ANTIOXIDANT CAPACITY OF BUFFALO MILK FROM TWO CZECH FARMS

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ABSTRACT

The aim of this study was to evaluate the antioxidant capacity of buffalo milk from two farms in the Czech Republic with respect to farm origin and sampling period. A total of 73 individual and pooled milk samples were collected between May and October 2025. Antioxidant capacity was determined using the DPPH radical scavenging assay. Buffalo milk from farm B showed significantly ($p < 0.05$) higher antioxidant capacity than milk from farm A. Seasonal variation was observed at both farms, with the lowest antioxidant capacity recorded in October and the highest values in June (farm A) and September (farm B). These results indicate that both farm-related factors and production period significantly affect the antioxidant capacity of buffalo milk.

Keywords: buffalo milk, antioxidant capacity, DPPH, seasonality

INTRODUCTION

Buffalo milk is the second most produced milk worldwide, accounting for approximately 11–15% of global milk production, with the highest output in Asia and increasing importance in Europe, particularly in Italy (Fantuz et al., 2022; Kopáček, 2025). Compared with cow's milk, buffalo milk is characterized by higher contents of protein, fat, minerals, and total solids, as well as by the presence of bioactive compounds such as δ-valerobetaine and acetyl-L-carnitine, which exhibit antioxidant and health-promoting properties (Ahmad et al., 2013; Liao et al., 2025). Its nutritional and functional quality is influenced by multiple factors, including breed, feeding regime, lactation stage, and season, which may affect the concentration of antioxidant-related components. Despite its growing popularity, information on the variability of antioxidant capacity of buffalo milk under different production conditions remains limited, particularly in Central Europe. Therefore, the aim of this study was to evaluate the antioxidant capacity of buffalo milk from two farms in the Czech Republic with respect to farm origin and sampling period.

MATERIALS AND METHODS

Sampling

Buffalo milk samples were collected from two farms (A and B) in the Czech Republic. A total of 50 samples were obtained from farm A across seven sampling times between May and October (first at May, second and third at June, fourth at July, fifth at August, sixth at September and seventh at October), while 23 samples were collected from farm B during five sampling times between June and October (first at June, second at July, third at August, fourth at September and fifth at October). Except for the first two sampling times, which involved only farm A, sampling was conducted on the same day at both farms. The buffalo milk samples obtained from both farms included both individual and pooled samples.

Antioxidant capacity determination

DPPH Radical Scavenging Assay was used for determination of antioxidant capacity. Sample extracts were prepared following the procedure described by Jung et al. (2010). Briefly, 3 g of the sample was homogenized

with 15 ml of 5% trichloroacetic acid, after which 10 ml of chloroform was added. A fresh DPPH radical stock solution was prepared by dissolving DPPH in methanol at a concentration of 0.025 $g \cdot l^{-1}$.

The absorbance of the DPPH solution was measured spectrophotometrically at 515 nm against a blank and recorded as A_0. Subsequently, 0.2 ml of the prepared sample extract was mixed with 3.8 ml of the DPPH solution and allowed to react for 10 min. After incubation, absorbance was measured again and denoted as A_{10}. All measurements were carried out in duplicate (Heilerová et al., 2003).

The percentage of DPPH radical inhibition was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{A_0 - A_{10}}{A_0} \times 100$$

where A_0 represents the absorbance of the control at 0 min and A_{10} corresponds to the absorbance after 10 min of reaction with the sample.

Statistical analysis

Means and standard deviations were calculated using Microsoft Office Excel 2016 (Redmond, WA, USA). Differences between buffalo milk from farms A and B were assessed by Student's *t*-test, while differences among sampling periods (May–October) were evaluated using ANOVA with Tukey's post hoc test. Statistical analyses were performed using SPSS Statistics 20 (IBM Corporation, Armonk, NY, USA), with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

The results of the antioxidant capacity of buffalo milk are presented in Figures 1–4. A comparison of all milk samples from farms A and B is shown in Figure 1. Buffalo milk from farm B exhibited significantly higher antioxidant capacity ($p < 0.05$) than milk from farm A.

