

*Full Length Research Paper*

## Gross antibodies, chemical composition of bovine milk and its influence by thermal stability

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Received 28 August, 2014; Accepted 17 December, 2014

**Immunoglobulin G (IgG), chemical composition contents of bovine milk during the first week of postpartum and the effect of heat treatments on bovine colostrum IgG contents were evaluated. Individual milk samples were collected from five cows at 0 to 0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum. The obtained results showed that the total solids, total protein, fat and ash contents decreased irregular with time after parturition, while the lactose content had an opposite trend. IgG concentrations were higher significantly during 0 to 0.5 and 1<sup>st</sup> days than those of other days postpartum, where the mean±SD of IgG concentrations were 122.60±5.24 and 118.44±5.90 g/L during 0-0.5 and 1<sup>st</sup> days postpartum, respectively. However, IgG concentrations dropped markedly with time progress of lactation at the end of the first week (7<sup>th</sup> day); it was 55.16±17.30 g/L that had dropped ratio of 55.01% when compared with its concentrations at 0 to 0.5 day. The IgG concentrations of thermally treated colostrum were decreased to 28.24, 30.27 and 30.18% at 63°C/30 min as well as 57.33, 73.54 and 95.1% at 72°C/15 s during 1, 2 and 3 days postpartum, respectively. On the other hand, the most thermal influence on IgG was at 100°C/10 min, where the percentage losses were 95.72% at 1<sup>st</sup> and 100% at 2 and 3 days postpartum. The total amino acids values of bovine milk immunoglobulins (IgS) were highest at 0 to 0.5 day and dropped markedly with time progress of lactation.**

**Key words:** Bovine milk, colostrum, immunoglobulin G (IgG), heat treatments, amino acids.

### INTRODUCTION

Colostrum is very important part of milk and lays down the immune system and confers growth factors and other protective factors for the young ones in mammals. Also, it is a unique food created by nature to sustain and protect the new born mammal. It is a pre-milk made available by the mother to the newborn in the first few days after birth has taken place. Colostrum contains high levels of

immunoglobulins, the self-defense mechanism by which the body fights infection as well as valuable growth factors to nourish the newborn (Radu Dragomirescu, 2013). Vetter et al. (2013) mentioned that the provision of quality colostrum with a high concentration of immunoglobulin S is critical for newborn calf health, because first colostrum may be low in overall

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concentration to effectively reduce the risk of newborn infections.

The nutritional and physiological needs of the neonate during this period of very early life are typically quite specialized. The composition of the maternal colostrum is tailored to meet these unique requirements (Tsioulpas et al., 2007; Chistiansen et al., 2010; Abd El-Fattah et al., 2012). The colostrum composition and its quality are influenced by a variety of factors, including maternal age, parity, breed, nutritional status, season, premature parturition, premature lactation, colostrum handling factors, induction of parturition and health status. During transition from colostrum to normal milk, gradual or sometimes sudden changes may occur in composition and properties (Gulliksen et al., 2008; Abd El-Fattah et al., 2012; Morrill et al., 2012).

Colostrum is not only a source of nutrients such as proteins, carbohydrates, fat, vitamins and minerals, but it also contains several biologically active molecules that are essential for specific functions. Most of the biologically active substances in complete bovine colostrum that can convey significant health benefits are proteins (Pakkanen and Aalto, 1997).

In recent years, bovine colostrum has become popular as a product for human consumption, because it is an excellent source of bioactive proteins. The latter would have the ability to prevent bacteria and viruses as well as to improve the gastrointestinal and body condition. Really, the exploitation of the beneficial properties of colostrum is not a new concept (Conte and Scarantino, 2013). The immunoglobulins S (IgS), or antibodies, found in colostrum or milk are the same as those found in the blood or mucosal secretions. They are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. For cattle, IgS are grouped into four isotypes, IgG (IgG<sub>1</sub> and IgG<sub>2</sub>), IgM, IgA, and IgE, based on the heavy chain they possess (Korhonen et al., 2000a, b; Gapper et al., 2007). IgG, IgM and IgA are present in high levels in milk, especially in colostrum. The IgG is dominant in colostrum, milk and blood (about 80 to 90%, 60 to 70% and 90% of total IgS, respectively) (Mix et al., 2006; Zhao et al., 2010).

IgG concentrations change throughout the first six milking's postpartum. The relatively high levels of IgG in early bovine colostrum thus provide an essential source of this nutrient to the calf immediately following parturition and until it can establish immunosufficiency. IgG antibodies express multifunctional activities, including complement activation, bacterial opsonisation and agglutination as well as act by binding to specific sites on the surfaces of most infectious agents or products, either inactivating them or reducing infection (Lilius and Marnila, 2001; Gapper et al., 2007).

