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Microbial and Nutritional Quality of Extended Shelf Life (ESL) Milk

Jee Young Imm[1], Jong Gun Kim[2], Ji Uk Kim[3], Soon Ok Park[3], Sejong Oh[4], Young Jin Kim[5], Ho Nam Chun[5], Hoo Kil Jung[5], Seungkwon You[6], Kwang Yeon Whang[2] and Sae Hun Kim[3]*

[1]*Department of Food & Nutrition, Kookmin University, Seoul 136-702, Korea*
[2]*Division of Bioscience and Technology, Korea University, Seoul 136-713, Korea*
[3]*Division of Food Science, Korea University, Seoul 136-713, Korea*
[4]*Department of Animal Science, Chonnam National University, Gwangju 500-757, Korea*
[5]*R&D Center, Maeil Dairy Industry Co., Ltd., Pyeongtaek, Gyeonggi 451-861, Korea*
[6]*Division of Biotechnology & Genetic Engineering, Korea University, Seoul 136-701, Korea*

Abstract Changes in milk quality during storage of extended shelf life milk (ESL milk) and non-ESL milk were evaluated. No significant differences were observed between ESL and typical ultra high temperature-treated (UHT) milk in physico-chemical properties including non-casein nitrogen (NCN) content, whey protein nitrogen index (WPNI), and L-ascorbic acid content. Low temperature and long time-treated milk (LTLT milk) had significantly higher NCN content and WPNI than those of UHT milk. In terms of microbial quality, yeast, molds, coliforms, and other bacteria were not detected in ESL milk during entire storage (21 days after expiration date) period at 4 and 25°C, while LTLT milk was more susceptible to microbial infection. Rats fed ESL milk resulted in significantly higher body weight, average daily gain, and feed efficiency than those given UHT milk. These results suggest ESL milk maintains better microbial quality than typical UHT milk, particularly during storage under extended refrigeration and at high temperature.

Keywords: extended shelf life, bovine milk, microbial quality, growth performance, physico-chemical properties

Introduction

Milk contains various nutritional components and biologically active substances necessary for both infants and adults (1, 16). However, although milk is an excellent source of nutrients for humans, it is also a good medium for the growth of undesirable microorganisms. The microbial and organoleptic qualities of milk are influenced by its processing, packaging, and storage conditions (2).

Extended refrigerated storage of pasteurized foods has become more common in industrialized countries, and there has been growing demand for high-quality pasteurized milk with an extended shelf life (ESL). Milk processing technology for ESL was developed to increase the typical 14 days refrigerated shelf life of pasteurized milk.

Although there is no clear legal definition of ESL milk, ESL milk means normal pasteurized (UHT) fluid milk sealed under hermetic conditions. In addition, the packing material is sterilized using H_2O_2 and UV exposure prior to use. The combination of the peroxide sterilant and UV exposure produces radicals, which effectively kills the microorganisms with their spores (3). Henyon (4) reported that ESL-milk reduced the risk of post-pasteurization contamination and was more palatable and nutritious than aseptic milk.

However, few comparison studies have been performed on the quality differences between typical pasteurized and ESL milk during storage. The objective of this study was to examine the quality differences between ESL milk and non-ESL milk during storage with a focus on microbial and nutritional qualities.

*Corresponding author: Tel: 82-2-3290-3055; Fax: 82-2-3290-3506
E-mail: saehkim@korea.ac.kr
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Materials and Methods

Milk samples All milk samples including commercial LTLT-treated (65°C for 30 min) milk, UHT-treated (130°C for 1~2 sec) milk, and an ESL® milk were purchased from a local supermarket (Seoul, Korea). According to the manufacturer, the ESL milk was processed by a typical UHT treatment in a controlled filling environment (4). For storage tests, the samples were placed in either a 4°C or a 25°C incubator for up to 21 days. The day after the designated expiration date was fixed as day 0 of storage, and samples were taken at 7-day intervals for analytical and microbial measurements.

