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Effect of Supplementation of Herbs Containing Essential Oils on Nutrients Digestibility, Rumen Fermentation and Blood Parameters in Cross Bred Calves

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A B S T R A C T

The present study was conducted to assess the effect of supplementing herbal feed additives (HFAs) Jaiphal Suva and Haldi at the rate of 1% of DM basis in TMR (R:C65:35) on the nutrient utilization, nitrogen retention, rumen fermentation and blood parameters of male crossbred calves by 4x4 latin square designThe herb supplementation did not have any significant effect on digestibility of various nutrients and percent nitrogen retention in male cross bred calves. The blood biochemical profile for various parameters did not show any significant effect on herb supplementation i.e. herbs have no deleterious effect on animal health except supplementation of herb suva in male cross bred calves significantly increased ($P<0.05$) the alkaline phosphatase activity (AKP). Herbs supplementation has stimulatory effect on rumen fermentation parameters (TN, TVFA, NPN NH_3-N) which were ($P<0.05$) higher than control group.

Introduction

Climate change is the major growing concern where greenhouse gas emissions have a major role. It has been reported that methane is one of the important greenhouse gas that cause21 times more global warming than carbon dioxide(IPCC 2007). It is normally produced during anaerobic fermentation of feeds in ruminants and share of 15% out of the total methane produced globally (Moss *et al*2000). Moreover, for methane production around 7-15% of their gross energy is consumed. So the emphasis is being drawn to inhibit methane production from nutritional aspect as well as for global warming aspect. Currently, many chemical additives as well as antibiotics are being used to inhibit methane production in

rumen however these chemical additives are either toxic to host animals or have a transient effect on methanogenesis (Moss *et al.,* 2000). In addition, an increasing awareness of hazards associated with chemical feed additives, i.e. presence of chemical residues in animal derived foods and development of bacterial resistance to antibiotics has diverted the research on feed additive technology towards exploiting natural products as feed additives.

Plants produce a range of plant secondary metabolites (PSM) to protect against microbial and insects attacks. These natural plant eco chemicals such as essential oils (EOs), saponins, tannins and organo sulphur compounds have been shown to selectively modulate the rumen microbial populations (Patra and Saxena 2009) thus resulting in improvement of rumen fermentation and nitrogen metabolism, and a decrease in methane production and thus improving the productivity and health of animals. Our previous *in vitro* study (Sharma et al 2018) on effect of herbs containing EOs from jaiphal, suva and haldi supplemented individually @ 1 to 3% levels on total mixed ration (TMR) where we have found that 1% level of herbs containing essential oils supplementation significantly reduced the methane production and improved the utilization of nutrients.

Hence this study is being planned to assess the effect of supplementation of EO from Jaiphal, Suva and Haldi on digestibility of nutrients, rumen fermentation and health status of cross bred calves.

Materials and Methods

Four cross bred male calves of 10 to 14 months age were selected and divided into four groups of one each. This experiment was approved by institute of animal ethics committee (IAEC), GADVASU, Ludhiana. The feeding trial was carried out in four periods in a switch over design as shown below. Each period lasted for four weeks with an adjustment period of three weeks and collection period of one week.

Each group was offered four different total mixed rations with or without supplementation of different herbs i.e. Jaiphal, Suva and Haldi @1% of DM intake. i.e TMR1 (control), TMR2 (1 % Jaiphal), TMR3 (1% Suva) and TMR4 (1% Haldi) for 120 days. At the termination of experimental period, a 7-day metabolism trial was conducted. During this period digestibility coefficients of various nutrients and nitrogen balance studies were conducted. The samples of concentrate mixture, wheat straw, green fodder, feed residue and faeces were analysed for their proximate constituents (AOAC, 2000) and other cell wall constituents (Robertson and Van Soest, 1981).

