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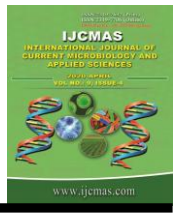

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In vitro Nutritional Evaluation and Digestion Kinetics of Concentrates Containing Varying Levels of *Moringa oleifera* Leaf Meal Supplementation as Protein Source for Goats

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A B S T R A C T

The present study was undertaken to study the effect of varying levels of *Moringa oleifera* leaf meal (MOLM) supplementation on *in vitro* utilization of concentrate mixtures replacing total crude protein of soybean @ 10,20,30,40 and 50%.The net gas production was significantly increased as the moringa supplementation level increased in the concentrate ration. It was significantly higher in 30% moringa based concentrate and significantly lowest in control concentrate. The partition factor (PF) was significantly lowest in 30% moringa concentrate (3.71) and significantly higher in 10% moringa containing concentrate mixture (4.26). The OMD was significantly higher in 30% moringa based ration and lowest in control ration but it was statistically comparable in moringa supplemented concentrate rations. The NDFD was significantly higher in 30% moringa concentrate ration but statistically comparable in moringa leaf meal supplemented concentrate rations. The MMP and EMMP was significantly higher in 10%. However, it was statistically comparable in all moringa leaf meal supplemented concentrate rations. The true digestibility was significantly higher in 30% moringa supplemented and it was significantly lower in control ration. The ME value was significantly lower in control concentrate and statistically higher in 30% moringa supplemented ration followed by 40 % moringa based ration. It can be concluded that *Moringa oleifera* leaf meal can replace up to30 percent protein of soybean meal without any adverse effect.

Introduction

Globally, agriculture plays a key role in improving livelihood, especially in rural communities. Livestock is the mainstay of the agricultural community. It provides 50% of the value of agricultural output globally and one third in developing countries. During the past 3 decades, rapid increase and development in the livestock sector have led to the livestock revolution. The livestock sector in India alone contributes nearly 25.6% of the value of output at current prices of the total value of output in Agriculture, Fishing & Forestry sector. The overall contribution of the Livestock Sector in total GDP is nearly 4.11% at current prices in 2012-13.

At present, the country faces a net deficit of 61.1% green fodder, 21.9% dry crop residues and 64% feeds. The deficit of Supply and demand scenario of forage and roughage at present is 696 (63.50) and dry 143 (23.56) million tonnes. The situation is further aggravated due to the increasing growth of livestock particularly that of genetically upgraded animals.

Livestock scientists are eager to explore and investigate good-quality fodders that can boost milk and meat production in an organic and economical way. *Moringa oleifera* is one of those plants that has been neglected for several years, but now is being investigated for its fast growth, higher nutritional attributes, and utilization as a livestock fodder crop. The *Moringa oleifera* is a multipurpose fast-growing tree and is high in protein and vitamins (pro-vitamin A, vitamins B and C) and minerals (Fe) and amino acids, methionine and cystine generally deficient in other feeds (Makkar and Becker 1996).

Leaves of the moringa tree are the preferred part for use in animal diets as leaf meal. Fresh leaves were found to contain 23 % crude protein (CP) in dry matter (DM), 12.3 ME/kg DM and had an *in vitro* DM digestibility of 79.7% (Becker 1995). The CP of Moringa is of better bioavailability for ruminants because of its high content of bypass protein (Becker 1995).

Materials and Methods

The *Moringa oleifera* leaf meal sample used in the *in vitro* study was obtained from the department of Animal Nutrition GADVASU, Ludhiana and was air-dried and then ground in a Wiley mill through a 2mm screen. The six concentrate mixtures were prepared by using various *Moringa oleifera* leaf meal (MOLM) levels replacing total crude protein of soybean meal at 10,20,30,40and 50% i.e. Concentrate 1(control), Concentrate 2(10%MOLM), Concentrate 3 (20%MOLM), Conc 4 (30% MOLM), Concentrate 5 (40% MOLM) and Concentrate 6(50% MOLM) as shown in Table 1. All the concentrate mixtures prepared were iso-nitrogenous having approximately 20% CP. The samples were analyzed for proximate (AOAC, 2005) and cell wall components (Robertson and Vansoest, 1981).