Interest has arisen on the effect of heat treatments on different Ig classes. Detectable IgG in colostrum or colostrum whey are reduced by heat treatment at a slower rate than the isolated IgG. Thermal treatments such as sugars or glycerol can increase the stability of isolated

IgG to heat treatment (Chen et al., 2000; Zagorska and Ciprovica, 2012). According to Chen and Chang (1998) IgS are thermo labile. Exposure to temperatures of 75°C/5 min can reduce detectable isolated bovine IgG by 40%, and by 100% at 95°C/15 s. The explanation of it is conformational changes in the IgG molecule caused by heat exposure (Calmettes et al., 1991). Donahue et al. (2012) demonstrates that batch heat treatment of colostrum at 60°C/60 min can be successfully conducted on commercial dairy farms by farm staff to decrease colostrum microbial counts while maintaining colostrum IgG concentrations. Also, Gelsinger et al. (2014) reported that heat treatment significantly reduced all types of bacteria and IgG concentration in colostrum at 60°C/30 min.

The aim of the current study was to analyze gross IgS, chemical composition of bovine milk during the first week postpartum and evaluate the effect of heat treatments on bovine milk IgG content.

## MATERIALS AND METHODS

### Sample collection

This study was conducted from February 2010 till December 2012 aiming at estimation of the concentrations of total protein and IgG in bovine milk during the first week postpartum.

Individual milk samples were collected from five Frisian cows of El-Sadeen village, Menia Al-kamh Center, Sharkia Governorate, Egypt. Milk samples were obtained at 0 to 0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum. Samples were collected in sterilized bottles by supervised manual expression at the end of the milking and transported to the laboratory in an ice box. All samples were stored at -20°C immediately on arrival and kept frozen till analysed.

### Determination of gross chemical composition

The total solids (TS), total protein (TP) and fat contents, lactose and ash contents were determined according to AOAC (2000).

### Determination of immunoglobulins in bovine milk

#### Samples preparation

Bovine milk samples were defatted by centrifugation at 4000 rpm/3 min. Milk whey was prepared from the skim milk by adjusting pH to 4.6 using 1 N HCl solution and centrifuging at 10000 rpm/15 min to remove casein precipitate. Total IgS were prepared from whey samples by using saturated ammonium sulphate solution according to the method described by Hebert (1974). The ammonium sulphate extract was dialysed against distilled water for 24 h, at refrigerator with several changes of distilled water during this period. The dialysed extract was kept at -20°C until analysed.

#### Immunoglobulin quantification by single radial immunodiffusion (SRID)

The immunoglobulin G (IgG) content was quantified using Single Radial Immuno Diffusion Technique (SRID) as described by Fahey and Mckelvey (1965). SRID plates containing antibodies to IgG

**Table 1.** Gross chemical composition content (%) with means $\pm$ SD\* of bovine milk (N=5) during the first week postpartum.

Lactation period (days)	TS	TP	Fat	Lactose	Ash
0-0.5	19.83 $\pm$ 2.12 <sup>a</sup>	10.65 $\pm$ 1.71 <sup>a</sup>	5.68 $\pm$ 0.72 <sup>a</sup>	2.50 $\pm$ 0.45 <sup>e</sup>	1.01 $\pm$ 0.09 <sup>a</sup>
1	17.55 $\pm$ 1.65 <sup>b</sup>	9.26 $\pm$ 1.32 <sup>b</sup>	4.76 $\pm$ 0.89 <sup>b</sup>	2.59 $\pm$ 0.34 <sup>e</sup>	0.94 $\pm$ 0.07 <sup>b</sup>
2	15.33 $\pm$ 0.96 <sup>c</sup>	7.36 $\pm$ 0.53 <sup>c</sup>	4.26 $\pm$ 0.86 <sup>bc</sup>	2.83 $\pm$ 0.38 <sup>de</sup>	0.88 $\pm$ 0.04 <sup>bc</sup>
3	14.63 $\pm$ 0.68 <sup>cd</sup>	6.67 $\pm$ 0.13 <sup>cd</sup>	3.90 $\pm$ 0.64 <sup>cde</sup>	3.22 $\pm$ 0.24 <sup>cd</sup>	0.85 $\pm$ 0.02 <sup>cd</sup>
4	13.76 $\pm$ 0.80 <sup>de</sup>	5.77 $\pm$ 0.36 <sup>de</sup>	3.70 $\pm$ 0.56 <sup>cdef</sup>	3.49 $\pm$ 0.15 <sup>bc</sup>	0.81 $\pm$ 0.02 <sup>def</sup>
5	13.18 $\pm$ 0.80 <sup>de</sup>	5.18 $\pm$ 0.74 <sup>ef</sup>	3.30 $\pm$ 0.48 <sup>def</sup>	3.90 $\pm$ 0.35 <sup>ab</sup>	0.79 $\pm$ 0.02 <sup>def</sup>
6	12.81 $\pm$ 0.70 <sup>e</sup>	5.00 $\pm$ 0.56 <sup>ef</sup>	3.10 $\pm$ 0.46 <sup>ef</sup>	3.94 $\pm$ 0.44 <sup>a</sup>	0.77 $\pm$ 0.04 <sup>ef</sup>
7	12.22 $\pm$ 0.78 <sup>e</sup>	4.48 $\pm$ 0.63 <sup>f</sup>	2.94 $\pm$ 0.50 <sup>f</sup>	4.04 $\pm$ 0.29 <sup>a</sup>	0.75 $\pm$ 0.03 <sup>f</sup>
LSD**	1.45	1.11	0.82	0.42	0.60

