Biochemical Profile of Milk of Buffalo (*Bubalus bubalis*), Cow (*Bos taurus*) and Goat (*Capra hircus*): Potential Candidates for Supporting the Growth of *Leishmania donovani* Promastigotes in Culture Medium as Alternative to Fetal Bovine Serum (FBS)

M. Muniaraj[1,*], S. Kumar[2], C.S. Lal[2] and P.K. Sinha[2]

[1]*Centre for Research in Medical Entomology (Indian Council of Medical Research), 4-Sarojini Street, Chinna Chokkikulam, Madurai – 625 002, Tamil Nadu, India*

[2]*Rajendra Memorial Research Institute of Medical Sciences (Indian Council of Medical Research), Agamkuan, Patna – 800 007, Bihar, India*

Abstract: The milk of cow (*Bos taurus*), buffalo (*Bubalus bubalis*), and goat (*Capra hircus*) were investigated for the biochemical profile such as glucose, total protein, triglycerides, cholesterol and calcium. Although used as potential candidate to replace fetal bovine serum in the culture medium for the cultivation of parasitic protozoan such as *Leishmania donovani* promastigotes, the biochemical profile of milk will provide information about which factor supports and essential for the growth of parasite. We found triglycerides were predominant in goat followed by cow and buffalo milk. The level of cholesterol was high in buffalo milk followed by goat and FBS. The level of calcium was high in buffalo milk followed by goat and cow milk.

Keywords: Biochemical profile, milk of buffalo, cow, goat, *Leishmania donovani*, culture medium, alternative, fetal bovine serum.

INTRODUCTION

In vitro cultivation of parasitic protozoa that cause human disease is invaluable, as it provides information on the development of parasites that could be used in the containment and eradication of the parasite [1]. The media used for the cultivation of *Leishmania donovani* require either fetal bovine serum (FBS) (also fetal calf serum) or blood lysate as one of their essential ingredients. FBS is highly expensive (top-quality FBS can cost up to £500/liter), and reliable supply is very difficult to obtain, especially in developing countries [2]. Serum can also harbor hazardous contaminants such as viruses, bacteria, prions, and mycoplasma [2]. Above all, the increasing concerns about animal suffering inflicted during collection add an ethical imperative to move away from the use of serum wherever possible [2]. Although several attempts have been made to replace FBS with bovine serum albumin or a mixture of purine bases, vitamins, large concentrations of certain amino acids, hormones, hemin, hemoglobulin, and, even human and animal urine [3-9], none of them is widely in practice. This is due to shortcomings such as cost, complicated procedures, and laborious preparation. More recently, milk of Cow (*Bos taurus*), Buffalo (*Bubalus bubalis*), and Goat (*Capra hircus*) was used as a better alternative to FBS in media for the primary isolation, *in vitro* cultivation, and maintenance of *Leishmania donovani* promastigotes [10]. It was expected that as a product from blood, milk could be a alternative than serum for not only parasite culture but also for tissue culture at large. Milk of common cattle has several advantages over serum viz. cheap, easily available and no ethical disputes etc. [10]. The main objective of this study was to investigate the biochemical profile of milk of common cattle such as buffalo, cow and goat in comparison with FBS.

MATERIALS AND METHODS

Four sets of three tubes were taken for each profile investigated such as glucose, total protein, triglycerides, cholesterol and calcium (15+15+15+15). The milk samples were collected from cow (*Bos taurus*), buffalo (*Bubalus bubalis*), and goat (*Capra hircus*) in separate sterile containers, brought to the lab, and processed fresh or stored at 5 to 8°C. The milk samples were tyndalized as described by Muniaraj *et al*. [10], briefly, The milk samples, if stored, were brought to room temperature (RT) and were then exposed to boiling water for 30 min. The caps of the glass tubes were kept loose to facilitate the release of steam. After the milk samples were brought down to RT (26 to 30°C), they were kept at 3 to 4°C. The next day, the milk samples were taken out from the

*Address corresponding to this author at the Centre for Research in Medical Entomology (Indian Council of Medical Research), 4-Sarojini Street, Chinna Chokkikulam, Madurai – 625 002, Tamil Nadu, India; Tel: +91 452 2525131; Fax: +91 452 2530660; E-mail: mmuniaraj@yahoo.com

