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Immunoglobulin G and Total Protein Concentration in Blood and Colostrum of Different Cattle Breeds and its Passive Transfer to Neonatal Calves

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MS and RK designed the study and planned the experiments. IA, JH, SUR and SU helped in the analysis. MS wrote the manuscript. RK, SJM and MJ revised the manuscript.

Key words
Refractometer, Achai, Pre-partum, Post-partum, IgG

ABSTRACT

The aim of this study was to determine the effect of cattle breed type on (a) cow serum IgG and serum total protein concentration (b) colostrum immunoglobulin level and (c) their respective calves' serum immunoglobulin and serum total protein concentration. Three breeds of cattle were observed: Jersey, Holstein Friesian (HF) and local Pakistani cow breed Achai. To assess serum IgG, sodium sulphite precipitation technique was used while IgG in colostrum were determined using digital Brix refractometer (Atago RX-1000) and serum total protein (STP) in cows and calves were analyzed by chemistry analyzer (Procan PS-520). Overall Achai breed of cattle showed highest mean value for pre and postpartum serum immunoglobulin (11.78 ± 0.92 mg/ml, 10.00 ± 1.09 mg/ml) than Jersey breed (9.80 ± 1.30 mg/ml, 7.86 ± 1.01 mg/ml) and HF breed (7.86 ± 1.30 mg/ml, 6.43 ± 0.92mg/ml) respectively. Calf serum IgG was higher ($P < 0.05$) for Achai cow breed than other two cow breeds. The mean Brix (%) value for colostrum of Achai breed was higher (24.03% Brix) than Jersey and HF breeds. In the current study, there was a positive significant ($P<0.05$) correlation found for pre-partum IgG with postpartum STP and calf serum IgG. The results revealed that Achai breed had better potentials for serum and colostrum immunoglobulins (IgG) and total proteins (STP) production under local environment than HF and Jersey. Calf serum immunoglobulin concentration was affected by cattle breed type and showed a positive correlation with cow serum IgG decrease before parturition. It was found that the Partum stage of different cattle breeds has a significant effect on IgG in colostrum and serum of their respective calves. Therefore, the present study provided a baseline for the improvement of passive transfer of immunity to newborn calves while manipulating IgG concentration in the pre-partum stage of cattle.

INTRODUCTION

Calf is one of the saleable products in dairy industry and therefore a basic management objective is to make sure that the calf survives and remains healthy. Tolerable resistance to infection after post-parturient period is vital for the health and existence of a calf (Woods and Roussel, 1993). Neonatal calf morbidity and mortality are the major economic losses to livestock industry. In Peshawar commercial dairy farms (17.98%) neonatal calves' mortality in buffalo and calves were reported by (Khan *et al*., 2007). Mortality rate in cattle and buffalo calves in Pakistan ranged from 29.1% to 39.8% (Afzal *et al*., 1983). (Martin) reported that calf mortality of 20% decrease the profit of livestock farm to 38%. The main cause of high mortality in commercial dairy farms is the inadequate feeding and health facilities like colostrum feeding, inadequate milk feeding, naval cord disinfection and timely treatment (Tiwari *et al*., 2007). Calf mortality can only be reduced by finding and targeting its specific

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reasons (Khan and Khan, 1995).

Neo-born calves in cattle are born lacking of circulating immunoglobulins, because of syndesmochorial type of placenta and due to the placental barriers the larger immunoglobulin G cannot pass through placenta so the neonatal calves achieve their passive immunity by feeding maternal colostrum (Blum and Baumrucker, 2008). It has been described by (Hernandez-Castellano *et al*., 2015), that the immune status can influence the transfer of immune components from blood to colostrum. According to (Arthington *et al*., 2000) unsuckled neonatal calves' serum IgG concentration is enormously low (<0.1 g/L). The ability of the new-born calf to absorb colostral IgG decreases rapidly after birth. Therefore, they must absorb maternal immunoglobulins via intestine from colostrum for passive immunity during the neonatal period. Transport of these immunoglobulins from maternal serum to colostrum starts 5 weeks prior to parturition and reach to its optimum peak level at 1 to 3 days before parturition while cessation of these immunoglobulins occur immediately just after parturition (Sasaki *et al*., 1976).

