IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 4, Issue 2 (Mar. - Apr 2018), PP 55-60
www.iosrjournals.org

Nutritive Analysis and Microbial Characterization of Bovine Colostrum

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Abstract: *Colostrum is the first milk produced by mammary gland of milk-producing animals soon after parturition and its composition varies considerably with the whole milk. In the present study, bovine colostrum was collected within 12 hours of parturition from a local breed of cow from Chennai city and its microbial and nutritive analysis was carried out. The nutritive analysis was compared with locally sourced bovine whole milk. Proteins were five-fold higher and immunoglobulins (Ig) nearly ten-folds higher in the colostrum sample, indicating its metabolic and protective role for the newborn calf. The average count of total heterotropic bacterial population through standard plate count method was found to be 11,000 colony forming units (cfu)/ml. Bacteriological load on colostrum can impact the bioactive protein quality and can have direct influence on the available Ig for the feeding infant. The microbial analysis also indicated the presence of Streptococcus bovis and Lactobacillus spp. in the colostrum sample, which may contribute to the gut flora of the newborn. The presence of coliforms through this study, pose health risk to the animal as well as the newborn calf and can contaminate the milk samples also, thereby bringing down the quality of the milk. Safer and better maintanence of the animal can avoid such risks and improve the quality of milk thereby providing better nourishment to the newborn.*

Keywords *- Bovine colostrum, LAB, immunoglobulins, lactose, gut flora*

Date of Submission: 11-05-2018 Date of acceptance: 26-05-2018

I. Introduction

Milk is opaque fluid secreted by mammary gland to meet the nutritional requirements of neonate of that species. The composition of milk is variable over the period of lactation from birth to weaning, indicating the changing metabolic needs of the growing infant [1]. Colostrum is the first fluid produced by the mammary gland of all milk-producing animals soon after parturition for 2-5 days [2] and has distinct appearance, volume and composition [3]. It is rich in immunologic components such as immunoglobulins, leukocytes, lactoferrin, lactoperoxidase as well as developmental factors such as epidermal growth factor. The protective function is ensured by the presence of a complex mixture of bioactive and antimicrobial proteins like IgG, lactoferrin, lactoperoxidase, lysozyme, and proline-rich polypeptides [4].

It also provides non-immunoglobulin proteins, which help in nutrient absorption and provide amino acids for development of the gastrointestinal tract [5]. Colostrum also contains relatively low concentrations of lactose, indicating its primary functions to be immunologic and trophic rather than nutritional. Levels of sodium, chloride and magnesium are higher and levels of potassium and calcium are lower in colostrum than later milk [6]. Colostrum, like milk also contains probiotics, which can bestow physiological benefits such as removal of carcinogens, lowering of cholesterol, immunostimulating and allergy lowering effects, synthesis and enhancing the bioavailability of nutrients, alleviation of lactose intolerance [7].

Colostrum can be contaminated by microorganisms, which can contribute to morbidity and mortality rates of infants [8,9]. These pathogens may originate from the mammary gland or may contaminate the colostrum during collection, manipulation, and storage processes [10]. These pathogenic microbes can bind to free immunoglobulins in the intestinal lumen and block their absorption [11].

The present study aims to conduct nutritional analysis and microbial characterization of bovine colostrum collected from Chennai city, Tamil Nadu, India.

II. Materials And Methods

Colostrum sample was collected soon after birth of calf within 12 hours of parturition, from a 24 months old cow of local breed from Veterinary Hospital, Chennai city, Tamil Nadu, India. Sample was collected in sterile containers and stored in aliquots of 50 ml at 4℃ and -20℃ for further analysis. Microbiological tests were conducted within 2-6 hours of sampling.

2.1 Microbial analysis

Enumeration of Total Heterotrophic Bacterial Population (THBP): This was done using standard plate count (SPC) method, by serially diluting the colostrum sample in pH adjusted Phosphate Buffered Saline (PBS) solution and spread plating 1.0ml of each dilution on nutrient agar medium. The plates were incubated under aerobic conditions at 37°C for 24 hours to determine the THBP. All bacterial plate counts were expressed as the number of colony forming units (cfu) per milliliter (ml).

Isolation and identification of microorganisms under Aerobic, microaerophilic and anaerobic conditions: Serially diluted colostrum samples were also plated on Nutrient Agar (NA) medium, Mac Conkey's agar medium and incubated at 37°C for 24- 48 hours in aerobic conditions and in candle jar for microaerophilic conditions. Another set of serially diluted samples were plated on Man Rogosa Sharpe (MRS) agar medium and incubated in anaerobic conditions at 37°C for 48 hours for determining and isolating *Lactobacillus* sp. Well isolated bacterial strains from each of these were then picked up and stored in NA and MRS agar slants for further studies.

