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Relationship Between the Values of Density and Immunoglobulin Concentrations of Buffalo's Colostrum and their Thermal Stability

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Hydrometer is a simple, rapid tester and little cost method to evaluate the quality of colostrum. Colostral immunoglobulins (Igs) concentrations measured from one partial to third consecutive complete milking at 12 h intervals postpartum decreased at different rates over time or milking number. The colostrum is thicker, stickier and slightly yellow compared to normal milk, the specific gravity was higher than that of normal milk and the pH is lower. The thermal stability and some physico-chemical properties of individual buffalo's colostrum (5 animals) were followed during three days after postpartum. The heat stability of first postpartum milk can be visually defined in terms of the time required to induce coagulation at boiling temperature. Gradually marked variations of heat coagulation time were detected up to 60 h postpartum milking when heated at boiling temperature. The thermal stability of individual buffalo's colostrum was gradually increased with progressive postpartum milking, namely, at 1 h (0.55 min), 6 h (3.53 min), 12 h (7.10 min), 24 h (12.30 min), 36 h (18.10 min), 48 h (21.05 min) and 60 h (24.13 min). But at 72 h postpartum, it was observed no visual change (coagulation) during heating at boiling temperature for one hour. It can be seen that first postpartum milk was very unstable to heat treatment with coagulation at boiling temperature. The total solids, total protein, fat and ash contents were highest after parturition and then decreased rapidly to reach values of normal milk almost after three days of parturition, where the protein content was the most variable constituent. But, the changes in the lactose content followed an opposite trend.

Key words: Buffalo's colostrum, immunoglobulins, hydrometer, density, thermal stability

INTRODUCTION

The world buffalo population amounts about 190 million of heads distributed as follows: Asia 96.4% (mainly concentrated in India, China and Pakistan), Africa 2.9% (with greater consistency in Egypt) and the rest in Europe (especially Italy, Romania, Georgia, Bulgaria and Turkey) and Latin America (Brazil, Venezuela, Colombia and Argentina). In the past 10 years, the buffalo population growth was around 20 million of heads and representing a growth of 12.5% (FAO, 2010).

Buffalo's milk is a backbone of the dairy industry in several regions of the world. Buffalo milk represents in south and East Asia (India, Pakistan and China), Egypt, Bulgaria and some area of southern Italy (Stefano, 1998).

Buffalo's milk is ranked second in the world after cow's milk, being more than 12% of the world's milk production. Buffaloes were known during the pharonic times, it is currently believed that the Egyptian buffaloes originated from the wild Indian water buffaloes and rapidly became the principle milk producing animal in Egypt. In 2002 the number of buffaloes in Egypt was estimated to be 3.72 million animals (Ahmad *et al.*, 2008; Borghese, 2005). Who belong to one breed and two types, Behairy and Seidie which vary in their general characteristics and milk production. Buffaloes contribute more than 65% of the milk production and 35% of red meat production in Egypt (Mourad, 1997).

The newborn calf should receive 4 L of colostrum in the first 12 h, although up to 6 L is often recommended for the first day (University of Sydney, 2007). Requirements will depend on the quality of colostrum usually determined by assessing its density associated with IgG content (Mastellone *et al.*, 2005). When calves consume insufficient amounts of IgG from colostrum within the first 24 h of life, calves are much more susceptible to developing disease and possibly dying. A major reason that pre-weaning mortality is higher than optimum (defined as less than 5% of calves born alive) is due to inadequate IgG intake (Quigley, 2005).

Colostrum is the first natural food produced by female mammals towards the end of pregnancy and possibly during the first 24-72 h after giving birth (Campbell and Petersen, 1963; Brambell, 1969; Butler, 1974). Chemically, colostrum is a very complex fluid, usually lemon-yellow, rich in nutrients profile (proteins, carbohydrates, lipids, vitamins and minerals), in addition to contain oligosaccharides, growth factors, anti-microbial compounds and immune-regulating constituents that are either not present in milk or present in substantially lower concentrations (Gopal and Gill, 2000). In adequate or improper colostrum feeding and management cause a

significant portion of the calf morbidity and mortality on dairy farms. The importance of adequate colostrum of suitable quality in the critical first 24 h of life is well documented (McGuire *et al.*, 1976; Bush and Staley, 1980; Matte *et al.*, 1982).

