Abstract

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 0-0.5, 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 0-0.5 and 1st 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 1st days postpartum, respectively. However, IgG concentrations dropped markedly with time progress of lactation. 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 sec 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 1st and 100% at 2 and 3 days postpartum. The total amino acids values of bovine milk immunoglobulins (IgS) were highest at 0-0.5 day and dropped markedly with time progress of lactation.

Keywords: Bovine milk; Colostrum; Immunoglobulin G (IgG); Heat treatments; Amino acids

Introduction

Colostrum is the first collection of a thick creamy liquid, without blood or infection, produced by the mammary gland of a parturient mother shortly after birth. It 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. This is the source of passive immunity achieved by the mother and is transferred to the baby. Also, it is a unique food created by nature to sustain and protect the newborn 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 [1]. Vetter et al. [2] mentioned that the provision of quality colostrum with a high concentration of IgG is critical for newborn calf health, because first colostrum may be low in overall concentration to effectively reduce the risk of newborn infections.

Colostrum is the secretion of the mammary gland produced immediately after parturition though three days post-parturition. Within each species, colostrum is the first natural food for the newborn calf. The nutritional and physiological needs of the neonate during this period of very early life are typically quite specialized and correspondingly. The composition of the maternal colostrum is tailored to meet these unique requirements [3-5]. 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, and colostral 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 [6,7].

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 [8].

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 [9]. The immunoglobulins (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 (IgG1 and IgG2), IgM, IgA, and IgE, based on the heavy chain they possess [10-12]. IgG, IgM and IgA are present in high levels in milk, especially in colostrum. The IgG is dominant in colostrum, milk and blood (about 80-90%, 60-70% and 90% of total IgS, respectively) [13,14].

IgG concentrations change throughout the first six milkings’ 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 immuno-sufficiency. 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 [15].

Interest has been given to the effect of heat treatments on different Ig classes. Detectable IgG in colostrum or colostral whey are reduced by heat treatment, however at a slower rate than for isolated IgG. Thermal Proteins' such as sugars or glycerol can increase the stability of isolated IgG to heat treatment [16,17]. According to Chen and Chang IgS are thermo labile. Exposure to temperatures of 75°C/5min can reduce detectable isolated bovine IgG by 40%, and by 100% at 95°C/15 sec. The explanation of it is conformational changes in the IgG molecule caused by heat exposure [18]. Donahue et al. [19] 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, Gelsing et al. [20] 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 analyse 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 0-0.5, 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 [21].

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 1000 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 [22]. 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 & Mackelvey [23]. SRIX plates containing antibodies to IgG and IgM (Cat. No. RK 200.3, RN 278.3, the Binding Site LTDDR, 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 sec. 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 [24]. 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. Twenty µl 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 0-150 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 [25]. All data were expressed by means & 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<0.01-0.05.

Results and Discussion

Gross chemical composition of bovine milk

It was noticed that the mean ± SD concentration of TS was 19.83 ± 2.12% during 0-0.5 day postpartum. TS content decreased to reach a mean ± SD 17.55 ± 1.65% at 1st 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 0-0.5 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. who observed that the TS contents decreased irregularly with time after parturition. TS content at 1st day postpartum in the present study was higher than those reported by Kleinsmith [26]. Bar et al. found that the mean of TS contents in bovine colostrum were 27.64 and 23.56% with ranges 18.3-43.3 and 21.6-29.15%, respectively, while in mature milk were 12.7 and 12.9% [27] found that the TS contents in bovine colostrum were 26.99, 20.46, 14.53, 12.77, 12.23 and 11.46% at 0, 6, 12, 24, 36 and 48 h postpartum, respectively. Also, Abd El-Fattah stated that the mean of TS content in bovine colostrum at calving was 24.19%. TP concentration of bovine milk was higher significantly during 0-0.5 day postpartum (10.65 ± 1.71%) than other days, followed by 1st day postpartum (9.26 ± 1.32%). No significant differences were found between the mean ± SD concentrations of TP at 2nd day (7.36 ± 0.53%) and 3rd day (6.67 ± 0.13%), but significant differences were found between 2nd (7.36 ± 0.53%) and 4th days (5.77 ± 0.36%). However, no significant differences were found between the mean ± SD concentrations of TP at 5 (5.18 ± 0.74%), 6 (5.00 ± 0.56%) and 7 (4.48 ± 0.63%) days postpartum (Table 1). These results are in agreement with those found by Tsiouplas et al.;
It was noteworthy found that the 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 [35] suggests 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., at 1st to 5th day; Kleinsmith at 1st and 2nd days; Klimes et al. at 3rd and 5th days postpartum. But, it was higher than those reported by Klimes et al. at 1st day postpartum. Bar et al. (2010); Conte and Scartantino showed that the mean of lactose contents in bovine colostrum were 2.49, 3.5 and 2.04% with ranges 1.2-5.2, 3.37-3.94 and 1.46-3.19%, in the same order, while in mature milk, it were 4.1 and 4.6%.

