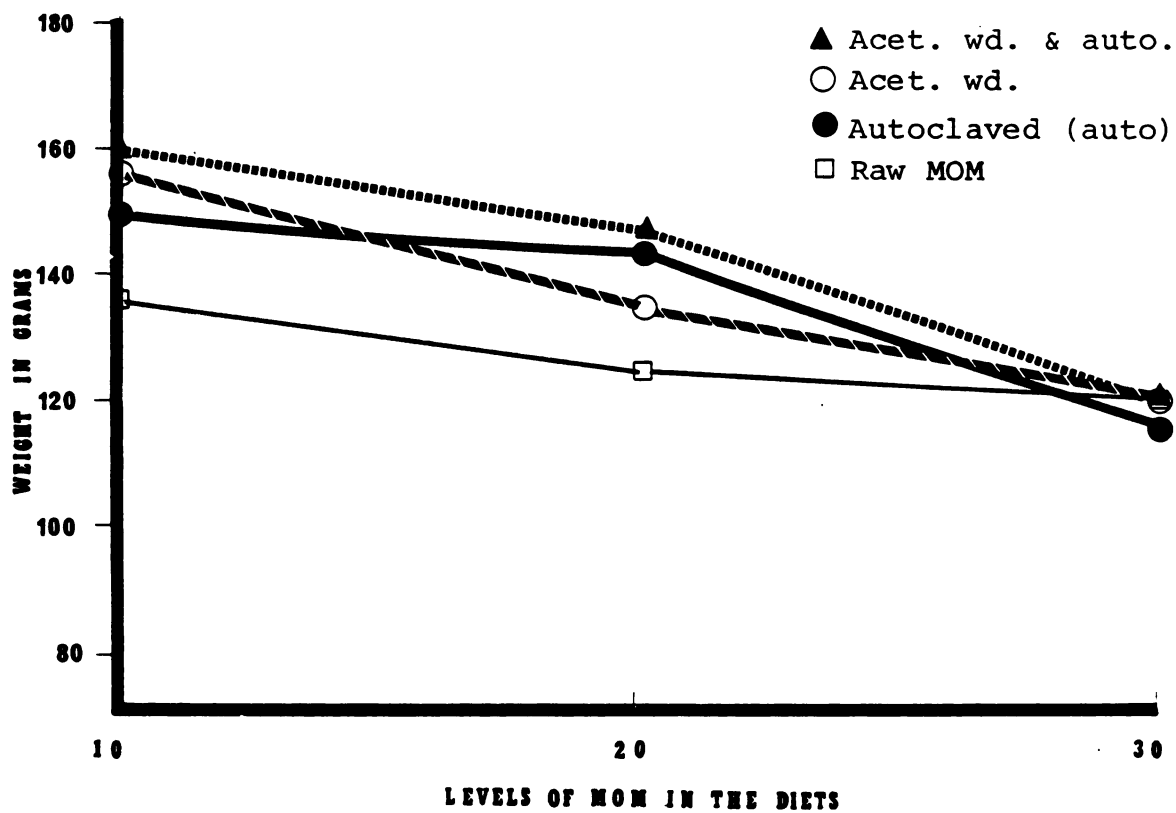


Figure 7. Weight gain of chicks fed raw, acetone washed, acetone washed and autoclaved and autoclaved mustard oil meal (MOM) at 3 levels of intake

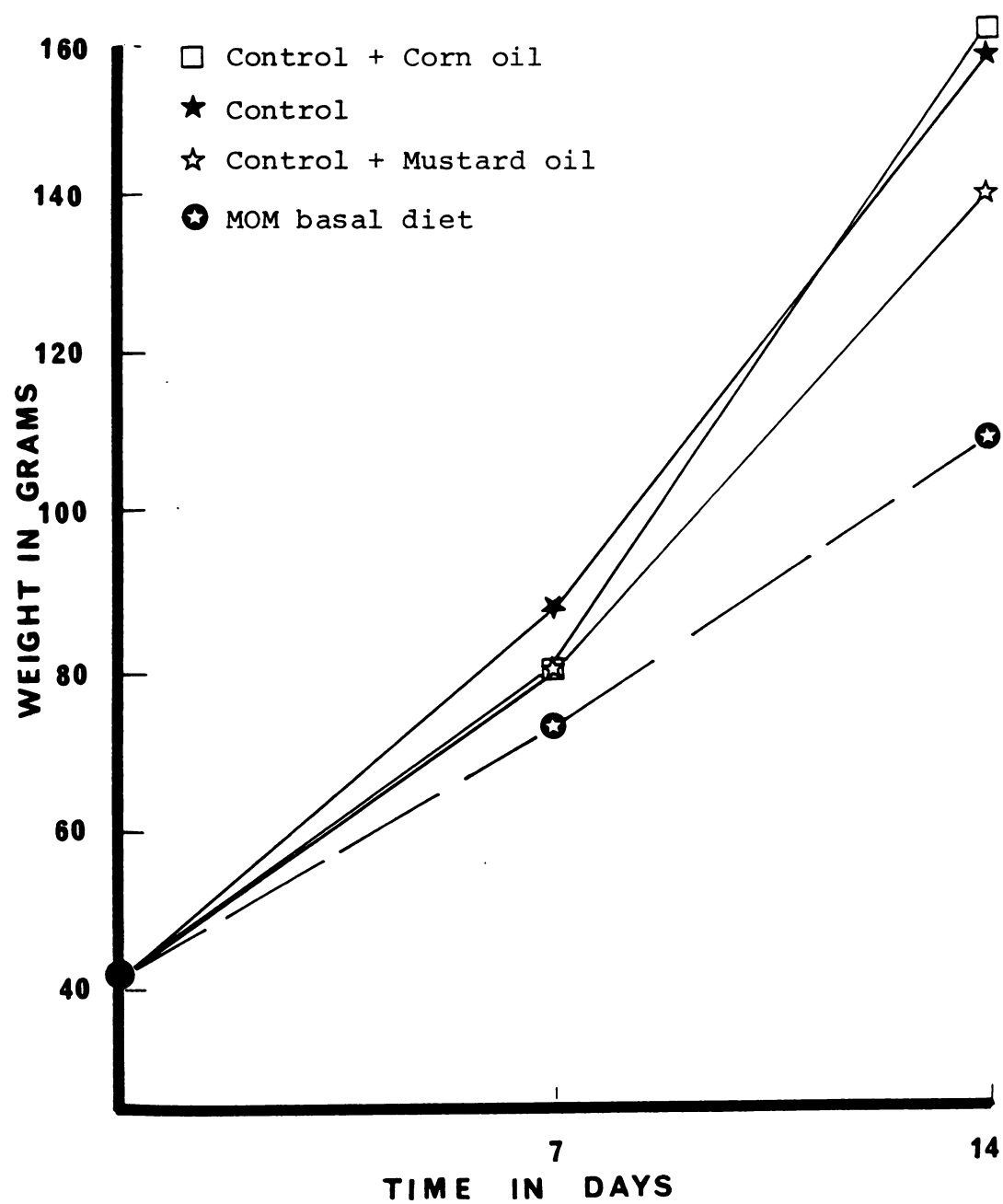


Figure 8. Weekly weight of chicks fed mustard oil added standard reference diet

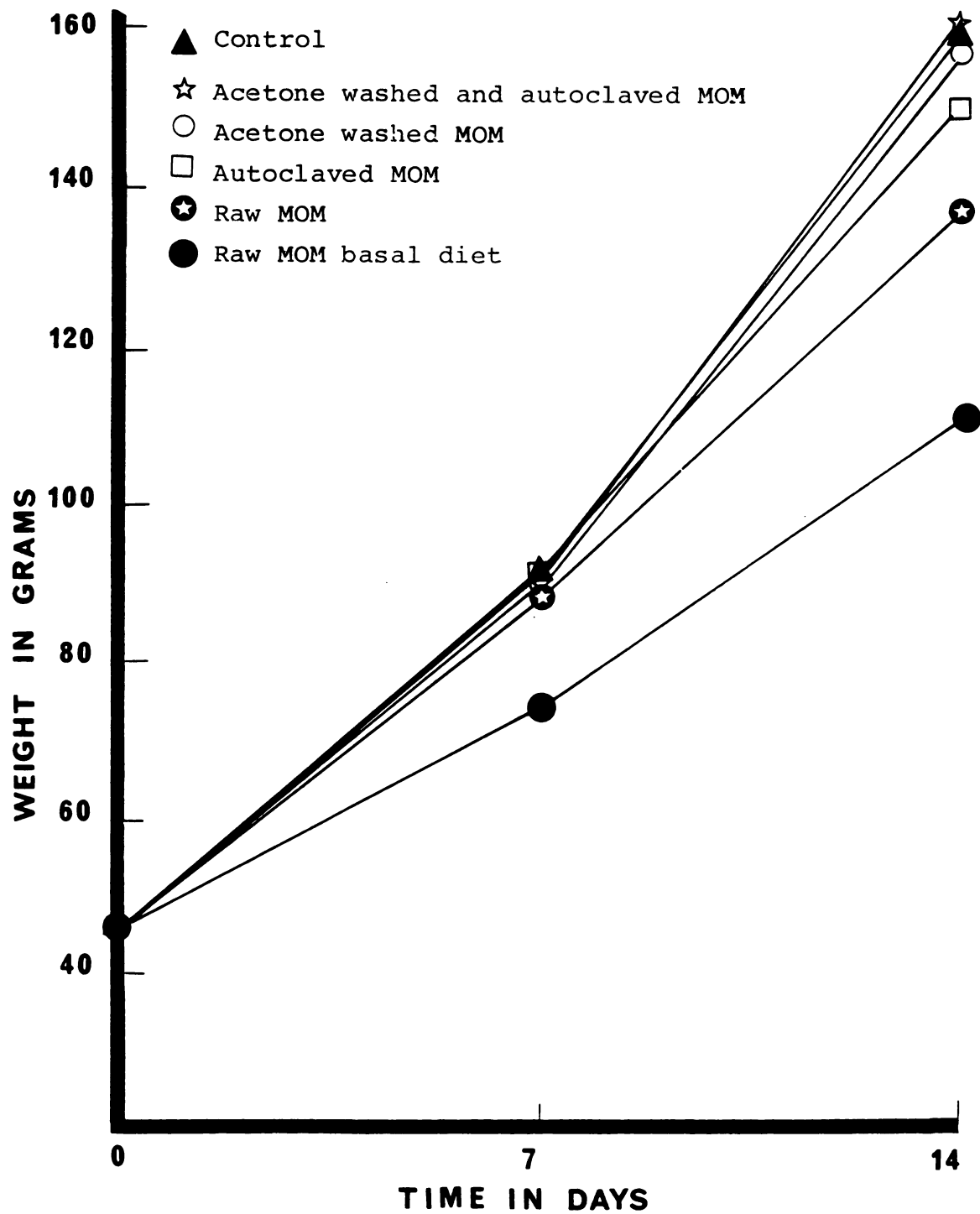


Figure 9. Weekly weight of chicks fed experimental diets at 10% level of treated or untreated MOM

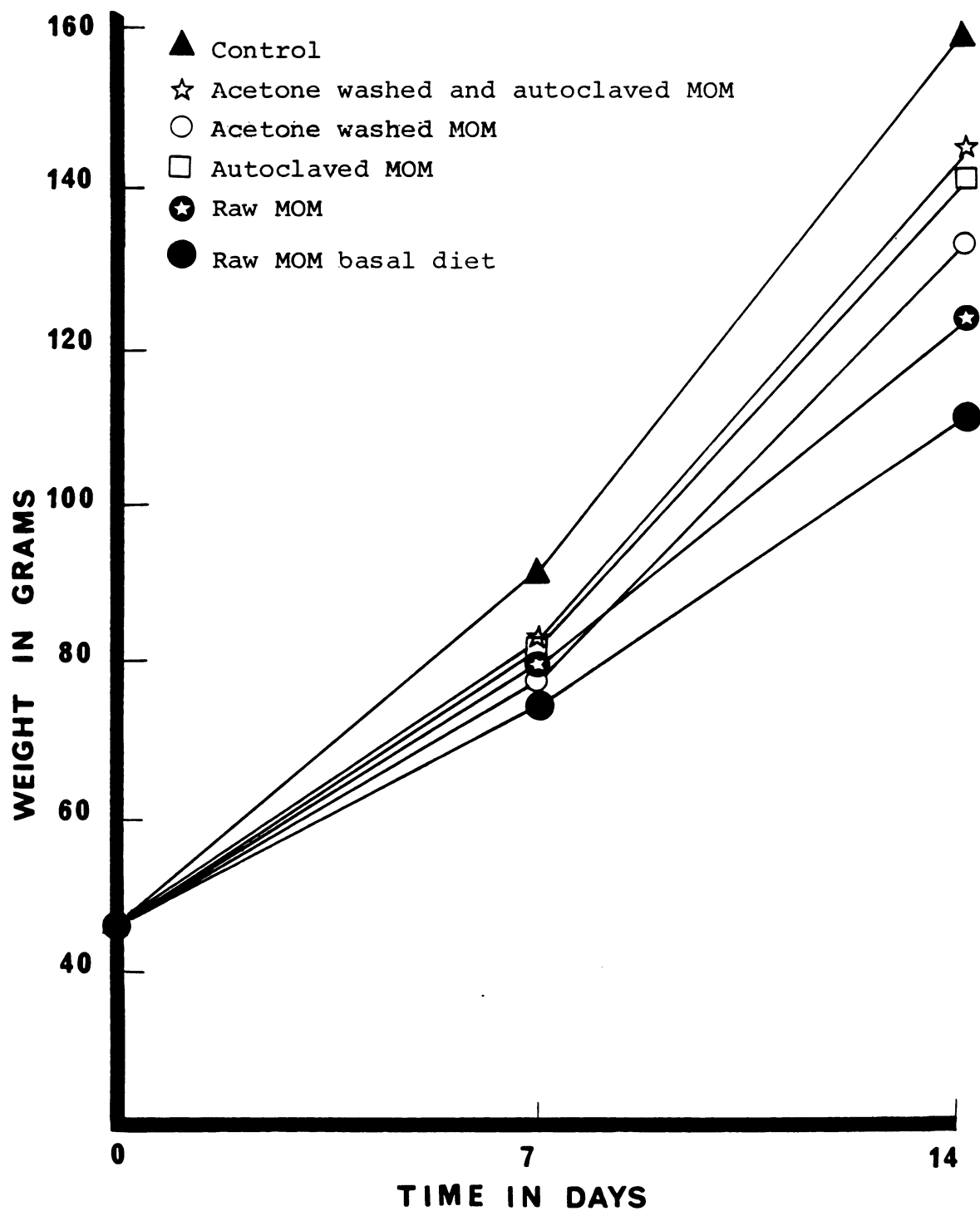


Figure 10. Weekly weight gain of chicks fed experimental diets containing 20% treated or untreated MOM

