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Effect of vegetable oil supplementation on growth performance and blood biochemicals parameters in crossbred calves in winter months

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Abstract
The present study was conducted on eighteen female crossbred calves for 120 days to examine the influence of vegetable oil (mustard oil) on growth performance and blood biochemical parameters in crossbred calves in winter months. The calves were blocked into two equal groups on the basis of average body weight. The calves in both groups received a basal diet and calves in treatment group additionally supplemented with vegetable oil @ 3% of drymatter intake (DMI). The DMI is not affected by supplementation with vegetable oil. The overall average gain was significantly more ($p<0.05$) in treatment group as compared to control. There was no effect of vegetable oil supplementation on FRAP assay, SOD and catalase activity in winter months. Plasma concentration of cortisol was also not affected by treatments. It was concluded that vegetable oil supplementation does not able to impose any effect on biochemical parameters, but there is significant effect on the growth performance as additional energy is provided by mustard oil to compensate for increased energy demands in cold stress.

Keywords: Vegetable oil, dry matter intake; crossbred calves, growth performance, cortisol

Introduction
Improved female calves growth performance is very important, because rearing of female calves involves significant financial input accounting for approximately 20% of total farm costs (Gabler *et al*., 2000) [8]. Age at first calving depends largely on early life management, nutrition and their overall performance. Thermal environment effect the ability of livestock to grow lactate and reproduce to their maximum genetic potential. Animals are homoeothermic so they have to maintain their temperature within normal range to maintain homeostasis. Cold stress (below the thermoneutral zone) increased maintenance requirement of livestock (Todini, 2007) [32]. At ambient temperature below the lower limit of the thermoneutral zone, animals convert more energy to heat and digest the feed less efficiently (NRC, 1981). Unless additional energy is provided to compensate for increased energy demands in cold environment, the availability of metabolizable energy (ME) for productive processes would be limited (McBride and Christopherson, 1984). Sufficient energy supply is very important in the ruminant diets especially in cold stress. The problems of energy supply in ruminants could be solved with dietary fat, which are commonly available (NRC, 2001; Adeyemi *et al*., 2016) [1]. In addition to increase energy supply, the addition of vegetable oil is used to serve as carriers and to facilitate the digestion process of fat-soluble ingredients (Palmquist, 1987) [23] and ruminal acidosis prevention (Chilliard, 1993) [4]. Mustard oil is a cheap source of fat supplement for dairy cows and buffaloes used traditionally in field conditions which contain 60% MUFA and 40% PUFA (Kathirvelan and Tyagi, 2013) [13]. The inclusion of vegetable oil on ruminant diets (4-8% of dry matter) had no effect on nutrient intake and nutrient digestibility, but it alter rumen fermentation, change microbial population and improvement has been observed in average daily gain (ADG) and feed efficiency (FE) (Fiorentini *et al*., 2014; Cleef *et al*., 2016; Hartanto *et al*., 2019) [7, 5]. Therefore, the present study was hypothesized that supplementation of vegetable oil (3% of DMI) during cold weather, increases energy requirement would not adversely affect dry matter intake, thereby improving energy intake and growth performance of young calves.

Materials and Method

Location, Animals and Feeding

Twelve female crossbred calves (4 to 6 months) maintained by Department of Livestock Farm Complex, CSKHPKV Palampur (H.P.), India, were divided into two equal groups on body weight basis and were fed as per NRC (2001) feeding standard. The energy and protein requirements of all the calves were met by feeding concentrate mixture and available green fodder. The experiment was conducted during winter months (1 October 2021 - 31 January 2022). Calves were housed on a concrete floor and no mechanical means were used to control the temperature. Experimental animals were fed iso-nitrogenous and iso-caloric diet as per NRC (2001) standard and requirements of animal were fulfilled by feeding concentrate mixture, wheat straw and maize/oats fodder. The mustard oil @ 3% of DMI was added to the concentrate mixture daily by pouring it on and mixing at feeding to ensure its equal distribution in the ration. The dietary treatments were continued for 120 days. Concentrate mixture (21.23% CP and 2.88 Mcal ME/kg DM) contained maize 33%, groundnut cake (oiled) 21%, mustard cake (oiled) 12%, wheat bran 20%, de-oiled rice bran 11%, mineral mixture 2% and common salt 1%. The DM content of offered and refusal feed was measured daily and the actual DM intake was derived. Body weight of the animals was recorded at fortnightly interval for two consecutive days before offering feed and water. Microclimatic data *viz*., dry bulb temperature, wet bulb temperature, minimum and maximum temperature and relative humidity was recorded at 7.30 AM and 2.30 PM using thermometer (GH Zeal Ltd., London, United Kingdom) every day during experimental period. Temperature humidity index (THI) was calculated using the formula (NRC 1981): THI = 0.72 (Tdb + Twb) + 40.6; where, Tdb = dry bulb temperature (ºC), Twb = wet bulb temperature (ºC).

Chemical analysis of feed samples

The roughage and concentrate were grounded individually, labeled and analyzed for proximate composition as per AOAC (2005) and cell wall constituents as per Goering and Van Soest (1970) [11].