Colostrum is abnormal milk, unsuitable and unprocessable milk which differs considerably in composition and properties from normal milk (Johnson, 1978; Walstra and Jennes, 1984). Therefore, it is not marketable product; the unique importance of colostrum is to serve as a feed for offspring to provide a much higher nutrition to the new born calf. During transition from colostrum to normal milk gradual or sometimes sudden changes may occur in composition and properties (Prasad, 1997). In additional, the inclusion of colostrum in normal milk affects the market milk industry due to denaturation of immunoglobulin and tends to gel during heating at home level or processing at dairy plant (Haggag *et al.*, 1991b; Harding, 1995). The differences in physico-chemical make behave differently when the milk is processed for the manufacturing of different products (Sindhu, 1996).

Fleener and Stott (1980) first developed a colostrometer test that incorporated the relationship between immunoglobulin concentration in bovine colostrum and the specific gravity of fresh whole colostrum into a conventional hydrometer.

Therefore, the aim of this study was carried out to evaluate the buffalo's colostrum quality, the thermal stability and some physico-chemical characteristics during transition to normal milk.

MATERIALS AND METHODS

Sampling of milk: Colostrum samples were collected from five healthy lactating dairy buffalo's in a private farm, Giza governorate, Egypt at 1, 6 and at 12 h intervals from 12-72 h after parturition. Immediately after complete milking of individual animals, the milk was thoroughly mixed and about 100 mL was taken in a clean stopper sample bottle in an ice box and transferred to the dairy laboratory, National Research Centre and kept frozen at -20°C until analyzed. The average of duplicate replicates was taken for each sample.

Heat treatments: Ten milliliter of each buffalo's colostrum samples in stopper glass tubes were heated in a water bath at boiling temperature to visually define in terms of the time required to induce coagulation.

Measuring colostrum quality: Colostrum quality was measured using hydrometer calibrated in immunoglobulin

concentration at intervals 5-50 mg mL⁻¹ and in increments of 0.002 with a range of 1.000-1.080 at 20°C according to Fleenor and Stott (1980).

Analyses of composition: Physico-chemical parameters were measured as follows: pH by a digital pH meter (HANNA Instruments, pH 211, Microprocessor) and specific gravity at 20°C gravimetrically using a hydrometer. Milk samples were analyzed for titratable acidity, Total Solids (TS), fat, Solids Not Fat (SNF), Total Protein (TP) and ash contents according the methods of Ling (1963). While lactose content was determined colorimetrically using the phenol-sulphoric acid method according to Barnett and Abd El-Tawab (1957).

RESULTS AND DISCUSSION

The thermal stability and physico-chemical composition of individual buffalo's colostrum (5 animals) were followed during three days after postpartum. Gradually marked variations of heat coagulation time were detected up to 60 h postpartum milking when heated at boiling temperature (Fig. 1). The thermal stability of individual buffalo's colostrum was gradually increased with successive postpartum milking, namely, at 1 h (0.55 min), 6 h (3.53 min), 12 h (7.10 min), 24 h (12.30 min), 36 h (18.10 min), 48 h (21.05 min) and 60 h (24.13 min). But at 72 h postpartum, no visual change (coagulation) during heating at boiling temperature for one hour was observed. Coagulation properties were strongly affected by milking number. Milk did not clotting during heating (boiling) after three milking. It can be seen that our samples reached the normal milk around third milking day postpartum.

Boiling of our samples showed that the milk from the first milking after calving behaved differently from more mature milk, this coagulation might attribute to the high concentration of total protein especially Igs content that low heat stability (denaturation). This finding is in line to those of Parry (1974) and Walstra and Jenness (1984), who decided that might be due to the low heat stability of the Igs or to the low pH in these samples. Thus, the first postpartum milk was more sensitive and very unstable to heat treatment with coagulation at boiling temperature.