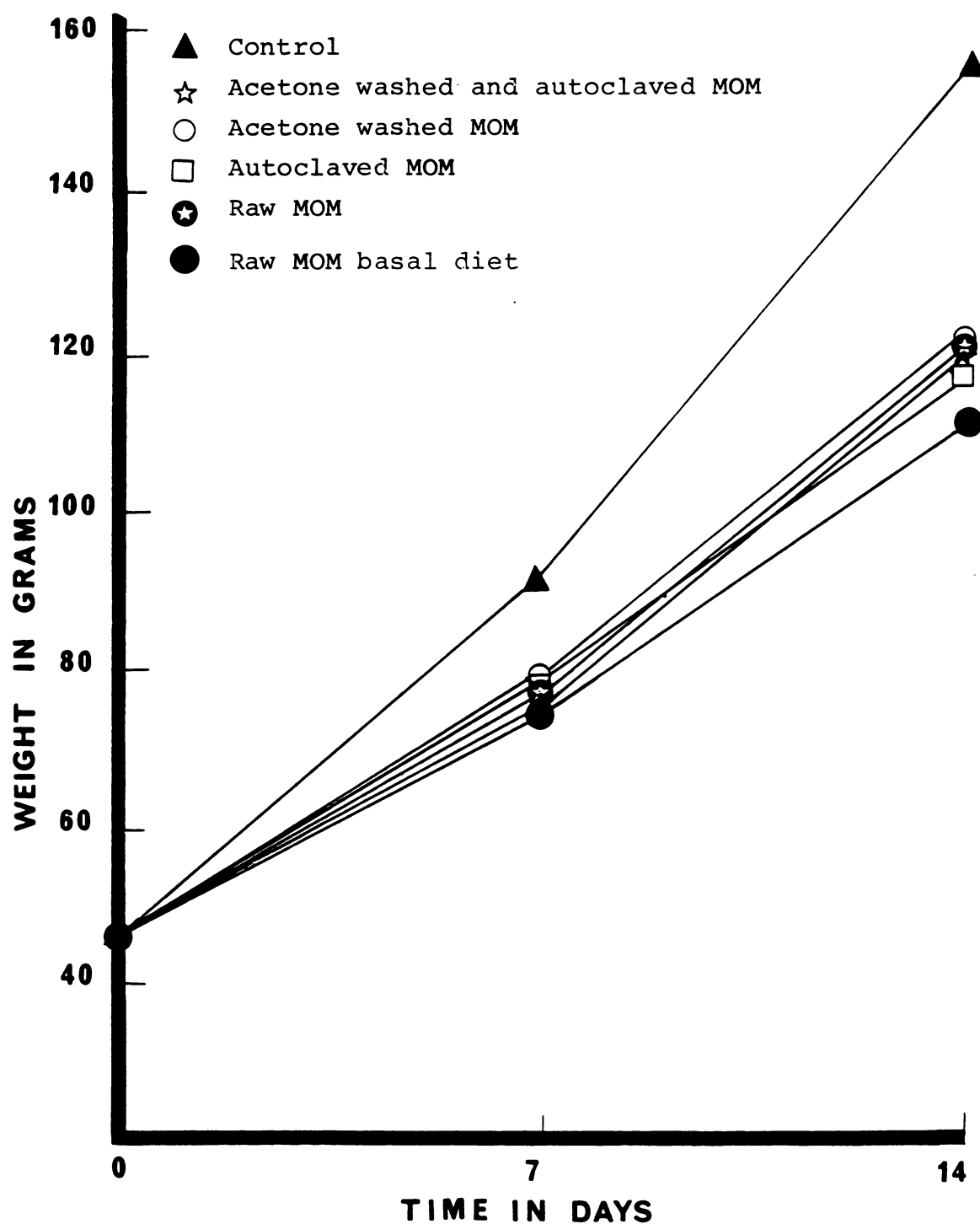


Figure 11. Weekly weight of chicks fed experimental diets at 30% level of treated or untreated MOM

PART I: EXPERIMENT 6: PARTITIONING THE EFFECT OF ERUCIC
ACID AND OTHER ANTINUTRITIVE COMPONENTS IN MUSTARD
OIL MEAL (MOM) USING THE CHICK GROWTH ASSAY

INTRODUCTION

Deleterious effect of erucic acid in MOM has been evidenced by several researchers (Abdellatif and Vles, 1970a; Abdellatif and Vles, 1970b; Abdellatif et al., 1972; Ray et al., 1979; Prasad and Rao, 1982). In experiment 4 of part 1 of this manuscript it was observed that acetone extraction of MOM resulted in better growth performance of the chicks compared to the chicks fed raw MOM in the diets. In experiment 5, part 1, it was observed that mustard oil addition to standard reference diet reduced growth significantly. However, corn-MOM diets, which contained equivalent erucic acid, supported significantly lower growth. The results suggested that in MOM, in addition to erucic acid other antinutritive factors are present (glucosinolates, tannins etc.)

This experiment was designed to partition the effect of the oil faction (erucic acid) and the meal fraction (combined effects of glucosinolates, tannins etc.).

Materials and Methods:

In this experiment MOM from batch 3 was used.

Oil extraction:

A Soxhlet type extractor was used to extract oil from MOM. Hexane was used as organic solvent. After the extraction the mixture was evaporated under the hood till no odor remained. Mustard oil was stored in brown glass bottles and kept in the refrigerator until used.

Acetone washing:

Mukherjee et al. (1979) removed glucosinolates by washing RSM with 70% acetone. Pure acetone was mixed in a vat with defatted MOM at the ratio of 3:1, in a vat, stirred occasionally for 2 to 3 hours and let stand for 24 hours under the hood. The supernatant liquid was siphoned off into a large beaker and evaporated under the hood until the smell of acetone was completely gone.

Diet preparation:

Combinations of dietary treatments are given in Table 27 and composition of the diets are given in Table 28. All the diets are equicaloric (ME; 3132 Kcal/kg) and equinitrogenous (CP; 21.32%). Defatted MOM, defatted and acetone washed MOM, and canola meal contribute crude protein in the diets equivalent to the percent crude protein contributed by the raw MOM in the corresponding diets.

TABLE 27. COMBINATION OF DIETARY TREATMENTS

Dietary treatments	Effects to be studied
1. Corn-soy diet	---
2. Corn-soy diet + Mustard oil	E
3. Corn-soy diet + Act. wd. rd.	(G+O)
4. Raw MOM diet	E+G+O
5. Defatted MOM	(G+O)
6. Acetone washed defatted MOM	(G+O)
7. Canola meal	(G+O)

G= Glucosinolate; E= Erucic acid; O= Others;

TABLE 28. DIETARY COMPOSITIONS

Ingredients	Diet #s						
	1	2	3	4	5	6	7
Yellow corn	54.56	54.56	54.16	47.95	47.95	47.95	49.38
SBM	37.00	37.00	37.00	---	---	---	---
CGM	---	---	---	6.32	6.32	6.32	5.70
Corn oil	4.46	---	4.46	2.25	6.07	6.52	5.30
Mustard oil	---	4.46	---	---	---	---	---
Acetone Wd. residue	---	---	0.30	---	---	---	---
MOM	---	---	---	39.04	---	---	---
Defatted MOM	---	---	---	---	35.53	---	---
Deftd. & Ac. Wd. MOM	---	---	---	---	---	34.59	---
Canola Meal	---	---	---	---	---	---	35.71
Dical	1.57	1.57	1.57	1.37	1.37	1.37	1.41
Limestone	1.43	1.43	1.43	1.20	1.20	1.20	1.17
Common Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Trace Min. Mix.	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Vitamin Mix.	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Dl-Meth.	0.25	0.25	0.25	0.10	0.10	0.10	0.08
Lysine-HCL	---	---	---	0.02	0.02	0.02	0.26
L-Arginine	---	---	---	0.12	0.12	0.12	0.26
Solkaflok	---	---	---	0.90	0.52	1.08	---

Experimental:

Day-old Hubbard broiler male chicks were wing banded, weighed and grouped in four weight groups. Upper and lower weight groups were discarded. From the two selected

groups, equal numbers of chicks were randomly assigned to 21 pens(10 birds/pen) in a battery brooder. Seven dietary treatments were randomly assigned to 21 pens (3 pens/ treatments). Chicks were fed ad libitum and fresh water was available at all times. Weekly weight gains and feed consumptions were recorded.

Results and discussion:

Initial weight, final weight, feed consumption and feed to gain ratio are given in Table 29. Growth patterns during the two week growth assay period are shown in Figure 12. Final weight was subjected to ANOVA (Appendix C-8; page 151).

TABLE 29. PERFORMANCE OF CHICKS IN EXPERIMENT 6

Diet #	Initial Weight	Final Weight	Weight Gain	Feed Intake	F/G Ratio	% Nitrogen Absorbability
1	40.0	344.3	304.3	420.0	1.4	51.8
2	39.8	300.3	260.5	379.9	1.5	47.4
3	39.8	321.2	281.3	402.6	1.5	46.9
4	40.5	249.4	208.8	349.6	1.7	53.8
5	40.0	272.0	232.0	343.9	1.5	52.2
6	40.1	283.8	243.7	368.2	1.5	46.1
7	40.1	303.4	263.3	381.2	1.4	52.6

SE = ± 8.43 ; 95% CI for group means = $\bar{Y} \pm 18.08$

The addition of mustard oil equivalent to the amount in diet 4 to the standard reference diet significantly reduced growth of chicks ($P < 0.01$). There was significant improvement in growth rate of chicks fed defatted MOM (diet #5) compared to the chicks fed diet 4 ($P < 0.01$). Defatting and acetone

was significantly lower than the growth rate of chicks fed the canola meal diet (diet # 7), which was low in glucosinolates and devoid of erucic acid ($P < 0.01$). There was numerical improvement in growth rate upon acetone washing of defatted MOM. Growth depression due to the addition of acetone washed residue to standard reference diet was not equal in magnitude to the growth improvement observed in chicks fed defatted and acetone washed MOM ($P < 0.01$).

The combined effect of the addition of mustard oil and acetone washed residue to the standard reference diet was significantly different ($P < .001$) from the combined effect of defatting and acetone washing and defatting of MOM. It therefore suggests that even after defatting and acetone washing of MOM it fails to achieve weight gain comparable to SBM diet, which is indicative of presence of growth depressing factors that can not be destroyed by deffating and or acetone washing of MOM.

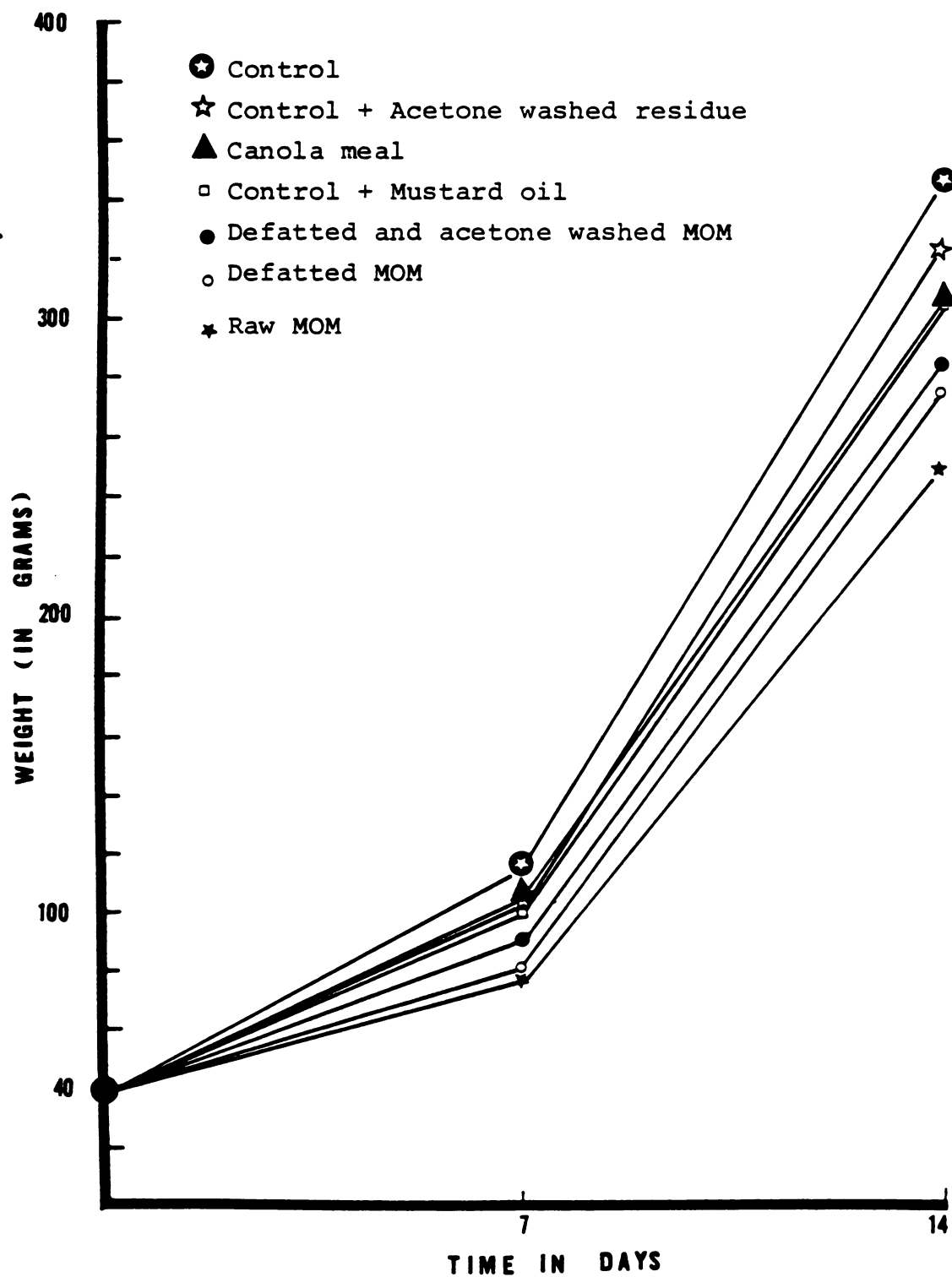


Figure 12. Weekly weight gain of chicks fed treated and untreated mustard oil meal (MOM)

