

Supplementation with rumen-inert fat in the growing phase altered adipogenic gene expression and the size and number of adipocytes in Hanwoo steers

Rajaraman Bharanidharan^{1,1}, Krishnaraj Thirugnanasambantham^{1,‡,||,1}, Jayeon Kim^{\$},

Panyavong Xaysana^{\$}, Napasirth Viengsakoun¹, Ridha Ibidhi^{**}, Joonpyo Oh^{††}, Na-Yeon Kim^{‡‡}, Seok-Hyeon Beak^[]], Stephen B. Smith^{\$\$,11}, and Kyoung Hoon Kim^{†,\$,2}

[†]Department of Eco-friendly Livestock Science, Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

[‡]Pondicherry Centre for Biological Science and Educational Trust, Puducherry 605004, India

^{II}Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai 602105, India ^SDepartment of International Agricultural Technology, Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

¹Department of Livestock and Fisheries, Faculty of Agriculture, National University of Laos, Vientiane Capital, 856, Lao People's Democratic Republic

**Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France

⁺⁺Cargill Animal Nutrition Korea, Seongnam, Republic of Korea

^{‡‡}Asia Pacific Ruminant Institute, Icheon 17385, Republic of Korea

^{III}Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea ^{SS}Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

^{III}Institute for Advancing Health through Agriculture, Texas A&M AgriLife, College Station, TX 77843, USA

¹Equal contributors.

²Corresponding author: khhkim@snu.ac.kr

Abstract

We hypothesized that the provision of rumen-inert fat (RIF) to growing cattle (9 to 13 mo of age) would affect the expression of genes involved in lipid metabolism and thereby affect the size and number of adipocytes of steers slaughtered at 30 mo of age. Thirty steers with an average initial body weight (BW) of 239 ± 25 kg were allocated to six pens, balanced for BW and genetic merit for marbling, and assigned to one of two treatment groups: control (only basal diet) or test diet (basal diet with 200 g of RIF per day, on an as-fed basis) for 5 mo. Biopsy samples of longissimus lumborum (LM) muscle were then collected for analysis of fatty acid composition and gene expression. Both groups were then fed the same basal diets during the early and late fattening phases, without RIF, until slaughter (average shrunk BW = 759 kg). Supplementation with RIF increased the longissimus thoracis (LT) intramuscular fatty acid concentration at slaughter (P = 0.087) and numerically increased the quality grade score (P = 0.106). The LM intramuscular relative mRNA expression of genes such as PPARα, ZFP423 and SREBP1, FASN, SCD, FABP4, GPAT1, and DGAT2 were downregulated (P < 0.1) following RIF supplementation. Supplementation of RIF decreased (P < 0.1) diameter and concomitantly increased intramuscular adipocytes per viewing section at slaughter. This likely was caused by promotion of triacylglycerol hydrolysis during the growing phase. Another possible explanation is that the relative mRNA expression of gene ATGL was upregulated by RIF supplementation during the growing (P < 0.1) and the fattening phases (P<0.05), while the genes associated with fatty acid uptake (FABP4) and esterification (DGAT2) were downregulated during the growing phase and upregulated (P < 0.1) during the fattening phase. This implies that the lipid turnover rate was higher for steers during the growing than fattening phase. This study demonstrated that RIF supplementation during the growing phase induced a carryover effect on the lipogenic transcriptional regulation involved in adipocyte lipid content of intramuscular adipose tissue; increased triacylalycerol hydrolysis during the growing phase subsequently was followed by increased lipid accumulation during the fattening phases.

Lay Summary

Rumen inert fat (RIF) is a type of fat supplement that is used in the diets of beef cattle as early as 6 mo of age in calves and continues through the finishing period to improve the dietary energy density which can be used by the animal to deposit more lipid in the muscle tissue. However, for Hanwoo beef cattle, the precise time of RIF supplementation has not yet been determined. This study hypothesized that supplementing RIF at the growing phase (9 to 13 mo of age) would have a positive influence on the marbling characteristics of meat at slaughter. The growth rate and performance of steers were not improved by RIF supplementation, however, an increase in intramuscular fatty acid content was noted that was accompanied by the increased number of intramuscular adipocytes and decreased intramuscular adipocyte diameter. Supportively, upregulation of the genes associated with fatty acid uptake and esterification during the fattening phase of RIF-fed animals was noted. Overall, supplementing RIF at the growing stage could improve the lipid content of the meat which is supported by the increased lipid hydrolysis during the growing phase and followed by increased lipid accumulation during the fattening phases.

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Received May 8, 2023 Accepted September 27, 2023.

Key words: adipocyte size, gene expression, imprinting, lipogenesis, rumen-inert fat

Abbreviations: ACACA, acetyl-CoA carboxylase; ADFom, acid detergent fiber excluding residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; ATGL, adipose triglyceride lipase; BSCL2, berardinelli-Seip congenital lipodystrophy2-seipin; BW, body weight; CP, crude protein; DGAT, diacylglycerol acyltransferase; DM, dry matter; DMI, dry matter intake; FABP, fatty acid binding protein; FAME, fatty acid methyl esters; FASN, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase; LPL, lipoprotein lipase; LT, longissimus thoracis; OM, organic matter; PPAR, peroxisome proliferator-activated receptors; RIF, rumen-inert fat; RPF, rumen-protected fat; SCD, stearoyl-CoA desaturase; SNAP, synaptosome-associated protein; SREBP, sterol regulatory element-binding proteins; VLCAD, very long chain acyl-CoA dehydrogenase

Introduction

Intramuscular adipocytes increase in both size and number in response to nutrients during the fetal and postnatal stages in ruminant animals (Smith et al., 2009; Brooks et al., 2011). An effect of late-gestation supplementation (i.e., fetal programming) on fetal adipose tissue development and intramuscular lipid deposition, during the postnatal growth and development of beef cattle, has previously been demonstrated (Larson et al., 2009; Radunz et al., 2012). Metabolic imprinting of epigenetic genes via postnatal feeding of high-concentrate feeds (Scheffler et al., 2014; Reddy et al., 2018) also has a substantial effect on carcass yield and beef quality, particularly marbling scores in later life. Furthermore, providing rumen-protected fat (RPF) to early-weaned steers positively impacts marbling deposition, without impacting other carcass measures (Mangrum et al., 2016). Despite the large number of previous studies, early dietary intervention has mostly been implemented at around 6 mo of age, while carcass quality has mainly been investigated at 16 to 18 mo of age.

In contrast, conventional feeding programs for enhancing beef production of Hanwoo steers start at around 7 mo of age, after castration, and finish at around 30 mo of age. Steers are supplied with three formulated diets based on their growth characteristics, at 7 to 13 mo of age (growing phase), 14 to 21 mo of age (early fattening phase) and 22 to 30 mo of age (late fattening phase). This is because, in Hanwoo steers, the greater daily dry matter intake (DMI) occurs at 15 to 18 mo of age, while the greatest daily body weight (BW) gain occurs at 19 to 21 mo of age (Kim et al., 2007). The inflection point of the ratio of daily retained energy to daily BW gain is also between 19 and 21 mo of age; it dramatically increases thereafter until 30 mo of age. The total digestible nutrient content of the concentrate feeds is gradually increased from 70% to 74% DMI by increasing the amount of maize grain to supply surplus energy, while the crude protein content gradually decreases from 16% to 12% DMI (Kim et al., 2005). RPF, as an energy source for the late fattening phase of Hanwoo steers (Lee et al., 2003; Park et al., 2010; Kim et al., 2012) or the finishing phase of Angus steers (Bakker et al., 2019), has been provided in an effort to induce high levels of marbling but all attempts have failed thus far. Recently, the provision of RPF rich in 18:2n - 6 to early weaned steers positively impacted marbling deposition, reflected in both the size and number of intramuscular adipocytes, with no impact on other carcass measures (Mangrum et al., 2016). It is now clear that saturated and unsaturated fatty acids derived from dietary fat exert positive or negative effects on the proliferation and differentiation of preadipocytes (Petersen et al., 2003; Madsen et al., 2005). Yanting et al. (2018) showed that the lipid content of bovine adipocytes was affected by RPF supplementation; 18:1n - 9 was most affected, followed by 18:2n - 6, 14:0, 18:0 and 16:0. However, it is likely that animal age during RPF or RIF (rumen-inert fat) supplementation influences its effects on carcass characteristics.

Preadipocyte proliferation is well recognized, however committed preadipocytes go through growth arrest and then final differentiation into adipocytes (Gregoire et al., 1998). It is possible that the faster proliferation of Wagyu fibro-adipogenic progenitors (FAPs) than Angus FAPs is a factor in the higher marbled fat deposition in Wagyu muscle (May et al., 1994; Wei et al., 2015). Consequently, reduced preadipocyte differentiation during the growth phase and an increase in the number of developed adipocytes thereafter boost triacylglycerol accumulation in adipocytes during the fattening phase.

To the best of our knowledge, no study has determined the effects of RIF supplementation during the growing phase of Hanwoo steers on age-associated changes in the fatty acid composition of the longissimus muscle, the expression of genes involved in lipid metabolism between the growing and late fattening phases, or the carcass parameters of steers slaughtered at 30 mo of age. The aim of this study was to determine the effects of postnatal metabolic imprinting during the growing phase, with RIF rich in 18:1n - 6 and 18:2n - 6, on adipogenesis and lipid deposition during a 17-mo period in a later life stage of Hanwoo steers.

