ASSAYING THE CONCENTRATION OF IMMUNOGLOBULIN G IN COLOSTRUM FROM FEMALES POSTPARTUM AND SERUM FROM NEONATAL CALVES OF BUFFALOES (*Bubalus Bubalis*)

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ABSTRACT

Neonatal ruminants are born without any humoral immunity due to lack of placental transfer of immunoglobulins during gestation. This predisposes the newborn buffalo calves to a high incidence of morbidity and mortality on exposure to infectious agents. Colostrum is the first milk produced by the females after calving and is a rich source of immunoglobulins, especially immunoglobulinG (IgG). The immunocompetence of the neonates can be boosted by feeding them sufficient amount of good quality colostrum within a few hours after birth. Optimal colostrum management at a farm not only reduces the occurrence of diseases among the younger stock but also enhances their growth performance and productivity once they are adults. In the present study, eighty animals at a Murrah buffalo farm were screened for the concentration of IgG in the colostrum collected from recently parturated females and in the serum collected from their calves within 6 to 12 h of colostrum consumption to determine the status of transfer of passive immunity. Indirect ELISA was used to estimate the IgG levels. The overall mean (range) of colostral and serum IgG concentration was found to be 50.44±3.36 (12.71 to 227.78) and 10.85±0.62 (0.25 to 19.88) mg/ml, respectively for all the 80 animals. Routine screening of buffaloes, in a similar way, will help to reduce calf deaths due to immunodeficiency.

Keywords: *Bubalus bubalis*, buffaloes, colostrum, failure of passive transfer, immunoglobulin G, serum

INTRODUCTION

In-utero transfer of antibodies from dam to the foetus does not occur in ruminants due to the presence of syndesmochorial placenta (Weaver *et al*., 2000; Borghesi *et al*., 2014). Hence, the calves born are devoid of any maternal immunity and are said to be aggamaglobulinemic (Jain *et al*., 2007). Newborn calves' naive immune

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system is functional but does not produce an effectual immune response for about 3 weeks of life (Franklin *et al*., 2003). During this period the calves entirely depend on the maternal immunoglobulins they acquire via consumption of colostrum to provide them protection against infectious diseases (Duhamel and Osburn, 1984; Tizard, 1996). Colostrum is the first milk produced by females after calving that contains micro- and macro-nutrients, immunoglobulins, growth factors and peptides with anti-microbial activity. Intake of good quality colostrum thus plays a vital role in the growth, development, and survival of neonates by enhancing their immunocompetence. Three major classes of immunoglobulins are present in bovine colostrum: IgG, IgA, IgM (Korhonen *et al*., 2000). Of these, the most abundant immunoglobulin is IgG, comprising approximately 85 to 90% of the total followed by IgM (7%) and IgA (5%). Further, IgG has two main isotypes: IgG1 and IgG2, with the former accounting for 80 to 90% of the total IgG (Larson *et al*., 1980; Godden *et al*., 2019). IgG can prevent binding of pathogens to host cells, stimulate T-cell and B-cell mediated immune responses, present pathogens to macrophages and induce the local production of IgA. Therefore, IgG not only provides passive immunity but also regulates the innate and adaptive immune systems (Ulfman *et al*., 2018; Playford and Weiser, 2021). In cows, colostrum is considered to be of optimum quality if the concentration of IgG is >50 mg/ml in dairy animals and >100 mg/ml in beef animals (McGuirk and Collins, 2004; Godden, 2008; Beam *et al*., 2009). However, the concentration of IgG varies in colostrum produced by different animals and is influenced by a variety of factors such as breed, parity, length of dry period, season of calving, number of milkings after calving and body condition score (Oyeniyi and Hunter, 1978; Muller and Ellinger, 1981; Pritchett *et al*., 1991; Filteau *et al*., 2003; Jaster, 2005; Kehoe *et al*., 2007). Colostral IgG levels have been seen to fall rapidly with each passing hour after birth (Morin *et al*., 2010).

