



Effect of molasses based multi-nutrient herbal supplements on hemato-biochemicals, serum lipid, antioxidants and hormonal profile in buffalo calves

PUNITA KUMARI¹, P SINGH², A K VERMA³, G K GAUR⁴ and A DAS⁵

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

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ABSTRACT

Effect of molasses based multivitamins 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) on hematobiochemicals, serum lipid, antioxidants and hormonal profile in male buffalo calves was assessed for 9 months. Fifteen male Murrah buffalo calves (10 to 15 months of age and mean body wt. 234–236 kg) were randomly distributed into 3 groups (5 each) according to Randomized Block Design (RBD). All animals were fed individually with conventional concentrate mixture, available green fodder (3–4 kg DM/d) and wheat straw *ad lib.* to meet out nutrients requirement. While animals in control group (C) were fed no supplement but animal's diet in group T₁ and T₂ 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}). The values of Hb, PCV, RBC, WBC, platelets count, serum total protein, uric acid, creatinine, urea level, total cholesterol, triglyceride, high density lipoprotein (HDL) and T₃ hormone level were comparable among 3 groups. Serum glucose, globulin, antioxidants and testosterone levels were significantly increased but serum albumin, A/G ratio, low density lipoprotein (LDL) and cortisol levels were significantly decreased among supplemented groups as compared to control group and hormone T₄ was significantly higher in T₁ group. The results showed that supplementation of MMS-I and MMS- II in the diet of buffalo calves improved serum glucose, globulin, antioxidants, T₄ and testosterone levels but lower albumin, A/G ratio, LDL and cortisol.

Key words: Blood biochemical, Buffalo calves, Deoiled mahua seed cake, Fenugreek seed, Haematology

The WHO encourages using medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to nature (WHO 2004). Using medicinal herbs and seeds as feed additives to ruminants seem to be a recent trend globally (Singh *et al.* 1993, Wanapat *et al.* 2015). Molasses based supplements have been developed as a scarcity feed (Ranjhan *et al.* 1973, Verma *et al.* 1995). 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 favorable 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).

Fenugreek is abundant in polyphenolic compounds

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

(Rayyan *et al.* 2010), has anti-microbial, hypoglycemic (Broca *et al.* 2000), hypolipidemic, hypocholesteremic (Sowmya and Rajyalakshmi 1999), anticancer, antiulcer, anthelmintic and antioxidant effect on animals (Xue *et al.* 2007), contains alkaloids like trigonelline, flavonoids and saponins (3–5%) (Singh and Garg 2006). Deoiled mahua seed cake (DMSC) is also good source of saponins, and tannins (Singh and Singh 1991). So fenugreek and DMSC can effectively be used as a functional feed and can be employed as excellent source of tannins and saponins as well as intact protein. Thus, the present experiment was designed to assess the effect of molasses based multi-nutrients herbal supplements (MMS-I and II) on hemato-biochemicals, serum lipid, antioxidants and hormonal profile in buffalo calves.

MATERIALS AND METHODS

Animal's management and experimental feeding: Healthy male Murrah buffalo calves (15) of about 10–15 months of age and mean body wt. 234±12.48 kg were used for the experiment. Proper health management and sanitation conditions were maintained and provision of both open and close enclosures 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 T₁ and T₂, MMS-I and MMS-II in form of laddoo (small ball shape) was given @ 44 g/100 kg body weight or 200 g/100 kgW^{0.75} respectively as serving by hand. MMS-I and II consisted (%) of molasses (49 and 49), ground fenugreek seed (24.50 and 12.25), DMSC (24.50 and 36.75) and mineral mixture (2 and 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).

Collection and analysis of blood: Blood from all animals was collected before feeding and watering at 0, 90, 180 and 270 days of experimental period by puncturing the jugular vein with the help of a clean sterilized needle into two separate test tubes. The first test tube contained sodium EDTA and second one without anticoagulant was used. By

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 and DMSC, Deoiled mahua seed cake.

