INTRODUCTION

Fat contributes to nutritional (source of essential fatty acids, liposoluble vitamins, and energy), organoleptic (flavour, texture, and mouthfeel), and technological properties (release of moisture) in meat products (Olivares et al., 2010). Nevertheless, fat in meat and meat products is considered a triggering factor in the development of some chronic diseases (World Health Organization, 2003). For this reason, health organizations all over the world have promoted lowering the intake of total dietary fat intake to less than 30% of total calories, particularly saturated fatty acids and cholesterol, as a result of preventing cardiovascular heart disease (Nutrition Committee, American Heart Association, 1986; Kris-Etherton et al., 1988). Thus, meat researchers have focused on developing new formulations by reducing fat levels.

Fermented sausages are meat products with a high fat content. Traditional sausages made with a normal recipe have fat contents around 32% directly after manufacture, but during the first week these values rise to about 40%, as a result of drying, and after 4 weeks to about 40% to 50% (Wirth, 1988). Therefore, it is valuable to reduce the amount of pork backfat used in fermented sausages, since it contains a high content of saturated fat. However, low-fat fermented sausages become not only harder, but have high weight loss and an unacceptable appearance due to intensely wrinkled surfaces (Muguerza et al., 2002). Consequently, fat reduction can affect the sensory characteristics of the products (Giese, 1996; Mendoza et al., 2001) and increase the toughness of meat products (Barbut and Mittal, 1996). Furthermore, the granulated fat in fermented sausages helps to loosen the sausage mixture and this aids the continuous release of moisture from the inner layers of the product; a process absolutely necessary for undisturbed fermentation and flavour/aroma development (Muguerza et al., 2002). For these reasons, fermented...
sauces are the most difficult of meat products as far as fat reduction is concerned (Wirth, 1988). Generally, fat reduction (10%) affected the external appearance and flavour intensity of fermented sausages (Liaros et al., 2009) while higher reduction percentages (20%) produced a suitable acceptability (Papadima and Bloukas, 1999; Olivares et al., 2010). Very few studies have dealt with fat reduction of fermented sausages during ripening and drying. The objective of this study was to investigate the effect of the fat reduction on the physicochemical and microbiological characteristics of fermented sausages during ripening and drying.

MATERIALS AND METHODS

Fermented sausages manufacture

For fermented sausages production, fresh beef, refrigerated pork, and frozen pork backfat were obtained in a vacuum packaged condition from a local meat packer. Pork and beef meat were trimmed of visible fat and pork backfat of adhering skin. Both meats and pork backfat were cut and weighed in appropriate amounts, vacuum packed and kept frozen at −24°C for 3 days. Four treatments of fermented sausages were prepared at different levels (30%, 20%, 10%, and 5%) of pork backfat. Fermented sausages were made with ground beef, pork, pork backfat, curing sodium chloride (2.8%), garlic (0.05%), mettwurst additive (0.1%), monosodium glutamate (0.05%), glucose (0.5%), and starter culture (0.4%). A commercially available frozen meat starter culture (Lyocarni RBL-73, Lyocarni, SACCO, Milan, Italy) consisting of Lactobacillus curvatus and Staphylococcus xylosus with 2.5×10^6 log colony-forming unit (CFU)/g was added at a concentration of 6 log CFU/g, according to the manufacturer’s instructions. Beef, pork, and backfat were cut in a bowl chopper (Fuje Co., Seoul, Korea) at low speed to the desired particle size, about 3 to 5 mm and mixed in a mixer (Fujee Co., Korea). Starter culture and ingredients were added during mixing. All treatments, about 10 kg each, were replicated three times in separate meat sources of each replicate. The mixture was then stuffed using a stuffer (H20E, TALSA Co., Northampton, EU) into 25 mm diameter hog casings (Seoul, Korea) at low speed to the desired particle size, about 3 to 5 mm and mixed in a mixer (Fujee Co., Korea). Starter culture and ingredients were added during mixing. All treatments, about 10 kg each, were replicated three times in separate meat sources of each replicate. The mixture was then stuffed using a stuffer (H20E, TALSA Co., Northampton, EU) into 25 mm diameter hog casings (Seoul, Korea). The casings had been pre-soaked in lukewarm water. Sausage filling was restrained and tied with a string by hand. Special cleaning and sanitizing measures were taken during sausage manufacture in a pilot plant. All equipment was rinsed thoroughly between treatments. Samples were dried and ripened in a laboratory smoke chamber (BTDS76P, Bradley, Galesburg, IL, USA). The following conditions of relative humidity (RH) and temperature were applied: day 0 until day 3, 93% RH and 20°C±1°C; day 3, 81% to 85% RH and 17°C±1°C; day 4 until day 7, 88% to 90% RH and 18°C±2°C; day 7 until day 10, 87% RH and 16°C; after day 10, 60% to 70% RH and 16°C. The sausages were smoked after 3 and 7 days in the chamber. Samples from each treatment were taken for physicochemical and microbiological analyses on the 0, 1, 2, 3, 4, 5, 7, 10, 14, and 21st day of ripening. All the results in proximate composition were expressed as the mean of triplicate trial at each sampling time.