SD\*: Standard deviation; LSD\*\*: The least significant difference. Means with different superscript within the same column are significantly different.

and IgM (Cat. No. RL 200.3, RN 278.3, the Binding Site LTTDR, UK) were used.

#### Heat treatments

To investigate the effect of heating methods on the IgG content of bovine milk, the milk samples were collected from individual cow's milk during the first three days of postpartum (colostrum) and defatted, the skim milk was heat treated as follows: Heating was carried out at 63°C / 30 min, 72°C / 15 s and 100°C / 10 min; then followed by rapid cooling to 37°C for all samples.

#### Amino acids analysis

Amino acids content were determined as described by Folkertsma and Fox (1992). The analysis was performed in Central Service Unit, National Research Centre, Egypt, using LC3000 amino acid analyzer (Eppendorf-Biotronik, Germany). The technique was based on the separation of the amino acids using strong cation exchange chromatography followed by the ninhydrine color reaction and photometric detection at 570 nm. Samples were hydrolysed with 6 N HCl at 110°C in Teflon capped vials for 24 h. After vacuum removal of HCl, the residues were dissolved in a lithium citrate buffer, pH 2.2. 20  $\mu$ l of the solution were loaded on to the cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7 respectively, were successively applied to the column at flow rate 0.2 ml/min. The ninhydrine flow rate was 0.2 ml/min and pressure of 0 to 150 bar. The pressure of buffer was from 0 to 50 bars and reaction temperature was 130°C.

#### Statistical analysis

Statistical analysis for the obtained data was carried out using SPSS version 20 computer program (Dominick and Derrick, 2001). All data were expressed by means and standard deviations of 3 replicates and were compared using One-way ANOVAs and least significant difference (LSD). Values with different letters within the same column differ significantly at  $p < 0.01$  to 0.05.

## RESULTS AND DISCUSSION

### Gross chemical composition of bovine milk

It was noticed that the mean $\pm$ SD concentration of TS was

19.83 $\pm$ 2.12% during 0 to 0.5 day postpartum. TS Total solids content decreased to reach a mean $\pm$ SD 17.55 $\pm$ 1.65% at 1<sup>st</sup> day postpartum. While gradual decreases of TS could be noticed on the following days at 2, 3, 4, 5, 6 and 7 postpartum, respectively. The mean concentrations of TS were higher significantly during 0 to 0.5 day than other days postpartum. But no significant differences were found between 3, 4 and 5 days. No significant differences were found between 4, 5, 6 and 7 days postpartum, in the same order (Table 1). Similar results have been reported by Abd El-Fattah et al. (2012) who observed that the TS contents decreased irregularly with time after parturition. TS content at 1<sup>st</sup> day postpartum in the present study was higher than those reported by Kleinsmith (2011). Bar et al. (2010) found that the mean of TS contents in bovine colostrum were 27.6 and 23.6% with ranges 18.3 to 43.3 and 21.6 to 29.15%, respectively, while in mature milk (0.05H) were 12.7 and 12.9%; Walstra et al. (2006) and Kleinsmith (2011) found that the TS contents in bovine colostrum were 27, 20.5, 14.5, 12.8, 12.2 and 11.5% at 0, 6, 12, 24, 36 and 48 h postpartum, respectively. Also, Abd El-Fattah et al. (2012) stated that the mean of TS content in bovine colostrum at calving was 24.2%. TP concentration of bovine milk was higher significantly during 0 to 0.5 day postpartum (10.7 $\pm$ 1.7%) than other days, followed by 1<sup>st</sup> day postpartum (9.3 $\pm$ 1.3%). No significant differences were found between the mean $\pm$ SD concentrations of TP at 2<sup>nd</sup> day (7.4 $\pm$ 0.5%) and 3<sup>rd</sup> day (6.7 $\pm$ 1.1%), but significant differences were found between 2<sup>nd</sup> (7.4 $\pm$ 0.5%) and 4<sup>th</sup> days (5.8 $\pm$ 0.4%). However, no significant differences were found between the mean $\pm$ SD concentrations of TP at 5 (5.2 $\pm$ 0.7%), 6 (5.00 $\pm$ 0.6%) and 7 (4.5 $\pm$ 0.6%) days postpartum (Table 1). These results are in agreement with those found by Tsioulpas et al. (2007); Kleinsmith (2011) and Abd El-Fattah et al. (2012) who found that the protein content decreases gradually with time after parturition. Protein content at 1<sup>st</sup> day postpartum in this study was higher than those reported by Klimes et al. (1986) and lower than those reported by Tsioulpas et al. (2007). The mean of TP content in bovine