EXPERIMENTAL PART II

1. Experiment 1: Effects of temperature, moisture and duration of heating on the nutritive value of mustard oil meal (MOM) using invitro and invivo assay techniques
2. Experiment 2: Development of a low lysine basal diet to establish a standard reference curve
3. Experiment 3: Effects of temperature, moisture and duration of heat treatment on the available lysine value of mustard oil meal (MOM) using chick growth assay
4. Experiment 4: Effect of amino acid supplementation on the utilization of mustard oil meal (MOM) by growing chicks

PART II: EXPERIMENT 1: EFFECTS OF TEMPERATURE, MOISTURE AND DURATION OF HEATING PERIOD ON THE NUTRITIVE VALUE OF MUSTARD OIL MEAL (MOM) USING VARIOUS IN VITRO AND IN VIVO TECHNIQUES

INTRODUCTION

In part 1 of this study it was seen that heating of MOM improves its feeding value for growing chicks. Wet heating was more effective in improving its utilization by the chicks compared to dry heat treated MOM. However, wet heating of the meal required added energy expense in terms of heating required to dry the slurry when MOM is boiled.

Toxicity in MOM seem to be thermally sensitive to some extent. It was seen that autoclaving of meal for 30 minutes at 121 C reduced several amino acids in the resultant meal. Especially lysine was reduced by 17.5%. It was also observed that autoclaving of the meal supported maximum growth compared to other treatments in several of the feeding trials conducted in part 1. The growth promoting effect of autoclave treated meal seem to be independent of lysine destruction (Gray et al., 1957). There seem to be a complex interaction of moisture, temperature and duration of heat treatment on the nutritive value of MOM. Josefsson (1975) found that the optimum temperature and time combinations for best nutritional value of low glucosinolate

rapeseeds were 100-110 C and 15-60 minutes at a moisture content of 8%. MOM used in this study had high moisture content (12.5%). Information on the effects of addition of high moisture levels in MOM at low and high temperatures is limited. This experiment was designed to study the effects of addition of high levels of moisture to MOM prior to the treatments with temperatures for different periods of time.

Materials and methods:

Heating System:

In this study, a drum heating system was used to heat MOM. The heating system is of infra red lamps (dimmer switch controlled). Drum rotates on a horizontal hollow rod, through which thermocouples are inserted to record temperature at different depth. Figure 13 and 14 show external diagram and placement of thermocouples inside of the drum respectively.

Design of Experiment:

Two temperature levels three moisture levels and three duration of the heat treatments were used. The experimental plan is given in Table 30. The following analyses were performed on treated MOM:

1. Dry matter
2. Crude protein
3. Ether extract
4. Gross energy
6. Total thioglucosides
7. Allylisoithiocyanate
8. Erucic acid
9. Total lysine

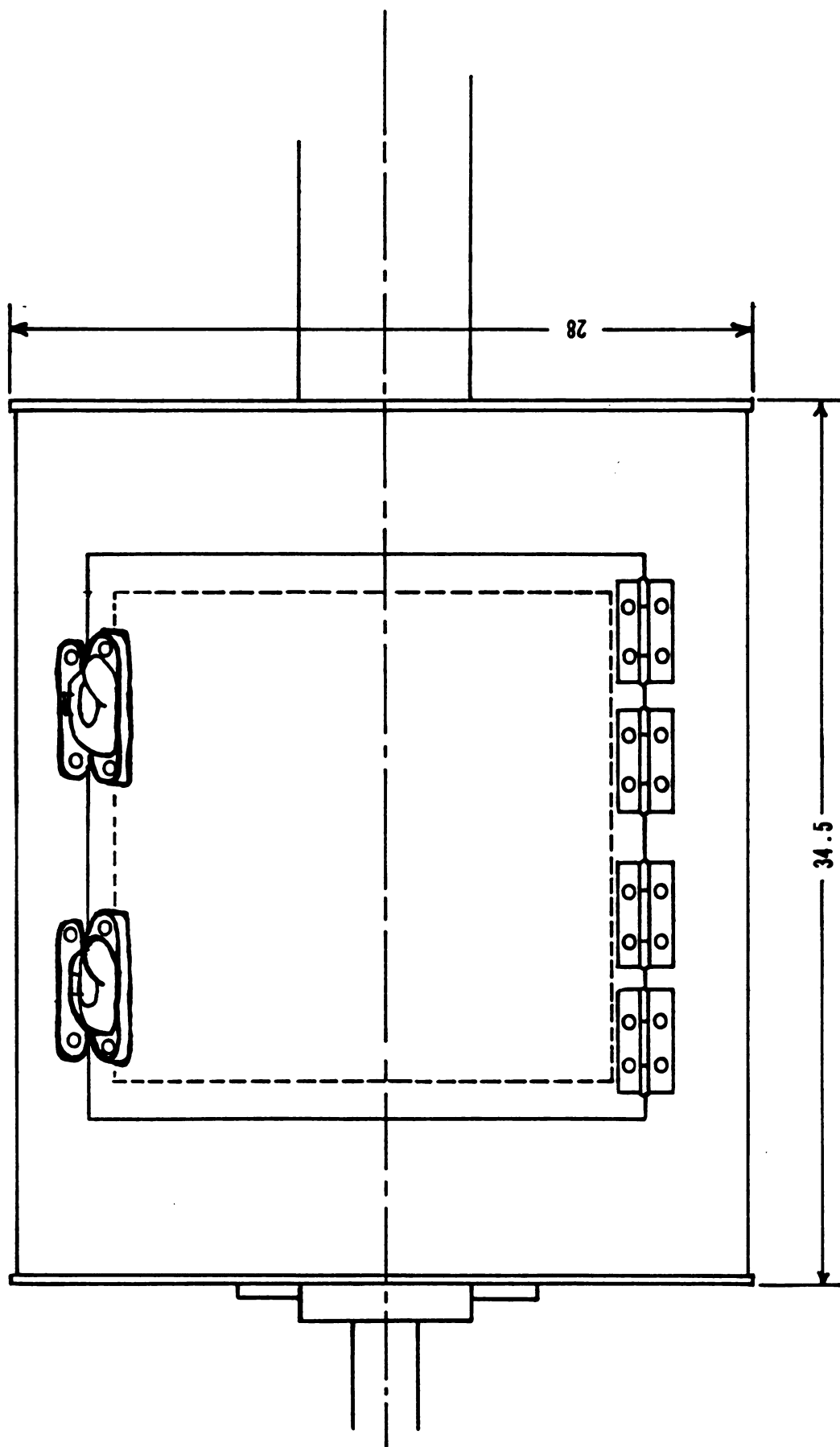


Figure 13. External diagram of the drum (dimensions in cm)

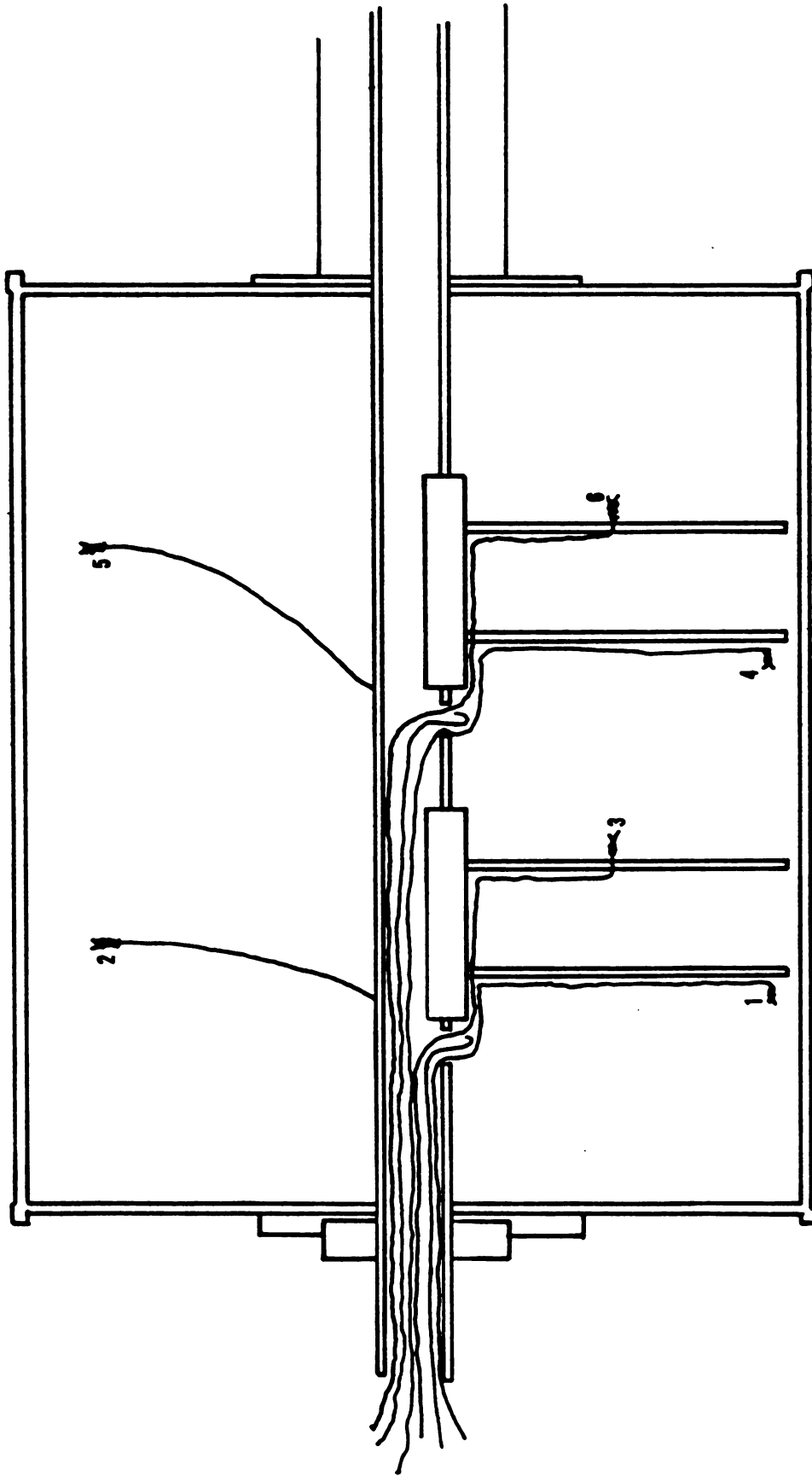


Figure 14. Placement of the thermocouples inside of the drum (tips are numbered)

Reactive lysine determination:

Reactive lysine was determined using a modified dye binding procedure developed in our laboratory (appendix A-I, page 136) similar to the methods used by Hurrell and Carpenter (1979) and Walker (1979). Samples were analyzed in duplicates. Experimental design was a 2 X 3 X 3 plus control (Gill, 1978). The analysis of variance table is presented in appendix C-9 (page 151).

ME determination:

The TME method of Sibbald (1982) was used with some modification (appendix A-VII, page 144). Twenty SCWL roosters were selected from a group of 29 roosters. Experimental design was split plot (Gill, 1978). Period was main plot and temperature was subplot. In the first period 20 dietary treatments (including a blank and a control) were randomly assigned to 20 roosters. In the second period only temperature was reversed eg. the rooster that was fed MOM treated at 85-12.5-15 in the first period was fed MOM treated at 100-12.5-15. ME was subjected to ANOVA (appendix C-10; page 152).

TABLE 30. TREATMENT COMBINATIONS

Temperature C	Moisture levels %	Heating Period min
85	natural (12.5)	15
		30
		60
	to 25.0	15
		30
		60
	to 37.5	15
		30
		60
100	natural (12.5)	15
		30
		60
	to 25.0	15
		30
		60
	to 37.5	15
		30
		60

Results and Discussion:

DM, CP and EE are presented in Table 31. In Table 32 erucic acid and total glucosinolate contents are presented. Total lysine, reactive lysine and available lysine values are presented in Table 33. Gross energy and metabolizable energy are provided in Table 34.