Effects of breed have been reported for blood immunoglobulin's concentration in cow serum (Norman *et al*., 1981; McGee, 1997; Guy *et al*., 1994) and serum immunoglobulin G concentration of calves (Vann *et al*., 1995a). Breed effects have also been reported for colostrum immunoglobulin concentration (Muller and Ellinger, 1981). They revealed that HF and Jersey breeds have significant effect on colostrum immunoglobulin G concentration compared with Arshyre cows. According to the results obtained by (Hernandez-Castellano *et al*., 2015; Poulsen *et al*., 2010; Tyler *et al*., 1996) if neonatal calf does not receive sufficient amount of colostrum or if the colostrum is deprived of immunoglobulin's, then the new born calf may suffer from the failure of passive immunity transfer (FPIT). Failure of passive immunity transfer is defined as that serum which have less than 10 mg/ml of immunoglobulin G (IgG) or calf serum which have less than 5.2 gm/dL serum total protein (STP) (Calloway *et al*., 2002; Moore *et al*., 2005; Tyler *et al*., 1996). The objective of this study was to determine the effect of cattle breed type on (a) cow serum and colostrum immunoglobulin level and (b) their respective calves' serum immunoglobulin concentration.

MATERIALS AND METHODS

Ethics statement

This study has been reviewed and approved by the institutional animal care and use committee of the University of Agriculture Peshawar, Faculty of Animal Husbandry and Veterinary Sciences, and was performed in accordance with the relevant guidelines and regulations.

Animals selection and sampling

A total of 168 mature advance pregnant cows were selected of Achai, Jersey and Holstein Friesian (HF) cattle breeds. Achai are small-sized dairy cattle breed found in Khyber Pakhtunkhwa province and are famous for its high resistant to extreme environmental conditions. Each breed of cattle group has been comprised of 56 cows with parity range from 2-5 with known expected dates of parturitions. The samples were collected from Cattle Breeding and Dairy Farm Hari Chand. Serum and colostrum samples were collected from April 2015 to September 2015. Two times of 5ml blood samples were collected from 168 cows at pre-partum (15 days prior to parturition) and post-partum stages (within 2 h of parturition) with no (EDTA) solution in test tubes. Immediately after post-partum and prior to suckling a 20 ml of fresh-pooled colostrum were collected from the right front quarter or hindquarter of the udder. The colostrum samples were placed in plastic bottles labeled with each cow identification number. Calves were fed whole milk at a rate of 5% of birth weight, twice daily. After calving, the calves received 1.5 liters of colostrum from their mother through teat bottle. After colostrum feeding to neonatal calves 5ml of blood samples were collected in test tubes with no anticoagulant from a jugular vein after 24 hours of their 1st colostrum feeding.

Centrifugation and transportation of samples

After collection of all blood samples, were then kept undisturbed at room temperature for 15 to 30 minutes for clot formation. Then the samples were centrifuged at 3,000 rpm for 15 minutes. Serum and colostrum samples were collected with universal safety measures and put into leak proof container. Samples were properly packed, labeled with a specific codes and collection date and kept in a container having dry ice pieces. The container was sent within 2 h to laboratory. All samples were transported to Pathology lab at UAP and kept frozen at -20˚Cuntil analysis.

Immunological measurements of serum

Analysis of immunoglobulin G (IgG) in cow and calves' serum were performed by sodium sulphite precipitation method as described by (Dawes *et al*., 2002). This test uses 14%, 16% and 18% sodium sulphite solutions prepared by dissolving 14, 16 and 18 gm of anhydrous salt each in a total volume of 100 ml distilled water. Then 9 ml of each solution samples were added in three test tubes. Finally, 0.9 ml of serum sample were added, in each test tube and properly mixed, then allowed to stand undisturbed for 30 min.

Analysis of serum total protein

For estimation of total protein (TP) in serum samples Chemistry Analyzer (Procan PS-520) and protein kit (Human Total Protein liquid color) was used. Serum total proteins (STP) analyzed by the Biuret method as described by (Josephson and Gyllensward, 1957).

Colostrum analysis through brix refractometer

Analysis of IgG in colostrum samples digital brix refractometer (ATAGO RX-1000) used to assess failure of passive transfer (FPT). For the digital Brix refractometer, approximate 250 µL of colostrum sample were used, and then the Brix score of the liquid determined by shining a light through the sample in the prism, measuring the refraction index, and representing the reading (%Brix) on a digital scale (Bielmann *et al*., 2010; Quigley *et al*., 2013).

Statistical analysis of data

The data was statistically analyzed for the effect of breed and partum stage on serum immunoglobulin and %Brix of colostrum using General linear model (GLM) technique in statistical program (SAS, 2002). Analysis of variance was carried out using PROC of SAS (SAS, 2002). The means compared by Duncan multiple range test (DMR). Computer software Excel (Microsoft 2016) was used for storage of data. Values considered significant when $P < 0.05$.

RESULTS

The immunoglobulin (IgG) concentration in serum samples of cows and calves.

