

# EFFECT OF GRADED LEVELS OF HIGH GLUCOSINOLATE MUSTARD (*BRASSICA JUNCEA*) MEAL INCLUSION ON NUTRIENT UTILIZATION, GROWTH PERFORMANCE, ORGAN WEIGHT, AND CARCASS COMPOSITION OF GROWING RABBITS

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ABSTRACT: Mustard (Brassica juncea) meal (MM) was incorporated at the levels of 80, 160 and 245 g/kg of rabbit diets in replacement of sovbean meal (SBM) and compared with a SBM based diet. The three levels of incorporated MM contributed total glucosinolate (TGLS) 3.6, 8.0 and 11.5 g/kg DM respectively. Forty-four weaning rabbits (4 weeks old, 314 ± 24 g live weight) of Soviet Chinchilla and White Giant breed comprising 24 males and 16 females were balanced for weight and sex, and randomly allocated to the four experimental diets. The feed intake and growth of the rabbits were monitored in an 8-week long growth study. The nutrient utilization was determined at the middle of the study. The MM used in the experimental diets contained 58 g TGLS/kg. MM incorporated diets had higher ME content ranging from 11.05 to 11.48 MJ/kg DM. The replacement of SBM protein at 33% amounting to incorporation of 8% MM in diet increased (P<0.05) the apparent digestibility of DM, OM, CP, ADF and GE, Quadratic increase was observed for nutrient digestibility except for GE, which showed both linear and quadratic increase. The MM incorporation in growing rabbit diets linearly reduced protein and increased fat content in muscle. The liver weight increased due to MM incorporation. Rabbits fed MM diets reduced feed intake whereas feed conversion efficiency was improved, which showed linear and quadratic effects. Average daily gain of rabbits reduced linearly (P<0.05) on MM diets. Further, rabbits in the present experiment tolerated up to 3.6 g TGLS/kg diet DM during active growth phase without any apparent effect on health and growth performance. It is concluded that MM cannot replace SBM in growing rabbit feeding due to growth and feed intake depression which could be attributed to the TGLS presence. depressing the liver function and affecting the muscle nutrient accretion pattern. However, partial replacement of SBM amounting to 80 g MM/kg diet could not have apparent adverse effects on growth and health of growing rabbits.

Key Words: mustard meal, glucosinolate, rabbit, growth performance, carcass composition.

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## INTRODUCTION

India produces one fifth of the world rapeseed-mustard and is the second largest producer of rapeseed-mustard in the world (KIRESUR, 1999). The mustard group of oil seed crops has the advantage of being drought and disease resistant, and the production of rapeseed-mustard has increased substantially in the country during the last decade. Mustard meal (MM) is a by-product that is obtained following oil extraction from mustard seeds. Mustard meal is cheaper than other conventional oil meals. The MM obtained from the Indian varieties contains 300 to 395 g CP/kg DM (TRIPATHI AND SINGHAL, 1994). The MM is also rich in sulphur, with content ranging from 14.3 to 23.0 g/kg DM (PAPAS et al., 1978; TYAGI et al., 1996). In spite of its well balanced amino acid composition (PASTUSZEWSK et al., 2000), the utilization of the MM in rabbit feeding is limited by its high content of glucosinolates. Glucosinolate (TGLS) content of MM made from Indian mustard varieties range from 12 to 90 g/kg DM (CHAUHAN et al., 1999). Glucosinolates themselves are nontoxic, whereas their degraded products thiocyanate, isothiocyanates, nitriles, etc. are toxic to animals. These end products suppress thyroidal uptake of iodine (DUNCAN, 1991; BARRETT et al., 1997) and induce metabolic disorders such as liver and thyroid hypertrophy (PAPAS et al., 1979; FENWICK and CRUITS, 1980; BELL, 1984). The information on response of rabbits towards dietary glucosinolates content is scanty, while these are studied in detail in other animal classes including ruminants.

The aim of the present work was to incorporate unconventional MM as a protein supplement in rabbit feed formulations to economize the cost of production and to study the effect of high glucosinolate containing MM as a substitute for conventional soybean meal (SBM) on intake, nutrient utilization, growth performance, organ weight, carcass composition and glucosinolates tolerance capabilities of growing rabbits.

