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Effect of molasses based multi-nutrient herbal supplements on *in vitro* digestibility, serum enzymes and minerals profile in buffalo calves

PUNITA KUMARI[1], P SINGH[2], A K VERMA[3], G K GAUR[4] and A DAS[5]

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

Molasses based multinutrients herbal supplements (MMS) containing ground fenugreek seed and de-oiled mahua seed cake at two different ratios (1:1; MMS I or 1:3; MMS II) was evaluated under *in vitro* as well as *in vivo* conditions. For *in vitro* evaluation, concentrate mixture and wheat straw in 50: 50 ratio (on DM basis) was taken as substrate for the experiment. MMS I and MMS II were added at 2, 3 and 4% of the substrate, respectively. The total gas production (ml/g DM), partitioning factor (PF) and microbial biomass production (MBP) were found comparable in all the groups but significantly improved the IVDMD and IVOMD in supplemented groups. Under *in vivo* feeding trial, serum enzymes and minerals profile in buffalo calves were assessed during 9 months of experimental period. Fifteen male Murrah buffalo calves (10 to 15 months of age and mean body wt. 234.0±12.5 kg) were randomly distributed into 3 groups (5 in each group) according to randomized block design. All animals were fed with conventional concentrate mixture, available chopped green fodder (3–4 kg DM/d) and wheat straw *ad lib* to meet out nutrients requirement. While control group (C) were not fed supplements but groups T_1 and T_2 supplemented with MMS I and MMS II, respectively at 44 g/100 kg body weight or 200 g/100 kg metabolic body weight ($kgW^{0.75}$). Serum AST, ALT activities and serum concentration of Ca, P, Fe, Mn, Cu and Co were comparable among 3 groups but serum concentration of Zn was significantly higher among supplemented groups. Thus, results show that supplementation of MMS improved IVDMD, IVOMD and serum Zn level resulting in to better health conditions of buffalo calves.

Key words: Buffalo calves, Deoiled mahua seed cake, Fenugreek seed, *In vitro* digestibility, Serum enzymes, Serum minerals

Mineral deficiency exist widely in livestock and have more significant consequences than any infectious disease, the severity of the deficiency depends upon the type of feed and soil, physiological status of the animals and the agro-climatic conditions of the region. Minerals act as structural components of body organs and tissues, constituents of body fluids and tissues as electrolytes and catalysts in enzyme and hormone systems. Deficiency and/or imbalance of nutrients that can alter the activity of certain enzymes and function of specific organs, thus impairing specific metabolic pathways as well as overall immune function. Thus, nutritional supplementation of minerals to the livestock is important to improve general, productive and reproductive health of animals (Kleczkowski *et al*. 2003) and to overcome the detrimental effect of deficiencies on animal performance (Underwood 1981). Medicinal herbs and seeds are used as feed additives to ruminants globally (Singh *et al*. 1993).

Plant secondary metabolites such as 8 hydroxyquinoline, bornyl acetate and thymoquinone have promising effects on rumen CH_4 production and also considerable impact on rumen bacterial communities even at the lowest concentrations that decreased CH_4 production (Joch *et al*. 2018). Phytogenic feed additives showed health promoting functions and also affected rumen ecosystem of ruminants which resulted in reduced methanogenesis and increased feed utilization efficiency (Kamra *et al*. 2008, Bodas *et al*. 2009). Saponins may decrease methane production *via* defaunation and/or decrease the activities (rate of methanogenesis or expression of methane producing genes) and numbers of methanogens (Patra and Saxena 2009).

Molasses based supplements have been developed as a scarcity feeds (Ranjhan *et al*. 1973, Verma *et al*. 1995). Fenugreek contains alkaloids such as trigonelline, flavonoids and saponins (3–5%) (Singh and Garg 2006) whereas, mahua seed cake is a rich source of saponins 9.8% (Singh and Singh 1991), and tannins 6.2% (Singh and Agarwala 1989). Therefore, ground fenugreek seeds and mahua seed cake can effectively be utilized as functional feeds containing tannins and saponins as active compounds.

Present address: [1]PhD Scholar (puneeta.kri@gmail.com), [2]Principal Scientist (putan60@gmail.com), [3]Head and Director (vermaak62@gmail.com), Animal Nutrition Division; [4]Principal Scientist (gyanendrakg@gmail.com), Livestock and Production Management Section. [5]Principal Scientist (drasitdas @rediffmail.com), ICAR-NDRI, Karnal.

