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Original Article

Performance of neonatal calves fed kitchen herbs and probiotics dissolved in whole milk up to weaning age

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Abstract

Background: Diarrhea is widespread in neonatal calves, often treated with antibiotics. However, prolonged use of these antibiotics may promote problems of antimicrobial resistance. **Aims:** The experiment was carried out to determine the effect of feeding cinnamon, turmeric, carom seed powder, and probiotics fortified milk on the health and growth performance of Jersey crossbred calves. **Methods:** A complete randomized block design was applied with 4 treatments of 10 calves in each experimental unit. All experimental groups except the control group were offered herbs, probiotics and a mixture of both, respectively for 3 months. **Results:** Calves fed herb-probiotic mixture had significantly higher dry matter intake (DMI) from concentrates ($P<0.05$, 0.72 kg), dry matter intake (DMI) per 100 kg body weight ($P<0.05$, 2.30 kg) and had better fecal scores ($P<0.05$, 1.19±0.07) when compared to the other treatment groups. Simultaneously, there was an increase in the *Lactobacillus* sp. ($P<0.05$, 7.58±0.44 CFU/g) with markedly reduced *E. coli* counts ($P<0.01$, 4.93±0.41 CFU/g) when the herb-probiotics combination was fed as compared to the control calves. Also, the average duration of illness was lowest in the herb-probiotic group (3.38±0.59 days), with higher serum total protein levels ($P<0.05$, 5.33±0.15 g/dL). **Conclusion:** It can be concluded that feeding herb-probiotic mixtures reduced calf diarrhea and improved dry matter intake in calves.

Key words: Calf, Diarrhea, Growth, Kitchen herbs, Probiotics

Introduction

Diarrhea is most common during the first 30 days of life, with a fatality risk of nearly 5% (Svensson *et al*., 2006; Windeyer *et al*., 2014), causing huge economic loss incurred due to the cost of treatment, reduced body weight, off-feed, and sometimes death of the animal. Some pathogenic *Escherichia coli* produce toxins that cause secretory diarrhea and dehydration (Sandhu and Gyles, 2002; Fleckenstein *et al*., 2010). Antibiotics are commonly used as therapeutics and growth promoters (Butaye *et al*., 2003), but the growing concern of antibiotic resistance in neonates is alarming (Rai e*t al*., 2018). Therefore, products such as probiotics, essential oils, phytochemicals, antioxidants, and immuno-modulators have been used as alternatives (Miguel, 2010). Additionally, in preruminant calves, the milk is bypassed in the rumen via the esophageal groove and flows directly to the abomasum, coagulated as a result of hydrolysis of kappa casein by chymosin, hence separating the curd and whey respectively. Delay in abomasal emptying of milk also increases the chance of gastrointestinal disease, such as abomasal bloat and diarrhea (Glenn and Miskimins, 2005). Some common culinary kitchen herbs available in every Indian household have many potential uses. Cinnamon obtained from the bark contains numerous essential oils, cinnamic acid, cinnamon aldehyde, eugenol, L-borneol, and many other active substances (Tung *et al*., 2008). Various studies have reported the use of cinnamon in regulating digestion, increasing immunity, anti-inflammatory effects (Koochaksaraie *et al*., 2011), and antimicrobial activity against bacteria, fungi, and molds (Abd El-Hack *et al*., 2020). Feeding cinnamaldehyde to calves did not affect average daily gain, dry matter intake (DMI), and feed efficiency, but controlled diarrhea and increased the ruminal bacteria (Yang *et al*., 2021). *Trachyspermum ammi* (carom seeds) has the chief constituent thymol, where 2.5 to 5.0% is present in essential oil (Ishikawa *et al*., 2001). Carom seed is traditionally used to treat gastrointestinal diseases, including intestinal disorders and colic or diarrhea (Bentely, 1983). In addition to