colostrum was 14.92% with range 7.1 to 22.6% within 4 h of calving (Kehoe et al., 2007); 9.5% with range 6.6 to 11.7% at first milked after calving (Bar et al., 2010); 13.45% at calving (Abd El-Fattah et al., 2012) and 12.2% with range 8.9 to 21.9% after 2 to 3 days postpartum (Conte and Scarantino, 2013), while in mature milk, the protein contents were 3.4% (Gopal and Gill, 2000), 2.9% (Fox and McSweeney, 2003) and 3.3% (Walstra et al., 2006).

The mean  $\pm$  SD concentration of fat was  $5.7 \pm 0.7\%$  during 0 to 0.5 day postpartum and dropped to a mean  $\pm$  SD  $4.8 \pm 0.9\%$  at 1<sup>st</sup> day postpartum. Thereafter, a gradually decreasing could be noticed on the following days, where the means  $\pm$  SD of fat concentrations were  $4.3 \pm 0.9$ ,  $3.9 \pm 0.6$ ,  $3.7 \pm 0.6$ ,  $3.3 \pm 0.5$ ,  $3.1 \pm 0.5$  and  $3 \pm 0.5\%$  at 2, 3, 4, 5, 6 and 7 days postpartum, respectively (Table 1). These results indicate that a significant difference was found between the fat content during 0 to 0.5 day and other days, but no significant differences were found between 1 and 2; 2, 3 and 4 and 4, 5, 6 and 7 days postpartum. Also, the results are in quite agreement with those of Abd El-Fattah et al. (2012) who observed that the fat content decrease with time after parturition, but in contrast with the study of Tsioulpas et al. (2007) and Kleinsmith (2011). Fat content at 1<sup>st</sup> day postpartum in the present study was higher than those reported by Tsioulpas et al. (2007). Fat content of the first milking colostrum varies over a wide range and was reflected in values for TS (Elfstrand, et al., 2002). The mean of fat contents in bovine colostrum were 6.7% with range 2.0 to 26.5% within 4 h of calving (Kehoe et al., 2007), 3.5 (4.6 to 5.8%) of first milked after calving (Bar et al., 2010), 8.0% at calving (Abd El-Fattah et al., 2012) and 7.9 (2.6 to 16.1%) after 2 to 3 days postpartum (Conte and Scarantino, 2013), while in mature milk, it was 3.7% (Jensen, 1995), 4.6% (Gopal and Gill, 2000), 4.5% (Fox and McSweeney, 2003) and 4.0% (Walstra et al., 2006).

It is evident from Table 1 that the mean  $\pm$  SD of lactose concentrations at 0 to 0.5, 1<sup>st</sup> and 2<sup>nd</sup> days were  $2.50 \pm 0.45$ ,  $2.59 \pm 0.34$  and  $2.83 \pm 0.38\%$ , respectively without significant differences between the first two days of lactation. The lactose content increased to a mean  $\pm$  SD  $3.2 \pm 0.2\%$  at 3<sup>rd</sup> day,  $3.5 \pm 0.2\%$  at 4<sup>th</sup> day and  $3.90 \pm 0.3\%$  at 5<sup>th</sup> day. On the other hand, the mean  $\pm$  SD concentrations of lactose at 6 and 7 days were  $3.9 \pm 0.4$  and  $4.04 \pm 0.3\%$ , respectively without significant differences between them. Our results are closely similar with those of Kleinsmith (2011) and Abd El-Fattah et al. (2012) who observed that the lactose content increase with time after parturition. This difference is an advantage because lactose can induce the young to scour (diarrhea) with subsequent death or unthriftiness (Roy, 1970). In contrast with the study of Elfstrand et al. (2002) who found that the lactose contents were 3.0, 2.9, 3.5, 3.2, 3.5, 3.5 and 3.8% during 0 to 6, 7 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50 and 51 to 80 h, respectively.

