RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 43 Issue 4: 728-735 (December 2024)

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

Tushar Rajendra Bhosale[1], Trupti R. Raypurkar[2], U.S. Gaikwad[1], D.K. Kamble[3], S.D. Mandakmale[3]

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,

[1]Department of Animal Husbandry and Dairy Science, College of Agriculture, Muktainagar, Jalgaon-425 306, Maharashtra, India.
[2]Department of Agricultural Statistics, College of Agriculture, Muktainagar, Jalgaon-425 306, Maharashtra, India.
[3]Department of Animal Husbandry and Dairy Science, Mahatma Phule Krishi Vidyapeeth, Ahmednagar, Rahuri-413 722, Maharashtra, India.

Corresponding Author: Tushar Rajendra Bhosale, Department of Animal Husbandry and Dairy Science, College of Agriculture, Muktainagar, Jalgaon-425 306, Maharashtra, India.
Email: tusharbhosale520@gmail.com

How to cite this article: Bhosale, T.R., Raypurkar, T.R., Gaikwad, U.S., Kamble, D.K. and Mandakmale, S.D. (2024). 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. 43(4): 728-735. 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

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; CSC: Cotton seed cake

(MJ/d/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.

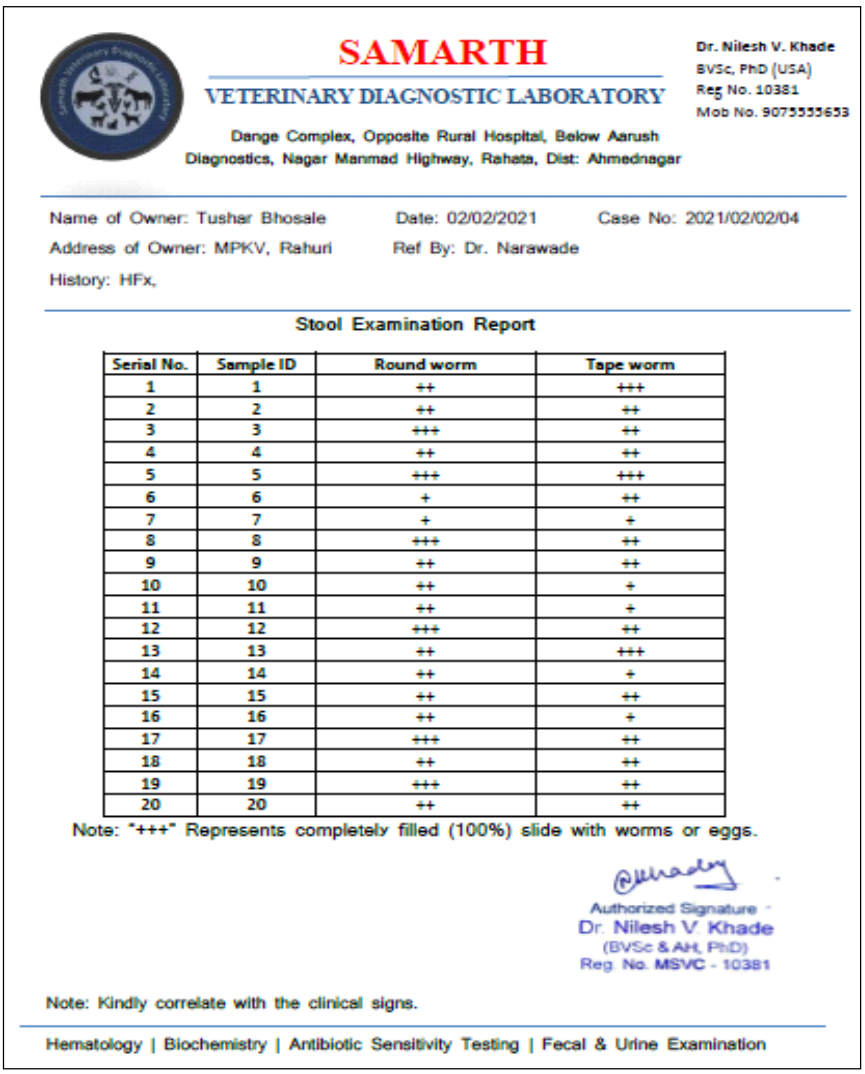
The current results concurred with those of Dong *et al*. (1997), who claimed that CO had the greatest impact, lowering CH_4 generation in diets high in grass hay by 59 per cent and in concentrate by 85 Per cent. Like this, Machmüllerand Kreuzer (1999) found that adding CO to the grass hay diet at the 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 (*P*>0.05) similar in all treatment groups at the end of the experimental period.