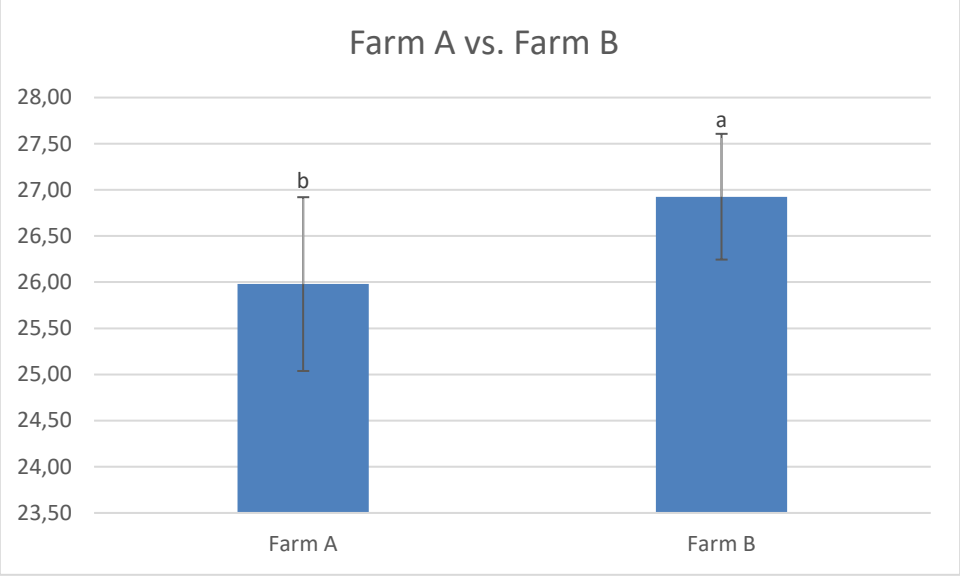

Figure 1: Antioxidant capacity (in % inhibition) of buffalo milk of farm A and farm B for all samples

The effect of sampling time on antioxidant capacity for farms A and B is illustrated in Figures 2 and 3, respectively. The results indicate that antioxidant capacity was influenced by the production period. For both farms, the lowest antioxidant capacity was observed at the final sampling in October, with values significantly

lower ($p < 0.05$) than those at earlier sampling times. In contrast, the highest antioxidant capacity was recorded in June (09 June 2025) for farm A and in September for farm B. A comparison of antioxidant capacity between farms A and B at individual sampling times is shown in Figure 4. At the June, September, and October samplings, buffalo milk from farm B showed significantly higher antioxidant capacity ($p < 0.05$) compared with milk from farm A.

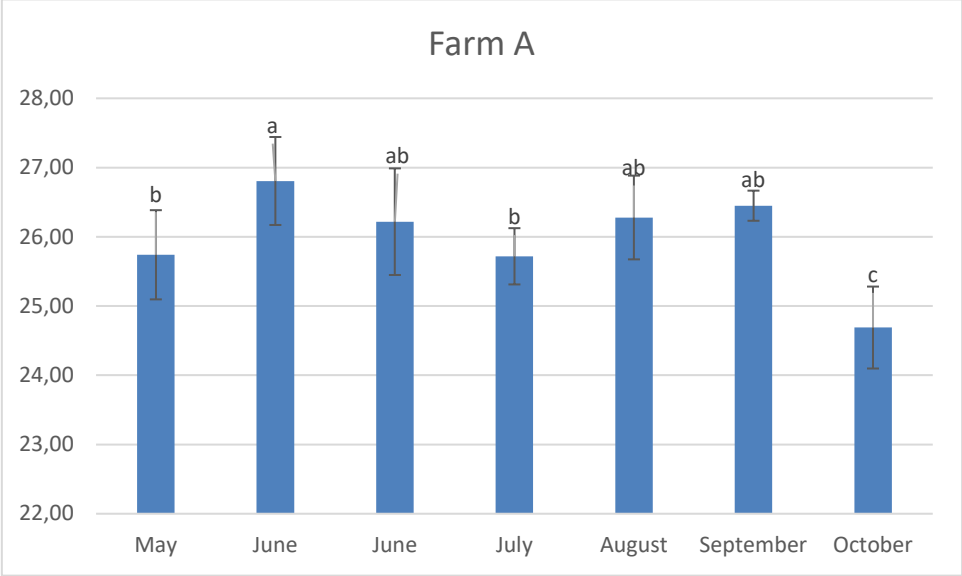

Figure 2: Antioxidant capacity (in % inhibition) of buffalo milk of farm A according to the sampling times