relieving stomach pain, it showed a significant reduction in gastric ulcer in an animal model (Ramaswamy *et al*., 2010; Komeili *et al*., 2012), also showing antispasmodic activity of the ileum in guinea pigs (Saini and Singh, 2015). Carom seed had an inhibitory effect on the contraction of the smooth muscle of the digestive tract, especially the intestines, causing increased activity of digestive enzymes and bile secretion (Sandhu and Gyles, 2002; Hejazian *et al*., 2007; Fleckenstein *et al*., 2010). *Curcuma longa* (Turmeric) is a traditional medicinal plant, which comprises mainly turmerones, curcuminoids (3-6%), curcumin (16.1%), bisdemethoxycurcumin (10.5%), and diacetyl curcumin, tetrahydrocurcumin (Faizal *et al*., 2009). There was membrane leakage in Gram-negative and Gram-positive bacteria on exposure to curcumin I by membrane permeabilization assays (Tyagi *et al*., 2015). Rai *et al*. (2023) reported that the antibacterial activity of cinnamon, carom seeds and turmeric extracts at higher concentration (500 µg/ml) was highly sensitive when tested on pathogenic *E. coli* isolates carrying heat-labile (LT), heat-stable (ST), Shiga toxins (Stx1 and Stx2) and *eAea* enterotoxins from calf diarrhea. Tannins and tannic acid in herbs denature the proteins in intestinal mucosa and form protein tannates, which make the intestinal mucosa more resistant to chemical alteration by reducing gastric acid secretion (Ashok and Upadhyaya, 2012). Prebiotics, probiotics, organic acids, phytogenic substances, and essential oils enhanced gut health and immunity (Calsamiglia *et al*., 2007). A meta-analysis report showed that probiotics consisting of lactic acid bacteria have protective effects against opportunistic intestinal pathogens, achieved by maintaining a favorable microbial balance, reducing the occurrence of diarrhea in calves (Signorini *et al*., 2012). Calves fed an essential oil-prebiotic combination demonstrated greater dry matter intake (1.63 and 1.74 kg/d), feed efficiency (0.62 and 0.65 kg of gain/kg of dry matter intake), and body measurements with a lower incidence of diarrhea. Blood concentrations of IgG, IgM, and total serum protein were also greater for calves fed an essential oil-prebiotic combination (Ting *et al*., 2020). Probiotics commonly used in the livestock and poultry industry are *Lactobacillus*, *Bacillus*, *Enterococcus*, and *Saccharomyces* (Gaggia *et al*., 2010). They are also known to stimulate the immune system and reduce the incidence of neonatal diarrhea caused by the enterotoxigenic *E. coli* (de Vaux *et al*., 2002). There is limited research on the use of herbs and probiotics to enhance body weight and health performance in calves, despite their potential as natural alternatives to traditional growth promoters.

We hypothesized that probiotics (*Lactobacillus fermentum* NCDC605 and *Lactobacillus rhamnosus* NCDC610 @ 10^9 CFU/ml), kitchen herbs (cinnamon, carom seed, and turmeric), or their combination dissolved in whole milk can improve the overall health, growth, immunity, and biochemical blood indices of dairy calves during the preweaning period. Therefore, this study aimed to evaluate whether probiotics, kitchen herbs, or their combination as feed additives can improve neonatal calf health, growth, and immunity status throughout the preweaning period.

Materials and Methods

The work was carried out at the Indian Council of Agricultural Research, National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal, India. Protocols for this study were approved by the Institute Research Committee (project number C-66) of the National Dairy Research Institute, India.

Experimental animals and feeding trial

Forty newborn healthy female Jersey crossbred (Jersey Red Sindhi cross) calves with an average body weight of 22.51±0.51 kg were selected for the study. The calves were divided into four groups (10/group):

Control group: Whole milk without additives.

Herb group: Whole milk supplemented with a mixture of cinnamon, carom seed, and turmeric powder in a 1:1:1 ratio, with 10 g dissolved per liter of milk.

Probiotic group: Whole milk supplemented with probiotics, including *Lactobacillus fermentum* NCDC605 and *Lactobacillus rhamnosus* NCDC610, at a 1:1 ratio and 10^9 CFU/ml concentration.

Herb-probiotic group: Whole milk supplemented with both the herb mixture (cinnamon, carom seed, and turmeric @ 10 g/L of whole milk) and the probiotic blend (*L. fermentum* NCDC605 and *L. rhamnosus* NCDC610 @ 10^9 CFU/ml) in the above-mentioned ratio.

The basal diet comprises an *Ad lib* supply of maize green fodder and concentrates introduced as early as the 1st week of age to enhance quick rumen development (Table 1). The experiment was conducted up to the milk feeding period of 90 days.

Table 1: Composition of feed and fodder offered

Parameter	Concentrates (% of DM)	Green fodder (Maize, % of DM)
Dry matter	92.05	18.05
Crude protein	20.04	8.02
Crude fibre	7.11	24.15
Total ash	9.10	8.00

Colostrum was fed during the first three days, after which the whole milk was fed at the rate of 10% body weight at each age (Qadeer *et al*., 2021). The mixed kitchen herbs (including cinnamon, turmeric, and carom seed powder at a 1:1:1 ratio) were dissolved at 10 g/L of whole milk and fed through feeding bottles. Probiotics, *Lactobacillus fermentum* NCDC605 and *Lactobacillus rhamnosus* NCDC610, were procured from the Dairy Microbiology Department of the National Dairy Research Institute, Karnal, Haryana, India. Both cultures were mixed at a 1:1 ratio, having a 10^{10} CFU/ml concentration in milk while feeding (Fig. 1).

