



PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF COLOSTRUM AND MILK UNDER TRANSITION STATE IN KUNDI AND NILI RAVI BUFFALO BREEDS OF PAKISTAN

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Abstract

Comparison of physicochemical properties of colostrum and transition milk of two breeds of buffalo (Kundi n=10 and Nili Ravi n= 10, total samples= 220) were investigated. Colostrum and transition milk were obtained 06, 12, 24,36, 48, 60, 72, 84, 96, 108, 120 hours after calving of each animal for analysis. The values of fat %, protein %age, lactose %age, SNF (Solids-Non-Fat)%age, Total solids (TS) %age, ash %age, pH and acidity% of Kundi buffalo colostrum after 6 hours of calving were 8.40±0.06, 13.82±0.33, 2.72±0.14, 20.07±0.01, 28.91±0.01, 1.04±0.10, 6.07±0.00, 0.32±0.00 and of Nili Ravi buffalo colostrum were found as 6.00±0.06, 12.34±0.72, 2.25±0.01, 19.79±0.01, 25.98±0.01, 0.98±0.04, 6.37±0.01 and 0.30±0.01 respectively. Viscosity of Kundi and Nili Ravi Buffaloes (K & NRB) 6.98±0.01b and 7.33±0.01a, respectively at 6 hours, Lysozyme (µg/ml) of buffalo Kundi colostrum after 6 hours of study was 0.32±0.01h and in Nili Ravi (0.34±0.00h). Lactoferrin (mg/ml) was decreased in both breeds of buffalo and at 120 hours after calving it was observed 0.59±0.01j and 0.47±0.01k (K & NRB) transition milk. Statistically results of Analysis of variance of fat %age, lactose %age, SNF %age, TS%, Immunoglobulins (mg/ml), lactoferrin (mg/ml), lysozyme (µg/ml) of (K & NRB) breeds p<0.001 were highly significant.

Key words: Colostrum, Transition milk, nutritional attributes, buffalo breeds (Nili Ravi & Kundi).

INTRODUCTION

Colostrum is very vigorous and energetic feed which is secreted from the mammary glands for the newly native individual in all mammals during first 5 to 7 days immediate after birth. It is the rich source of various nutrients like; protein, fats, glucose, water soluble vitamins, fat soluble vitamins and minerals. It also comprises of some biological and antibacterial active substances such as maternal antibodies, immunoglobulins and growth promoters (Elfstrand, Lindmark-Mansson, Paulsson, Nyberg, & Akesson, 2002). Colostrum is defined by various scientists, as the secretion of the mammary gland produced instantaneously & subsequently after parturition (Levieux, & Ollier, 1999) or collectively the secretion of first limited days after birth as mentioned by (Tsioulpas, Grandison, & Lewis, 2007). Others described colostrum is through two days' post parturition (Martin-Sosa, Martin,

García-Pardo, & Hueso, 2003). Colostrum is the first natural food obtained through the mammals after parturition time which is help full for newborn during 24-72 hours after birth of calf (Campbell, & Petersen, 1963).

The study on composition varies from colostrum to milk and post colostrum secretion which helps in establishing the fact that milk is suitable for use (Tsioulpas, Grandison, & Lewis, 2007). Composition of colostrum and milk are very different, properties are also different (Walstra, & Jenness, 1984). Colostrum is not a saleable foodstuff and its distinctive importance is that serve as a feed for offsprings to utilize much developed nutrition. Composition of colostrum changes from colostrum to normal milk (Prasad, 1997). The changes in colostrum composition and its properties are alteration in milk, may be sudden or gradual (Arain, Khaskheli, Arain, Soomro, & Nizamani, 2008). The protein segments of bovine colostrum have been comprehensively premeditated for instinctive husbandry, economic and nutritional causes. Colostrum comprises high contents of whey proteins as compared to mature milk (Levieux, & Ollier, 1999). Colostrum contains extra protein, fat, non-protein nitrogen, peptides, ash, minerals, vitamins and hormones, cytokines and nucleotides, growth factors and less lactose compared to mature milk. All these content levels reduced fastly throughout the first three days of lactation, except in the case of lactose which increased as colostrum changes into transition milk (Blum, & Hammon, 2000).

The Colostrum is a complex physiological fluid loaded with antimicrobial and immune-enhancing constituents that are unique as compared to normal milk (Aurelia, Cristian, Camelia, Vioara, & Gheorghie, 2009). Colostrum remains most important liquid material for the new native for its early nourishment. It also has significance for baby at early age to get immune against infectious diseases since they have no any antibodies which fight against the microbes (Quigley, 2002). Colostrum has fat, macro nutrients, micronutrients and it also contains vitamins (Zachwieja et al., 2007).

Colostrum comprises plenty of physiologically and immunologically active constituents, which contain immunoglobulins, lactoferrin, leucocytes, lysozyme, cytokines and further immune-modulatory factors, growth factors and hormones etc. The previous bioactive constituents partake in the preceding period attracted an increasing interest in commercial manipulation of colostrum for pharmaceutical and dietary practices (Roginski, Fuquay, & Fox, 2003). Plasma cells made Immunoglobulins existing in the body and these cells are originally obtained from bone marrow cells. The plasma cells are existing in various positions in body. These cells also secrete immunoglobulins that gather in the blood. The cells can be applied through the calf for the mandatory response of immunity (Sing, Chandra, Huozha, & Kushwaha, 2011).

MATERIALS AND METHODS

Research Station: Research was conducted in the laboratory of Department of Dairy Technology and in Central laboratory complex, A-Block in University of Veterinary and Animal Sciences (UVAS), Ravi Campus, Pattoki, District Kasur.

Collection of samples: After calving colostrum and transition milk from Nili Ravi and Kundi buffaloes (10 animals each of 2nd calving) were collected from Buffalo Research Institute, Tehsil Pattoki, District Kasur and Tehsil Ali Pur District Muzaffargarh, Province Punjab Pakistan, respectively in sterile bottles. The samples of first five days with morning and evening milking and 6 hours after birth of calves, total sample (n= 220) were collected and analysed. These samples were labeled, ice-packed and then transferred to the laboratory of Department of Dairy Technology, UVAS, Ravi Campus, Pattoki. All colostrum and transition milk samples were kept at 4°C in refrigerator and used for further analysis. The detail of experimental plan is provided in Table-1

Table-1: Detail of sampling.