Material and Methods

Animals, treatments, and biopsy sampling

The methods and protocols for all animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (Approval no. SNU-171211-1) and were performed in accordance with relevant guidelines and regulations. After an adaptation period of 20 d to the basal diets, a total of 28 Hanwoo steers with an average initial live weight of 239 ± 25 kg (average age = 9 mo) were allocated to six pens based on origin and sire, to maintain the genetic lineage according to the Korea Animal Improvement Association (KAIA). Steers were allocated to one of two treatments (three pens for each treatment) according to a completely randomized design: control group (basal diets for growing phase: 54% growing concentrate and 46% timothy hay on a DM basis), and RIF group (basal diet supplemented with 200 g RIF/d on an as-fed basis) for 5 mo. All the animals were fed according to the feeding program of the Korean feeding standard for beef cattle (NIAS, 2017). The RIF group received 400 g less concentrate than the control group, on an as-fed basis, to ensure an isocaloric balance between groups. The fatty acid composition of RIF, and the average DMI (kg/d) of the animals throughout the RIF supplementation period (5 mo) are presented in Table 1.

After feeding RIF for 5 mo, longissimus lumborum (LM) muscle tissue samples (approximately 2 g/steer) were collected via biopsy of the left-side rear of the third lumbar vertebra randomly from seven animals (two animals from two pens plus three animals from the third pen) in each group using a spring-loaded biopsy instrument (DB Biotech, Košice, Slova-kia), as described by Cheah et al. (1997), under intramuscular (xylazine, 20 mg/steer; Kepro B.V, Deventer, Netherlands)

Table 1. Daily DMI of concentrate and hay by Hanwoo steers during each growth phase

| Items | Growing ¹ | | Early | Late |
|------------------------------------|----------------------|-------|-----------|-----------|
| | Control | RIF | fattening | fattening |
| Average DMI ² , kg/d | 8.3 | 8.1 | 10.4 | 8.4 |
| Concentrate, g/ kg DMI | 538.0 | 507.0 | 806.0 | 887.0 |
| Hay³, g/kg DMI | 462.0 | 470.0 | 194.0 | 113.0 |
| RIF ⁴ , g/kg DMI | - | 23.0 | - | - |

Data are means for n = 14 steers per group. ¹Steers fed experimental diets without RIF (control) or with RIF (200 g/d as fed basis) during 5 mo of the growing phase.

²Values represent the average DMI at the growing stage of 5 mo, the early

fattening stage of 8 mo, and the late fattening stage of 9 mo. ³Timothy hay was fed for the growing phase and rye grass hay was fed for the early to fattening phases.

⁴Rumen-inert fat (Bypass Mate L; Yuka Sangyo Co., Ltd, Tokyo, Japan) composed of calcium soap of soybean and rapeseed oil, and contained (% total fatty acids) 11.1% 16:0, 3.4% 18:0, 49.4% 18:1n - 9, 28.9% 18:2n -6, 1.7% 18:3n – 3, 1.0% 18:3n – 6, 1.1% 20:1n – 9, and 3.4% other fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 11:0 + 12:0 + 14:0 + 14:1 + 15:0 + 15:1n -6 + 16:1n - 7 + 17:0 + 17:1n - 8 + 18:1n - 9t + 18:2n - 6t + 20:0 + 20:2n-6 + 20:3n - 6 + 20:3n - 3 + 20:5n - 3 + 21:0 + 20:4n - 6 + 22:0 + 22:1n9 + 22:2n - 6 + 23:0 + 24:0 + 24:1n - 9 + 22:6n - 3).Chemical composition (% DM) includes: 92% DM as fed basis, 2.6%

crude protein, and 33.5% ether extract.

and line block (2% lidocaine, 10 mL/head; Cheil Bio, Ansan, Republic of Korea) injections of a local anaesthetic. The tissue samples were snap-frozen in liquid nitrogen and stored at -80 °C for further analysis of fatty acid composition and relative mRNA expression of lipid metabolic genes. Immediately after collecting the LM muscle samples, the animals were intramuscularly injected with procaine penicillin G (4,500 IU/kg, G.C. GPS Inj.; Green Cross Veterinary Products, Yongin-Si, Republic of Korea). For an additional 3 d, the animals were intramuscularly injected with 3 mg/kg of ketoprofen (New-Procop Inj., Shinil Biogen, Anyang, Republic of Korea).

After RIF supplementation, all steers were fed the same restricted diets of early fattening concentrate for 8 mo, and late fattening concentrate for 9 mo. The ingredients and chemical compositions of the experimental diets fed during the whole period are shown in Table 2. Throughout the whole experimental period, twice a day (8:00 and 18:00 h) steers were restrained for 1 h using selflocking stanchions as part of the feed restriction program and were fed the concentrate first and then the hay according to the feeding program of Korean feeding standard for beef cattle (NIAS, 2017). The DMI was restricted in all periods and the amount (kg DMI) was constant between the groups throughout each feeding period until slaughter. The average DMI of the animals during early fattening and late fattening periods are given in Table 1. Each pen was equipped with one automatic drinking trough to ensure that all steers had free access to fresh water. The live BW of the Hanwoo steers was measured every 2 mo throughout the whole experimental period.

Carcass evaluation

All the animals were transported to the local abattoir at an average of 30 mo of age and then starved (but with access to water) for approximately 12 h prior to slaughter. On the following day, the animals were slaughtered following the commercial slaughtering procedure. Following 24 h chilling,

carcasses were evaluated for quality and quantity by an official grader according to the Korean Carcass Grading System of the Korea Institute of Animal Products Quality Evaluation (KAPE, 2017). Two slices of muscle (thickness of 1 cm) between the 12th and 13th right thoracic vertebrae (LT) of the cold carcasses, free of subcutaneous fat and connective tissue were collected from all the 28 steers. One slice was homogenously ground through a 5-mm plate in a grinder (MGB-32, Hankook Fujee Industries Co. Ltd., Suwon, Korea) and immediately snap-frozen in liquid nitrogen for analysis of fatty acid composition and relative mRNA levels of lipid metabolic genes. Another LT slice was stored intact on ice to process further for histological analyses as described later.

Total RNA extraction and real-time quantitative polymerase chain reaction

Total RNA was isolated from biopsy LM muscle samples and grounded LT muscle taken at slaughter using RNeasy Lipid Tissue Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and quantity of total RNA were analysed using a NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States). The integrity of total RNA was confirmed based on the 28S and 18S bands using nucleic acid dye stained (EcoDye, Biofact, Daejeon, South Korea) agarose gel electrophoresis. Total RNA (1 µg) was reverse-transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. Realtime PCR was performed using SYBR Green real-time-PCR Master Mix (Bioneer, Daejeon, South Korea) and the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, United States). Briefly, PCR was carried out in 20 µL total reaction volumes containing 30 ng cDNA, 10 µL SYBR Green RT-PCR Master Mix, and 1.0 µL of each 10 µM primer. The thermal cycling parameters were as follows: 95 °C for 15 min, followed by 40 cycles at 94 °C for 15 s, 60°C for 30 s, and 72°C for 30 s. Primers targeting genes synaptosome-associated protein 23 (SNAP23) and berardinelli-seip congenital lipodystrophy2-seipin (BSCL2) were designed using the Primer-BLAST tool (Ye et al., 2012), based on National Center for Biotechnology Information (NCBI) published sequences (www.ncbi.nlm.nih.gov; Table 3). All other primers were adapted from previously published articles (Jeong et al., 2012; Oliveira et al., 2014). The $2^{-\Delta\Delta CT}$ method was used to determine the relative fold changes (Livak and Schmittgen, 2001), and all data were normalized based on the housekeeping β -actin gene.

Histological analysis

Three random areas in LT muscle taken for histological analvsis at slaughter showing marbling flecks were selected, and an area of 2×2 cm (breadth and width) of each carcass was sectioned using surgical blades. Each collected section was subsequently transferred to a 50-mL Falcon tube (Corning, NY, United States) filled with 40 mL of 10% formalin, and stored at 4 °C for 8 h. Then, the tube was refilled with fresh 10% formalin until all blood had drained from the tissue. The fixed sections were stored in formalin until staining. The fixed tissue sections embedded in paraffin were sectioned with a microtome at 4 µM (RM2255; Leica Microsystems, Inc., Wetzlar, Germany) and placed on a microscopic slide. The paraffin in the tissue sections was then removed in xylene and dehydrated in graded ethanol. Subsequently, the sections