Table 2. Haematological parameters in male buffalo calves

Treatment	Period (days)				Mean±SE	SEM	T	P	T*P
	0	90	180	270					
<i>Hb (g/dl)</i>									
C	13.04±0.13	13.22±0.32	13.26±0.34	13.29±0.32	13.20±0.14	0.09	0.22	0.17	0.99
T ₁	13.12±0.25	13.62±0.36	13.76±0.29	13.82±0.16	13.58±0.14				
T ₂	13.08±0.36	13.44±0.49	13.60±0.28	13.72±0.25	13.46±0.17				
Mean	13.08±0.14	13.43±0.22	13.54±0.17	13.61±0.15					
<i>PCV (%)</i>									
C	33.79±3.95	34.01±2.33	34.13±2.99	34.19±1.27	34.03±1.29	0.83	0.68	0.76	1.00
T ₁	33.90±2.58	35.28±3.90	36.36±2.39	37.67±2.55	35.80±1.38				
T ₂	33.82±3.95	34.24±3.27	35.91±1.95	36.72±1.76	35.17±1.35				
Mean	33.84±1.90	34.51±1.73	35.47±1.35	36.19±1.10					
<i>RBC (10¹²/L)</i>									
C	6.27±0.33	6.36±0.25	6.46±0.46	6.79±0.22	6.47±0.16	0.10	0.30	0.02	0.99
T ₁	6.36±0.45	6.71±0.53	6.82±0.27	7.50±0.26	6.85±0.20				
T ₂	6.32±0.40	6.61±0.39	6.70±0.14	7.39±0.35	6.76±0.18				
Mean	6.32 ^B ±0.21	6.56 ^B ±0.22	6.66 ^{AB} ±0.17	7.23 ^A ±0.17					
<i>WBC (10⁹/L)</i>									
C	13.42±0.30	13.58±0.39	13.62±0.32	13.76±0.40	13.59±0.17	0.11	0.57	0.37	1.00
T ₁	13.50±0.38	13.82±0.35	14.02±0.44	14.14±0.30	13.87±0.18				
T ₂	13.46±0.34	13.68±0.36	13.86±0.34	14.04±0.46	13.76±0.18				
Mean	13.46±0.18	13.69±0.20	13.83±0.20	13.98±0.21					
<i>Platelets (10⁹/L)</i>									
C	226.40±22.43	227.40±8.60	228.20±7.68	228.80±13.82	227.70±6.60	3.45	0.95	0.98	1.00
T ₁	227.20±4.85	229.80±12.38	231.20±5.41	233.20±10.70	230.35±4.14				
T ₂	227.80±5.07	228.40±15.38	230.20±3.73	231.40±17.08	229.45±5.48				
Mean	227.13±7.26	228.53±6.65	229.87±3.14	231.13±7.56					

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

centrifuging the second test tube samples at 2,000 rpm for 20 min, serum was separated carefully so that no hemolysis occurred and was stored at -20°C for further analysis. Whole blood was analyzed using BC-2800 Vet Auto Haematological Analyzer. The haematological parameters determined by analyzer were haemoglobin, PCV, RBC, WBC and platelets immediately within one hour of blood collection. Serum samples were analyzed for glucose, uric acid, total cholesterol, triglyceride, HDL and LDL (Trinder 1969), total protein (Doumas 1975), albumin and determination of globulin is by difference between total protein and albumin (Doumas *et al.* 1971), creatinine (Bowers 1980) and urea (Wynbenga *et al.* 1971) using

commercial kits (Coral Clinical Systems, India). Superoxide dismutase (SOD) (Marklund 1980), catalase (CAT) (Wheeler *et al.* 1990) and glutathione reductase (GR) (Inoue *et al.* 1987) were determined by using Cayman's Assay kits. Triiodothyronine (T_3), thyroxine (T_4), cortisol and testosterone were determined by using RIA kits supplied by Immunotech, France.

Statistical analysis: Data pertaining to hemato-biochemical, serum lipid, antioxidants and hormonal profile was 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

