



## EFFECT OF LACTOFERRIN LEVELS ON TBARS AND NONHEME IRON OF GROUND PORK

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### Background

Lactoferrin is an iron-binding glycoprotein that belongs to the transferring family, and mainly exists in mammalian milk. Iron ions are an important catalyst of lipid oxidation which could be bound by lactoferrin (Naidu, 2002). Probably due to this reason, the natural bovine lactoferrin could inhibit lipid oxidation in corn oil emulsions and lecithin liposome systems (Huang *et al.*, 1999). Lactoferrin could be used as a dual-purpose additive in infant formulas and similar food products for its antioxidant and antimicrobial properties (Satué-Gracia *et al.*, 2000). In the USA, lactoferrin is permitted at levels of 65.2 mg/kg of beef. In Taiwan, lactoferrin may be used in special nutritional foods under the condition “only for supplementing foods with an insufficient nutritional content and may be used in appropriate amounts according to actual requirements”. Currently, the European Union does not have a specific regulation for lactoferrin. Higher levels of bovine lactoferrin had been administered orally to mice and rats, as high as 20 g/L of milk for fourteen days and 20 g/kg for thirty weeks, respectively, with no known side effects (Naidu, 2002).

### Objectives

Lactoferrin was reported to inhibit various microorganisms, but only limited information has been reported on the effect of lactoferrin on lipid oxidation in food systems and to what extent the antioxidative effect of this additive exerts when used in ground pork and what influence it could have. The purpose of this study was to investigate the effect of lactoferrin levels on TBARS values, total and nonheme iron and pH of ground pork during storage at 4 °C for 9 days.

### Materials and methods

Bovine lactoferrin was obtained from DMV International (Veghel, the Netherlands). Ground meat samples were divided into three batches (1 kg/batch). To each batch was added 0, 40 or 80 mg lactoferrin/kg meat, respectively. Samples were stored at 4 °C for 0, 3, 6 and 9 days. TBARS (2-thiobarbituric acid reactive substances) values of meat samples were determined by using the distillation method (Ockerman, 1985). TBARS values were expressed as mg malonaldehyde/kg meat. Total iron concentration of the samples was determined on dry ashed samples by an atomic absorption spectrophotometry (Hitachi Z-8000, Tokyo, Japan) according to the AOAC method (1995). A modification of the method of Rhee and Ziprin (1987) as described by Schricker *et al.* (1982) was used to determine nonheme iron. The pH values of ground pork were determined according to Ockerman (1985).

### Results and discussion

Mean total and nonheme iron concentration of samples is shown in Fig. 1. Due to that lactoferrin is an iron-containing protein; therefore, the treatments with the addition of lactoferrin (40 and 80 mg/kg) had higher ( $P<0.05$ ) total iron concentration than the controls. The higher the levels of lactoferrin added, the higher the total iron. However, the differences in total iron concentration between the treatments with the addition of 40 and 80 mg/kg lactoferrin were not significant ( $P>0.05$ ). On the contrary, nonheme (free) iron concentration was higher for the controls (14.2 µg/g), compared to the treatments with the addition of 40 mg/kg (12.0 µg/g) or 80 mg/kg lactoferrin (10.5 µg/g). The higher the levels of lactoferrin added, the lower were the nonheme iron. The differences in nonheme iron between the controls and the treatment with the addition of 80 mg/kg lactoferrin were significant ( $P<0.05$ ). Lactoferrin used in this study is partially saturated with iron, which has the ability to bind free iron; therefore, nonheme iron concentration was lower, but total iron concentration was higher, in the treatments with the addition of lactoferrin when compared to the controls. In general, nonheme iron in all treatments increased slightly during storage at 4 °C for 9 days; however, the results were not consistent (data not shown). Kanner *et al.* (1988) reported that free iron in raw turkey and chicken muscles increased during storage at 4 °C, and suggested that this free iron increase was important in lipid oxidation in stored meat.