Qualitative assessment of kitchen herbs

Extract yield

All the kitchen herbs, cinnamon, turmeric and carom

Fig. 1: Herb-Probiotics mixture: Kitchen herb powder (cinnamon, carom seed, and turmeric in a 1:1:1 ratio and 10 g of the herb mixture was dissolved/L of milk) and probiotics (*L fermentum* NCDC605 and *L rhamnosus* NCDC610 at a 1:1 ratio with a concentration of 10^9 CFU/ml)

seed, were purchased from Kalyani (District Nadia, West Bengal- India), dried and ground to fine powder and mixed at the ratio of 1:1:1. Then it was extracted by cold aqueous percolation at 1:10 ratio, which was further dried in vacuum evaporator at 40°C. The dried extract was calculated with the below formula (Sukhdev *et al*., 2008; Nagappan, 2012):

$R/S \times 100$

Where,
R: Weight of extracted plant residue
S: Weight of plant raw sample

The extract was dissolved in Dimethyl Sulphoxide (DMSO) at 100 mg/ml concentration and filtered through syringe filters (Sigma®, 0.45 micron size) for further assessment.

Total phenolic content

Total phenolic content of the ground kitchen herb extract was determined by Folin-Ciocalteu reagent (Sigma®) as described by Singleton and Rossi (1965). Readings were made using a UV Spectrophotometer (Labman®) at 765 nm wavelength. The phenolic content was measured as Gallic acid equivalents (GAE/g) of dry plant material based on the standard curve of Gallic acid (5-500 mg/L, Y=0.0027x-0.0055, R^2=0.9999) (Chandra *et al*., 2014).

Total flavonoid content

The aluminum chloride colorimetric method was used for total flavonoid content determination (Marinova *et al*., 2005). The absorbance was measured by a spectrophotometer at 420 nm. Concentration of the flavonoid content was calculated from the calibration plots (Y=0.0162x + 0.0044, R^2=0.999) and expressed as mg quercetin equivalent/g of herb powder.

2, 2-Diphenyl picrylhydrazyl assay (DPPH)

The DPPH assay of the herb extract was measured at 515 nm absorbance. Gallic acid was used as the positive control. The percentage of DPPH radical scavenging activity was calculated as follows (Cheng *et al*., 2006).

$$\% \text{ Radical scavenging activity} = \left\{1 - \frac{(\text{sample} - \text{blank})}{(\text{control} - \text{blank})}\right\} \times 100$$

Antimicrobial and MIC assay of the kitchen herb extract

These *E. coli* isolates, positive for the virulent enterotoxins, heat-labile (LT) and heat-stable (ST), and Shiga toxins (Stx1 and Stx2) were acquired from the Veterinary Microbiology Division of IVRI, Eastern Regional Station, Belgachia, West Bengal. Antimicrobial potency of the herb extracts at different concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, and 32.6 µg/ml) was determined by agar well diffusion method (Rai *et al*., 2023). Positive pathogenic *E. coli* was grown in nutrient broth and adjusted to 0.5 McFarland. 10 µL of the culture was spread over a sterile agar plate. A 6-8 mm hole was punched aseptically, and the herb extract at different concentrations was poured into the hole. Then, agar plates were incubated at 37°C for 18-20 h. The zone of inhibition was measured in mm (Valgas *et al*., 2007). Minimum inhibitory concentration (MIC) was determined by the Microplate method. Selected *E. coli* was grown overnight in nutrient agar and adjusted to McFarland standard 0.5, equivalent to 10^8 CFU/ml. The herb extracts were dissolved in Dimethyl Sulphoxide (DMSO) to a concentration of 10 mg/ml, and 100 µL was added to the first well of the microtitre plate and serially diluted with water. *E coli* grown overnight (100 µL) is added to each well. Gentamycin was used as a positive control, and DMSO was the solvent control. As a growth indicator of the microorganism, 40 µL of 0.2 mg/ml p-iodonitrotetrazolium violet (INT) (Sigma) was added to the microplate wells and incubated at 37°C for 2 h. Therefore, the yellow tetrazolium dye was reduced by viable microorganisms to a pink/purple color observed by the naked eye (Elisha *et al*., 2017).

Acceptance of formulated milk by the calves

To study the acceptance of herbs and probiotics dissolved in milk, the suckling time (time taken by the calves to drink milk, min), sucking frequency (number of bouts taken to drink milk, number of time) and amount of milk consumed (L) by the calves were noted.

Health recording of animals

Daily health parameters, including rectal temperature, nasal discharge, lameness, fecal consistency, and days of illness, were observed daily by an attendant before morning and evening feeding. Fecal score of the animal depended on the fecal consistency daily (Table 2). In case of any sickness, antibiotics were prescribed to maintain the welfare of the animals.