Sr. #	Species	Breed	Days	Milking time	Experimental unit
1.	Buffalo	Nili Ravi	1	Morning + Evening	(10 animals of each breed)
			2	Morning + Evening	(Nili Ravi + Kundi)
			3	Morning + Evening	Species = 1
			4	Morning + Evening	Breeds = 2
			5	Morning + Evening	Colostrum & Transition milk = 5 days
		Kundi	1	Morning + Evening	Timing= 2
			2	Morning + Evening	10 x 1 x 2 x 5 x 2 =200
			3	Morning + Evening	After calving 6 hours
			4	Morning + Evening	(10 animals of 2 breeds =10x2= 20)
			5	Morning + Evening	Total samples (n)= 200+20= 220

Nutritional Attributes: Nutritional attributes of colostrum and transition milk contains chemical and physical analysis including pH, acidity, protein, fat, TS and ash contents and these were measured by the methods (AOAC, 2005). The method described (Pearson, 1976) was applied to obtain the SNF value of colostrum and transition milk. Lactose contents of colostrum and transition milk were determined by the anthrone method (Richard, 1959). Viscosity of colostrum and transition milk was measured according to the method described by (AOAC, 2000). Immunoglobulins of colostrum and transition milk were determined through the method described by (Fleenor, & Stott, 1980), in Bio-imaging laboratory, Central laboratory Complex, A-Block, Ravi Campus. Lactoferrin and lysozyme of colostrum and transition milk were determined through HPLC (Model 1260, infinity II, Agilent tech., Germany) in Central laboratory complex, A- Block, Ravi campus UVAS by the method as described by (Billakanti, Fee, Lane, Kash, & Fredericks, 2010).

Statistical analysis: The experiment was performed in (CRD) completely randomized design. Factorial analysis of variance technique (ANOVA) was used for data analysis. DMR Test was made for significant difference among the treatment means (Steel, Torrie, & Dickey, 1997).

RESULTS AND DISCUSSIONS

Fat contents (%age): Fat contents of (K & NRB) colostrum were studied at different time intervals. After calving 6 hours' fat%age of Kundi buffalo (8.4%) while Nili Ravi buffalo colostrum contains (6%). Fat %age of Kundi buffalo colostrum after 12 hours was (9.3%) and then decreased gradually upto 120 hours study. Fat %age in Nili Ravi was also decreased from 6 hours' study to 120 hours of study. Study of 120 hours (5th day Evening) milking fat %age was 5.70±0.06 of Kundi buffalo transition milk and 4.97±0.02 of Nili Ravi buffalo transition milk. Mean values of (K & NRB) fat %age were 7.38±0.03 and 4.97±0.02 respectively. Fat %age of Kundi buffalo colostrum and transition milk was higher as compared to fat %age of colostrum and transition milk of Nili Ravi buffalo colostrum. Analysis of variance of fat of (K & NRB) breeds p values were source: breeds p<0.001, time p<0.001, breeds*time interaction p<0.001 and model p<0.001.

Fat % of buffalo colostrum to transition milk was decreased slowly (Rifaat, Hassan, El-Alamy, & Abd El-Salam, 1972), (El-Loly, 2005) and (El-Loly, & Salim, 2008) found that the fat contents of colostrum were higher first but it was reduced gradually after about 3rd milking. Literature of colostrum composition and reported the mean percentage of fat, protein, and lactose in cow colostrum that were 6.7, 14.9, and 2.5, respectively (Kehoe, Jayarao, & Heinrichs, 2007). The changes in physico-chemical characteristics from colostrum to milk showed a wide range of values as suggested (Arain, Khaskheli, Arain, Soomro, & Nizamani, 2008). These changes might be due to alterations in the configuration of colostrum and milk such as colour and appearance of colostrum was yellowish while it was white in milk which might be due to the variation in protein contents in colostrum of black and white cows (Cziszter et al., 2008). Changes in proteins, fat, lactose and TS concentration of buffalo colostrum showed similar trend as reported by (Nawar, 2006) in case of Egyptian buffalo's colostrum. As the transition period advances, these components in both colostrum and transition milk decreased gradually as suggested by (Qyenyiyi, & Hunter, 1978).

Solid Not Fat (SNF): Mean \pm SD values of (K & NRB) colostrum were (13.88 \pm 0.01) and (12.59 \pm 0.01), respectively. Overall SNF %age of (K & NRB) colostrum to transition milk decreased. Overall SNF %age at different study hours of Kundi buffalo colostrum to transition milk was higher as compared to SNF %age of Nili Ravi buffalo colostrum to transition milk (Table No. 1). Analysis of variance of SNF %age of (K & NRB) breeds ($p < 0.001$) highly significant. Solids-not-fat contents was higher after calving about (23.08%); then gradually reduced till it approached at a level of 10.45% at the 72h after calving. Also, obtained the mean value of SNF %age for normal milk was 9.44%; which is lower than our results at 3rd day (El-Agamy, Abou-Shloue, & Abdel-Kader, 1998). These wide differences in SNF %age contents of colostrum may be due to the individuality in buffalo's breeds (Andrew, 2001).

Proteins: Protein %age contents of Kundi buffalo colostrum after 6 hours (13.82 \pm 0.33) and Nili Ravi (12.34 \pm 0.72) were obtained. Protein %age of Kundi buffalo colostrum in first 12 hours were higher as compared to first 12 hours' study of Nili Ravi buffalo colostrum. Then Nili Ravi colostrum after 24hours was higher values as compared to Kundi buffalo colostrum. Protein %age of (K & NRB) colostrum decreased from calving to 5th day. After 120 hours' protein contents of Kundi buffalo transition milk was (3.97 \pm 0.10) and Nili Ravi buffalo transition milk was (4.13 \pm 0.50). Nili Ravi protein %age was decreased less after 120 hours as compared to protein %age of Kundi buffalo protein %age (Table No. 1). Analysis of variance of protein %age of (K & NRB) breeds p values were; source: breeds $p > 0.05$ (0.5239 non-significant), time $p < 0.001$ (highly significant), breeds*time interaction $p > 0.05$ (0.3764 non-significant) and model $p < 0.001$.