Ideal colostrum management implies that the calves consume sufficient quantity of clean and high-quality colostrum within the first few hours after birth (Godden, 2008). The optimum amount of colostrum that a calf should receive in the first feeding is about 10 to 12% of their body weight (Godden, 2019). Serum IgG concentration was found to be higher in calves that consumed 4 L of colostrum as compared to those that consumed 2 L of colostrum (Morin *et al*., 1997). The time of colostral intake is also crucial for the neonates as the permeability of the intestinal mucosa for immunoglobulin molecules rapidly reduces after birth and maximum IgG absorption occurs within first 6 to 8 h of life (Blom, 1982). Intake of colostrum with adequate levels of immunoglobulins by the calves soon after birth reduces the risk of mortality and morbidity caused by enteric and respiratory diseases and even due to leg injuries (Norheim *et al*., 1985; Raboisson *et al*., 2016; Todd *et al*., 2018). Such calves also exhibit a superior growth performance and greater milk production in adlulthood (Faber *et al*., 2005; Mastellone *et al*., 2011; Elsohaby *et al*., 2019a). According to some recent reports 2 vs 1 feeding of colostrum after birth decreased the risk of failed passive transfer in calves, thus reducing morbidity and promoting growth (Abuelo *et al*., 2021). Though the use of commercial colostrum and milk replacers as a strategy to alleviate the ill effects of passive transfer failure is being explored, they still remain deficient in immunological components leading to occurrence of infectious diseases at higher rates (Vlasova and Saif, 2021). The acquisition of passive immunity by ingestion of colostrum, ensuring absorption of at the very least 20 to 25 mg/ml of IgG, by neonatal bovine calves can be evaluated through determination of their serum IgG levels (Souza *et al*., 2020).

Inadequate transfer of colostral IgG from dam to the newborn calves is referred to as Failure of Passive Transfer (FPT). In cow calves, serum IgG levels of < 10 mg/ml at 24 to 48 h of age is considered to be an indicator of FPT (Besser *et al*., 1991; Jaster, 2005; Chigerwe *et al*., 2014; Mugnier *et al*., 2020; Sutter *et al*., 2020). The risk of mortality and morbidity in calves suffering from FPT is 6 times higher than those with successful transfer of passive immunity (McGuirk and Collins, 2004). More than 30% of mortality in pre-weaned calves was directly related to deficient acquisition of passive immunity (Stilwell and Carvalho, 2011). Thus, FPT leads to severe economic losses to calf husbandry and makes finding sufficient herd replacements difficult. On an average 25% early calf mortality withholds any chance of regular replacement of animals with low production (Afzal *et al*., 1983). An incidence of FPT at <10% can be contemplated as an acceptable goal in dairy farming (Meganck *et al*., 2014). Routine screening of animals on the basis of IgG concentration measured in colostrum and blood serum of dams and their calves, respectively will facilitate efficient colostrum management.

Although the contribution of buffaloes to world dairy industry is immense, there is a dearth of research work on occurrence of FPT in buffalo calves. Not many studies evaluating the concentration of IgG in buffalo colostrum and calf serum are reported. Unlike cows, optimum IgG levels for quality colostrum and calf serum, ensuring successful transfer of passive immunity in buffaloes, are yet to be specified. Therefore, the present study was undertaken to measure the IgG content in colostrum and calf serum of Murrah buffaloes.

MATERIALS AND METHODS

Experimental animals

Eighty recently parturated Murrah buffaloes reared in the cattle and buffalo farm at ICAR-Indian Veterinary Research Institute, Izatnagar (U.P.), and their respective neonatal calves were included in the study. Among the females, there were primiparous buffaloes and multiparous buffaloes with 2^{nd} to 5^{th} parity. All the animals were stall fed with requisite amount of green fodder, dry fodder and concentrate and had access to clean drinking water at all times. They were healthy and under no medication except for small ailments such as a short spell of diarrhoea. Animals were kept under loose housing during the day and secured in closed sheds at night. A female showing signs of parturition was isolated to an individual pen and closely monitored for assistance in case of any difficulty. All females conceived through artificial insemination and only the calves born out of normal calvings were included in the study.