TABLE 31. DRY MATTER, CRUDE PROTEIN AND ETHER EXTRACT
CONTENT OF TREATED MUSTARD OIL MEAL (MOM)

Treatments	DM %	CP %	EE %
Raw MOM	87.50	37.92	13.22
85-12.5-15*	94.48	35.99	9.73
30	94.72	37.67	10.31
60	92.66	37.61	10.39
25.0-15	92.04	37.29	9.28
30	91.36	37.59	8.82
60	91.79	37.62	9.34
37.5-15	87.36	36.64	9.23
30	87.21	37.53	9.61
60	92.77	36.21	9.41
100-12.5-15	94.71	36.34	11.09
30	95.69	35.72	10.51
60	94.06	37.77	10.73
25.0-15	90.32	38.51	9.72
30	91.86	38.84	9.17
60	91.78	37.24	10.07
37.5-15	91.13	38.01	9.70
30	91.06	37.24	8.69
60	91.27	37.89	9.32

* Expressed as Temp - Moisture - Time; Values are means
of two replicates expressed on DMB

TABLE 32. ERUCIC ACID AND TOTAL GLUCOSINOLATE CONTENT

Treatments	Erucic* acid %	Total** Thioglucosides %
Raw MOM	37.01	4.75
85-12.5-15	28.67	3.96
30	27.58	3.87
60	24.04	3.79
25.0-15	23.34	3.99
30	24.93	3.27
60	23.43	3.75
37.5-15	27.61	4.09
30	24.31	3.83
60	27.66	4.05
100-12.5-15	25.08	3.74
30	24.08	4.31
60	27.66	3.74
25.0-15	25.68	3.95
30	22.53	3.64
60	26.02	4.37
37.5-15	24.38	3.87
30	25.31	4.00
60	19.69	4.16

* Percent of residual oil; ** Fat free DMB

Average effect of heat treatment on the erucic acid content in residual oil was compared with raw MOM using Studentized t-test (Gill, 1978). Heat treated MOM contained significantly less erucic acid in its residual oil ($P < 0.05$). However, there were no significant differences among the heat treated MOM.

ANOVA for total glucosinolates is presented in appendix C-11 (page 153). Average effect of heat treated MOM was compared to the raw MOM using Studentized t-test (Gill, 1978). Heat treated MOM contained significantly lower levels of total glucosinolate compared to the raw MOM ($P < 0.05$).

TABLE 33. TOTAL, REACTIVE AND AVAILABLE LYSINE CONTENT

Treatments	Total (g/kg CP)	Reactive (g/kg CP)	Available (%)
Raw MOM	60.19	46.16	76.68
85-12.5-15	60.29	50.65	84.01
30	56.62	47.59	84.05
60	57.59	47.72	82.86
25.0-15	59.31	48.45	81.66
30	53.63	47.89	89.29
60	53.80	41.76	77.62
37.5-15	52.53	53.93	102.66
30	51.42	52.13	101.36
60	49.59	43.72	88.16
100-12.5-15	56.27	41.67	74.03
30	59.79	41.47	69.34
60	54.77	38.66	70.56
25.0-15	49.33	44.61	90.00
30	47.68	42.61	89.36
60	48.38	41.00	84.75
37.5-15	51.30	39.71	77.41
30	52.22	38.54	73.80
60	49.40	32.13	65.04

Availability of lysine using dye binding assay technique for heat treated MOM was factorially analyzed (Gill, 1978). It has to be noted that availability figures are based on means of two replicates of RL and TL values (Table 33). ANOVA for reactive lysine is presented in appendix C-9 (page 151) and for available lysine in appendix C-19 (page 157).

Average available lysine value of treated MOM was significantly greater than for raw MOM ($P < 0.05$). Similarly, average reactive lysine value for raw MOM was significantly smaller compared to the average of treated MOM ($P < 0.05$).

TABLE 34. GROSS ENERGY AND METABOLIZABLE ENERGY CONTENT

Treatments	GE (KCal/kg)	ME* (KCal/kg)
Raw MOM	4.65	3.29
85-12.5-15	4.81	2.95
30	4.88	2.92
60	4.88	2.83
25.0-15	4.75	3.13
30	4.77	3.03
60	4.73	2.87
37.5-15	4.55	2.77
30	4.56	2.78
60	4.82	2.68
100-12.5-15	4.92	2.89
30	4.84	2.97
60	4.87	2.91
25.0-15	4.77	3.07
30	4.75	2.90
60	4.75	2.72
37.5-15	4.79	3.17
30	4.79	3.12
60	4.77	2.95

* DMB

ANOVA for ME values is presented in appendix C-10 (page 152). Data was analyzed in a split plot design (Gill, 1978). Only the effect of period of heat treatment was significantly different ($P < 0.05$). Average effect of heat treated MOM was compared to raw MOM using Studentized t-test (Gill, 1978). There was no significant difference between heat treated and raw MOM in ME value. Similar result was reported by Hijikuro and Takemasa (1979a).

PART II: EXPERIMENT 2: DEVELOPMENT OF A LOW LYSINE BASAL
DIET TO ESTABLISH A STANDARD REFERENCE CURVE

INTRODUCTION

An important step is to develop a standard curve for a chick growth assay for available lysine. To do that, selection of a basal diet that is low in lysine but adequate in all other nutrients is needed. This experiment was conducted to establish reference points for a standard curve using a practical type diet without the use of crystalline amino acid mixtures.

Materials and methods:

Protein sources that are low in lysine but adequate in other amino acids were used to balance a basal diet that contains less than half the recommended level of lysine but is adequate in all other nutrients. Composition of the basal diet is presented in table 35. The diet contained 21.13% CP; 3217 Kcal/kg ME and lysine content was 0.51% on an as fed basis.

TABLE 35. COMPOSITION OF BASAL DIET

Ingredients*	%
Corn gluten meal	12.00
Sesame seed meal	18.24
Yellow corn	64.76
Dical (Ca,21%; P,18.5%)	1.68
Limestone (ca,38%)	0.70
Common salt	0.40
Trace min. mix ¹	0.03
Vitamin premix ²	0.30
Solkafloc	1.89

* Detailed compositions in appendices B-1,2
(Page 145); 1 & 2 appendices B-3,4 (Page 146)

Reference diets:

Two reference diets were constructed by adding 0.1 and 0.2% lysine (L-Lysine.HCL,78.4%) to the basal diet at the cost of corn.

Experimental:

Sixty Hubbard broiler male, day old chicks were fed a basal diet for 5 days. Thirty chicks were selected and randomly assigned to six pens (5 chicks/pen). Three dietary treatments were randomly assigned to six pens (2pens/treatment). Five day weight was used as the initial weight. Chicks were fed ad libitum and fresh water was available at all times.

Results and Discussion:

Initial weight, two week weight gain and feed consumption data are presented in Table 36. In Table 37, Weight gain, lysine consumed and weight gain as a function of lysine in the diets are presented. Linear regression

equations were developed using two week weight and weight gain of chicks on Basal, Basal+ .1% Lys and Basal+.2% Lys diets. ANOVA is presented in appendix C-12 (page 153). Linear equation $Y = 71.51 + 79.41(X)$ was developed using cumulative gain (5 chicks/pen), in which $Y = \text{weight gain(g)}$ and $X = \text{lysine consumed}$. (appendix C-13; page, 153)

TABLE 36. PERFORMANCE OF CHICKS IN EXPERIMENT 2

Diet #	Initial wt (in gm)	Two wk wt (in gm)	Feed Intake	F/G ratio
1 Basal	39.5	122.73	177.28	2.13
2 Basal+0.1	38.9	162.97	233.98	1.89
3 Basal+0.2	39.7	225.50	302.85	1.63

TABLE 37. LYSINE CONSUMPTION AND WEIGHT GAIN OF CHICKS IN EXPERIMENT 2

Diet #	Lysine consumed (gm)	Weight gain (gm)	Wt/lys ratio
1 Basal*	0.816	79.80	97.77
2 Basal +0.1	1.280	114.90	89.73
3 Basal +0.2	2.156	186.00	86.25

* Basal diet contain 0.512% lysine

It is apparent from Table 37 that efficiency of lysine utilization decreases as the amount of lysine consumed increases. Two week weight and percentage of lysine in the diets showed linear response ($P < 0.0009$).

Figure 14 shows the weekly and cumulative weight gain as a function of amount of lysine consumed. There was a linear relationship between cumulative weight gain and

lysine consumption ($P < 0.0001$). Lysine consumption per pen and weight gain per pen are presented. In each pen there were 5 chicks. Weight gain was highly correlated to lysine consumed ($r = .98$). These results suggest that at .5 to .7 % lysine in the diets there is a linear relationship between lysine consumption and weight gain. Usefulness of a practical type of low lysine basal diet was also evidenced. For the experiments to examine lysine availability in a given feedstuff, basal diet tested in this experiment will be useful. Test ingredients should contribute .1 to .2% lysine in the experimental diets, to stay in the linear portion of the response curve.

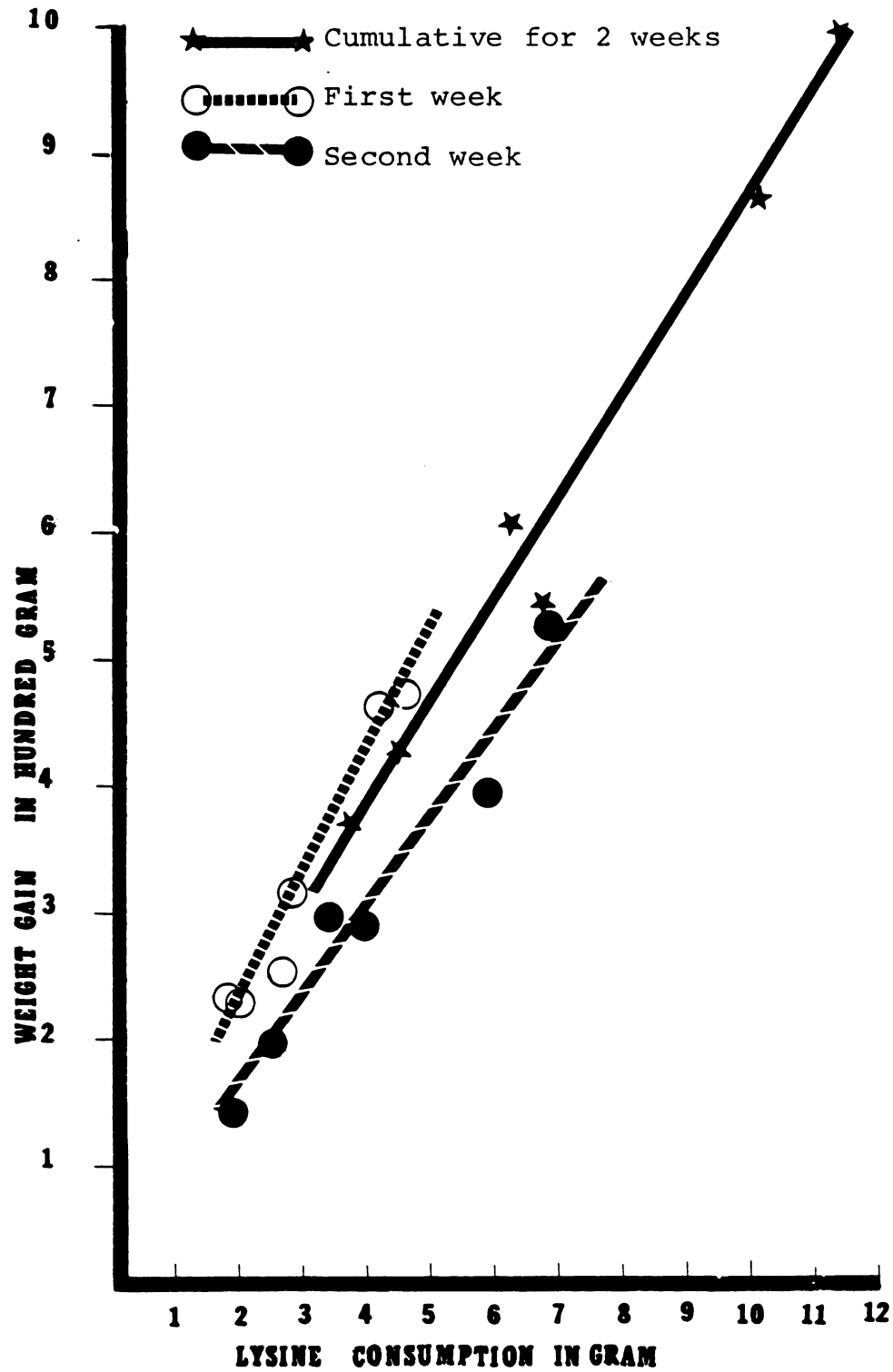


Figure 15. Group weight gain (5 birds/pen) of chicks as a function of amount of lysine consumed

PART II: EXPERIMENT 3: EFFECT OF TEMPERATURE, MOISTURE AND DURATION OF HEAT TREATMENT ON THE AVAILABLE LYSINE VALUE OF MUSTARD OIL MEAL USING CHICK GROWTH ASSAY

INTRODUCTION

Many researchers have reported that some form of heating improves the nutritive value of plant proteins (Olsen et al., 1975; Bayley and Summers, 1975; Srihara and Alexander, 1984). Severe heating destroys basic amino acids especially lysine (Wolzak et al., 1981). Progressive decline in available lysine value of RSM, upon autoclaving at 120 C for 15 to 120 minutes was reported (Rayner and Fox, 1976). Lower temperature heating (98.9 C) of rapeseeds prior to oil extraction resulted in better lysine content (Clandinin and Tajcner, 1961). Capper et al.(1982) reported severe decline in lysine content as well as availability upon open pan roasting of mustard seeds prior to oil extraction. In experiment 3 of part 1 it was observed that autoclaving at 121 C for 30 minutes decreased the lysine content by 17% and by heating in an oven (at 121 C for 30 min) by 10%. Improvement in weight gain observed in experiments 1, 2 and 4 of part 1 of this manuscript is supported by the findings of Gray et al. (1957). They attributed the improvement in gain to something other than lysine

destruction

The basic amino acids, lysine , arginine and histidine are most heat labile in MOM (McGhee et al.,1964). MOM is relatively balanced in all amino acids. However, it is low in Lysine. Lysine is of most interest due to its importance in total nutritive value for the animals. This experiment was conducted to investigate the availability of lysine in MOM upon heat treatments.