Results of recent study are similar with other scientist's work; they reported the very 1st milking of colostrum contained 18.44 percent proteins, an average of within 12 hours of parturition. Protein rapidly reduced and reached to an average value of (5.83) percent at 48hours postpartum. Protein %age progressively reduced to an average value of (4.18) percent at 72 hours after calving. This declined trend is generally because of higher level of globulins that helps as the transporter of antibodies for suckling calf against disease producing organisms (Nickerson, 1995). The results of current study are in according to that attained (El-Loly, & Salim, 2008). Colostrum composition; the mean values of fat%, protein%, and lactose% in cow colostrum (6.7, 14.9, and 2.5) respectively (Kehoe, Jayarao, & Heinrichs, 2007). The changes in physicochemical properties from colostrum to milk displayed a wide range (Arain, Khaskheli, Arain, Soomro, & Nizamani, 2008). These changes might be due to variances in the conformation of colostrum and milk such as colour and appearance of colostrum was yellowish while it was white in milk which might be due to the variation in protein contents as described by (Cziszter et al., 2008) in colostrum of black and white cows. Changes in proteins %, fat %, lactose % and TS concentration of colostrum of buffalo were similar as reported by (Nawar, 2006) in case of Egyptian buffaloes' colostrum. As the transition period passed these constituents in colostrum's declined gradually as mentioned by (Qyenyi, & Hunter, 1978).

Lactose: Lactose %age of Kundi buffalo colostrum was higher after 6 hours of calving as compared to Nili Ravi buffalo colostrum. Lactose %age of Kundi buffalo colostrum to transition milk and Nili Ravi Buffalo colostrum to transition milk enhanced as time increased and these values reached (4.49 \pm 0.01 and 4.19 \pm 0.01) whereas initially after 6 hours these were (2.72 \pm 0.14 and 2.25 \pm 0.01), respectively. Mean \pm SD of (K & NRB) colostrum to transition milk were 3.66 \pm 0.01 and 3.25 \pm 0.01, respectively (Table No. 2). Analysis of variance of lactose %age of (K & NRB) breeds p values were source: breeds $p < 0.001$, time $p < 0.001$, breeds*time interaction $p < 0.001$ and model $p < 0.001$.

Results of recent study correlated to (Roy, 1970). He reported that lactose concentration was lower (3.27) % in colostrum, formerly slowly increased to reach its normal level (5.44) percent on the 3rd day of calving. The alteration is an improvement because lactose can induce the young to scour (diarrhea) through subsequent death or unthriftiness (Roy, 1970). These results were in agreement with (Rifaat, Hassan, El-Alamy, & Abd El-Salam, 1972) and (El-Loly, & Salim, 2008). These results also correlated to (El-Agamy, Abou-Shloue, & Abdel-Kader, 1998). Colostrum composition (mean

%ages) of fat, protein, and lactose in cow colostrum were 6.7, 14.9 and 2.5, respectively (Kehoe, Jayarao, & Heinrichs, 2007). The variations in physico-chemical characteristics from colostrum to milk presented a wide range of ethics (Arain, Khaskheli, Arain, Soomro, & Nizamani, 2008). These deviations might be due to alterations in the composition of colostrum and milk such as colour and appearance of colostrum was yellowish although it was white in milk which might be due to the difference in protein substances (Cziszter et al., 2008) in colostrum of black and white cows. Changes in lactose concentration of buffalo colostrum were similar as stated by (Nawar, 2006) in Egyptian buffaloes. As the transition period passed these constituents in both colostrum and transition milk decreased regularly as suggested by (Qyeniyi, & Hunter, 1978)

TS: TS %age of Kundi buffalo colostrum and transition milk at 6 hours of calving were 28.91 ± 0.01 and TS %age of Nili Ravi buffalo colostrum and transition milk at 6 hours of calving were 25.98 ± 0.01 . TS of both breeds decreased from 6 to 120 hours of study. After 120 hours TS of transition milk of (K & NRB) were 16.67 ± 0.01 and 14.46 ± 0.01 , respectively. TS of Kundi buffalo colostrum and transition milk were higher all time as compared to the TS of colostrum and transition milk of Nili Ravi buffalo. Average mean values of Kundi buffalo colostrum and transition milk (21.18 ± 0.01) were also higher as compared to colostrum and transition milk (17.73 ± 0.01) of Nili Ravi buffalo colostrum (Table No. 2). Analysis of variance of TS %age of (K & NRB) breeds p values were source: breeds $p < 0.001$, time $p < 0.001$, breeds*time interaction $p < 0.001$ and model $p < 0.001$.

TS declined rapidly in colostrum throughout the transition period from colostrum to milk. Rates of changes were comparable to (El-Loly, & Salim, 2008). The most differences of TS contents in colostrum dissimilarity to normal milk; could be the consequence of raised content of antibodies of colostrum (Nickerson, 1995). As the transition period succeeded these constituents in both colostrum's reduced increasingly as suggested by (Qyeniyi, & Hunter, 1978).

Ash: Ash %age of Kundi buffalo colostrum after 6 hours of calving (1.04 ± 0.10) and of Nili Ravi colostrum (0.98 ± 0.04) were determined. Ash %age of (K & NRB) transition milk at 120 hours 0.76 ± 0.01 and 0.79 ± 0.02 , respectively were obtained. Ash %age of Kundi buffalo colostrum after 6, 12, 24 and 36 hours was higher as compared to sister breed. After 48 and 60 hours of study ash %age was equal. After 120 hours of study ash %age of Kundi buffalo transition milk 0.76 ± 0.01 was lower as compared to Nili Ravi buffalo transition milk (0.79 ± 0.02). Mean \pm SD of Kundi buffalo colostrum and transition milk (0.88 ± 0.04) and Nili Ravi buffalo colostrum and transition milk (0.87 ± 0.02) were noticed. Average mean of Kundi buffalo colostrum and transition milk were higher as compared to colostrum and transition milk of Nili Ravi (Table No. 2). Analysis of variance of ash %age of (K & NRB) breeds p values were source: breeds $p > 0.05$ (0.7177 non-significant), time $p < 0.001$, breeds*time interaction $p > 0.05$ (0.9895 non-significant) and model $p < 0.0018$ (highly significant) colostrum and transition milk were determined. Present study results when compared to other scientists results as ash was higher after parturition; then declined gradually as days passed after parturition. These results were similar to (El-Loly, 2005).

