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Effect of molasses based multi-nutrient herbal supplements on the performance of buffalo calves

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ABSTRACT

An experiment was conducted to study the effect of molasses based multi-nutrient herbal supplements (MMS) containing ground fenugreek seed and de-oiled mahua seed cake at two different ratios (1:1; MMS-I and 1:3; MMS-II) on growth performance, nutrient utilization and immune response of male buffalo calves. Fifteen male Murrah buffalo calves (10 to 15 months of age and mean body wt. 234–236 kg) were randomly distributed into 3 groups of 5 each. All animals were supplied with conventional concentrate mixture, green forages (3–4 kg DM/d) and wheat straw *ad lib.* to meet out nutrient requirement. Animals in control group were not fed supplement but animals in group T_1 and T_2 were fed MMS-I and MMS-II, respectively @ 200 g/100 kg metabolic body weight (kg $W^{0.75}$). The intake and digestibility of DM, OM, EE, NDF, ADF, hemicelluloses, cellulose and purine derivatives in urine were comparable among 3 groups. Digestibility of crude protein (CP) was higher in supplemented groups (T_1 and T_2) than control. Total dry matter intake, body weight gain, average daily gain (ADG) and feed conversion ratio (FCR) during 270 days were comparable among 3 groups. Both cellular and humoral immune response improved in T_1 and T_2 groups. The results revealed that molasses based multi-nutrient herbal supplement containing fenugreek and DMSC improved the growth performance, nutrient utilization and immune responses.

Key words: Buffalo calves, Deoiled mahua seed cake, Fenugreek seed, Growth performance, Immune response, Nutrient utilization

Molasses based supplements have been developed as a scarcity feed (Ranjhan *et al.* 1973, Verma *et al.* 1995). But urea molasses mineral block are used to improve the productivity of livestock fed on poor quality roughages (Mohini and Gupta 1993, Sahoo *et al.* 2004, Sihag *et al.* 2007). Molasses based multi-nutrient liquid supplements have been formulated that contained urea (10%), molasses (74%), deoiled mahua seed cake (5%), guar meal (5%), mineral mixture (2% in MMLS and 4% in MMLS plus), salt (1%) and their supplementation in the diet of buffalo heifers improved the growth performance and immunity besides saving of 20% concentrate mixture (Ankita *et al.* 2016). Moreover, attention is being paid on herbal feed supplements using fenugreek seed and deoiled mahua seed cake (DMSC) that may either influence feeding pattern, growth of favourable microorganisms in the rumen or stimulate the secretion of various digestive enzymes, which in turn may improve the efficiency of nutrients utilization, resulting in improved production and reproductive performance of animals (Ojha *et al.* 2012, Inamdar *et al.* 2015, Kumar 2015, Ankita *et al.* 2016). Keeping this in mind, the present experiment was designed to assess the effect of molasses based multi-nutrients herbal supplements (MMS-I and II) on growth performance, nutrient utilization and immune response in buffalo calves.

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MATERIALS AND METHODS

Experimental site, animals and housing: Healthy male buffalo calves (15) of about 10 to 15 months of age and mean body wt. 234 to 236 kg were selected. All the animals were ear tagged and housed in the Cattle and Buffalo Farm in a well maintained sheds having provision of both open and close enclosure. Prior to the experimental feeding, all animals were dewormed with albendazole @ 5 mg/kg body weight. Proper health management and sanitation conditions were maintained throughout the experimental period.

Experimental design, sampling and dietary treatments: Animals were randomly divided into 3 groups of 5 each. All animals were supplied with green forages (3–4 kg DM/d) and wheat straw *ad lib*. In addition, animals in all groups were fed a conventional concentrate mixture (wheat bran 45%, deoiled soybean meal 17%, crushed maize 17%, crushed barley 18%, mineral mixture 2%, common salt 1%) to meet out their nutrient requirement (ICAR 2013). Additionally, MMS-I and MMS-II in form of laddoo was fed @ 200g/100kg$W^{0.75}$ in groups T_1 and T_2, respectively.

The MMS-I consisted of molasses (49%), ground fenugreek seed (24.50%), DMSC (24.50%) and mineral mixture (2%). The MMS-II consisted of molasses (49%), ground fenugreek seed (12.25%), DMSC (36.75%) and mineral mixture (2%). 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. All the animals were weighed at the beginning of the experiment and subsequently at fortnightly intervals before feeding and watering in the morning (9:00 AM). The dry matter intake was adjusted at every fortnight with the change in body weight. Feed conversion ratio was calculated by dividing the total quantity of feed consumed by the live weight gained (kg).