The mean ± SD concentrations of ash were higher significantly during 0-0.5 day (1.01 ± 0.09%) than those of other days postpartum, then dropped to range from 0.84 to 0.10% at 1st day postpartum with a mean ± SD 0.94 ± 0.07%, while at 2nd day postpartum had a mean ± SD 0.88 ± 0.04%, without significant differences between 1st and 2nd day. A gradual decrease could be observed on the following days namely, 0.85 ± 0.02, 0.81 ± 0.02, 0.79 ± 0.02, 0.77 ± 0.04 and 0.75 ± 0.03% at 3, 4, 5, 6 and 7 days postpartum, in order. There were significant differences between ash content at 2nd and 4th 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.; Tsioulpas et al. and Abd El-Fattah et al. who observed that the ash content decrease with time after parturition. This may be attributed to decrease 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 [36]. Bar et al. showed that the mean values of ash content in bovine colostrum were 0.05 and 1.48% with ranges 0.02-0.07 and 1.10-1.33%, respectively, while in mature milk it were 0.7 and 0.8%.

**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 0-0.5, 1, 2, 3, 4 and 7 days postpartum. The relation between the IgG concentrations and the diameter of the precipitated antigen-antibody reaction are found in (Figures 1-5) (wells No. 1 & 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 0-0.5 and 1st 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 1st days postpartum, respectively (Table 2). However, IgG concentrations dropped markedly with time progress of lactation. At 2nd, 3rd and 4th days, the mean ± SD values of IgG were 93.74 ± 7.57, 79.34 ± 10.02 and 67.72 ± 22.83 g/L, respectively, which had dropped ratios of 23.54, 35.29 and 44.77%, in order. At the end of the first week (7th day), the mean ± SD of IgG concentration was 55.16 ± 17.30 g/L that had dropped ratio 55.01% when compared with its concentrations at 0-0.5 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 [37]. These results are in good agreement with those of Saucedo-Quintero and Avendano-Reyes [38]. Elstrand et al. stated that the major IgS present in bovine milk are IgG with 85% ratio, among which 95% belong to the sub classes IgG1 and 5% to the IgG2.

The mean concentrations of IgG (IgG1+IgG2) were 92.8, 80.9, 68.9, 25.1, 17.30 and 9.27 g/L, respectively, during 0-0.5, 1st, 2nd, 3rd, 4th and 7th days postpartum, respectively. The IgG 1 and 2 were obtained as 80.2 ± 17.30, 70.6 ± 15.02, 60.0 ± 12.97 and 30.9 ± 9.86 g/L during 0-0.5, 1st, 2nd and 3rd days postpartum respectively (Table 2). These results are closely similar with those of Kleinsmith and Abd El-Fattah et al. 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 [34].
31.5, 17.7 and 12.2 g/L during 0-6, 7-10, 11-20, 21-30, 31-40, 41-50 and 51-80 h, respectively. Similar results were reported by Bar et al.; Morrill et al, and Quigley et al. [39], while in mature milk, it was 0.72 and 0.556 mg/ml.

**Effect of heat treatments on bovine colostrum IgG**

It could be noticed that the concentrations of IgG during thermal treatments were reduced from 125.70, 107.70 and 84.79 g/L in control individual milk samples at 1, 2 and 3 days postpartum to 90.20, 75.10 and 59.20 g/L in thermally treated milk at 63°C/30 min, in order, it were decrease to 28.24, 30.27 and 30.18%, respectively (Table 3). These different as a result of using various temperatures, it has occurred loss (denaturation) in IgG content at different rates.

Increasing temperature to 72°C/15 sec., the IgG concentrations of heated individual milk samples were reduced to 66.20, 28.50 and 4.23 g/L, it were decreased by 52.67, 26.46 and 4.99% 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 95.72% at 1° 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 [40]; While Mainer et al. [41] had different research results, HTST pasteurization (72°C/15 sec.) led to 25-40% loss of IgG concentration.