Changes in lactose content of colostrum showed the

opposite trend than the corresponding values in the mature milk, probably due to the knowledge of the mechanisms of lactose synthesis. Kuhn (1983) suggested that the lower availability of plasma glucose and colostral Lactalbumin is a possible cause of the lower percentage of lactose in colostrum immediately after parturition. Lactose contents in the present study were lower than those reported by Tsioulpas et al. (2007) at 1<sup>st</sup> to 5<sup>th</sup> day; Kleinsmith (2011) at 1<sup>st</sup> and 2<sup>nd</sup> days; Klimes et al. (1986) at 3<sup>rd</sup> and 5<sup>th</sup> days postpartum. But, it was higher than those reported by Klimes et al. (1986) at 1<sup>st</sup> day postpartum. Bar et al. (2010) and Conte and Scarantino (2013) showed that the mean of lactose contents in bovine colostrum were 2.49, 3.5 and 2.04% with ranges 1.2 to 5.2, 3.37 to 3.94 and 1.46 to 3.19%, in the same order, while in mature milk, it were 4.1 and 4.6% (Walstra et al., 2006).

The mean  $\pm$  SD concentrations of ash were higher significantly during 0 to 0.5 day ( $1.0 \pm 0.1\%$ ) than those of other days postpartum, then dropped to range from 0.8 to 1.0% at 1<sup>st</sup> day postpartum with a mean  $\pm$  SD  $0.9 \pm 0.1\%$ , while at 2<sup>nd</sup> day postpartum had a mean  $\pm$  SD  $0.9 \pm 0.04\%$ , without significant differences between 1<sup>st</sup> and 2<sup>nd</sup> day. A gradual decrease could be observed on the following days namely,  $0.9 \pm 0.02$ ,  $0.8 \pm 0.02$ ,  $0.8 \pm 0.02$ ,  $0.8 \pm 0.04$  and  $0.75 \pm 0.03\%$  at 3, 4, 5, 6 and 7 days postpartum, in order. There were significant differences between ash content at 2<sup>nd</sup> and 4<sup>th</sup> days postpartum, but insignificant variations were found between 4, 5, 6 and 7 days postpartum (Table 1). These results are in agreement with the previous reports by Klimes et al. (1986), Tsioulpas et al. (2007) and Abd El-Fattah et al. (2012) who observed that the ash content decrease with time after parturition. This may be attributed to increase of mineral in colostrum compared to mature milk. However, in colostrum, high protein and salt, low sugar content are ideal for the neonate's immature digestive system (Hamosh, 1996). Bar et al. (2010) showed that the mean values of ash content in bovine colostrum were 0.05 and 1.5% with ranges 0.02 to 0.1 and 1.10 to 1.3%, respectively, while in mature milk were 0.7 and 0.8% (Fox and McSweeney, 2003).

### **IgG concentrations of bovine milk during the first week postpartum**

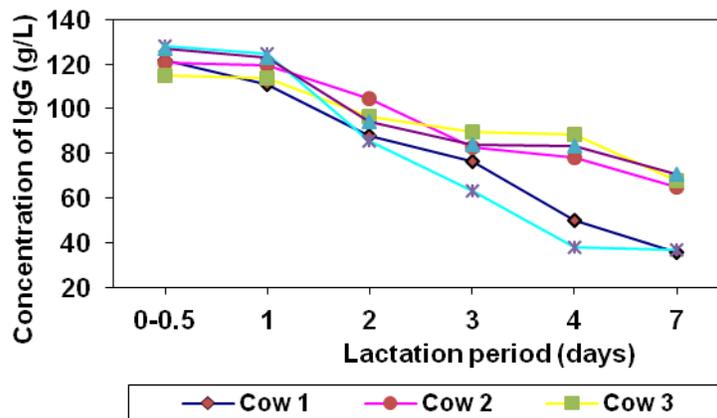
Individual milk samples were taken from five cows within 7 days postpartum. The IgG concentrations of bovine milk samples were quantified by the SRID technique at 0 to 0.5, 1, 2, 3, 4 and 7 days postpartum. The relation between the IgS concentrations and the diameter of the precipitated antigen-antibody reaction are found in Figures 1, 2, 3, 4 and 5 (wells No. 1 and 2). It is clear that the IgG concentrations were highest in colostrum, which falls drastically with the first few days of lactation. The IgG concentrations were higher significantly during 0 to 0.5



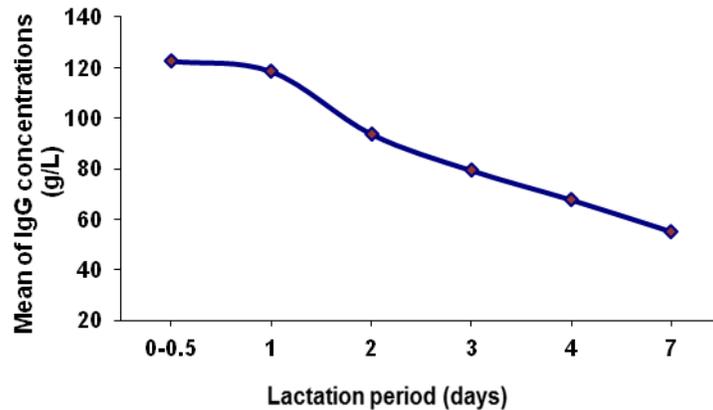
**Figure 1.** Single radial immunodiffusion analysis of IgG for individual bovine milk samples during the first week postpartum. Wells No: 1, 2, 3, 4, 5 and 6 represent samples of cow number 1 at 0-0.5, 1, 2, 3, 4 and 7 days postpartum. Wells No: 7, 8, 9, 10, 11 and 12 represent samples of cow number 2 at 0-0.5, 1, 2, 3, 4 and 7 days postpartum. Wells No: 13 and 14 represent samples of cow number 3 at 0-0.5 and 1 day postpartum.