Collection and analysis of rumen liquor samples

The rumen studies were conducted on three rumen fistulated animals for assessing the effect of supplementing herbs containing essential oils on the rumen metabolites. After 30 days adaptation on a particular ration, the rumen liquor samples from each animal were collected for 3 consecutive days at 2 hourly intervals, starting from zero and continuing up to 12 h post-feeding. The rumen liquor samples were strained through four layered muslin cloth and few drops of saturated mercuric chloride solution were added to stop the microbial activity. The samples of strained rumen liquor (SRL) were pooled for the respective animal and the samples were stored in a refrigerator till analyzed. The SRL samples were analyzed for TCA- precipitable nitrogen, non-protein nitrogen (NPN), ammonical nitrogen (AOAC, 2000) and individual and total Volaltile fatty acids

(VFAs) were estimated by (Cottoyn and Boucque, 1968) using gas liquid chromatography (GLC) technique using Net Chrom-9100 model

Collection and analysis of blood samples

At the termination of metabolism trial blood samples were collected (in heparin and sodium flouride + oxalate vials) from the juglar vein of animals at 4 h post parandial. The serum was separated and stored at -20^{o}Ctill analyzed. All biochemical parameters were estimated by using diagnostic kits from Siemens Autopack and analyze at RA-50 blood analyzer.

Statistical analysis

Data were analysed by ANOVA (Snedecor and Cochran 1994), by using SPSS Version 19. The differences in means were tested by Tukey B and Duncan.

Results and Discussion

Chemical composition of the feed stuffs

The values of different principles and fibre fractions in concentrate mixture, green fodder and wheat straw fed to the cross bred calves during experiment are presented in Table 1.

Intake and digestibility of nutrients

Our data showed that dry matter intake (DMI) (Kg/d) was similar in three herbal supplemented and control groups. (Table 2). This may be attributed to the comparable body weight of experimental cross bred calves among the groups as no effect of cinnamaldehyde supplementation was observed. But in case of suva supplemented group it was statistically lower and comparable to other groups. Cardozo *et al* (2006) observed no change in DMI when dietary supplementing with a mixture of 600 mg/d of cinnamaldehyde and 300 mg/d of eugenol in beef heifers fed a high concentrate diet. In a study with growing lambs, Chaves*et al* (2008b) found that addition of carvacrol or cinnamaldehyde (200 mg/kg of dietary DM) had no effect on DMI in the barley or corn concentrate based diets. Similar results were observed by Benchaar*et al* (2006a) who reported no change in DMI of beef cattle fed a silage based diet supplemented with 2 or 4 g/d of a commercial mixture of EO compounds consisting of thymol, eugenol, vanillin and limonene.

There was no significant difference ($P<0.05$) in the dry matter digestibility (DMD), ADF, Ether extract (EE), Crude protein (CP) digestibility, total carbohydrate and total non-fibre carbohydrate digestibility in control and herbs supplemented groups.

No significant difference in the digestibility (%) of organic matter (OM) was found among the four groups. It varied from 57.32 to 67.03 in control and herbs suva group. In herbs supplemented group it varied from 62.03 to 67.03 % respectively. This might be due to the corresponding comparable intake and digestibility (%) of DM among the groups.

The CP digestibility (%) was not modified by herbs supplementation. It varied from 70.34-75.77 % in all the four groups. There were no significant differences in the mean values of EE digestibility (%) among the groups. The digestibility coefficients values for EE (%) were 73.47, 72.47, 77.73 and 74.92 respectively in control and herbs supplemented groups.

The total carbohydrate (TCHO) digestibility (%) did not differ significantly among the herbs supplemented and control groups. This may be due to similar OM, CP and EE intake (g/d), digested (g/d) and digestibility (%)

among the four groups. The corresponding values for digestibility (%) were 54.32, 57.11, 62.38 and 57.45 in groups I, II, III and IV respectively. The digestibility (%) of NDF was similar in four groups. The NDF digestibilities (%) were 49.32, 52.36, 58.36 and 54.47 respectively in all four groups respectively.