Hence, the present study envisaged to assess the influence of molasses based multi-nutrients herbal supplements (MMS I and II) on *in vitro* digestibility, serum enzymes and minerals profile in buffalo calves.

MATERIALS AND METHODS

In vitro *analysis*: MMS-I (T_1) and MMS-II (T_2) were added to the substrate containing concentrate and wheat straw in 50:50 ratio @ of 2, 3 and 4% of DM respectively and different types of rations were formulated. Air equilibrated samples (200 mg of substrate along with supplement) were incubated with 30 ml buffered rumen inoculum under continuous flushing with CO_2 into 100 ml calibrated glass syringes (Menke and Steingass 1988). The partitioning factor (PF) was calculated as the ratio of mg true digested organic matter (TDOM) to ml gas produced at 24 h. Microbial biomass production (MBP) was calculated (Blümmel *et al*. 1997). The contents of syringes were quantitatively transferred into a 500 ml spoutless beaker for recovering undigested NDF residue (Goering and Van Soest 1970). *In vitro* true dry matter digestibility (IVDMD) and *in vitro* true organic matter digestibility (IVOMD) were estimated.

Animal's management, experimental feeding and feed analysis: Healthy male buffalo calves (15) of about 10 to 15 months of age and mean body wt. 234.0±12.5 kg were used for the experiment. Proper health management and sanitation conditions were maintained throughout the experimental period of 9 months. Animals were randomly divided into 3 groups of 5 each following randomized block design. All animals were supplied with available green fodder (3–4 kg DM/d), wheat straw *ad lib*. and a conventional concentrate mixture (45% wheat bran, 17% deoiled soybean meal, 17% crushed maize, 18% crushed barley, 2% mineral mixture, 1% common salt) to meet out their nutrients requirement (ICAR 2013). In groups T_1 and T_2, MMS-I and MMS-II in form of Laddoo (ball shape) was given @ 44 g/100 kg body weight or 200 g /100 kg metabolic body weight ($kgW^{0.75}$). The MMS I and II consisted (%) of molasses 49 & 49, ground fenugreek seed 24.50 & 12.25, DMSC 24.50 & 36.75, mineral mixture 2 & 2, respectively. Each animal received weighed amount of feed (concentrate mixture, green fodder and wheat straw) once daily at 9-11 AM. All animals had free access to clean drinking water throughout the day. Feed samples were analyzed for dry matter and crude protein following standard procedures (AOAC 2005). Calcium content (Talapatra *et al.* 1940) and total inorganic phosphorus (spectrophotometric methods, AOAC 1995) was estimated from mineral extract of feeds, MMS I and MMS II. Zinc, copper, iron, manganese and cobalt content in mineral extract of feeds, MMS I and MMS II samples were estimated by Atomic Absorption Spectrophotometer (ECIL, Hyderabad).

Collection and analysis of blood: Blood from all animals was collected at 0, 90,180 and 270 days of experimental periods to study the serum enzymes and minerals profile by puncturing the jugular vein with the help of a clean sterilized needle into test tubes and allowed to clot. By centrifuging the test tube samples at 2000 rpm for 20 min, serum was separated and stored at –20°C for further chemical analysis. The AST and ALT enzymes were estimated by the method described by Reitman and Frankel (1957) using commercial kit (Coral Clinical Systems, India). The concentrations of calcium and phosphorus in the serum were estimated by the OCPC method (Gitelman 1967) and Molybdate UV method (Goodwin 1970) respectively using commercial kit (Coral Clinical Systems, India). The concentrations of serum micro minerals Zn, Cu, Fe, Mn and Co was done by Atomic Absorption Spectrophotometer (ECIL, Hyderabad). Samples for trace element analysis were processed according to the wet digestion procedure (Kolmer 1951).

Statistical analysis: Data pertaining to *in vitro* digestibility were analyzed by one-way ANOVA and data pertaining to serum enzymes and minerals profile were subjected to general linear model (GLM)-univariate or multivariate analysis to separate the effect of treatment, day of sampling and their interaction. Treatment means were separated by Duncan's multiple range test and the differences were considered to be significant ($P<0.05$). All data analyses were performed using statistical package of SPSS (20.0) (2012).