Physicochemical analyses Non-casein nitrogen (NCN) concentration was determined according to the method reported by Ashworth and van Orden (5). Milk samples (5 mL) were added to 5 mL acetate buffer (1 M, pH 4.6) and filtered through Whatman No. 23 filter paper (Whatman International Ltd., Maidstone, UK). Subsequently, 4 mL alkaline copper solution was added into 1 mL milk filtrate. After folin-ciocalteau reagent (Sigma Chemical Co., St. Louis, MO, USA) was added to the sample, absorbance at 750 nm was measured using a spectrophotometer (Optizen 2120 UV, Mechasys, Daejun, Korea). A standard curve was made using BSA (Sigma Chemical Co.).

Whey protein nitrogen index (WPNI) was determined based on the method of ADMI Bulletin 916 (Method No. A21a) (6). Briefly, NaCl (8 g) was added to the sample (20 mL), and the mixture was placed in a 37°C water bath for 30 min. After filtration through Whatman No. 42, the filtrates (1 mL) were mixed with 10 mL saturated NaCl

solution. Within 10 min after the addition of 2 drops HCl solution (23 mL of 37% HCl), the transmission of the samples was measured at 420 nm using a spectrophotometer. WPNI was calculated using a WPNI conversion curve (ADMI bulletin 916).

Changes in L-ascorbic acid content during storage were determined by HPLC method (7). For sample preparation, milk (1 mL) was dissolved in an equal volume of 10% metaphosphoric acid (Sigma Chemical Co.) and was brought to a final volume of 100 mL with 5% metaphosphoric acid. The samples were centrifuged at 1,500 g for 15 min, and the supernatants were filtered through a 0.22 μm membrane filter (Satorious, Goettingen, Germany). Aliquots of the samples were injected into the column (SynChropak, 4.6 × 150 mm, 5 μm) in the HPLC system (Gilson 506C) using a Gilson 234 auto injector. The mobile phase (0.05 M KH_2PO_4: acetonitrille = 6:4) was delivered at 1.0 mL/min. The L-ascorbic acid content was determined at 245 nm by a UV detector using standard L-ascorbic acid (Sigma Chemical Co.).

Microbiological analyses Total microbial growth during storage was determined by the standard plate count method (8). Dilutions were made using a 0.1% peptone solution, and the total microbes were enumerated after incubation of plates at 35 ± 1°C for 48 hr.

Acidified potato dextrose agar (Difco Laboratory, Detroit, MI, USA) was used as the medium for the total yeast and mold counts after incubation at 25 ± 1°C for 5 days (9). Coliforms were counted during storage to check for post contamination. Serial 10-fold diluted milk samples were cultured using McConkey agar (Becton Dickinson, Cockeysville, MD, USA) after incubation at 35 ± 1°C for 24 hr (9).

Growth performance test using rats A total of 64 male rats (Sam: TacN (SD) BR) were obtained from Samtako Ltd. (Kyungki-do, Korea). After 1 week adjustment period, 48 rats were selected based on their body weights and health status. The average body weight of the rats used in the experiment was 228 ± 13.02 g. The rats were individually caged and randomly assigned to 8 treatments (Table 1). The experiment was conducted for 2 weeks, and all treatments were replicated six times. Food and water were given to rats *ad libitum*, and the rats were force-fed milk twice a day (at 10:00 and 18:00) using a disposable

Table 1. Experimental treatments of ESL and UHT milk

Treatment code	Storage Condition	
	Temperature (°C)	Period (day)
ESL[1] 4-0	4	0
ESL 4-14	4	14
ESL 4-21	4	21
ESL 25-14	25	14
UHT[2] 4-0	4	0
UHT 4-14	4	14
UHT 4-21	4	21
UHT 25-14	25	14