In visual appearance, color of the samples changed with time from parturition, it is thicker, stickier, golden and slightly yellow compared to normal milk, similar observation was reported by Edelsten (1988). These properties were slightly decreased and stabilized after the second milking, while, yellowness decreased with progressive lactation time, it became slightly lighter which increased over the first three milking, then it take

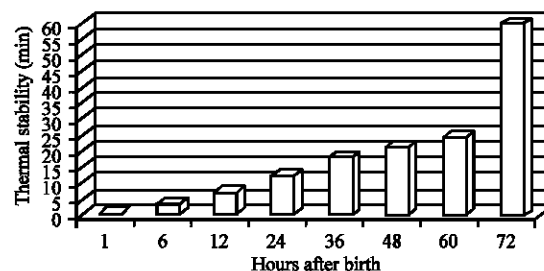


Fig. 1: Changes in thermal stability of buffalo's colostrum during three days postpartum at boiling temperature

opaque-white in normal milk, thus, the colour is a useful tool to identify abnormal milk. However the color and appearance of the colostrum or milk are not indicative of the good quality (high in antibodies) which play an important role in transferring passive immunity to the young calf, this transfer mechanism starts to decline approximately 12 to 23 h after birth and ceases on average at 24 h (Stott *et al.*, 1979) and play an important role at the localized intestinal level. Robinson *et al.* (1988) reported that the importance of achieving adequate levels of colostrum Igs to protect the neonate from enteric disease and septicemia has long been recognized.

Hydrometer enable to the dairyman to establish the quality of colostrum that should be used to select colostrum that has an adequate immunoglobulin concentration (high quality) for feeding the newborn calf which are absorbed intact from colostrum to blood during the first day after suckling to effect reliable transfer of passive immunity or for a colostrum bank and extra colostrum can be saved and freeze-dried to used as raw materials for human food supplements which participate to improve immunity of human bodies by raising the Igs concentrations, because it is produce usually far more than a calf can consume. The use of colostrum or milk as a source of Igs, whether intended for the neonate of the species producing the secretion or for a different species (Walter and Peter, 2011).

The commercially available hydrometer readings of colostrum Igs concentrations had averages 39.45, 34.05, 30.30, 26.95, 24.20 and 23.20 mg mL⁻¹ at 0-12, 24, 36, 48, 60 and 72 h postpartum, respectively (Fig. 2). These values were the same trend but higher than that reported by Mahran *et al.* (1997), who it determined by Single Radial Immunodiffusion (SRID) technique. Also, similar data were observed in bovine colostrum by Pritchett *et al.* (1994) the mean globulins reading was 82.1 mg mL⁻¹ and mean Igs by SRID was 48.1 mg mL⁻¹. For these results, hydrometer was considered to be the diagnostic test value, while SRID was considered to be the true value.

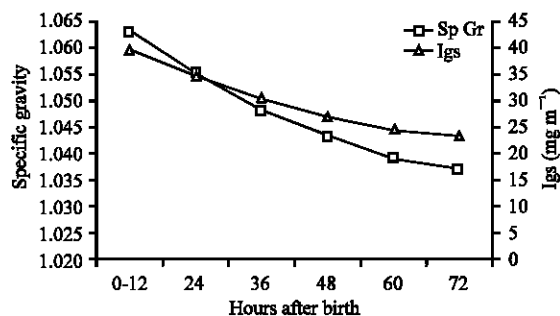


Fig. 2: Effect of lactation time on specific gravity values and its relation to immunoglobulins (Igs) concentration

Immunoglobulins were highest in the colostrum and then gradually decreased in the first three days after parturition. This parallels the transition from colostrum to normal milk. Similar trend in bovine colostrum was decided by Oyeniyi and Hunter (1978), Stott *et al.* (1981) and Mahran *et al.* (1997).

Specific gravity of buffalo's colostrum ranged from 1.035-1.065 with an average of 1.048. It is dependent on temperature at the time of measurement and composition of material, especially fat content (Ganguli, 1974). Density of milk declined during the first to three milking (Fig. 3a). Also, Haggag *et al.* (1991a) found that specific gravity of buffalo's colostrum decreased from 1.040-1.032 within 48 h postpartum which is lower than present study. In addition, Madsen *et al.* (2004) mentioned that the mean value of specific gravity for cow was 1.038 at third milking postpartum which is corresponding to our results (1.037). Moreover, El-Agamy *et al.* (1998) found that the mean value of specific gravity for normal buffalo milk was 1.035 which is in agreement with our results at third day.

The pH value of buffalo's colostrum increased from 6.28-6.47 with an average of 6.38 during the first three days postpartum. These changes in this parameter are presented in Fig. 3b. Haggag *et al.* (1991a) found that the pH of colostrum was 6.43 ± 0.02 in comparison with 6.57 ± 0.04 for normal milk which is slightly higher than our results (6.38). Also, Madsen *et al.* (2004) mentioned that the mean value of pH for cow was 6.42 at third milking postpartum which is corresponding to our results (6.42). Moreover, El-Agamy *et al.* (1998) found that the mean value of pH for normal buffalo milk was 6.70.