Table 3. Serum biochemical parameters in male buffalo calves

Treatment	Period (days)				Mean±SE	SEM	T	P	T*P
	0	90	180	270					
<i>Glucose (mg/dl)</i>									
C	42.45±1.60	42.57±9.72	43.39±2.19	43.78±1.67	43.05 ^b ±2.35	1.26	0.03	0.20	0.94
T ₁	44.23±3.51	50.35±4.73	53.47±2.69	55.91±4.34	50.99 ^a ±2.05				
T ₂	44.16±3.40	49.66±4.66	51.99±3.62	53.01±4.01	49.71 ^a ±1.98				
Mean	43.61±1.60	47.52±3.75	49.62±1.95	50.90±2.34					
<i>Protein (g/dl)</i>									
C	7.49±0.23	7.51±0.15	7.53±0.21	7.54±0.22	7.52±0.10	0.07	0.40	0.31	0.95
T ₁	7.47±0.35	7.60±0.25	7.82±0.20	8.04±0.08	7.73±0.12				
T ₂	7.48±0.25	7.59±0.30	7.73±0.23	7.89±0.15	7.67±0.11				
Mean	7.48±0.15	7.57±0.13	7.69±0.12	7.82±0.10					
<i>Albumin (g/dl)</i>									
C	3.81±0.08	3.82±0.08	3.80±0.08	3.82±0.07	3.81 ^a ±0.04	0.02	<0.01	<0.01	0.13
T ₁	3.80±0.07	3.53±0.10	3.49±0.12	3.35±0.06	3.54 ^b ±0.06				
T ₂	3.80±0.07	3.59±0.08	3.52±0.08	3.39±0.05	3.57 ^b ±0.05				
Mean	3.80 ^A ±0.04	3.64 ^B ±0.06	3.60 ^B ±0.06	3.52 ^B ±0.07					
<i>Globulin (g/dl)</i>									
C	3.68±0.30	3.70±0.10	3.72±0.21	3.72±0.22	3.71 ^b ±0.10	0.07	0.02	0.03	0.64
T ₁	3.67±0.42	4.07±0.34	4.33±0.15	4.68±0.12	4.19 ^a ±0.16				
T ₂	3.68±0.30	4.01±0.29	4.22±0.23	4.50±0.19	4.10 ^a ±0.14				
Mean	3.68 ^B ±0.18	3.93 ^{AB} ±0.15	4.09 ^{AB} ±0.13	4.30 ^A ±0.15					
<i>A/G ratio</i>									
C	1.07±0.12	1.03±0.03	1.04±0.07	1.04±0.07	1.05 ^a ±0.04	0.02	0.01	<0.01	0.46
T ₁	1.11±0.16	0.90±0.09	0.81±0.04	0.72±0.03	0.88 ^b ±0.05				
T ₂	1.07±0.10	0.91±0.07	0.84±0.06	0.76±0.04	0.90 ^b ±0.04				
Mean	1.08 ^A ±0.07	0.95 ^{AB} ±0.04	0.90 ^B ±0.04	0.84 ^B ±0.05					
<i>Uric acid (mg/dl)</i>									
C	2.03±0.25	1.54±0.14	2.25±0.19	1.99±0.14	1.95±0.10	0.06	0.65	0.12	0.56
T ₁	2.21±0.13	1.89±0.20	2.04±0.21	1.69±0.23	1.96±0.10				
T ₂	2.00±0.25	1.65±0.25	1.77±0.10	1.92±0.32	1.84±0.12				
Mean	2.08±0.12	1.70±0.11	2.02±0.11	1.87±0.13					
<i>Creatinine (mg/dl)</i>									
C	0.71±0.23	0.55±0.07	1.07±0.16	1.05±0.15	0.84±0.09	0.05	0.95	<0.01	0.85
T ₁	0.56±0.16	0.76±0.22	0.88±0.18	1.03±0.10	0.81±0.09				
T ₂	0.51±0.06	0.75±0.10	0.92±0.21	1.11±0.16	0.82±0.08				
Mean	0.59 ^B ±0.09	0.69 ^B ±0.08	0.96 ^A ±0.10	1.06 ^A ±0.07					
<i>Urea (mg/dl)</i>									
C	29.32±0.60	30.35±1.33	31.17±0.82	32.22±1.08	30.77±0.52	0.38	0.17	0.01	0.99
T ₁	30.33±2.19	31.93±0.88	33.36±1.05	33.78±0.86	32.35±0.70				
T ₂	29.61±2.55	31.51±0.85	33.81±0.94	33.99±0.97	32.23±0.80				
Mean	29.76 ^B ±1.06	31.27 ^{AB} ±0.58	32.78 ^A ±0.59	33.33 ^A ±0.56					

^{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 ($P<0.01$) ($P<0.05$).