Proximate composition and physicochemical analyses

Before analysis, the fat was manually removed from the sausages slices by a knife. All determinations were carried out on the homogenized sample, in triplicate. Moisture, fat, protein and ash were determined on samples using with a slightly modified method of AOAC (Horwitz, 2000). The pH of samples was determined with a pH meter (PHM201, Radiometer, Villeurbanne, France). The pH values of samples were measured by blending a 10 g sample with 90 mL distilled water for 60 seconds in a homogenizer (Ultra-turrax, T25-S1, IKA, Staufen, Germany). For measurement of weight loss, two strings of sausages from each treatment were weighed just before the sausages were put into the fermentation room. The same strings were reweighed on the 0, 1, 2, 3, 4, 5, 7, 10, 14, and 21st day of ripening. Weight loss was expressed as a percentage of the initial weight. The mean of the two measurements was recorded. Color measurements were taken with a Minolta chromameter (Model CR-410, Minolta Co. Ltd., Tokyo, Japan). Commission Internationale de l'Eclairage L*, a*, and b* values were determined with measurements standardized with respect to a white calibration plate (L* = 94.4, a* = 0.313, b* = 0.319) after 30 minutes blooming at room temperature. Color measurements for each of three replicates, always trying to avoid areas with excess fat, were taken and the value was recorded. The TBARS of samples were analyzed by the modification method described by the procedure of Witte (Witte et al., 1970). Readings were made on a spectrophotometer (X-MA 3000, Human Ltd., Seoul, Korea) at 530 nm. A micro-diffusion method described by Conway (1950) was modified for the determination of volatile basic nitrogen (VBN) in sausage samples. Each sample (3 g) was homogenized (Ultra-turrax, T25-S1) for 30 seconds with 27 mL of distilled water. The supernatant solution was filtered using a filter paper (No. 4, Whatman). A 0.01 N of boric acid was placed in the inner section of a Conway micro-diffusion cell (Sibata Ltd., Tokyo, Japan). A 1 mL sample solution and 1 mL of saturated K_2CO_3 were also placed into the outer section of the same cell, and the lid was immediately closed. The cell was incubated at 25°C for 60 minutes, and it was then titrated against 0.02 N H_2SO_4. The VBN value was reported as mg%. Shear force values were analyzed by the method described by the procedure of Bourne (1978). The samples were prepared a cubic form (30×30×20 mm) and were cut.
perpendicular to the longitudinal orientation of the muscle fiber with a Warner-Bratzler shear attachment on a texture analyzer (TA-XT2, Stable Micro System Ltd., Godalming, UK). The maximum shear force value (kg) was recorded for each sample. Test and post-test speeds were set at 1.0 mm/s.

**Microbiological analysis**

Ten grams of samples from each treatment was weighed and then homogenized with 90 mL distilled water using a stomacher (STOMACHER 400 CIRCULATOR, Seward Ltd., Worthing, UK) for 90 seconds at room temperature. Total aerobic plate counts (TACs) were analyzed according to the Standards for Processing and Ingredients Specifications of Livestock Products, Animal, Plant and Fisheries Quarantine and Inspection Agency Notification (Quarantine and Inspection Agency, 2014). Homogenized microbial extracts were serially diluted with distilled water by 10-fold. Portions of the samples (0.1 mL) were plated separately on each plate and spread thoroughly. TACs were enumerated on plate count agar (Difco, Difco Laboratories, Detroit, MI, USA) and colonies were counted after incubation at 35°C±1°C for 48 hours. *Pseudomonas* spp. were assessed by spread technique on *Pseudomonas* Agar (Difco, USA), incubation at 30°C±1°C for 48 hours. All analyses were performed in duplicate, and results expressed as logarithm colony-forming units per gram of samples (log CFU/g).

