DR-2055
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RESEARCH ARTICLE | Asian Journal of Dairy and Food Research

Effect of Anti-nutritional Factors as Feed Additives on Enteric Methane, Blood Serum Indices and Worm Load in Crossbred Calves

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10.18805/ajdfr.DR-2055

ABSTRACT

Background: Ruminants have the distinct advantage of being able to eat forages and graze on land that is not suitable for growing crops. During breakdown of feed in rumen, 2 to 12 per cent of the gross energy consumed is converted to enteric CH_4, which accounts for about 6 per cent of the world's anthropogenic greenhouse gas emissions. Anti nutritional factor (ANF) plays a vital role in rumen manipulation. It helps in mitigation of methane production and improves the feed efficiency.

Methods: Twenty calves of 6-7 months old with an average body weight of 90.2±4.0 kg were distributed randomly into four dietary treatment groups. The calves of the control group (T0) were fed on a basal diet as per ICAR (2013) feeding standard. The basal diet of T1, T2 and T3 were supplemented with NLP, MOP, CO@ 2 per cent on DM basis, respectively. Blood serum and fecal samples were collected and analyzed on 30th, 90th and 120th day of the experiment. The methane emission per animal was measured over fortnightly.

Result: It was depicted that supplementation of ANFs as feed additives to the crossbred calves didn't not affect the blood serum indices but the worm load and methane emission in calves differ significantly (P<0.05).

Key words: Anti- nutritional factors, Blood serum, Methane, Methanogenesis, Worm load.

INTRODUCTION

A very wide class of molecules with low molecular weights known as anti-nutritional factors (ANF) are designed to guard against insects, pathogens and herbivores as well as to adapt to harsh climatic conditions. These ANFs have significant functions in animal husbandry; among them, antibacterial activity is one of the most remarkable contributing qualities. For calves and monogastric animals, ANFs are typically thought of as plant secondary metabolites.

ANFs have been postulated to have a role in altering the rumen microbial pattern to prevent methanogenesis due to their antibacterial properties (Patra *et al*., 2010). ANFs are naturally occurring substances that can be used in kitchens and are both safe for animals and socially acceptable. By changing the rumen's microbial fermentation system, ANFs such as tannins, saponins, essential oils (EOs), flavonoids have been found to reduce methane emissions and increase feed utilization efficiency (Rira *et al*., 2015; Inamdar *et al*., 2015). ANFs are quite effective in reducing methane at higher doses and speeding up development, but their fiber digestibility is drastically reduced (Pawar *et al*., 2014).

Neem leaves powder (NLP) was also found to increase plant productivity and lessen the acute feed shortage that occurred during the lean season in other studies (Neeti, 2017). *Moringa oleifera* powder (MOP) has been found as a possible beneficial animal feed due to its high protein content, carotenoids, various minerals and vitamins and certain phytochemicals. An efficient method for reducing CH_4 without impacting feed intake or rumen fermentation is to combine anti-methanogenic agents with complementary modes of action (Patra *et al*., 2017).

Essential amino acids, carotenoids, vitamin-A, tocopherol, ascorbic acid and possibly healthy phytochemicals are all present in a balanced ratio in the *Moringa* tree (Ma *et al*., 2020). Feeding *Moringa* at 5 per cent or less of the total DMI has been found to improve the health, feed conversion ratio (FCR) and growth performance of many livestock species (Falowo *et al*., 2018). Additionally,

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How to cite this article: Bhosale, T.R., Raypurkar, T.R., Gaikwad, U.S., Kamble, D.K. and Mandakmale, S.D. (2023). Effect of Anti-nutritional Factors as Feed Additives on Enteric Methane, Blood Serum Indices and Worm Load in Crossbred Calves. Asian Journal of Dairy and Food Research.doi:10.18805/ajdfr.DR-2055

Submitted: 09-12-2022 **Accepted:** 18-04-2023 **Online:** 24-05-2023

it improved growth efficiency of animals (Sreelatha and Padma, 2009).

Dietary fats have been utilized to enhance ruminant development and alter the properties of meat with advantages for human health (Fiorentini *et al*., 2015). Additionally, adding more Medium chain fatty acids (MCFAs) to growing calves diets is thought to be an effective way to change their rumen microbiota (Bhosale *et al*., 2022). MCFAs are abundant in coconut oil (CO), which is a highly saturated oil (Bhatnagar *et al*., 2009). Additionally, the anti-methane properties of CO were also seen in dairy cows and swamp buffalo (Liu, 2019; Faciola, 2014) and neither research found that CO had a deleterious impact on DMI, nutritional digestibility or ruminal fermentation.