## **MATERIALS AND METHODS**

#### Animals

Forty weaning rabbits (4 weeks old,  $314 \pm 24$  g live weight (LW)) comprising

of Soviet Chinchilla and White Giant were used. The animals were housed in individual cages of angle iron supported wire mesh (45 cm x 50 cm x 37 cm) with provision of feeder and watering bowl. The animals were raised inside a covered shed that had a concrete floor with cement roofing sheets. The house was designed to ensure cross ventilation and to exclude rodents and other pests.

The study was carried out at the Central Sheep and Wool Research Institute, Avikanagar, located at 26° 17' N latitude, 75° 28' E longitude and 320 m above sea level. The climate is typically semi-arid with yearly mean minimum and maximum temperatures of 6°C and 41°C, respectively. The ambient temperature and relative humidity of the animal shed during experimental period were  $9.0 \pm 0.4$ °C and 43.0% minimum and  $30.0 \pm 0.8$ °C and 80% maximum, respectively. The feeding trial began in February 2000 and lasted for 8 weeks.

### Diets

Complete pelleted diets using ground ingredients were prepared (Table 1) at the Feed Technology Unit of the Institute. The diets were balanced for crude protein. The weighed feed ingredients were mixed and moistened with water to achieve 25 to 30% moisture content. The feed mixtures were pelleted in a horizontal minipelleting machine specially manufactured for experimental use. The pellets were sun dried after pelleting.

#### Feeding trial

The weaning rabbits were balanced for weight and sex, and randomly allocated to the four experimental diets containing 0 (contained SBM as protein source and served control), 80, 160 and 245 g MM/kg diet. Thus, ten weaning rabbits (6 males and 4 females) were allocated to each of the four treatments. The three levels of MM replaced SBM protein 33, 66 and 100% respectively.

The feeding trial lasted 8 weeks. The animals were fed on the experimental diets *ad libitum*. The diets were offered at 10:00 h daily with allowance of 10% above the previous day's consumption. The unconsumed feed was weighed and

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	E	Diets (Level o	MM	CDM		
	0	80	160	245	MM	SBM
Ingredients (g/kg	)					
Barley grain	250	250	250	250		
Deoiled Rice Bran	110	100	105	100		
Wheat Bran	150	140	120	105		
Soybean meal	190	130	65	-		
Mustard meal	-	80	160	245		
Fish meal	50	50	50	50		
Cowpea hay	150	150	150	150		
Molasses	80	80	80	80		
Vitamin-mineral premix <sup>1</sup>	15	15	15	15		
Salt	5	5	5	5		
Chemical composition (g/	kg DM) <sup>2</sup>					
Dry matter	958	958	963	965	963	953
Organic matter	850	852	846	848	901	913
Crude protein	186	191	187	189	376	464
Ether extract	11	15	21	23	77	0.8
NDF	553	532	515	471	340	468
ADF	219	22	219	219	192	173
ADL	79	80	86	98	61	32
Ash	149	148	154	152	99	87
GE (MJ/kg DM)	16.67	17.36	17.68	18.41	17.99	17.12
TGLS (g/kg DM)	-	3.6	8.0	11.5	58.0	-

## Table 1: Ingredients and chemical composition of experimental diets.

MM: Mustard meal, SBM: Soybean meal, 'Contained: Calcium 320 g/kg premix, phosphorus 62 g/kg, manganese 2.7 g/kg, zinc 2.6 g/kg, iron 1000 mg/kg, fluorine 900 mg/kg, iodine 100 mg/kg, copper 100 mg/kg, thiamine 0.25 g/kg, riboflavin 1.5 g/kg, calcium pantothenate 5.0 g/kg, pyridoxine 0.1 g/kg, nicotinic acid 12.5 g/kg, cholecalciferol 1 g/kg, -tocopherols 15 g/kg, cyanocobalamin 6.0 mg/kg and choline chloride 100 g/kg, <sup>2</sup>Mean of three determination.

discarded before offering fresh feed to determine the intake for the previous day. Weaning rabbits had free access to clean drinking water. Daily records of feed intake were maintained throughout the experiment. The animals were weighed at weekly intervals to assess their growth performance.

## **Carcass evaluation**

The growing rabbits were slaughtered after 8 weeks of experimental feeding. The weights of liver, kidney and spleen were recorded. Samples of dorsal muscle (*Longissimus dorsi*) were collected for chemical analysis.