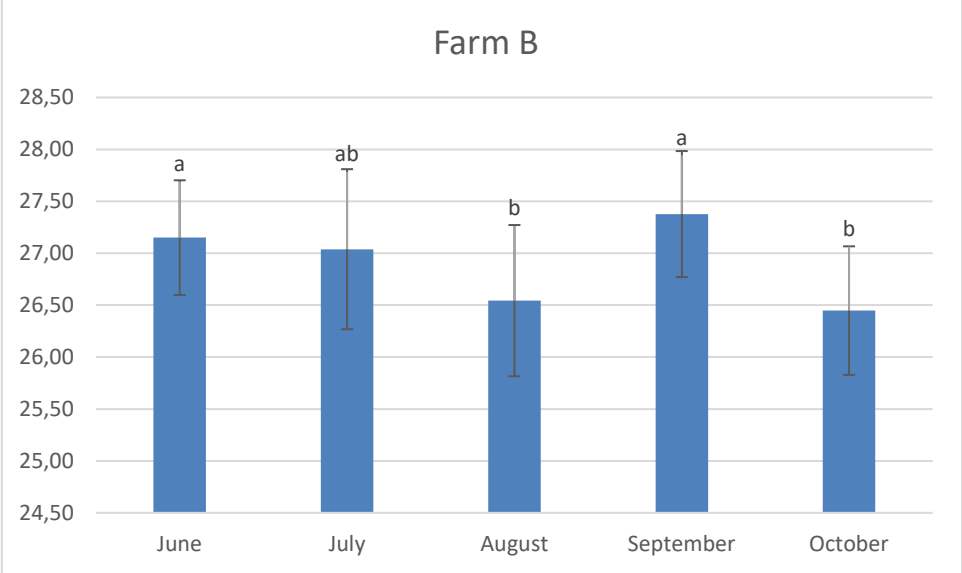

Figure 3: Antioxidant capacity (in % inhibition) of buffalo milk of farm B according to the sampling times

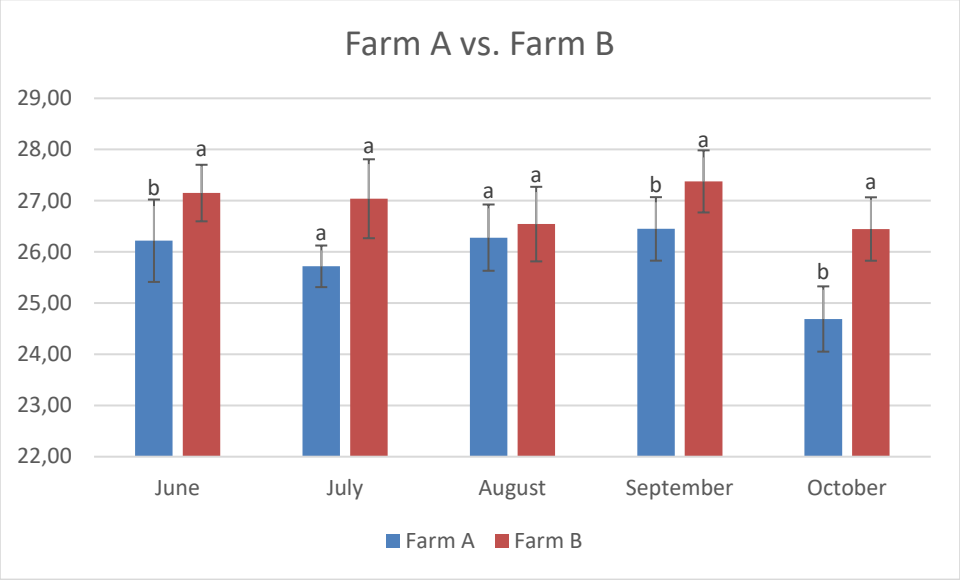

Figure 4: Antioxidant capacity (in % inhibition) of buffalo milk of farm A and farm B for each sampling time

CONCLUSION

This study demonstrates that the antioxidant capacity of buffalo milk produced in the Czech Republic is significantly influenced by both farm origin and sampling period. Milk from farm B consistently showed higher antioxidant capacity than milk from farm A, indicating the importance of farm-specific factors such as feeding regime, management, or environmental conditions. Temporal variation in antioxidant capacity was observed at both farms, with the lowest values recorded at the final sampling in October, while higher antioxidant capacity was detected during earlier sampling periods. Overall, these findings highlight the variability of antioxidant properties in buffalo milk under Central European conditions and provide valuable baseline data for further research focused on identifying the underlying factors affecting its functional quality.

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