This study was conducted to look into the effect of different feed additives on growing calves in light of the conflicting findings regarding the effect of supplementation with NLP, MOP and CO on blood serum indices, worm infestation and ruminal fermentation.

MATERIALS AND METHODS

Experimental design

The study procedures were adopted after approval by the Department of Animal Husbandry and Dairy Science, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra province of India in the academic year of 2021-22. The methane gas measured by using the model of Ellis *et al*. (2007). A total of 20 calves (6-7 months of age), with similar body weight, were selected from Research Cum Development Project (RCDP), MPKV, Rahuri. After an acclimatization period of seven days, all calves were assigned randomly to each of the four treatment groups: Control (T0), NLP (T1), MPO (T2) and CO(T3) @ 2% of DM basis. Each group had five replicates. The packaged feed additives were procured from commercial sources (Shivanand Ayurvedalaya, Ahmednagar and Sanket agencies, Rahuri). In addition to these chelated minerals, 1% of the concentrate mixture was fed to the experimental animals (Bhosale *et al*., 2021). The experiment lasted for 120 days. The ingredients used and feed formulation have been presented in Table 1. All the calves in four treatment groups were maintained at 25-30°C throughout the experiment.Serum was harvested from centrifugation of the blood samples at 5000 RPM for 5 min and stored at -20°C until further analysis by using automatic blood analyzer *Miura* 200 (Germany made). Fecal samples was examined at Samarth laboratory, Rahata, Dist. Ahmednagar, MH. To detect the presence of nematode and cestode eggs in the samples, modified Sheather's salt floatation technique as described by Soulsby (1982) was used.

Digestion trial

The complete amount of feces from each calf was physically collected after the trial ended, gathered by an experimental unit (individual calf per group) and frozen. Additionally, representative feed samples were taken over a seven-day period and kept in moisture-proof plastic bags at 4°C until analysis using a standard analytical method (AOAC, 2012). The stated methodology was used to analyze the content of neutral detergent fiber (NDF) (Van Soest *et al*., 1991).

Methane production

Methane production was estimated using the equation given by Ellis *et al*. (2007).

$$CH_4 \text{ (MJ/d)} = 2.70\ (\pm 1.38) + 1.16\ (\pm 0.271) \times \text{DMI (kg/d)} - 15.8\ (\pm 6.86) \times \text{E.E (kg/d)}$$

Statistical analysis

The results are presented as mean± standard error of mean (SEM). Statistical differences between groups were evaluated by one-way analysis of variance (ANOVA) to investigate effects of various factors on methane production. Blood serum parameters were analyzed by using two-way analysis of variance (ANOVA) with SPSS 22.0 statistical software (SPSS Inc., Chicago, USA). Significant differences were set as *P*<0.05.

RESULTS AND DISCUSSION

Enteric methane

The results related with the methane emission and its relation with the different groups have been presented in Table 2 and 3 respectively. The average methane emission (MJ/d/

Table 1: Physical composition of concentrate mixture.

Ingredients	T0	T1	T2	T3
Maize grain	33	33	33	33
GNC	21	21	21	21
CSC	12	12	12	12
Wheat bran	20	20	20	20
Soybean meal	11	11	11	11
Chelated mineral	2	2	2	2
Salt	1	1	1	1
Total	100	100	100	100

ANF was added to T1, T2 and T3 @ 2 per cent.

GNC: Groundnut cake and CSC: Cotton seed cake animal) in different groups was 5.27±0.08, 4.86±0.11, 4.82±0.05 and 4.15±0.08 for T0, T1, T2 and T3, respectively. The results showed about a 21.26 per cent reduction in methane production in CO treatment groups with comparison to control groups. Similarly methane production in the MOP treatment group showed 8.53 per cent reduction with comparison to the control group. The NOP treated group had 7.77 percent reduction over the control group. The highest methane emission was found in control group (T0) and coconut oil treated group (T3) showed lowest methane

Table 2: Effect of feeding ANF containing feed additives on methane (MJ/day) emission in growing calves (7 days digestion trial).

