

ESTIMATION OF IMMUNOGLOBULIN G LEVELS IN COLOSTRUM OF MURRAH BUFFALOES

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Abstract: Individual variation in Immunoglobulin content of first colostrum may be responsible for differences in susceptibility of calves to infections. So, the present study was undertaken to analyze immunoglobulin G levels by ELISA in the colostrum of buffalo dams before first milking after calving. Colostrum (before first milking) of 40 newly parturated Murrah buffaloes maintained at Livestock Production Management (LPM) Section of IVRI, Izatnagar, was digested with rennet and then analysed by indirect ELISA. Levels of IgG in buffalo colostrum (before first milking) estimated by Indirect ELISA ranged from 11.22 to 185.1 mg/ml and mean IgG concentration in colostrum was 51.71 ± 5.99 mg/ml.

Keywords: absorption, calf, ELISA, immunity, rennet digestion

Introduction

Colostrum, the protein rich “first milk,” is considered to be “liquid gold” as it contains maternal immunoglobulins that protect neonates from invading pathogens (Larson *et al.*, 1980). Besides this, colostrum is also a rich source of proteins, vitamins and minerals. The immunoglobulins present in colostrums are absorbed through ruminant neonatal gut; provide passive immunity to young animals. However, both the concentration of immunoglobulin in colostrum and the permeability of the gut decrease rapidly and progressively over the first 48 h after birth (Bush and Staley, 1980 and Moore *et al.*, 2005). The ability of a calf’s small intestine to absorb immunoglobulins drops rapidly over the first few hours of life. By 24 hr of age, the ability to absorb immunoglobulins is nearly nonexistent. As only less than 40% immunoglobulins are absorbed, an ample supply of colostrum, with abundant immunoglobulins, is essential during the initial period of life for the young to gain sufficient passive immunity to be able to survive until its own immune system is fully developed.

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The immunoglobulins present in colostrum or milk are the same as those found in the blood or mucosal secretions (Duncan *et al.*, 1972). IgG is the primary immunoglobulin present in ruminant milk, in contrast to IgA being the key immunoglobulin present in human milk (Stewlagen *et al.*, 2009).

There are many studies in which IgG levels were estimated in cattle colostrum (Zhang *et al.*, 2001 and Butler, 1973) while similar studies on buffalo milk are very scanty (Dang *et al.*, 2009). Considering it, this study was designed to estimate IgG levels in colostrum of Murrah buffaloes.

Material and Methods

Experimental animals: 40 newly parturated Murrah buffaloes maintained at Livestock Production Management (LPM) Section of IVRI, Izatnagar, were included in the present study.

Collection of colostrum samples: Approximately 10ml of fresh colostrum samples were collected in 15 ml polypropylene tube from each dam before first milking. These were then transported to laboratory in an icebox and stored at -200C till estimation of IgG concentration.

Digestion of Colostrum: Before performing actual ELISA all the colostrum samples were digested with rennet (Sigma, USA). For this, 10ml of each colostrum sample was taken in 50ml beaker and heated to 37°C in a water bath. 0.5ml of 0.5% rennet (250mg in 50ml distilled water) was added. After 10 minutes clotted colostrum was mixed with glass rod and then filtered through Whatman filter paper no. 42 (quantitative grade) overnight. Filtered sample was further used for ELISA.

ELISA: Indirect ELISA was performed on colostrum whey with the help of commercial kit (Koma Biotech) with slight modifications. The OD values obtained were analyzed using GraphPad Prism 6 software. Figure 1 shows the curve obtained by plotting standard concentrations against absorbance at 450nm.

Results and Discussion

IgG levels in colostrum ranged from 11.22 to 185.1 mg/ml (Table1) and mean IgG concentration in colostrum was found to be 51.71 ± 5.99 mg/ml. The mean colostrum IgG concentration observed in our studies (51.71 mg/ml) was in accordance with the reports of Agrawal (2015) *i.e.*, 50.44 ± 3.36 mg/ml, Verma (2016) *i.e.*, 56.71 ± 5.76 mg/ml and Dang *et al.* (2009) *i.e.*, 54.0 mg/ml who employed the same breed (Murrah) of buffalo and method of estimation of IgG (indirect ELISA using Koma Biotech kit) as that of our study. Our mean

colostral IgG concentration was lower than those published by Zhang *et al.* (2001) in bovines (67.2 mg/ml) however Butler (1973) has reported 50.0 mg/ml colostral IgG concentration as estimated by radial immunodiffusion method. This variation could be attributable to species difference as well as different method of estimation. The greater variation observed in colostral IgG in our buffalo dams is in accordance with Kehoe *et al.* (2011) who also observed a wide range of IgG concentration in colostrum (11–221 mg/ml). Alike our observations in buffalo Kehoe *et al.* (2007) has also observed extremely high animal variation in IgG concentration ranging from 11.8–74.2 mg/ml in cows.

Conclusion

Our results present the levels of IgG in buffalo colostrum. Levels of IgG in buffalo colostrums (before first milking) estimated by Indirect ELISA ranged from 11.22 to 185.1 mg/ml and mean IgG concentration in colostrum was 51.71 ± 5.99 mg/ml.

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Fig 1: Standard curved obtained by plotting standard concentration against absorbance at 450nm

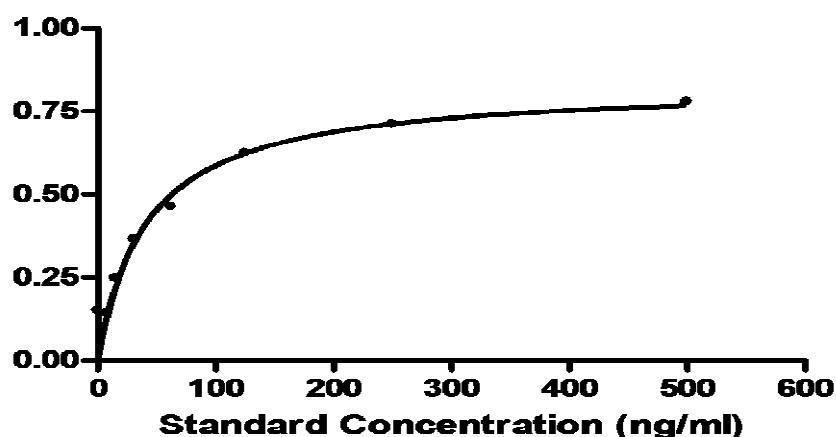


Table 1: IgG concentrations in colostrum

S. No.	Animal #	IgG concentration (mg/ml)	S. No.	Animal #	IgG concentration (mg/ml)
1	713	18.84	21	905	23.84
2	680	51.66	22	715	87.34
3	944	48.29	23	895	81.17
4	989	134.43	24	799	28.24
5	911	22.7	25	704	27.91
6	869	34.14	26	698	70.59
7	982	48.67	27	812	11.22
8	734	46.25	28	899	41.16
9	908	21.33	29	913	50.61
10	700	20.75	30	1055	29.16
11	571	20.83	31	900	50.35

12	972	88.97	32	905	31.29
13	1022	30.96	33	717	23.06
14	1012	32.21	34	895	24.71
15	766	54.29	35	799	185.01
16	893	43.29	36	812	128.81
17	903	51.67	37	725	76.05
18	712	17.85	38	712	37.06
19	725	34.16	39	704	132.94
20	1007	35.62	40	668	70.76