Table 1. Chemical composition (% on DM basis) of different feed ingredients

Particular	CM	GF	WS	MMS-I	MMS-II	FS	DMSC
Dry matter (%)	88.55	23.84	92.76	83.57	84.47	88.61	90.03
OM	94.09	89.10	92.13	86.22	84.60	96.45	78.48
CP	17.73	9.18	3.49	21.94	22.25	29.53	32.65
EE	2.44	2.90	1.04	1.33	1.19	3.28	0.43
NDF	38.18	63.77	78.26	29.22	30.20	37.26	41.99
ADF	13.03	46.97	56.11	16.07	17.54	15.80	32.35
Hemicellulose	25.15	16.80	22.15	13.15	12.66	21.46	9.64
Cellulose	7.17	39.46	47.43	10.03	9.92	13.34	24.89
Lignin	5.86	7.50	8.68	6.04	7.62	2.47	7.46

CM, Concentrate mixture; GF, Green fodder; WS, Wheat straw; MMS, Molasses based multi-nutrients herbal supplement; FS, Fenugreek seed; DMSC, Deoiled mahua seed cake.

Table 2. Effect of graded level of MMS-I on *in vitro* gas production, DM and OM digestibility and microbial biomass production

Parameter	Control	MMS I (T_1)			SEM	P value
		2%	3%	4%		
Total gas (ml/g DM)	155.47±9.48	166.67±1.67	168.33±3.33	163.33±11.67	3.62	0.67
TG (ml/0.2 g DM)	31.09±1.90	33.33±0.33	33.67±0.67	32.67±2.33	0.72	0.67
TG (ml/g DDM)	294.57±15.64	281.80±5.69	274.22±11.08	270.73±19.15	6.51	0.64
IVDMD (%)	52.74^{b}±0.73	59.17^{a}±0.60	61.50^{a}±1.53	60.33^{a}±0.88	1.11	Â0.01
IVOMD (%)	56.56^{b}±2.64	63.58^{a}±0.79	64.29^{a}±1.19	63.35^{a}±1.26	1.17	0.03
PF (mg/ml)	3.41±0.18	3.55±0.07	3.66±0.15	3.73±0.28	0.09	0.66
MBP (mg)	36.09±5.24	43.06±1.70	43.54±3.29	43.96±5.63	2.06	0.54

^{a,b}Mean bearing different superscripts in a row differ significantly.

Table 3. Effect of graded level of MMS-II on *in vitro* gas production, DM and OM digestibility and microbial biomass production

Parameter	Control	MMS II (T_2)			SEM	P value
		2%	3%	4%		
Total gas (ml/g DM)	155.47±9.48	155.00±2.89	158.33±1.67	155.00±0.00	2.18	0.96
TG (ml/0.2 g DM)	31.09±1.90	31.00±0.58	31.67±0.33	31.00±0.00	0.44	0.96
TG (ml/g DDM)	294.57±15.64	265.02±5.47	263.36±6.71	261.24±0.73	5.61	0.09
IVDMD (%)	52.73^{b}±0.73	58.50^{a}±0.76	60.17^{a}±0.93	59.33^{a}±0.17	0.93	0.01
IVOMD (%)	56.56^{b}±2.64	62.65 ^{a}±2.41	63.89 ^{a}±2.21	62.22 ^{a}±0.32	1.24	0.05
PF (mg/ml)	3.41±0.18	3.78±0.08	3.80±0.10	3.83±0.011	0.07	0.09
MBP (mg)	36.09±5.24	46.43±4.97	47.13±4.77	45.43±0.58	2.29	0.31

^{a,b}Mean bearing different superscripts in a row differ significantly.

Table 4. Serum enzyme profile of male buffalo calves

Parameter	Days				Mean	SEM	T	P	T*P
	0	90	180	270					
AST (IU/L)									
C	92.94±3.95	93.28±2.13	93.94±3.04	94.17±2.31	93.58±1.36	0.95	0.90	0.95	1.00
T_1	93.06±4.05	94.28±2.73	94.83±4.62	95.17±0.79	94.33±1.56				
T_2	93.94±4.88	94.50±2.18	94.94±3.58	95.06±2.75	94.61±1.61				
Mean	93.31±2.31	94.02±1.27	94.57±2.03	94.80±1.14					
ALT (IU/L)									
C	25.90±0.81	26.19±3.61	26.76±4.08	26.38±2.52	26.31±1.39	0.74	0.88	0.90	1.00
T_1	26.10±3.11	27.14±2.22	27.43±2.55	28.10±2.06	27.19±1.17				
T_2	25.81±2.71	26.86±1.71	27.33±2.16	27.81±1.35	26.95±0.96				
Mean	25.94±1.30	26.73±1.42	27.17±1.63	27.43±1.11					