The data of serum IgG concentration of cows with different partum stages and calves at 24 h after colostrum feeding is presented in Table I. In prepartum stage of cattle, the highest mean concentration of IgG was found in Achai breed of cattle followed by Jersey breed while lowest mean concentration was found in Holstein Friesian breed of cattle. Breed effect on postpartum serum IgG was found significant (P<0.05). Mean value of IgG for Achai breed of cattle was higher than the mean value of Jersey breeds of cattle, while the lowest concentration was recorded in Holstein Friesian breed of cattle. Effect of breed on calf serum immunoglobulin was found significant (P<0.05) as shown in Table I. The mean value of IgG in Achai calves showed highest concentration followed by Jersey calves and the lowest mean value of IgG was found in HF calves.

Serum total protein (STP) concentration of cows and calves and Brix% values of fresh pooled colostrum.

The data of STP concentration in serum samples of cows and calves and Brix% values of colostrum is presented in Table II. Effect of breed have found highly significant (P<0.001) on prepartum and postpartum STP. Highest mean value of serum total protein was found for Achai breed followed by Jersey breed and the lowest mean value was found for Holstein Friesian breed of cattle at pre and postpartum stages respectively. While the effect of breed on calf serum total protein was found highly significant (P<0.05) for calves' serum total protein. Mean value for Jersey calves was highest in concentration followed by Achai calves and the lowest mean value was found in HF.

For the estimation of colostrum IgG Brix refractometer was used. The data of Brix (%) values are presented in Table II. Similarly effect of breed on colostrum IgG %Brix value was found significant (P<0.05). Mean %Brix value of colostrum in Achai breed of cattle was found highest, second highest %Brix value was found for Jersey breed of cattle, while the lowest mean value was found for HF breed of cattle.

Table I. The immunoglobulin (IgG) concentration (mg/ml) (Mean±SEM) in serum samples of cows and calves.

Breed	Prepartum serum IgG in Cows	Postpartum serum IgG in Cows	Calves serum IgG at 24 h
Jersey	9.80 ± 0.92	7.86 ± 1.01	11.43 ± 0.92
HF	7.86 ± 1.30	6.43 ± 0.92	9.29 ± 1.30
Achai	11.78 ± 0.92	10.00 ± 1.09	12.14 ± 1.49
P-Value	<0.05	<0.05	<0.05

Abbreviations: IgG, immunoglobulin G; SEM, standard error of mean; significant difference (P<0.05).

DISCUSSION

The transition period is considered the most critical and challenging for the dam health status during the lactation cycle (Hernandez-Castellano *et al*., 2017) and (Kessel *et al*., 2008). In the present study effect of different breeds was evaluated on cow serum IgG and serum total protein concentration, colostrum IgG and their respective calves' serum immunoglobulin and serum total protein concentration. The findings of the present study are in consistent with the findings of (Murphy *et al*., 2009) and (Bayram *et al*., 2016). They studied that there is significant variation of breed in the concentration of pre-partum serum immunoglobulin G. According to Hernandez-Castellano *et al*. (2018) and Barrington *et al*. (1997) variations are present in serum immunoglobulin's concentration for different cattle breeds. The high concentration of serum immunoglobulin concentration in Achai breed may be due to their high adoptability and acclimatization to local

Table II. Serum total protein (STP) concentration (mg/ml) (Mean±SEM) of cows and calves and Brix% values of fresh pooled colostrum.

Breed	Prepartum serum total protein (STP) in Cow	Postpartum serum total protein (STP) in Cows	Calves serum total protein (STP) at 24 h	Colostrum Brix (%) for Cow
Jersey	5.71 ± 0.42	5.28 ± 0.26	7.57 ± 0.37	21.84 ± 1.05
HF	5.28 ± 0.46	4.85 ± 0.57	5.00 ± 0.53	17.13 ± 1.49
Achai	7.28 ± 0.58	7.00 ± 0.48	7.14 ± 0.51	24.03 ± 0.72
P-Value	<0.05	<0.05	<0.05	<0.05

Abbreviations: STP, serum total protein; SEM, standard error of mean; significant difference ($P<0.05$).

environment compared with HF and Jersey breed of cattle. The current study is in consistent with the arguments of (Tao and Dahl, 2013). They studied that high ambient temperature effect the performance of dairy cattle's. The present findings are in line with the results of (Hernandez-Castellano *et al*., 2017; Logan *et al*.,1981; Shell *et al*., 1995). Their results show that immunoglobulin G concentration in serum of cow decreases after 1-day postpartum and relatively low up to 2 to 4 weeks. (Norman *et al*., 1981) also justify our findings. He found that breed differences are present in cow serum IgG concentration.