Kinetics of gas production

Air equilibrated feed samples (200 ± 10 mg) of RH, CS and mixed diets were incubated in 100 ml calibrated glass syringes in triplicate according to Menke and Steingass,(1988) with 30 ml mixed rumen suspension with three blank incubations and standards. Cumulative gas production was recorded at 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60 and 72 h of incubation. The rate and extent of gas production were calculated by non-linear regression using the model Y = D (1 – e-k*t) where, Y is gas volume (ml) at time t, D is potential gas production (ml) and k is rate (per hour) at which gas is produced (Orskov model, 1979; Krishnamoorthy *et al.*, 1995). The time at half asymptotic gas production (t1/2) was calculated as ln2/k.

Animal feeding and rumen analysis

Rumen liquor was collected in morning (6 am) from the goats before feeding and watering into a pre-warmed thermo-flask and brought to the laboratory. The *in vitro* gas production was done according to Menke *et al.*, (1979). The amount of net gas produced (NGP) was used to calculate the metabolizable energy (ME) value. Neutral Detergent Fibre (NDF) of the residue was also determined. Total degradable sample (TDS), organic matter degradability (% OMD), partition factor (PF), neutral detergent fiber degradability (% NDFD), microbial mass

production (mg, MMP), efficiency of microbial mass production (% EMMP), true digestibility (% TD) and short chain fatty acids (mmol, SCFA) were calculated according to Makkar (2004). Volatile fatty acids (VFAs) were estimated by (Cottoyn and Boucque, 1968) using gas liquid chromatography (GLC) technique using Net Chrom-9100 model. The gas column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 was used for the estimation of VFA. The gas flow for nitrogen hydrogen and zero air were 30, 30, and 320 µl/ min, respectively. Temperature of injector oven, column oven and detector were 270°C, 172°C respectively.

Statistical analysis

Data found from *in vitro* study were analyzed 1x3 factorial design (Snedecor and Cochran, 1994), by using SPSS Version 19. The differences in means were tested by Tukey B.

Results and Discussion

Ingredient and chemical composition of concentrates fed to male goats

The ingredient and chemical composition of different concentrates supplemented with varying levels of moringa leaf meal is given in (Table 1 and 2). The CP content of control concentrate and moringa supplemented concentrate varied from 20.10% to 20.93%. All the concentrate rations prepared were isonitrogenous in nature. The NDF content varied from 40.50 % to 42.6 %. The fat content of ration was between 3.88% and 4.3 %. The ash content in control concentrate ration was 7.57 %while in moringa supplemented concentrates ration it varied from 7.10 to 7.40 % and OM varied from 92.5% to 92.8% in moringa supplemented concentrate ration and in control concentrate it was 92.40%. The total carbohydrates (TCHO) in control concentrate ration was 66.23% while in moringa concentrate mixtures it varied from 56.30 to 62.63%. The non-fibre carbohydrates (NFC) of the moringa supplement concentrates varied from 14.25% to 21.43 and in control, it was 26.63%.

In-vitro gas production data on *in vitro* utilization of nutrients of concentrates

Concentrate mixtures containing different levels of replacement of CP of soybean with *Moringa oleifera* leaf meal is shown in Table 3. The control concentrate mixture has significantly produced lower ($p<0.05$) net gas production (75.25 ml) and highest in 30% moringa based concentrate mixture (88.0 ml). However, the 40% moringa concentrate produced net gas production (84.50ml) and in 50% moringa concentrate it was (81.0 ml). There was no significant effect seen on a truly degraded substrate (TDS). It was statistically comparable in control, 40% and 50% moringa based concentrates mixtures. The partitioning factor (PF) is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher partitioning factor means that proportionally more of the degraded matter is incorporated into microbial mass i.e. the efficiency of microbial protein synthesis is higher. The partition factor calculated *in vitro* provides useful information for predicting the dry matter intake, microbial mass production in the rumen and the methane emission of the whole ruminant animal. The partition factor was significantly higher in Conc 2 (4.26) and Conc 3 (4.20) however, it was significantly lower in 30% moringa concentrate mixture (3.71). The Organic matter digestibility was statistically higher in 30% moringa based concentrate mixture (93.26 %) and significantly lowest in the control group (87.16%). However, it was statistically comparable in all moringa containing concentrate mixtures as it varied from 89.00% to 92.13 %. A significant difference has been seen on neutral detergent fibre digestibility (NDFD%) in all