RESULTS AND DISCUSSION

The chemical composition of the different feed ingredients is shown in Table 1. The total gas production (ml/g DM), partitioning factor (PF) and microbial biomass production was found comparable (P>0.05) in all groups supplemented with different levels of MMS I and MMS II (Table 2 and Table 3). Addition of MMS I and MMS II at 2%, 3% and 4% of ration significantly (P<0.01) improved the IVDMD and IVOMD (%), which were highest at 3% levels as compared to control (Tables 2 and 3). In case of total gas production result are well collaborated with finding of Inamdar *et al.* (2015), Sachan *et al.* (2014) but was contradictory with findings of Kumar *et al*. (2016). The similar total gas production in all treatment groups thus indicated similar rate of fermentation in all three diets. Gas production is an indirect measurement of substrate degradation and is a good predictor for the production of VFA, but it is not always positively related to microbial mass production. In case of IVDMD (%), IVOMD (%) the findings are well collaborated with results of Kumar *et al*. (2016) and Ankita (2016) who reported an improvement in the *in vitro* DM and OM digestibility. This might be due to increase in level of fermentable sugars or carbohydrates (Kumar 2015). The findings of partitioning factor (PF) and microbial biomass production (MBP) are in agreement with Ankita (2016).

In the present study, there was no variation in the activity of ALT and AST among the treatments across various time intervals in treatments C, T1 and T2, respectively (Table 4). The findings were in agreement with Ojha *et al.* (2013) in male crossbred calves, Ishtiyak *et al.* (2013) in kids, Inamdar

Table 5. Mineral composition (% on DM basis) of different feed ingredients

Parameter	CM	GF	WS	MMS-I	MMS-II	FS	DMSC
Dry matter (%)	88.55	23.84	92.76	83.57	84.47	88.61	90.03
On DM basis							
Ca (%)	0.83	0.60	0.43	1.58	1.78	0.62	0.82
P (%)	0.66	0.49	0.14	0.60	0.67	0.43	0.92
Fe (ppm)	173.90	375.89	182.12	239.10	243.40	201.70	214.50
Zn (ppm)	74.50	27.80	11.35	77.50	79.90	25.78	27.16
Mn (ppm)	68.70	61.40	38.30	107.50	113.90	21.30	35.54
Cu (ppm)	17.60	13.71	4.27	45.60	49.90	17.50	21.28
Co (ppm)	0.42	0.34	0.24	0.43	0.50	0.22	0.28

CM, Concentrate mixture; GF, Green fodder; WS, Wheat straw; MMS, Molasses based multi-nutrients herbal supplement; FS, Fenugreek seed; DMSC, Deoiled mahua seed cake.