Table 4. Serum lipid profile in male buffalo calves

Treatment	Period (days)				Mean±SE	SEM	T	P	T*P
	0	90	180	270					
<i>Cholesterol (mg/dl)</i>									
C	60.76±4.41	73.95±2.97	78.89±0.89	73.40±4.31	71.75±2.21	0.97	0.37	<0.01	0.33
T ₁	64.99±3.52	71.85±2.43	76.24±2.50	70.12±2.65	70.80±1.59				
T ₂	67.02±4.00	72.04±5.11	71.11±3.65	63.59±1.04	68.44±1.89				
Mean	64.26 ^C ±2.25	72.61 ^{AB} ±1.99	75.42 ^A ±1.64	69.04 ^{BC} ±1.93					
<i>Triglyceride (mg/dl)</i>									
C	34.84±3.74	27.45±3.24	30.52±1.61	39.78±1.72	33.15±1.65	0.74	0.11	<0.01	0.06
T ₁	36.27±1.19	28.70±3.19	31.71±2.77	28.45±1.25	31.28±1.27				
T ₂	36.59±3.99	26.13±1.63	27.45±2.78	26.70±1.08	29.22±1.55				
Mean	35.90 ^A ±1.74	27.43 ^B ±1.52	29.89 ^B ±1.39	31.64 ^B ±1.71					
<i>HDL (mg/dl)</i>									
C	36.93±3.80	43.37±1.29	44.60±1.45	38.13±2.10	40.76±1.33	0.74	0.32	<0.01	0.98
T ₁	37.78±2.32	44.16±1.79	45.25±0.79	41.78±2.97	42.24±1.17				
T ₂	39.13±2.17	47.07±4.12	47.99±1.02	39.93±3.97	43.53±1.69				
Mean	37.95 ^B ±1.55	44.87 ^A ±1.50	45.95 ^A ±0.72	39.95 ^B ±1.71					
<i>LDL (mg/dl)</i>									
C	16.86±1.91	25.10±3.24	28.19±1.78	29.31±4.59	24.86 ^a ±1.81	0.78	0.02	0.18	0.15
T ₁	19.96±2.43	21.95±2.16	24.65±2.49	22.65±1.49	22.30 ^{ab} ±1.07				
T ₂	20.57±2.00	19.74±2.50	17.64±2.91	18.33±3.52	19.07 ^b ±1.31				
Mean	19.13±1.22	22.26±1.54	23.49±1.75	23.43±2.20					

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

Table 5. Serum antioxidant status of male buffalo calves in different groups

Parameter	Days				Mean	SEM	T	P	T*P
	0	90	180	270					
<i>SOD (U/l)</i>									
C	23.41±2.81	21.25±1.77	21.86±1.17	22.08±2.64	22.15 ^b ±1.03	0.74	<0.01	0.04	0.36
T1	25.66±4.25	29.46±1.83	32.62±2.66	34.28±2.66	30.50 ^a ±1.56				
T2	22.01±3.05	26.16±1.76	30.02±1.70	31.92±2.80	27.53 ^a ±1.41				
Mean	23.69 ^B ±1.88	25.62 ^{AB} ±1.31	28.17 ^A ±1.61	29.42 ^A ±2.02					
<i>Catalase (nmol/min/ml)</i>									
C	30.15±3.50	30.96±7.14	32.17±4.37	34.94±1.57	32.05 ^b ±2.15	1.25	0.02	0.00	0.27
T1	30.93±2.31	33.35±3.12	44.10±5.16	53.44±2.14	40.46 ^a ±2.59				
T2	29.12±3.17	32.82±1.57	42.37±4.17	53.14±7.90	39.36 ^a ±3.07				
Mean	30.07 ^C ±1.63	32.38 ^C ±2.47	39.55 ^B ±2.83	47.17 ^A ±3.46					
<i>Glutathione reductase (nmol/min/ml)</i>									
C	55.52±2.19	56.03±1.80	56.54±2.19	57.05±5.84	56.29 ^b ±1.58	0.91	0.05	<0.01	0.55
T1	55.01±2.50	59.09±3.16	62.14±3.08	68.77±2.55	61.25 ^a ±1.74				
T2	54.50±3.47	59.60±2.36	61.64±4.06	67.75±2.62	60.87 ^a ±1.83				
Mean	55.01 ^B ±1.49	58.24 ^B ±1.40	60.11 ^{AB} ±1.84	64.52 ^A ±2.55					