pH: pH of Kundi buffalo colostrum was 6.07 ± 0.00 after 6 hours of calving and Nili Ravi buffalo colostrum 6.37 ± 0.01 was obtained. pH of both breeds increased from 6 hours to 120 hours (5th day) after calving. Results of pH of (K & NRB) transition milk after 120 hours (6.58 and 6.67 ± 0.01 , respectively). pH values of Nili Ravi buffalo colostrum and transition milk were higher as compared to Kundi buffalo colostrum and transition milk pH. Mean \pm SD values of Kundi buffalo colostrum and transition milk (6.34 ± 0.01) was higher as compared to Mean \pm SD values of pH of Nili Ravi buffalo colostrum and transition milk 6.56 ± 0.01 (Table No. 3). Analysis of variance of pH of (K & NRB) breeds p values were source: breeds $p > 0.05$ (0.3247 non-significant), time $p > 0.05$ (0.4215 non-significant), breeds*time interaction $p > 0.05$ (0.4542 non-significant) and model $p > 0.05$ (0.4542 non-significant). Results of present study were compared with the study of other scientists; pH value of buffalo colostrum increased as studied by (Haggag, Mahran, Ali, & Hamzawi, 1991), they found that

the pH value of colostrum was (6.43±0.02). Madsen, Rasmussen, Nielsen, Wiking, & Larsen (2004) also reported that the mean value of pH in cow colostrum was 6.42 at 3rd milking at parturition which are correlated to our results. The mean value of pH for normal milk of buffalo was 6.70 (El-Agamy, Abou-Shloue, & Abdel-Kader, 1998).

Acidity: Acidity% of Kundi buffalo colostrum after 6 hours was higher as compared to Nili Ravi Buffalo colostrum 0.32±0.00 and 0.30±0.01, respectively. After 12 hours study acidity% of Kundi buffalo colostrum was also higher as compared to acidity% of Nili Ravi buffalo colostrum 0.28±0.00 and 0.27±0.01, respectively. Acidity values of Kundi buffalo transition milk after 120 hours (0.17±0.01) and Nili Ravi Buffalo transition milk (0.18±0.01). Mean±SD values of colostrum and transition milk of Kundi buffalo (0.22±0.01) were lower as compared to Mean±SD values of colostrum and transition milk of Nili Ravi buffalo 0.23±0.01 (Table No. 3). Analysis of variance of acidity% of (K & NRB) breeds p values were source: breeds p>0.05 (0.6980 non-significant), time p<0.0001 (highly significant), breeds*time interaction p (0.0274) and model p<0.0001 highly significant colostrum and transition milk were observed. Acidity %age of milk during colostrum periods ranges between 0.22 and 0.32. Data of acidity %age remained higher as mean value of buffalo milk acidity was 0.175% while in cattle milk acidity was 0.172%, as reported by (Hofi, Rifaat, & Khorshid, 1966). Acidity in colostrum and milk were 0.20% and 0.15% were observed (Haggag, Mahran, Ali, & Hamzawi, 1991), these values are lesser than our study results which were (0.22 and 0.23%). The mean value 0.18% of acidity in normal milk of buffalo had been reported and its clear that the pH value took an opposite trend of acidity %age (El-Agamy, Abou-Shloue, & Abdel-Kader, 1998).

Viscosity: Viscosity in colostrum and transition milk of different breeds of buffaloes at different hours (6, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120) for 5 days after calving was determined (Table No. 4). Viscosity of K & NRB 6.98±0.01cP and 7.33±0.01cP, respectively at 6 hours after calving was measured. Viscosity of both breeds (K & NRB) colostrum to transition milk decreased from 6 hours to 120 hours after calving. Viscosity of (K & NRB) at 120 hours after calving was 1.61±0.00cP and 1.70±0.01cP, respectively. Viscosity of Kundi buffalo colostrum and transition milk was lower at 6 hours and 120 hours after calving as compared to Nili Ravi colostrum and transition milk. Mean±SD values of (K & NRB) colostrum and transition milk were 3.04±0.01cP and 3.27±0.01cP, respectively. Mean±SD values of Nili Ravi buffalo colostrum and transition milk was greater as compared to Kundi buffalo colostrum and transition milk. Highest viscosity was measured in buffalo Nili Ravi colostrum at 6 hours after calving 7.33±0.01cP. Results of our study were agreed with that throughout the transition from colostrum to normal milk the viscosity drops. The change was being fastest through the first 3 milking (Kadian, 2000). These differences appeared to be correlated to changes in specific gravity, solids-not-fat, albumin-globulin and total protein fractions. The higher serum protein content level causes colostrum to gel like egg white when heated. Coagulation temperature of colostrum is usually less than normal milk (Kadian, 2000). ANOVA of viscosity %age of Nili Ravi and Kundi Breeds were p<0.0001.

LR/Specific Gravity: LR value in colostrum and transition milk of different breeds of buffaloes at different hours (6, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120) for 5 days after calving was noticed (Table No. 4). LR values of (K & NRB) colostrum and transition milk after 6 hours of calving were measured as 70.00±0.58 and 71.00±0.58, respectively. These values shows that LR values of Nili Ravi buffalo colostrum was higher as compared to LR values of colostrum and transition milk of Kundi buffalo. LR values of both breeds (K & NRB) colostrum to transition milk declined gradually from 6 - 120 hours after parturition. LR of (K & NRB) at 120 hours after calving was 36.00±0.58 and 33.00±0.58, respectively. These values showed that LR values of Kundi buffalo colostrum and transition milk was higher at 120 hours as compared to LR values of Nili Ravi colostrum and transition milk. Nili Ravi and Kundi buffaloes p<0.0001 in breeds and time but p>0.05 (p=0.1440) non-