Collection and digestion of colostrum samples

Within 1 to 2 h of parturition, approximately 10 ml of colostrum was collected from each dam in a 15 ml polypropylene tube. It was then taken to the laboratory in an icebox and stored at -20°C till its digestion by rennet (R5876-50G: Sigma, USA). Each colostrum sample was poured into a 50 ml beaker and heated to a temperature of 37°C in a water bath. Following that, 0.5 ml of 0.5% rennet (250 mg per 50 ml distilled water) was added to the colostrum to improve clotting and aid in immunoglobulin release. Rennet is a complex set of enzymes that occurs naturally in the stomach of ruminant mammals, the active enzyme being chymosin or rennin, which assists in the release of immunoglobulins from the colostrums and milk ingested by causing curd formation. About 10 minutes later, the clotted colostrum was mixed using a glass rod, followed by overnight filtration through Whattman filter paper no. 42 (quantitative grade). The filtrate obtained thereafter was used for ELISA.

Collection of blood samples from calves

About 6 to 12 h after a newborn buffalo calf was fed colostrum for the first time, 3 ml of blood was collected from its jugular vein in vaccutainer tubes (without anticoagulant). The tubes were kept in a slanting position at room temperature for the isolation of serum from clotted blood. They were then centrifuged at 2000 rpm for 10 minutes and the serum was aliquoted in eppendorf tubes which were stored at -20°C untill the estimation of IgG concentration.

Estimation of IgG concentration

The quantitative measurement of IgG in collected samples was performed by indirect ELISA using a commercial kit (Koma Biotech) with some modifications in the manufacturer's instructions. The OD values obtained by measuring absorbance at 450 nm were analyzed using GraphPad Prism 6 software for evaluating the IgG concentration of each colostrum and serum sample. The curve derived by plotting standard IgG concentrations against absorbance at 450nm is depicted in Figure 1.

RESULTS AND DISCUSSIONS

The concentration of IgG in colostrum produced by female Murrah buffaloes are presented in Table 1. The mean value of IgG content in colostrum samples was found to be 50.44±3.36 mg/ml. A large variation was observed in the colostral IgG levels ranging from 12.71 to 227.78 mg/ml (Table 3). The values obtained in the present study are in agreement with the findings of Chaudhary *et al.* (2016a) who reported a mean value of 51.71±5.99 mg/ml in colostrum collected from 40 Murrah buffalo dams and Dang *et al.* (2009) who estimated a mean IgG level of 54 mg/ml in colostrum samples from eight Murrah buffaloes at NDRI, Karnal. Verma *et al.* (2018) also estimated the colostral IgG content in 23 Murrah buffaloes and obtained a mean value of 56.98±5.71 mg/ml. All the above workers had used the same technique of IgG estimation (Indirect ELISA) and worked on the same breed of buffalo though the animals studied were maintained at different farms under varying managemental conditions. In another study on 72 Murrah buffaloes in different parities, a much higher mean concentration of 71.4±2.81 mg/ml was reported, determined by SDS-PAGE. It was supposed to be an outcome of vaccination during gestation and division of females on the basis of their parity to ensure proper nutrition and management catering to each animal depending on its physiological status (Souza *et al*., 2020). Recently, Giammarco *et al*. (2021) estimated a mean colostral IgG level of 64.9±29.3 mg/ml in Italian mediterranean buffaloes. Though they measured a higher mean level with the help of indirect ELISA, the range of variation (21 to 110 mg/ml) was large, as observed by us. The lowest mean concentration of IgG at 33 mg/ml was obtained in 18 Egyptian buffaloes using single radial immunodiffusion method (SRID) (Abd El-Fattah *et al*., 2012).

Not many studies have been conducted to assess the IgG levels of buffalo colostrum. However, on comparing several studies on cattle, factors such as breed, parity, yield, month of calving, length of dry period and pre-parturient vaccination of dams appears to have a significant effect on colostral IgG content (Muller and Ellinger, 1981; Gomes *et al*., 2011; Conneely *et al*., 2013; Silva-del-rio *et al*., 2017; Ahmann *et al*., 2021). Abd El-Fattah *et al*. (2012) compared the IgG concentration in colostrum from Egyptian buffaloes and Holstein cows but found no significant difference between the two species. The number of animals involved in the study were small and similar experiments with larger number of animals should be carried out for a true comparison. The composition of buffalo and cow milk varies considerably with respect to a number of nutrients. Therefore, the composition of their colostrum is also expected to vary and to be influenced by different genetic and environmental factors to a variable extent. Colostral IgG levels should be assessed in various buffalo breeds raised under diverse agro-climatic, nutritional and managemental conditions so that a standard value denoting colostrum of optimum immunological quality can be determined for buffaloes as well, alike cattle, promoting efficient colostrum management.