Growth and dry matter intake

Body weight (kg) and body measurement (cm, body length, withers height, and heart girth) (Siddiqui *et al*., 2015) were taken at fortnightly intervals. The dry matter

intake from concentrates and green fodder (maize) was sampled at every 14-day interval and quality was assessed as per AOAC (2005).

Table 2: Fecal scores used to determine fecal consistency (Morrison *et al*., 2010)

Fecal score	Description of the score
1	Normal consistency
2	Slightly liquid consistency
3	Moderately liquid consistency
4	Primarily liquid consistency

Hematology

Blood sample was collected in an evacuated tube (BD Vacutainer®) at every 14-day interval to assess the hematological parameters (blood glucose, total erythrocyte count, total leukocyte count, packed cell volume, and hemoglobin) according to Brar *et al*. (2000). Serum total protein (STP) was estimated after clotting the blood and harvesting serum by low-speed centrifugation (1600 g for 15 min). The STP concentration was measured in a Brix scale using a hand refractometer (Labart Erma®) by placing 0.3 ml serum on the prism surface instrument has a measurement range from 0 to 50%. Distilled water was used for calibration before each measurement, which was performed twice for each sample (Nikolaos *et al*., 2018).

Enumeration of fecal Lactobacillus and E. coli bacteria

The fecal sample was collected aseptically from the rectum at weekly intervals to enumerate *Lactobacillus* (MRS Agar media) and *E. coli* (McConkey Agar) bacteria by serial dilution (Hasunuma *et al*., 2011).

Ultrasonography of the abomasum in calves

The clotting properties of whole milk and the ones fortified with ground kitchen herbs and probiotics were assessed by *in vitro* rennet coagulation test (Miyazaki *et al*., 2009) where milk clot was caught in the sieve (Fig. 2). The abomasum is the largest organ in the pre ruminant calves and its appearance via ultrasonography depends upon milk ingestion. The curd formation in the abomasum was assessed using Ultrasonography (Digi 1100 CD-E Vet, SS Medical Systems (I), Pvt. Ltd, Uttarakhand, India) with a 5.0 MHz linear transducer of high resolution. The calf was restrained in a standing position, hair was clipped short and swabbed clean with alcohol; contact gel was applied on the transducer and placed in the region specified. The site of the abomasum was scanned between the 5th and 12th intercostal space, starting from the ventral midline, progressing laterally and dorsally with the transducer held parallel to the ribs (Braun *et al*., 1997). Five calves in each group were analyzed after feeding the herbs, probiotics, and herb+probiotics every hour up to 4 h of feeding.

Statistical analysis

The experiment was a randomized complete block design with four treatments: Control (C), Herb (H), Probiotic (P), and Herb Probiotic mix (HP) fed calves. The body weight, body measurements (length, withers height, and heart girth), dry matter intake, and blood parameters of the calves were analyzed by ANOVA for repeated-measures test using SPSS (ver. 20) in which the treatment, sampling time, and their interaction were considered fixed effects. Results were demonstrated as mean values with the standard error of the means. Differences between dietary treatment means were determined using Duncan's Multiple Range Test. The significance was determined at a $P<0.05$ level.

Fig. 2: *In vitro* Rennet Coagulation Test: The clotting properties of whole milk with ground kitchen herbs and probiotics were assessed by the *in vitro* rennet coagulation test, which was comparable to the control

Results

Qualitative analysis of Herb

The extracts of the kitchen herbs (cinnamon, carom seed and turmeric) showed the antimicrobial properties as presented in Fig. 3. The extract yield of these herbs was 2.01±0.12 (%w/w), total phenolic content of 0.43±0.18 (mg/g GAEq), total flavonoid content of 1.95±0.27 mg/g Quercetin Equivalent, DPPH scavenging activity of 80.61±2.24 (%), antimicrobial zone of inhibition of 28.50±1.43 (mm) and minimum inhibitory concentration of 0.04±0.01 (MIC, µg/ml).

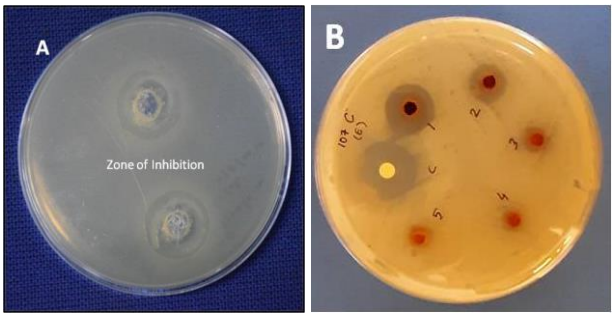

Fig. 3: Zone of inhibition, antimicrobial screening of probiotics and herbs on pathogenic *E coli*: A good zone of inhibition (above 20 mm) was observed with probiotics (**A**) and kitchen herb extracts (**B**)

Acceptance of herbs by the calves

The suckling time sucking frequency (times/min)

milk consumed (L) in control, herb, probiotics and herb+probiotics fed calves is presented in Table 3. No effect of treatments on suckling time, sucking frequency and amount of milk consumed were observed among groups.