[1]ESL; an extended shelf life fluid milk product
[2]UHT; UHT-treated fluid milk

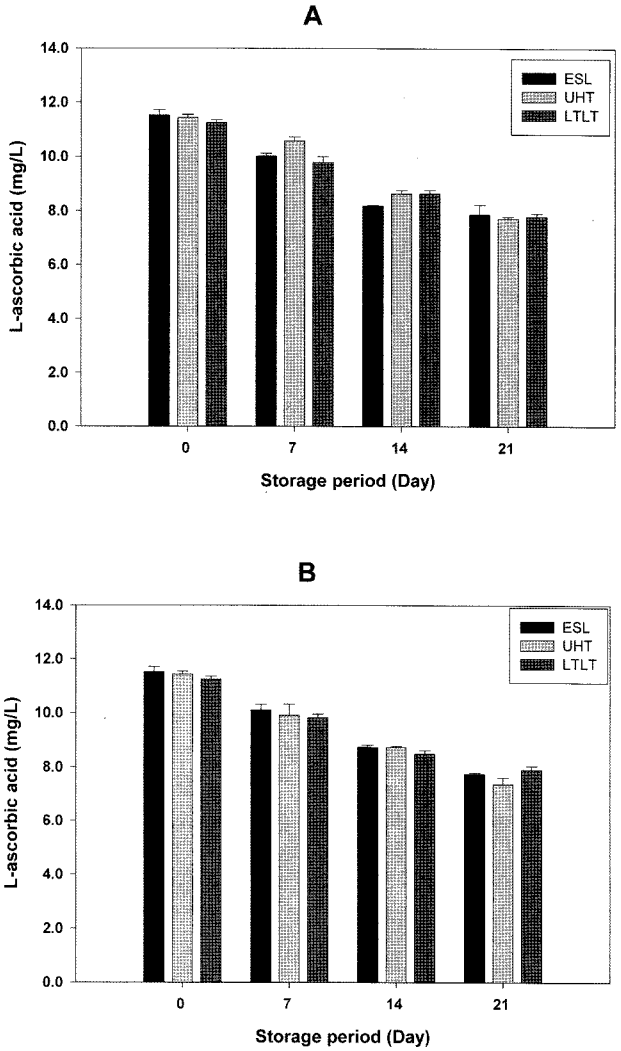
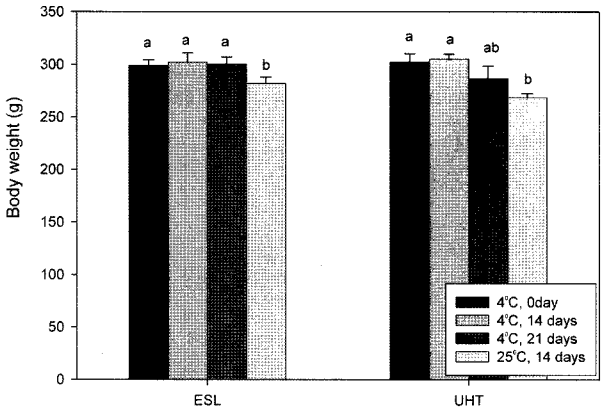


Fig. 1. Changes in L-ascorbic acid content of commercial market milk during storage at 4°C (A) and 25°C (B) for 21 days.

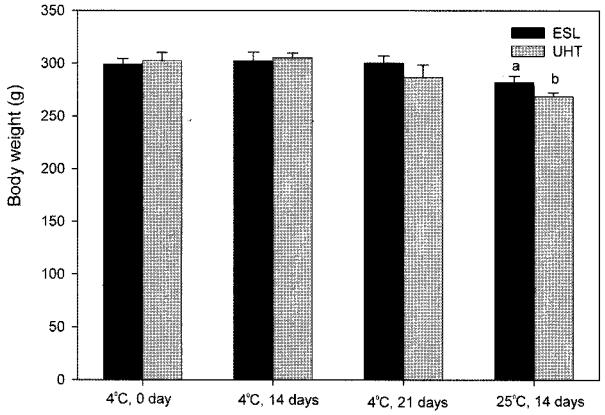
syringe and a feeding needle. Daily milk intake was 1% of their body weight (body weight was measured at days 0 and 7). Body weight and feed consumption were measured at days 0, 7, and 14. Average daily gain (ADG) and average daily feed intake (ADFI) were then calculated from these values. During the experiment, the room temperature was maintained at 25 ± 2°C with a 12 hr light (08:00 to 20:00) and a 12 hr dark (20:00 to 08:00) cycle.