**Figure 2.** Single radial immuno diffusion analysis of IgG for individual bovine milk samples during the first week postpartum. Wells No: 1, 2, 3 and 4 represent samples of cow number 3 at 2, 3, 4 and 7 days postpartum. Wells No: 5, 6, 7, 8, 9 and 10 represent samples of cow number 4 at 0-0.5, 1, 2, 3, 4 and 7 days postpartum. Wells No: 11, 12, 13 and 14 represent samples of cow number 5 at 0-0.5, 1, 2 and 3 days postpartum.



**Figure 3.** IgG concentrations of individual bovine milk samples during the first week postpartum.



**Figure 4.** Mean IgG concentrations of bovine milk samples during the first week postpartum.



**Figure 5.** Single radial immunodiffusion analysis of IgG for bovine milk samples affected by different heat treatments. Wells No: 1 and 2 represent samples of cow number 5 at 4 and 7 days postpartum. Wells No: 3, 4, 5 and 6 represent control, 63°C/30 min; 72°C/15 s and 100°C/10 min at 1st day postpartum. Wells No: 7, 8, 9 and 10 represent control, 63°C/30 min., 72°C/15 s and 100°C/10 min at 2nd day postpartum. Wells No: 11, 12, 13 and 14 represent control, 63°C/30 min., 72°C/15 s and 100°C/10 min at 3rd day postpartum.

and 1<sup>st</sup> days than those of other days postpartum, where the mean±SD of IgG concentrations were 122.6±5.2 and 118.4±5.9 g/L during 0 to 0.5 and 1<sup>st</sup> days postpartum, respectively (Table 2). However, IgG concentrations dropped markedly with time progress of lactation. At 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days, the mean ± SD values of IgG were 93.7±7.6, 79.3 ± 10.0 and 67.7±22.4 g/L, respectively, which had dropped ratios of 23.5, 35.3 and 44.8%, in order. At the end of the first week (7<sup>th</sup> day), the mean ± SD of IgG concentration was 55.2±17.3 g/L that had dropped ratio 55.0% when compared with its concentrations at 0 to 0.5 day. This change in IgG content indicates that the significance of colostrum for the

health of the newborn calf where the absorption of IgG during the first 24 h, after birth was reported occur excessively (Butler, 1971). These results are in good agreement with those of Saucedo-Quintero and Avendano-Reyes (2004) and Abd El-Fattah et al. (2012). Elfstrand et al. (2002) stated that the major IgS present in bovine milk are IgG with 85% ratio, among which 95% belong to the sub classes IgG<sub>1</sub> and 5% to the IgG<sub>2</sub>. The mean concentrations of IgG (IgG<sub>1</sub>+IgG<sub>2</sub>) were 92.8, 80.9, 66.8, 25.1, 31.5, 17.7 and 12.2 g/L during 0 to 6, 7 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50 and 51 to 80 h, respectively. Similar results were reported by Bar et al. (2010), Morrill et al. (2012) and Quigley et al. (2013),

**Table 2.** IgG concentrations of bovine milk during the first week postpartum.

Lactation period (days)	Concentration of IgG (g/L)					Mean±SD <sup>*</sup>	% of decrease
	Cows						
	1	2	3	4	5		
0-0.5	122.00	120.80	115.10	128.20	126.90	122.60±5.24 <sup>a</sup>	-
1	110.80	119.70	114.00	124.50	123.20	118.44±5.90 <sup>a</sup>	3.39
2	88.07	104.70	96.50	85.42	94.00	93.74±7.57 <sup>b</sup>	23.54
3	76.60	83.00	89.50	63.40	84.18	79.34±10.02 <sup>bc</sup>	35.29
4	50.00	78.20	88.80	38.00	83.58	67.72±22.38 <sup>cd</sup>	44.77
7	35.80	64.80	67.70	36.90	70.60	55.16±17.30 <sup>d</sup>	55.01
<b>LSD<sup>**</sup></b>	<b>16.986</b>						

SD<sup>\*</sup>: Standard deviation; LSD<sup>\*\*</sup>: The least significant difference. Means with different superscript within the same column are significantly different.