A digestion trial of 6 days was conducted after 150 days of the experimental period to determine intake and digestibility of nutrients. Dry matter content of feed, refusals and faecal samples was determined by drying to a constant weight in an oven at 100±2ºC for 24 h. At the end of trial, each animal's refusals and faecal samples were pooled across the 6-day trial period. Dried samples of feed, refusals and faecal samples were grinded to pass through a 1 mm screen and stored for further laboratory analysis. Samples of feed offered, residues left and faeces voided were analyzed for proximate principles (AOAC 2012) and fibre fractions (Van Soest *et al.* 1991). A representative sample of spot urine excreted daily after 5–6 h of feeding by individual animal was collected separately in a small container keeping the pH below 3. The representative samples of urine from all animals were brought to the laboratory and pooled daily into a properly marked, well stopper sample bottle maintaining pH below 3 and stored at –20°C for estimation of purine derivatives.

Towards the end of the experimental feeding, the cell mediated immune (CMI) response of male buffalo calves was assessed by a cutaneous delayed-type hypersensitivity (DTH) reaction to intra-dermal inoculation of phytohaemagglutinin-P (PHA-p; Himedia, India). After completion of CMI, humoral immune response was assessed and all buffalo calves were injected with 1 ml of 20% suspension of washed chicken red blood cells (CRBC) via intravenous route. Blood collection was done at 0, 7, 14, 21 and 28 days post inoculation and serum was separated and stored at –20ºC for further analysis.

Statistical analysis: Data pertaining to feed intake, body weight, nutrient intake and utilization were subjected to one way ANOVA while the data pertaining to immunity 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 at $P<0.05$. All data analyses were performed using statistical package of SPSS (20.0).

RESULTS AND DISCUSSION

The chemical composition of the different feed ingredients is shown in Table 1. Nutrient composition of the different feed ingredients used in this experiment was comparable to the reference value of feed ingredients (ICAR 2013).

The intake and digestibility of DM, OM, EE, NDF, ADF, hemicelluloses, cellulose were comparable among 3 groups (Table 2). This is in agreement with the findings of earlier workers in buffalo heifers (Ankita *et al.* 2016), male buffaloes (Inamdar *et al.* 2015), and crossbred calves supplemented with deoiled mahua seed cake (DMSC) (Ojha *et al.* 2012) as well as in kids supplemented with amla (*Phyllanthus emblica*) fruit powder and fenugreek (*Trigonella foenum-graecum*) seeds (Kumar 2015). In this study, CP intake (g/d) was comparable among 3 groups but its digestibility (%) was higher ($P<0.01$) in both T_1 and T_2 groups than control (Table 2). This corroborates well with the observations of Kumar (2015) who found digestibility (%) of CP significantly ($P<0.05$) higher in kids supplemented with amla (*Phyllanthus emblica*) fruit powder and fenugreek (*Trigonella foenum-graecum*) seeds. This is also in agreement to the findings of Allam *et al.* (1999) who observed improvement ($P<0.05$) in digestibility of CP in Zaraibi goats fed diets supplemented with medicinal herbs (garlic, *Nigella sativa*, fenugreek and comomile). But contrary to the finding, Inamdar *et al.* (2015) who reported non-significant difference in CP digestibility (%) in male buffaloes supplemented with deoiled mahua seed cake

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. Mean intake (kg/d) and digestibility (%) of nutrients