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

**Table 2:** IgG Concentrations of Bovine Milk During the First Week Postpartum

<table>
<thead>
<tr>
<th>Lactation period (days)</th>
<th>Concentration of IgG (g/L)</th>
<th>Mean ± SD</th>
<th>% of decrease</th>
<th>Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.5</td>
<td>122.00</td>
<td>120.80</td>
<td>115.10</td>
<td>128.20 126.90 126.90</td>
</tr>
<tr>
<td>1</td>
<td>110.80</td>
<td>119.70</td>
<td>114.00</td>
<td>124.50 123.20 94.00</td>
</tr>
<tr>
<td>2</td>
<td>88.07</td>
<td>104.70</td>
<td>96.50</td>
<td>85.42 38.00 84.18</td>
</tr>
<tr>
<td>3</td>
<td>76.80</td>
<td>83.00</td>
<td>89.50</td>
<td>63.40 84.18 67.70</td>
</tr>
<tr>
<td>4</td>
<td>50.00</td>
<td>78.20</td>
<td>88.80</td>
<td>38.00 83.58 70.60</td>
</tr>
<tr>
<td>7</td>
<td>35.80</td>
<td>64.80</td>
<td>67.70</td>
<td>36.90 64.80 55.16</td>
</tr>
</tbody>
</table>

LSD*: Standard deviation; LSD**: The least significant difference. Means with different superscript within the same column are significantly different. SD*: Standard deviation; LSD**: The least significant difference.
### Table 3: Effect of Heat Treatments on Bovine Colostrum IgG Concentrations (G/L)

<table>
<thead>
<tr>
<th>Lactation period (days)</th>
<th>Control</th>
<th>Heat treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>63°C/30min.</td>
</tr>
<tr>
<td>1</td>
<td>125.70</td>
<td>90.20</td>
</tr>
<tr>
<td>2</td>
<td>107.70</td>
<td>75.10</td>
</tr>
<tr>
<td>3</td>
<td>84.79</td>
<td>59.20</td>
</tr>
</tbody>
</table>

### Table 4: Amino Acids Composition (Mg/100 Ml) of Bovine Milk Igs During the First Week Postpartum

<table>
<thead>
<tr>
<th>Lactation period (days)</th>
<th>Control</th>
<th>Heat treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>63°C/30min.</td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td></td>
<td>0-0.5</td>
</tr>
<tr>
<td>Valine</td>
<td>1.08</td>
<td>0.98</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.42</td>
<td>1.91</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.72</td>
<td>0.57</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.89</td>
<td>0.87</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.61</td>
<td>0.43</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.37</td>
<td>1.17</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.93</td>
<td>0.78</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.52</td>
<td>1.40</td>
</tr>
<tr>
<td>Total</td>
<td>9.54</td>
<td>8.11</td>
</tr>
<tr>
<td>Aspartic</td>
<td>1.59</td>
<td>1.54</td>
</tr>
<tr>
<td>Serine</td>
<td>1.15</td>
<td>1.19</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3.25</td>
<td>2.84</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.23</td>
<td>1.07</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.98</td>
<td>0.84</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.01</td>
<td>0.93</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.04</td>
<td>0.96</td>
</tr>
<tr>
<td>Proline</td>
<td>2.21</td>
<td>1.85</td>
</tr>
<tr>
<td>Total</td>
<td>12.70</td>
<td>11.46</td>
</tr>
</tbody>
</table>

**ND:** Not detected
The essential and non-essential amino acids composition of bovine milk IgS from parturition to 7th 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 0.89-0.38, 2.42-1.00, 1.37-0.60, 0.93-0.33, 1.59-0.69, 3.25-1.29, 0.25-0.12 and 1.01-0.47 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 0-0.5 day that then decreased at 7th day postpartum, but these decreases were un-gradually. These proline concentrations were the highest at 0-0.5 day that then decreased methionine, isoleucine, lysine, serine, alanine, cystine, arginine and proline concentrations were the highest at 0-0.5 day that then decreased at 7th day postpartum, but these decreases were un-gradually. These results are in contrast with those reported by El-Loly et al. (2010) Changes in physical properties of bovine milk from the colostrum period to early lactation. J Dairy Sci 90: 5012-5017.

Furthermore, it is very clear that glutamic acid (non-essential) was found in the largest amount of bovine milk IgS in amount 2.20 mg/100 ml. These results are in contrast with those reported by El-Loly 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 632°C for 30 min., 72°C for 15 sec. and 100°C for 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 sec. 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 sec. and 100°C/10 min. compared to control sample except proline value at 72°C/15 sec. 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 sec. 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 sec.), 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.

References