**Statistical methods**

Experimental design used was 4 (fat level)×10 (ripening time) factorial analysis. An analysis of variance were performed on all the variables measured using the General Linear Model procedure of the SAS statistical package (SAS, 2002). The Duncan’s multiple range test (p<0.05) was used to determine differences among the treatment means.

**RESULTS AND DISCUSSION**

**Proximate composition, physicochemical characteristics, and microbial analysis**

Effect of fat level for pork backfat on proximate composition of fermented sausages is shown in Table 1. The differences between the fat level on proximate composition were significant (p<0.05). The moisture, protein and ash contents were inversely proportional to the fat content during ripening and drying. The lower the added fat level, the higher moisture, protein and ash contents and the lower fat content were as expected. A similar finding was reported by Muguerza et al. (2002) and Olivares et al. (2010), who found that fat reduced sausages were lower in fat content and higher in moisture, protein and ash contents. The moisture content ranged from 51.48% to 63.91% at day 0 and from 17.69% to 25.03% on the 21st day. At day 0, the moisture content was the highest (p<0.05) in fat reduced samples (T3). At the end of the ripening and drying process (day 21), the sausages reached a fat content of 47.27%, 34.82%, 25.71%, and 22.49% for the 30%, 20%, 10%, and 5% formulations, respectively. Protein contents were 29.78%, 33.29%, 41%, and 43.42% for the 30%, 20%, 10%, and 5% formulations, respectively at day 21. The storage conditions had significant effect on the proximate composition of the sausages (p<0.05). During ripening and drying, the moisture contents of sausages were significantly decreased, while the fat, protein and ash content was increased on the 21st day compared to day 0 (p<0.05). A similar trend has been reported by Olivares et al. (2010), who found the reduction in moisture during ripening caused the increase in fat and protein contents.

Effect of fat level for pork backfat on physicochemical
Characteristics of fermented sausages is shown in Table 2. Weight losses were significantly affected by the fat level (p<0.05). During ripening and drying, the weight losses of sausages were significantly increased on the 21st day compared to day 0 (p<0.05). This agrees with the results of Liaros et al. (2009), who found the weight loss of high and low-fat non-packaged sausages continuously increased during ripening. While the weight losses of fermented sausages were higher in 30% and 20% formulations than in 10% and 5% during the first 4 days, they were lower in 30% and 20% after 10 days of storage. This is in agreement with the results of other studies (Papadima and Bloukas, 1999; Liaros et al., 2009), which have indicated low-fat sausages had higher weight loss than high-fat ones at the end of ripening. Weight losses depend on various factors including the temperature and RH of the ripening room, the air movement and the ripening time, and the width of the casings, the material of which the casings are made and the fat content of the sausages (Muguerza et al., 2002). The initial pH of fermented sausages ranged from 5.28 to 5.36 and the final pH on the 21th day from 4.62 to 4.92. No differences in pH value were found between the high-fat (30%) and low-fat (5%) treatments during ripening and drying. Similar results have been reported by Bloukas et al. (1997) and Muguerza et al. (2002). Papadima and Bloukas (1999) mentioned the higher weight loss of sausages stored in the ripening room may also be attributed to their lower pH values. During ripening and drying, the pH of sausages were significantly decreased on the 21st day compared to day 0 (p<0.05).