The Non-fibre carbohydrate (NFC) digestibility (%) was not significantly different. The corresponding values were 67.50, 68.67, 72.09 and 64.50 in groups I, II, II and IV respectively. Similar results were reported by Castillejos*et al* (2005), who observed no change in DM, OM, NDF, and CP digestibility, when a CRINA Ruminants EO mixture (a commercial blend of EO) was added at the dose of 3.8 mg/L of ruminal fluid in continuous-culture fermenters.

However, Benchaar*et al* (2006) observed that ADF digestibility was significantly increased (3 percentage points) when diets were supplemented with EO @ 2 g/ cow per day (48.9 vs. 46.0%). Benchaar*et al* (2008) observed no effect on DMI or digestibility when supplementing lactating dairy cows with 1 g/d of cinnamaldehyde (one of the active compounds found in the current blend). Digestibility of nutrients was also unaffected with the addition of CRINA EO (a commercial blend of essential oils that contains eugenol) in several *in vivo* trials at various inclusion levels (Benchaar*et al* 2006, 2007). Such variability in the results could be due to many factors such as species, age, breed and body condition of the animals. There was no significant difference in DM digestibility (%) in four groups.

Nitrogen balance in buffalo calves

The intake of N (g/d) in the groups I, II, III and IV were 147.16, 124.49, 130.70 and 121.45 respectively. Nitrogen intake (g/d) was significantly lower in herbs supplemented groups as compared to control group. The mean values of total N excreted through urine (g/d) were 67.74, 70.45, 74.17 and 63.90 respectively in all the four groups. The N excreted (g/d) through faeces were 44.98, 35.16, 32.98 and 34.75 four groups respectively (Table3). Faecal N excretion was statistically higher in control group as compared to herbs supplemented groups. However, urinary and total nitrogen excreted (g/d) were statistically similar among the four groups as no significant effect was seen in herbs supplemented groups. Animals in all the four groups were in positive N balance. There were statistically lower N retention in jaiphal supplemented group as compared to other groups. However, it was comparable in control and suva and haldi supplemented groups. The N retention (g/d) was 34.43, 18.86, 23.54 and 22.79 in all four groups (Table 3).

The daily total excretion of nitrogen was highest in control non-herb supplemented groups as compared to herbs supplemented groups. The urinary nitrogen excretion was highest in animals feed suva supplemented group and lowest UN excretion was observed in haldi supplemented groups. The nitrogen retention percentage was highest in control group than herbs supplemented groups though the results were non-significant.

Rumen fermentation parameters

The results recorded during rumen fermentation study in crossbred calves are presented in figure 2. Mean NH_3-N concentration (mg/dl SRL) was significantly ($p<0.05$) lower in control group (19.87) as compared to group II (27.07) group III (28.17) and group IV (28.79) which may be due to its more incorporation into microbial protein as rumen microbes mostly prefer ammonia or peptides as nitrogen source

(Bryant 1977). The mean NH_3-N concentration recorded in this study was higher than the minimum threshold of 5-8 mg/100 ml strained rumen liquid (SRL), as proposed by Maynard *et al* (1979) for optimum microbial growth in all the four groups.

In the conversion of dietary N to microbial protein, NH_3 is a prime intermediate in the rumen. Ingestion of large amount of protein can cause excessive NH_3 production in rumen. If rate of production of NH_3 is more than its utilization by rumen microbes, then concentration of NH_3 in rumen increases which is particularly evident when diet is lacking readily available carbohydrate.

In group I there was significant ($p<0.05$) lower NH_3-N in comparison to herbs supplemented groups II to IV (Table 4). This was probably due to better utilization of NH_3 –N by rumen microbes for the synthesis of microbial protein in the presence of supplemented herbs. But it is not evident from increased microbial protein (TCA-ppt N) in all groups as non-statistically difference was observed in all the groups.