# Metabolism trial

A metabolism trial was carried out at the middle of the feeding experiment and lasted for 5 days. Rabbits were placed in metabolic cages with facility for separate quantitative collection of urine and faeces. Daily records of feed offered, unconsumed feed, faeces and urine voided were maintained during the collection period of 5 days. Total faeces voided was weighed and dried in a forced draught oven at 60 °C until constant weight. The dried samples for each animal over the 5-day collection period were pooled and ground for analysis. Urine voided was measured, mixed and 1% aliquot was preserved in sulfuric acid for nitrogen (N) estimation. The growing rabbits were weighed before and at the end of the trial to assess growth and level of feeding during metabolic trial.

# Chemical analysis

The pooled representative samples of SBM, MM, experimental diets, faeces, urine and muscles were analysed following the method of AOAC (2000), crude protein by Kjeldahl method, organic matter (OM) by muffle furnace incineration method and ether extract (EE) by solvent extraction method. The method of VAN SOEST *et al.* (1991) was used for NDF, and for ADF and ADL method of GOERING AND VAN SOEST (1970) was followed. The TGLS content of MM and experimental diets were determined using the method of THOLEN *et al.* (1989). Gross energy (GE) contents of samples were determined by Ballistic bomb calorimeter (Gallenkemp, UK), while metabolisable energy (ME) was calculated by using the equation proposed

by BOLIS et al. (1996):

ME = DE - 37 Nu - 6.6

Where, ME = Metabolisable energy, kJ/d DE = Digestible energy, kJ/d

Nu = Nitrogen content of urine (g/d).

#### Statistical analysis

Data on feed and nutrient intake, feed conversion efficiency, level of feeding, N-balance, organ weights and carcass composition were subjected to one way analysis of variance procedure of SPSS Base 10.0. Data were subjected to regression analysis utilizing a polynomial design, which tested for linear and quadratic relationship.

### RESULTS

#### Total glucosinolate and chemical composition

The MM used in the test diets contained 58 g TGLS/kg DM. Diets formulated using 80, 160 and 245 g MM had 3.6, 8.0 and 11.5 g TGLS/kg DM respectively (Table 1). All the diets were isonitrogenous and similar in chemical constituents except for EE content, which increased with levels of MM incorporation. The higher EE of MM was reflected in MM incorporated diets. The CP content of MM was lower to that of SBM. The MM also had lower NDF and higher ADL to that of SBM.

### Feed intake and growth performance

The data on live weight gain, feed intake and feed conversion efficiency (g feed/g weight gain) are given in Table 2. The rabbits on the four diets gained 27 to 31 g/d, which was linearly (P<0.05) lower on MM diets. Total live weight gain attained during 8 week feeding period varied from 1519 to 1710 g, showed both linear and quadratic decrease for MM diets. Feed intake on MM diets was

	Diets (Level of MM, g/kg)				SEM	Regression analysis	
	0	80	160	245	- SEM	L	Q
Total weight gain (g)	1710	1710	1706	1519	73.9	NS	NS
Daily weight gain (g/d)	31	31	30	27	1.3	*	NS
Total feed intake (kg)	7.4	7.1	6.9	5.7	0.34	*	*
Daily feed intake (g/d)	132	127	124	102	6.1	*	*
Feed conversion efficiency	4.3	4.2	4.1	3.8	0.10	*	*

 Table 2: Growth performance and feed intake of growing rabbits on experimental diets.

L: significance of linear effect of MM incorporation, Q: significance of quadratic effect of MM incorporation. SEM: standard error of mean, NS: not significant, \*P < 0.05; \*\*P < 0.01.

significantly (P < 0.01) lower than the feed intake on SBM diet. However, feed conversion efficiency was significantly (P < 0.01) better on MM diet compared to that of SBM. Linear and quadratic effects were observed for reduced feed intake and improved feed conversion efficiency due to MM incorporation.

## Nutrient intake and digestibility

The nutrient intake and coefficients of total tract apparent digestibility obtained during the metabolism trial are given in Table 3. The intakes of DM, OM, CP, ADF and GE were similar among the four groups. The TGLS intake was significantly (P<0.05) higher on MM diets and tended to increase linearly with increasing levels of MM.