TBARS values were lower ( $P < 0.05$ ) in lactoferrin-treated samples (40 and 80 mg/kg) than in controls at 3, 6 and 9 days of storage, except at day 0 (Fig. 2). This suggested that lactoferrin addition could inhibit lipid oxidation and decrease TBARS values in ground pork. This was due to the iron-binding properties of lactoferrin (Naidu, 2002). From our results, it seemed that nonheme (free) iron was probably more important than total iron in lipid oxidation in ground pork. Satué-Gracia *et al.* (2000) reported that lactoferrin could be used as an additive in infant formulas and similar food products for its antioxidant properties. The differences in TBARS values between the treatments with the addition of 40 and 80 mg/kg lactoferrin were not significant at each interval of storage time. Increasing the concentration of lactoferrin at 80 mg/kg could only slightly enhance its antioxidant activity in ground pork. This suggested that the addition of 40 mg/kg lactoferrin in ground pork (20% fat) was probably sufficient to retard the lipid oxidative rancidity. TBARS values in controls increased as the storage time increased up to 9 days. TBARS values in the treatments with the addition of 40 and 80 mg/kg lactoferrin were not significantly changed at 3 days of storage; however, the TBARS values significantly increased at 6 and 9 days of storage. It seemed that lactoferrin could be more effective in inhibiting lipid oxidation in ground pork at the early storage time. All samples with or without the addition of lactoferrin had TBARS values below 0.5 during 9 days of storage, which were relatively low and well below the threshold value (1.0 mg malonaldehyde/kg meat) for detection of warm-over flavor (Boles and Parrish, 1990).

The differences in pH values among the treatments were not significant ( $P > 0.05$ ) at each storage interval (Table 1). The results indicated that lactoferrin addition has little effect on pH values of ground pork. The pH values of all meat samples slightly increased (from 6.19 to 6.31) during 9 days of storage; however, the differences were very small.

## Conclusions

Ground pork containing 20% fat was treated with 0, 40 and 80 mg/kg bovine lactoferrin and stored at 4 °C for 0, 3, 6 and 9 days. Total iron concentration was lower, but nonheme iron was higher in controls than in lactoferrin-treated samples. The higher the levels of lactoferrin added, the higher were the total iron, and the lower were the nonheme iron. The addition of lactoferrin decreases TBARS (thiobarbituric acid reactive substances) in ground pork. The antioxidant activity increased with lactoferrin concentration.

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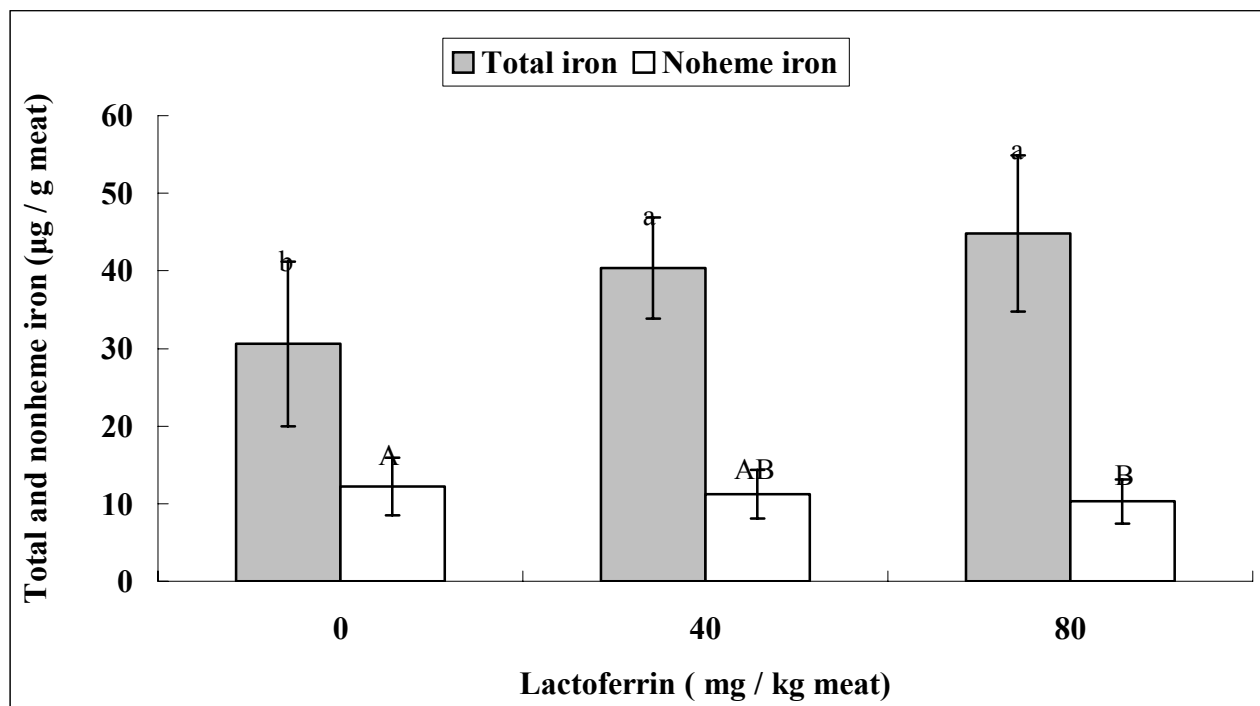