Statistical analysis: Statistical analysis of the data was by Student‘t’ test as per Snedecor and Cochran (1994) [27] with the SPSS (1998) [28].

Result and Discussion

Chemical composition of the experimental diet

Chemical and mineral composition of feedstuffs offered to the calves during the experimental period of 120 days is presented in Table 1.

Table 1: Chemical composition of feed ingredients DM basis (%)

Feed/fodder	CP	NDF	ADF	EE	Ash
Concentrate mixture	21.23	34.17	21.83	4.72	5.04
Maize fodder	8.93	54.38	23.47	1.62	7.85
Oats fodder	11.26	48.84	30.65	1.95	12.76
Wheat straw	3.17	67.85	40.20	0.76	12.04

Environmental conditions during the experiment

The maximum and minimum temperature (^{0}C) ranged from 13.51-26.45 and 3.81-15.67, respectively throughout the experimental period (October – January; Table 2) The minimum morning temperatures were observed in fortnight VII (Jan., 16-Jan., 31). The relative humidity in morning and afternoon ranged from 71-84% and 46-69%, respectively. The THI throughout the experimental period varied from 52.90-70.92, all other values during the whole experiment were below 72, indicating cold stress on the animals (LPHSI, 1990) [15]. Srikandakumar *et al*. (2003) [29] and Gantner *et al*. (2011) [9, 10] stated that THI value below 72 is used to indicate cold stress on the animals. The experimental animals were under more cold stress during the months of December to January as the lowest THI values were observed in these months.

Table 2: Environmental parameters recorded during the winter months

Fortnight	Max. Temp (^{0}C)	Min. Temp (^{0}C)	Relative Humidity (%)	Relative Humidity (%)	THI
			M	E	
0 (Oct. 1,- Oct.15,2021)	26.45	15.67	79.20	65.73	70.92
I (Oct. 16,- Oct.31,2021)	23.58	11.50	76.25	59.31	65.85
II (Nov. 1,- Nov. 15,2021)	21.95	8.51	71.07	50.20	62.37
III (Nov. 16,- Nov. 30,2021)	19.99	6.23	76.20	46.47	59.5
IV (Dec. 1,- Dec. 15,2021)	17.61	5.98	76.33	56.20	54.39
V (Dec. 16,- Dec. 31,2021)	14.95	4.00	79.69	47.75	54.29
VI (Jan. 1,- Jan.15,2022)	14.15	4.79	83.60	65.13	54.31
VII (Jan. 16,- Jan.31,2022)	13.51	3.81	84.00	69.44	52.90

M= observations recorded at 7:00 am, E= observations recorded at 2:30 pm Temperature humidity index (THI) was calculated using the formula: THI = 0.72 (Tdb + Twb) + 40.6 (NRC, 1981) Where, Tdb = dry bulb temperature (ºC) Twb = wet bulb temperature (ºC)

Dry matter intake

In the beginning of the experiment, DMI averaged 2.87 and 2.85 kg/ d which increased progressively to 3.82 and 3.50 kg/ d at the end of 120 days of experimental feeding in control and treatment group respectively (Table 3). There was no significant difference (p>0.05) of feeding of vegetable oil on DMI in control and treatment group. Similarly, Shingfield *et al.* (2011) [26] and Polviset *et al.* (2014) [25] reported that adding oil in animal feeds below 4% DM had no effect on feed intake and nutrient digestibility. Mao *et al*., 2010 [17] and Peng *et al*., 2016 [24], also reported that addition of soybean oil 3% had no effect on DMI of lambs.

Table 3: Effect of vegetable oil supplementation on DM intake (kg/d) in crossbred calves during winter months.

Fortnight	DMI (kg/d)		
	Control	Treatment	SEM
0	2.87	2.85	0.08
I	2.88	3.05	0.08
II	3.07	3.00	0.08
III	3.61	3.41	0.12
IV	3.82	3.50	0.11
Mean	3.25	3.16	0.05

Growth performance

The overall average weight gain/ month were 5.33 and 6.57 kg respectively in control and treatment group respectively (Table 4). Statistical analysis revealed that there was significantly more (p<0.05) overall average gain (g/d) in treatment group as compared to control showing the positive effect of feeding of vegetable oil in treatment groups. Similarly to this significant gain in the growth rate by feeding 4% added fat was observed by the Engstrom *et al*., 1994 [6]. It has been found that the inclusion of vegetable oil on ruminant diets (4-8% of dry matter) had no effect on nutrient intake and nutrient digestibility, but improvement has been observed in average daily gain (ADG) and feed efficiency (FE) (Fiorentini *et al*., 2014; Cleef *et al*., 2016; Hartanto *et al*., 2019) [7, 5]. In cold stress Sufficient energy supply is very important in the ruminant diets and the problems of energy supply in ruminants could be solved with dietary fat, which are commonly available (NRC, 2007; Adeyemi *et al*., 2016) [1]. So by feeding mustard oil to calves increase growth performance by reducing cold stress.

Table 4: Body weights (kg) and average daily gain (g) of crossbred calves in control and treatment group.