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



SAMARTH
VETERINARY DIAGNOSTIC LABORATORY
Dange Complex, Opposite Rural Hospital, Below Aarush Diagnostics, Nagar Manmad Highway, Rahata, Dist: Ahmednagar

Dr. Nilesh V. Khade
BVSc, PhD (USA)
Reg No. 10381
Mob No. 9075555653

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.

Authorized Signature
Dr. Nilesh V. Khade
(BVSc & AH, PhD)
Reg. No. MSVC - 10381

Note: Kindly correlate with the clinical signs.

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

Pic 1: Stool examination reports at 30th 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.

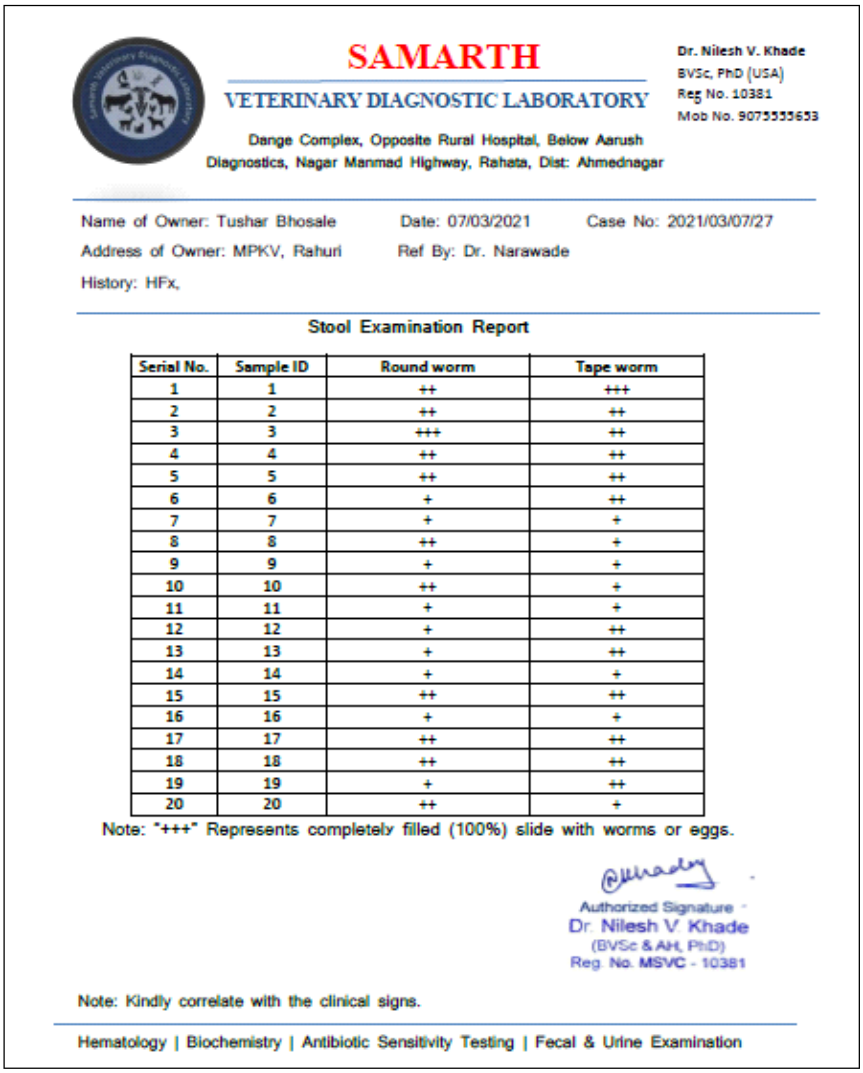
Present findings were more related with Adelusi *et al*. (2018) who reported blood serum biochemistry of grazing cattle drenched with various levels of CO. Blood glucose and total bilirubin contents of cattle were not significantly affected (P<0.05) by CO inclusion. However, there was significant difference (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 Pic 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 per cent efficacy of NLP (*A. Indica*), tobacco, barbados lilac, betel leaf, pineapple, jute, turmeric, garlic, dodder and bitter gourd @ 100 mg/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