Particular	C	T_1	T_2	SEM	P value
		DM intake			
Conc. mixture	2.17± 0.04	2.19± 0.02	2.20± 0.05	0.04	0.06
MMS-I/ MMS-II (g/d)	0.00	135.4± 4.87	138.5± 6.32	3.80	0.61
Green oats	3.63± 0.10	3.74± 0.29	3.87± 0.15	0.11	0.70
Wheat straw	0.53± 0.06	0.56± 0.06	0.69± 0.03	0.04	0.12
Total DMI	6.32± 0.12	6.63± 0.25	6.90± 0.19	0.12	0.15
Digestibility	59.74± 1.75	64.93± 2.25	61.90± 3.01	1.40	0.34
		Organic matter			
Intake	5.76± 0.11	6.03± 0.22	6.27± 0.17	0.11	0.15
Digestibility	62.74± 1.57	67.63± 1.82	64.85± 2.83	1.27	0.31
		Crude protein			
Intake (g/d)	735.71± 9.47	781.22± 25.57	799.79± 16.25	12.15	0.07
Digestibility	52.91[b]± 2.69	66.68[a]± 2.74	65.997[a]± 2.34	2.19	0.004
		Ether extract			
Intake (g/d)	163.7± 3.04	169.6± 7.90	174.8± 4.73	3.23	0.40
Digestibility	66.48± 1.76	73.25± 1.16	69.34± 2.63	1.28	0.08
		NDF			
Intake	3.55± 0.08	3.70± 0.15	3.89± `0.12	0.07	0.19
Digestibility	52.74± 2.29	60.91± 2.96	57.90± 2.71	1.69	0.13
		ADF			
Intake	2.28± 0.06	2.38± 0.11	2.52± 0.09	0.05	0.22
Digestibility	50.87± 1.91	56.66± 2.90	55.43± 3.33	1.63	0.33
		Hemicellulose			
Intake	1.27± 0.02	1.32± 0.04	1.37± 0.03	0.02	0.13
Digestibility	56.08± 3.36	68.45± 3.44	62.35± 3.00	2.21	0.06
		Cellulose			
Intake	1.84± 0.05	1.91± 0.09	2.03± 0.07	0.04	0.24
Digestibility	53.49± 1.57	61.12± 2.05	60.70± 3.03	1.55	0.06

C, Control; T_1, MMS I supplemented group; T_2, MMS II supplemented group; SEM, standard error of mean; NDF, neutral detergent fibre; ADF, acid detergent fibre; [a,b]Means bearing different superscripts in a row differ significantly (P<0.01).

(*Madhuka longifolia*) either alone or in combination with harad seed pulp (*Terminalia chebula*). The improvement in digestibility of nutrients in present study could be justified on the basis that fenugreek seeds and deoiled mahua cake contain saponins, which stimulate anaerobic fermentation of organic matter that improve efficiency of utilization of nutrients (Abo El-Nor *et al.* 2007). In addition, fenugreek seeds also increase bacterial population in the rumen of lactating cows as reported by Valdez *et al.* (1986).

DMI (kg/d, g/kg $W^{0.75}$/d and % of body weight), DOM intake (kg/d and g/kg $W^{0.75}$/d), CP intake (g/d and g/kg $W^{0.75}$/d) and TDN (kg/d, g/kg $W^{0.75}$/d) were comparable among the 3 groups (Table 3). However, DCP intake (g/d and g/kg $W^{0.75}$/d) was significantly higher (P<0.01) in both treatment groups (T_1 and T_2) as compared to control group. This is because of the significantly higher digestibility (%) of CP (Table 2). This is well corroborated with the results of Ankita *et al.* (2016) who supplemented MMLS in buffalo heifers. At 300 kg body weight and ADG of 0.6 kg in male buffalo calves, the requirement of DM, CP and TDN has been worked out to be 6.9 kg, 675 g and 4.0 kg/day, respectively (ICAR 2013) and thus, the findings of present study was comparable and matching with the requirement of ICAR (2013).

The nutritive value of diet (CP and TDN) was comparable among the 3 groups but its DCP values were significantly higher (P<0.01) in both T_1 and T_2 as compared to control group (Table 3). Present results are in agreement with the findings of Allam *et al.* (1999) who reported improvement (P<0.05) in the nutritive value (DCP) for all additive groups as compared to control when diets were supplemented with 4 medicinal herbs (garlic, *Nigella sativa*, fenugreek and comomile) in Zaraibi goats. This also corroborated well with the results of Ankita *et al.* (2016) who supplemented MMLS in buffalo heifers.

Mean concentration of urinary allantoin, uric acid, purine derivatives, creatinine and purine derivatives index (PDI) showed non-significant (P>0.05) difference among 3 groups (Table 4). These findings are concomitant with the results of Ojha *et al.* (2012) where male crossbred calves were fed concentrate mixture containing deoiled mahua seed cake and guar meal @ 10% and Ankita *et al.* (2016) in buffalo heifers fed with molasses based multi-nutrient liquid supplements (MMLS). The comparable PDs excreted in the urine in all three groups indicated that nutrients from different diets were utilized effectively and incorporation of molasses based multi-nutrient herbal supplement (MMS-I and MMS-II) had beneficial effect on rumen microbial protein synthesis/supply.