concentrates rations. It varied from 73.79% (30 % moringa concentrate mixture) to 57.91% (control concentrate mixture). The efficiency of microbial mass production was significantly higher ($p<0.05$) in 10 % moringa based (48.33%) concentrates and lowest in 30 % moringa rations (40.71%). However, it was statistically comparable in control and 50% moringa leaf meal concentrate mixture. There were statistically significant higher difference in true or dry matter digestibility in control (87.60%) and moringa containing concentrate mixture (93.20%) The control concentrate ration has produced significantly lower ($p<0.05$) short-chain fatty acids (1.62 mmole) and highest in 30% moringa based concentrate mixture (1.91mmole). The control concentrate rations had significantly higher ($p<0.05$) ammonia concentration (41.66 mg/dl) and lowest in 40% moringa based concentrate (33.03 mg/dl)however, it was statistically comparable in 10 and 30% moringa containing concentrate. Metabolizable energy (ME) was statistically comparable in 10, 20 and 50% moringa based concentrate mixtures. The ME value was significantly higher in 30% moringa based concentrate mixture and was found significantly lower in control concentrate rations. The amount of fermentable methane (0.1820 mmol) was lower ($p<0.05$) in 10 and 20% moringa based rations and higher in 30 % moringa (0.507 mmol) based rations, whereas fermentable carbon dioxide was significantly higher ($p<0.05$) in 40% moringa ration (0.207 mmol) and lowest in control rations (0.182 mmole) (Fig. 1).

In vitro volatile fatty acids production (mM/dl) of different concentrates containing different levels of moringa leaf meal

The effect of different concentrate mixtures containing varying levels of moringa leaf meal on total and individual volatile fatty acids *in vitro* is presented in (Table 4 and Fig. 2). The TVFA was significantly lowest ($p<0.05$) in 10 and 20% moringa based ration (4.86 mM/dl) and was significantly higher in 30% moringa based concentrate mixture (7.10 mM/dl). The relative percent of acetate was significantly lowest (49.15%) in 10% moringa supplemented concentrate mixture and highest in 30% moringa based concentrate ration (58.62%) and 50% moringa concentrate mixture (56.01%).

The propionate percent was statistically higher in 10% moringa concentrate (41.16%) and lowest in 30 % moringa based concentrate mixture (31.68%). The percent isobutyric was significantly higher ($p<0.05$) in 20% moringa concentrate mixture (2.2 %) whereas it was significantly lower in 30% moringa concentrate (1.53%). The butyrate percent was observed to be significantly highest in 40% moringa concentrate ration (6.17%) followed by 50% moringa (5.99 %) and 30% moringa based concentrate ration (5.61%) and lowest percent in control ration (4.19%). The isovalerate percent was significantly higher in 40% moringa concentrate ration (1.39%) and lowest in 20% moringa concentrate mixture (0.69%). The acetate to propionate ratio was significantly lowest ($p<0.05$) in 20% moringa supplemented ration (1.20) and highest in 30% moringa supplemented concentrate ration (1.89).

Digestion kinetics parameters *in vitro* gas production of concentrates

Cumulative gas production profiles from the *in vitro* fermentation of concentrates are shown in Figure 3 and the estimated parameters are given in Table 5. The cumulative volume of gas production increased with increasing time of incubation. The gas produced after 72 h incubation ranged between 56.83 and 65.33 ml per 0.200 g of dry matter.