SAMARTH
VETERINARY DIAGNOSTIC LABORATORY
Dange Complex, Opposite Rural Hospital, Below Aarush Diagnostics, Nagar Manmad Highway, Rahata, Dist: Ahmednagar

Dr. Nilesh V. Khade
BVSc, PhD (USA)
Reg No. 10381
Mob No. 9075555653

Name of Owner: Tushar Bhosale Date: 07/03/2021 Case No: 2021/03/07/27
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	++	+

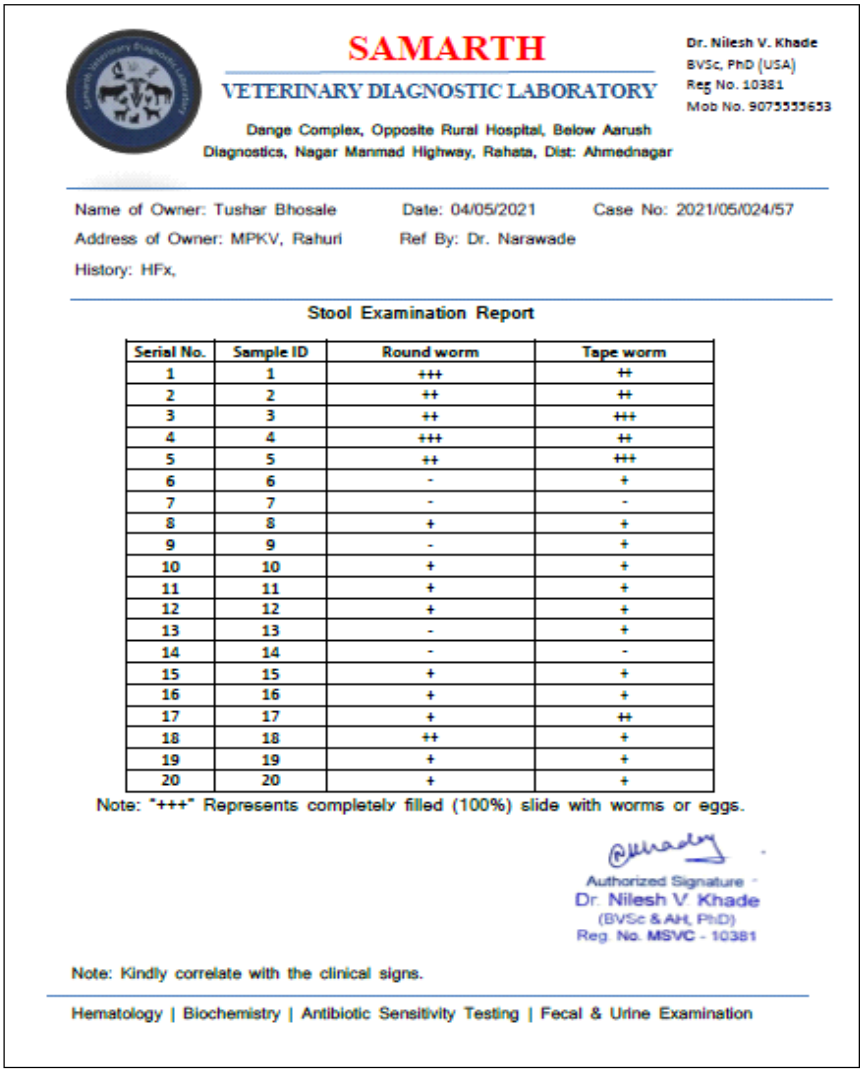
Note: "+++" Represents completely filled (100%) slide with worms or eggs.

Authorized Signature
Dr. Nilesh V. Khade
(BVSc & AH, PhD)
Reg. No. MSVC - 10381

Note: Kindly correlate with the clinical signs.