Coliforms in the cecum At the end of the experiment, all animals were sacrificed to examine the population of coliforms in their ceca. The cecum contents were collected, and diluted ten times with a 0.1% peptone solution. The number of coliforms in the cecum was determined by a serial dilution technique using McConkey agar after incubation at 37°C for 24 hr under anaerobic conditions, which were maintained using an anaerobic jar with a gas generator envelope (GasPak Plus, disposable H_2 and CO_2 generating system).

Statistical analysis Data were analyzed by an analysis of the variance using the GLM (General Linear Model)

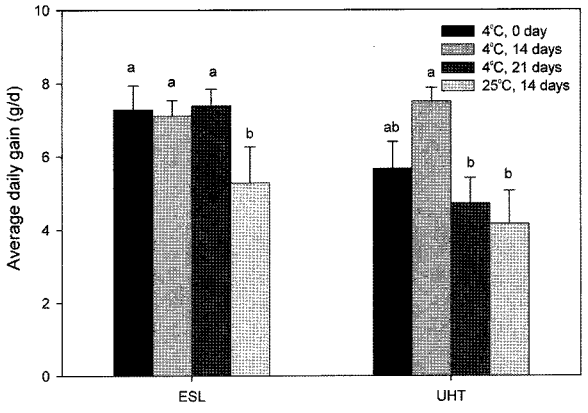


(a) Milk sources

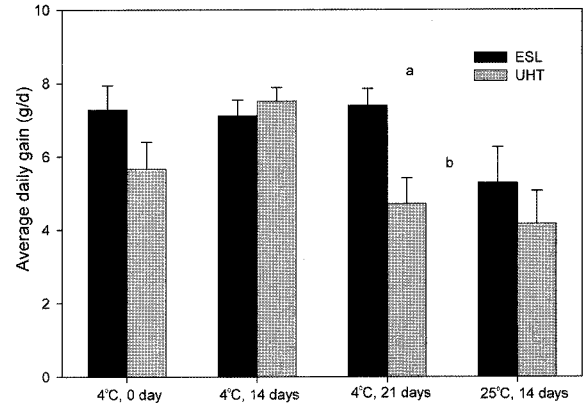


(b) Storage condition

Fig. 2. Comparison of body weight according to the milk source or storage condition. [a-b]bars with different letters are significantly different ($p<0.05$).



(a) Milk sources



(b) Storage condition

Fig. 3. Comparison of average daily gain according to milk source or storage condition. [a-b]bars with different letters are significantly different ($p<0.05$).

procedure in SAS (10). Duncan's multiple range test was used to determine the difference in means among the treatments. All data are represented as means ± standard error values.

Results and Discussion

Physicochemical analyses Heating is a principle process applied to raw milk for obtaining microbial stability and shelf life extension. Higher extent of heating improves the safety of the final product, but the taste and nutritional quality of the milk are sacrificed. To compare the extent of heating given to the milk samples, NCN content and WPNI were determined, because casein is barely affected by the pasteurization conditions typically applied in the dairy industry.

Previous reports (11) showed NCN contents of ESL and UHT milk were similar at approximately 3.0 mg/mL, LTLT milk showed a significantly higher NCN content (about 5.4 mg/mL, $P<0.05$). A similar trend was observed with WPNI. WPNI of the UHT pasteurized commercial milk samples (ESL and UHT milk) was 1.7 mg/mL, whereas that of the LTLT milk was 5.8 mg/mL. The levels of NCN and WPNI were not significantly altered by storage period and storage temperature (data not shown).

The changes in the L-ascorbic acid content of all milk samples were examined during 21 days of storage. At day 0, no significant differences were observed among the samples. However, the L-ascorbic acid content steadily decreased as the storage period increased, regardless of the storage temperature (Fig. 1), consistent with the results of Jandal (12). Milk has a relatively low L-ascorbic acid content, and the amount of ascorbic acid could be altered by factors such as heat treatment, sunlight, and storage conditions. The level of ascorbic acid in raw milk was about 20 mg/L (data not shown) and decreased by 40% after heat treatment, regardless of temperature. In addition, the concentration of ascorbic acid decreased by an additional 30% during 21 days of storage. In addition, no significant differences were observed in the ascorbic acid content among commercial milk samples within the same storage period. This result indicates that ascorbic acid content may not be substantially influenced by manufacturing processes for ESL milk.