Attributes	T0	T1	T2	T3	SEM	C.D.
Day 1	5.22±0.10[a]	4.81±0.10[b]	4.83±0.04[b]	4.13±0.07[c]	0.07	0.20
Day 2	5.33±0.07[a]	4.83±0.10[b]	4.84±0.06[b]	4.16±0.06[c]	0.08	0.22
Day 3	5.21±0.12[a]	4.90±0.11[b]	4.81±0.08[b]	4.14±0.05[c]	0.08	0.23
Day 4	5.36±0.06[a]	4.79±0.13[b]	4.83±0.03[b]	4.08±0.10[c]	0.08	0.23
Day 5	5.35±0.07[a]	4.92±0.11[b]	4.82±0.05[b]	4.1±0.11[c]	0.09	0.27
Day 6	5.19±0.08[a]	4.94±0.13[b]	4.83±0.06[b]	4.22±0.12[c]	0.08	0.24
Day 7	5.25±0.08[a]	4.82±0.12[b]	4.8±0.03[b]	4.25±0.06[c]	0.08	0.22
Overall mean	5.27±0.08[a]	4.86±0.11[b]	4.82±0.05[b]	4.15±0.08[c]	0.08	0.25

Means without a common superscript within a row differs (P<0.05).

Table 3: Effect of feeding ANF containing feed additives on methane gas (MJ/day) emission in growing calves.

Attributes	T0	T1	T2	T3	SEM	C.D.
DMI kg/day	3.637±0.06[b]	3.694±0.06[b]	3.917±0.03[a]	3.639±0.1[b]	0.065	0.18
EEI kg/day	0.105±1.06[d]	0.134±1.47[c]	0.153±1.85[b]	0.175±1.5[a]	1.53	4.21
*CH_4 (MJ/Day/Calve)	5.27±0.08[a]	4.86±0.11[b]	4.82±0.05[b]	4.15±0.9[c]	0.08	0.23
% Reduction	-	7.77	8.53	21.26	-	-

Means without a common superscript within a row differs (P<0.05).

Table 4: Effect of feeding ANF containing feed additives on blood serum indices in growing calves.

Glucose (ml/dl)	T0	T1	T2	T3	Average	SEM	P Value		T*P
							Treatment	Period	
Day 30	62.08±2.79	62.41±3.37	61.06±2.88	60.44±2.39	61.50±0.62	0.958	0.914	0.434	0.358
Day 60	59.80±1.29	60.08±1.09	60.74±1.19	61.89±2.26	60.63±1.63				
Day 120	61.05±1.98	59.92±1.23	61.05±2.08	61.70±1.63	60.93±1.75				
Period Mean	60.98±2.18	60.80±2.32	60.95±2.01	61.34±2.08	61.02±2.10				
Total protein (gm/dl)									
Day 30	6.64±0.41	6.96±0.23	6.60±0.18	6.88±0.12	6.77±0.28	0.136	0.388	0.202	0.726
Day 60	6.99±0.21	6.98±0.20	6.79±0.44	6.89±0.22	6.91±0.28				
Day 120	6.97±0.46	6.90±0.44	6.90±0.23	6.95±0.22	6.93±0.33				
Period mean	6.86±0.39	6.95±0.29	6.76±0.31	6.91±0.18	6.87±0.30				
Albumin (gm/dl)									
Day 30	3.28±0.14	3.23±0.10	3.27±0.23	3.29±0.23	3.27±0.17	0.105	0.971	0.542	0.081
Day 60	3.32±0.20	3.38±0.22	3.08±0.12	3.32±0.23	3.28±0.22				
Day 120	3.31±0.24	3.28±0.21	3.58±0.47	3.20±0.22	3.34±0.32				
Period mean	3.30±0.18	3.30±0.18	3.31±0.36	3.27±0.22	3.29±0.24				
Globulin (gm/dl)									
Day 30	3.36±0.49	3.73±0.25	3.33±0.09	3.59±0.32	3.50±0.34	0.17	0.478	0.545	0.522
Day 60	3.66±0.32	3.60±0.23	3.71±0.45	3.57±0.26	3.63±0.31				
Day 120	3.66±0.58	3.62±0.64	3.32±0.26	3.75±0.28	3.59±0.46				
Period mean	3.56±0.46	3.65±0.39	3.45±0.34	3.64±0.28	3.58±0.37				
A:G ratio									
Day 30	1.00±0.19	0.87±0.07	0.98±0.09	0.93±0.15	0.94±0.13	0.075	0.727	0.631	0.379
Day 60	0.92±0.13	0.94±0.11	0.84±0.12	0.93±0.12	0.91±0.12				
Day 120	0.94±0.25	0.95±0.29	1.09±0.22	0.86±0.11	0.96±0.22				
Period mean	0.95±0.19	0.92±0.17	0.97±0.18	0.91±0.12	0.94±0.16				

emission. The statistical analysis of the data shows highly significant (P<0.01) reduction in methane emission among the treatments.