Table 2. Ingredient, chemical, and fatty acid composition of basal diets fed to Hanwoo steers during the growing, early fatting and late fattening phases

| Constituents | Concentrates ¹ | | Hay | | |
|--|---------------------------|-----------------|----------------|----------------------|-----------------------|
| | Growing | Early fattening | Late fattening | Timothy ³ | Ryegrass ⁴ |
| Ingredients, g/kg DM | | | | | |
| Steam-flaked corn | - | 300 | 370 | - | _ |
| Broken corn | 45.3 | 6.8 | 95.4 | - | _ |
| Corn gluten feed | 250.0 | 180.0 | 52.0 | - | _ |
| Wheat flour | - | 100.0 | 90.0 | - | _ |
| DDGS | 163.0 | 27.0 | 82.6 | - | - |
| Palm kernel meal | 121.0 | 140.0 | 90.0 | - | - |
| Soybean hull | 111.0 | - | - | - | - |
| Wheat | 100.0 | 85.0 | 70.0 | - | - |
| Rice bran | 60.0 | 40.0 | 30.0 | - | - |
| Wheat bran | 40.0 | 25.0 | 20.0 | - | - |
| Alfalfa | 40.0 | - | - | - | - |
| Flaked lupin | - | - | 30.0 | - | - |
| Limestone | 23.2 | 29.0 | 6.4 | - | _ |
| Condensed molasses solubles | 23.0 | 19.0 | 18.4 | - | _ |
| Molasses | 15.0 | 28.0 | 23.0 | - | _ |
| Baking soda | _ | 8.2 | 13.1 | _ | _ |
| Urea | _ | 5.5 | 2.9 | _ | _ |
| Sodium chloride | 3.0 | 2.0 | 1.7 | - | _ |
| Mineral and vitamin premix ² | 2.0 | 2.0 | 2.0 | - | _ |
| Ammonium chloride | 1.5 | 1.5 | 1.5 | - | _ |
| Yeast culture (Diamond XPC) | 1.0 | 1.0 | 1.0 | - | - |
| Chemical, g/kg DM (unless otherwise stated) | | | | | |
| DM (in fresh matter) | 889.0 | 889.0 | 877.0 | 954.0 | 955.0 |
| СР | 190.0 | 166.0 | 145.0 | 82.0 | 57.0 |
| Ether extract | 47.0 | 39.0 | 47.0 | 20.0 | 12.0 |
| Ash | 72.0 | 65.0 | 53.0 | 56.0 | 44.0 |
| aNDFom | 290.0 | 225.0 | 180.0 | 601.0 | 671.0 |
| ADFom | 143.0 | 105.0 | 72.0 | 364.0 | 408.0 |
| Fatty acid, g/100 g identified FAME | | | | | |
| 16:0 | 15.4 | - | - | 23.3 | - |
| 18:0 | 2.5 | - | - | 1.8 | - |
| 18:1 <i>n</i> – 9c | 25.1 | - | - | 4.0 | _ |
| 18:2 <i>n</i> – 6c | 41.7 | - | - | 20.4 | _ |
| 18:3 <i>n</i> 3 | 2.6 | - | - | 41.0 | _ |
| Others ⁵ | 12.7 | - | - | 9.5 | - |
| Total fatty acids, g/100 g DM | 4.6 | | | 1.1 | |

FAME, fatty acid methyl esters.

¹Growing (9–13 mo of age); early fattening (14–21 mo of age); late fattening (22–30 mo of age). ²Containing the following nutrients per kg of additive (Grobic-DC; Bayer Heath Care, Leverkusen, Germany): vitamin A, 2,650,000 IU; vitamin D₃,

530,000 IU; niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg.

³Provided with timothy hay in the growing phase.

⁴Provided with rye grass hay in the early and late fattening phases.

Includes 4:0 + 6:0 + 8:0 + 10:0 + 11:0 + 12:0 + 14:0 + 14:1 + 15:0 + 15:1n - 6 + 16:1n - 7 + 17:0 + 17:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 8 + 18:1n - 9t + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 8 + 18:1n - 8 + 18:2n - 6t + 18:3n - 6 + 20:0 + 20:1n - 8 + 18:1n - 8 + 189 + 20:2n - 6 + 20:3n - 6 + 20:3n - 3 + 20:5n - 3 + 21:0 + 20:4n - 6 + 22:0 + 22:1n - 9 + 22:2n - 6 + 23:0 + 24:0 + 24:1n - 9 + 22:6n - 3.

were stained with hematoxylin and eosin. Each section was imaged at 400× magnification with a Leica SCN400F digital pathology slide scanner (Leica Microsystems, Inc., Wetzlar, Germany). Three images of each section showing adipose tissue were spot-checked for appropriate quantification. The scanned TIF files were analyzed in terms of adipocyte area and number using the Adiposoft plugin (v. 1.15) for Image] Fiji (v 2.0.0). The Adiposoft plugin identified adipocytes with a diameter of 10 to 300 µ m, using a "µ m per pixel" ratio of 2.5 based on the calibration of the microscope. All images were assigned random identifiers to blind the operator to the test group of the animal.

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Table 3. Gene names, GenBank accession numbers, sequences, and amplicon sizes of primers for Bos taurus used in real-time quantitative PCR