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

Pic 2: Stool examination reports at 60th day.



SAMARTH
VETERINARY DIAGNOSTIC LABORATORY
Dange Complex, Opposite Rural Hospital, Below Aarush Diagnostics, Nagar Manmad Highway, Rahata, Dist: Ahmednagar

Dr. Nilesh V. Khade
BVSc, PhD (USA)
Reg No. 10381
Mob No. 9075555653

Name of Owner: Tushar Bhosale Date: 04/05/2021 Case No: 2021/05/024/57
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.

Authorized Signature
Dr. Nilesh V. Khade
(BVSc & AH, PhD)
Reg. No. MSVC - 10381

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

All authors declare that they have no conflict of interest.

REFERENCES

Adelusi, O.O., Oni, A.O., Aderinboye, R.Y., Arigbede, O.M. and Onwuka, C.F.I. (2018). Effects of coconut oil on the weight and blood status of grazing cattle fed concentrate as supplementary feed. Nigerian Journal of Animal Production. 45(2): 316-324.

Ahmad, A.E., Ibrahim, A.A.S., Ebtehag, I.M.A.E, Mohamed, S.A. and Hassan M.S. (2017). Effect of feeding dry *Moringa oleifera leaves* on the performance of suckling buffalo calves. Asian J. Anim. Sci. 11(1): 32-39.

Akbar, M.A., Ahmed, T.U. and Mondal, M.H. (2003). Study on the Efficacy of Different Herbal Plants Against Gastro-Intestinal Nematode Infection in Cattle. In Proceedings of the Sixth International Symposium on the Nutrition of Herbivores: Merida, Yuc Annona reticulata, Mexico. pp: 19-24.

Alexander, G., Singh, B., Sahoo, A. and Bhat, T. (2008). *In vitro* screening of plant extracts to enhance the efficiency of utilization of energy and nitrogen in ruminant diets. Anim. Feed Sci. Technol. 145: 229-244.

Amin M.R., Mostofa, M., Hoque, M.E. and Sayed, M.A. (2009). *In vitro* anthelmintic efficacy of some indigenous medicinal plants against gastrointestinal nematodes of cattle J. Bangladesh Agril. Univ. 7(1): 57-61.

AOAC. (2012). Official Methods of Analysis, 19th ed. Association of Official Analytical Chemists, Washington, DC.

Bhatnagar, A.S., Prasanth Kumar, P.K., Hemavathy, J., Gopala Krishna, A.G. (2009). Fatty acid composition, oxidative stability and radical scavenging activity of vegetable oil blends with coconut oil. J. Am. Oil. Chem. Soc. 86: 991-999.

Bhosale, T.R., Antre, G.R., Chavan, M.K., Lawar, V.S., Mandakmale, S.D. and Kumar, D. (2022). Effect of anti-nutritional factors containing feed additives on growth performance in growing calves. The Pharma Innovation Journal. 11(4S): 1861-1864.

Bhosale, T.R., Antre, G.R., Kumar, D. and Pandey, R.K. (2021). Effect of chelated minerals supplement on milk yield and composition of sahiwal and hariana cows. Asian Journal of Dairy and Food Research. 40(2): 189-192. doi: 10.18805/ajdfr.DR-1598.

Blood, D.C., Radostis, O.M., Handerson, J.A., Arunded, J.H., Gay, C.C., (1983). Normal Laboratory Values. In: Veterinary Medicine, 6th ed. ELBS and Baillière, Tindall, Eastbourne, UK.

Cabardo Jr, D.E. and Portugaliza, H.P. (2017). Anthelmintic activity of *Moringa oleifera* seed aqueous and ethanolic extracts against Haemonchus contortus eggs and third stage larvae. International Journal of Veterinary Science and Medicine. 5(1): 30-34.

Dohme, F., Machmuller, A., Wasserfallen, A. and Kruzer, M. (2001). Ruminal methanogenesis as influence by individual fatty acids supplemented to complete ruminant diets. Lett. Applied Microbiol. 32: 47-51.

