Breast milk is changing with the progression of lactation and during a 24-h period. To determine the effect of diurnality or nocturnality on total nitrogen and protein content of the breast milk. We collected human milk samples from health mothers living throughout Community of Extremadura (Spain) from January 2008 to December 2008 with less than two months of lactation. We divided the samples in three groups: calostral group (1-5 days postpartum), transitional group (6-15 days postpartum) and mature group (> 15 days postpartum). All samples were stored in a freezer at -80°C. We considered as day period between 08:00-20:00h and night period 20:00-08:00h. Analysis of the human milk samples was based on the Kjeldahl method. Protein contents were calculated from total nitrogen x 6,25. The statistical analysis of the data was descriptive (mean ± standard deviation) and inferential (T-Student test). No differences (P > 0,05) were found to exist among the contents of individual human milk samples. The mean contents of each component were as follows: Total nitrogen of calostral, transitional and mature group was 0,30 ± 0,06 g/dL (night period), 0,29 ± 0,05 g/dL (day period); 0,26 ± 0,04 g/dL (night period), 0,25 ± 0,04 g/dL (day period); 0,22 ± 0,05 g/dL (night period), 0,20 ± 0,04 g/dL (day period) respectively, in this mature group with a statistical variation (P<0,05). Protein content of calostral, transitional and mature group was 1,88 ± 0,4 g/dL (night period), 1,81 ± 0,3 g/dL (day period); 1,62 ± 0,3 g/dL (night period), 1,59 ± 0,3 g/dL (day period); 1,35 ± 0,3 g/dL (night period), 1,26 ± 0,3 g/dL (day period) respectively, in this mature group with a statistical variation (P < 0,05). Although we observed differences in the nitrogen and protein content during the individual stages of lactation, it is just in the population of mature lactating women, where the components analyzed varied significantly between day and night.

**Abstract**

**Keywords**

Protein, Total nitrogen, Kjeldahl, Breast milk, Cronobiology.