| Tanscription factors: Parac RP CAATGGAGATGGTGGACACA 95 Peroxisome proliferator-activated receptors γ NM_001034036.1 PPARα RP TGGAGCATGGCGGACACA 207 Peroxisome proliferator-activated receptors γ NM_181024 PPAR γ RP ACATCGCCAAGGAAGGCAC 207 Sterol regulatory element-binding proteins NM_001113302.1 SREBP RP GGCTCCTCCAACGAA 88 Zinc finger protein 423 NM_001101893 ZFP423 RP GGCTCCTCGATGAAGAGAA 120 Lipogenesis: Zerv21 RP CGTCATGTGGGACGAGTGGACAGCA 92 Fatty acid synthase NM_001012669.1 FASN RP TGGGACATCCCGAAGGCAAG 92 Fatty acid synthase NM_00107510.2 LPL RP TGTGTGCAGAGAGCAGCA 93 Fatty acid binding protein 4 NM_010107510.2 LPL RP GTCCTGCGGAGAGAGCAGCA 93 Fatty acid translocase NM_17401.3 GPAR PF GGCCATCCCGAAGAGCAC 93 Fatty acid translocase NM_001075120.2 LPL RP CTCCAGGAGAGGAGGAGGAGGAGGAG 94 Fatty acid translocase NM_17401.3 GPAR PF GGTCCTTACACATACAGAGAGGGG 96 Fatty acid translocase NM_00101228.2. <th>Gene</th> <th>NCBI acc. no.</th> <th>Primer name</th> <th>Primer sequence (5'-3')</th> <th>Product size (bp)</th> | Gene | NCBI acc. no. | Primer name | Primer sequence (5'-3') | Product size (bp) |
|--|--|----------------|-------------|-------------------------|-------------------|
| Peroxisome proliferator-activated receptors a NM_001034036.1 PPAR a FP CAATGGAAGTGGTGGACACA 95 Peroxisome proliferator-activated receptors y NM_181024 PPAR v RP TGGCACATCGAAGGCACC 207 PPAR v RP ACATCCCCACAGCAAGGCAC PPAR v RP ACATCCCCACAGCAAGGCACC 207 Sterol regulatory element-binding proteins NM_001113302.1 SREB PF GAGCACCCTTCAACGCAA 88 Zinc finger protein 423 NM_001101893 ZFP423 RP TGGTCCTCATGCGTGACAGCA 10 Lipogenesis: | Transcription factors: | | | | |
| PARα RPIARα PTIGTAGGAAGTCTGCCGAGAGPara γ RPPAR γ RCGCCACGATGAATCCCGAGT207PAR γ RPAR γ RCATCCCCACGAGAGGCACPAR γ RPAR γ RCATCCCCACGAGAGGCAC88Steal regulatory element-binding proteinNM_00113320.1SERP FGAGCCACCCTCAACGAA88Zin car portein 423NM_00110189ZFP423 RGAGTCCCCGTGATGAGAGA120Lipoensis:VCGTCGCTGTGTGGAGGAAT117Actar PCGTCGGGGGGGAAT117Star of syntheseNM_001012661FASN FRGGTCGGTGGAGGAAT117Star of syntheseNM_001012661FASN FRGTGGAGCACTCGGAGAGCA98Star of syntheseNM_001012661FASN FRTGTGAGGACATCTGGAAGCAC98Star of syntheseNM_0010751201PLF RPGTGTGGTGGTGGAGAT116Faty acid binding protein 4NM_010102281PLF RPGTGTGTGAGGGGGGAGACC98Faty acid translocaseNM_17401ACD36 FPGTGCTGTGGTGGAGAGCA116Faty acid translocaseNM_17401ACD36 FPGTGCTGTGCGGGGAGAGGG116Faty acid translocaseNM_0010122821GATC PCGTGCGGGGGCAACAGGG88Gogerol-3-phosphate acyltransferase-1NM_0010122821GGTCTGTGCGTGTGTGGTGAGAG116Gaty Car Disposphate acyltransferase-2NM_0010122821GGTCTGTGCGGGGGCAACAGGG16Gogerol-3-phosphate acyltransferase-3NM_0010122821GGTCTGTGCGGGGGCACAGGGGGGGAGAGG16Gaty Car Disposphate acyltransferase-3NM_001012821 </td <td>Peroxisome proliferator-activated receptors α</td> <td>NM_001034036.1</td> <td>PPARa FP</td> <td>CAATGGAGATGGTGGACACA</td> <td>95</td> | Peroxisome proliferator-activated receptors α | NM_001034036.1 | PPARa FP | CAATGGAGATGGTGGACACA | 95 |
| Peroxisome proliferator-activated receptors Y NM_181024 PPAR Y FP TGGCCATTGAATGCCGGGTC 207 PAR y RP ACATCCCCACAGCAAGGCAC PAR y RP ACATCCCCACAGCAAGGCAC 88 Sterol regulatory element-binding proteins NM_001113302.1 SREBP FP TGCTCTTCATGTGCGTCAGCA 120 Zinc finger protein 423 NM_001101893 ZFP423 RP GATTCCTCCGTGAAGGAA 117 Acetyl-CoA carboxylase NM_174224.2 ACACA RP CGTCATGTGGACGATGAAGAA 117 Fatty acid synthase NM_001012669.1 FASN RP TGGGACATCTCGGAAGCAA 92 Fatty acid synthase NM_0173959.4 SCD PP TTATTCCGTTATGCGCTGGA 83 Stearoyl-CoA desaturase NM_0174314.2 FABP RP GGATGGAAGAGCAC 98 Fatty acid binding protein 4 NM_174010.3 CD3 FP GGATGGATAAGAGGGGGTGCAAAGGAG 115 Fatty acid translocase NM_174010.3 CD3 FP GGATGGCAAGAGAGGAGGAGGAGGAGGAG 116 Fatty acid translocase NM_174010.3 CD3 FP GGTGCTATAGCAGCTCCCCAAGGGAGGAGGAGGAGGA 116 Fatty acid translocase NM_174010.3 CD3 FP GGTGCTATAGCAGCTCTCCAAAGGAGGGGGGAGGAGGAGGAGGAGGAGGAGGAGGAG | | | PPARα RP | TTGTAGGAAGTCTGCCGAGAG | |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | Peroxisome proliferator-activated receptors γ | NM_181024 | PPARy FP | TGGCCATTGAATGCCGGGTC | 207 |
| Sterol regulatory element-binding proteins NM_001113302.1 SREBP PP GAGCCACCCTTCAACGAA 88 Zinc finger protein 423 NM_001101893 ZFP423 FP GGATTCCTCAGTGACAGCA 120 Lipogenesis: ZFP423 RP TCGTCTCATTGCATCCCTCCTCTCCTCT 17 Accea RP CGCTCAGGAGAGAGAA 117 Accea RP CGCTCAGTGGACAGGCAGGAGA 117 Fatty acid synthase NM_001012669.1 FASN RP ATCGAGAGCACAGGCAAGGCA 92 Fatty acid synthase NM_0173959.4 SCD FP TTATTCCGTTATGCCCTTGG 83 Stearoyl-CoA desaturase NM_001075120.1 LPL FP CTCAGGAGTCCCCGAAGACAC 98 Lipoprotein lipase NM_174314.2 FABP4 RP ATCCCTTGGCTTATGCTGGA 80 Fatty acid translocase NM_174010.3 CD36 FP GGTCTTACAACAAGAGGGTCG 116 Gycerol-3-phosphate acyltransferase-1 NM_205793.2 GPAT1 FP TGTGCGCCACTTAAGAGAGA 116 Gipalyleycerol acyltransferase-2 NM_205793.2 GPAT1 FP TGTGCGCCACTTACAGAGATG 126 Diacylelycerol acyltransferase-2 NM_205793.2 GPAT1 FP TGTGCGCGCCTTACTGCA 66 Diacylelyce | | | PPAR y RP | ACATCCCCACAGCAAGGCAC | |
| SREBP RPTGTCTTCTATGTCGGTCAGCA12Zine finger protein 423NM_001101893ZFP423 RPGGATTCCTCCGTGACAGCA120Lipogenesis:CGTCGCTCATTCCTCTCCTCTCCTCTCCTCCCCCCCCCC | Sterol regulatory element-binding proteins | NM_001113302.1 | SREBP FP | GAGCCACCCTTCAACGAA | 88 |
| Zinc finger protein 423NM_001101893ZFP423 FP ZFP423 RPGGATTCCTCGGTGACAGCA120Lipogenesis: | | | SREBP RP | TGTCTTCTATGTCGGTCAGCA | |
| ZFP423 RP TCGTCCTATTCCTCTCCTCTCT Lipogensis: ACACA RP CGCTCAGTGAAGAAAAAAAAAAAAAAAAAAAAAAAAAAA | Zinc finger protein 423 | NM_001101893 | ZFP423 FP | GGATTCCTCCGTGACAGCA | 120 |
| Liogenesis: Acetyl-CoA carboxylase NM_174224.2 ACACA RP CGCTCGGTGATTGAAGAGAA 117 Actyl-CoA carboxylase NM_01012669.1 FASN FP ATCGAGTGCATCAGGCAAGT 92 Fatty acid synthase NM_0101012669.1 FASN FP ATCGAGTGCATCGGGAAGAGC 92 Stearoyl-CoA desaturase NM_173959.4 SCD FP TTATTCCGTTATGCCCTTGG 83 Fatty acid uptake: IIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII | | | ZFP423 RP | TCGTCCTCATTCCTCTCCTCT | |
| AceqJ-CoA carboxylaseNM_174224.2ACACA FPCGCTCGGTGATTGAAGAGAA117Fatty acid synthaseNM_001012669.1FASN FPATCGAGTGCATCAGGCAAGT92Fatty acid synthaseNM_173959.4SCD FPTTATTCCGTTATGCCCTTGGA83Stearoyl-CoA desaturaseNM_173959.4SCD RPTTGTCATAAGGGCGGTATCC72Fatty acid uptake:Image: Component of the synthageNM_001075120.1LPL FPCTCAGGACTCCCGAAGACAC98Fatty acid binding protein 4NM_174314.2FABP4 FPGGATGATAAGATGGTGCTGGA80Fatty acid translocaseNM_0101012282.1GPAT1 FPGTCCGCGAGGGTTCAAAGATGG115Fatty acid translocaseNM_001012282.1GPAT1 FPCTCCGCCACATAAGAAGTG116Gipcerol-3-phosphate acyltransferase-1NM_001012282.1GPAT1 FPCTCCGCCACATAAGAAGTG116Diacylglycerol acyltransferase-2NM_001046005.2AGT1 FPCTCCGCGACCACTGTCACACA86DGAT2 FPAGCTTCCGGACCACTGTGAA116116116Lipolysis:Image: Component of the synthesis of the synthage of the synt | Lipogenesis: | | | | |
| ACACA RPCGTCATGTGGACAATGGAATFatty acid synthaseNM_001012669.1FASN RPATCGAGTGCATCAGGCAAGT92Stearoyl-CoA desaturaseNM_173959.4SCD FPTTATTCCGTTATGCCCTTGG83SCD RPTGTGAGCACATCCCGAAGACAC98SCD RPTGTGAGCAGTGCGAGAGACAC98Fatty acid uptake:Lipoprotein lipaseNM_174314.2FABP4 FPGGATGATAAGAGGGGGTGCTGGA80Fatty acid binding protein 4NM_174314.2FABP4 RPGGCCTTACACATACAGAGGTGC 116115Fatty acid translocaseNM_174010.3CD3 FPGGTCGTATGCGTGTGAAAGATGG 116116Fatty acid sterification:CCCCGCCACATATAAGAATGG116116Glycerol-3-phosphate acyltransferase-1NM_2001012282.1GPAT1 FPCTCCGCCACATATAAGAATGG116Glycerol-3-phosphate acyltransferase-2NM_200759.3.2DGAT2 FPCATGCGCGCGCTCTACTCTG86Diacylglycerol acyltransferase-2NM_2001046005.2ATGL CPCATGCCGCACATGTGAGGA106Lipopsi:XXXXXXXVery long chain acyl-CoA dehydrogenaseU30817.1UCAD FPTCTCCGCAGCAAATGGC116XVCAD FPCTCTCCGAGGGGCAAATGGC116XXXXXXXLipopsiCCTCTCGGGGGCAAAATGGC116XXXXXXXXXXXXXXXXXXXXXXXXXXX <t< td=""><td>Acetyl-CoA carboxylase</td><td>NM_174224.2</td><td>ACACA FP</td><td>CGCTCGGTGATTGAAGAGAA</td><td>117</td></t<> | Acetyl-CoA carboxylase | NM_174224.2 | ACACA FP | CGCTCGGTGATTGAAGAGAA | 117 |
| Fatty acid synthaseNM_001012669.1FASN FPATCGAGTGCATCAGGCAAGT92Stearoyl-CoA desaturaseNM_173959.4SCD FPTTATTCCGTTATGCCCTTGG83Stearoyl-CoA desaturaseNM_001075120.1LPL FPTTCGAGACTCCCGAAGACAC98Fatty acid uptake:LPL RPGTTTTGCTGTGTGGTTGAA90Fatty acid binding protein 4NM_174314.2FABP4 FPGGATGATAAGATGGTGCTGGA80Fatty acid translocaseNM_174010.3CD36 FPGTCCTTACACATAAGATGGTCCT115Fatty acid translocaseNM_001012282.1GPAT1 FPTGTGGCTATCTGCTCTCAATGATG116Glycerol-3-phosphate acyltransferase-1NM_001012282.1GPAT1 FPCTCCGCCACTATAAGAATG86Diacylglycerol acyltransferase-2NM_0010146005.2ATGL FPCATTGCCGTGCTCCAACA100Atfogs triglyceride lipaseNM_001046005.2ATGL FPTGACCACACTCTCCAACA100Atfogs triglyceride lipaseNM_001046005.2ATGL FPAAGCGGATGGTGAAGGA116Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPTCTTCGAGGGGACAAATGAC116VLCAD RPAGCATTCCCAAAAGGGTTCT116116116VLCAD RPAGCATTCCCAAAAGGGTTCT116116VLCAD RPAGCATTCCCAAAAGGGTTCT116116AGCATTCCCAAAAGGGTTCTVLCAD RPAGCATTCCCAAAAGGGTTCT116 | | | ACACA RP | CGTCATGTGGACGATGGAAT | |
| Stearoyl-CoA desaturaseNM_173959.4FASN RPTGTGAGCACATCTCGAAAGCCAStearoyl-CoA desaturaseSCD FPTTATTCCGTTATGCCCTTGG83Fatty acid uptake:TGTCAGGACTCCCGAAGACAC98Lipoprotein lipaseNM_001075120.1LPL FPCTCAGGACTCCCGAAGACAC98Fatty acid binding protein 4NM_174314.2FABP4 FPGGATGATAAGATGGTGCTGGA80Fatty acid translocaseNM_174010.3CD36 FPGTCCTTGACATACAGAGATGG115Fatty acid esterification:CTCCGCCACTATAAGATGGTGCTCCAATG116Fatty acid esterification:GPAT1 RPCTCCGCGCACTATAAGATG116Diacylglycerol acyltransferase-1NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86Diacylglyceride lipaseNM_001046005.2ATGL FPTGACCACACTCTCCAACA100Adipose triglyceride lipaseNM_001046005.2ATGL FPTGACCACACTCTCCAAAGAGGA116Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPCTCTGCGAGGACAAATGAC116VLCAD RPCTCTCGAGAGGACAAATGAC116VLCAD RPAGCATTCCCAAAAGGGTTCT | Fatty acid synthase | NM_001012669.1 | FASN FP | ATCGAGTGCATCAGGCAAGT | 92 |