The acidity percentages milk during colostral period ranged between 0.20 and 0.40 with mean of 0.29 (Fig. 3c). These data of acidity percentages were higher but lower for pH than that reported by Hofi *et al.* (1966) who observed that the mean value of acidity and pH for herd

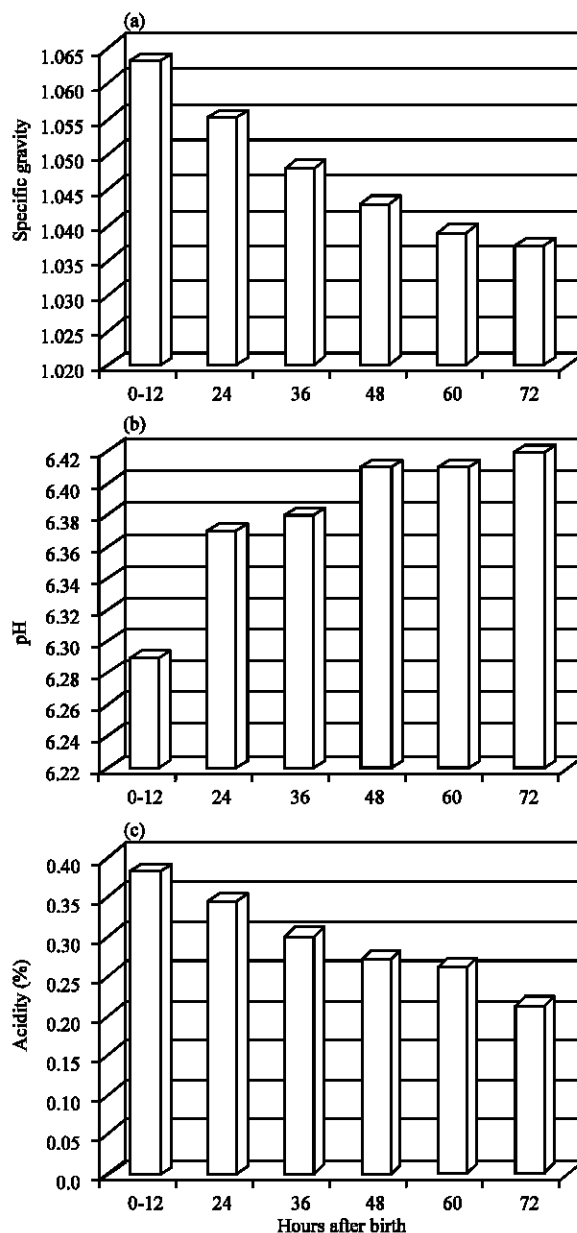


Fig. 3(a-c): Changes in (a) Specific gravity, (b) pH values and (c) Acidity percentage, of buffalo's colostrum during three days postpartum

samples of buffalo's milk to be 0.175% and 6.58 compared to 0.172% and 6.53 for milk from cattle, respectively. Also, Haggag *et al.* (1991a) found that the acidity of colostrum was $0.20\% \pm 0.01$ in comparison with $0.15\% \pm 0.006$ for normal milk which is lower than our results (0.29). Moreover, El-Agamy *et al.* (1998) was fond that the mean value of acidity for normal buffalo milk was 0.18. It is clear that the pH values took an opposite trends of acidity percentages.

Table 1: Chemical characteristics of buffalo's colostrum during three days of parturition

Hours after birth	Components (%)					
	Total solids	Total protein	Fat	Solids not fat	Lactose	Ash
0-12	30.40	18.44	7.32	23.08	3.27	1.07
24	27.68	15.23	6.36	21.32	4.20	0.96
36	22.74	11.08	5.83	16.91	4.85	0.89
48	17.42	5.83	5.29	12.13	5.17	0.84
60	15.81	4.57	5.00	10.81	5.34	0.80
72	15.15	4.18	4.70	10.45	5.44	0.79

*The average of duplicate was taken for each sample

Table 1 shown that the total solids in buffalo's colostrum which consist essentially of protein, carbohydrate (mainly lactose), fat and minerals (ash) (Roy, 1970). These nutrients are needed for the survival of the newborn until it is weaned. Total solids decreased rapidly in the colostrum milk during the transition period. Content of solids in first milking colostrum of buffalo was appreciably higher (30.40%) than that from the third milking of postpartum (15.15%). Rates of change were similar to those observed by Rifaat *et al.* (1972), El-Loly (2005) and El-Loly and Salim (2008). The greatest differences in the total solids content of colostrum contrast to normal milk could be the result of elevated content of antibodies of colostrum (Nickerson, 1995).