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

differences were considered to be significant (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. The overall means for Hb (g/dl), PCV (%), RBC, WBC and platelets count were similar among the groups (Table 2). However, significant

period effects (P<0.05) were evident with regards to RBC. The values were within normal physiological range throughout the experimental period (Abd Ellah *et al.* 2013). Saponins having the haemolytic properties (Hassan *et al.* 2007) and they may lower the Hb level in the blood of the animal but levels of Hb observed in the present study were within the normal physiological range (Abd Ellah *et al.* 2013), which was also reported previously (Hb, 9–15 g/dl and PCV, 27–45%) in crossbred calves (Radostits *et al.*

2007, Ojha *et al.* 2012) and in male buffaloes (Inamdar *et al.* 2015).

There was significantly ($P<0.05$) higher level with respect to serum glucose (mg/dl) among the supplemented groups (Table 3). This is in agreement to the findings of El-alamy *et al.* (2001) and Abo El Nor *et al.* (2007) who reported significantly increased ($P<0.05$) blood glucose in buffaloes. Conversely, Ojha *et al.* (2012) in male crossbred calves, Inamdar *et al.* (2015) in male buffaloes and Ankita (2016) in buffalo heifers did not find any change in serum glucose levels.

There was non-significant difference with respect to serum total protein, uric acid, creatinine and urea level among different groups but serum creatinine and urea level varied significantly periodically. The values obtained were within the normal range. Albumin (g/dl) level was significantly lower ($P<0.05$) among supplemented groups (T_1 and T_2) than control but in case of globulin, levels were significantly increased ($P<0.05$) among supplemented group with respect to control. The A/G ratio depends upon albumin and globulin concentration and levels significantly decreased ($P<0.01$) among supplemented groups and it was also varied periodically (Table 3). Higher globulin production can be considered as an improvement in the immune status of animals (Matanovic *et al.* 2007, Kumari 2017). This is in well agreement to the findings of Hassan and Abdel-Raheem (2013) in buffalo calves. Ojha *et al.* (2012) in male crossbred calves and Inamdar *et al.* (2015) in male buffaloes did not get any difference in total protein, albumin, globulin, A/G ratio, uric acid, creatinine and urea level.

There were nonsignificant differences with respect to total cholesterol, triglyceride and HDL levels among the 3 groups but varied significantly periodically. LDL (low-density lipoprotein), sometimes called 'bad' cholesterol, makes up most of body's cholesterol. High levels of LDL cholesterol raise your risk for heart disease and stroke. But in present study, LDL (mg/dl) was found significantly lower ($P<0.05$) in supplemented groups (T_1 and T_2) than control group (Table 4). The finding was well corroborated with the results of Abo El Nor *et al.* (2007) in lactating buffalo and Kumar (2015) in kids. Fenugreek seeds contain compounds known as steroidal saponins that inhibit both cholesterol absorption in the intestine and cholesterol production by the liver. Hence, it is possible that fenugreek lowers serum lipids because it contains saponins that are transformed in the gastrointestinal tract into sapogenins. Thus, saponins reduced the more harmful LDL-cholesterol selectively in the serum of rats, gerbils and human subjects (Potter *et al.* 1993).

In the present study, the mean value of SOD, catalase and glutathione reductase was found significantly higher in T_1 and T_2 groups (Table 5). The present finding was well correlated with the result of Ankita (2016) and Patil (2017) in buffalo heifers and lactating Murrah buffaloes respectively. Beneficial effects on serum antioxidant status were related to a direct saponin antioxidant activity.