| Stearoyl-CoA desaturaseNM_173959.4SCD FPTTATTCCGTTATGCCCTTGG83Fatty acid uptake:Lipoprotein lipaseNM_001075120.1LPL FPCTCAGGACTCCCGAAGACAC98Fatty acid binding protein 4NM_174314.2FABP4 FPGGATGATAAGATGGTGCTGGA80Fatty acid translocaseNM_174010.3CD36 FPGTCCTTACACATACAGAGTCG115Fatty acid esterification:CD36 RPATAGCGAGGGTCAAAGATGG116Glocerol-3-phosphate acyltransferase-1NM_001012282.1GPAT1 RPCTCCGCCACTATAAGAATG116Diacylglycerol acyltransferase-2NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86Diacylglyceride lipaseNM_001046005.2ATGL FPTGACCACACTCTCCAACA100Adipose triglyceride lipaseNM_001046005.2ATGL FPTGACCACACTCTCCAACA100Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPTCTTCGAGGGGACAAATGAC116VLCAD RPAGCATTCCCAAAAGGGTTCT116VLCAD RPAGCATTCCCAAAAGGGTTCT | | | FASN RP | TGTGAGCACATCTCGAAAGCCA | |
| SCD RPTTGTCATAAGGGCGGTATCCFatty acid uptake:Lipoprotein lipaseNM_0010751201LPL RPCTCAGGACTCCCGAAGACAC98LPL RPGTTTTGCTGCTGTGGGTTGAA8080Fatty acid binding protein 4NM_174314.2FABP4 RPGGATGATAAGATGGTGCTGGA80Fatty acid translocaseNM_174010.3CD36 FPGGTCCTTACACATACAGAGTTCG115Fatty acid sterification: | Stearoyl-CoA desaturase | NM_173959.4 | SCD FP | TTATTCCGTTATGCCCTTGG | 83 |
| Fatty acid uptake:Lipoprotein lipaseNM_001075120.1LPL FPCTCAGGACTCCCGAAGACAC98LPL RPGTTTTGCTGCTGTGGTTGAALPL RPGGATGATAAGATGGTGCTGGA80Fatty acid binding protein 4NM_174314.2FABP4 RPATCCCTTGGCTTATGCTCTCT10Fatty acid translocaseNM_174010.3CD36 FPGGTCCTTACACATACAGAGTTGG115Fatty acid esterification:CD36 RPATAGCGAGGGTTCAAAGATGG116Glycerol-3-phosphate acyltransferase-1NM_001012282.1GPAT1 FPTGTGCTATCTGCTCTCCAATG116Diacylglycerol acyltransferase-2NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86Diacylglyceride lipaseNM_001046005.2ATGL FPTGACCACACTCTCCAAACA100Atipose triglyceride lipaseU30817.1VLCAD FPTCTTCGAGGGGACAAATGAC116Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD RPAGCATTCCCAAAAGGGTTCT116 | | | SCD RP | TTGTCATAAGGGCGGTATCC | |
| Lipoprotein lipase NM_001075120.1 LPL FP CTCAGGACTCCCGAAGACAC 98 LPL RP GTTTTGCTGCTGTGGTTGAA Fatty acid binding protein 4 NM_174314.2 FABP4 FP GGATGATAAGATGGTGCTGGA 80 FABP4 RP ATCCCTTGGCTTATGCTCTCT Fatty acid translocase NM_174010.3 CD36 FP GGTCCTTACACATACAGAGTTCG 115 CD36 RP ATAGCGAGGGTTCAAAGATGG 115 CD36 RP ATAGCGAGGGTTCAAAGATGG 116 Fatty acid esterification: Glycerol-3-phosphate acyltransferase-1 NM_001012282.1 GPAT1 FP TGTGCTATCTGCTCTCCAATG 116 Diacylglycerol acyltransferase-2 NM_205793.2 DGAT2 FP CATTGCCGTGCTCTACATTAAGAATG Lipolysis: Adipose triglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCAACA 100 ATGL RP AAGCGGATGGTGAAGGA Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 VLCAD RP AGCATTCCCAAAAGGGTTCT | Fatty acid uptake: | | | | |
| LPL RPGTTTTGCTGCTGTGGTTGAAFatty acid binding protein 4NM_174314.2FABP4 FPGGATGATAAGATGGTGCTGGA80FAtty acid translocaseNM_174010.3CD36 FPGGTCCTTACACATACAGAGTTCG115CD36 RPATAGCGAGGGTTCAAAGATGG115CD36 RPTGTGCTATCTGCTCTCCAATG116Fatty acid esterification:GPAT1 FPTGTGCTATCTGCTCTCCAATG116116CD3c PLNM_001012282.1GPAT1 RPCTCCGCCACTATAAGAATG116Diacylglycerol acyltransferase-2NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86Diacylglyceride lipaseNM_001046005.2ATGL RPTGACCACACTCTCCAACA100Adipose triglyceride lipaseNM_001046005.2ATGL RPAAGCGGATGGTGAAGGA116Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPTCTTCGAGGGGACAAATGAC116VLCAD RPAGCATTCCCAAAAGGGTTCT116116116CD30 RDAGCATTCCCAAAAGGGTTCT116116CD30 RDAGCGGATGGTGAAGGA116116CD30 RDAGCGGATGGTGAAGGA116116CD30 RDAGCGGATGGTGAAGGA116116CD30 RDAGCGGATGGTGAAGGA116116CD30 RDAGCGGATGGTGAAGGA116116CD30 RDAGCGGATGGTGAAGGA116116CD30 RDAGCGGATGGTGAAGGA116116CD30 RDAGCATTCCCAAAAGGGTTCT116116CD30 RDAGCATTCCCAAAGGGGACAAATGAC116116CD30 RDAGCATTCCCAAAAGGGTTCT< | Lipoprotein lipase | NM_001075120.1 | LPL FP | CTCAGGACTCCCGAAGACAC | 98 |
| Fatty acid binding protein 4NM_174314.2FABP4 FPGGATGATAAGATGGTGCTGGA80Fatty acid translocaseNM_174010.3CD36 FPGGTCCTTACACATACAGAGTTCG115Fatty acid esterification:CD36 RPATAGCGAGGGTTCAAAGATGG116Glycerol-3-phosphate acyltransferase-1NM_001012282.1GPAT1 FPTGTGCTATCTGCTCTCCAATG116Diacylglycerol acyltransferase-2NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86Lipolysis:ATGL RPAGCCCACACTCTCCAACA100Adipose triglyceride lipaseNM_001046005.2ATGL FPTGACCACACTCTCCAACA100Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPTCTTCGGAGGGGACAAATGAC116VLCAD RPAGCATTCCCCAAAAGGGTTCT116 | | | LPL RP | GTTTTGCTGCTGTGGTTGAA | |
| Fatty acid translocaseNM_174010.3FABP4 RPATCCCTTGGCTTATGCTCTCTFatty acid esterification:CD36 RPGGTCCTTACACATACAGAGTGG115Glycerol-3-phosphate acyltransferase-1NM_001012282.1GPAT1 FPTGTGCTATCTGCTCTCCAATG116GPAT1 RPCTCCGCCACTATAAGAATGGPAT1 RPCTCCGCCACTATAAGAATG116Diacylglycerol acyltransferase-2NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86DGAT2 RPAGTTTCGGACCCACTGTGAC100100Lipolysis:ATGL RPTGACCACACTCTCCAACA100Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPTCTTCGAGGGGACAAATGAC116VLCAD RPAGCATTCCCAAAAGGGTTCT116116116 | Fatty acid binding protein 4 | NM_174314.2 | FABP4 FP | GGATGATAAGATGGTGCTGGA | 80 |
| Fatty acid translocaseNM_174010.3CD36 FPGGTCCTTACACATACAGAGTTCG115CD36 RPATAGCGAGGGTTCAAAGATGGCD36 RPATAGCGAGGGTTCAAAGATGGI16Fatty acid esterification:GPAT1 FPTGTGCTATCTGCTCTCCAATG116Glycerol-3-phosphate acyltransferase-1NM_001012282.1GPAT1 RPCTCCGCCACTATAAGAATGI16Diacylglycerol acyltransferase-2NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86DGAT2 RPAGTTTCGGACCCACTGTGACDGAT2 RPAGTTTCGGACCCACTGTGAC100Lipolysis:ATGL RPTGACCACACTCTCCAACA100Very long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPTCTTCGAGGGGACAAATGAC116VLCAD RPAGCATTCCCAAAAGGGTTCT116116 | | | FABP4 RP | ATCCCTTGGCTTATGCTCTCT | |
| CD36 RP ATAGCGAGGGTTCAAAGATGG Fatty acid esterification: GPAT1 FP TGTGCTATCTGCTCTCCAATG 116 Glycerol-3-phosphate acyltransferase-1 NM_001012282.1 GPAT1 FP TGTGCCACTATAAGAATG 116 Diacylglycerol acyltransferase-2 NM_205793.2 DGAT2 FP CATTGCCGTGCTCTACTTCA 86 Diacylglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCAACA 100 Atigo chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 VLCAD RP AGCATTCCCAAAAGGGTTCT AGCATTCCCAAAAGGGTTCT 116 | Fatty acid translocase | NM_174010.3 | CD36 FP | GGTCCTTACACATACAGAGTTCG | 115 |
| Fatty acid esterification: Glycerol-3-phosphate acyltransferase-1 NM_001012282.1 GPAT1 FP TGTGCTATCTGCTCTCCAATG 116 GPAT1 RP CTCCGCCACTATAAGAATG GPAT1 RP CTCCGCCACTATAAGAATG 86 Diacylglycerol acyltransferase-2 NM_205793.2 DGAT2 FP CATTGCCGTGCTCTACTTCA 86 Lipolysis: Diacylglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCAACA 100 Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 | | | CD36 RP | ATAGCGAGGGTTCAAAGATGG | |
| Glycerol-3-phosphate acyltransferase-1 NM_001012282.1 GPAT1 FP TGTGCTATCTGCTCTCCAATG 116 Diacylglycerol acyltransferase-2 NM_205793.2 DGAT2 FP CATTGCCGTGCTCTACTTCA 86 Diacylglycerol acyltransferase-2 NM_205793.2 DGAT2 RP AGTTTCGGACCCACTGTGAC 100 Lipolysis: Adipose triglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCAACA 100 Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 | Fatty acid esterification: | | | | |
| GPAT1 RPCTCCGCCACTATAAGAATGDiacylglycerol acyltransferase-2NM_205793.2DGAT2 FPCATTGCCGTGCTCTACTTCA86DGAT2 RPAGTTTCGGACCCACTGTGACDGAT2 RPAGTTTCGGACCCACTGTGACLipolysis:NM_001046005.2ATGL FPTGACCACACTCTCCAACA100Atgl RPAAGCGGATGGTGAAGGAATGL RPAAGCGGATGGTGAAGGAVery long chain acyl-CoA dehydrogenaseU30817.1VLCAD FPTCTTCGAGGGGACAAATGAC116VLCAD RPAGCATTCCCAAAAGGGTTCTAAGCATTCCCAAAAGGGTTCT116 | Glycerol-3-phosphate acyltransferase-1 | NM_001012282.1 | GPAT1 FP | TGTGCTATCTGCTCTCCAATG | 116 |
| Diacylglycerol acyltransferase-2 NM_205793.2 DGAT2 FP CATTGCCGTGCTCTACTTCA 86 DGAT2 RP AGTTTCGGACCCACTGTGAC Lipolysis: Adipose triglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCAACA 100 ATGL RP AAGCGGATGGTGAAGGA Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 VLCAD RP AGCATTCCCAAAAGGGTTCT | | | GPAT1 RP | CTCCGCCACTATAAGAATG | |
| DGAT2 RP AGTTTCGGACCCACTGTGAC Lipolysis: Adipose triglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCCAACA 100 ATGL RP AAGCGGATGGTGAAGGA 100 Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGGACAAATGAC 116 VLCAD RP AGCATTCCCCAAAAGGGTTCT | Diacylglycerol acyltransferase-2 | NM_205793.2 | DGAT2 FP | CATTGCCGTGCTCTACTTCA | 86 |
| Lipolysis: Adipose triglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCAACA 100 ATGL RP AAGCGGATGGTGAAGGA 100 Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 VLCAD RP AGCATTCCCAAAAGGGTTCT | | | DGAT2 RP | AGTTTCGGACCCACTGTGAC | |
| Adipose triglyceride lipase NM_001046005.2 ATGL FP TGACCACACTCTCCAACA 100 ATGL RP AAGCGGATGGTGAAGGA 100 Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 VLCAD RP AGCATTCCCAAAAGGGTTCT | Lipolysis: | | | | |
| ATGL RP AAGCGGATGGTGAAGGA Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 VLCAD RP AGCATTCCCAAAAGGGTTCT VLCAD RP AGCATTCCCAAAAGGGTTCT | Adipose triglyceride lipase | NM_001046005.2 | ATGL FP | TGACCACACTCTCCAACA | 100 |
| Very long chain acyl-CoA dehydrogenase U30817.1 VLCAD FP TCTTCGAGGGGACAAATGAC 116 VLCAD RP AGCATTCCCAAAAGGGTTCT | | | ATGL RP | AAGCGGATGGTGAAGGA | |
| VLCAD RP AGCATTCCCAAAAGGGTTCT | Very long chain acyl-CoA dehydrogenase | U30817.1 | VLCAD FP | TCTTCGAGGGGGACAAATGAC | 116 |
| | | | VLCAD RP | AGCATTCCCAAAAGGGTTCT | |
| Adipoctye size: | Adipoctye size: | | | | |
| Synaptosome-associated protein 23 BT030678.1 SNAP23 FP GGAGGGGAGGCAAGAGATAA 148 | Synaptosome-associated protein 23 | BT030678.1 | SNAP23 FP | GGAGGGGAGGCAAGAGATAA | 148 |
| SNAP23 RP AAACCAAGCACTGGCCTAAA | | | SNAP23 RP | AAACCAAGCACTGGCCTAAA | |
| Berardinelli-Seip congenital lipodystrophy2-seipin BC105396.1 BSCL2 FP CGAAAGGTCTCTGCCCATC 140 | Berardinelli-Seip congenital lipodystrophy2-seipin | BC105396.1 | BSCL2 FP | CGAAAGGTCTCTGCCCATC | 140 |
| BSCL2 RP GTTTTCTCCTCCGGACAG | | | BSCL2 RP | GTTTTCTCCTCCTCGGACAG | |
| Housekeeping: | Housekeeping: | | | | |
| Beta-actinBC142413.1β-Actin FPGTCCACCTTCCAGCAGATGT90 | Beta-actin | BC142413.1 | β-Actin FP | GTCCACCTTCCAGCAGATGT | 90 |
| β-Actin RP CAGTCCGCCTAGAAGCATTT | | | β-Actin RP | CAGTCCGCCTAGAAGCATTT | |