Health parameters of calves

Out of 40 calves, 67% suffered from diarrhea, followed by fever (11%), weakness (6%), lameness (5%), joint ill (5%), naval ill (3%), and pneumonia (3%). The average durations of illnesses ($P<0.05$) were 7.25±2.32, 7.57±1.73, 5.80±0.82, and 3.38±0.61 days in control, herbs, probiotics, and herb+probiotic groups, respectively. Calves fed herb+probiotic had the lowest fecal score ($P<0.05$, 1.19±0.07), and the highest was recorded for the control group (1.79±0.08).

Intake and growth performance

No difference was observed in the initial or final body weight and body measurements of the calves in all the groups. Supplementation of herb+probiotics significantly increased ($P<0.05$) the DMI from concentrates and DMI/100 kg body weight when compared with the herb, probiotic, and control groups (Table 4).

Blood parameters

The effect of experimental treatments on blood parameters is shown in Table 5. Feeding herbs and probiotics did not influence blood parameters except for the serum total protein concentration, which was significantly higher in the herb-fed group (5.33 g/dL, $P<0.05$) compared to the control group.

Enumeration of *Lactobacillus* sp. and *E. coli* bacteria

Calves fed herb+probiotics had better fecal counts of *Lactobacillus* (7.58, $P<0.05$) and lower *E. coli* (4.93, $P<0.01$) compared with calves fed herb and probiotics (Table 6).

Table 3: Milk acceptance behavior in calves

Behaviour parameter	Control	Herb	Probiotics	Herb + Probiotics	SEM	Sig.
Suckling time (min)	1.60	1.92	1.56	1.77	0.06	NS
Sucking frequency (No.)	120.44	117.78	117.22	119.50	2.29	NS
Amount of milk consumed (L)	2.16	2.18	2.19	2.23	0.03	NS

Table 4: Dry matter intake and growth performance of calves fed herb, probiotics, herb+probiotics, and the control group

Parameter (treatments)	Herb	Probiotic	Herb+Probiotic	Control	SEM	P-value
Initial body weight (kg)	22.01	23.00	23.60	21.80	0.52	0.59
Final body weight (kg)	54.80	52.60	52.00	50.60	1.48	0.80
Total body weight gain (kg)	32.79	29.70	28.40	28.80	1.27	0.62
Average daily gain (g)	364.66	330.00	315.55	320.00	14.11	0.60
Body length (cm)	70.37	69.59	68.69	69.31	0.465	0.64
Heart girth (cm)	78.97	78.60	79.19	78.19	0.56	0.93
Height (cm)	75.21	75.09	74.54	73.84	0.41	0.64
DMI from concentrates (kg)	0.62	0.58	0.72	0.50	0.20	0.03*
Total DMI from maize green (kg)	0.14	0.11	0.12	0.15	0.01	0.11
Total DMI from milk (kg)	0.06	0.06	0.06	0.05	0.00	0.77
Total DMI (kg)	0.82	0.75	0.89	0.70	0.02	0.09
DMI/100 kg body weight	2.15	2.00	2.30	1.94	0.48	0.04*

* The mean difference is significant at the 0.05 level. DMI: Dry matter intake

Table 5: Blood parameters of calves under different feed supplements

Parameters	Control	Herb	Probiotic	Herb+Probiotic	SEM	P-value
Glucose (mg/dL)	107.29	108.94	112.17	109.08	8.08	0.39
Total protein (g/dL)	4.75^{b}	5.33^{a}	4.94^{b}	4.96^{b}	0.14	0.04*
TEC (10^6/µL)	14.18	16.69	17.68	16.02	2.01	0.92
TLC (10^3/µL)	5.19	6.73	4.74	4.59	1.12	0.61
PCV (%)	34.00	37.00	31.25	35.33	1.93	0.46
Hb (%)	10.75	8.51	8.32	10.15	1.02	0.47

* The mean difference is significant at the 0.05 level. TEC: Total erythrocyte count, TLC: Total leucocyte count, PCV: Packed cell volume, and Hb: Hemoglobin

Table 6: Microbial load count (Log10 CFU/ml) and fecal scores

Parameter	Control	Herb	Probiotic	Herb+Probiotic	SEM	P-value
E. coli	8.66^{c}	7.14^{b}	7.11^{b}	4.93^{a}	0.40	0.00**
Lactobacillus sp.	6.06^{a}	5.76^{a}	7.19^{c}	7.58^{bc}	0.43	0.04*
Fecal scores	1.79^{a}	1.43^{b}	1.35^{b}	1.19^{c}	0.10	0.04*