Materials and Methods:

MOM used in this experiment was from batch 3. Treatment combinations for MOM was selected from the experiment 1 of part 2. Two levels of temperature, two levels of moisture and two levels of duration of heating were selected for this experiment.

Basal diet:

A pretested diet (expt 2 partII) with slight modification was used as a basal diet. Reference diets were constructed by adding incremental levels of lysine HCl (78.4%). Composition of the basal diet is presented in Table 38. From the results of experiment 2, of part 2 it was apparent that 0.5 to 0.7 % lysine gave linear relationships between weight gain and lysine in the diet. Combinations of reference diets are presented in Table 39.

Experimental diets:

Experimental diets were constructed by adding

treated MOM at two levels (0.1% and 0.25%) of lysine supplementation at the expense of corn starch. Solkafloc was adjusted to balance crude fiber content of the diets. Heat treatment was in a 2 X 2 X 2 factorial design (temperature, moisture and period of heating). Treatment combinations are presented in Table 40.

TABLE 38. COMPOSITION OF BASAL DIET

Ingredients	%
Corn starch	13.00
CGM	12.61
SSM	20.00
Ground yellow corn	50.24
Dical	1.75
Limestone	0.59
Trace mineral mix	0.03
Vitamin mix	0.30
Common salt	0.40
Solkafloc	1.08

TABLE 39. COMBINATIONS OF REFERENCE DIETS

Diet #	+ lysine (%)
1 Basal	
2 Basal	+0.10
3 Basal	+0.20
4 Basal	+0.30
5 Basal	+0.60

Experimentals:

Four hundred and fifty, day-old male Hubbard broiler chicks were wing banded and kept on a low lysine basal diet for 3 days. After 3 days chicks were weighed and grouped in 4 weight groups. Upper and lower weight groups were

discarded. Birds weighing 46 to 57 gm were selected. Five chicks were randomly selected and placed in each of the sixty four pens. Diets 1 thru 5 had two replicates and diets 6 thru 23 had 3 replicates each. Dietary treatments were randomly assigned to the pens. Chicks were fed ad libitum and fresh water was available at all times.

TABLE 40. AMOUNTS OF TREATED MOM IN THE DIETS*

Diet #	Treatment combination	For +0.1 Lys (%)	For +0.25 Lys (%)
6	85-12.5-15	4.61	
7	15		11.52
8	85-12.5-60	4.62	
9	60		11.54
10	25.0-15	4.52	
11	15		11.30
12	25.0-60	4.94	
13	60		12.35
14	100-12.5-15	4.89	
15	15		12.22
16	60	4.83	
17	60		12.08
18	25.0-15	5.26	
19	15		13.16
20	60	5.55	
21	60		13.87
22	Raw MOM	4.97	
23	Raw MOM		12.44

* As fed basis

Results and discussion:

Average initial weight, final weight (17 days), feed intake (average) and feed efficiency ratios of the chicks fed basal and reference diets and experimental diets are presented in Table 41.

TABLE 41. PERFORMANCE OF CHICKS ON EXPERIMENTAL DIETS
IN EXPERIMENT 3

Diet #	Treatment	Initial Weight (gm)	Final Weight (gm)	Feed Intake (gm)	F/G Ratio
1	Basal diet	50.60	104.00	174.00	3.26
2	Basal + 0.1 Lys*	51.60	125.00	214.50	2.92
3	Basal + 0.2 Lys	51.70	171.62	273.50	2.28
4	Basal + 0.3 Lys	52.10	226.00	345.20	1.99
5	Basal + 0.6 Lys	52.90	339.75	442.25	1.54
6	Raw MOM +0.10 Lys	50.00	121.00	190.80	2.69
7	" " +0.25 "	51.00	175.00	268.00	2.16
8	85-12.5-15 +0.10 "	52.20	134.33	219.33	2.67
9	" " " +0.25 "	52.50	170.66	260.00	2.20
10	" " 60 +0.10 "	51.60	126.66	202.67	2.70
11	" " " +0.25 "	51.90	184.33	289.60	2.19
12	" 25.0-15 +0.10 "	50.70	129.66	200.67	2.54
13	" " " +0.25 "	51.40	188.66	292.67	2.13
14	" " 60 +0.10 "	51.30	114.33	177.67	2.82
15	" " " +0.25 "	51.40	182.33	280.00	2.14
16	100-12.5-15 +0.10 "	51.70	126.66	196.67	2.62
17	" " 15 +0.25 "	51.90	180.23	284.00	2.21
18	" " 60 +0.10 "	51.50	128.00	202.00	2.64
19	" " " +0.25 "	52.10	173.33	259.00	2.14
20	" 25.0-15 +0.10 "	51.40	134.83	194.67	2.33
21	" " " +0.25 "	52.20	199.66	292.67	1.98
22	" " 60 +0.10 "	51.30	131.33	210.67	2.63
23	" " " +0.25 "	51.80	194.00	300.00	2.11

It is apparent that chicks on diets with higher lysine concentration ate more and grew more. Feed efficiency ratios followed similar patterns. Feed efficiency was poorest for the basal diet.

Daily gains above the basal diet for the chicks fed reference diets and supplemented lysine percentage were used to develop a regression equation $Y = -0.530 + 23.81 (X)$, where Y = gain above basal (g) and X = % of lysine from L-Lysine.HCL. The response was linear for the levels tested in this experiment ($P < 0.0001$). Regression equations for the

treatments were also developed in a similar manner. Regression parameters are presented in Table 42. ANOVA is presented in appendix C-14 (page 154)

TABLE 42. REGRESSION PARAMETERS OF EXPERIMENT 3

Treatments	Intercept	Slope	SE (slope)	r	r ²	SE ^a (est.)
Standard	-0.530	23.81	1.16	0.99	0.98	0.76
Raw MOM	-1.230	21.53	6.25	0.93	0.86	0.94
85-12.5-15	0.280	14.13	2.03	0.96	0.92	0.37
60	-0.980	22.58	5.13	0.91	0.83	0.94
25.0-15	-0.790	22.89	3.72	0.95	0.90	0.68
60	-2.090	26.58	2.43	0.98	0.97	0.45
100-12.5-15	-0.830	21.00	2.12	0.98	0.96	0.39
60	-0.400	17.56	2.22	0.97	0.94	0.41
25.0-15	-0.730	24.96	3.15	0.97	0.94	0.58
60	-0.870	24.04	2.79	0.97	0.94	0.51

^a Standard error of estimates

Final weight of the chicks (Table 41.) were used to compute daily gain and available lysine from MOM was determined using regression equation for the reference diets. Daily gain, dietary lysine from MOM, percent available lysine from MOM and lysine availability are presented in Table 43. A ninety five % confidence interval was established using inverse regression as described by Gill (1978). Effects of temperature, moisture, period of heating and levels of lysine from MOM were examined by Yates' method (Gill,1978). Detailed analysis is presented in appendix C-15 (page 154).

TABLE 43. DAILY GAIN, AVAILABLE LYSINE IN THE DIETS,
AND LYSINE AVAILABILITY OF MOM

Treatments	Lysine From MOM (%)	Daily Gain (gm)	available Lysine (%)	(95% CI)	Availability (%)
Raw MOM	0.10	1.036	0.066	(.008 -.127)	65.80
" "	0.25	4.154	0.197	(.141 -.254)	78.64
85-12.5-15	0.10	1.691	0.093	(.044 -.145)	93.30
" " "	0.25	3.810	0.182	(.135 -.231)	72.92
" " 60	0.10	1.275	0.076	(.027 -.129)	75.80
" " "	0.25	4.650	0.217	(.170 -.266)	87.04
"-25.0-15	0.10	1.505	0.085	(.037 -.137)	85.50
" " "	0.25	4.934	0.229	(.179 -.273)	91.80
" " 60	0.10	0.568	0.046	(.003 -.100)	46.20*
" " "	0.25	4.562	0.214	(.167 -.261)	85.56
100-12.5-15	0.10	1.269	0.076	(.027 -.128)	75.60
" " "	0.25	4.409	0.207	(.161 -.255)	83.00
" " 60	0.10	1.360	0.079	(.031 -.132)	79.40
" " "	0.25	3.991	0.189	(.143 -.238)	75.96
"-25.0-15	0.10	1.768	0.096	(.048 -.148)	96.50
" " "	0.25	5.538	0.255	(.207 -.302)	101.88
" " 60	0.10	1.568	0.088	(.039 -.140)	88.10
" " "	0.25	5.225	0.242	(.194 -.289)	96.68

* Lower extreme of the treatment means

The mean lysine availability values were factorially analyzed using Yates' method (Gill, 1978). The detailed analysis is given in appendix C-15 (Page 154). The main effects (temperature (T), moisture (M) and period of heat treatment (P)) and 4 two way interactions were significantly different ($P < 0.1$). Evidence of treatment effects are not strong as there were fewer degrees of freedom. It is to be noted that availability figures were computed using average of three replicates.

Combined average effect of temperature, moisture and period of heating was compared with the raw MOM using

Studentized t-test (Gill, 1978). Heat treated MOM was significantly higher in lysine availability compared to raw MOM ($P < 0.05$). The average availability of lysine in raw MOM was 72.22% and for heat treated MOM it was 83.45%.

Dunnett's t-test (Gill, 1978) was used to compare raw MOM to individual heat treated MOM across 0.1% lys and 0.25% lysine levels. Only MOM treated at 85-12.5-15 and 100-25.0-15 were significantly higher in available lysine compared to raw MOM ($P < 0.1$).

Average lysine availability value for MOM treated at 85 C was 79.76% compared to 87.14% for 100 C. Average lysine availability for 12.5% moisture was 80.37% compared to 86.5% for 25% moisture level. Availability of lysine at 0.1% lysine was 80.05% and at the 0.25% level it was 86.65%. Availability of lysine averaged across the duration of heat treatment was 87.05% for 15 min compared to 79.34% for 60 min.

It is apparent from these data that availability of lysine is higher when MOM is treated at 100 C, at moisture level of 25.0% and treated for 15 min.

Table 44 is constructed by extracting information on lysine availability values from Table 33 (page 108) determined by dye binding assay and from Table 43 (page 121) determined by chick growth assay to establish the correlation between the two methods of lysine availability.

TABLE 44. LYSINE AVAILABILITY VALUES DETERMINED BY TWO DIFFERENT METHODS

Treatments	Dye Binding Assay (DBA)	Chick growth Assay (CGA)
Raw MOM	76.68	72.22
85-12.5-15	84.01	83.11
" " 60	82.86	81.42
" 25.0-15	81.65	88.65
" " 60	77.62	65.88
100-12.5-15	74.03	79.30
" " 60	70.56	77.68
" 25.0-15	90.00	99.19
" " 60	84.75	92.39

Data from Table 43 was used to determine correlation between dye binding assay (DBA) and chick growth assay (CGA) using product moment correlation (Gill, 1978). DBA was found to be significantly ($P < 0.05$) correlated with CGA ($r^2 = 0.74$). Omitting of the treatment 85-12.5-15 from the correlation analysis stronger correlation was observed ($r^2 = 0.81$). Ranking of the treatments are given in appendix C-19 (page 156). Rank correlation showed stronger correlation ($r^2 = 0.86$). It is to be noted that the top three treatments are top three in both of the methods applied, middle three and bottom three also were similar in ranking. It is evidenced from these analysis that DBA method could be used to analyse available lysine in feedstuffs for routine analysis.

PART II: EXPERIMENT 4: EFFECT OF AMINO ACID SUPPLEMENTATION
ON THE UTILIZATION OF MUSTARD OIL MEAL(MOM) BY
GROWING CHICKS

INTRODUCTION

Mustard oil meal (MOM) is generally low in lysine and arginine (Klain, et al., 1956). In current study, variation in lysine content of MOM from one sample to another were observed. MOM from batch 3 was stored in vaccum packed bags in a freezer at below -40 C. Descrepancy in amino acid analysis was observed in the same batch of MOM.

When analyzed fresh upon recieving MOM from Nepal in September 1984, lysine content was 2.576% (as is basis). After one and half year of storage, lysine content was only 2.01% (as is basis). This descerepancy in lysine value suggests that even under the condition described above, amino acid losses do occur.

In experiment 6 of part 1, MOM basal diet was computed using the value of 2.576% lysine. There was severe growth depression in chicks fed MOM basal diet. Even after defatting and acetone washing of MOM, growth improvement was significantly lower than for corn-soy standard referance diet. This experiment was designed to see if there is improvement in growth in chicks fed MOM basal diet supplemented with amino acids, lysine and arginine. Supplementation of

arginine and lysine were the differences in two determinations.

Materials and Methods:

MOM from batch 3 was used in this experiment. MOM basal diet and standard reference diets were selected from experiment 6 of part 1. Experimental diets were constructed by supplementing lysine and/or arginine to MOM-basal diet.

To appreciate the discrepancy in amino acid composition of MOM from same batch analyzed at two different times are presented in Table 44. Dietary combinations are presented in Table 45.