Chemical and fatty acid analysis

The feed samples were collected at the beginning of every month throughout the experimental period and stored at -20 °C until further analysis. The concentrate and timothy hay samples were dried in a forced-air oven at 65 °C for 72 h to estimate the DM content, and then ground to pass through a 1-mm screen (Model 4; Thomas Scientific, Swedesboro, NJ, United States). The ground samples were assayed for crude protein via combustion (Method 990.03, AOAC, 2016) using the rapid N-cube protein and nitrogen apparatus (Elementar Americas, Ronkonkoma, NY, United States), ash (Method 942.05, AOAC, 2016), and ether extract (EE; Method 960.39, AOAC, 2016). The neutral detergent fiber content was assayed with a heat-stable amylase (without sodium sulphite) and expressed exclusive of residual ash (aNDFom) using the method of Van Soest et al. (1991). Contents of acid detergent fiber excluding residual ash (ADFom) were determined according to Van Soest, (1973).

The intramuscular lipid content of the muscle samples was measured as described previously (Folch et al., 1957) with modifications. In brief, 3.0 g of sample was homogenized in 27 mL of Folch I solution (chloroform:methanol, 2:1, v/v) and filtered through a Whatman No. 1 paper into a 100-mL cylinder after being stirred at 4 °C for 2 h. Filtrates were separated into two layers using a 0.88% NaCl and Folch II solution (chloroform:methanol:deionized water, 3:47:50, v/v). The upper layer was removed and the lower layer was evaporated under nitrogen gas. Intramuscular lipid is presented as a percentage of the sample.

The fatty acid composition in feed, RIF and muscle samples was measured by the method described by O'Fallon et al. (2007) and analyzed using a gas chromatography system (7890B: Agilent Technologies, Santa Clara, CA, United States) with a flame ionization detector (FID). A commercial 37-component FAME standard mixture (CRM47885) and the internal standard 13:0 were obtained from Supelco Co. (United States). The inlet and detector temperatures were maintained at 250 °C and 260 °C, respectively. Aliquots (1 µL) were injected with a split ratio of 30:1 into a 100 m \times 0.25 mm × 0.20 µm SP-2560 biscyanopropyl polysiloxane capillary column (Cat. No: 24056 Supelco; Sigma-Aldrich, St. Louis, MO, United States), with helium carrier gas set to a flow rate of 1.18 mL/min and an initial oven temperature of 100 °C. The oven temperature was held constant at the initial temperature for 5 min and increased thereafter by 4 °C/min to a final temperature of 240 °C and held for 14 min. The fatty acids were quantified after normalization with an internal standard using the AOCS Official Method Ce 1j-07 (AOCS, 2018). Theoretical flame ionization detector correction factors reported in AOCS Official Method Ce 1 h-05 (AOCS, 2009) were applied to individual fatty acid methyl esters.

Statistical analysis

A Shapiro-Wilk test was performed to check the normality of the data. When data were not normally distributed, they were transformed using the PROC RANK procedure of SAS 9.4 (SAS Institute, Cary, NC, United States). Data on fatty acid composition, relative mRNA expression, and carcass characteristics were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC, United States). The experiment followed a complete randomized design. Because the experimental treatments were applied at the pen level, the pen was used as the experimental unit. The model included the fixed effect of treatment. Individual animal data were included in the model with a random effect of pen and animal nested within treatment (St-Pierre, 2007). Thus for all statistical analyses of treatment effects, the error term is based on a number of pen replicates, not the number of animals (df = 2). Means were calculated using the LSMEANS statement. The least squares means were separated using the PDIFF option. Treatment differences were considered significant at P < 0.05, and trends were considered at $0.05 < P \le 0.10$.