There was non-significant (P>0.05) difference in the overall intake of concentrate mixture, green fodder, wheat straw among 3 groups during 270 days of experimental period. Also, total DMI (kg/d), total body weight gain, ADG and FCR during experimental period of 270 days was comparable among 3 groups (Table 5). The optimum DMI ensures availability of various nutrients for maintenance and physiological functions (Kumar *et al.* 2016). Several factors like breed, age, season, quality of feed, physiological state, etc. influence feed consumption in ruminants. In this experiment, all the animals were of similar age, productive stage and were maintained under uniform environmental conditions. The present findings are in agreement with Ojha *et al.* (2012) who reported comparable DMI among

Table 3. Plane of nutrition of male buffalo calves in different groups during trial period

Particular	C	T_1	T_2	SEM	P Value
		Mean body weight			
Body weight (kg)	335.5± 27.30	340.5± 19.03	339± 18.82	11.81	0.99
$kgW^{0.75}$	78.20± 4.78	79.17± 3.33	78.92± 3.25	2.06	0.98
		DM intake			
DMI (kg/d)	6.32± 0.12	6.63± 0.25	6.90± 0.19	0.12	0.15
$g/kgW^{0.75}/d$	81.84± 4.18	83.78± 0.99	87.71± 2.26	1.63	0.35
DMI (% of BW)	1.93± 0.14	1.95± 0.04	2.05± 0.07	0.05	0.63
		DOM intake			
DOMI (kg/d)	3.61± 0.11	4.07± 0.17	4.07± 0.23	0.11	0.14
$g/kgW^{0.75}/d$	46.90± 3.12	51.55± 1.55	51.90± 3.44	1.63	0.40
		CP intake			
CP (g/d)	735.71± 9.47	781.22± 25.57	799.79± 16.25	12.15	0.07
$g/kgW^{0.75}/d$	9.54± 0.55	9.89± 0.16	10.17± 0.27	0.21	0.49
		DCP intake			
DCP (g/d)	389.46^{b}± 21.56	519.34^{a}± 19.05	527.62^{a}± 19.98	20.07	0.001
$g/kgW^{0.75}/d$	5.07^{b}± 0.44	6.59^{a}± 0.29	6.73^{a}± 0.39	0.29	0.017
		TDN intake			
TDN (kg/d)	3.75± 1.13	4.23± 1.74	4.23± 2.33	1.14	0.14
$g/kgW^{0.75}/d$	48.67± 3.24	53.51± 1.58	53.83± 3.55	1.69	0.40
		Nutritive value of diet (%)			
CP	11.64± 0.1	11.80± 0.08	11.60± 0.08	0.05	0.25
DCP	6.16^{b}± 0.33	7.87^{a}± 0.33	7.65^{a}± 0.27	0.26	0.005
TDN	59.29± 1.47	63.86± 1.70	61.15± 2.64	1.18	0.30

C, Control; T_1, MMS I supplemented group; T_2, MMS II supplemented group; SEM, standard error of mean; DMI, dry matter intake; DCP, digestible crude protein; CP, crude protein; TDN, total digestible nutrients; ^{a,b}Means bearing different superscripts in a row differ significantly (P<0.01).

treatment groups of male crossbred calves. On the contrary, Ankita *et al.* (2016) reported that overall average dry matter intake during 240 days of experiment was significantly (P<0.05) higher in both treatment groups of buffalo heifers where basal diets were supplemented with MMLS. The present findings in total body weight gain, average daily gain (ADG) and FCR are well collaborated with findings of Ankita *et al.* (2016) who also reported non-significant difference (P>0.05) in total body weight gain and ADG among the groups of buffalo heifers. Conversely, Ojha *et al.* (2012) reported significant (P<0.05) improvement in average daily gain (g) and feed conversion ratio in male

Table 4. Urinary excretion of allantoin, uric acid, purine derivatives, creatinine and PDC index in male buffalo calves

Parameter (mmol/l)	C	T_1	T_2	SEM	P value
Allantoin	2.33±0.24	2.42±0.17	2.45±0.18	0.11	0.91
Uric acid	0.57±0.01	0.59±0.01	0.61±0.01	0.01	0.05
Purine derivative	2.90±0.24	3.01±0.17	3.06±0.19	0.11	0.85
Creatinine	6.07±0.08	6.13±0.14	6.17±0.10	0.06	0.83
PDC index	36.97±2.59	38.75±2.39	39.08±2.67	1.39	0.82

C, Control; T_1, MMS I supplemented group; T_2, MMS II supplemented group; SEM, standard error of mean; PDC index, purine derivatives and creatinine index.