Dong, Y., Bae, H.D., McAllister, T.A., Mathison, G.W. and Cheng, K.J. (1997). Lipid-induced depression of methane production and digestibility in the artificial rumen system (RUSITEC). Canadian Journal of Animal Science. 77(2): 269-278.

Ellis, J.L., Kebreab, E., Odongo, N.E., McBride, B.W., Okine, E.K. and France, J. (2007). Prediction of methane production from dairy and beef cattle. Journal of Dairy Science. 90(7): 3456-3466.

Faciola, A.P. and Broderick, G.A. (2014). Effects of feeding lauric acid or coconut oil on ruminal protozoa numbers, fermentation pattern, digestion, omasal nutrient flow and milk production in dairy cows. Journal of Dairy Science. 97(8): 5088-5100.

Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Lorenzo, J.M., Afolayan, A.J., Muchenje, V. (2018). Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. Food Res. Int. 106: 317-334.

Fiorentini, G., Carvalho, I.P., Messana, J.D., Canesin, R.C., Castagnino, P.S,, Lage, J.F. (2015). *et al.* Effect of lipid sources with different fatty acid profiles on intake, nutrient digestion and ruminal fermentation of feedlot Nellore steers. Asian-Australas J. Anim. Sci. 28: 1583-1591.

Inamdar, A.I., Chaudhary, L.C., Agarwal, N. and Kamra, D.N. (2015). Effect of *Madhuca longifolia* and *Terminalia chebula* on methane production and nutrient utilization in buffaloes. Animal Feed Science and Technology. 201: 38-45.

Johnson, W.A. and Satterlee, D.G. (1988). Selection of Japanese quail for contrasting blood corticosterone response to immobilization. Poultry Science. 67(1): 25-32.

Liu, H., Puchala, R., LeShure, S., Gipson, T.A,, Flythe, M.D., Goetsch, A.L. (2019). Effects of lespedeza condensed tannins alone or with monensin, soybean oil and coconut oil on feed intake, growth, digestion, ruminal methane emission and heat energy by yearling Alpine doelings. J. Anim Sci. 97: 885-899.

Ma, Z.F., Ahmad, J., Zhang, H., Khan, I., Muhammad, S. (2020). Evaluation of phytochemical and medicinal properties of Moringa *(Moringa oleifera)* as a potential functional food. South Afr. J. Bot. 129: 40-46.

Machmüller, A. and Kreuzer, M.C.J.A.S. (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. Canadian Journal of Animal Science. 79(1): 65-72.

Machmuller, A., Dohme, F., Soliva, C.R., Wanner, M. and Kreuzer, M. (2001). Diet composition affects the level of ruminal methane suppression by medium chain fatty acids. Aust. J. Agric. Res. 52: 713-722.

Neeti (2017). Effect of feeding a rumen modifier on methane production and performance of buffalo calves. M.V.Sc. Thesis, Deemed University, Indian Veterinary Research Institute, Izatnagar, India.

Njidda, A.A. and Nasiru, A. (2010). *In vitro* gas production and dry matter digestibility of tannin- containing forages of semi-arid regions of north-eastern Nigeria. Pakistan Journal of Nutrition. 9(1): 60-66.

Patra, A.K. and Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry. 71(11-12): 1198-1222.

Patra, A., Park, T., Kim, M. and Yu, Z. (2017). Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. Journal of Animal Science and Biotechnology. 8(1): 1-18.

Pawar, M.M., Kamra, D.N., Agarwal, N. and Chaudhary, L.C. (2014). Effects of essential oils on *in vitro* methanogenesis and feed fermentation with buffalo rumen liquor. Agricultural Research. 3(1): 67-74.

Rira, M., Chentli, A., Boufenera, S. and Bousseboua, H. (2015). Effects of plants containing secondary metabolites on ruminal methanogenesis of sheep *in vitro*. Energy Procedia. 74: 15-24.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. 7th ed. Bailliere Tindall, London. 809p.

Sreelatha, S., Padma, P.R., (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum. Nutr. 64: 303-311.

Van Soest, P.V., Robertson, J.B. and Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74(10): 3583-3597.

Wang, L., Chen, X. and Wu, A. (2016). Mini review on antimicrobial activity and bioactive compounds of *Moringa oleifera*. Med. Chem. 6(9): 2161-0444.