Evaluation of Physico-Chemical Properties and Nutrient Components of Dairy Water Buffalo (Bubalus bubalis) Milk Collected during Early Lactation

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ABSTRACT

The study determined the physico-chemical properties and nutrient component of dairy water buffalo (Bubalus bubalis) milk collected during early lactation. Specifically, it determined the association of the physicochemical properties and milk composition to physiological parameters (age, number of parities, history of mastitis) of the animals. Results showed that the properties of the buffalo milk collected during the early lactation have gravity of 1.035% titratable acidity of 0.14% and pH 6.63. Alcohol precipitation test for protein stability was positive. MBRT was 7 hours which means that the quality of milk was excellent. The mean milk components of dairy buffaloes were as follows: fat was 6.17%, protein was 4.56%, lactose was 4.73%, total solid (TS) was 15.53% and solid not fat (SNF) was 9.36% while the mean somatic cell count was 122 x 1000 cell/ml. Among the physiological parameters, history of mastitis was significantly associated with pH and% lactose, parity was significantly associated with protein stability and age was significantly associated with% fat and% TS.

Key words: Milk quality, Physico-chemical properties, Milk composition, Early lactation, Water buffalo

INTRODUCTION

In the Philippines, dairy production is gaining popularity. The local supply of liquid milk is only about 1.12%. The total dairy animal population was 6.34% higher than the last year’s headcount. The country produced 20.4 million liters of fresh milk, 3.34% higher than last year’s level of 19.7 million liters, 7.1 million liters or 36% were water buffalo milk, 0.34 million liters or 1.7% were goat’s milk and 65.5% or 12.9 million liters were cow’s milk. The production from individual dairy farms shared around 38% in the total water buffalo milk output while the contributions to the total milk production from cooperative, commercial and institutional dairy farms were 30.98%, 3.55% and 25.35%, respectively (PSA, 2016).

In Japan, the quality of cow’s milk was standardized based on the genetic performance and breeds, feeding style and state of milk consumption. Tests such as physical and chemical testing (specific gravity, alcohol precipitation, titratable acidity, pH), microbiological testing (total aerobic plate count and total coliform count, Methylene Blue Reduction test (MBRT), milk component assessment and somatic cell count (SCC) help established the cow’s milk quality standard (Ichikawa et al., 1998). In the Philippines, cow’s milk standards for milk quality are being used to evaluate the produce of water buffaloes. Although water buffalo’s milk has higher quality compared to cow’s milk, there are no established standard that is being followed in ranking or grading the milk produced by water buffaloes.

Milk quality standards can be achieved through evaluation of milk quality such as physical and chemical testing (specific gravity, titration acidity, alcohol precipitation test, MBRT and pH) and milk component assessment (using Milkoscan) and SCC. Any failure at the farmer’s stage, deterioration on the quality of milk might occur and practically milk quality is not obtained, even though milk is basically a high quality food and good for the health (Ichikawa et al., 1998).

Studying milk quality would develop and establish standards on buffalo’s milk serving as future reference data to rank or grade the milk. Prerequisite is to make further studies before quality standards can be well-established.
regarding the milk quality standard for dairy buffaloes. This study was conducted to determine the physico-chemical properties and milk components of dairy buffalo milk collected during early lactation. Specifically, it determined the association of the physicochemical properties and milk composition to physiological parameters (age, number of parities, history of mastitis) of the animals.

**MATERIALS AND METHODS**

**Animal Selection and Identification**

A total of five water buffaloes at their early lactation were used as experimental animals. Early lactation according to Oliveira et al. (2010) is approximately one month after the start of lactation but not exceeding four months. The animal profile such as age, number of parity, and history of mastitis record were gathered.

**California Mastitis Test (CMT)**

The CMT is routine practice in the milk collection site, and it is conducted before milking. About one teaspoon (5 ml) of milk from each quarter was collected from each of the animal and an equal amount of CMT solution was added and mixed to each cup in the paddle. The milk from the animals with mastitis has a visible gel-like formation. This formation has an equivalent CMT scores which is directly related to average somatic cell counts present in the milk (Table 1).

**Evaluation of the physico-chemical properties and MBRT of milk samples**

Collection of milk samples was done on the 5th week, 9th week, 13th week, and 17th week of lactation. The collection was done twice a day (early morning and afternoon) in the whole duration of the experiment. A total of 400 ml of milk samples per animal were collected using a milking machine. Samples were placed in a clean and sterile bottle to avoid contamination and transported immediately in the laboratory.