significant. ANOVA of LR and Specific gravity were same. LR values of colostrum to transition milk in breeds of buffalo decreased at 6 hours to 120 hours (1st day to 5th day) after calving. SG values of (K & NRB) colostrum and transition milk after 6 hours of calving 1.07 ± 0.00 and 1.072 ± 0.00 , respectively were measured. These values showed that SG values of Nili Ravi buffalo colostrum after 6 hours were higher as compared to SG values of colostrum of Kundi at 6 hours. SG values of both breeds of buffaloes colostrum to transition milk declined gradually from 6 hours to 120 hours after parturition. SG of (K & NRB) at 120 hours were 1.036 ± 0.00 and 1.033 ± 0.00 , respectively. These values showed that SG values of Kundi buffalo colostrum and transition milk was higher at 120 hours as compared to SG values at 120 hours after calving of Nili Ravi colostrum and transition milk. Mean \pm SD values of (K & NRB) colostrum and transition milk were 1.046 ± 0.00 and 1.043 ± 0.00 , respectively. Mean \pm SD values of Nili Ravi buffalo colostrum and transition milk was lower as compared to Kundi buffalo colostrum and transition milk. ANOVA of LR %age and specific gravity of Nili Ravi and Kundi buffaloes $p < 0.0001$ in breeds and time but $p > 0.05$ ($p = 0.1440$) non-significant. Results of present research agreed with the study of (Madsen, Rasmussen, Nielsen, Wiking, & Larsen, 2004) who described that colostrum density reduced fastly from 1048kg.m^{-3} - 1034kg.m^{-3} throughout the 1st two days' after calving, it was monitored through a more continuing reduction to 1030kg.m^{-3} through 6 days' after calving. Specific gravity of colostrum was observed and it was mentioned that its decreased from 1.048-1.032 during the first 5 to 6 milking and then its became stable after the 6 milking (Madsen, Rasmussen, Nielsen, Wiking, & Larsen, 2004). The average density of colostrum from 1st-calf heifers was 1059kg.m^{-3} but density of 3rd and 4th calf of cow's time was 1068kg.m^{-3} ; the density of colostrum decreased fastly in winter then in spring (Strekozov, Motova, & Fedorov, 2008). The specific gravity (1.030-1.035) ranged of whole milk at 15.5°C was measured (Jenness, & Patton, 1959). The SG value of colostrum was higher primarily, reductions fastly amongst the 1st & 4th milking and gradually declined after the 4th milking and then decreased at a slow rate. The lowest SG is in milk of Holstein cows, although noticeable dissimilarities were observed in the milk of Ayrshire cow, Jersey cow and Guernsey cows (Parrish, Wise, Highes, & Atkeson, 1950). The SG of colostrum was observed in the ranges 1.028-1.074 and mean value of SG of colostrum was 1.052. It was also studied that positive correlation among SG, total N and protein N (Quigley, Martin, Dowlen, Wallis, & Lamar, 1994).). The high SG of colostrum may be due to high SNF content as compared to mature milk (Kulkarni, & Pimpale, 1989).

Immunoglobulins (Ig): The prime protein fractions of colostrum are the immunoglobulins (Roginski, Fuquay, & Fox, 2003). Immunoglobulins are a family of globular proteins with antimicrobial and other protective bioactivities and these protected the gut mucosa against pathogenic microorganisms. Immunoglobulins divided into 5 classes, named IgG, IgM, IgD, IgA and IgE. Immunoglobulin G (IgG: IgG1 & IgG2) is the chief immune constituent whereas IgG1 established about 80 percent of the total Ig substances of milk (Seth, & Das, 2011).

Immunoglobulins in colostrum and transition milk of different breeds of buffaloes at different hours (6, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120) for 5 days after calving were obtained (Table No. 5). Immunoglobulins values (mg/ml) of (K & NRB) colostrum and transition milk after 6 hours of calving $110.75\pm 0.01\text{mg/ml}$ and $113.50\pm 0.06\text{mg/ml}$, respectively. These values showed that immunoglobulins values of Nili Ravi buffalo colostrum after 6 hours was higher as compared to immunoglobulin values of colostrum and transition milk of Kundi at 6 hours after calving. Immunoglobulin values of both breeds (K & NRB) colostrum to transition milk decreased gradually from 6-120 hours after parturition. Immunoglobulins of (K & NRB) at 120 hours after calving was $24.35\pm 0.01\text{mg/ml}$ and $16.70\pm 0.06\text{mg/ml}$, respectively. ANOVA of immunoglobulins of Nili Ravi and Kundi breeds $p < 0.0001$ are very highly significant.

Results of current study showed immunoglobulins were reduced gradually and the results were in agreement with other researcher's findings that immunoglobulins were higher at 3rd day after calving but as the time passes they decreases. Same results were mentioned by (Qyeniyi, & Hunter, 1978) total Immunoglobulins in the 1st milking after calving ranged between 20-200 g/L and it was also

described that IgG concentration in postpartum arrays from 15-180 g/L. The mean value was about 60 g/L; in this study it was observed that the IgG concentration declined fastly to approximately 1g/L at the 12th and 14th milking. Results of these studies agreed with the results of our study. Immunoglobulins in colostrum and milk of bovine was 20-200 and 0.7 g/L, respectively (Roginski, Fuquay, & Fox, 2003).

The level of Ig and growing factors were more in the 1st colostrum percentages instantaneously after calving then fastly decreased as time passed after calving (Blum, & Hammon, 2000). The concentration of antibodies in colostrum reduces rapidly. A lower concentration of immunoglobulins in the first colostrum in cows, amounting to an average of 58.4 g/L (Błaszowska, & Twardoń, 2006). The immunoglobulins influenced by a cow's consecutive lactations. It was measured that the highest level of IgG, up to 76.2 g/L, in colostrum from cows in the 3rd and late lactations (Prichett, Gay, Besser, & Hancock, 1991). Higher levels of immunoglobulins in colostrum from cows in the second lactation than from primiparous cows (Guliński, Niedziałek, SalaMończyk, & Górski, 2006). Normally, the data on the basic configuration of colostrum determined in our study do not differ from the information described (Sobczuk-Szul, Wielgosz-Groth, Wronski, & Rzemieniewski, 2013).