TABLE 45. AMINO ACID COMPOSITION OF MOM*

Amino acid	Sept/1984	Feb/1986
Arginine	2.42	2.04
Lysine	2.57	2.01
Histidine	1.01	0.89
Isoleucine	1.72	1.35
Leucine	2.95	2.49
Methionine	0.63	0.73
Cystine	0.59	0.91
Phenylalanine	1.61	1.31
Tyrosine	1.06	0.97
Threonine	1.85	1.41
Tryptophan	0.41	0.50
Valine	2.27	1.70

* As is basis; analyzed at Supersweet Research Farms, Courtland MN, through the courtesy of Multifood Intl. Corp.

TABLE 46. DIETARY COMBINATIONS

#	Diets	Lysine	Arginine
1.	Std. Ref. Diet		
2.	MOM basal Diet		
3.	MOM basal Diet	+	
4.	MOM basal Diet		+
5.	MOM basal Diet	+	+

Experimental:

Sixty uniformly weighing day old Hubbard male chicks were selected from 450 chicks and were randomly assigned to 10 pens (6 chicks/pen). Five dietary treatments were randomly assigned to ten pens (2 pens/treatment). Chicks were fed ad libitum and fresh water was available at all times. Weekly weight , feed intake and mortality were recorded weekly for two weeks.

Results and Discussions:

Two week weights, feed consumption and feed to gain ratios are presented in Table 46. Weekly weights of chicks on experimental diets are presented in Figure 17. Final weight was subjected to ANOVA (appendix C-17; page 155).

TABLE 47. PERFORMANCE OF CHICKS IN EXPERIMENT 4

Diet #	Treatment	Initial Weight	Final Weight	Feed Intake	F/G Ratio
1	Std. Ref. Diet	38.85	351.00	435.35	1.39
2	MOM-Basal "	39.05	264.40	353.75	1.59
3	# 2 + Lys "	39.35	284.60	355.85	1.45
4	# 2 + Arg "	38.65	274.70	372.50	1.57
5	# 2 + Lys + Arg "	38.80	290.55	353.30	1.40

After performing ANOVA on final weight, interesting comparisons were made using Bonferoni t test (Gill, 1978). The addition of lysine supported slightly higher growth above basal or arginine supplemented diets. Addition of arginine and lysine had an additive effect on growth and was significantly higher compared to the basal diet ($P < 0.1$). The growth supported by the reference standard diet was significantly higher than the diet supplemented with lysine and arginine. ($P < 0.05$). Addition of lysine alone to raw RSM diets did not improve growth or feed efficiency, however, lysine addition to diets in which autoclaved RSM was incorporated improved growth significantly (Hijikuro and Takemasa, 1979a).

Thyroid weights of chicks on experimental diets are presented in Table 48. Thyroid weight was taken after fixing 24 hr with formaldehyde. ANOVA on thyroid size is presented in appendix C-18 (page 156).

TABLE 48. THYROID WEIGHT OF CHICKS FED
EXPERIMENTAL DIETS

Diet #	Treatments	Thyroid Weight (mg)	mg/100 g of body wt
1	Ref. Std. Diet	10.70	3.00
2	Raw MOM	34.70	13.10
3	# 2 + Lys	24.05	8.50
4	# 2 + Arg	20.40	7.40
5	# 2 + Lys + Arg	14.60	5.00

Chicks fed raw MOM basal diet had thyroids (3.2 X) compared to the chicks fed control diet. Addition of lysine or arginine slightly reduced the thyroid size but it was significantly larger than the controls ($P < 0.01$). However, the addition of lysine and arginine together reduced the thyroid size to a level not significantly different from the controls. Leeson et al. (1976) observed that supplementation of RSM with arginine and methionine reduced thyroid size. Summer et al. (1971) also observed similar results.

There was no change in thyroid histology in contrast to the results of Summers et al. (1971). They observed enlargement of the follicles and sparsely distributed colloid (evidence of inhibition of thyroxine biosynthesis). However, their study was in laying hens. It is therefore suggested that thyroid hyperplasia may not be visible at early stage of the development of chicks.

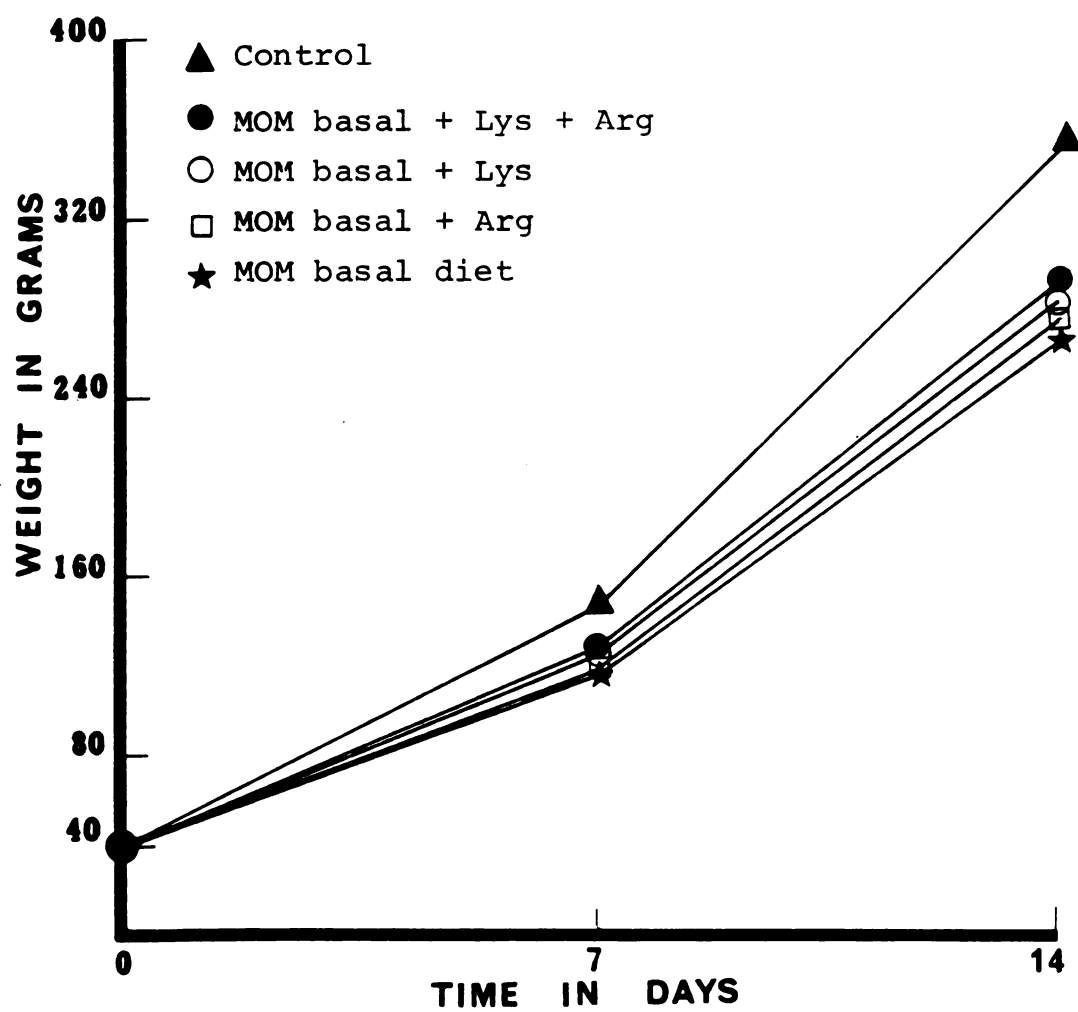


Figure 16. Weekly weight of chicks fed experimental diets in experiment 4

SUMMARY AND CONCLUSIONS

Under the experimental conditions and procedures described in this manuscript, the following conclusions are drawn.

1. MOM is a rich source of protein (38 - 40 %) with moderately balanced amino acid profile. Due to its high level of residual oil (10 - 14 %), GE and ME values are also moderately high (4.65 and 3.29 Kcal/kg respectively). It is also rich in minerals (calcium, phosphorous and iron).
2. Antinutritive components in MOM are glucosinolates (4.75% on fat free DMB), erucic acid (37.01% in residual oil) and tannins (2.73% as quercitannic acid). By defatting MOM it can be made erucic acid free and washing with acetone reduces tannic acid and glucosinolates. However, complete destruction of all antinutritive factors does not occur.
3. Wet heating (autoclaving at 121 C for 30 min) was effective in improving its nutritive value to growing chicks whereas dry heating (121 C for 30 min in an oven) was deleterious and boiling water treatment was not as effective.
4. At lower levels of incorporation (10%) effect of heat treatment is significantly greater. However, at higher levels (30% or more) difference between heat treated

and raw MOM is less distinct. It is apparent that there is a thresh hold response to the levels of MOM that can be raised to some extent upon heat treatment.

5. Defatting of MOM improves its nutritive value and further improvement occurs upon acetone washing after defatting.
6. There was only slight improvement upon autoclaving of acetone washed MOM.
8. Wet heating (autoclaved at 121 C) reduces lysine content compared to dry heating (oven heated at 121 C). However, there was improvement in growth of chicks independent of lysine destruction.
9. Heating of MOM in a rotating drum heating system at 85 C to 100 C for 15 min at 12.5 to 25% moisture level improve its lysine availability.
10. Supplementation of lysine and arginine improved chick growth over MOM basal diet. It indicates the possibility of further improvement in chick growth upon amino acid supplementation to defatted and acetone washed MOM.
11. Dye binding assay (DBA) was well correlated with chick growth assay (CGA)
12. Thyroids of chicks fed raw MOM basal diet were 3.2 times larger than the control and amino acid supplementation apparently reduced thyroid size.

Importance of this study:

1. MOM can be incorporated in poultry diets. The levels that can be incorporated in the rations will depend on the economic returns. It can partially replace SBM and GNC, which are imported and are relatively expensive, from poultry rations.
2. Low temperature heat treatment of MOM has been found to improve lysine availability and also lowers the levels of glucosinolates and erucic acid.
3. Erucic acid has been found to influence the metabolic process, in that thyroxine biosynthesis has been adversely affected by MOM in growing chicks. Others have found undesirable changes in the histology of cardiac and skeletal muscles. This issue raises the question whether, there is adverse effect in human beings. There may be a threshold effect of erucic acid in the diets of human beings. Since in Nepal, most commonly used cooking oil is mustard oil it may be of interest for researchers to further investigate effect of mustard oil in the diets of human beings.

RECOMMENDATIONS

As a outcome of this study, the following suggestions for further studies are made:

1. The MOM used in this study came from a commercial source. It's chemical characteristics as seed is not known. Research in this area should begin with the seed itself in order to understand what is occurring in the various stages of processing and storage. Therefore it is proposed that chemical characteristics of known varieties of mustard seed before and after oil extraction be carried out. This will establish a baseline for the evaluation of nutritive value of MOM.
2. In the current study, it was found that there is an influence of moisture level, temperature and duration of heat treatment on the nutritive value of MOM as indicated by the changes in available lysine and partial destruction of antinutritive factors like glucosinolates and erucic acid. Lower temperature heating was more effective in improving the availability of lysine in MOM. It is proposed that studies be conducted to determine the effect of low temperature treatment of mustard seeds prior to oil extraction on its nutritive value as a meal.

3. MOM in this study was stored in a freezer at below -40 C. It was found that even under such conditions there was substantial reduction of lysine. There is limited information on this aspect. Therefore it is suggested that study of storage on loss of lysine will be useful in understanding the nature of MOM under different storage conditions.
4. Due to the limitation in the amounts of MOM available for this study, short experiments were carried out. Although trends of the results indicated consistency, it is prudent to understand the effect of long term feeding on the performance of poultry and swine. Therefore it is proposed that long term feeding trials with poultry and swine be carried out to understand it's behaviour under such conditions.
5. Currently use of MOM in animal rations is very limited. Our analysis indicate that MOM has a relatively well balanced essential amino acid profile compared to GNC, which is a relatively expensive protein supplement. MOM may replace GNC partially or completely in poultry rations. Therefore it is proposed that studies be conducted to establish , how much of GNC could be replaced by MOM in poultry and swine rations.
6. Complete defatting improved the feeding value of MOM to growing chicks. Almost all oil extraction in Nepal is

expeller processed. Prepress solvent extraction or solvent extraction processes extract most of the oil and that oil is what is of interest to the farmers. Introduction of such a process alters the characteristics of the resultant meal. It is proposed that economically feasible methods of defatting MOM be studied, as adoption of such technology will depend upon economic returns. If it is economically beneficial to adapt a technology that extracts almost all oil from the seeds, it would have eliminated an important antinutrient from the meal, namely erucic acid.

7. Feeding of MOM increased the size of the thyroid in growing chicks. A rather significant observation lies in the fact that thyroid size was reduced by supplementation of amino acids in the diets. There is limited information available on this aspect of MOM feeding. Since thyroid has an important role in metabolic processes affects the performance of chicks. This area of research would appear to merit further study.
8. The national oilseed coordination project, the national foods laboratory and the Institute of Agriculture and Animal Science could collaborate the studies in various aspects of mustard seed production and utilization.

APPENDICES

APPENDIX A

APPENDIX A

METHODS OF DETERMINATION

A-I. Dye Bindung Assay: A Modified dye binding procedure adapted from Hurrell and Carpenter (1979):

Preparation of reagents:

1. Dye Buffer Reagent :

Crocein orange	1.36 g
Oxalic acid	20.00 g
Potassium Phosphate	
Monobasic	3.40 g
Acetic acid	60.00 g

2. 2.2 M Sodium Acetate:

Crystalline sodium acetate	182.49 g
Distilled water to	1000.00 ml

Procedure:

1. Determination of Dye Binding Capacity:

- A. Weigh 65 to 80 mg of sample for determining dye binding capacity and 110 to 135 mg for the determination of dye binding capacity (DBC), into screw capped 125 ml polypropylene bottles.
- B. Add 10 6mm glass beads and 3 marbles.
- C. Add 0.2 ml propionic anhydride for the determination of (DBCAP).
- D. Add 4.0 ml of sodium acetate solution.