All the pure cultures isolated from colostrum sample using suitable medium under aerobic , microaerophilic and anaerobic conditions were studied for their morphological, cultural and biochemical characteristics. Morphological examination was carried out by examining colony morphology characteristics of all the isolates. Cultural and biochemical characterization of all isolates were done by Gram (1984) staining, motility test, catalase, oxidase, MR-VP and sugar fermentation test. Catalase test was performed with the help of hydrogen peroxide.(3% H_2O_2 reagent grade). MR-VP tests were performed in MRVP broth. For MR test, methyl red was used as a reagent whereas for VP test alpha naphthol and KOH were used as reagents. Nitrate test was performed in Nitrate broth and tested with alpha- naphthylamine and sulphanilic acid. Indole test was performed in tryptone broth and tested with the help of Kovac's reagent. Citrate test was performed on Simon's Citrate agar. Carbohydrate utilization for *Lactobacillus* spp. was checked in 1% (w/v) MRS broth containing the specific sugar and devoid of glucose and beef extract containing phenol red as indicator Acid and gas production from glucose was checked with the help of inverted Durham's tube containing 1% (w/v) glucose in MRS broth without beef extract.

Antibiotic sensitivity test for the isolated organisms were done using disc-diffusion method on Muller Hinton Agar (MHA) plates using known set of antibiotics. Each isolated strain was swabbed on previously prepared MHA plates and different antibiotics were placed to record the sensitivity of the organism towards the tested antibiotics through sensitivity zone formation. The cultures was incubated at 37° C for 24 hours and results were recorded with the annotation and percentage of susceptibility calculation as described by Bauer et al. [12] through the size of sensitivity zone around each disc.

The plates were incubated at 37° C for 24 hours for zone formation under aerobic and anaerobic conditions.

2.2 Nutritive analysis

2.2.1 Physicochemical analysis to evaluate the nutritive content was carried out on the colostrum sample. The tests included estimation of amount of moisture, ash, protein, fat, Sulphur as S, and Iron as Fe, performed in accordance with Methods of analysis:AOAC Official method [13] AOAC 927.05-1927 (moisture), AOAC 945.46 (ash), AOAC 991.20 (protein), AOAC 989.05 (Fat) and AOAC 2011.14-2011(Sulphur and Iron) and FSSAI Manual, methods of analysis of milk, 2015.

2.2.2 Estimation of Lactose: Lactose in colostrum was estimated quantitatively by modified method of crystallization of lactose after separation from casein. 100ml of colostrum sample was warmed to 40° C and mixed with 10% acetic acid solution until precipitation of casein. Casein was separated by centrifugation and supernatant was mixed with 3g of Calcium carbonate. The mixture was heated by gentle boiling over a hotplate for 10 mins until precipitation of remaining proteins was achieved. Proteins and undissolved $CaCO_3$ was removed again by filteration and the filtrate was then boiled down to 10 ml. To this, 80ml of 95% ethanol was added and gently heated the solution to 70° C. The solution was cooled in a petridish at room temperature to collect crystals of Lactose. Lactose crystals were dried and weighed.

2.2.3 Estimation of Immunoglobulins (Ig): Colostrum is known to be rich in Ig. Estimation of Ig in the give colostrum sample was done by simple salting out procedure as described by Duong-Ly and Gabelli [14].

III. Results And Discussion

The collected bovine colostrum was deep yellow in colour (**Fig.1**). The deep coloration is indicator of high levels of carotenoids from fat [15] and presence of red blood cells. The increased permeability of the mammary gland membranes in the pre-partum phase is responsible for more blood constituents gaining access to the milk [16]. This coloration gradually changes as mammary secretions changes to normal milk in subsequent milkings due to drop in levels of carotenoids and RBCs.

3.1 Microbial analysis

3.1.1 THBP of the bovine colostrum sample was done by serial dilution and SPC and the result is presented in **Table-1**.

The average count of THBP in the bovine colostrum sample was found to be 11,000 colony forming units (cfu)/ml. According to study by Catellani et al., [17], colostrum of water buffaloes showed SPC mean value of 14,000 cfu/ml.

While there are no set standards available for colostrum, Pasteurized Milk Ordinance (US FDA=CFSAN) enforces an SPC standard of 10,000-100,000 cfu/ml for raw milk leaving the dairy farm. Bacteriological load on colostrum can impact the bioactive protein quality and can have direct influence on the available Ig for the feeding infant.