Lysozyme and lactoferrin in colostrum and transition milk of buffalo: Lysozyme ($\mu\text{g/ml}$) in colostrum and transition milk of (K & NRB) at different hours was studied. Study hours were started after 6 hours of calving and investigated the lysozyme in colostrum from 06 to 120 hours (1-5days) in both breeds. Lysozyme of buffalo Kundi colostrum after 06 hours of study was $0.32\pm 0.01\mu\text{g/ml}$ and in Nili Ravi ($0.34\pm 0.00\mu\text{g/ml}$). Lysozyme values decreased from 06 to 120 hours ($0.11\pm 0.00\mu\text{g/ml}$) after calving, gradually in Nili Ravi buffalo. But there was a fluctuation observed in lysozyme values of colostrum and transition milk in Kundi breed, 6 hours after calving to 84 hours' lysozyme ($\mu\text{g/ml}$) level decreased and then level increased at 96 hours of study which then again decreased at 120 hours ($0.73\pm 0.01\mu\text{g/ml}$) (Table No. 5). Lysozyme was observed in bovine colostrum and noted that the lysozyme concentration ranges from 0.3mg/L to 0.8 mg/L, its further studied that lysozyme value decreased to 0.1mg/liter of mature milk as described by (Roginski, Fuquay, & Fox, 2003). The existence of lactoferrin improves the anti-bacterial action of lysozyme against the E.coli (Seth, & Das, 2011). It was studied that lysozyme activity was higher in colostrum as compared to lysozyme activity in milk (Korohnen, 1977). Lysozyme obtained the level of 867.53mgL^{-1} in the 1st milking of colostrum after calving (Demkowicz, 2012). The concentration of lysozyme in colostrum and normal milk was in the range 0.14 to 0.7mg/ml & 0.07 to 0.6mg/ml lysozyme was found in colostrum and normal milk, respectively (Tripathi and Vashishtha, 2006). Lysozyme activity was found in the cows having high yields of milk and low yields of milk ($0.401\mu\text{gmL}^{-1}$ in high yield and $0.327\mu\text{gmL}^{-1}$ in low yield) at parturition but the values were $0.096\mu\text{gmL}^{-1}$ and $0.073\mu\text{gmL}^{-1}$ on 3rd day of postpartum, correspondingly (Gueorguiev, Bivolarski, Kutsarov, Iliev, & Gueorguieva, 1996).

Statistically Analysis of Variance (ANOVA) of lysozyme of (K & NRB) breeds is $p<0.0001$ highly significant results in breeds, time, breeds*time interaction and in model all results of analysis of variance were $p<0.0001$ in breeds of buffalo. All results were highly significant.

Lactoferrin in colostrum and transition milk: Samples of Colostrum and transition milk of two breeds (K & NRB) at different hours 6 to 120 hours after calving were obtained and then lactoferrin was determined. Lactoferrin (mg/ml) at 6 hours in Kundi buffalo was $1.47\pm 0.01\text{mg/ml}$ which was higher as compared to Nili Ravi lactoferrin at 6 hours ($1.32\pm 0.01\text{mg/ml}$). Lactoferrin in Kundi buffalo from 6 hours to 120 hours was higher as compared to lactoferrin present in colostrum and transition milk of Nili Ravi buffalo. Lactoferrin found decreased in both breeds of buffalo at 120 hours after calving it was observed $0.59\pm 0.01\text{mg/ml}$ and $0.47\pm 0.01\text{mg/ml}$ in (K & NRB) transition milk. (Table No. 5). The results of our present study were compared with the study of other scientists that in colostrum of cow, it contains the lactoferrin concentration changes from 1g/L to 2g/L. These values of lactoferrin decreased rapidly after the calving as time passed out and values of lactoferrin reached

to low level of 0.1g/L in normal milk (Roginski, Fuquay, & Fox, 2003). The level of lactoferrin of milk (0.02mg/ml to 0.75mg/ml) (Hahn, Schulz, Schaupp, & Jungbauer, 1998). A higher level of lactoferrin in the colostrum was found (Sobczuk-Szul, Wielgosz-Groth, Wronski, & Rzemieniewski, 2013). The 30-fold higher level of lactoferrin in colostrum researched as compared to milk lactoferrin (Reiter, 1978). A 100-fold higher lactoferrin was found in colostrum as compared to level of lactoferrin in milk (Cheng et al., 2008). Normally, the level of lactoferrin in colostrum was 1.5mg/ml to 5mg/ml as studied (Korohnen, 1977). Statistically analysis of variance (ANOVA) of lactoferrin of (K & NRB) breeds was $p < 0.0001$ highly significant results.

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Table No. 1 Physicochemical properties of colostrum and transition milk of (K & NRB) breeds

Parameters (Time)	Fat %age		SNF %age		Protein %age	
	Kundi	Nili Ravi	Kundi	Nili Ravi	Kundi	Nili Ravi
6	8.40±0.06f	6.00±0.06g	20.07±0.01a	19.79±0.01a	13.82±0.33c	12.34±0.72d
12	9.30±0.06e	5.40±0.06g	16.77±0.01b	14.66±0.01c	11.82±0.25d	11.32±0.12d
24	8.50±0.06f	5.13±0.01g	15.60±0.01b	13.85±0.01c	8.59±0.35f	10.00±0.63e
36	8.01±0.01f	5.09±0.01g	14.73±0.01c	13.34±0.01c	7.10±0.36f	8.55±0.50f
48	7.16±0.01f	4.95±0.01g	13.55±0.01c	12.56±0.01d	6.69±0.33g	8.12±0.34f
60	6.94±0.01g	4.87±0.01g	13.03±0.04c	11.79±0.01d	6.45±0.29g	6.14±0.67g
72	6.81±0.01g	4.61±0.01g	12.72±0.01d	11.23±0.01d	5.78±0.53g	5.32±0.95g
84	6.51±0.01g	4.57±0.01g	12.15±0.01d	10.72±0.01e	5.49±0.58g	4.95±0.93h
96	6.23±0.01g	4.55±0.01g	11.84±0.01d	10.47±0.01e	5.18±0.43g	4.75±0.94h
108	5.89±0.01g	4.53±0.01g	11.27±0.01d	10.22±0.01e	4.64±0.56h	4.63±0.87h
120	5.70±0.06g	4.41±0.01g	10.97±0.01e	09.94±0.01e	3.97±0.10h	4.13±0.50h
Mean±SD	7.38±0.03f	4.97±0.02g	13.88±0.01c	12.59±0.01d	7.23±0.37f	7.30±0.65f