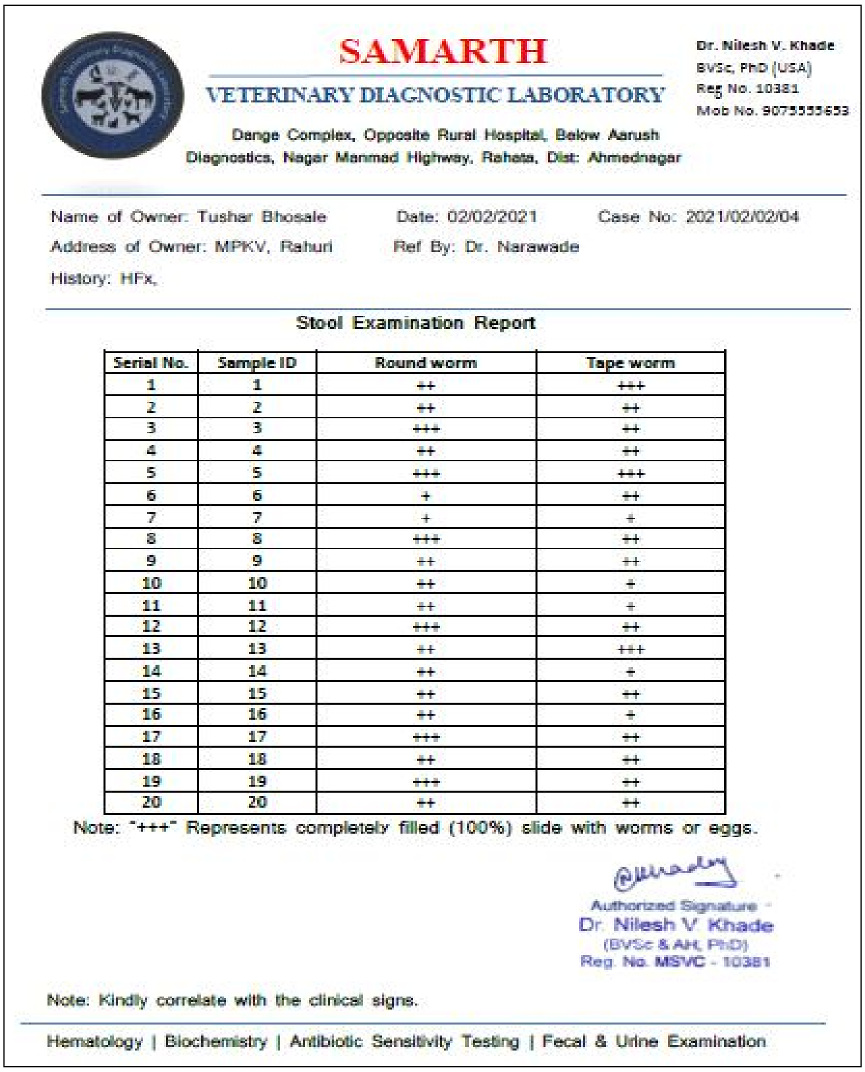
The current results concurred with those of Dong *et al*. (1997), who claimed that CO had the greatest impact, lowering CH_4 generation in diets high in grass hay by 59 per cent and in concentrate by 85 Per cent. Like this, Machmüllerand Kreuzer (1999) found that adding CO to the grass hay diet at the ratios of 3.5 and 7 percent reduced methane generation by 28 and 73 per cent, respectively. According to their findings, supplementing with lauric acid on its own had a greater methane-suppressing impact than doing so in conjunction with myristic acid. This result can be a result of the MCFA in CO additive action.