The fat content of buffalo's colostrum ranged from 4.55-8.80 with an average of 5.75% during three days. Milk fat percentages were 7.32, 5.29 and 4.70% at 0-12, 48 and 72 h milking after parturition respectively (Table 1). A similar trend was noted as reported by Rifaat *et al.* (1972), El-Loly (2005) and El-Loly and Salim (2008), where the fat content of colostrum initially was high, then reached it lowest levels after about a third milking.

Solids not fat content was high after parturition (23.08 %) and then dropped gradually until it reached a level of 10.45 % at the 72 h after parturition. Also, El-Agamy *et al.* (1998) found that the mean value of SNF for normal milk was 9.44% which is lower than our results at third day. These wide variations in SNF content of colostrum may be to the evidence of individuality in buffaloes (Andrew, 2001).

In addition, the very first-milking colostrum is comprised largely of proteins, where it had an average of 18.44% within 12 h of parturition, followed by rapid decrease to reach an average value of 5.83% at 48 h postpartum, then gradually decreased to an average value of 4.18% at 72 h postpartum. This decline trend is normally due to higher concentration of globulin that serves as the carrier of antibodies for suckling calf against disease producing organism (Ebina *et al.*, 1985; Nickerson, 1995). The obtained results are in accordance trend with that obtained by Rifaat *et al.*

(1972), El-Agamy *et al.* (1998), Walstra *et al.* (1999), El-Loly (2005) and El-Loly and Salim (2008).

Bar *et al.* (2010) illustrated that the gamma-globulin represents approximately 47% of the total protein so can be found a significant relationship between specific density and gamma-globulin. Also, they reported that the gamma-globulin had the largest coefficient of variation of all cow's colostrum constituents related to specific density that may be the primary factor in variation of colostrum specific density.

On contrary, the lactose concentration was lower (3.27%) in colostrum and then gradually increased to reach its normal level 5.44% on the third day after calving (Table 1). This difference is an advantage because lactose can induce the young to scour (diarrhea) with subsequent death or unthriftiness (Roy, 1970). These results were in accordance with pervious finding of Rifaat *et al.* (1972), Foley and Otterby (1978), Walstra *et al.* (1999), El-Loly (2005) and El-Loly and Salim (2008). But it was higher than those of El-Agamy *et al.* (1998).

Moreover, the change in ash content of buffalo's colostrum throughout the first three days postpartum was observed (Table 1). It was high after parturition (1.07%) and then decreased gradually up to three days parturition (0.96, 0.89, 0.84, 0.80 and 0.79% at 24, 36, 48, 60 and 72 milking, respectively). These results were in agree to that reported by Abd El-Salam and El-Shibiny (1966), Kholif (1989) and El-Loly (2005).

In general, very large variations occurred in physical properties of the milk and these changes were related to chemical composition. For example, the total solids, total protein, fat and ash contents were highest after parturition and then decreased rapidly to reach values of normal milk almost after three days of parturition. But the changes in the lactose content followed an opposite trend to other constituents. Also, coagulation properties were affected by milking number.

CONCLUSION

This study was carried out to evaluate the quality and the heat stability characteristics of buffalo's colostrum during its transition to normal milk. Gradual change as coagulation time was observed up to sixth postpartum milking when heated at boiling temperature. No coagulation was occurred in third day postpartum milk heated for 1 h at boiling temperature.

The results demonstrate that the colostrum specific gravity was more strongly correlated with colostrum total protein concentrations especially Igs that were primary factor in variation of colostrum.

These data showed that great variations occur in both physical properties and composition of the mammary secretion during transition from colostrum to milk. Because of these variations seen between animals, it might be better to separate milk based on the composition of the milk instead of time from calving. This period should not be shorter than three days if milk composition is the criterion as it is not until this time come close to levels.

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