The rabbits fed MM incorporated diets had quadratic increase (P<0.05) for digestibility of DM, OM, CP, ADF and GE (Table 3). The GE digestibility improved 5-7 units in MM diets over SBM diet, which showed both linear and quadratic increase. The ME of SBM diet (9.4 MJ/kg DM) was lower than that of MM diets (11.0 – 11.5 MJ/kg DM).

#### Nitrogen balance and level of feeding

The N intake, excretion and its retention, digestible protein (DCP), DE and ME

	Ľ	iets (Level o	of MM, g/kg	SEM	Regression analysis		
	0	80	160	245	SEM	L	Q
Intake							
DM (g/d)	149	140	142	128	7.2	NS	NS
DM (% LW)	10.5	9.6	9.6	10.9	0.63	NS	NS
OM (g/d)	127	119	120	109	6.1	NS	NS
CP (g/d)	28	27	27	24	1.4	NS	NS
GE (kJ/d)	2483	2422	2515	2361	101.2	NS	NS
ADF (g/d)	33	31	31	28	1.6	NS	NS
TGLS (g/d)	0.0	0.5	1.2	1.5	0.04	*	*
TGLS (g/kg LW)	0.0	0.4	0.8	1.3	0.06	**	NS
Digestibility coeffici	ent						
Dry matter	0.57	0.61	0.58	0.55	0.010	NS	**
Organic matter	0.60	0.63	0.64	0.59	0.014	NS	**
Crude protein	0.69	0.72	0.71	0.67	0.014	NS	*
ADF	0.19	0.33	0.23	0.18	0.035	NS	**
Gross energy	0.58	0.65	0.65	0.63	0.008	*	**
ME (MJ/kg DM)	9.38	11.01	11.28	11.50	0.341	*	*

Table 3: Intake and digestibility during metabolism trial.

L: significance of linear effect of MM incorporation, Q: significance of quadratic effect of MM incorporation. SEM: standard error of mean, NS: not significan, \*P < 0.05, \*\*P < 0.01.

intakes are shown in Table 4. The N-intake and its excretion in faeces were similar in rabbits fed SBM and MM diets. The urinary-N excretion decreased linearly (P<0.05) in rabbits fed MM diets. Apparent N-retention was similar whereas Nretention as percentage of ingested and absorbed N showed a linear increase with increasing MM levels. The DE and ME intakes were significantly (P<0.01) higher on MM diets. However rabbits on 100% replacement of SBM protein, i.e. 245 g

	Diets (Level of MM, g/kg)				OFM	Regression analysis	
	0	80	160	245	SEM	L	Q
Live weight (g)	1423	1475	1512	1221	98.3	NS	NS
N-balance							
N-intake (g/d)	4.4	4.3	4.3	3.9	0.22	NS	NS
N- faeces (g/d)	1.4	1.2	1.3	1.3	0.10	NS	NS
N-urine (g/d)	1.0	1.0	0.8	0.7	0.08	*	NS
N-retention (g/d)	2.1	2.1	2.3	1.9	0.14	NS	NS
N-retention (% N-intake)	46.3	48.8	53.3	49.2	2.03	*	NS
N-retention (% N-absorbed)	67.6	67.5	75.0	73.6	2.41	*	NS
Level of feeding							
DCP intake (g/d)	19.0	19.1	18.9	16.2	0.71	*	NS
DE intake (kJ/d)	1440	1584	1636	1506	45.1	*	*
DE intake (kJ/kg LW)	1088	1074	1082	1232	31.6	NS	*
ME intake (kJ/d)d	1397	1541	1602	1474	37.7	*	*
ME intake (kJ/kg LW)	982	1045	1060	1207	38.1	*	NS
Nutrient intake (per g we	eight gair	ı)					
DCP intake (g)	0.6	0.6	0.8	0.5	0.09	NS	*
DE intake (kJ)	46	51	54	56	2.1	*	NS
ME intake (kJ)	45	50	53	55	2.6	*	NS

Table 4: Nitrogen balance and level of feeding metabolism trial.

L: significance of linear effect of MM incorporation, Q: significance of quadratic effect of MM, incorporation. SEM: estandard error of mean, NS: not significant, \*P < 0.05, \*\*P < 0.01.