* The mean difference is significant at the 0.05 level, and ** 0.01 level

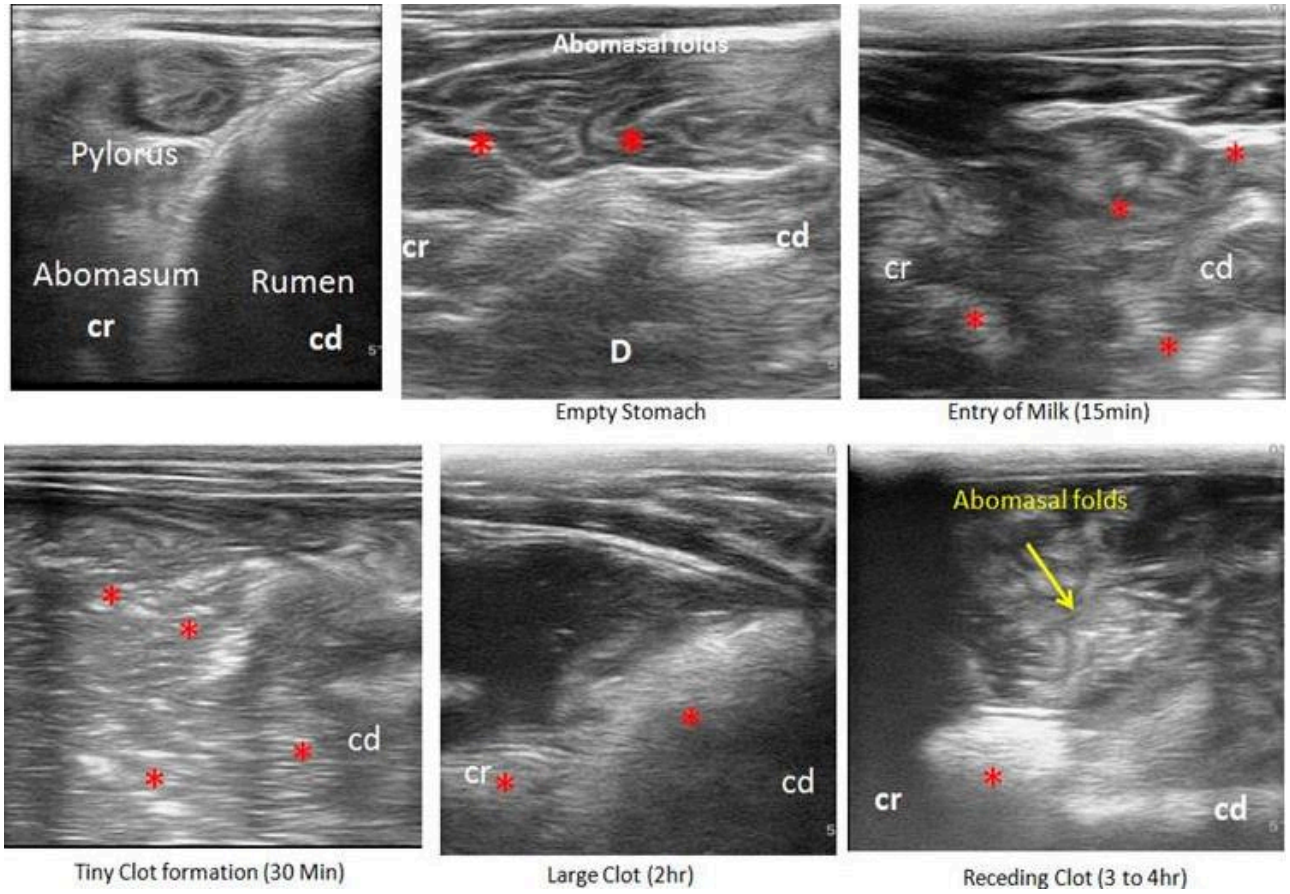

Fig. 4: USG of Abomasum with herb+probiotic formulation in milk

Ultrasonography of abomasum in calves

The formation of milk curd in the abomasum after consuming herbs and probiotics is presented in Fig. 4. The pH (6.0-6.5) remained unchanged in all the groups. Softer clots formed in the whole milk compared to the probiotics herb mix. After 30 min ingestion of whole milk or with fortification (probiotics or kitchen herbs), milk clots started forming visible as tiny hyperechoic clots. The contents appear heterogeneous with gas formation, and abomasal folds were slowly masked by these tiny clots. The flow of the milk contents was easily visible with strong contractions of the abomasum, which was not measured. After 1 h of milk ingestion, large hyperechoic clumps of curd and whey were formed. The clot was firmer, where, during 2 h of milk ingestion, a very clear demarcation of hyperechoic abomasal folds was visualized. At 3 to 4 h of milk ingestion, the movement of whey was visible with stronger contractions, and there was the presence of abomasal folds.