There was non-significant differences with respect to T_3 (nM/L) levels among the groups but varied significantly periodically. T_4 (nM/L) significantly higher ($P<0.01$) in T_1 than control and T_2 groups. Cortisol (nM/L) level was significantly lower ($P<0.05$) and testosterone (ng/mL) level

Table 6. Serum hormone profile of male buffalo calves

Parameter	Period (days)				Mean±SE	SEM	T	P	T*P
	0	90	180	270					
T_3 (nM/L)									
C	2.07±0.10	2.30±0.11	2.14±0.09	1.68±0.04	2.05±0.07	0.04	0.47	<0.01	0.09
T_1	2.11±0.19	2.20±0.19	1.97±0.17	2.06±0.06	2.09±0.08				
T_2	2.32±0.08	2.07±0.17	1.68±0.10	1.80±0.20	1.97±0.09				
Mean	2.17 ^A ±0.08	2.19 ^A ±0.09	1.93 ^B ±0.08	1.84 ^B ±0.08					
T_4 (nM/L)									
C	51.73±2.74	43.65±6.80	36.85±3.07	34.75±3.63	41.74 ^b ±2.52	1.39	0.01	<0.01	0.07
T_1	56.79±3.61	48.31±6.62	42.73±5.03	59.76±2.52	51.90 ^a ±2.66				
T_2	41.95±6.47	44.58±4.70	33.38±4.33	54.71±5.49	43.65 ^b ±2.30				
Mean	50.16 ^A ±2.94	45.52 ^{AB} ±3.31	37.65 ^B ±2.48	49.74 ^A ±3.61					
Cortisol (nM/L)									
C	88.03±14.83	84.16±18.81	91.32±6.04	97.47±6.37	90.24 ^a ±5.96	3.20	<0.05	0.36	0.47
T_1	85.69±12.34	79.33±9.17	72.96±13.30	50.16±5.23	72.03 ^b ±5.71				
T_2	87.32±13.83	74.91±7.39	69.01±9.98	64.59±6.30	73.96 ^b ±4.91				
Mean	87.01±7.33	79.47±6.92	77.76±6.05	70.74±6.18					
Testosterone (ng/mL)									
C	0.03±0.01	0.05±0.006	0.08±0.02	0.09±0.006	0.06 ^b ±0.007	0.01	<0.01	<0.01	0.82
T_1	0.03±0.006	0.13±0.04	0.15±0.03	0.16±0.03	0.12 ^a ±0.02				
T_2	0.06±0.04	0.14±0.04	0.16±0.03	0.18±0.04	0.14 ^a ±0.02				
Mean	0.04 ^B ±0.01	0.11 ^A ±0.02	0.13 ^A ±0.02	0.14 ^A ±0.02					

^{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 ($P<0.01$) ($P<0.05$).

significantly higher ($P < 0.05$) in both supplemented groups (Table 6). Effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone T_4 (Sauvaire *et al.* 1991) or impaired peripheral conversion of thyroid hormones resulting in a significant decrease in serum T_3 with a concomitant increase in T_4 levels (Tahilant and Kar 2000). Choubey *et al.* (2015) also reported reduced ($P < 0.05$) serum cortisol in Jamunapari goats. The linear decline in serum cortisol level in the present study could be supposed to have an ameliorative effect against the various types of stresses. The declining levels of serum cortisol by MMS supplementation could be due to the strong anti-oxidative principles (saponins, phenolics and flavonoids) of the constituent herbs feed ingredients in the formulations. In present study, the testosterone levels in three respective groups after 9 months experimental feeding were 0.09 ± 0.006 , 0.16 ± 0.03 and 0.18 ± 0.04 ng/ml, respectively. However, Ahmad *et al.* (1989) observed that mean serum testosterone concentrations remained low from birth to 12 months of age (0.3 ± 0.1 ng/ml) while a marked rise in testosterone was observed at 14 months of age (2.7 ± 0.9 ng/ml). It has been theorized that the anabolic potential of the Fenugreek seed may be due to its ability to block enzymes which breakdown testosterone. Those enzymes are aromatase and $5-\alpha$ reductase. If that is true, it would mean it's not triggering more testosterone to be produced, but rather promoting preservation of that which your body is already making. Wilborn *et al.* (2010) reported that Fenugreek seed appears to act as an aromatase inhibitor, which may help to keep men's estrogen levels low, thereby freeing up more testosterone and also block $5-\alpha$ -reductase, which converts testosterone into dihydro-testosterone (DHT) so blocking it, may sustain testosterone-levels.

Based on the results it may be deduced that supplementation of molasses based multi-nutrient herbal supplements in the diet of buffalo calves improved level of serum glucose, globulin, antioxidants, T_4 and testosterone and lowered level of low density lipoprotein (LDL) and cortisol resulting into an improvement in the health status.

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