Milk tests include specific gravity, titratable acidity, alcohol precipitation, pH (Draaiyer et al., 2009) and MBRT (Atherton and Newlander, 1977). The raw data were analyzed using Tukey's Studentized Range (HSD), Pearson Correlation Coefficient, and graphical analysis.

**Assessment of milk component and somatic cell count**

Pooled milk samples per animal were collected from the 2nd, 3rd, 4th, 5th, 9th, 13th and 17th week. A total of 50 ml milk per animal was collected directly from the milk machine receiver using conical tube to accommodate all the tests involved. Iodine was used as an antiseptic using teat dips after milking. A 0.5 ml of 10% Potassium Dichromate was added to the samples that were not processed within the day of collection.

Automatic milk analyser was used to evaluate the milk composition such as fat, protein, lactose, total solids (TS) and solids not fat (SNF) and the SCC. Association, descriptive and graphical analysis were used to interpret the data.

**RESULTS AND DISCUSSION**

**Physico-chemical Properties and MBRT of milk samples**

The overall mean specific gravity for the early lactation was 1.033, % titratable acidity was 0.14%, pH was 6.63, MBRT was seven hours. APT test for protein stability was positive. The results are in conformity to the standard range provided by the FAO (1989) for physico-chemical properties and MBRT by Wehr and Frank (2004) except protein stability. This means that the water buffalo milk passed the physico-chemical properties and microbial quality and was fit for human consumption. The results on the specific gravity, titratable acidity, pH are shown in Figure 1. Variation existed possibly due to various factors like the age of the animal, number of lactation cycles, breed, diet, and diseases (Tsoulpas et al., 2007).

The specific gravity was highest during the fifth week of collection at 1.034 and lowest during the 13th and 17th week at 1.032. Titratable acidity was highest during 5th, 13th, and 17th week of collection at 0. 15% and lowest during the 9th week, 0. 12%. Titratable acidity increases in the presence of acid-producing bacteria and decreases by the addition of water or neutralization (Ichikawa et al., 1998). The highest pH was recorded on the 5th week at 7.03 and lowest on the 13th week at 6.13. The results are in congruence of the study of Mahmood and Usman (2010) with few numerical differences, which was 6.53 - 7.00. The shortest time for the methylene blue to disappear was 5.1 hours on the 13th week and the longest was 8 hours on the 17th week (Figure 1).

**Table 1:** Interpretation of CMT scores

<table>
<thead>
<tr>
<th>CMT score</th>
<th>Somatic cell range</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (Negative)</td>
<td>0 – 200,000</td>
<td>Healthy Quarter</td>
</tr>
<tr>
<td>T (Trace)</td>
<td>200,000 – 400,000</td>
<td>Subclinical Mastitis</td>
</tr>
<tr>
<td>1</td>
<td>400,000 – 1,200,000</td>
<td>Serious Mastitis Infection</td>
</tr>
<tr>
<td>2</td>
<td>1,200,000 – 5,000,000</td>
<td>Serious Mastitis Infection</td>
</tr>
<tr>
<td>3</td>
<td>Over 5,000,000</td>
<td>Serious Mastitis Infection</td>
</tr>
</tbody>
</table>

**Table 2:** Overall mean of the physico-chemical properties and MBRT

<table>
<thead>
<tr>
<th>Property</th>
<th>Mean</th>
<th>FAO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>1.0350</td>
<td>1.030-1.036</td>
</tr>
<tr>
<td>% Titratable acidity</td>
<td>0.14</td>
<td>0.13-0.16</td>
</tr>
<tr>
<td>pH</td>
<td>6.63</td>
<td>6.6-6.8</td>
</tr>
<tr>
<td>MBRT (hrs.)</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Low specific gravity would mean that the milk has more milk fat or water was added. According to Ichikawa et al. (1998), milk with more SNF has higher specific gravity. The buildup of acids in milk could be due to the presence of acid-producing bacteria and decreases by the addition of water or neutralization. The specific gravity, titratable acidity, pH are shown in Figure 1.
50,000 (Wehr and Frank, 2004). Furthermore, according to Michael (2012), the microbial quality of milk is influenced by the hygiene and sanitary conditions or practices of milk collection at the farm. Microflora present on teat skin, milking machine and milker’s hand, water and the milking environment are different sources that could influence contamination of microorganisms in milk. Such factors affect the quality of milk; thus, increase in numbers of microorganism is linked to hygiene practices during milking. The MBRT is a faster way to detect the quality of milk; however, it can be applied to aerobic bacteria. The result of APT was positive contrary to the negative result set by FAO (1989) (Table 2). This means that the milk samples could be high-acidity alcohol-unstable milk. There was build-up of lactic acid and other acids in milk, calcium has liberated from milk proteins, and calcium lactate and other calcium acid salts were formed. The milk proteins then become unstable and coagulate in the presence of 70% alcohol (Ichikawa et al., 1998).