During ripening and drying, the shear force values of sausages were increased on the 21st day compared to day 0 (p<0.05). The shear force values of fermented sausages were higher in 5% fat formulations than in other treatments after 3 days of storage (p<0.05). Previous studies have shown that sausage texture in low fat sausages caused an increase in chewiness and hardness at longer ripening times (Mendoza et al., 2001; Olivares et al., 2010). Total VBN concentration was used as an index of meat freshness, because it is increased by the levels of microbial

Table 2. Effect of fat level for pork backfat on physicochemical traits of fermented sausages

| Variable                  | Days of ripening |          |          |          |          |          |          |          |          |          |          |          |
|---------------------------|------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Weight loss (%)           |                  |          |          |          |          |          |          |          |          |          |          |          |
| C1                        | 0                | 3.23 AF  | 4.21 AF  | 8.74 AC  | 13.52 BD | 21.63 CD | 28.91 BC | 39.04 AB | 45.33 BA | 47.34 BA |          |          |
| T1                        | 1                | 3.14 AF  | 4.12 AF  | 8.90 AC  | 14.09 BD | 23.14 CD | 29.99 BC | 41.81 BA | 48.36 BA | 50.32 BA |          |          |
| T2                        | 2                | 3.17 AF  | 4.21 AF  | 9.16 AC  | 14.79 BD | 24.53 CD | 30.47 BC | 45.56 AA | 54.09 AA | 55.94 AA |          |          |
| T3                        | 3                | 2.69 BF  | 3.06 BF  | 7.65 BE  | 12.52 BD | 21.66 CD | 28.29 BC | 47.24 AA | 53.68 AA | 55.64 AA |          |          |
| pH                        |                  | C        | 5.35 A   | 5.42 A   | 4.61 B   | 4.38 B   | 4.54 B   | 4.50 B   | 4.67 B   | 4.52 B   | 4.61 B   | 4.64 B   |
|                           | T1               | 5.36 A   | 5.45 A   | 4.69 B   | 4.46 B   | 4.55 B   | 4.52 B   | 4.64 B   | 4.71 B   | 4.82 B   |          |          |
|                           | T2               | 5.34 A   | 5.38 A   | 4.63 B   | 4.49 B   | 4.64 B   | 4.65 B   | 4.74 B   | 4.94 B   | 4.93 B   | 4.92 B   |          |
|                           | T3               | 5.28 B   | 5.38 B   | 4.76 B   | 4.35 B   | 4.43 B   | 4.38 B   | 4.46 B   | 4.53 B   | 4.60 B   | 4.62 B   |          |
| Shear force (kg)          |                  | C        | 0.19 C   | 0.18 C   | 0.71 C   | 1.34 DC  | 1.43 SC  | 1.43 SC  | 1.72 SC  | 2.20 BC  | 3.50 A   | 5.23 A   |
|                           | T1               | 0.19 C   | 0.22 C   | 0.91 C   | 1.45 SC  | 1.46 SC  | 2.19 SB  | 2.48 BB  | 3.22 SB  | 3.49 A   | 7.08 A   |          |
|                           | T2               | 0.25 D   | 0.24 D   | 1.28 B   | 1.60 BD  | 1.68 BD  | 3.58 AC  | 2.72 BC  | 5.72 AB  | 8.63 BB  | 17.67 A  |          |
|                           | T3               | 0.28 C   | 0.26 C   | 1.35 C   | 2.49 AC  | 2.07 AC  | 3.03 AC  | 3.24 AC  | 4.33 AC  | 13.29 AB  | 20.14 A  |          |
| VBN (mg malonaldehyde/kg) |                  | C        | 2.32 SC  | 4.75 BC  | 4.87 BC  | 7.12 BD  | 8.02 BB  | 8.11 BD  | 12.45 BA | 17.72 A  | 19.13 A  | 16.21 A  |
|                           | T1               | 2.46 BD  | 6.91 AC  | 8.94 AC  | 7.13 SC  | 10.27 BB | 9.80 BB  | 13.85 BB | 21.93 A  | 20.45 A  | 27.33 A  |          |
|                           | T2               | 4.96 AC  | 7.42 AC  | 8.85 AC  | 10.21 AB  | 12.12 AB  | 14.13 AB  | 18.88 AB  | 27.12 A  | 28.30 A  | 28.11 A  |          |
|                           | T3               | 4.98 AC  | 8.89 AB  | 9.61 AB  | 10.20 AB  | 11.61 AB  | 11.90 AB  | 15.11 AB  | 26.27 AB  | 26.06 AB  | 28.71 AB  |          |
| TBA (mg malonaldehyde/kg) |                  | C        | 0.38 B   | 0.52 B   | 1.00 A   | 1.44 A   | 1.49 A   | 1.52 A   | 1.50 A   | 1.62 A   | 1.68 A   | 1.57 A   |
|                           | T1               | 0.36 B   | 0.53 B   | 0.80 A   | 1.30 A   | 1.30 A   | 1.13 A   | 1.14 A   | 1.26 A   | 1.35 A   | 1.10 A   |          |
|                           | T2               | 0.39 B   | 0.48 B   | 0.59 B   | 0.61 B   | 0.73 B   | 0.73 B   | 0.57 B   | 0.78 B   | 0.84 B   | 0.74 B   |          |
|                           | T3               | 0.40 B   | 0.48 B   | 0.60 B   | 0.59 B   | 0.62 B   | 0.63 B   | 0.37 B   | 0.70 B   | 0.77 B   | 0.77 B   |          |