- E. Shake for 15 minutes in a shaker.
- F. Add 40.00 ml Dye reagent buffer.
- G. Shake for 15 minutes.
- H. Centrifuge @ 15 ml of the supernatant from each bottle at 3000 X g for 15 minutes.
- I. Dilute contents of centrifuge tube by 1:65 ratio using distilled and deionized water.
- J. Determine absorbance in a spectrophotometer and calculate dye concentration from a standard curve.
- K. Prepare a blank in the manner described omitting the sample.
- L. Calculate DBCAP and DBC

$$\text{DBCAP} = \frac{\text{Reagent Blank Conc.} - \text{Conc. of Filtrate}}{1000/44.2} \times \frac{1000}{\text{Sample wt.}}$$

$$\text{DBC} = \frac{\text{Reagent Blank Conc.} - \text{Conc. of filtrate}}{1000/44.0} \times \frac{1000}{\text{Sample Wt.}}$$

2. Determination of Dye bound lysine (DBL)

$$\text{DBL} = \frac{\text{DBC} - \text{DBAP} \times 128.17}{1000} \times \frac{1000}{\text{g of protein/kg sample}}$$

Note: Only samples expressing filtrate concentration in the range 1.2 to 1.8 M MOL/L can be accepted as valid. Assays resulting in filtrate concentrations outside this range must be repeated after adjustment of sample size.

A-II. Total lipid extraction: Adapted from Folch and Sloanstanley (1956):

Reagents: 1. Chloroform
2. Methanol

Preparation of reaction mixtures:

1. Chloroform and methanol is mixed in a ratio of 2:1 (V/V).

Preparation of upper and lower phase solvents:

1. Upper phase solvent consists of 3 part chloroform
2. Lower phase solvent consists of 86 parts of chloroform, 14 parts of methanol and 1 part of DD water

Extraction of lipid:(Work under the hood)

1. Take 2 g of sample in a flask and add to it 34 - 40 ml of reagent mixture.
2. Use magnetic stirrer and extract over night.
3. Filter the homogenate thru fat free filter paper in a glass stoppered vessel.
4. The crude extract is washed thru with 5-fold its volume of DD water and let stand over night.
5. Remove upper phase by pipette as much as possible.
6. Wash at least 3 times using upper phase pure solvent without disturbing the lower phase.
7. Finally, add methanol to combine the two phases into single phase.
8. Add reagent mixture to desired volume.

A-III. Erucic acid determination: (AOAC, 1984)

Reagents:

1. Hexane
2. Methanol
3. Sodium methoxide
4. Methyl erucate standard (2mg/ml of hexane)
5. Methyltetrasonoate standard (2mg/ml of hexane)
6. 1 N HCL

Apparatus:

1. HP-5848 model GC with flame ionization detector.
2. 3' x 1/8" OD stainless steel column containing 15% Diethylene glycol succinate on 80 -100 mesh, chromosorb W.
3. Injector 250. C, detector 290. C, column 190. C. Use helium as carrier gas and adjust the flow rate to give retention time of ca. 17 min. for methyl tetracosenoate.
4. Measure peak areas by electric integrator.
5. 125 ml conical flask with T 24/40 joint and neck elongated to 8 - 10 mm i.d.

Standard preparation:

1. Me erucate standard is prepared by mixing 100 mg of Me erucate in 50 ml volumetric flask and add hexane to volume.
2. Me tetracosanoate standard solution is prepared by mixing 100 mg of Me tetracosanoate in a 50 ml volumetric flask and add hexane to volume.

Determination:

A. Weight response factor for Me-docosanoate:

1. Pipette 1, 2 and 3 ml Me-erucate standard solution into 3 separate conical flasks.
2. Pipette 3 ml of Me-tetracosanoate standard into each of the above flasks.

3. Add hexane to make 8 ml total volume.

4. Inject 2 micro liter of each solution into GC.

$R_{22:1} = (W_{22:1}/W_{24}) * (P_{24}/P_{22:1})$; Where;

$R_{22:1}$ = Wt. response factor for erucate relative to tetracosanoate.

W_{24} = mg Tetracosanoate

$W_{22:1}$ = mg docosanoate (erucate)

P_{24} = Peak area for tetracosanoate

$P_{22:1}$ = Peak area for docosanoate

B. Docosanoic acids in fats and oils;

1. Weigh 50 mg oil into dry transesterification flask.
2. Add 3 ml Me-tetracosanoate std. soln
3. Add 10 ml NaOMe soln.
4. Reflux for 1 hour and cool
5. Add 5 ml hexane and swirl
6. Add 7 ml 1N HCL, stopper and shake vigourosly for 1 minute.
7. Add H₂O until hexane reaches constricted neck and inject 2 micro liter hexane into the GC.

Calculation:

% Docosanoec acids = $(P_{22:1}/P_{24}) * (W_{24}/W_{oil}) * 100 * R_{22:1}$.

A-IV. Total glucosinolate determination: Adapted from

McGhee and Mustakas (1965):

Reagents: 1. 5% BaCl₂ solution

- Procedure:
1. Take 10 g defatted air dried meal and add to 250 ml distilled water.
 2. Hydrolyze at 54 C for 1 hour.
 3. Boil the mixture for 2 hours keeping the volume constant.
 4. Filter the mixture.
 5. Slurry the cake with additional 25 ml hot water and filter.
 6. Repeat step 5 twice.
 7. The filtrate volume is made to 600 ml.
 8. Add excess volume of BaCl₂ solution.
 9. Digest on steam bath for 6 to 12 hours.
 10. Filter the precipitate and ash in ash-less filter paper.

Calculation:

$$\% \text{Thioglucoside} = \frac{[(\text{MW of thioglucoside}) \times (\text{Wt. of BaCl}_2) \times 100]}{[(\text{MW of BaCl}_2) \times (\text{Sample Wt.})]}$$

A-V. Allyl isothiocyanate: Adapted from AOAC, Official

Methods of Analysis (1984):

Reagents: 1. 5% Alcohol

2. 10% Alcohol

3. Allylisothiocyanate

Preparation of reagents:

1. 5 ml of ethyl alcohol and 95 ml of double distilled (DD) water.
2. 10 ml of ethyl alcohol and 90 ml of DD water.
3. 30 micro liter of allylisothiocyanate and 50 ml 10% alcohol in a 100 ml volumetric flask and shake intermittently until dissolved. Dilute to volume with DD water

Apparatus: HP 5848 model GC.

Column: 12' X 4 mm id stainless steel.

Packing: 5% carbowax 4000 on flouropak 80,
20 - 40 mesh.

Optimum conditions: Column :- 145. C

Detector:- 200. C

Injector:- 160. C

Optimum conditions are achieved when peak height of 10 cm or greater for 8 micro liter std solution, is obtained. Carrier gas N flow rate 100 ml/min.

A-VI. Tannin determination: (AOAC, 1984).

Reagents: 1. 0.1N oxalic acid
 2. Potassium permanganate std. soln.
 3. Indigo carmine

Preparation of the reagents:

1. 0.1N $C_2H_2O_4$
 Mix 6.3025 g oxalic acid (crystalline)
 in a liter of DD water.
2. $KMnO_4$ std solution
 - a. Dissolve 1.33 gm $KMnO_4$ in a liter of
 DD H_2O .
 - b. Standardize with oxalic acid.
3. Indigo solution
 - a. Dissolve 6g sodium indigotin
 disulfonate in 500 ml DD H_2O .
 - b. Heat the solution to dissolve.
 - c. Cool and add 50 ml H_2SO_4 and dilute
 to 1 liter and filter.

- Procedure: 1. Extract 2 g sample 20 hours with anhydrous ether.
2. Boil residue with 300 ml DD H_2O and cool,
 dilute to 500 ml and filter.
 3. Measure 25 ml of this infusion into 2 liter
 porcelain dish and add 20 ml indigo
 solution and 750 ml of DD H_2O .
 4. Add std. $KMnO_4$ solution 1 ml at a time
 until blue color of the solution changes
 to green, then add few drops until color
 changes to golden yellow.
 5. Similarly titer the mixture of 20 ml
 indigo solution and 750 ml of DD H_2O for
 blank.

Calculation:

Multiply the difference between 4 and 5 with desired
 factor (derived from the factor 1 ml oxalic acid =
 0.006235 g quercitannic acid).

A-VII. Metabolizable energy determination: Adapted from TME
method as described by Sibbald (1983):

Animal used: SCWL roosters in individual cages.

Equipment: Syringe, polyethylene tubing, mixer.

Procedure: 1. Fast the roosters for 24 hours.

2. Force feed the roosters in duplicate
groups with test material (25 g/rooster).

3. Two roosters are used for determining
the endogenous excretion.

4. Collect excreta quantitatively and
freeze dry.

Determinations:

1. Metabolizable energy

Calculations:

$$\text{ME} = \frac{[\text{GE intake} - (\text{GE excreted} - \text{GE of endogenous excreta})]}{\text{GE intake}}$$

APPENDIX B

APPENDIX B

COMPOSITION OF FEED INGREDIENTS

TABLE B-1. COMPOSITION OF FEED INGREDIENTS USED

Ingredients	DM %	ME Kcal/kg	CP %	EE %	CF %	Ca %	P %
Yellow corn	89	3350	8.80	3.80	2.20	0.02	0.28
Soybean meal	89	2230	44.00	0.80	7.30	0.29	0.65
Wheat middlings	88	1800	16.00	3.00	7.50	0.12	0.90
Corn gluten meal (60% CP)	90	3720	62.00	2.50	8.00	0.40	0.80
Corn gluten meal	91	2940	41.00	2.50	7.00	0.23	0.55
Canola meal	93	2000	38.00	3.80	11.10	0.68	1.17
Sesame seed meal	93	2210	43.80	8.60	9.70	1.99	1.37

Source: (NRC, 1977 & 1984)

TABLE B-2. AMINO ACID COMPOSITION OF FEED INGREDIENTS

Amino acids	Ingredients				
	Corn	SBM	CGM (40%)	SSM	CM
Arg	0.50	3.28	1.93	4.93	2.32
Lys	0.24	2.93	1.00	1.30	2.45
His	0.20	1.15	1.22	1.09	1.07
Ile	0.37	2.39	2.29	2.12	1.51
Leu	1.10	3.52	10.11	3.33	2.65
Met	0.20	0.65	1.91	1.20	0.68
Cys	0.15	0.69	1.11	0.59	0.47
Phe	0.47	2.27	3.77	2.22	1.52
Tyr	0.45	1.28	2.94	2.00	0.93
Thr	0.39	1.81	1.97	1.65	1.71
Trp	0.09	0.62	0.25	0.80	0.44
Val	0.52	2.34	2.74	2.41	1.94

TABLE B-3. COMPOSITION OF TRACE MINERAL MIX

Elements	Forms	Content (mg/g)
Manganese (Mn)	Manganous oxide	239.40
Zinc (Zn)	Zinc oxide	229.50
Iron (Fe)	Iron carbonate	79.90
Copper (Cu)	Copper oxide	9.90
Iodine (I)	Calcium iodide	4.97
Cobalt (Co)	Cobalt carbonate	1.98
Calcium (Ca)	Calcium carbonate	

Roche Chemical Division, Hoffman-La roache Inc.
Nutley, New Jersey 07110.