The association of physiological parameters with physico-chemical properties and microbial quality are shown in Table 3. Results showed that during early lactation, history of mastitis has significant effect on the pH of milk. This could imply that the animal used in the study may be infected with subclinical mastitis. According to Hassan (2013), the pH is significantly higher in the infected animal’s milk than uninfected milk. These changes were linked to increased permeability of the mammary epithelium cell which leads to the transfer of the components from blood to milk such as citrates, bicarbonates that cause elevated pH levels. The probable reason of pH increase due to severity of mastitis may be attributed to the lowered acidity as found in mastitic milk. The lowered acidity in mastitic milk is due to the reduction in lactose contents as the lactic acid formation is minimum in this case (Ahmad et al., 2005).

Furthermore, parity was significantly associated with the protein stability. According to Bonfatti et al. (2012), parity affected the content of protein fractions as well as protein composition. Contents TCN, WH, αS2-CN, β-CN, and κ-CN decreased as parity increased. Decreased contents of all casein and whey protein fractions were observed for parity greater than three (Ahmad et al., 2005). No previous study investigated parity effects on water buffalo milk protein composition, but their results largely agree with those reported for protein composition of bovine milk.

### Table 3: Association of age, parity and history of mastitis to physico-chemical properties and microbial quality.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>% Titratable Acidity</th>
<th>Specific gravity</th>
<th>Protein stability</th>
<th>MBRT (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td><strong>Correlation</strong></td>
<td>-0.0841</td>
<td>0.1540</td>
<td>0.0970</td>
<td>0.0000</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.7235</td>
<td>0.5166</td>
<td>0.6841</td>
<td>1.0000</td>
<td>0.1149</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td><strong>Correlation</strong></td>
<td>-0.02070</td>
<td>-0.13940</td>
<td>-0.19939</td>
<td>0.49237</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.9310</td>
<td>0.5578</td>
<td>0.3993</td>
<td>0.0274*</td>
<td>0.7544</td>
</tr>
<tr>
<td><strong>History of mastitis</strong></td>
<td><strong>Correlation</strong></td>
<td>-0.353</td>
<td>-0.0105</td>
<td>-0.1695</td>
<td>0.0773</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.0265*</td>
<td>0.823</td>
<td>0.3115</td>
<td>0.3775</td>
<td>0.793</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

### Table 4: Mean with standard deviation and range of milk components and SCC obtained during early lactation with reference to other studies.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>%Fat</td>
<td>6.17±1.43</td>
<td>3.07-9.15</td>
<td>6.71</td>
<td>7.97±0.44</td>
</tr>
<tr>
<td>%Protein</td>
<td>4.56±0.58</td>
<td>3.41-5.59</td>
<td>4.52</td>
<td>4.36±0.23</td>
</tr>
<tr>
<td>%Lactose</td>
<td>4.73±3.39</td>
<td>3.27-5.26</td>
<td>4.45</td>
<td>5.41±0.54</td>
</tr>
<tr>
<td>%TS</td>
<td>15.53±1.38</td>
<td>12.50-18.81</td>
<td>16.82</td>
<td>18.45±0.85</td>
</tr>
<tr>
<td>%SNF</td>
<td>9.36±0.55</td>
<td>8.25-10.32</td>
<td>10.11</td>
<td></td>
</tr>
<tr>
<td>SCC (1000cells/ml)</td>
<td>122±109</td>
<td>23.6-481.88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Association of age, parity and history of mastitis to milk components and SCC