The microbiological analyses Changes in the total microbial numbers during storage of the samples were examined (Table 2). In all samples except the ESL milk, microorganisms were detected by a standard plate count

Table 2. Changes in the bacterial numbers of commercial market milk during storage at 4°C and 25°C[1)]

Samples	Mean bacterial counts (log cfu/mL)							
	Storage (day) at 4°C				Storage (day) at 25°C			
	0	7	14	21	0	7	14	21
ESL[3)]	ND[2)y]	ND ^{y}	ND ^{z}	ND ^{z}	ND^{ay}	ND^{az}	ND^{az}	ND^{az}
UHT[4)]	ND^{cy}	ND^{cy}	1.54±0.02^{by}	2.24±0.01^{ay}	ND^{dy}	1.61±0.02^{cy}	1.98±0.02^{by}	4.54±0.05^{ay}
LTLT[5)]	1.50±0.02$^{d\,x}$	1.87±0.04^{cx}	3.58±0.02^{bx}	4.36±0.02^{ax}	1.50±0.04^{dx}	3.65±0.02^{cx}	3.88±0.02^{bx}	5.28±0.01^{ax}

[1)]All data are represented as mean ± standard error values
[2)]Not detected
[3)]ESL; an extended shelf life fluid milk product, [4)] UHT; UHT-treated fluid milk, and [5)] LTLT; LTLT-treated fluid milk
[a-d]Means with different letters within the same row at the same storage temperature are significantly different ($p<0.05$).
[x-z]Means with different letters within the same column at the same storage temperature are significantly different ($p<0.05$).

(SPC) after storage for 14 days or longer, even under refrigerated storage conditions. In refrigerated storage, SPC of UHT milk increased to 10^2 cfu/mL and that of the LTLT milk increased to 10^4 cfu/mL after 21 days storage. Similar results were observed when the samples were stored at 25°C. No microorganisms were detected in the ESL product until the end of the tested storage period. SPCs of the UHT and LTLT milk increased to 10^4 and 10^5 cfu/mL after 21 days storage at 25°C, respectively.

At 4°C, yeast and molds were detected in LTLT milk after 7 days of storage and after 14 days of storage in UHT milk (Table 3). When UHT and LTLT milk were stored at 25°C, yeast and molds were detected after 7 days in both samples, and the level increased to 10^3 cfu/mL for UHT milk and 10^4 cfu/mL for LTLT milk after 21 days. Yeast and molds were not detected in ESL milk during the entire storage period.

As few as 10^0 cfu/mL coliforms were detected in LTLT milk after 21 day storage at 4°C (Table 4). At 25°C, coliforms were detected after 7 days in LTLT milk and 14 days in UHT milk. Interestingly, no coliforms were detected in ESL milk during the tested storage period. Overall, there were no observed evidences of microbial contamination and/or multiplication in the ESL milk samples during the tested storage period.

Considering that the heat treatment processes for UHT milk and ESL milk are almost identical, the reduced microbial contamination found in ESL milk was probably due to the subsequent ESL process rather than the extent of heat treatment.

Growth performance test using rats The reduced microbial quality during the storage of milk could induce undesirable nutritional consequences. Therefore, a growth performance study was conducted on rats fed milk stored under different conditions. The growth rates of UHT milk- and ESL milk-fed rats were compared, because these two commercial milk samples had undergone similar heat treatment processes.