According to previous research by Alexander *et al*. (2008), adding *Moringa oleifera* aqueous methanol extract (2 mg/ml), which contained 1.11 per cent hydrolysable tannin and 4.09 per cent saponin, reduced total gas generation by 6.8 percent compared to control. The net gas production of tannin-rich forages was also reported by Njidda and Nasiru (2010) to be 2.83 ml in Acacia tortilis, 1.14 ml in *Leucaena leucocephala*, 8.16 ml in *Moringa oleifera* and 25.50 ml in *Ziziphus mucronata*, respectively. Additionally, they reported that the increasing levels of total condensed tannin in the forages of *Leucaena leucocephala*, *Ziziphus mucronata*, *Acacia tortilis and Moringa oleifera*, respectively, decreased the total gas generation.

Blood serum indices in growing calves fed ANF containing feed additives

The blood serum glucose (ml/dl), total protein (gm/dl), albumin (gm/dl), globulin (gm/dl) and A:G Ratio *etc*. parameters of crossbred calves in different groups have been given in Table 4. Blood serum globulin concentration was statistically similar in all treatment groups at the end of the experimental period ($P>0.05$).

The results was in agreement with Blood *et al*. (1983) who observed the serum glucose concentration of cattle



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Name of Owner: Tushar Bhosale | Date: 02/02/2021 | Case No: 2021/02/02/04
Address of Owner: MPKV, Rahuri | Ref By: Dr. Narawade
History: HFx,

Stool Examination Report

Serial No.	Sample ID	Round worm	Tape worm
1	1	++	+++
2	2	++	++
3	3	+++	++
4	4	++	++
5	5	+++	+++
6	6	+	++
7	7	+	+
8	8	+++	++
9	9	++	++
10	10	++	+
11	11	++	+
12	12	+++	++
13	13	++	+++
14	14	++	+
15	15	++	++
16	16	++	+
17	17	+++	++
18	18	++	++
19	19	+++	++
20	20	++	++

Note: "+++" Represents completely filled (100%) slide with worms or eggs.

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Note: Kindly correlate with the clinical signs.

Hematology | Biochemistry | Antibiotic Sensitivity Testing | Fecal & Urine Examination

Pic 1: Stool examination reports at 30^{th} Day.

on CO supplementation in a study was within the normal range for healthy ruminants. In a study reported by Johnson *et al*. (1988), it was observed that no effects were recorded in glucose concentration when dairy cows were fed with supplementary dietary fat. Present finding of this study was more related with Ahmad *et al*. (2017) who reported the effect of supplementation of MOP on blood constituents of suckling calves. No significant difference was observed in the value of total protein, globulin, urea, creatinine and ALT enzyme levels. However, significant changes were observed in values of total serum albumin and AST enzyme levels among the groups.

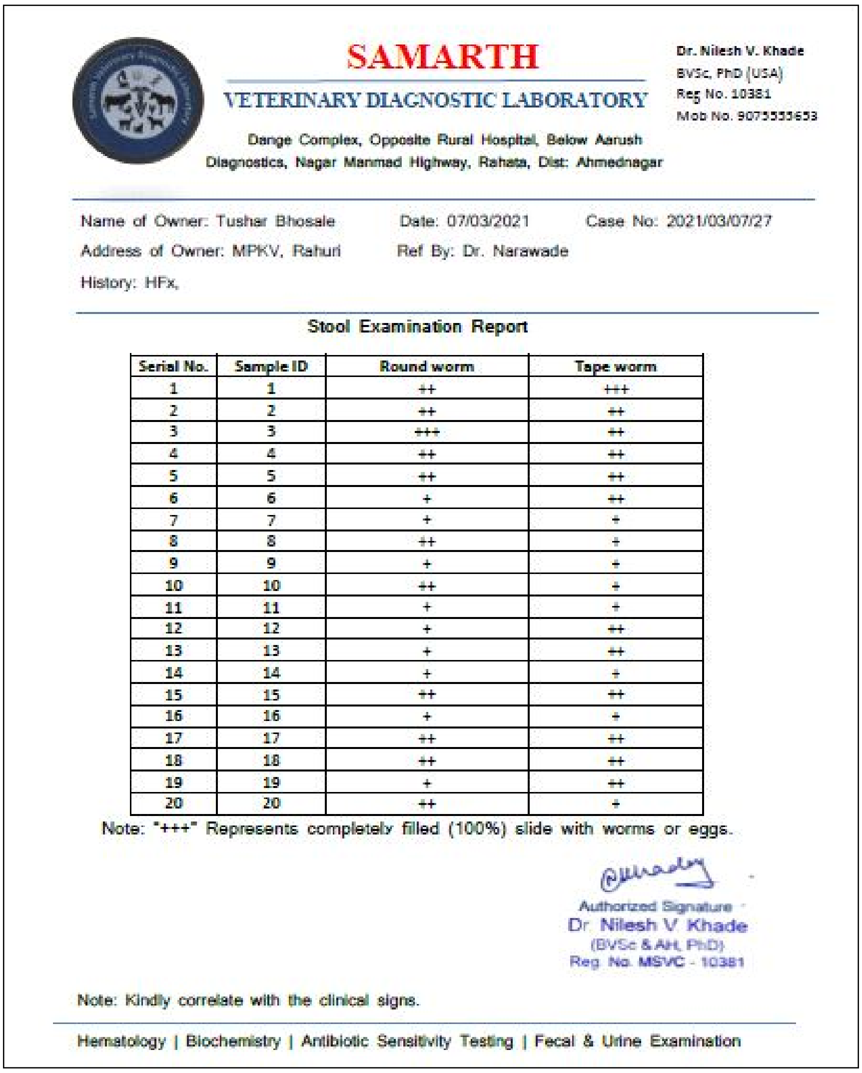
Present findings were more related with Adelusi *et al*. (2018) who reported blood serum biochemistry of grazing cattle drenched with various levels of CO. Blood glucose and total bilirubin contents of cattle were not significantly affected (P<0.05) by CO inclusion. However, there was significant difference (P<0.05) in blood urea nitrogen, creatinine, conjugated bilirubin and total protein content.