Months	Body weights (kg)		Average daily gain (kg)	
	Control	Treatment	Control	Treatment
0	52.53±6.60	52.60±4.59		
I	58.60±6.22	60.47±4.15	6.07±1.75	7.87 ±0.76
II	64.10±6.32	67.30±3.87	5.50±0.719	6.83±0.95
III	68.69±5.62	73.03±3.70	4.59±1.12	5.73±0.86
IV	73.86±5.23	78.87±4.28	5.17^a ±0.87	5.83 ±0.95
Mean	59.65±2.58	62.75±2.72	5.33^b ±0.45	6.57^b ±0.50

Plasma glucose

The overall average glucose level (mg/dl) was 60.49 and 60.64 in control, and treatment groups, respectively exhibiting no significant difference (*p*>0.05) of feeding of vegetable oil (Table 5). The blood glucose levels observed in crossbred calves in the present study are in the normal physiological range (40.00 to 80.00 mg/dl) as reported by other workers (Upadhyay and Rao 1985; More *et al*. 2008 and Bhooshan *et al*. 2010) [33, 20, 2].

Table 5: Effect of vegetable oil supplementation on glucose level (mg/dl) in growing crossbred calves during winter months

Fortnight	Glucose(mg/dl)		
	Control	Treatment	SEM
0	60.04	59.02	2.18
I	59.27	58.37	1.84
II	60.09	59.89	2.14
III	61.31	63.32	1.95
IV	61.72	62.60	1.67
Mean	60.49	60.64	0.68

Means having different superscripts within a row differ significantly (*p*<0.05)

Effect of feeding of vegetable oil on antioxidant status

Plasma total antioxidant activity

Average plasma total antioxidant activity (µmol/l) in the blood samples drawn monthly in control and treatment groups has been presented in Table 6. The overall mean of FRAP value averaged 1946.18 and 2042.84 µmol/ l in control and treatment groups respectively showing no significant (*p*>0.05) effect of feeding of vegetable oil. The observed FRAP values are within the range reported by various workers (Vaidya *et al*., 2012, Suman *et al*., 2019) [34, 30].

Superoxide dismutase and catalase activity: Effect of vegetable oil supplementation on plasma overall SOD (U/ml) and catalase activity (mmol/min/ml) in growing calves in control and treatment group was 2.58, 2.83 and 51.27, 51.75 respectively, no significant effect (*p*>0.05) of dietary treatments has been observed on the plasma SOD and catalase levels (Table. 6). The values of SOD and catalase were in normal range as reported by earlier workers (Manish *et al*. 2011, Suman *et al*. 2018, 2019) [16, 31, 30].

Table 6: Effect of vegetable oil supplementation on FRAP level (µMol/l), SOD (U/ml) and catalase activity (nmol/min/ml) in crossbred calves during winter months

Fortnight	FRAP level (µMol/l)			SOD (U/ml)			Catalase activity (mmol/min/ml)		
	Control	Treatment	SEM	Control	Treatment	SEM	Control	Treatment	SEM
0	2033.13	2035.02	49.48	2.97	2.99	0.13	51.76	53.2	1.16
I	1841.76	1971.01	62.03	2.38	2.5	0.11	52.23	54.91	0.96
II	1867.7	2134.58	37.71	2.62	2.74	0.10	46.85	53.69	0.77
III	1961.09	2036.32	76.23	2.19	2.84	0.11	55.14	48.53	1.40
IV	2027.24	2037.27	56.92	2.74	3.06	0.06	50.39	48.43	1.25
Mean	1946.184	2042.84	56.47	2.58	2.83	0.10	51.27	51.75	1.11

Plasma cortisol: Effect of feeding of vegetable oil on plasma cortisol in crossbred calves during winter season is presented in Table 7. Cortisol levels at the start of experiment averaged 3.05 and 3.01 ng/ml in control and treatment group. The cortisol numerically increased gradually up to due to the increase in cold stress. Similar to this, Kim *et al*. (2009) [14] and Suman *et al*., 2019 [30] also reported that exposure of animals to cold, increased plasma cortisol level. The overall average plasma cortisol (ng/ml) was 3.56 and 3.46 in control and treatment groups, respectively. There was no significant difference (*p*>0.05) found among the groups. Plasma cortisol levels observed during the present study were within the normal physiological range (Block *et al*. 2001, Maurya *et al*. 2011, Suman *et al*., 2019) [3, 18, 30]. Results of winter trial surmise that vegetable oil supplementation is not able to affect plasma cortisol.

Thus it can be concluded that, feeding of vegetable oil increase the energy density of the diet and can be used as a effective tool to enhance the body weight gain and growth performance of growing calves in the winter stress, which ultimately going to effect the productive and reproductive performance of the animals,

Table 7: Effect of of vegetable oil supplementation on Cortisol activity (nmol/min/ml) in crossbred calves during winter months

Fortnight	Cortisol (ng/ml)		
	Control	Treatment	SEM
0	3.05	3.01	0.25
I	3.20	3.00	0.21
II	3.3	3.85	0.32
III	4.24	3.85	0.32
IV	4.2	3.64	0.18
Mean	3.56	3.46	0.25

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