MM diet, had a quadratic decreased DCP intake. Substitution of SBM with MM protein linearly improved DE and ME intakes, quadratic increases were also observed for daily DE and ME intake. Intakes of DCP, DE and ME/g weight gain was higher for rabbits fed MM incorporated diets. Both linearly and quadratic increase for DE

	Diets (Level of MM, g/kg)					Regression analysis		
	0	80	160	245	SEM	L	Q	
Live weight (g)	2390	2600	2810	2420	13.9	NS	NS	
Organ weight								
Liver (g)	47.7	63.7	69.4	59.4	2.03	NS	*	
Liver (g/kg LW)	20.0	24.2	24.3	24.5	1.15	NS	*	
Kidney (g)	11.7	12.0	12.5	13.3	1.03	NS	NS	
Kidney (g/kg LW)	4.9	4.8	4.5	5.5	0.37	NS	NS	
Spleen (g)	1.0	0.9	0.7	0.9	0.19	NS	NS	
Spleen (mg/kg LW)	425	368	233	369	83.1	NS	NS	
Muscle composition (g/kg fresh muscle)								
Water	732.0	731.4	742.5	735.4	4.10	NS	NS	
Protein	251.1	255.7	242.0	233.9	3.21	NS	*	
Fat	6.8	12.8	15.2	14.5	2.11	*	*	

Table 5: Organ weight and muscle composition of rabbit.

L: significance of linear effect of MM incorporation, Q: significance of quadratic effect of MM incorporation SEM: standard error of mean, NS: not significan, \* P<0.05, \*\* P<0.01.

and ME, and quadratic increase for DCP intakes/g gain were observed. Growing rabbits consumed 0.5 to 0.8 g DCP, 40 to 56 kJ DE and 45 to 55 kJ ME/gram of weight gain, however linear and quadratic effects were observed for increased intakes due to MM incorporation.

#### Organ weight and carcass composition

The weight of liver, kidney and spleen, and muscle composition are given in Table 4. Quadratic increase for liver weight (P<0.05) was observed in rabbits fed MM diets. Whereas kidney and spleen weight were similar on the four diets. Water content of dissected dorsal muscle was similar on MM and SBM diets. However quadratic decrease for muscle protein, both linear and quadratic increases (P<0.05)

were for muscle fat content in rabbits fed MM diets.

# DISCUSSION

Rapeseed/mustard meal containing more than 10 mg/kg TGLS is toxic to pigs and poultry (FENWICK and CRUITS, 1980), and is responsible for poor growth and lower thyroid hormone levels in ruminants (PAPAS et al., 1979; TRIPATHI, 1999). The TGLS content of MM used in this experiment was within the normal range for Indian rapeseed/mustard meal (CHAUHAN et al., 1999; TYAGI, 2002). Although the level of adverse effects on animal health and production is associated with the level of rapeseed/mustard meal incorporated in diet and its TGLS content, neither mortality nor the adverse effect of TGLS was observed on health, however growth rate of rabbits was poor on diets containing 160 or 245 g MM/kg diets in the present study. The process of complete feed pelleting involves inclusion of 30% moisture, that could favour activation of the myrosinage enzyme present in rapeseed-mustard meal and responsible for TGLS degradation (MUKHERJEE et al., 1976). Myrosinase could be deactivated during pelleting due to the high temperature (70-80°C) which existed inside the pelleting chamber of the pelletizer, as at this temperature the enzyme is denatured (GIL AND MACLEOD, 1980). The TGLS degradation products that arose due to activation of the myrosinase in the presence of moisture (TYAGI, 2002) would have evaporated during pellet processing and drying (VERKERK et al., 1997), thus the TGLS content of the MM incorporated diets was lower than that expected. The TGLS content of unpelleted feed mixture was 4.2, 8.7 and 13.0 g/kg DM respectively in the diets containing 80, 160 and 245 g MM/kg.

The ME of the SBM diet (9.4 MJ) was below 10 MJ/kg DM, which is considered slightly inadequate for growing rabbits (BUTCHER *et al.*, 1983), while MM incorporated diets had adequate ME. The MM had higher fat compared to SBM used, and the lower GE digestibility of SBM diet further reduced its ME. Although ADF contents of SBM and MM diets were similar, the significantly higher ADF digestibility in the rabbits fed MM diet compared to SBM supports the fact that

higher sulphur content of MM favoured the caecum fibre digestion (PAPAS *et al.*, 1978) and that the rabbits are able to efficiently digest fibrous materials in MM diets (DE BLAS *et al.*, 1978). The lower urinary-N excretion and better N-retention on MM diets could be due to the better energy supply.