VBN, volatile basic nitrogen; TBA, thiobarbituric acid.
Values are presented as means ± standard deviation of three replicate experiments with three samples analyzed per replicate (n = 9).

1 C, 30% fat; T1, 20% fat; T2, 10% fat; T3, 5% fat.
2 Figures with different letters within a same row differ significantly (p<0.05).
3 Figures with different letters within a same column differ significantly (p<0.05).
4 Figures with different letters within a same row differ significantly (p<0.05).
contamination (Lee and Joo, 1999). During ripening and drying, fermented sausages with low-fat had significantly higher VBN than those with high-fat (p<0.05) (Table 2). The high content of VBN in low fat sausage seems due to the higher content of protein in low fat sausage. According to the studies of Egan et al. (1981), the higher VBN of sausages with low-fat is explained by accelerated protein degradation. The VBN value of sausages continuously increased during the ripening period (p<0.05). However, it remained up to 21st day at values less than 30 mg/%. The range in VBN values is 7 to 18 mg (VBN/100 g) in Chinese style dry-cured sausages during the ripening period (Lin and Lin, 2002). As presented in Table 2, the lipid oxidation of sausages steadily increased during fermentation and ripening (p<0.05). The casings used for stuffing are permeable to air. Therefore, fermented sausages are exposed to oxygen and, in consequence, to increase lipid oxidation (Liaros et al., 2009). After 7 days of storage, the TBA values of fermented sausages were higher in high-fat (C and T1) compared to low-fat (T2 and T3) sausages (p<0.05). This could be explained by differences in lipid stability as the result of higher fat content. Low-fat fermented sausages had TBA values lower than 1 mg malonaldehyde/kg, which is considered the limit of acceptability for rancidity for fresh meat (Ockerman, 1976).

The influence of fat level for pork backfat on bacteria count of fermented sausages is shown in Table 4. The initial count of total plate bacteria and *Pseudomonas* counts in the sausage mixture was about 10^6 log CFU/g and remained unchanged up to the 21st day. This attribute is due to a lack of fermentable carbohydrates (Papadima and Bloukas, 1999). Total plate bacteria and *Pseudomonas* counts of sausages were not significantly affected by fat level (p>0.05). Previous studies have shown that fat level had no effect on lactic acid bacteria counts (Muguerza et al., 2002; Liaros et al., 2009). Bacteria counts of sausages appeared to