<table>
<thead>
<tr>
<th></th>
<th>% Fat</th>
<th>% Protein</th>
<th>% Lactose</th>
<th>% TS</th>
<th>% SNF</th>
<th>SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td><strong>Correlation</strong></td>
<td>-0.497**</td>
<td>-0.184</td>
<td>0.126</td>
<td>-0.527**</td>
<td>-0.033</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.002</td>
<td>0.289</td>
<td>0.471</td>
<td>0.001</td>
<td>0.849</td>
<td>0.036</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td><strong>Correlation</strong></td>
<td>-0.275</td>
<td>-0.271</td>
<td>0.16</td>
<td>-0.341*</td>
<td>-0.143</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.111</td>
<td>0.116</td>
<td>0.359</td>
<td>0.045</td>
<td>0.414</td>
<td>0.646</td>
</tr>
<tr>
<td><strong>history of mastitis</strong></td>
<td><strong>Correlation</strong></td>
<td>-0.083</td>
<td>-0.147</td>
<td>-0.218</td>
<td>-0.193</td>
<td>-0.269</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.634</td>
<td>0.399</td>
<td>0.209</td>
<td>0.266</td>
<td>0.118</td>
<td>0.816</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

### Table 6: T-test result of age, milk components and SCC

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Protein</th>
<th>Lactose</th>
<th>TS</th>
<th>SNF</th>
<th>SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age 1-5</strong></td>
<td>6.71**</td>
<td>4.61</td>
<td>4.70</td>
<td>16.05**</td>
<td>9.34</td>
<td>82.86**</td>
</tr>
<tr>
<td><strong>Age 6-10</strong></td>
<td>5.36**</td>
<td>4.49</td>
<td>4.79</td>
<td>14.75**</td>
<td>9.39</td>
<td>180.76**</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

### Table 7: T-test result of parity, milk components and SCC

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Protein</th>
<th>Lactose</th>
<th>TS</th>
<th>SNF</th>
<th>SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parity 0-2</strong></td>
<td>6.20</td>
<td>4.69</td>
<td>4.68</td>
<td>15.64</td>
<td>9.44</td>
<td>131.16</td>
</tr>
<tr>
<td><strong>Parity 3-6</strong></td>
<td>6.13</td>
<td>4.37</td>
<td>4.82</td>
<td>15.38</td>
<td>9.25</td>
<td>108.31</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).
Fig. 1: Physico-chemical properties and MBRT of the milk samples during early lactation. A) Specific gravity, B) Titratable acidity, C) pH and, D) MBRT.

Fig. 2: A) Trends in milk composition during early lactation (1 week post calving) and B) SCC during the time of collection.

(NG-Kwai-Hang et al., 1987). According to them, the content of αS-CN progressively increased from the first to the third parity and decreased in later parities, β-CN decreased when parity number increased, and the sum of αS-CN, β-CN and κ-CN decreased when cows got older.

Milk Components and SCC

Results showed that the mean fat content of water buffalo milk was 6.17±1.43% which was nearly similar with the results of Aneja et al. (2002) and Mahmood and Usman (2010). Milk fat of water buffalo milk normally varies from 4.4 to 8.9%. Mean SNF content from milk collected was slightly lower than the results of Aneja et al. (2002). The concentration of protein, lactose and TS were 4.56±0.58%, 4.73±0.39%, and 15.53±1.38%, respectively. Results from the study are nearly the same as the results of Aneja et al. (2002) but% lactose and% total solids were lower than the results of Mahmood and Usman (2010). Furthermore, the concentration of fat, protein, lactose, TS and SNF was in range of 3.07-9.15%, 3.41-5.59%, 3.27-5.26%, 12.50-18.81% and 8.25-10.32%, respectively. Results of the study have a wider range except for the% TS compared to the results of Mahmood and Usman (2010) for fat, protein, lactose and TS which is 6.99-8.41%, 4.01-4.78%, 4.56-6.21% and 16.99-20.18%, respectively (Table 4).

Wider range of results can be attributed to the variations of the milk composition of individual animals. These variations depend on genetic factors such as breed and pedigree, physiological factors such as the stage of lactation, feeding factors such as the quality and quantity of feed, and environmental factors such as summer heat and disease such as mastitis (Ichikawa et al., 1998).