The current results of serum total protein are similar with the previous findings (Calloway *et al*., 2002; Tyler *et al*., 1996). They recommended that serum total protein cut-point ranges of 5.0 to 5.5 gm/dL equal to serum immunoglobulin G level of 8.9 to 13.4mg/ml (Tyler *et al*., 1998) found that serum total protein below 5.0m/dL will be at risk of failure of passive immunity transfer. Previous studies of Ozcelik *et al*. (2017), Ali *et al*. (2019) and Tao and Dahl (2013) showed that performance of dairy animals are affected by several factors including season, latitude. The high concentration of serum total protein concentration in Achai breed may be due to their high adoptability and familiarization to native environment compared with HF and Jersey breed of cattle.

Ambient temperature and stress are important factors in the absorption of immunoglobulin and total protein in newborn calves. The present findings are in line with the arguments of Campbell *et al*. (2007). They opined that environmental stress has a significant effect on the absorption of immunoglobulin and total protein in newborn calves. The probable reason of high serum immunoglobulin in newborn Achai calves may be due to their adoptability to local environment. These finding supports the study of Deelen *et al*. (2014). He concluded that the calves of Jersey cattle had highest concentration of serum total protein (4.4 to 8.8 g/dl). Benavides-Varela *et al*. (2013) also studied the concentration of total serum protein in calves. He concluded that the calves of HF breed have lowest concentration of serum total protein (5.4 g/dl) compared with other cross breed. Villarroel *et al*. (2013) results shows that Jersey calves have significantly higher serum total protein than HF calve. Tendency exist that Jersey breed calves have higher STP concentration than HF calves after ingestion of colostrum (Tennant *et al*., 1969).

Effect of breed on colostrum IgG was not significant ($P>0.05$). Mean value for jersey breed was found highest, second highest mean value of colostrum for Achai breed of cattle while mean value for HF cattle breed was lowest than the other two breed. These findings are in line with the findings of Muller and Ellinger (1981). They concluded that there is no significant effect of breed on colostrum IgG however trends were existed that Jersey breed having highest IgG concentration. Similarly, Hernandez-Castellano *et al*. (2017) and (Hernandez-Castellano *et al*., 2018) also concluded that colostrum IgG in did not differ between two groups of cattle throughout the experimental period. (Vann *et al*., 1995b) observed the same findings that any breed type did not affect IgG concentration in colostrum. However, breed variations for colostrum immunoglobulin G in Holstein Friesian were found by (Pritchett *et al*., 1991) and in Jersey Cows (Logan *et al*., 1981).

The present study mean Brix% for HF cow's colostrum is similar to the findings of Quigley *et al*. (2013) and Hernandez *et al*. (2016). He concluded that when Brix refractometer is used for colostrum IgG than 21% Brix will be measured the break point for high quality (>50 gm of IgG/L) maternal colostrum. However, variation may exist in colostrum Brix% value among different cattle breeds (Morrill *et al*., 2012; Bielmann *et al*., 2010). Their findings of Brix% values for maternal colostrum are identical in line with the current study's findings. Brix % for Achai was found higher than other two breeds. There are many possible explanations for the difference between brix% reported in the present study and previous studies. In previous studies of Morrill *et al*. (2012), Bielmann *et al*. (2010), Quigley *et al*. (2013) a wide range of environmental conditions, feeding practices and management were involved which are known to affect colostrum quality. In the current study 168 milking animals were studied whereas the Morrill *et al*. (2012) having flock size of 80 to 5000 dairy cows were studied. Previous studies show that time variation in colostrum collection may also affect colostrum brix%. Chigerwe *et al*. (2008) and Moore *et al*. (2005) showed that colostrum collected more than 2 h after parturition than brix% in colostrum is significantly lower than the early collection.

CONCLUSION

In this study, the partum stage of different cattle breeds has a significant effect on IgG and total protein in colostrum and serum of their respective calves. Therefore, the current study provided a base line for the enhancement of passive transfer of immunity to newborn calves while manipulating IgG andserum total protein level in the pre-partum stage of cattle. Similarly, it was concluded that Achai and Jersey calves were in low risk for failure of immunity transfer (>10 mg/ml of IgG) while in HF calves medium risk of failure of immunity transfer (<10 mg/ml) was found. Brix refract meter was used among different cow breeds for colostrum IgG analysis which was considered easy and good indicator for IgG estimation in the field.

RECOMMENDATIONS

1. Research needed to be conducted to find out the sources of IgG and total protein supplementation for the cattle's in advance pregnant stages.
2. IgG estimation of cow and calves' serum through sodium sulfite precipitation test and serum total protein was considered good indicators of passive immunity in calves at laboratory.
3. Brix refractometer for colostrum analysis is considered easy and good indicator for estimation of passive immunity transfer.
4. In the current study HF calves were on risk due to low serum IgG in their dam serum and colostrum so supplementation is needed to the dam at advance pregnancy or treated colostrum is given to their calves to prevent from the failure of passive immunity transfer.

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Conflict of interest

The authors declare that they have no conflict of interest.

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