Discussion

Qualitative analysis of herbs

In the present study, the qualitative determination of herb powder (cinnamon, carom seed and turmeric) had phenolic, flavonoid and DPPH scavenging activity (0.43±0.18 mg/g GAEq; 1.95±0.27 mg/g QEq, and 80.61±2.24%) quite low than the ones reported by Shahid *et al*. (2018) in cinnamon extract where total phenolic contents (TPC) and DPPH values were 355.01±8.34 (mg GAE/g) and 90.18±2.12 (%) respectively. In another study, the total phenolic content and radical scavenging activities of turmeric in aqueous solution were 496.76 mg GAE/100 g and 31.33%, respectively (Tanzeela *et al*., 2015), while the total flavonoid content ranged from 22.52±0.015 to 79.36±0.01 mg QE/g (Sahu and Saxena, 2013). However, in carom seed, Modareskia *et al*. (2022) reported the total phenolic content of 54.3±0.001 mg GAE/g DW, total flavonoid content as 3.68±0.00 mg QE/g DW, and DPPH of 121.7±0.49 µg/ml. This difference in the recovery of flavonoid and phenolic content from plant sources may depend on the solvents used, preparation of samples, particle size, and density of the extracts (Dvorackova *et al*., 2015). Cinnamon, Turmeric and Carom seeds showed remarkable antimicrobial properties against *E. coli* (MIC 0.04±0.01 µg/ml; ZOI 28.5±1.43 mm) in the present study while, Gupta and Girija (2015) reported MIC of 0.1 µg/ml and ZOI (mm) of 11.00±0.1, 12.0±0.01, and 12.00±0.00 for cinnamon, turmeric and carom seed, respectively. Cinnamon and cloves are rated among the top 100 oxygen radical absorbance capacity (ORAC) value antioxidant foods (Jorgustin, 2015). Cinnamaldehyde, 4-methoxy cinnamaldehyde, eugenol, d-cadinene, and coumarin in cinnamon may be attributed to its greater

activity (Singh *et al*., 2007). Cinnamon has the potential to change the bacterial cell microstructure, where the surface of the *E. coli* was wrinkled and irregular, changing the cell permeability, cell morphology, and membrane potential, leading to leakage of small electrolytes, hence death of the cell (Zang *et al*., 2016).

Feed intake, growth, and health performances

In the present study, the calves fed herb-probiotics showed increasing dry matter intake through concentrates and DMI/100 kg BW. Sada *et al*. (2003) also observed higher total DMI in Holstein steers with herbs like Peppermint (2,469.5±217.5 g), Clove (2,469.9±217.4 g), and Lemongrass (2,469.8±217.7 g) when fed in addition to the total diet. A polyherbal mixture of *Achyrantes aspera*, *Trachyspermum ammi*, *Andrographis paniculata*, *Azadirachta indica*, and *Citrullus colocynthis* @ 4 g/d improved the growth and health status of the pre-weaning calves (Galvan *et al*., 2021). Ghosh *et al*. (2011) also found that garlic extract, when fed to crossbred calves, has led to significant improvement in feed intake and feed conversion efficiency, while Vakili *et al*. (2013) reported that thyme or cinnamon did not affect the DMI, ADG, or feed efficiency in calves. It may be that the presence of secondary metabolites in the form of saponins, essential oils, tannins, and flavonoids in herbs can improve digestibility and feed utilization by modulating the rumen microbial fermentation process (Patra, 2011). Weight gain improved in calves fed herbs (cinnamon, carom seed, and turmeric powder) compared to the group receiving probiotics and the control group. When calves were supplemented with essential oils (menthol crystal, eucalyptus oil, mint oil) in milk replacer demonstrated an increase in growth performance and better general health, as well as reduced antibiotic usage before weaning (Soltan, 2009). The findings are in agreement with Dezfouli *et al*. (2007) and Bayatkouhsar *et al*. (2013), who found no significant difference in the body height, body length, heart girth, and hip width in calves fed probiotics at an early age. While, increase in average daily weight gain has been reported in calves fed *Saccharomyces cerevisiae* @ 2 g/head/day (1×10^{10} CFU) for 16 weeks (Nehru *et al*., 2017).