3.1.2 The following organisms were isolated and identified by aerobic, microaerophilic and anaerobic conditions, as listed out in **Table-2**:

On blood agar, Group D streptococci was first identified as non-haemolytic pin-point colonies. Further confirmation was then done using Bile Esculin Agar test. **Figure-2** shows negative control *Escherichia coli* showing growth but absence of black colouration, while *Streptococcus bovis* and *Lactobacillus* spp. isolated from the bovine colostrum sample showed positive reaction with black coloration in the medium after 24 hours of incubation at 37°C.

3.1.3 Antimicrobial sensitivity: The antimicrobial sensitivity patterns for the organisms isolated in this study have been presented in **Table-3.**

The two coliform species, *E.coli* and *Klebsiella* spp isolated from the bovine colostrum sample were mild to moderately sensitive to basic first and second generation antibiotics indicated that these could be contamination through human handlers of the animal. They pose health risk to the animal as well as the newborn calf and can contaminate the milk samples also.

Streptococcus bovis (Group D streptococci) isolated from bovine colostrum was moderate to highly sensitive to most of the antimicrobials tested in this study, indicating it to be a normal flora of the gut of the animal and can confer health benefit to the animal as well as the new born calf.

Lactobacillus spp., isolated by anaerobic methods was moderate to highly sensitive to common antimicrobials except Methicillin, indicating that this organism could be the normal microflora of the gut of the animal conferring health benefits to the animal as well as the new born calf. It may also serve as a useful probiotic in the milk sample providing protective benefits to the users.

3.2 Nutritive Analysis

Nutritive properties of the bovine colostrum sample taken for this study, in comparison with bovine whole milk obtained from animal of similar breed from local diary farm has been listed in **Table-4**. From the result, it is clear that bovine colostrum is rich in proteins, especially immunoglobulins (Ig), to provide the passive immunity to the new born calf. The level of Ig observed in this study is average. A minimum level of 50g/1000ml of colostrum has been recommended as threshold value for quality colostrum [18] and this value can vary from breed to breed as well as the general wellbeing of the animal [19, 20]. The higher protein content observed in the colostrum also correlates with the higher need of proteins for the rapid growth and metabolism in the newborn. The iron content (5.0mg/kg) is indirect indicator of Lactoferrin present in the colostrum. Lactoferrin is a cationic iron-binding glycoprotein secreted by mammary gland and plays a key role in defence [21].This again is a very useful ingredient in Colostrum conferring health benefits to the users. Lactose sugar (6.5mg/1000ml) is lower in colostrum than normal milk. Our values also correlate with previous studies by several authors indicating the presence of lower sugars in colostrum immediately at post-partum followed by steady increase subsequently. Lactose intolerance is caused by the deficiency of the enzyme lactase. Without lactase, the body cannot breakdown lactose for digestion. Lactose being naturally low in Colostrum helps for those who have lactose intolerance and safe to use.

IV Conclusion

The present study provides a comprehensive microbial and nutritive analysis of bovine colostrum sample collected immediately post-partum from local breed. The study brings out the marked variation in composition of colostrum from milk, indicating differences in the biological function of these two two

secretions. The higher levels of proteins, especially immunoglobulins (Ig) in colostrum evokes interest in developing it either as a health-promoting product holistically or a source of individual components. The average count of total heterotropic bacterial population through standard plate count method in the bovine colostrum sample was found to be 11,000 colony forming units (cfu)/ml. Bacteriological load on colostrum can impact the bioactive protein quality and can have direct influence on the available Ig for the feeding infant. The microbial analysis also indicated the presence of *Streptococcus bovis* and *Lactobacillus* spp. in the colostrum sample, which may contribute to the gut flora of the newborn. The presence of coliforms pose health risk to the animal as well as the newborn calf and can contaminate the milk samples also, thereby bringing down the quality of the milk. Safer and better maintanence of the animal can avoid such risks.

Acknowledgements

The authors are thankful to the Management of Women's Christian College, Chennai, for providing the infrastructure to conduct this research work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict Of Interest

The authors declare that they have no conflict of interest.