### Table 3. Effect of fat level for pork backfat on meat color of fermented sausages

| Variable | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 10 | 14 | 21 |
|----------|---|---|---|---|---|---|---|----|----|----|
| **L***  |
| C         | 52.96 | 51.30 | 51.98 | 53.21 | 53.62 | 51.07 | 49.02 | 48.28 | 48.65 | 49.10 |
| T1        | 52.19 | 50.49 | 51.07 | 51.99 | 53.50 | 52.04 | 50.22 | 48.46 | 47.81 | 46.87 |
| T2        | 50.74 | 48.45 | 49.85 | 50.35 | 53.21 | 49.67 | 49.65 | 49.40 | 45.90 | 46.97 |
| T3        | 47.10 | 46.35 | 47.75 | 48.64 | 50.54 | 49.00 | 49.39 | 49.39 | 46.56 | 46.49 |
| **a***  |
| C         | 3.79 | 2.32^b | 4.08^b | 5.65^b | 6.75^c | 6.35^c | 4.54^b | 4.97^b | 3.66^b | 3.47^b |
| T1        | 3.54^b | 2.42^b | 4.10^b | 5.86^b | 7.73^b | 8.78^b | 7.15^b | 5.62^b | 4.46^b | 4.22^b |
| T2        | 5.44^a | 5.98^a | 6.92^a | 8.35^a | 9.31^a | 9.88^a | 8.78^a | 6.90^a | 6.61^a | 6.98^a |
| T3        | 5.55^a | 5.78^a | 6.44^a | 9.26^a | 10.30^a | 10.08^a | 8.63^a | 8.63^a | 5.79^a | 6.10^a |
| **b***  |
| C         | 6.30^A | 4.83^A | 4.22^A | 4.95^A | 4.52^A | 3.57^B | 3.19^B | 3.54^B | 2.51^C | 2.40^C |
| T1        | 5.27^A | 4.58^A | 4.50^A | 4.47^A | 5.41^A | 5.36^A | 4.39^A | 3.22^B | 2.44^C | 2.38^C |
| T2        | 5.91^A | 4.07^A | 4.16^A | 4.11^A | 5.51^A | 5.06^A | 4.37^A | 3.06^B | 3.08^B | 3.29^B |
| T3        | 4.04^A | 3.95^A | 3.92^A | 4.08^A | 4.76^A | 5.11^A | 4.32^A | 4.32^A | 3.45^B | 2.58^B |

L, lightness; a, redness; b, yellowness.
Values are presented as mean±standard deviation of three replicate experiments with three samples analyzed per replicate (n = 9).

1 C, 30% fat; T1, 20% fat; T2, 10% fat; T3, 5% fat.

*a* Figures with different letters within a same column differ significantly (p<0.05).

**a** Figures with different letters within a same row differ significantly (p<0.05).
be not related to higher fat content in this study.

**CONCLUSION**

This result showed that the fat level for pork backfat significantly affects the proximate composition and physicochemical characteristics of fermented sausages during ripening and drying. This was also able to produce low fat fermented sausages without negative effects on the bacteria counts, except for only a higher VBN and weight loss. Further research is needed to improve the sensory attributes of these products, particularly their fatty acid. The result of this study could provide useful information to produce healthier reduced fat fermented sausages.

**CONFLICT OF INTEREST**

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

**ACKNOWLEDGMENTS**

This research was supported by research funds of Sangji University.

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**Table 4.** Effect of fat level for pork backfat on total plate counts and *Pseudomonas* of fermented sausages

| Variable |  | 0   | 1   | 2   | 3   | 4   | 5   | 7   | 10  | 14  | 21  |
|----------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|          |   | Total plate counts (log CFU/g) |     |     |     |     |     |     |     |     |     |
|          |   | C1  | 6.74| 6.84| 6.31| 6.03| 6.13| 5.94| 6.45| 6.25| 6.25| 6.04|
|          |   | T1  | 6.68| 6.80| 6.24| 5.92| 5.96| 5.81| 6.31| 6.41| 6.31| 6.48|
|          |   | T2  | 6.61| 6.90| 6.21| 6.01| 5.96| 6.01| 6.11| 6.43| 6.41| 6.01|
|          |   | T3  | 6.79| 6.85| 6.44| 6.08| 5.96| 6.01| 6.34| 6.45| 6.42| 6.07|
|          |   | T3  | 6.79| 6.85| 6.44| 6.08| 5.96| 6.01| 6.34| 6.45| 6.42| 6.07|
|          |   | Pseudomonas (log CFU/g) |     |     |     |     |     |     |     |     |     |
|          |   | C   | 6.71| 6.66| 6.23| 5.70| 5.77| 5.63| 6.21| 5.95| 6.07| 5.95|
|          |   | T1  | 6.61| 6.65| 6.00| 5.82| 5.89| 5.64| 6.10| 6.04| 6.30| 6.35|
|          |   | T2  | 6.51| 6.70| 6.21| 5.74| 5.56| 5.69| 5.80| 6.24| 6.23| 6.23|
|          |   | T3  | 6.71| 6.82| 6.60| 6.19| 5.74| 5.92| 6.30| 6.22| 6.32| 6.26|

CFU, colony-forming unit.

Values are presented as means±standard deviation of three replicate experiments with three samples analyzed per replicate (n = 9).

1 C, 30% fat; T1, 20% fat; T2, 10% fat; T3, 5% fat.
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