The concentration of IgG in the calf sera from neonatal buffalo calves are presented in Table 2. The average serum IgG concentration for all the calves measured was 10.85±0.62 mg/ml ranging from 0.25 to 19.88 mg/ml (Table 3). These values are quite similar to those reported by Chaudhary *et al*. (2016b) (11.68±0.75 mg/ml) and Verma *et al*. (2018) (11.23±0.70 mg/ml) in Murrah buffalo calves at 6 to 12 h and 12 to 18 h post first colostrum consumption, respectively. However, Souza *et al*. (2020) deduced a greater average value of 34.5±1.48 mg/ml in Murrah neonates at 24 h after suckling colostrum. In contrast to the present study, a better immune status exhibited by these calves could be a consequence of intake of colostrum with higher IgG content and measurement of sera IgG levels after a longer duration allowing more time for absorption of colostral IgG. A correlation was also found between IgG content in colostrum at parturition and IgG content in sera of calves ingesting it (Osaka *et al*., 2014; Souza *et al*., 2020).

Kwatra *et al*. (2019) placed the buffalo calves into groups *viz*. normal calves (serum IgG >10 mg/ml) and FPT calves (serum IgG <10 mg/ml), with mean serum IgG levels of 14.15±0.65 mg/ml and 4.41±1.22 mg/ml, respectively. They reported a higher severity of illness and lower average daily weight gain (ADG) in calves of the FPT group. Similar effect of IgG concentration in calf serum on growth and ADG was observed in 12 neonatal buffalo calves with a mean IgG content of 31.0±2.4 mg/ml at 24 h after birth (Mastellone *et al*., 2011). A correlation between the serum IgG concentration and disease occurrence or growth rate in buffalo calves was not investigated in the present study but it was noted that 30/80 buffalo calves had serum IgG levels <10 mg/ml (ranging from 0.25 to 9.88 mg/ml), accounting for 37.5% cases of FPT in the sample population. However, most of them thrived well during the period of the study. The same was also noted by Giammarco *et al*. (2021) in Italian Meditarranean buffalo calves, which had serum IgG levels of 6.0 to 14.5 mg/ml but none of them showed signs of any disease or discomfort during the course of study. Also, they assessed a mean serum IgG concentration of 11.1±2.0 mg/ml, which is very close to our findings. The serum IgG level of <10 mg/ml, which indicates FPT in cow calves, should not be considered as a standard value to analyze passive transfer of immunity in buffalo calves as well. Instead, an appropriate value for minimum IgG content in the sera of neonatal buffaloes, that denotes adequate transfer of passive immunity, should be determined to minimize the buffalo calf mortality due to immunodeficiency.

Apparent efficiency of absorption (AEA) is a measure of the IgG content in calf's blood at 24 h expressed as a fraction of total IgG mass fed to the calf (Lopez and Heinrichs, 2022). Various factors affect AEA such as quality of colostrum (IgG content), feeding method, volume fed, time at which colostrum is first fed, calf's sex, calf's body weight at birth and calf's hydration status (Quigley *et al*., 1998; Godden *et al*., 2009). Since, all these factors may vary from one study to another, so will the AEA and for that reason the calf serum IgG levels reported by each should be compared judiciously. Further research on passive immunity transfer in buffaloes should be directed towards determination of ideal values for those factors which are under human control. In the present study, the percent absorption of IgG was calculated as the concentration of IgG in the calf serum expressed as a proportion of the IgG concentration in the colostrum of its dam. Its value ranged from 0.29 to 89.93% with an overall mean of 27.69±2.23%. The time period between birth of calf and first consumption of colostrum can be predicted as a major determinant of percent absorption owing to the fact that the permeability of the neonatal gut to macromolecules such as immunoglobulins declines with each passing hour of life (Weaver *et al*., 2000). Therefore, to achieve a higher percent absorption of IgG, colostrum should be fed to the calves within the first few hours of life.