There were no differences in the body weight among the experimental groups at day 7; however, the UHT-fed (14 days storage at 25°C) (UHT 25-14) group showed a

Table 3. Changes in the yeast and mold counts of the commercial market milk during the storage at 4°C and 25°C[1)]

Samples	Mean value of fungal counts (log cfu/mL)							
	Storage (day) at 4°C				Storage (day) at 25°C			
	0	7	14	21	0	7	14	21
ESL[3)]	ND[2)]	ND ^{y}	ND^{z}	ND^{z}	ND^{y}	ND^{z}	ND^{z}	ND^{z}
UHT[4)]	ND^{c}	ND$^{c\,y}$	1.67±0.03^{by}	2.35±0.01^{ay}	ND^{d}	1.29±0.03^{cy}	2.10±0.02^{by}	3.09±0.01^{ay}
LTLT[5)]	ND^{d}	1.67±0.01^{cx}	2.28±0.01^{bx}	3.13±0.01^{ax}	ND	2.19±0.01^{cx}	2.40±0.01^{bx}	4.10±0.01^{ax}

[1)]All data are represented as mean ± standard error values
[2)]Not detected
[3)]ESL; an extended shelf life fluid milk product, [4)] UHT; UHT-treated fluid milk, and [5)] LTLT; LTLT-treated fluid milk
[a-d]Means with different letters within the same row at the same storage temperature are significantly different ($p<0.05$).
[x-z]Means with different letters within the same column at the same storage temperature are significantly different ($p<0.05$).

Table 4. Changes in the coliforms of the commercial market milk during storage at 4°C and 25°C for 21 days[1)]

Samples	Mean value of bacterial counts (log cfu/mL)							
	Storage (day) at 4°C				Storage (day) at 25°C			
	0	7	14	21	0	7	14	21
ESL[3)]	ND[2)]	ND	ND	ND^{y}	ND	ND^{ay}	ND^{ay}	ND^{z}
UHT[4)]	ND	ND	ND	ND^{y}	ND ^{c}	ND^{cy}	0.59±0.06^{bx}	1.64±0.03^{ay}
LTLT[5)]	ND^{b}	ND^{b}	ND ^{b}	0.49±0.12^{ax}	ND ^{d}	0.90±0.03^{cx}	0.63±0.07^{bx}	1.93±0.02^{ax}

[1)]All data are represented as mean ± standard error values
[2)]Not detected
[3)]ESL; an extended shelf life fluid milk product, [4)] UHT; UHT-treated fluid milk, and [5)] LTLT; LTLT-treated fluid milk
[a-d]Means with different letters within the same row at the same storage temperature are significantly different ($p<0.05$).
[x-z]Means with different letters within the same column at the same storage temperature are significantly different ($p<0.05$).

significantly lower body weight ($p<0.05$) at day 14 (Table 5). The effects of milk source and storage condition on body weight are shown in Fig. 2. A reduced body weight was observed only in the group fed ESL milk (14 days storage at 25°C), whereas the other groups maintained their body weights. In the UHT-treated fluid milk feeding groups, the body weights of the UHT (21 days storage at 4°C) 4-21 group were generally lower than those of the UHT (0 day storage at 4°C) 4-0 and UHT (14 days storage at 4°C) 4-14 groups. However, only the UHT (14 days storage at 25°C) 25-14 group showed a statistically lower body weight. ($p<0.05$).

No significant differences were observed in body weight regardless of the milk sources when the samples were stored at 4°C. However, after 14 days storage at 25 °C, the body weight of the UHT-fed group was significantly lower than that of the ESL-fed group ($p<0.05$).

ADG of the rats is shown in Table 6. No difference was found among the experimental groups during the first week (day 0 to 7). In the second week, ADGs of both the UHT 4-21 and UHT 25-14 test groups were significantly lower than those of the other groups ($p<0.05$).

There were no differences in ADG among rats fed ESL milk stored at 4°C; however, a significantly lower ADG was observed in the ESL 25-14 group ($p<0.05$). In contrast, ADG was significantly lower ($p<0.05$) in the UHT 4-21 and UHT 25-14 groups (Fig. 3a). Under the same storage conditions, ESL and UHT milk had similar effects on ADG up to 14 days storage at 4°C. A significantly higher ADG was observed in the rats fed ESL milk stored for 21 days at 4°C and 14 days at 25°C (Fig. 3b). During the experimental trials, the feed intake was equivalent in all dietary treatments at the first week, while varied at the second week (Table 6). The ESL milk-fed groups showed slightly higher feed intake; however, under the same storage conditions, no clear differences were observed depending on the milk source. In terms of the feed efficiency, the UHT 25-14 group showed a significantly lower feed efficiency than the other groups at the second week of the experiment ($p<0.05$) (Table 6).