**Table 3.** Effect of heat treatments on bovine colostrum IgG concentrations (g/l).

Lactation period (days)	Control	Heat treatment					
		63°C/30min	%Loss	72°C/15 s	%Loss	100°C/10 min	%Loss
1	125.70	90.20	28.24	66.20	47.33	5.38	95.72
2	107.70	75.10	30.27	28.50	73.54	-	100
3	84.79	59.20	30.18	4.23	95.01	-	100

while in mature milk, it was 0.72 and 0.556 mg/ml (Zagorska and Ciprovica, 2012).

### Effect of heat treatments on bovine colostrum IgG

It could be noticed that the concentrations of IgG during thermal treatments were reduced from 125.7, 107.7 and 84.8 g/L in control individual milk samples at 1, 2 and 3 days postpartum to 90.2, 75.1 and 59.20 g/L in thermally treated milk at 63°C/30 min, in order, it were decrease to 28.2, 30.3 and 30.2%, respectively (Table 3). Increasing temperature to 72°C/15 s, the IgG concentrations of heated individual milk samples were reduced to 66.2, 28.5 and 4.2 g/L, it were decreased by 52.7, 26.5 and 5% at 1, 2 and 3 days postpartum, respectively. On the other hand, the most influence on IgG content at 100°C/10 min, where the percentage losses were 95.7% at 1<sup>st</sup> and 100% at 2 and 3 days postpartum. It could be concluded that the stability of IgG in bovine milk was influenced by thermal treatments. These results are in accordance with those reported by El-Loly (1996) and Zagorska and Ciprovica (2012). While Mainer et al. (1997) had different research results, HTST pasteurization (72°C/15 s) led to 25 to 40% loss of IgG concentration.

### Amino acids composition of bovine milk IgS during the first week postpartum

The essential and non-essential amino acids composition

of bovine milk IgS from parturition to 7<sup>th</sup> day postpartum are presented in Table (4) Threonine, leucine, phenylalanine, histidine, aspartic, glutamic, glycine and tyrosine concentrations were gradually decreased during the first week of lactation, values being 0.9 to 0.4, 2.4 to 1.0, 1.4 to 0.6, 0.9 to 0.3, 1.6 to 0.7, 3.25 to 1.3, 0.25 to 0.1 and 1.0 to 0.5 mg/100 ml at 0-0.5 and 7 days postpartum, respectively. Whereas, valine, methionine, isoleucine, lysine, serine, alanine, cystine, arginine and proline concentrations were the highest at 0 to 0.5 day that then decreased at 7<sup>th</sup> day postpartum, but these decreases were progressively. These values at 0 to 0.5 and 7<sup>th</sup> days postpartum were valine (1.1 and 0.5), methionine (0.6 and 0.2), isoleucine (0.7 and 0.3), lysine (1.5 and 0.6), serine (1.15 and 0.54), alanine (1.2 and 0.5), cystine (0.10 and 0.4), arginine (1.04 and 0.5) and proline (2.2 and 0.8) mg/100 ml, respectively. From the obtained data, it is evident that the generally total of amino acids values were highest at 0 to 0.5 day and gradually decreased at the following days, these values were 22.2, 19.6, 19.1, 16.6, 13.65, 12.4, 10.7 and 9.2 mg/100 ml at 0 to 0.5, 1, 2, 3, 4, 5, 6 and 7 days postpartum, respectively.

Furthermore, it is very clear that glutamic acid (non-essential) was found in the largest amount of bovine milk IgS in amount 2.2 mg/100 ml of the generally total amino acids with a ratio 14.25% of them. While in the essential amino acids, the leucine acid was presented in the highest content with ratio 10.2% of the generally total in a mean 1.6 mg/100 ml. These results are in contrast with