The total nitrogen in SRL is mainly expression of solubility of ingested protein in rumen and may also vary in relation to amount of protein intake. The total nitrogen (mg/dl)was significantly lower in control group (group I) and was statistically higher ($P<0.05$) in herbs supplemented groups (group 1I, III and IV) where it was 85.61 and 9, 63.07 and 69.18 respectively (Table 4). The TCA-ppt N mainly represents microbial N. It is evident from figure 2 that on supplementation of herbs there were no significant ($P<0.05$) increase in the concentration of TCA-ppt N (mg/100 ml) in all the groups as compared to control. The TCA-N values were 20.19, 20.50, 19.09 and 21.75 respectively in group I to Group IV.

The non-protein nitrogen (NPN) fraction of nitrogen contains chiefly NH_3-N, small quantity of amides and amino acids etc., and thus NPN concentration in the rumen fluid depends mainly on the production of ammonia, its uptake by microbes and absorption through rumen wall. NPN concentration (mg/100ml SRL) was also significantly ($p<0.05$) lower in control group (36.62) and highest in jaiphal group II (65.11) as compared to other herbs supplemented groups III (43.98) and IV (47.42) (Table 4).

The total production of volatile fatty acid (TVFA) where a significant ($P<0.05$) increase in concentration in haldi supplemented group (11.04mM/dl) and significantly lower in suva group (10.37mM/dl). This showed the stimulatory effect of herbs on rumen microorganism in the haldi supplemented groups. (Table 5)

The A: P ratio was significantly ($P<0.05$) reduced in jaiphal supplemented group (3.07) may be due to a reduced proportion of acetic acid and an enhanced percentage of propionic acid and found highest in non-herb supplemented group i.e control group. (Table5).

The mean percentage of acetate where it was statistically lower ($P<0.05$) in TMR group II (65.85) and highest in control group (69.81). The propionate was statistically higher ($P<0.05$) in all herbs supplemented groups as compared to control group. The values were 19.77, 21.40, 21.67 and 20.29 in group I, II, III and IV respectively.

The butyrate percentage was statistically lower in suva group (8.31) and highest in haldi group (9.79). However, the percent isobutyrate, isovalerate and valerate in rumen liquor were statistically higher ($P<0.05$)in jaiphal supplemented groups and lowest found in control group (Table6).

Yang *et al* (2010a) used cinnamaldehydeat three doses (400, 800 and 1600 mg/animal per d) in growing beef heifers and observed no changes in concentrations of total VFA and the molar proportions of acetate, propionate, BCVFA, and the acetate to propionate ratio. In an another study, Chaves*et al* (2008b) evaluated the effects of carvacrol and cinnamaldehyde in growing lambs and reported an increase in concentration of total VFA, but molar proportions of acetate, propionate, BCVFA, and the acetate to propionate ratio were not affected.

Blood biochemical aspects

Effect of herbs supplementation on blood glucose, creatinine, urea-nitrogen, cholesterol, aspartate aminotransferase (AST) and alkaline kinase phosphate (AKP)are presented in Table 7. There was no significant effect of herbs supplementation on glucose, creatinine, total protein, blood urea nitrogen and AST in all groups. The AST values (U/L) were 154.31, 107.14, 107.86 and 107.70 in groups I. II, III and IV respectively. The serum urea nitrogen concentration is closely associated with the breakdown of protein to amino acids and their deamination in rumen and the rate of utilization of NH_3 for bacterial protein synthesis. An increase in serum urea level may reflect an accelerated rate of protein catabolism rather than decrease in urinary excretion. The serum urea level also increases in renal tubular necrosis and decreases in hepatic insufficiency and low protein intake. Concentrations of urea-N in blood serum are indicator of the adequacy or inadequacy of the nitrogen in the diet of animals and results revealed no statistically significant difference in 4 groups. The blood urea concentration (mg/dl) were13.40, 18.35, 13.10 and 12.15 in all groups respectively. The serum creatinine concentration (mg/dl) varied from 0.97 to 1.06 in all four groups.

There was no significant ($P<0.05$) increase in blood glucose (mg/dl) level after herbs supplementation. The serum glucose (mg/dl) values varied from 80.50, 79.96, 76.28 and 77.26 in group I, II, III and IV respectively (Table 7).