Same letters show non-significant results

Table No. 2 Physicochemical properties of colostrum and transition milk of (K & NRB) breeds

Parameters (Time)	Lactose %age		TS %age		Ash %age	
	Kundi	Nili Ravi	Kundi	Nili Ravi	Kundi	Nili Ravi
6	2.72±0.14k	2.25±0.01k	28.91±0.01a	25.98±0.01b	1.04±0.10l	0.98±0.04l
12	2.94±0.01k	2.55±0.01k	26.35±0.01b	21.17±0.01d	0.98±0.10l	0.96±0.03l
24	3.08±0.01j	2.67±0.01k	24.19±0.01c	19.08±0.01e	0.95±0.07l	0.93±0.03m
36	3.34±0.01j	2.73±0.01k	22.89±0.01d	18.49±0.01f	0.92±0.06m	0.89±0.01m
48	3.48±0.01j	2.91±0.01k	20.18±0.01e	17.65±0.01g	0.90±0.03m	0.90±0.04m
60	3.63±0.01j	3.07±0.01j	19.94±0.01e	16.85±0.00g	0.87±0.03n	0.87±0.03n
72	3.89±0.01j	3.66±0.01j	19.59±0.01e	16.11±0.01g	0.86±0.04n	0.83±0.02n
84	4.12±0.01i	3.78±0.01j	18.78±0.01f	15.38±0.01h	0.83±0.05n	0.82±0.02n
96	4.26±0.01i	3.94±0.01j	18.21±0.01f	15.07±0.01h	0.81±0.03n	0.83±0.03n
108	4.33±0.01i	4.03±0.01i	17.29±0.01g	14.83±0.01h	0.78±0.03o	0.82±0.04n
120	4.49±0.01i	4.19±0.01i	16.67±0.01g	14.46±0.01h	0.76±0.01o	0.79±0.02o
Mean±SD	3.66±0.01j	3.25±0.01j	21.18±0.01d	17.73±0.01g	0.88±0.04n	0.87±0.02n

Same letters show non-significant results

Table No. 3 Physicochemical properties of colostrum and transition milk of (K & NRB) breeds

Time	pH		Acidity%	
	Kundi	Nili Ravi	Kundi	Nili Ravi
6	6.07±0.00d	6.37±0.01b	0.32±0.00e	0.30±0.01e
12	6.19±0.00c	6.43±0.01b	0.28±0.01f	0.27±0.01f
24	6.24±0.01c	6.48±0.01b	0.26±0.00f	0.25±0.01f
36	6.29±0.01c	6.51±0.01a	0.25±0.01f	0.24±0.01f
48	6.33±0.01b	6.55±0.01a	0.23±0.01f	0.24±0.00f
60	6.35±0.01b	6.59±0.01a	0.21±0.01g	0.22±0.01g
72	6.37±0.01b	6.61±0.01a	0.20±0.01g	0.21±0.01g
84	6.39±0.01b	6.63±0.01a	0.19±0.00g	0.21±0.01g
96	6.45±0.00b	6.62±0.00a	0.18±0.01h	0.20±0.01g
108	6.52±0.01a	6.65±0.01a	0.18±0.01h	0.19±0.00g
120	6.58±0.00a	6.67±0.01a	0.17±0.01h	0.18±0.01h
Mean±SD	6.34±0.01b	6.56±0.01a	0.22±0.01g	0.23±0.01f

Same letters show non-significant results

Table No. 4 physical properties of colostrum and transition milk of (K & NRB) breeds

(Time)	Viscosity cP		LR		SG	
	Kundi	Nili Ravi	Kundi	Nili Ravi	Kundi	Nili Ravi
6	6.98±0.01b	7.33±0.01a	70.00±0.58a	71.00±0.58a	1.07±0.00a	1.072±0.00a
12	5.89±0.01c	5.49±0.01c	56.00±0.58c	51.00±0.58c	1.056±0.00c	1.051±0.00c
24	3.78±0.01e	4.66±0.01d	52.00±0.58c	48.00±0.58d	1.052±0.00c	1.048±0.00d
36	3.13±0.01e	3.60±0.01e	49.00±0.58d	46.00±0.58d	1.049±0.00d	1.046±0.00d
48	2.56±0.01f	3.38±0.01e	45.00±0.58d	43.00±0.58d	1.045±0.00d	1.043±0.00d
60	2.26±0.01f	2.29±0.01f	43.00±0.58d	40.00±0.58d	1.043±0.00d	1.04±0.00d
72	1.94±0.01g	2.01±0.01f	42.00±0.58d	38.00±0.58e	1.042±0.00d	1.038±0.00e
84	1.84±0.01g	1.89±0.01g	40.00±0.58d	36.00±0.58e	1.04±0.00d	1.036±0.00e
96	1.79±0.01g	1.83±0.02g	39.00±0.58e	35.00±0.58e	1.039±0.00e	1.035±0.00e
108	1.68±0.01g	1.75±0.01g	37.00±0.58e	34.00±0.58e	1.037±0.00e	1.034±0.00e
120	1.61±0.01g	1.70±0.01g	36.00±0.58e	33.00±0.58e	1.036±0.00e	1.033±0.00e
Mean±SD	3.04±0.01e	3.27±0.01e	46.27±0.58d	43.27±0.58d	1.046±0.00d	1.043±0.00d

Same letters show non-significant results in table

Table No. 5 lysozyme, Immunoglobulins and lactoferrin in colostrum and transition milk of (K & NRB) breed