Effect of feeding ANF on roundworms (Nematodes) and tapeworms (Cestodes)

The egg counts (per g feces) were almost nil in all the dietary treatments and it has been presented in Picture 1, 2 and 3 respectively. The degree of infection was observed to be negligible. It indicated that natural infection was not able to establish in calves. The NLP treated group was found strongly superior in reducing roundworms or eggs count over the T0, T2 and T3 groups.

Present finding was close to the results of Amin *et al*. (2009) who evaluated aqueous extract of 20 indigenous plants against adult gastrointestinal parasites of ruminants and observed 100 percent efficacy of NLP (*A. Indica*), tobacco, barbados lilac, betel leaf, pineapple, jute, turmeric, garlic, dodder and bitter gourd @ 100mg/ml.

Cabardo and Portugaliza (2017) also evaluated the anthelmintic potential of *Moringa oleifera seed* ethanolic and aqueous extracts against *Haemonchus contortus* eggs and infective stage larvae (L3s). In the ovicidal assay, the



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Address of Owner: MPKV, Rahuri   Ref By: Dr. Narawade
History: HFx,

Stool Examination Report

Serial No.	Sample ID	Round worm	Tape worm
1	1	++	+++
2	2	++	++
3	3	+++	++
4	4	++	++
5	5	++	++
6	6	+	++
7	7	+	+
8	8	++	+
9	9	+	+
10	10	++	+
11	11	+	+
12	12	+	++
13	13	+	++
14	14	+	+
15	15	++	++
16	16	+	+
17	17	++	++
18	18	++	++
19	19	+	++
20	20	++	+

Note: "+++" Represents completely filled (100%) slide with worms or eggs.