The effect of herb supplementation on blood haemotology shows no significant effect on different parameters like WBC, RBC, Hb, MCH, and MCV (Table 8). In accordance to previous researches (Yang *et al* 2010b), EO supplementation did not significantly affect blood glucose concentration.

Table.1 Chemical composition of total mixed ration fed to calves, % dry matter basis

Parameter	Concentrate	Green	Wheat straw
Total ash	10.3	10.2	10.35
Organic matter	89.7	89.8	89.65
Crude protein	23.45	21.10	4.41
Ether extract	4.16	2.26	0.9
Cellulose	6.8	20.70	39.10
NDF	35.4	44.0	77.6
ADF	18.30	31.10	53.70
Hemicellulose	17.1	12.9	23.9

Table.2 Effect of supplementing total mixed rations with essential oils containing herbs on dry matter intake and digestibility of nutrients

Parameters	Group 1 (Control)	Group 2 (Jaiphal)	Group 3 (Suva)	Group 4 (Haldi)	SEM
Dry Matter Intake	5.88^{b}	5.48^{ab}	5.06^{a}	5.60^{ab}	0.097
Dry Matter Digestibility	57.46	60.15	61.03	60.60	1.23
Neutral Detergent Fibre Digestibility	49.32	52.36	58.36	54.47	1.34
Acid Detergent Fibre Digestibility	44.38	41.77	46.14	42.30	1.57
Ether Extract Digestibility	73.47	72.47	77.73	74.92	0.93
Hemi cellulose Digestibility	58.83^{a}	72.89^{b}	82.35^{c}	77.40^{bc}	1.65
Organic Matter Digestibility	57.32	62.20	67.03	62.38	1.17
Crude protein Digestibility	70.34	72.68	75.77	72.21	0.79
Total carbohydrate Digestibility	54.32	57.11	62.38	57.45	1.31
Non fibre carbohydrate Digestibilty	67.50	68.67	72.09	64.50	1.43
Cellulose Digestibility	71.43	68.87	71.08	77.43	1.29

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

Table.3 Effect of supplementation of Herbs containing essential oils on Nitrogen Retention in male crossbred calves

Parameter	Group 1 (Control)	Group2 (Jaiphal)	Group3 (Suva)	Group 4 (Haldi)	SEM
Total N intake g/day	147.16^{b}	124.49^{a}	130.70^{a}	121.45^{a}	2.37
Urinary N excretion g/d	67.74	70.45	74.17	63.90	1.65
Faecal N output g/d	44.98^{b}	35.16^{a}	32.98^{a}	34.75^{a}	1.07
Total N outgo g/d	112.73	105.62	107.15	98.65	1.99
N-retention g/day	34.43^{b}	18.86^{a}	23.54^{ab}	22.79^{ab}	2.54
%N retention	22.80	14.94	17.78	18.06	1.76

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

T**able.4** Effect of supplementing total mixed rations with essential oils containing herbs on rumen fermentation in male cross bred calves

Parameter	TMR 1 Control	TMR2 Jaiphal	TMR 3 Suva	TMR4 Haldi	SEM
Total nitrogen, mg/dl	56.81^{a}	85.61^{d}	63.07^{b}	69.18^{c}	4.07
NPN, mg/dl	36.62^{a}	65.11^{d}	43.98^{b}	47.42^{c}	3.96
TCA-N, mg/dl	20.19	20.50	19.09	21.75	0.65
NH_3-N, mg/dl	19.87^{a}	27.07^{b}	28.17^{b}	28.79^{b}	1.37

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

Table.5 Effect of supplementation of herbs containing essential oils on rumen volatile fatty acids production (mM/dl)