The fat % was highest during the second week of lactation which is 7.58% and was lowest during the fifth week which is 5.09%. Sudden decrease was observed from second week to fifth week of lactation and gradual increase from fifth week to seventeenth week of lactation is shown in Figure 2. Changes in the milk fat % can be due to the amount of roughage fed to the animal and the ambient
temperature during the collection. It is known that decrease in roughage consumption can lead to lower rumen pH, and this can decrease the number of protozoa and cellulose degrading bacteria. This will be followed by the increased generation of propionic and valeric acids and decreased generation of acetic and butyric acids for use in milk fat synthesis (Ichikawa et al., 1998). Daily rations for lactating cows consist of 25 kg/hd/d of napier/silage, 5.4 kg/hd/d of rice straw, 7.5 kg/hd/d of spent grain and 4.30kg/hd/d of dairy concentrate. Changes in the fat components may not be attributed to their feeds because there was no change in the composition and quantity of their diet during the period of collection. Therefore, as the temperature in the rumen increased due to an increased in the ambient temperature, the bacterial flora changed and the synthesis of short-chain fatty-acids also decreased, resulting in decreased fat, especially in unsaturated fat (Ichikawa et al., 1998).

The protein % has its highest % age on the second week of lactation. This can be due to the secretion of colostrum milk which is high in immunoglobulins content during the first week of lactation. Slight change in protein % as the lactation progresses was shown in Figure 2. Lactose was observed to have a low age % in the second week of lactation and showed a gradual increase as lactation progresses. TS indicating all solid contents present in milk were also high at the second week of lactation and showed a decrease as the lactation progresses. These results conform to Hurley (2010) that the composition of colostrum is considerably different from the composition of normal milk. As observed, it took 3 to 5 days post-partum for the secretions to change in component of milk. During this period the TS, especially the immunoglobulins, were elevated. Generally, lactose content is depressed in colostrums. High lactose in the intestine can cause scours in calves, and presumably the reduced lactose content of colostrum helps to prevent this disease. Therefore, it is imperative to let the calves suck milk from the dam during those days.

SNF was measured by subtracting fat % to TS. SNF was higher during second week of lactation due to high protein content which has great contribution to the TS. SNF was directly proportional to protein and lactose (Figure 2) but was inversely proportional to fat.

SCC mean result was 122 x1000 cell/ml, which has no effect on milk components according to Ichikawa et al. (1998) and does not exceed the limit of 400,000 cells per ml for SCC set by European Union Directives (Barbosa et al., 2010). It also showed that SCC did not exceed the normal count of milk which is <200,000 cell/ml during early lactation. In general, results showed that milk collected in early lactation (2nd–17th week) were within the range of acceptable values for water buffaloes as well as that of cattle.

The association of physiological parameters with milk components and SCC were also assessed. Results showed that during early lactation stage, age has a significant effect on milk fat % and TS % content and SCC. Table 5 shows that as the animals became older the milk fat % and TS % decreased while SCC increased. According to Ichikawa et al. (1998), age is negatively correlated to milk yield and as the milk yield increases fat content decreases. Significant effect on TS was due to decrease in fat which is a component of TS. Number of parity of an animal has a significant correlation to percent TS. California mastitis test as a basis for history of mastitis shows no significant correlation on milk content and SCC.

Table 6 shows that t-test revealed that both age group of 1-5 and 6-10 has a significant correlation on fat %, TS % and SCC while both sets of parity had no significant correlation to milk components and SCC. This means that the older the animal the lower could be the fat % and TS % and higher SCC.

Among the physiological parameters, age was significantly associated with fat %, TS % and SCC while history of mastitis was highly significantly associated with lactose. The older the animal the lower could be the fat % and TS % and the higher the SCC.

In conclusion, the milk quality of water buffaloes at early stage of lactation was evaluated in this study. The physico-chemical properties of dairy water buffalo milk collected during early lactation were as follows: specific gravity was 1.033% titratable acidity was 0.14%, pH was 6.63 which are all under the normal range set by FAO (1989). MBRT for microbial quality was seven hours. Alcohol precipitation test for protein stability was positive. Among the physiological parameters, history of mastitis was significantly associated with pH and parity was significantly associated with protein stability.

The milk components of dairy water buffaloes during early lactation were as follows: fat was 6.17%, protein was 4.56%, lactose was 4.73%, TS was 15.53% and SNF was 9.36% while the mean SCC was 122 x 1000 cell/ml. Among the physiological parameters, age was significantly associated with fat %, TS % and SCC while history of mastitis was highly significantly associated with lactose. The older the animal the lower could be the fat % and TS % and the higher the SCC. Age and history of mastitis have significant effect on milk components. Thus, these physiological changes should be monitored for the selection of animal that would produce quality milk and to prevent economic loss.

It is suggested that further study should be conducted regarding quality of milk in order to develop and establish standards that could serve as future reference data to rank or grade the water buffalo’s milk.

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