Results

Growth performance and carcass characteristics

Supplementation with RIF for 5 mo during the growing phase of Hanwoo steers did not significantly affect growth performance throughout the whole experimental period (P > 0.05). (Table 4). Steers fed the RIF diet had no effect on intramuscular lipid deposition in the longissimus muscle samples (P > 0.05) but numerically increased the quality grade score (P = 0.106) compared to control muscle samples, while no differences were observed for carcass weight, back fat thickness, ribeye area, dressing percentage, yield grade score, or yield index score (P > 0.05; Table 4).

 Table 4. Effects of RIF supplementation for 5 mo during the early growing phase on the carcass characteristics of Hanwoo steers slaughtered at 30 mo of age.

| Characteristics | Treatment ¹ | SEM | P-value | |
|---|------------------------|---------|---------|-------|
| | Control | RIF | | |
| Growth performance | | | | |
| Initial body weight, kg | 242.9 | 235.3 | 9.4 | 0.552 |
| After 5 mo RIF treatment, kg | 423.3 | 405.1 | 10.2 | 0.127 |
| Final body weight, kg | 762.0 | 757.3 | 21.9 | 0.892 |
| Average daily gain, kg/d | | | | |
| (initial to final) | 0.8 | 0.8 | 0.1 | 0.679 |
| Yield grade traits | | | | |
| Carcass weight, kg | 452.0 | 449.0 | 12.8 | 0.868 |
| Back fat thickness, mm | 16.0 | 15.0 | 1.8 | 0.845 |
| Ribeye area, cm ² | 91.4 | 90.5 | 3.6 | 0.336 |
| Dressing, % | 59.0 | 59.0 | 0.7 | 0.901 |
| Yield index, retail cut % ² | 63.0 | 63.0 | 1.0 | 0.777 |
| Yield grade (A:B:C, head) ³ | 0:4:10 | 0:5:8 | _ | - |
| Yield grade score ⁴ | 1.3 | 1.4 | 0.2 | 0.727 |
| Quality grade traits | | | | |
| Intramuscular lipid, % | 16.9 | 19.9 | 1.7 | 0.141 |
| Marbling Score ⁵ | 6.2 | 7.0 | 0.5 | 0.171 |
| Quality grade (1++:1+:1:2, head) ⁶ | 1:10:3:0 | 5:7:0:1 | - | - |
| Quality grade score ⁷ | 3.9 | 4.6 | 0.4 | 0.106 |

¹Steers fed experimental diets without RIF (control; n = 14) or with RIF (n = 14) during the growing phase (to 14 mo of age), after which the steers were fed fattening rations.

The yield index was calculated using the following equation: 68.184(0.625 × back fat thickness (mm)) + (0.130 × rib eye area (cm²); 0.024 × dressed weight amount (kg)).

³Carcass yield grades range from C (low yield) to A (high yield).

⁴Yield grade score: A = 3, B = 2, and C = 1.

⁵Marbling score ranges from 1 to 9, with higher numbers indicating better quality (1 = devoid, 9 = abundant). Marbling scores of 8 and 9, 6 and 7, 4 and 5, 2 and 3, and 1 were roughly categorized as 1++, 1+, 1, 2, and 3, respectively.

⁶Quality grades range from 2 (low quality) to 1++ (very high quality). ⁷Quality grade score: 1++ = 6, 1+ = 4, 1 = 3, 2 = 2 and 3 = 1.

Histological analysis of marbling adipocytes

Histological analysis of LT muscle sections obtained during slaughter indicated that supplementation with RIF during the growing phase of Hanwoo steers decreased the diameter (P = 0.061) of intramuscular adipocytes (Figure 1). Furthermore, the number of adipocytes captured in the histological sections from the RIF-supplemented group was greater (P = 0.076) than in the control group.

Fatty acid composition of feed, RIF, muscle biopsy tissue, and carcass longissimus muscle

The fatty acid composition of concentrate and timothy hay fed during the growing phase is presented in Table 2. Fatty acid data



Figure 1. Effects of RIF supplementation for 5 mo during the growing phase on the diameter (A) and total number (B) of adipocytes in steaks from Hanwoo steers slaughtered at 30 mo of age. Values are leastsquare means (n = 14 per treatment) with standard errors affixed to the bars

for both the LM muscle biopsy samples obtained at 14 mo of age, and LT samples obtained at slaughter (30 mo of age) are expressed as g fatty acid/100 g total fatty acid methyl esters (Tables 5 and 6). In biopsy samples obtained after 5 mo of RIF supplementation, a decrease in the proportion of 18:1n - 9c(P < 0.05), and a tendency to decrease in the proportion of 16:0 (P = 0.104) were noted. An increase (P < 0.05) in the proportions of 18:1n - 9t, 18:3n - 6, 18:3n - 3, and 21:0 were also noted in the RIF group. However, no changes (P > 0.05) in total fatty acid content were noted between the groups.

In LT samples obtained after slaughter, the RIF treatment increased (P < 0.05) the proportions of 14:1n - 5, 15:0 and tended to increase the proportions of 14:0 (P = 0.075) and 18:3n - 3 (*P* = 0.066). A tendency to decrease in proportions of 18:0 (P = 0.090) and 18:3n - 6 (P = 0.062) was also noted in the RIF group. The total fatty acid concentration in the muscle tended (P = 0.087) to be greater in the RIF group relatively when compared to the control group.

Relative mRNA expression of lipid metabolic genes in muscle biopsy tissue and carcass longissimus muscle

In LM biopsy samples obtained after 5 mo of RIF supplementation (14 mo of age), the mRNA relative expression of genes encoding transcriptional factors peroxisome proliferator-activated receptors (*PPARa*; P < 0.05), zinc finger protein 423 (ZFP423; P = 0.074) and Sterol regulatory element-binding proteins (SREBP1; P = 0.081) were downregulated in RIF compared to the control diet (Figure 2). Among the genes encoding proteins involved in fatty acid transport, the relative mRNA expression of fatty acid binding protein-4 (FABP4) was downregulated (P = 0.067) by RIF supplementation, whereas lipoprotein lipase (LPL) and fatty acid translocase (CD36) were not affected in the LM muscle biopsy samples (P > 0.05). The relative mRNA expression of LM muscle fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) genes were downregulated (P < 0.05) by RIF supplementation but the level of acetyl-CoA carboxylase (ACACA) gene was not affected by RIF. Relative mRNA expression of glycerol-3-phosphate acyltransferase-1 (GPAT1) and diacylglycerol acyltransferase-2 (DGAT2) was downregulated (P < 0.05) by RIF compared to the control diet. However, RIF supplementation did not affect the LM muscle levels of

| Fatty acids, g/100 g identified FAME | Treatme | nt | SEM | P-value |
|--------------------------------------|---------|-------|------|---------|
| | Control | RIF | - | |
| 14:0 | 3.91 | 3.78 | 0.17 | 0.953 |
| 16:0 | 28.58 | 27.17 | 0.36 | 0.104 |
| 16:1 <i>n</i> – 7 | 4.07 | 3.12 | 0.22 | 0.127 |
| 18:0 | 14.36 | 16.98 | 0.63 | 0.143 |
| 18:1n - 9t | 2.19 | 3.56 | 0.23 | 0.011 |
| 18:1n - 9c | 39.50 | 36.56 | 0.65 | 0.041 |
| 18:2 <i>n</i> 6 <i>t</i> | 0.06 | 0.08 | 0.01 | 0.016 |
| 18:2n - 6c | 2.30 | 3.33 | 0.27 | 0.220 |
| 18:3n-6 | 0.04 | 0.06 | 0.00 | 0.015 |
| 18:3n-3 | 0.20 | 0.27 | 0.01 | 0.031 |
| 20:1n-9 | 0.19 | 0.18 | 0.01 | 0.742 |
| 21:0 | 0.47 | 0.63 | 0.04 | 0.011 |
| 20:4n-6 | 0.56 | 0.78 | 0.13 | 0.923 |
| Others ¹ | 3.58 | 3.50 | 0.09 | 0.584 |
| SFA | 48.80 | 47.37 | 0.59 | 0.176 |
| MUFA | 47.71 | 49.19 | 0.67 | 0.235 |
| Omega-6 | 3.15 | 3.12 | 0.42 | 0.427 |
| Omega-3 | 0.34 | 0.32 | 0.04 | 0.742 |
| PUFA | 3.49 | 3.43 | 0.46 | 0.452 |
| Trans fatty acids | 2.25 | 4.02 | 0.25 | 0.011 |
| Total fatty acids, g/100 g muscle | 12.49 | 11.21 | 1.98 | 0.649 |

FAME, fatty acid methyl esters.

Data are means for n = 7 steers per treatment.

¹Includes 4:0 + 6:0 + 8:0 + 10:0 + 11:0 + 12:0 + 14:1 + 15:0 + 15:1n-6

+ 17:0 + 17:1n - 8 + 18:2n - 6t + 20:0 + 20:2n - 6 + 20:3n - 6 + 20:3n - 3

+20:5n - 3 + 22:0 + 22:1n - 9 + 22:2n - 6 + 23:0 + 24:0 + 24:1n - 9

- + 22:6n 3
- MUFA = 14:1n5 + 16:1n7 + 17:1n7 + 18:1n7 + 18:1n9 + 20:1n9

+ 22:1n9 + 24:1n9. Omega-6 = 18:2n6 + 18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:2n6 + 22:4n6.

Omega-3 = 18:3n3 + 22:6n3.