Table 6. Serum mineral profile of male buffalo calves

Parameter	Days				Mean	SEM	T	P	T*P
	0	90	180	270					
Ca (mg/dl)									
C	9.55±0.18	9.64±0.35	9.78±0.12	9.81±0.84	9.70±0.22	0.09	0.99	0.65	1.00
T_1	9.54±0.22	9.70±0.22	9.80±0.22	9.87±0.17	9.73±0.10				
T_2	9.53±0.22	9.66±0.12	9.81±0.15	9.84±0.24	9.71±0.09				
Mean	9.54±0.11	9.67±0.13	9.80±0.09	9.84±0.28					
P (mg/dl)									
C	6.46±0.10	6.53±0.05	6.54±0.12	6.56±0.08	6.52±0.04	0.02	0.98	0.14	1.00
T_1	6.41±0.09	6.55±0.07	6.57±0.09	6.61±0.10	6.53±0.04				
T_2	6.43±0.09	6.53±0.02	6.55±0.05	6.59±0.10	6.53±0.04				
Mean	6.43±0.05	6.54±0.03	6.56±0.05	6.59±0.05					
Fe (ppm)									
C	4.29±0.06	4.30±0.04	4.20±0.03	4.18±0.05	4.24±0.03	0.01	0.84	0.22	0.74
T_1	4.26±0.05	4.27±0.07	4.27±0.02	4.23±0.04	4.26±0.02				
T_2	4.28±0.02	4.25±0.03	4.26±0.01	4.24±0.04	4.26±0.01				
Mean	4.27±0.03	4.28±0.03	4.24±0.01	4.21±0.02					
Zn (ppm)									
C	2.28±0.006	2.24±0.019	2.28±0.006	2.25±0.030	2.26^{b}±0.009	0.004	≤0.01	0.45	0.20
T_1	2.29±0.007	2.30±0.006	2.30±0.005	2.26±0.019	2.29^{a}±0.006				
T_2	2.29±0.008	2.30±0.017	2.30±0.004	2.31±0.020	2.30^{a}±0.007				
Mean	2.28±0.004	2.28±0.011	2.29±0.003	2.27±0.015					
Cu (ppm)									
C	0.93±0.012	0.94±0.008	0.95±0.013	0.94±0.012	0.94±0.006	0.003	0.47	0.02	0.49
T_1	0.92±0.005	0.94±0.005	0.95±0.006	0.97±0.003	0.95±0.004				
T_2	0.94±0.007	0.95±0.016	0.96±0.006	0.95±0.006	0.95±0.005				
Mean	0.93^{B}±0.004	0.94^{AB}±0.006	0.95^{A}±0.005	0.96^{A}±0.005					
Mn (ppm)									
C	0.84±0.008	0.85±0.005	0.85±0.013	0.84±0.010	0.85±0.005	0.003	0.50	0.23	0.97
T_1	0.85±0.013	0.86±0.014	0.87±0.009	0.85±0.013	0.86±0.006				
T_2	0.86±0.012	0.85±0.014	0.86±0.007	0.84±0.005	0.85±0.005				
Mean	0.85±0.006	0.86±0.007	0.86±0.005	0.84±0.005					
Co (ppm)									
C	0.52±0.027	0.50±0.018	0.53±0.039	0.51±0.017	0.51±0.013	0.007	0.73	0.74	0.98
T_1	0.51±0.026	0.53±0.025	0.54±0.023	0.52±0.012	0.53±0.010				
T_2	0.51±0.027	0.52±0.020	0.53±0.023	0.51±0.026	0.52±0.011				
Mean	0.51±0.014	0.52±0.012	0.53±0.016	0.51±0.010					

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.01) (P<0.05). ^{A,B}Mean values with different superscripts within a row differ significantly (P<0.01) (P<0.05).

et al. (2015) in male buffaloes. Increased levels of aminotransferases in buffaloes may be attributed to liver damage (Sihag *et al*. 2009). In our experiment the level of ALT and AST were comparable to the control group, depicting that supplementation of MMS I and MMS II has no any harmful and degenerative effect on hepatic cells and muscle tissues.

Mineral composition (% on DM basis) of different feed ingredients is shown in Table 5. In the present study, mean serum Ca, P, Fe, Mn, Cu and Co values in groups C, T_1 and T_2 did not differ statistically (P>0.05) (Table 6). However, the serum concentration (ppm) of Zn was significantly higher in T_1 and T_2 (MMS-I and MMS-II) groups. The overall periodic mean values of Cu differed significantly during 0, 180 and 270 days. Livestock have presented unique requirements and toxicity issues depending on the species for the various concentrations of Cu and Zn and their interactions with other nutrients especially Fe, Se, Mo, and S and soil concentrations of these elements and their availability to crops influence the health of the crop and the amount found in vegetative tissues and seeds (Hill and Shannon 2019). Moreover, Kumar (2015) also showed no significant differences with respect to calcium and phosphorus (mg/dl) levels among the groups of kids. Our findings also correlated well with the results of Ankita *et al.* (2018) in buffalo heifers. Our result corroborated well with that of Mohapatra *et al*. (2012) who reported elevated serum concentration of Zn in mineral supplemented group as compared to non supplemented group. Use of organic copper and zinc may be beneficial for improving growth, nutrient utilization and health of female buffalo heifers (Singh *et al*. 2018). Zinc is a structural component in superoxide dismutase enzyme (SOD), which quenchs free radicals produced from various processes in the body during an immune response (Murray *et al.* 2000). During Zn deficiency, immune function is impaired through a decrease in T-cell function as well as a decrease in function of many other key components of the immune system such as thymus, natural killer T-cell, and neutrophils (Hambridge *et al.* 1986).

On the basis of these results it can be concluded that supplementation of molasses based multi-nutrient herbal supplements (MMS I & II) has improved *in vitro* dry matter digestibility (IVDMD), IVOMD (%) and serum Zn level in buffalo calves resulting in improvement in the health status.

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