**Table 4.** Amino acids composition (mg/100 ml) of bovine milk IgS during the first week postpartum.

Amino acids	Lactation period (days)								
	0-0.5	1	2	3	4	5	6	7	
Essential	Valine	1.08	0.98	1.02	1.08	0.71	0.68	0.47	0.47
	Leucine	2.42	1.91	1.81	1.68	1.24	1.24	1.31	1.00
	Isoleucine	0.72	0.57	0.45	0.49	0.38	0.35	ND	0.30
	Threonine	0.89	0.87	.081	0.70	0.56	0.48	0.42	0.3.8
	Methionine	0.61	0.43	0.48	0.23	0.29	0.23	0.31	0.19
	Phenylalanine	1.37	1.17	1.16	1.13	0.85	0.80	0.79	0.60
	Histidine	0.93	0.78	0.68	0.67	0.53	0.51	0.40	0.33
	Lysine	1.52	1.40	1.25	1.24	0.85	0.81	0.61	0.60
	Total	9.54	8.11	7.65	7.22	5.41	5.11	4.31	3.87
Non-essential	Aspartic	1.59	1.54	1.07	1.31	0.90	0.89	0.75	0.69
	Serine	1.15	1.19	1.12	1.30	0.85	0.69	0.59	0.54
	Glutamic acid	3.25	2.84	2.74	2.29	1.90	1.68	1.63	1.29
	Glycine	0.25	0.24	0.23	0.21	0.20	0.18	0.12	0.12
	Alanine	1.23	1.07	1.05	0.74	0.78	0.64	0.65	0.53
	Cystine	0.98	0.84	1.37	0.02	0.72	0.75	0.70	0.42
	Tyrosine	1.01	0.93	0.91	0.90	0.73	0.60	0.46	0.47
	Arginine	1.04	0.96	1.06	1.29	0.78	0.70	0.55	0.48
	Proline	2.21	1.85	1.88	1.31	1.37	1.20	1.00	0.76
Total	12.70	11.46	11.44	9.39	8.23	7.33	6.43	5.30	
<b>Overall total</b>	<b>22.24</b>	<b>19.57</b>	<b>19.09</b>	<b>16.61</b>	<b>13.65</b>	<b>12.44</b>	<b>10.74</b>	<b>9.18</b>	

ND: Not detected.

those reported by El-Loly (1996) who observed that buffalo's milk IgS at the first 12 h postpartum contained higher values of all essential amino acids except leucine and lysine.

#### Effect of some heat treatments on amino acids contents of bovine colostrum IgS

Composite colostrum samples collected throughout the first milking (1, 2 and 3 days) after calving in dairy cows. Heating was carried out at 63°C /30 min, 72°C / 15 s and 100°C /10 min., and then followed by rapid cooling to 37°C for all samples. As known, milk is a heat labile material and the thermal treatments of milk are to improve quality. Therefore, it is very important to understand the changes happening in the amino acids composition of milk IgS during the applied thermal treatments. It could be noticed that the values of all essential amino acids were reduced in thermal treated milk samples at 63°C/30 min, 72°C/15 s and 100°C/10 min. compared to control sample except histidine value at 63°C/30 min. Non-essential amino acids values were decreased in thermal treated milk samples at 63°C/30 min., 72°C/15 s and 100°C/10 min. compared to control

sample except proline value at 72°C/15 s. On the other hand, the highest influence on all amino acids values was at 100°C/10 min. because this protein is sensitive to heating. The effect of heat treatment at 72°C/15 s on values of aspartic, glutamic and glycine were lower than that effect of heated samples at 63°C/30 min. (Table 5). El-Loly (1996) observed that the lysine concentration of the IgS content of buffalo's milk was decreased at sterilization treatment (130°C/15 s), while aspartic and glutamic values were increased at the boiling and sterilization methods. Isoleucine, leucine and phenylalanine values were increased, but glycine and alanine were decreased from control to sterilized milk samples.

#### Conclusion

Immunoglobulins an important component of the immunological activity found in colostrum and milk. They are central to the immunological link that occurs when the mother transfers passive immunity to the offspring. Cattle provide a readily available immune rich colostrum and milk in large quantities, making those secretions important potential sources of immune products that may benefit humans.

**Table 5.** Effect of heat treatments on amino acids composition (mg /100 ml) of bovine milk IgS.

Amino acids	Control	Heat treatment			
		63°C/30 min	72°C/15 s	100°C/10 min	
Essential	Valine	0.66	0.58	0.44	0.18
	Leucine	1.09	0.94	0.88	0.42
	Isoleucine	0.33	0.30	0.25	0.16
	Threonine	0.52	0.41	0.35	0.26
	Methionine	0.27	0.27	0.24	0.19
	Phenylalanine	0.70	0.64	0.56	0.30
	Histidine	0.42	0.52	0.35	0.21
	Lysine	0.81	0.62	0.60	0.30
	<b>Total</b>	<b>4.80</b>	<b>4.28</b>	<b>3.67</b>	<b>2.02</b>
Non-essential	Aspartic	0.82	0.63	0.73	0.48
	Serine	0.80	0.59	0.57	0.29
	Glutamic acid	1.67	1.28	1.50	0.91
	Glycine	0.20	0.15	0.15	0.05
	Alanine	0.68	0.56	0.51	0.17
	Cystine	0.58	0.41	0.10	0.10
	Tyrosine	0.72	0.58	0.45	0.17
	Arginine	0.71	0.45	0.39	0.21
	Proline	1.01	0.99	1.17	0.25
<b>Total</b>	<b>7.20</b>	<b>5.64</b>	<b>5.58</b>	<b>2.62</b>	
<b>Overall total</b>	<b>12.00</b>	<b>9.92</b>	<b>9.25</b>	<b>4.63</b>	

ND: Not detected.

## Conflict of Interest

The authors have not declared any conflict of interest.

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