Table.1 Ingredient composition of different concentrate mixtures containing *Moringa oleifera* leaf meal

Ingredients	Control	30% MOLM	40% MOLM	50% MOLM
Maize	35	35	35	35
Soybean meal	28	19.6	16.84	14
MOLM	0	14	18.66	23.33
Wheat bran	17	12	11	11
Rice bran	14.75	14.9	14	12.5
Mineral mix	2	2	2	2
Salt	1	1	1	1
Urea	0	0.5	0.5	0.6
By-pass fat	2.25	1	1	0.57

Table.2 Chemical composition of concentrates fed to goats, % DM basis

Parameters	CONC 1 (control)	CONC 2 (30%)	CONC 3 (40%)	CONC 4 (50%)
DM	91	90.5	92	90
Ash	7.575	7.1	7.125	7.475
OM	92.425	92.9	92.875	92.525
CP	20.27	20.1	20.26	20.93
NDF	42.6	42.4	42	40.5
ADF	10.5	10.75	12.3	12.6
HC	32.1	31.65	29.7	27.9
ADL	2.25	3.1	3.55	3.35
EE	3.88	4.03	4.5	4.3
Cellulose	8.25	7.65	8.75	9.25
TCHO	68.275	68.77	68.115	67.295
NFC	25.675	26.37	26.115	26.795

Table.3 Effect of different levels of *Moringa oleifera* leaf meal-based concentrate mixtures on *in-vitro* utilization of nutrients

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	CONC 6	SEM
NGP, ml	75.25^{a}	72.75^{a}	76.00^{ab}	88.00^{d}	84.50^{cd}	81.00^{bc}	1.69
TDS, mg	346.68	347.25	349.87	350.06	349.68	345.75	0.51
PF	4.01^{ab}	4.26^{b}	4.20^{b}	3.71^{a}	3.81^{ab}	3.87^{ab}	0.066
OMD, %	87.16^{a}	89.20^{ab}	91.42^{bcd}	93.26^{d}	92.13^{cd}	90.88^{bc}	0.63
NDFD, %	57.91^{a}	59.01^{a}	67.49^{ab}	73.79^{b}	69.94^{b}	70.68^{b}	1.90
MMP, mg	136.63^{a}	149.70^{b}	152.67^{b}	132.96^{a}	136.28^{a}	136.05^{a}	2.53
EMMP, mg	45.21^{abc}	48.33^{c}	47.72^{bc}	40.71^{a}	42.28^{ab}	43.29^{abc}	0.89
TD, %	87.60^{a}	89.73^{b}	91.60^{bcd}	93.20^{d}	92.13^{cd}	91.06^{bc}	0.57
SCFA, mmol	1.62^{a}	1.57^{a}	1.64^{ab}	1.91^{d}	1.83^{cd}	1.75^{bc}	0.037
ME, MJ/Kg DM	9.58^{a}	10.05^{b}	10.13^{b}	10.78^{d}	10.49^{c}	10.16^{b}	0.11
NH_3-N, mg/dl	41.66^{c}	36.90^{b}	32.14^{a}	35.12^{ab}	33.03^{a}	32.14^{a}	0.001
Ferm. CO_2, mmol	0.1820^{a}	0.1895^{ab}	0.1891^{ab}	0.1914^{b}	0.2077^{c}	0.2036^{c}	0.027
Ferm. CH_4, mmol	0.416^{b}	0.388^{a}	0.389^{a}	0.507^{d}	0.470^{c}	0.476^{c}	0.013

Means bearing different superscripts in a row differ significantly (P<0.05)

Table.4 *In vitro* volatile fatty acids production (mM/dl) of different concentrates containing different levels of MOLM