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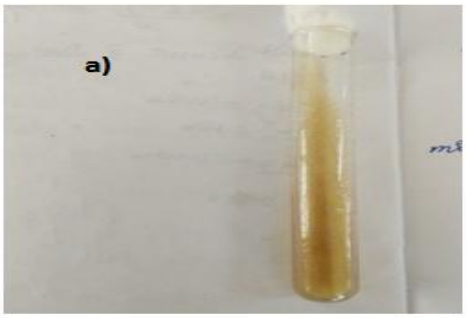
Figures

Fig.1: Bovine colostrum sample with deep yellow coloration collected on first day post-parturition**.**

Fig.2: Bile esculin agar test: a) *Escherichia coli* showing absence of dark colouration indicating negative reaction b)*Streptococcus bovis*(left) and *Lactobacillus* spp. showing dark coloration indicating positive reaction.



TABLES

Table-1: Enumeration of Total Heterotrophic Bacterial Population (THBP) at various dilutions of the colostrum sample

S.No.	SAMPLE	COLONY COUNT	No. of CFU/ml
1.	Undiluted colostrum	High	High
2.	1:10 diluted colostrum	1109	11,090
3.	1:100 diluted colostrum	110	11,000
4.	1:1000 diluted colostrum	10	10,000
5.	1:10,000diluted colostrum	1	10,000

Table-2: Identification of various organisms isolated and identified in the bovine colostrum sample

Tests/ Characteristics	*Escherichia coli*	*Klebsiella* spp.	*Streptococcus bovis*	*Lactobacillus* spp.
Gram's stain	Negative	Negative	Positive	Positive
Morphology	Bacilli	Bacilli	Cocci in chains	Cocco-bacilli
Motility	Motile	Non motile	Non motile	Non motile
Oxidase	Negative	Negative	Negative	Negative
Catalase	Positive	Positive	Positive	Negative
Indole	Positive	Negative	Negative	Negative
Methyl-Red (MR)	Positive	Negative	Positive	Negative
Vogues Proskaur (VP)	Negative	Positive	Negative	Negative
Simmons Citrate Agar	Negative	Positive	-	Negative
Urease test	Negative	Positive	-	-
Bile Esculin Agar	Negative	Negative	Positive	Positive

Table-3: Antimicrobial susceptibility pattern of various organisms

Bacterial Species	Antibiotic disc used	Sensitivity Zone (mm)	Percentage of sensitivity	Degree of sensitivity
Escherichia coli	Amikacin	9mm	50%	Moderately Sensitive
	Amoxicillin	9mm	50%	Moderately Sensitive
	Ampicillin	10mm	56%	Moderately Sensitive
	Cephalexin	10mm	56%	Moderately Sensitive

	Chloramphenicol	13mm	72%	Highly Sensitive
	Tetracycline	No zone	0%	Resistant
	Ceftriaxone	18mm	100%	Highly Sensitive
Klebsiella spp.	Amikacin	5mm	28%	Mildly Sensitive
	Amoxicillin	5mm	28%	Mildly Sensitive
	Ampicillin	8mm	44%	Moderately Sensitive
	Cephalexin	10mm	56%	Moderately Sensitive
	Chloramphenicol	4mm	22%	Mildly Sensitive
	Tetracycline	No zone	0	Resistant
	Ceftriaxone	18mm	100%	Highly Sensitive
Streptococcus bovis	Amikacin	8mm	44%	Moderately Sensitive
	Amoxicillin	6mm	33%	Mildly Sensitive
	Ampicillin	10mm	56%	Moderately Sensitive
	Cephalexin	18mm	100%	Highly Sensitive
	Chloramphenicol	6mm	33%	Mildly Sensitive
	Tetracycline	6mm	33%	Mildly Sensitive
	Gentamycin	10mm	56%	Moderately Sensitive
Lactobacillus spp	Tetracycline	15mm	68%	Moderately Sensitive
	Azithromycin	22mm	100%	Highly Sensitive
	Methicillin	No zone	0%	Resistant
	Doxicilin	20mm	91%	Highly sensitive
	Ofloaxone	13mm	59%	Moderately sensitive

Table-4: Nutritive properties of colostrum sample in comparison with bovine whole milk

S. No.	Test parameter	Colostrum	Whole milk
1.	Fat (g/kg)	36	35
2.	Protein (g/kg)	163	33
3.	Ash (g/kg)	8.98	7.5
4.	Total Solids (g/kg)	236	129
5.	Calcium (mg/kg)	2.4	1.1
6.	Sulphur (mg/kg)	5.0	2.4
7.	Iron (mg/kg)	5.0	1.2
8.	Lactose (mg/1000ml)	6.5	42
9.	Immunoglobulins (mg/1000ml)	60	0.7

IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

Pavithra A[1] "Nutritive Analysis And Microbial Characterization Of Bovine Colostrum." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 4.3 (2018): 55-60.