Fecal score and bacterial counts

In the present study, the days of illness and fecal score were reduced when an herb and probiotics mix was fed to the calves compared to the control. Also, there was a reduction in the fecal *E. coli* with the increase of *Lactobacillus* sp. in probiotics and the herb+probiotic fed group compared to the control. It may be due to the inherent antimicrobial and antioxidant properties of the herbs and probiotics that improved the enteric health, higher weight gains, and feed intake (Nayemeh *et al*., 2022). There are reports that oregano water (Ozkaya *et al*., 2018) and *Lactobacillus* (Fernández *et al*., 2020) improved the fecal scores while, Santos *et al*. (2015) found no effect on growth, gut microbiota and fecal scores when a mixture of cineole, carvacrol, pepper, cinnamaldehyde oil was fed to the calves. Castillo *et al*. (2006) stated that a mixture of cinnamaldehyde, capsicum, oleoresin, and carvacrol enhanced the ratio of *Lactobacilli* to *Enterobacteria*. Similarly, a significant increase in *Lactobacillus acidophilus* and *Bifidobacterium longum* was found in the gut when herbs were fed (Qian *et al*., 2016) to the calves. Roodposhti and Dabiri (2012) also reported ($P<0.05$) lower *E. coli* count (CFU/g of wet digesta) in calves fed probiotic (7.44), prebiotic (7.11), and symbiotic (7.04) when compared to the control (7.58) group on day 56. When calves were treated with probiotic fermented milk (*Lactobacillus acidophilus* NCDC15 @ 300 ml/calf/day), fecal *Lactobacillus* (10.08±0.02) and *Bifidobacterium* (9.98±0.05) CFU/g count increased when compared to the control calves (Lamella *et al*., 2021). While, the flavonoids present in certain herbs possess anti-diarrheal activity, inhibiting intestinal motility and hydro electrolytic secretion known to change in diarrhea conditions (Venkatesan *et al*., 2005); probiotics produce organic acids, hydrogen peroxide and bacteriocins that prevent colonization of pathogenic bacteria, form natural biofilm, increase γ-interferon, increase activity of lymphocytes and macrophages (Smulski *et al*., 2020). Besides, they are also known to stimulate the immune system and reduce the incidence of neonatal diarrhea caused by the enterotoxigenic *E. coli* (De Vaux *et al*., 2002) thereby maintaining the overall calf health.

Biochemical profile

The calves fed an herbal mixture and probiotics showed no change in blood glucose, total erythrocyte count, total leukocyte count, packed cell volume, and hemoglobin, except for the total protein. However, Nahid *et al*. (2020) reported that the blood glucose and insulin increased significantly in calves fed chavil and a mixture of rosemary-chavil. Others have reported that the mixture of herbs, including garlic powder, combined with probiotics improved blood glucose, beta-hydroxybutyrate, and total serum proteins in dairy calves (Seifzadeh *et al*., 2016). There is no change in the blood total protein of the calves as they have consumed sufficient colostrum to prevent them from failure of passive transfer and successful absorption of colostral gamma globulins from the colostrum fed immediately after birth (Csilla *et al*., 2016). Similarly, Bombik *et al*. (2012) also reported significant improvement in total erythrocyte count, hemoglobin, hematocrit value, and mean corpuscular volume when the herbal extract was fed to Holstein Friesian calves. Studies on other herbs such as oils of oregano and garlic, etc (Ozkaya *et al*., 2018) found an increase in hemoglobin, hematocrit, total erythrocyte, and leucocyte cell in calves. Dar *et al*. (2017) found a significant increase in hemoglobin and packed cell volume (PCV) amongst probiotic-fed crossbred calves. Herbs have shown promising results on biochemical profiles of calves, as studies of Kozyr *et al*. (2019) also reported increased total protein concentration in the blood associated with adequate transfer of passive immunity, where in the present study it was more than

the cut-off level of 5.0 g/dL (Peter *et al*., 2017).

Ultrasonography observation

After feeding probiotics and herbs, the curd and clot formation were visualized under USG. Gastric emptying of the abomasum could be visualized in the probiotics herb mixture at a faster pace. Several authors reported that clot formation in the abomasum could be assessed within 1 to 2 h of milk feeding when the pH is just sufficient for milk coagulation by chymosin and then decreased at 4 h (Ahmed *et al*., 2001; Miyazaki *et al*., 2009). There are reports that milk and milk replacers with oral rehydration solution (ORS) did not affect abomasal curd formation (Constable *et al*., 2009). It may be true that some kitchen herbs help in digestion, reducing problems of bloating, flatulence, abdominal pain, and gas formation (Boskabady *et al*., 2014). Abomasal bloat is a problem in farms feeding milk replacers with higher protein and fat or faulty management in the calf feeding schedule (Marshall, 2009).

In support to our hypothesis, supplementation of probiotics (*Lactobacillus fermentum* NCDC605 and *Lactobacillus rhamnosus* NCDC610 @ 10^9 CFU/ml), kitchen herbs (cinnamon, carom seed and turmeric) or their combination dissolved in whole milk improved the fecal *sc*ores, reduces days of illness, serum total protein and dry matter intake of dairy calves during the preweaning period. Since the study was subjected to some limitations, including a lack of biochemical composition of herbs and a small sample size, further studies with larger experimental animals considering daily records are essential to confirm these results.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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