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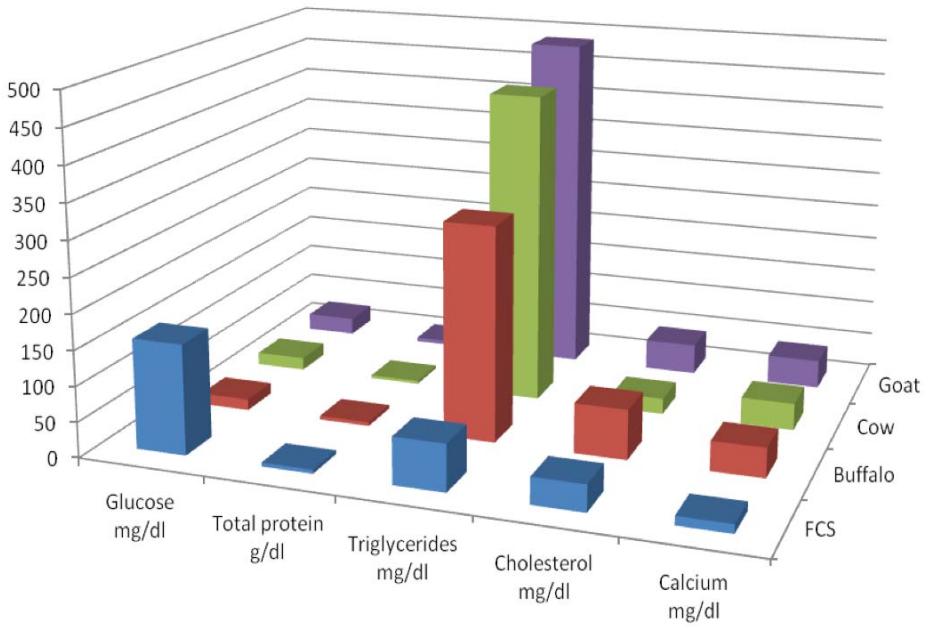

Figure 1: Biochemical profile of various parameters in the milk of buffalo, cow and goat and FBS.

refrigerator and kept at RT for 2 h before being exposed to boiling water. This was done on three successive days, with an incubation of 24 h between each exposure to boiling water.

Bacterial endospores that survive the initial heating will germinate in the milk while it is kept at room temperature. The resulting vegetative bacteria will be killed when the milk is reheated. It was noticed that, after every heat exposure, the colour of the milk slowly turned to yellow.

The tyndalized milk samples were centrifuged at 3,000 rpm for 30 min to remove fat globules, as they may disturb the microscopic examination for parasites when it is used as supplement in culture medium. Without disturbing the upper creamy layer and bottom deposits, the centrifuged milk samples were collected in separate glass tubes by a Pasteur pipette and stored in the refrigerator at 3 to 4°C. The milk samples were collected from three animals from each group and FBS (HiMedia, Mumbai, India) were collected from three different lots for this triplicate study. The stored milk was used for this investigation. The average value was calculated from the obtained value for each parameter tested for FBS (heat inactivated at 56°C for 30 min) and milk samples. The values used to plot graphs using excel software (Figure **1**).

RESULTS AND DISCUSSION

The level of glucose was exceptionally high (156 mg/dl) in FBS when comparing with milk samples (<24.4 mg/dl). The goat milk was found to have high level of both total protein and triglycerides viz. 5.8 g/dl and 485.8 mg/dl respectively.

The buffalo milk was found exceptionally high in cholesterol and calcium 70.7 mg/dl and 42.4 mg/dl. In the milk of other animals, the parameters such as cholesterol and calcium was found <43.3 and <40.44 mg in other animals respectively. The milk of cow did not show any extremes (Table **1**).

Table 1: Biochemical Profile of Various Tested Parameters of Milk of Buffalo, Cow, Goat and FBS in Numerical Form

	FBS	Buffalo	Cow	Goat
Glucose mg/dl	**156**	15.7	18.2	24.4
Total protein g/dl	5.5	5.3	4.2	**5.8**
Triglycerides mg/dl	66.6	304.5	444.5	**485.8**
Cholesterol mg/dl	37.8	**70.7**	22.5	43.3
Calcium mg/dl	12.8	**42.4**	38.3	40.44

It was reported by Muniaraj *et al*. [10] that the goat milk was found superior than all the two other tested milk and FBS. The milk of buffalo and cow are more or less equally supported the growth of *Leishmania* promastigotes. The efficiency of FBS was comparably less in supporting the growth of the parasite. From the presented investigation, it is clear that the goat milk was having rich triglycerides followed by buffalo and cow milk. This shows that triglycerides are very much essential for the development of parasites. Similarly,

the cholesterol and calcium level were found high in buffalo milk suggests its important role in the parasite development. Although total protein was high (5.5g/dl) in FBS when comparing with cow and buffalo milk, it gave less growth of parasite. This study shows that, the triglycerides followed by cholesterol, calcium and protein are essential for the parasite development than glucose which requires comparably less quantity.

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