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	CONC 6	SEM
Acetic acid	3.284^{b}	2.390^{a}	2.397^{a}	4.167^{d}	3.627^{c}	3.100^{b}	0.193
Propionic acid	2.501^{d}	2.002^{ab}	1.994^{ab}	2.251^{c}	2.203^{bc}	1.857^{a}	0.066
Iso butyric acid	0.117^{b}	0.101^{ab}	0.107^{b}	0.108^{b}	0.107^{b}	0.085^{a}	0.003
Butyric acid	0.267^{b}	0.210^{a}	0.211^{a}	0.398^{d}	0.404^{d}	0.331^{c}	0.024
Iso valeric acid	0.056^{bc}	0.026^{a}	0.033^{ab}	0.069^{cd}	0.091^{d}	0.070^{cd}	0.007
Valeric acid	0.149^{e}	0.132^{d}	0.124^{cd}	0.111^{bc}	0.108^{b}	0.091^{a}	0.005
TVFA	6.37^{c}	4.86^{a}	4.86^{a}	7.10^{d}	6.54^{c}	5.53^{b}	0.26
Relative proportion, %							
Acetate	51.477^{b}	49.153^{a}	49.237^{a}	58.621^{d}	55.439^{c}	56.017^{c}	1.08
Propionate	39.231^{c}	41.160^{d}	40.967^{d}	31.680^{a}	33.677^{b}	33.516^{b}	1.16
Iso butyrate	1.848^{ab}	2.078^{bc}	2.212^{c}	1.529^{a}	1.647^{a}	1.537^{a}	0.083
Butyrate	4.199^{a}	4.334^{a}	4.336^{a}	5.610^{b}	6.177^{c}	5.991^{c}	0.253
Iso valerate	0.896^{ab}	0.555^{a}	0.692^{a}	0.983^{abc}	1.399^{c}	1.278^{bc}	0.097
Valerate	2.347^{b}	2.717^{c}	2.554^{bc}	1.574^{a}	1.658^{a}	1.658^{a}	0.143
A:P ratio	1.31^{c}	1.19^{a}	1.20^{a}	1.85^{d}	1.64^{c}	1.67^{c}	0.77

Means bearing different superscripts in a row differ significantly (P<0.05)

Table.5 Effect of different levels of MOLM on fermentation kinetics of concentrates

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	CONC 6	SEM
2h	5.00^{a}	4.66^{a}	4.33^{a}	3.83^{a}	6.16^{b}	6.83^{b}	0.27
4h	11.33^{bc}	11.00^{ab}	10.16^{ab}	9.00^{a}	12.16^{bc}	13.33^{b}	0.37
6h	20.50^{b}	21.00^{b}	20.50^{b}	16.83^{a}	19.83^{b}	20.66^{b}	0.40
8h	26.83^{b}	27.83^{b}	28.50^{b}	24.00^{a}	27.06^{b}	28.00^{b}	0.42
10h	30.83	31.66	33.16	30.50	34.50	33.16	0.46
12h	33.83^{a}	34.50^{ab}	36.50^{ab}	35.16^{ab}	39.16^{b}	37.16^{ab}	0.56
24h	46.83^{a}	47.16^{a}	49.83^{ab}	50.16^{ab}	54.50^{c}	52.00^{bc}	0.70
36h	52.00^{a}	52.16^{a}	55.66^{ab}	55.50^{ab}	60.16^{c}	57.50^{bc}	0.75
48h	54.66^{a}	54.83^{a}	59.00^{abc}	57.83^{ab}	63.00^{c}	60.00^{bc}	0.78
60h	56.00^{a}	56.33^{a}	61.00^{bc}	59.16^{ab}	64.83^{c}	62.00^{bc}	0.83
72h	56.83^{a}	57.16^{a}	65.33^{b}	59.66^{ab}	65.50^{b}	62.66^{ab}	1.03

Means bearing different superscripts in a row differ significantly ($P<0.05$)

Table.6 Estimated parameters of concentrates containing varying levels of moringa leaf meal when incubated with rumen fluid at different incubation times