Parameter	TMR 1 Control	TMR2 Jaiphal	TMR 3 Suva	TMR4 Haldi	SEM
Acetic Acid	7.54^{d}	7.06^{b}	7.02^{a}	7.45^{c}	0.086
Propionic Acid	2.13^{a}	2.29^{c}	2.24^{b}	2.24^{b}	0.022
IsoButyric acid	0.0530^{a}	0.067^{d}	0.567^{b}	0.0615^{c}	0.002
Butyric Acid	0.916^{b}	1.037^{c}	0.862^{a}	1.08^{d}	0.033
IsoValeric Acid	0.085^{a}	0.119^{b}	0.094^{b}	0.097^{b}	0.005
Valeric Acid	0.072^{a}	0.139^{d}	0.87^{b}	0.110^{c}	0.011
Acetate/Propionate	3.53^{d}	3.07^{a}	3.12^{b}	3.32^{c}	0.083
Total Volatile Fatty Acid	10.80^{c}	10.72^{b}	10.37^{a}	11.04^{d}	0.091

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

Table.6 Effect of supplementation of herbs containing essential oils on rumen volatile fatty acids production, % Relative Proportion

Parameter	TMR 1 Control	TMR2 Jaiphal	TMR 3 Suva	TMR4 Haldi	SEM
AA	69.81^{d}	65.85^{a}	67.67^{c}	67.48^{b}	0.53
PA	19.77^{a}	21.40^{c}	21.67^{d}	20.29^{b}	0.29
IB	0.49^{a}	0.63^{c}	0.55^{b}	0.56^{b}	0.021
BA	8.45^{b}	9.67^{c}	8.31^{a}	9.79^{d}	0.24
IV	0.79^{a}	1.11^{d}	0.91^{c}	0.88^{b}	0.043
VA	0.66^{a}	1.30^{d}	0.84^{b}	1.00^{c}	0.081

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

Table.7 Effect of herbs supplementation on blood parameters of cross bred calves

Parameters	TMR1 Control	TMR 2 Jaiphal	TMR 3 Suva	TMR 4 Haldi	SEM
Glucose, mg/dl	77.26	80.50	79.96	76.28	1.12
Creatinine, mg/dl	1.04	1.06	0.98	0.97	0.05
Aspartate aminotransferase, U/L	107.70	154.31	107.14	107.86	11.15
Alkaline kinase phosphate, U/L	130.39^{a}	116.35^{a}	161.11^{b}	121.32^{a}	6.70
Total protein g/dl	7.10	7.72	7.97	7.68	0.19
Albumin g/dl	3.55	3.71	3.30	3.43	0.08
Blood Urea Nitrogen, mg/dl	12.15	13.40	18.35	13.10	1.27

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

Table.8 Effect of supplementation of herbs containing essential oils on blood hematology parameters

Parameters	TMR1 Control	TMR 2 Jaiphal	TMR 3 Suva	TMR 4 Haldi	SEM
WBC*10^3	12.39	13.57	14.03	14.92	0.68
RBC*10^6	6.94	6.90	7.15	6.90	0.32
HB	9.55	9.61	9.90	9.67	0.41
HCT	29.22	28.12	29.22	28.80	1.14
MCV	41.32	40.55	41.12	40.80	0.49
MCH	14.00	13.92	13.52	13.80	0.19
MCHC	33.92	34.75	33.20	34.17	0.32
CHCM	34.02	34.57	34.02	34.85	0.27
CH	14.02	13.90	14.00	14.12	0.16
RDW	17.20	18.85	17.75	17.87	0.47
HDW	2.03	2.12	2.06	2.22	0.033
PLT *10^3	728.75	651.50	687.75	651.50	57.5
MPV	7.15	6.82	6.75	6.65	0.20

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

The present study concluded that herbs containing essential oils supplementation did not have any significant effect on digestibility of various nutrients and percent nitrogen retention in male cross bred calves. The blood biochemical profile for various parameters did not show any significant effect on herb supplementation i.e. herbs have no deleterious effect on animal health except supplementation of herb Suva in male cross bred calves significantly increased (P<0.05) the AKP. Herbs supplementation has stimulatory effect on rumen fermentation parameters (TN, TVFA, NPN NH_3-N) which were (P<0.05) higher than control group.

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