**Table 1**

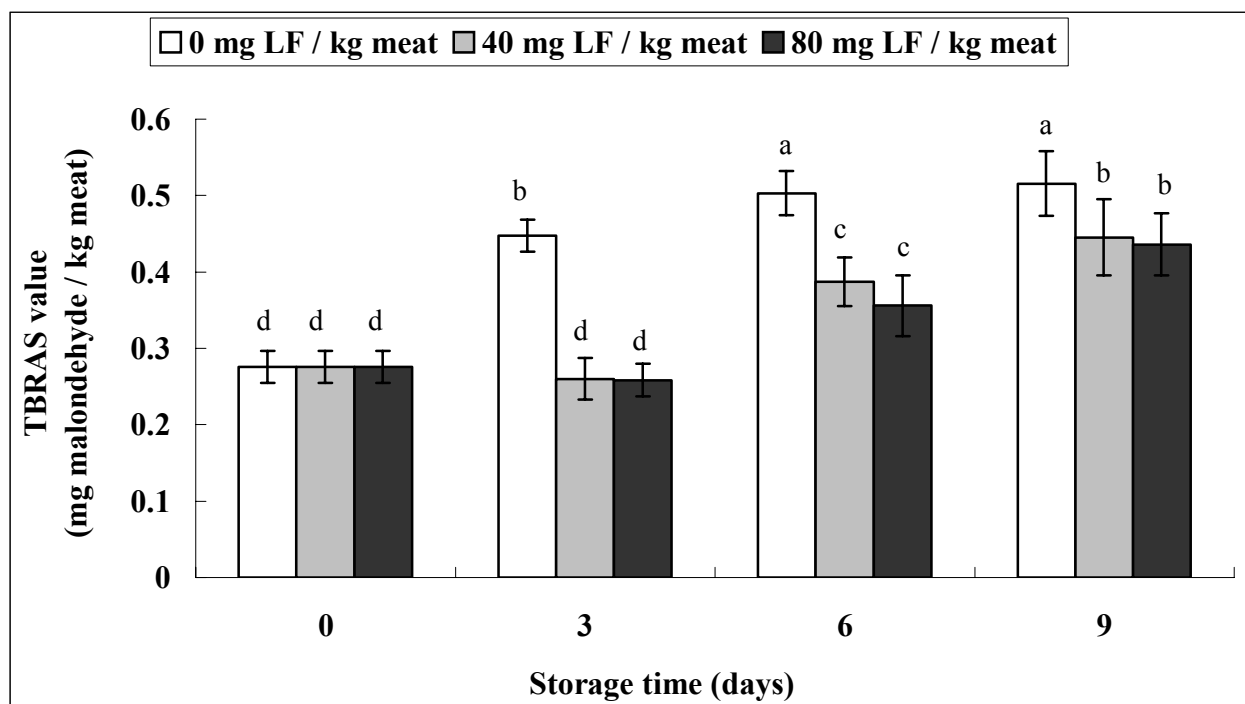
Effect of lactoferrin on pH value of ground pork during storage at 4 °C (n=12)

Days	Lactoferrin (mg / kg meat)		
	0	40	80
0	6.19±0.16	6.19±0.16	6.19±0.16
3	6.26±0.17	6.26±0.17	6.26±0.18
6	6.28±0.18	6.26±0.18	6.26±0.18
9	6.31±0.16	6.31±0.16	6.31±0.16

Each value is the mean ± 1 standard deviation.



**Figure 1.** Effect of lactoferrin levels on total and nonheme iron of ground pork during storage at 4 °C (n=27). Error bars represent ± 1 standard deviation. Each bar represents the average of three replicates. Bars with different letters are significantly different ( $P < 0.05$ ).



**Figure 2.** Effect of lactoferrin (LF) levels on TBARS values of ground pork during storage at 4 °C (n=8). Error bars represent  $\pm 1$  standard deviation. Each bar represents the average of three replicates. Bars with different letters are significantly different ( $P < 0.05$ ).