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	CONC 6
Yo (a)	-4.457	-5.471	-5.782	-7.984	-5.656	-3.33
Plateau (a+b)	55.87	55.91	61.72	59.58	65.10	62.13
K (c)	0.08526	0.08969	0.08056	0.08128	0.07956	0.07904
Tau (V min)	11.73	11.15	12.41	12.3	12.57	12.65
Half life	8.13	7.728	8.604	8.528	8.712	8.769
Span (b)	60.33	61.38	67.50	67.57	70.76	65.45
R square	0.995	0.9936	0.9856	0.9968	0.9856	0.99
Lag time	0.6hr	0.4hr	0.5hr	0.6hr	0.5hr	0.2hr

c = gas production rate, a = gas production (ml) from quickly soluble fraction, b = gas production (ml) from insoluble fraction, (a + b) = potential gas production

Fig.1 Effect of different levels of *Moringa oleifera* leaf meal-based concentrate mixtures on *in-vitro* utilization of nutrients

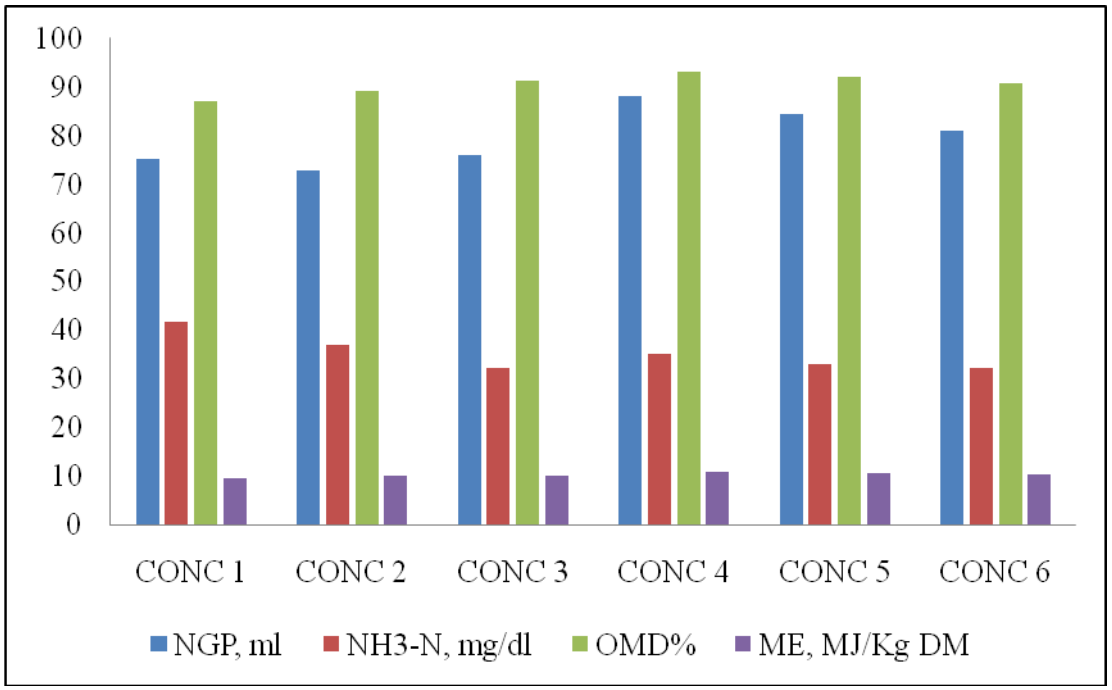


Fig.2 *In vitro* volatile fatty acids production (mM/dl) of concentrates containing different levels of MOLM