Overall, no significant differences were observed in the growth performance among milk sources at the early storage period (no longer than 14 days). However, differences in the growth performance, including average daily body weight gain and feed efficiency, became apparent under prolonged and unrefrigerated storage conditions such as 21 days at 4°C or 14 days at 25°C. Under such storage conditions, the ESL milk-fed groups showed a better growth performance than the UHT milk-fed group.

Table 5. Changes in the body weight (g) of the rats throughout the experimental period

Treatment	Day 0	Day 7	Day 14
ESL[1] 4-0	220.00±9.93	248.00±4.26	299.00±5.43[a]
ESL 4-14	231.60±6.12	252.40±6.83	302.20±8.59[a]
ESL 4-21	232.25±6.66	248.50±6.23	300.25±6.72[a]
ESL 25-14	220.80±10.66	245.00±10.31	282.00±5.93[ab]
UHT[2] 4-0	233.00±5.19	262.66±2.66	302.33±7.88[a]
UHT 4-14	231.60±6.66	252.60±2.15	305.20±4.55[a]
UHT 4-21	226.75±12.04	253.75±9.25	286.75±11.68[ab]
UHT 25-14	224.50±10.79	239.50±6.68	268.66±3.83[b]

[1]ESL; an extended shelf life fluid milk product
[2]UHT; UHT-treated fluid milk
[a-b]Means with different letters within the same column are significantly different ($p<0.05$).

Coliform population in cecum According to Rogers *et al.* (13), coliforms can produce endotoxins, and the level of endotoxin is positively related to the population of coliforms. Endotoxin has also been implicated in the pathogenesis of a variety of diseases (14, 15). Although the mechanisms behind the difference in the growth performances observed in the present study are not clear, changes in coliform populations in the cecum might suggest a clue.

A significantly higher number of coliforms were present in the ceca of the UHT 4-21 and UHT 25-14 groups, which showed reduced growth performance (Table 7). The increased coliform population might have disturbed the microbial balance of the gut environment, which led to reduced nutrient absorption. Considering that there were no differences in the population of lactic acid bacteria in the guts (data not shown), it is most probable that the oral administration of milk led to changes in the population of coliforms in the guts.

In conclusion, the microbial quality of milk can be affected by either heat-resistant psychotrophs or their

Table 6. Changes in the average daily gain, feed intake, and feed efficiency of the rats throughout the experimental period

Treatments	Daily gain (g/d)		Daily feed intake (g/d)		Feed efficiency (body weight gain / feed intake)	
	Day 0 to 7	Day 7 to 14	Day 0 to 7	Day 7 to 14	Day 0 to 7	Day 7 to 14
ESL[1] 4-0	4.00±1.48	7.28±0.66[a]	19.96±1.13	26.75±0.56[ab]	0.19±0.07	0.27±0.02[ab]
ESL 4-14	2.97±0.69	7.11±0.43[a]	19.71±0.28	27.65±0.34[a]	0.15±0.03	0.25±0.01[ab]
ESL 4-21	2.32±0.44	7.39±0.46[a]	18.25±1.67	26.96±1.14[ab]	0.12±0.02	0.27±0.02[ab]
ESL 25-14	3.45±0.70	5.28±0.98[ab]	18.91±0.45	26.62±0.61[ab]	0.18±0.03	0.19±0.03[ab]
UHT[2] 4-0	4.23±0.83	5.66±0.74[ab]	21.04±0.66	24.09±0.74[b]	0.19±0.03	0.23±0.03[ab]
UHT 4-14	3.00±0.94	7.51±0.37[a]	19.74±0.66	23.88±0.74[b]	0.14±0.04	0.31±0.02[a]
UHT 4-21	3.85±1.14	4.71±0.70[b]	21.89±0.92	25.57±1.22[ab]	0.17±0.05	0.18±0.03[ab]
UHT 25-14	2.14±1.80	4.16±0.90[b]	20.69±0.88	24.50±1.35[ab]	0.12±0.09	0.16±0.03[b]

[1]ESL; an extended shelf life fluid milk product
[2] UHT; UHT-treated fluid milk
[a-b]Means with different letters within the same column are significantly different ($p<0.05$).