Table 5. Feed conversion ratio of male buffalo calves

Parameter	C	T_1	T_2	SEM	P value
Initial BW (kg)	234± 26.14	236± 14.61	236± 27.17	12.48	1.00
Final BW (kg)	415± 21.97	435± 24.39	424± 19	11.91	0.81
Total BW gain (kg)	174± 14.09	191± 12.39	180± 9.35	6.74	0.61
No. of days	270	270	270		
ADG (g)	644.44± 52.18	707.41± 45.89	666.67± 34.65	24.95	0.61
Mean feed intake (kg)	6.78± 0.24	7.05± 0.24	6.96± 0.24	0.14	0.72
FCR	10.85± 1.03	10.15± 0.71	10.55± 0.50	0.42	0.82

C, Control; T_1, MMS I supplemented group; T_2, MMS II supplemented group; SEM, standard error of mean; BW, body weight; ADG, average daily gain; FCR, feed conversion ratio

crossbred calves fed on diet having 10% deoiled mahua seed cake, which is a rich source of tannins and saponins.

The cell mediated and humoral immune responses were significantly higher (P<0.01) in treatment groups (T_1 and T_2) as compared to control group (Tables 6 and 7) and these responses were significantly higher at 24, 48 and 72 h in case of CMI and 7, 14, 21 days in case of HI. This is well corroborated with the results of Ankita (2016) in buffalo heifers. These findings may be due to immune-modulatory action of saponins containing feeds/oil cakes in MMS-I and MMS-II according to Rao and Gurfinkel (2000) and also due to supplemented mineral mix in MMS-I and MMS-II.

The results revealed that molasses based multi-nutrients herbal supplement containing fenugreek and DMSC improved the growth performance by 9.77% (MMS-I), nutrient utilization, and immune responses. Further work is needed to be done to standardize the combination levels of the two to get optimum response of the supplement.

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Table 6. Cell mediated immune response (thickness, mm) of male buffalo calves

Group	Periods (h)					Mean±SE	SEM	T	P	T*P
	0	24	48	72	96					
C	17.20±0.67	19.78±0.49	18.88±0.32	17.92±0.44	17.31±0.64	18.22^{b}±0.33	0.21	0.001	0.00	0.002
T_1	15.96±0.22	25.59±0.31	22.80±0.20	19.84±0.50	17.17±0.65	20.27^{a}±0.96				
T_2	15.66±0.72	25.01±1.72	22.68±1.67	19.36±1.09	16.62±0.37	19.86^{a}±0.16				
Mean±SE	16.27^{D}±0.37	23.46^{A}±1.06	21.45^{B}±0.81	19.04^{C}±0.47	17.03^{D}±0.30					

C, Control; T_1, MMS I supplemented group; T_2, MMS II supplemented group; SEM, standard error of mean; ^{a,b}Mean values with different superscripts within a column differ significantly (P<0.01); ^{A,B,C,D}Mean values with different superscripts within a row differ significantly (P<0.01).

Table 7. Humoral immune response (HA titre) of male buffalo calves

Group	Period (days)					Mean±SE	SEM	T	P	T*P
	0	7	14	21	28					
C	2.33±0.33	3.00±0.00	4.33±0.33	3.33±0.33	2.67±0.33	3.13^{b}±0.22	0.11	0.00	0.00	0.78
T_1	3.00±0.00	4.33±0.33	6.33±0.88	5.00±0.58	3.67±0.33	4.47^{a}±0.36				
T_2	2.67±0.33	3.33±0.33	6.00±0.58	4.33±0.67	3.33±0.33	3.93^{a}±0.36				
Mean±SE	2.67^{D}±0.17	3.56^{BC}±0.24	5.55^{A}±0.44	4.22^{B}±0.36	3.22^{CD}±0.22					

C, Control; T_1, MMS I supplemented group; T_2, MMS II supplemented group; SEM, standard error of mean; ^{a,b}Mean values with different superscripts within a column differ significantly (P<0.01); ^{A,B,C,D}Mean values with different superscripts within a row differ significantly (P<0.01).

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