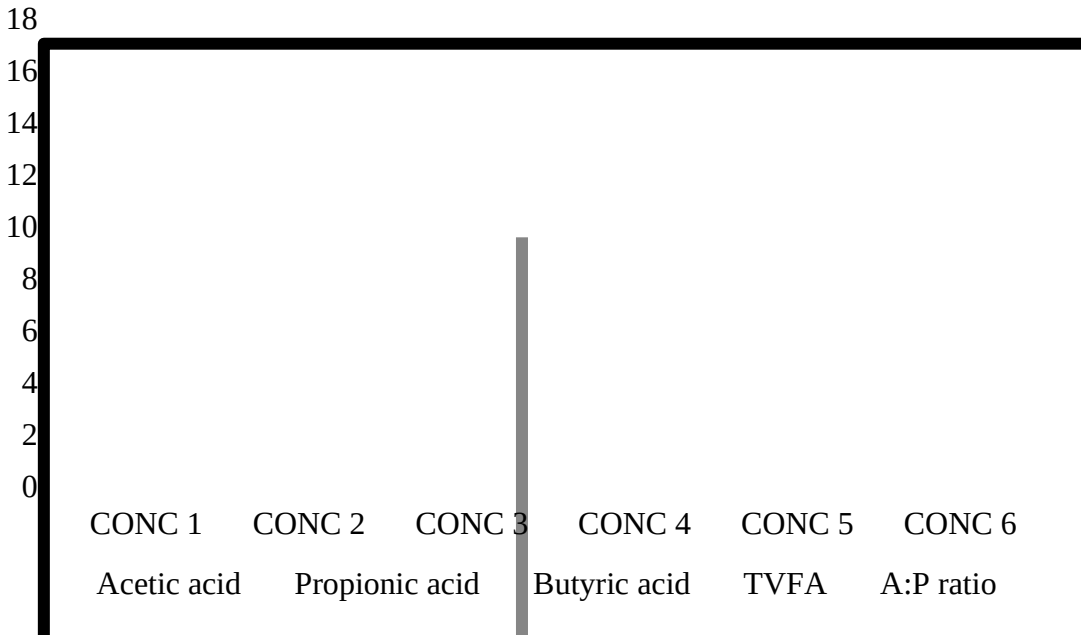
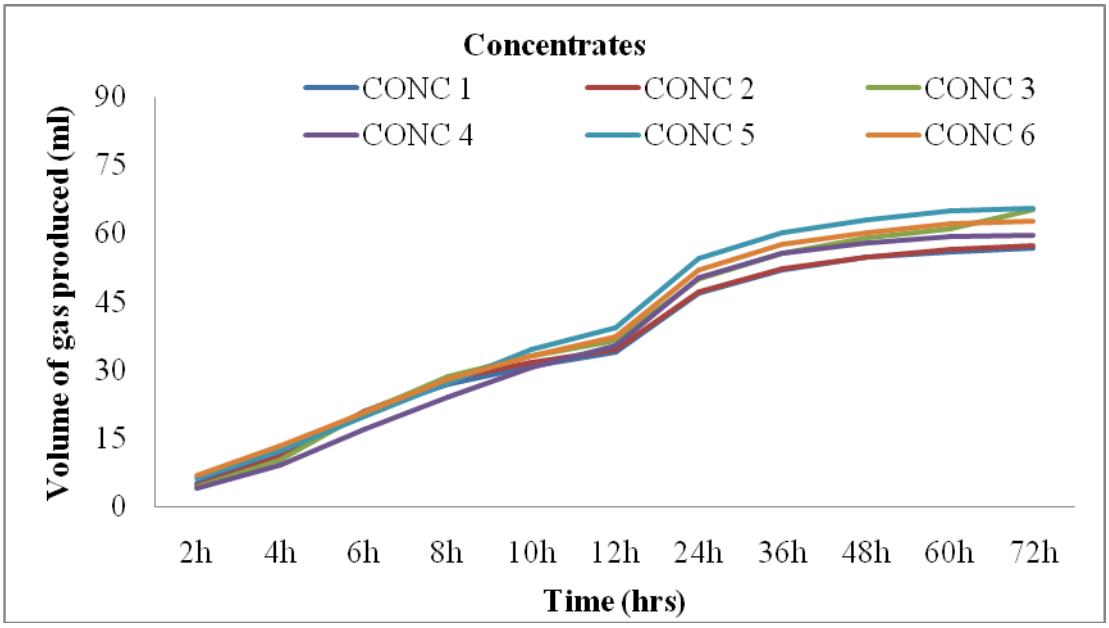


Fig.3 Effect of different levels of MOLM on fermentation kinetics of concentrates



At 72 h incubation times, cumulative gas productions (ml) of control and 10% moringa based concentrates have significantly ($P < 0.05$) lower than those of 30 % moringa based concentrate. However, in all moringa based concentrates the cumulative gas production at 72 h is statistically comparable and on the higher side, as compared to control concentrate ration. The gas production in all the concentrates at 24 h has differed statistically non significantly (Fig. 3).