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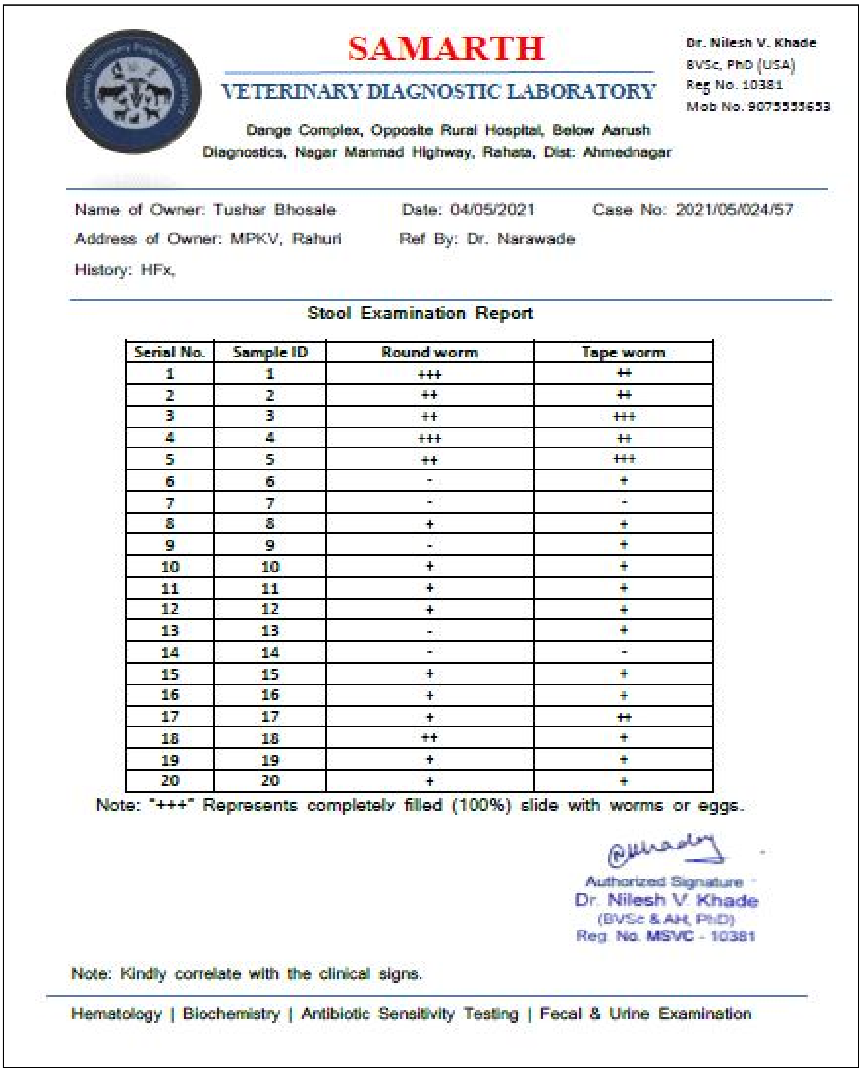
Note: Kindly correlate with the clinical signs.

Hematology | Biochemistry | Antibiotic Sensitivity Testing | Fecal & Urine Examination

Pic 2: Stool examination reports at 60th day.



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History: HFx,

Stool Examination Report

Serial No.	Sample ID	Round worm	Tape worm
1	1	+++	++
2	2	++	++
3	3	++	+++
4	4	+++	++
5	5	++	+++
6	6	-	+
7	7	-	-
8	8	+	+
9	9	-	+
10	10	+	+
11	11	+	+
12	12	+	+
13	13	-	+
14	14	-	-
15	15	+	+
16	16	+	+
17	17	+	++
18	18	++	+
19	19	+	+
20	20	+	+

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Note: Kindly correlate with the clinical signs.

Hematology | Biochemistry | Antibiotic Sensitivity Testing | Fecal & Urine Examination

Pic 3: Stool examination reports at 120th day.

ethanolic and aqueous extracts showed 95.89 percent and 81.72 per cent egg hatch inhibition at 15.6 mg/mL, respectively. In the larvicidal assay, the ethanolic and aqueous extracts exhibited 56.94 and 92.50 per cent efficacy at 7.8 mg/mL, respectively. *M. oleifera* is reported to contain various bioactive compounds with pharmacological activities such as anthelmintic property (Wang, 2016). He also evaluated the anthelmintic activity of *M. oleifera* seeds against *H. contortus* eggs and L3s and determined the different secondary metabolites that are possibly responsible for the anthelmintic activity of *M. oleifera* seed extracts.

The NLP treated group was found to be surprisingly better in reducing tapeworms or eggs count over the treatment T0, T2 and T3. Present results of this study was more or less comparable with Akbar and Ahmed, 2003 who reported the per cent reduction in fecal worm egg counts for albendazole and pineapple (88% and 82% reduction) were higher than that for neem leaves (56% reduction; $P<0.05$).

CONCLUSION

This present findings highlights that NLP, MOP and CO could be fruitfully used as an effective natural growth promoter as well as a rumen manipulating agent in growing calves ration. Although NLP, MP and CO were used in the experimental diets of calves, further study was recommended by various researchers regarding the doses of NLP, MOP and CO on optimum performance and sound health in calves. In this study, we have suggested future research with NLP, MOP and CO as an alternative for antibiotics in cattle so that it may be used as an effective strategy for organic milk production. It could be concluded that NLP, MOP and CO can be used as environmentally friendly feed additives in growing calves ration. The inclusion level of NLP, MP and CO up to 2 per cent DMB in a growing calves diet could be recommended.

Conflict of interest: None.

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