TABLE B-4. COMPOSITION OF VITAMIN PREMIX

Vitamins	Form	Content
Vitamin A	vitamin A acetate	3333 USP/g
Vitamin D	vitamin D3 premix	333 ICU/g
Vitamin E	vitamin E premix	3 IU/g
Vitamin K	menadione dimethyl pyrimidinol bisulfite	1.3 mg/g
Thiamin	Thiamin mononitrate	1.3 mg/g
Riboflavin	riboflavin premix	2.0 mg/g
C-pantothenate	d-calcium pantothenate	6.6 mg/g
Niacin	niacin premix	13.3 mg/g
Pyridoxine	pyridoxine hydro- chloride	1.6 mg/g
G-biotin	d-biotin	66.7 mg/g
Folic acid	folic acid	0.3 mg/g
Vitamin B12	calcium carbonate diluted with rice hulls	5.0 mcg/g

APPENDIX C

APPENDIX C

STATISTICAL ANALYSES

TABLE C-1. TWO WAY TABLE FOR TWO WEEK WEIGHT OF CHICKS
IN EXPERIMENT 2, PART I

Type of heat (B)	Levels of mustard oil meal(A)			Total
	10 %	20 %	30 %	
Unheated MOM	218.00	214.63	196.00	
	185.88	219.25	214.86	
	-----	-----	-----	
Sub total=		433.88	410.86	= 844.74
Dry heated MOM	187.14	211.63	201.00	
	225.38	216.25	202.00	
	-----	-----	-----	
Sub total=		427.88	403.00	= 830.88
Autoclaved MOM	232.14	231.00	205.88	
	224.00	237.88	229.50	
	-----	-----	-----	
Sub total=		468.88	435.38	= 904.26
Total	=	1330.64	1249.24	= 2579.88

TABLE C-2. ANALYSIS OF VARIANCE FOR EXPERIMENT 2
OF PART I

Sources of variation	df	SS	MS	F
Level of MOM (A)	1	552.16	552.16	8.52
Treatments (B)	2	759.94	379.97	5.71
Experimental Error	8	517.94	64.74	
	---	-----	-----	
Total	11			

*(F .05, 1, 8 =5.32 ; F .05, 2, 8 = 4.46)

Contrasts	coefficients				
1. Unheated Vs heated	+2	-1	-1	0	0
2. Autoclaved Vs Oven heated	0	+1	-1	0	0
3. 20% Vs 30% MOM	0	0	0	+1	-1
Q1 = (+2) (+844.74)+(-1) (+830.88)+(-1) (+904.26)	= -45.66				
Q2 = (+1) (+904.26)+(-1) (+830.64)	= 73.88				
Q3 = (+1) (+1330.64)+(-1) (+1249.24)	= 81.40				
t1 = -1.44					
t2 = 4.01					
t3 = 4.44					
(Critical value t.025,6=+-2.447)					

TABLE C-3. TWO WAY TABLE FOR TWO WEEK WEIGHT OF CHICKS
IN EXPERIMENT 3, PART I

Types of heat =B	Levels of mustard oil meal= A		
	15 %	30 %	Total
Autoclaved MOM	205.25	198.20	
	210.40	206.00	
	-----	-----	
Sub total =	415.65	404.20	= 819.85
Boiled MOM	199.40	195.80	
	205.00	192.40	
	-----	-----	
Sub total =	404.40	388.20	= 792.60
Total =	820.05	792.40	= 1621.45

TABLE C-4. ANALYSIS OF VARIANCE FOR EXPERIMENT
3 PART I

Sources of variation	df	SS	MS	F
Levels of MOM (A)	1	95.57	95.57	7.03*
Types of heat (B)	1	92.82	92.82	6.83*
Experimental Error	5	67.96	13.59	
	--	-----		
Total =	7	256.35		

* (F .05, 1, 5 = 6.61)

Comparisons of interest

1. Autoclave vs hot water

2. levels 15% vs 30%

	Means	se
	-----	-----
For: Autoclave	204.96	+/-2.606
Hot water	198.15	+/-2.606
15% MOM	205.01	+/-2.606
30% MOM	198.00	+/-2.606

TABLE C-5. ANALYSIS OF VARIANCE FOR EXPERIMENT 4
PART I

Sources of variation	df	SS	MS	F
Defatting (A)	1	300.19	300.19	7.44*
Autoclaving (B)	1	384.96	384.96	9.54**
Levels (C)	2	3890.91	1945.45	48.21***
A X B	1	28.65	28.65	0.71
A X C	2	319.40	159.70	3.96*
B X C	2	177.18	88.59	2.19
A X B X C	2	104.15	52.07	1.29
Error	12	484.24	40.35	

(F.01 ,2,12= 6.93; F.05,2,12= 3.89)

Orthogonal polynomials for levels

1. Linear {-1, +0, +1}

2. Quadratic {+1, -2, +1}

$$Q1 = (-1)(+1220.6) + (+1)(+953.32) = -267.28$$

$$Q2 = (+1)(+1220.6) + (-2)(+1099.98) + (+1)(+953.32) = -26.04$$

$$t1 = -21.03$$

$$t2 = -1.18$$

(Critical value $t_{0.025,12} = \pm 4.339$)

Effect of defatting to be tested at 3 levels

1. At 10% MOM $t1 = (159.50 - 145.93) = 3.02$

2. At 20% MOM $t2 = (145.18 - 129.81) = 3.42$

3. At 30% MOM $t3 = (118.04 - 120.29) = -0.51$

Critical value $\pm t(0.05), 12 = 2.179$



TABLE C-8. ANALYSIS OF VARIANCE FOR EXPERIMENT
6 PART I

Source	SS	df	MS	F
Treatment	17801.18	6	2986.86	13.88
Experimental error	2991.58	14	213.70	
Total	20793.04	20		

(F.01, 6, 14 = 4.46)

Nonorthogonal contrast (Bonferoni t test)

Contrasts		Coefficients						
-----		C1k	C2k	C3k	C4k	C5k	C6k	C6k
1. 1 Vs 2		+1	-1	0	0	0	0	0
2. 3 Vs 6		0	0	+1	0	0	-1	0
3. 4 Vs 5		0	0	0	+1	-1	0	0
4. 6 Vs 7		0	0	0	0	0	+1	-1
5. 5 Vs 6		0	0	0	0	+1	-1	0
6. 4+1 Vs 2+3+6		-2	+1	+1	-1	0	+1	0

Bt1 = 11.05

Bt2 = 9.38

Bt3 = -5.69

Bt4 = -4.91

Bt5 = -2.97

Bt6 = -8.16

(Critical value t.005,6,14 = +-3.88)

(Critical value t.05,6,14 = +-3.069)

TABLE C-9. ANALYSIS OF VARIANCE FOR RL, EXPERIMENT
1, PART II

Sources	SS	df	MS	F
Temperature(T)	559.795	1	559.795	101.19 (P<0.001)
Moisture(M)	9.856	2	4.928	0.89 ns
Period(P)	185.913	2	92.956	16.80 (P<0.001)
T X M	151.601	2	75.80	13.70 (P<0.001)
T X P	8.555	2	4.277	0.77
M X P	57.479	4	14.369	2.59
T X M X P	82.024	4	20.506	3.70 (P<0.05)
Error	99.571	18	5.531	

Treatment Means

 111 : 50.39
 112 : 47.59
 113 : 47.72
 121 : 48.45
 122 : 47.89
 123 : 41.76
 131 : 53.93
 132 : 52.01
 133 : 43.72
 211 : 41.29
 212 : 41.46
 213 : 38.65
 221 : 44.61
 222 : 42.61
 223 : 42.61
 231 : 39.97
 232 : 39.14
 233 : 32.12

From a three way table when means were plotted to detect interactions it was found that the main cause of interaction was at 85 C and 12.5% moisture level. Three factor interaction was ignored and A X C was examined at two temperature separately for 3 levels of moisture , averaged across the duration.

TABLE C-10. ANALYSIS OF VARIANCE FOR ME, EXPERIMENT 1, PART II

Sources	SS	df	MS	F
Moisture (M)	0.01234	2	0.00617	0.367
Period (P)	0.17396	2	0.08698	5.177 *
M X P	0.06648	4	0.01662	0.989
Error 1	0.15085	9	0.01680	
Rooster/(MH)				
Temperature(T)	0.0622	1	0.0622	2.338
M X T	0.0630	2	0.3150	1.184
P X T	0.0015	2	0.0008	0.030
T X M X P	0.3540	4	0.0885	3.327
Error 2				
Rooster X T	0.2400	9	0.0266	

(F.05,2,9 = 4.26; F.05,1,9 = 5.12; F.05,4,9 = 3.63)

TABLEC-11. ANALYSIS OF VARIANCE FOR TOTAL GLUCOSINOLATES IN EXPERIMENT 1, PART II

Sources	df	SS	MS	F	P
Temperature (T)	1	0.208	0.208	2.34	0.14
Moisture (M)	2	0.136	0.068	0.77	
Period (P)	2	0.131	0.066	0.74	
T X M	2	0.123	0.061	0.69	
T X P	2	0.316	0.158	1.77	0.19
M X P	4	1.032	0.258	2.89	0.05
T X M X P	4	0.666	0.166	1.87	0.15
Error	18	1.602	0.089		

Studentized t-test for raw and heat treated MOM:

$$t = 3.875$$

$$\pm t(0.05), 18 = 2.10$$

TABLE C-12. ANALYSIS OF VARIANCE FOR WEIGHT GAIN AND PERCENTAGE OF LYSINE, EXPERIMENT 2, PART II

Source	SS	df	MS	F
Total	11144.56	5		
Regression	10603.85	1	10603.77	78.42*
Residual	540.71	4	135.18	

* ($P < 0.0009$); $Y = -144.63 + 514.87(X)$; $SE = 11.63$
 $r = 0.99$; $r^2 = 0.98$

TABLE C-13. ANALYSIS OF VARIANCE FOR WEIGHT GAIN AND LYSINE CONSUMPTION IN EXPERIMENT 2, PART II

Sources	Sum of squares	df	MS	F
Total	305153.500	5		
Regression	299731.977	1	299731.977	221.14
Residual	5421.523	4	135.380	

* ($P < 0.0001$); $Y = 71.42 + 79.42(X)$; $SE = 36.82$
 $r = 0.99$; $r^2 = 0.98$

TABLE C-14. ANALYSIS OF VARIANCE FOR EXPERIMENT 3,
PART II

Source	df	SS	MS	F	<P
Total	9	244.981			
Regression	1	240.418	240.418	421.537	.00000
Residua	8	4.563	.570		

Similarly, for all the treatments analysis of variance was performed. Only regression parameters are presented in the text.

TABLE C-15. YATES' FACTORIAL ANALYSIS IN EXPERIMENT 3,
PART II

Ordered	Totals	1	2	3	4	Mean factorial (qK) effects
(1)	93.30	166.22	329.06	638.12	1335.24	=Y... Y=83.45
a	72.92	162.84	309.06	697.12	54.44	=qA = 6.80
b	75.80	177.30	313.96	36.52	-66.14	=qB = -8.27
ab	87.04	131.76	383.16	17.92	57.04	=qAB = 7.13
c	91.80	158.60	-9.14	-49.54	49.20	=qC = 6.15
ac	46.20	155.36	45.66	-16.60	72.72	=qAC = 9.09
bc	46.20	198.38	3.96	64.68	-60.14	=qBC = -7.52
abc	85.56	184.78	13.96	-7.64	15.48	=qABC = 1.93

d	75.60	-20.38	-4.00	-20.00	59.00	=qD = 7.37
ad	83.00	11.24	-45.54	69.20	-18.60	=qAD = -2.32
bd	79.40	6.30	-3.00	54.80	32.94	=qBD = 4.11
abd	75.96	39.36	-13.60	17.92	-72.32	=qABD = -9.04
cd	96.50	7.40	31.62	-49.54	89.20	=qCD = 11.15
acd	101.88	-3.44	33.06	-10.60	-36.88	=qACD = -4.61
bcd	88.10	5.38	-10.84	1.44	38.94	=qBCD = 4.87
abcd	96.68	8.58	3.20	14.04	12.60	=qABCD = 1.57

MSE = 51.17 (Pooled sums of square of (qk) for 3
three factors and 1 four factor interactions)
Critical value $\pm t(0.1), 4 = 1.533$

$$S^{\wedge}D = 3.3576$$

$$^{\wedge}D \pm (1.533)(3.576) = 5.48$$

TABLE C-16. TWO WEEK WEIGHT OF CHICKS (g) IN
EXPERIMENT 4, PART II

Rep #	1	2	Diet #		
			3	4	5
1	352.2	256.7	273.8	272.5	293.3
2	349.8	272.2	295.5	277.0	287.8
Total =	702.0	528.9	569.3	549.5	581.1
Mean =	351.0	264.4	284.6	274.7	290.5

TABLE C-17. ANALYSIS OF VARIANCE FOR
EXPERIMENT 4, PART II

Sources	SS	df	MS	F
Treatment	9175.72	4	2293.93	29.89
Experimental				
Error	383.69	5	76.73	
Total	9559.41	9		

Comparisons of interest:

Contrast	Coefficients				
	C1k	C2k	C3k	C4k	C5k
1. Diet 1 Vs Diet 5	+1	0	0	0	-1
2. Diet 1 Vs Diet 2	+1	-1	0	0	0
3. Diet 2 Vs Diet 5	0	+1	0	0	-1

$$tB1 = 120.9/\text{Sqr. rt.}(4 \times 76.73) = 6.90$$

$$tB2 = 173.1/\text{sq. rt.}(4 \times 76.73) = 9.87$$

$$tB3 = -52.2/\text{sq. rt.}(4 \times 76.73) = 2.98$$

Critical value from Bonferoni type table

$$+-\text{Alpha } 0.025, 3, 5 = 3.518$$

$$+-\text{Alpha } 0.05, 3, 5 = 2.882$$

TABLE C-18. ANALYSIS OF VARIANCE FOR THYROID WEIGHT OF CHICKS IN EXPERIMENT 4, PART II

Source	SS	df	MS	F	P(<)
Treatment	688.68	4	172.17	38.91	(0.01)
Exp. Error	22.12	5	4.42		
Total	710.81	9			

Treatment effects were compared with the control using Dunnett's t-test.

Critical value = ($f_{0.025, 4, 5} = \pm 4.71$)

1. Control Vs Raw MOM $+t_D = -14.43$
2. Control Vs Raw MOM + Lys = -8.03
3. Control Vs Raw MOM + Arg = -5.83
4. Control Vs raw MOM + Lys + Arg = -2.34

TABLE C-19. RANKING OF LYSINE AVAILABILITY BY DBA AND CGA IN EXPERIMENT 3, PART II

Treatments	DBA	CGA
1 Raw MOM	6	5
2 85-12.5-15	5	4
3 85-12.5-60	4	6
4 85-25.0-15	-	-
5 85-25.0-60	2	3
6 100-12.5-15	1	2
7 100-12.5-60	8	8
8 100-25.0-15	7	7
9 100-25.0-60	3	1

Rank correlation was $r = 0.86$

TABLE C-20. ANALYSIS OF VARIANCE FOR AVAILABLE LYSINE
IN EXPERIMENT 1, PART II

Source	df	SS	MS	F
Temperature (T)	1	526.82	526.82	319.28
Moisture (M)	2	233.61	116.80	70.18
Period (P)	2	173.86	86.93	52.68
T X M	2	702.36	351.18	212.83
T X P	2	23.88	11.94	7.23
M X P	4	104.71	26.17	15.86
T X M X P (Error)	4	6.59	1.65	

(f0.01, 1, 4 = 21.20; f0.01, 2, 4 = 18.0;
f0.01, 4, 4 = 16.0)

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