The gas production profiles presented in table 6 indicated that Y max (maximum potential of gas production) varied amongst the different concentrates evaluated. The Y max (ml) was lower in control TMR (55.87) and was higher in 40% moringa based concentrate (65.10 ml) followed by 50% (62.13) and 20% (61.72) moringa supplemented concentrates. However, the V min (ml) ranged from 11.15 to 12.65 ml in concentrates evaluated. It was lowest in 10% moringa supplemented concentrate (11.15) and highest in 50% moringa supplemented concentrate (12.65). The maximum rate of degradation (k) was observed in 10% moringa supplemented concentrate (8.96%, h) and lowest in 50% moringa concentrate (7.90 %h). The value of 'a' was found lowest in 50% moringa supplemented concentrate (3.33) and highest in 30% moringa supplemented concentrate (7.98)

If 'a' is positive, then there is a component which is degraded rapidly and/or a component which is soluble. When a negative value for 'a' is obtained this means that there has to be an initiation period for degradation to start (termed the lag phase).

The t ½ (time taken to reach half of the asymptote) was lowest for 10% moringa concentrate (7.73 h) and highest for 50% moringa concentrate (8.76 h). The value of "b" (gas production from insoluble fraction was lowest in control concentrate (60.33ml) and was highest in 40% moringa concentrate (70.76ml). In all the moringa based concentrates, the gas production from insoluble fraction was higher as compared to control concentrate (Table 5). The lag time

was lowest in 50% moringa concentrate (0.7hr) and was highest in 30% moringa supplemented concentrate (1.5hr)

In conclusions, the net gas production was significantly increased as the moringa supplementation level increased in the concentrate ration. It was significantly higher in 30% moringa based concentrate and significantly lowest in control concentrate. The partition factor (PF) was significantly lowest in 30% moringa concentrate (3.71) and significantly higher in 10% moringa containing concentrate mixture (4.26). The OMD was significantly higher in 30% moringa based ration and lowest in control ration but it was statistically comparable in moringa supplemented concentrate rations. The NDFD was significantly higher in 30% moringa concentrate ration but statistically comparable in moringa leaf meal supplemented concentrate rations. The MMP and EMMP was significantly higher in 10%. However, it was statistically comparable in all moringa leaf meal supplemented concentrate rations. The true digestibility was significantly higher in 30% moringa supplemented and it was significantly lower in control ration. The ME value was significantly lower in control concentrate and statistically higher in 30% moringa supplemented ration followed by 40 % moringa based ration. The A:P ratio and TVFA was significantly lowest ($p<0.05$) in 10 and 20% moringa based ration(4.86 mM/dl) and was significantly higher in 30% moringa based concentrate mixture (7.10 mM/dl). At 72 h incubation times cumulative gas productions (ml) of control was significantly ($p<0.05$) lower than that of 30% moringa based concentrate. The Y max (ml) was lower in control concentrate (55.87) and was higher in 40%moringa based concentrate (65.10 ml) followed by 50% (62.13) and 20% (61.72) moringa supplemented concentrates. In all the moringa based concentrates the gas production from insoluble fraction (b) was significantly higher as compared to control concentrate. The lag time was lowest in 50% moringa concentrate (0.2hr) and was highest in control and 30% moringa supplemented concentrate(0.6hr) It can be concluded that *Moringa oleifera* leaf meal can be replaced up to 30% of total crude protein of soybean meal without any adverse effect.

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