The kidney and spleen weight remained unaffected by MM incorporation. Whereas quadratic increase was for liver weight in rabbits fed MM incorporated diets. An increase of the weight of liver could be related to the TGLS intake as shown in growing pigs (Bourdon and Aumaitre, 1990). The bitterness of MM responsible for intake depression effect on MM diets might have been overridden by added molasses, which induce a sweet taste. Reduced feed intake on MM diets in this study agree with the finding of DUNCAN and MILNE (1990) and BRUEL et al. (2000), who also reported lower feed intake on discrete doses of dietary glucosinolates. The lower feed intake on MM diets is the effect of depressed appetite associated with the TGLS degradation products (PUSZTAI, 1989). In spite of lower urinary-N excretion and better amino-acid composition of MM (PASTUSZEWSK et al., 2000), the carcass protein content was lower than the SBM diet. The TGLS degradation products impair liver function (RABOT et al., 1993; TRIPATHI et al., 2001), which reduces protein biosynthetic activities of the liver (BARRETT et al., 1997), hence reduced muscle protein content. Higher muscle fat content is the reflection of excess energy availability to rabbits on MM diets.

The average daily gain (27 to 30 g/d) of rabbits maintained on SBM and MM incorporated diets was comparatively lower than those in the reports emanating from temperate locations (AL-BAR and AL-AGHBARI, 1996; BIELANSKI *et al.*, 1996; DE BLAS *et al.*, 1996); however, these gains are in agreement with the reports available from tropical locations (ABOUL-ELA *et al.*, 1996; PRASAD *et al.*, 1996 and 1998; RAHIM *et al.*, 1996; BHATT and SWAIN, 2003). Consumption of dietary DM was higher (9.5-10.8% LW) than the normal requirement (4.6-7.0% LW) of DM for growth (PRUD'HON, 1968). The ADL content of test diets was higher than normal dietary ADL requirements of growing rabbits (LEBAS, 1989). This higher lignin might be related to the higher feed intake per unit of body weight and a decrease of feed efficiency (NICODEMUS, *et al.*, 1999). Dietary lignin and fibre levels are major enhancing factors

of rate of passage and then of intake capability (VAN SOEST, 1994). Lower feed intake and reduced growth rate of rabbits fed MM incorporated diets are associated with breakdown of MM glucosinolate. Glucosinolate metabolites are known to depress appetite and impair growth if the diet contains glucosinolate more than tolerable limits (PUSZTAI, 1989; KLOSS *et al.*, 1994; WALLIG *et al.*, 2002). Amongst terrestrial animals, ruminants are considered to be less sensitive than single stomached animals (MAWSON *et al.*, 1994a and 1994b), growth, feed intake and feed conversion are reduced by a dietary glucosinolate content of approximately 1.2-1.65 g/kg in rat, 0.25-1.2 g/kg in young pig, 1.65-4.12 g/kg in growing poultry, and 2.9-6.2 g/kg in young calves. Thyroid function is affected by dietary level of 0.2-1.7 g/kg in the rat, 0.8-1.24 g/kg in swine, 0.6 g/kg in growing poultry and 3.3 g/kg in calves. However tolerable limits and effects of dietary glucosinolate on production and health of rabbits are not known. Growing rabbits in present experiment tolerated up to 3.6 g TGLS/kg diet DM during active growth phase without any apparent effect on health and growth.

# CONCLUSIONS

The MM incorporated diets reduced feed intake, depressed growth rate, reduced muscle protein, and increased muscle fat content and liver weight. Therefore MM cannot replace SBM in growing rabbit feeding, because the observed growth and feed intake depression could be attributed to the concomitant presence of TGLS, depressing the liver function and affecting the muscle nutrient accretion pattern. However partial replacement of SBM amounting to 80 g MM with 3.6 g TGLS/kg diet DM increased the digestibility of DM, OM, CP, ADF and GE. The incorporated MM increased dietary energy intake with higher N-retention. Further, the rabbits in the present study tolerated 3.6 g TGLS/kg diet DM in an 8 week long feeding period. This short feeding duration might not have caused mortality and morbidity in growing rabbits as expected.

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