In a comparative analysis of the serum IgG concentration in buffalo calves and cow calves, the mean values observed at 6 h post colostrum feeding were 5.99 ± 0.4 mg/ml and 14.7 0.5 mg/ml, respectively, which indicated poor immune-competence of newborn buffaloes than that of cows (Barmaiya *et al*., 2009). Similar findings were revealed by another study, though the average sera IgG levels for buffalo and cow calves were remarkably higher at 122.0 ± 3.0 and 153.0 ± 34.0 mg/ml (Jain *et al*., 2008). However, the IgG level of buffalo colostrum has been found to be higher than that of cow colostrum (Jain *et al*., 2008). The inconsistency observed with respect to the IgG content in dam colostrum and calf serum when buffalo is compared with cow is a subject matter of research. The average serum IgG content estimated in buffalo calves in this study is lower to that reported in cow calves by several workers (Laegrid *et al*., 2002; Johnsen *et al*., 2019; Martin *et al*., 2021). But the mortality rate in neonates, especially during the initial months of life, is higher for buffaloes as compared to cows (Jain *et al*., 2008; Uttam *et al*., 2015). Poor immune status and high mortality rate of newborn buffaloes indicates that FPT is a much serious concern than in this species than assessed so far.

The methods used in different studies for estimation of IgG levels in colostrum and serum samples may also be responsible for the variation in the obtained values. Direct method of radial immunodiffusion (RID) is still considered to be the gold standard (Godden *et al*., 2019; Weaver *et al*., 2000) but, is expensive and time consuming. The technique of ELISA used in the present study is indirect and cheaper to RID (Gelsinger *et al*., 2015). The results obtained using ELISA are lower but highly correlated with those derived from RID (Lee *et al*., 2008; Dunn *et al*., 2018). Several new techniques have been introduced and compared to determine their suitability for regular use on a farm (de Souza *et al*., 2021). Lab based methods

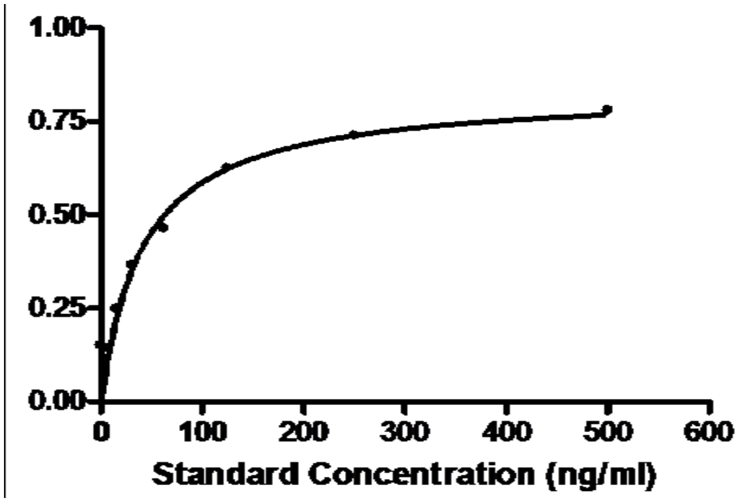

Figure 1. Standard curve with IgG concentration (ng/ml) along X-axis and absorbance at 450 nm along Y-axis.

are difficult to adopt in field, hence, cheaper and faster techniques employing portable instruments such as BRIX refractrometer and TP (Total protein) refractrometer are gaining popularity (Deelen *et al*., 2014; Elsohaby *et al*., 2019b; Giammarco *et al*., 2021).

The measures obtained for colostral and sera IgG levels can also be incorporated as a criteria while performing selection among buffaloes to ensure greater economic returns in the present as well as the future generations. A wide range of heritability estimates have been suggested for the IgG content in the colostrum and calf serum of cows (Conneely *et al*., 2013; Puppel *et al*., 2019; Maltecca *et al*., 2009; Norman *et al*., 1981; Martin *et al*., 2021). Accurate heritability values for the same should be determined in buffalo which will require phenotypic measurement of a large reference population.

CONCLUSION

The concentration of IgG in colostrum collected from recently parturated Murrah buffalo dams and serum samples from their calves was determined. The colostral IgG levels exhibited a wide variation emphasizing on the need for colostrum management in the herd. Several calves had a low serum IgG content raising concern over the successful transfer of passive immunity among neonates. Regular screening of adult females buffaloes and their calves for IgG content in their colostrum and serum, respectively can play a crucial role in reducing morbidity and mortality among the young ones and at the same time boost their productivity and growth as they grow up. Consequently, prevention of FPT can maximise the economic returns through better productivity, lowered veterinary expenses and fewer deaths.