Table 7. Coliform count in cecum contents of rats

Treatment	Coliforms (log cfu/g)
ESL[1] 4-0	5.95 ± 0.39[b]
ESL 4-14	6.18 ± 0.17[b]
ESL 4-21	6.06 ± 0.19[b]
ESL 25-14	6.25 ± 0.47[b]
UHT[2] 4-0	6.04 ± 0.14[b]
UHT 4-14	6.18 ± 0.38[b]
UHT 4-21	7.46 ± 0.25[a]
UHT 25-14	7.48 ± 0.23[a]

[1]ESL; an extended shelf life fluid milk product
[2] UHT; UHT-treated fluid milk
[a-b]Means with different letters within the same column are significantly different ($p<0.05$).

metabolites during the refrigerated storage of milk and by recontamination after pasteurization. This study demonstrated that ESL milk maintained a better microbial quality than typical UHT milk, particularly under conditions of extended or unrefrigerated storage.

References

1. Shah NP. Effects of milk-derived bioactives: an overview. Brit. J. Nutr. 84: S3-S10 (2000)
2. Allen JC, Joseph G. Deterioration of pasteurized milk on storage. J. Dairy Res. 52: 469-487 (1985)
3. Mans J. ESL fillers promote long shelf life. Packag. Digest. 39: 28-30 (2002)
4. Henyon DK. Extended shelf-life milks in North America: a perspective. Int. J. Dairy Tech. 52: 95-101 (1999)
5. Ashworth US, van Orden HO. The nitrogen distribution in dried milk. J. Milk Technol. 6: 272-273 (1943)
6. American Dry Milk Institute. Standards for Grades of Dry Milk Including Methods of Analysis. Bulletin 916 (1971)
7. Polydera AC, Stoforos NG, Taoukis PS. Comparative shelf life study and vitamin C loss kinetics in pasteurized and high pressure processed reconstituted orange juice. J. Food Eng. 60: 21-29 (2003)
8. Messer JW, Behney HM, Leudecke LO. Microbiological count methods. 15 th Ed. pp. 133-150. In: Standard Methods for the Examination of Dairy Products. Richardson GH (ed). American Public Health Association, Washington DC, USA (1985)
9. Christen GL, Davidson PM, McAllister JS, Roth LA. Coliform and other indicator bacteria. pp. 247-269. In: Standard Methods for the Examination of Dairy Products. Marshall RT (ed). American Public Health Association, Washington DC, USA. (1993)
10. SAS Institute, Inc. SAS User's Guide. Statistical Analysis Systems Institute, Cary, NC, USA (1990)
11. Morales FJ, Romero C, Jimenez-Perez S. Characterization of industrial processed milk by analysis of heat-induced changes. Int. J. Food Sci. Tech. 35: 193-200 (2000)
12. Jandal JM. Factors affecting ascorbic acid content and keeping quality of Shammi goat milk. Small Ruminant Res. 21:121-125 (1996)
13. Rogers MN, Moore R, Cohen J. The relationship between endotoxin and faecal microflora of the C57BL mouse. J. Hyg. Camb. 95: 397-402 (1985)
14. Morrison DC, Ulevitch RJ. The effects of bacterial endotoxins on host mediation systems: A review. Am. J. Pathol. 93: 526-617 (1978)
15. Reitschel ET, Schade U, Jensen M, Wollen weber HW, Luderitz O, Griesman SG. Bacterial endotoxin: chemical structure, biological activity and role in septicaemia. Scand. J. Infect. Disease. 31: 8-21 (1982)
16. Lee GH, Lee JS, Shin MG. Sensory attribute comparison of consumer milk using descriptive analysis. Food Sci. Biotechnol. 12: 480-484 (2003)