Time	Lysozyme (µg/mL)		Immunglobolins (mg/ml)		Lactoferrin (mg/ml)	
	Kundi	Nili Ravi	Kundi	Nili Ravi	Kundi	Nili Ravi
6	0.32±0.01h	0.34±0.00h	110.75±0.01b	113.50±0.06b	1.47±0.01b	1.32±0.01c
12	0.29±0.00i	0.30±0.00h	75.29±0.01f	62.55±0.01g	1.42±0.01b	1.27±0.01c
24	0.25±0.00i	0.30±0.00h	65.10±0.06g	54.91±0.01h	1.38±0.01c	1.11±0.01d
36	0.22±0.00i	0.27±0.00i	57.46±0.01h	49.82±0.01i	1.28±0.01c	0.99±0.01f
48	0.17±0.00j	0.22±0.00i	47.27±0.01i	42.18±0.01i	1.19±0.01d	0.89±0.01g
60	0.15±0.00j	0.20±0.00i	42.18±0.01i	34.53±0.01j	0.93±0.01f	0.87±0.01g
72	0.14±0.00j	0.18±0.00j	39.63±0.01j	29.44±0.01k	0.90±0.01f	0.67±0.01i
84	0.10±0.00j	0.14±0.00j	34.53±0.01j	24.35±0.01k	0.83±0.01g	0.61±0.01i
96	0.91±0.01e	0.13±0.00j	31.99±0.01j	21.80±0.06k	0.78±0.00h	0.59±0.01j
108	0.85±0.01f	0.12±0.00j	26.89±0.01k	19.25±0.01l	0.65±0.01i	0.53±0.01j
120	0.73±0.01f	0.11±0.00j	24.35±0.01k	16.70±0.06l	0.59±0.01j	0.47±0.01k
Mean±SD	0.38±0.00h	0.21±0.00i	50.50±0.01h	42.64±0.01i	1.04±0.01d	0.85±0.01g

Same letters show non-significant results

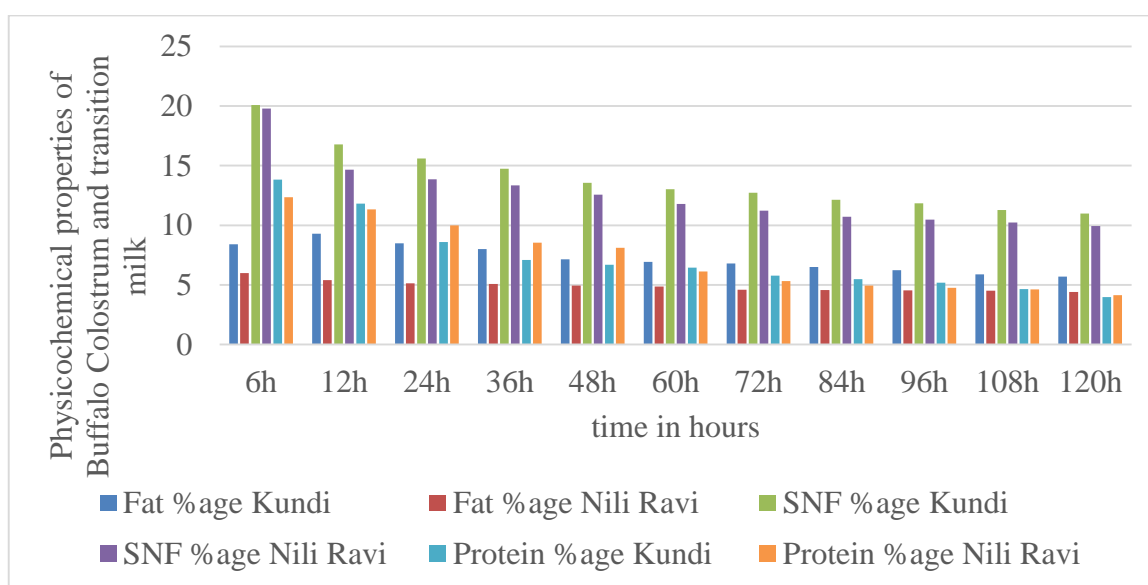


Figure No. 1 Physicochemical properties of Buffalo Colostrum and transition

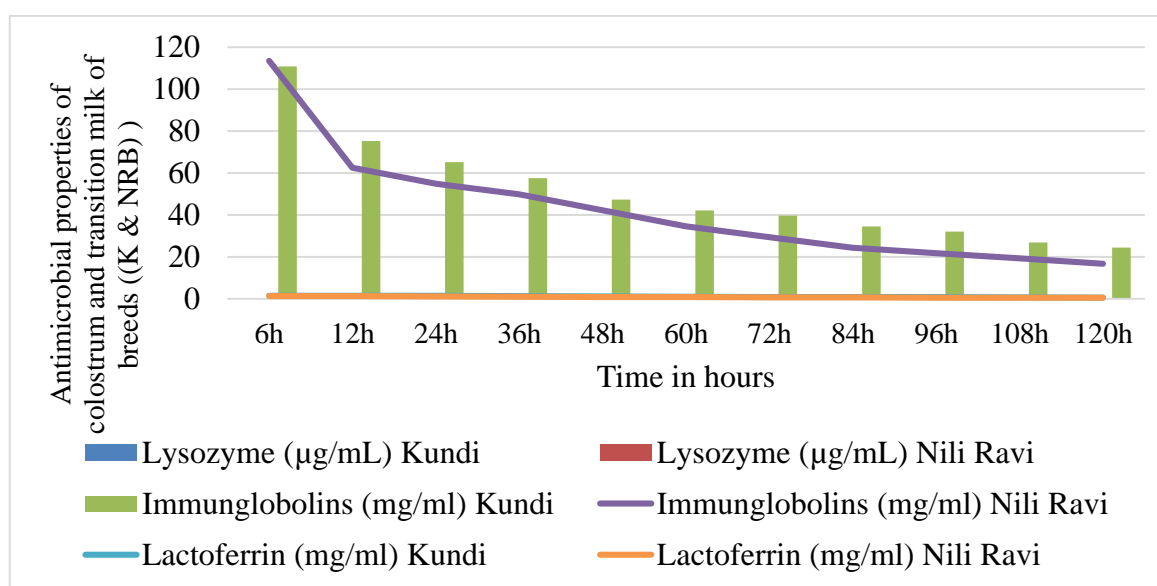


Figure No. 2 Antimicrobial properties of colostrum and transition milk of (K & NRB) breeds