Table 1. Concentration of IgG (mg/ml) in colostrums samples from Murrah buffalo dams.

Animal ID	**IgG Content**	**Animal ID**	**IgG Content**
B1	42.02	B41	18.84
B2	12.71	B42	51.66
B3	78.03	B43	48.29
B4	38.86	B44	134.43
B5	40.48	B45	22.7
B6	57.27	B46	34.14
B7	38.43	B47	48.67
B8	36.31	B48	46.25
B9	43.51	B49	21.33
B10	32.93	B50	20.75
B11	41.01	B51	20.83
B12	29.15	B52	88.97
B13	42.35	B53	30.96
B14	47.38	B54	32.21
B15	34.22	B55	54.29
B16	42.31	B56	43.29
B17	42.3	B57	51.67
B18	38.24	B58	17.85
B19	47.45	B59	34.16
B20	41.38	B60	35.62
B21	45.8	B61	23.84
B22	45.43	B62	87.34
B23	48.39	B63	81.17
B24	55.02	B64	28.24
B25	48.88	B65	27.91

Table 1. Concentration of IgG (mg/ml) in colostrums samples from Murrah buffalo dams. (Continue)

Animal ID	**IgG Content**	**Animal ID**	**IgG Content**
B26	97.48	B66	70.59
B27	37.84	B67	11.22
B28	39.83	B68	41.16
B29	53.6	B69	50.61
B30	40.4	B70	29.16
B31	55.73	B71	50.35
B32	31.74	B72	31.29
B33	56.87	B73	23.06
B34	64.2	B74	24.71
B35	227.78	B75	185.01
B36	70.54	B76	128.81
B37	119.01	B77	76.05
B38	72.75	B78	37.06
B39	66.11	B79	132.94
B40	40.65	B80	70.76

Table 2. Concentration of IgG (mg/ml) in serum samples of neonatal calves.

Animal ID	**IgG content**	**Animal ID**	**IgG content**
C1	18.06	C41	13.94
C2	7.15	C42	14.12
C3	19.52	C43	14.39
C4	14.92	C44	14.01
C5	16.45	C45	14.12
C6	9.17	C46	14.88
C7	15.16	C47	13.72
C8	8.08	C48	14.23
C9	17.24	C49	10.88
C10	2.9	C50	9.22
C11	3.46	C51	13.64
C12	4.55	C52	1.41
C13	6.28	C53	0.59
C14	3.35	C54	13.58
C15	3.74	C55	13.58
C16	2.82	C56	13.58
C17	19.45	C57	0.25
C18	4.64	C58	13.97
C19	3.8	C59	13.97
C20	5.53	C60	13.97
C21	6.25	C61	13.63
C22	3.97	C62	14.24
C23	4.34	C63	1.05
C24	1.71	C64	14.15
C25	19.17	C65	14.1

Table 2. Concentration of IgG (mg/ml) in serum samples of neonatal calves. (Continue)

Animal ID	IgG content	Animal ID	IgG content
C26	19.88	C66	13.75
C27	9.88	C67	10.09
C28	4.57	C68	13.85
C29	6.98	C69	13.95
C30	15.35	C70	13.66
C31	11.11	C71	13.91
C32	6.47	C72	1.01
C33	14.4	C73	13.71
C34	18.44	C74	13.49
C35	13.72	C75	0.54
C36	3.83	C76	13.88
C37	4.51	C77	13.51
C38	16	C78	13.98
C39	19.31	C79	14.38
C40	14.78	C80	14.35

Table 3. Mean, standard deviation, minimum and maximum value of IgG content in colostrum and serum.

Type of sample	Mean (mg/ml)	SD	Minimum (mg/ml)	Maximum (mg/ml)
Colostrum	50.44	3.36	12.71	227.78
Calf serum	10.85	0.62	0.25	19.88

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Ethics approval

Due approval was obtained from Institutional Animal Ethics Committee prior to collection of blood samples from buffalo calves.

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