PUFA = 18:2*n*6 + 18:2*c*9,*t*11 + 18:3*n*3 + 18:3*n*6 + 20:2*n*6 + 20:3*n*3

+ 20:3*n*6 + 20:4*n*6 + 20:5*n*3 + 22:2*n*6 + 22:4*n*6 + 22:5*n*3 + 22:6*n*3. Trans fatty acids = 18:1*n*7*t*11 + 18:1*n*9t + 18:2*n*6t + 18:2*c*9, *t*11.

very long-chain acyl-CoA dehydrogenase (VLCAD) transcripts (P > 0.05). The RIF downregulated the relative mRNA expression of SNAP23 (P < 0.05) and upregulated the expression of *BSCL2* (P = 0.076) and *ATGL* (P = 0.089).

The relative mRNA expression of PPARa, PPARy and ZFP423 in LT muscle taken at 30 mo of age at slaughter was not different between RIF and control (P > 0.05), whereas expression of SREBP1 was suppressed by RIF (P = 0.076; Fig. 3). There were no differences in the relative mRNA expression of FASN and SCD between the RIF and control (P > 0.05). Steers supplemented with RIF showed a significant (P < 0.05) increase in the relative mRNA expression of LPL, ATGL, VLCAD, DGAT2, ATGL, SNAP23, and BSCL2; RIF also tended to increase the relative mRNA expression of ACACA (P = 0.064), FABP4 (P = 0.091), and CD36 (P = 0.072).

Discussion

In normal beef cattle farms in Korea, 80% of steers are fed forage hays and concentrates separately, and concentrates are manufactured by commercial feed milling companies Table 6. Fatty acid composition of LT muscle taken from Hanwoo steers at slaughter following 5 mo of RIF supplementation followed by 16 mo of fattening (30 mo of age)

| Fatty acids, g/100 g identified FAME | Treatment | | SEM | P-value |
|--------------------------------------|-----------|-------|------|---------|
| | Control | RIF | | |
| 14:0 | 3.33 | 3.85 | 0.13 | 0.075 |
| 14:1 | 0.93 | 1.19 | 0.08 | 0.030 |
| 15:0 | 0.25 | 0.32 | 0.01 | 0.028 |
| 16:0 | 28.04 | 28.75 | 0.28 | 0.335 |
| 16:1 <i>n</i> -7 | 5.22 | 5.90 | 0.18 | 0.133 |
| 17:0 | 0.65 | 0.60 | 0.02 | 0.249 |
| 17:1 <i>n</i> -8 | 0.58 | 0.56 | 0.02 | 0.525 |
| 18:0 | 9.72 | 8.72 | 0.23 | 0.090 |
| 18:1 <i>n</i> -9 <i>t</i> | 1.13 | 1.17 | 0.05 | 0.830 |
| 18:1 <i>n</i> –9 <i>c</i> | 46.82 | 45.59 | 0.46 | 0.273 |
| 18:2 <i>n</i> –6 <i>c</i> | 2.11 | 2.20 | 0.04 | 0.268 |
| 18:3 <i>n</i> –6 | 0.20 | 0.13 | 0.02 | 0.062 |
| 18:3 <i>n</i> -3 | 0.33 | 0.40 | 0.02 | 0.066 |
| Others ¹ | 0.71 | 0.63 | 0.03 | 0.402 |
| SFA | 42.19 | 42.40 | 0.44 | 0.907 |
| MUFA | 54.83 | 54.57 | 0.44 | 0.876 |
| Omega-6 | 2.42 | 2.44 | 0.06 | 0.982 |
| Omega-3 | 0.55 | 0.59 | 0.01 | 0.283 |
| PUFA | 2.97 | 3.02 | 0.06 | 0.778 |
| Trans fatty acids | 1.23 | 1.28 | 0.05 | 0.843 |
| Total fatty acids, g/100 g muscle | 14.07 | 17.58 | 1.75 | 0.087 |

Data are means for n = 14 steers per treatment.

SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids.

 $\frac{1}{10} - \frac{1}{10} + \frac{1}{10}$

MUFA = 14:1*n*5 + 16:1*n*7 + 17:1*n*7 + 18:1*n*7 + 18:1*n*9 + 20:1*n*9 + 22:1*n*9 + 24:1*n*9.

Omega-6 = 18:2n6 + 18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:2n6 + 22:4n6.

Omega-3 = 18:3n3 + 22:6n3.

PUFA = 18:2*n*6 + 18:2*c*9,*t*11 + 18:3*n*3 + 18:3*n*6 + 20:2*n*6 + 20:3*n*3 + 20:3*n*6 + 20:4*n*6 + 20:5*n*3 + 22:2*n*6 + 22:4*n*6 + 22:5*n*3 + 22:6*n*3. Trans fatty acids = 18:1n7t11 + 18:1n9t + 18:2n6t + 18:2c9, t11.



Figure 2. Effect of RIF supplementation for 5 mo during the growing phase on the relative mRNA expression of genes in intramuscular tissue of Hanwoo steers at 14 mo of age (biopsy). Values are least-square means (n = 7 per treatment) with standard errors affixed to the bars, and are expressed as relative expressions to control. $^{+}P \le 0.1$; $^{*}P \le 0.05$.

(Bharanidharan et al., 2021). Conventionally, the proportion of concentrate in the diet of Hanwoo steers is recommended to be at least 50% for the growing phase, and 80% to 90% of the total DM intake during the early and late fattening phases (NIAS, 2017). This has resulted in high levels of energy in intramuscular, subcutaneous, and visceral adipose tissues (Kim et al., 2007), and a customer preference for Hanwoo beef over imported beef due to its high degree of marbling (Moon



Figure 3. Relative mRNA expression of genes in longissimus thoracic muscle samples from Hanwoo steers supplemented with RIF, obtained after slaughter at 30 mo of age. Values are least-square means (n = 14 per treatment) with standard errors affixed to the bars and are expressed as relative expressions to control. [†] $P \le 0.1$; ^{*} $P \le 0.05$.

and Seok, 2021). The feed restriction programs applied in this experiment resulted in no differences in growth performance between control and RIF treatments, with an average final BW (760 kg) similar to those reported previously (Reddy et al., 2018; Lee et al., 2020).

Supplementation with RPF or RIF or bypass fat is considered a useful strategy to supply energy directly to the hindgut and alter the fatty acid profile of longissimus muscle in beef cattle (Zinn et al., 2000; Gilbert et al., 2003; Lee et al., 2003). Gilbert et al. (2003) showed that supplementing beef cattle diets with rumen-protected canola oil resulted in an increased proportion of 18:2n - 6 and 18:3n - 3 in intramuscular adipose tissue while decreasing the proportion of 16:0 and 16:1n- 7. The canola oil also tended to increase the proportion of 18:0 and depress the proportion of 18:1n - 9. Similarly, Choi et al. (2013) demonstrated that feeding beef cattle with palm oil (which contains high levels of 16:0, 18:1n - 9, and 18:2n - 6) decreased the proportion of 16:1n - 7 and tended to increase 18:0 and 18:1n - 9 in longissimus muscle. The palm oil supplementation also downregulated the expression of *PPAR* γ and *SCD* in subcutaneous adipose tissue (Choi et al., 2016). In addition to the dietary supplementation studies, Choi et al. (2015) conducted experiments with bovine intramuscular adipocytes in culture and found that treatment with 18:1n - 9 or 18:2n - 6 strongly decreased the transcript levels of SCD. Based on the similarities between the effects of rumen-protected canola oil and RIF treatment on the fatty acid profile and gene expression in beef cattle, the current study concluded that the lower relative mRNA expression of SCD and potentially other genes responsible for adipocyte differentiation in RIF-supplemented steers were likely influenced by the presence of 18:1n - 9 and 18:2n - 6 in the RIF supplement during the growing phase.

Several studies involving supplementation with RPF or RIF in the late fattening phase did not show significant differences in growth performance and carcass yield compared to control groups. This suggests that late-phase supplementation with RPF or RIF may not have a substantial impact on traits such as back fat thickness and ribeye area in Hanwoo steers (Lee et al., 2003; Park et al., 2010; Kim et al., 2012). However, in contrast, Mangrum et al. (2016) and our current study observed greater fatty acid content in the longissimus muscle of steers that were supplemented with RPF or RIF in the early phase. Additionally, both studies found that the RPF or RIF-supplemented steers tended to have smaller but more numerous adipocytes compared to the control group. This suggests that the RIF supplementation during the early phase might lead to increased intramuscular fat deposition and a different adipocyte profile, potentially influencing the meat quality and composition of Hanwoo beef. The discrepancies in results between different studies may be attributed to various factors, including differences in the specific types and amounts of RIF used, the duration and timing of supplementation, the diet composition, and the genetic variability of the animals.

The hypothesis is that RIF supplementation during the growing phase (5 mo) might decrease intramuscular adipocyte size and suppress adipose differentiation. This, in turn, could lead to increased triacylglycerol accumulation in adipocytes during the fattening phase (17 mo) when a high-energy concentrate is provided. 18:1n - 9 and 18:2n - 6 found abundant in RIF used in our current study are mentioned as factors that inhibit preadipocyte differentiation (Madsen et al., 2005). These fatty acids are able to activate PPAR and play a role in the proliferation of preadipocyte cells with a minor impact on terminal adipocyte differentiation (Hansen et al., 2001; Madsen et al., 2005). This suggests that RIF supplementation, which contains these fatty acids, could influence the process of adipocyte differentiation. The suppression of adipose differentiation during the growing phase might lead to an increase in the proliferation of preadipocytes. This could result in an increased number of adipocytes, contributing to greater intramuscular fat accumulation during the fattening phase. The proliferation of preadipocytes and differentiation into adipocytes is a prerequisite for the growth and development of adipose tissue (Hausman et al., 2009). In a previous study, an increase in the number of cells of adult rats fed a high-fat diet was a consequence of general cellular proliferation rather than the filling of existing preadipocytes (Klyde and Hirsch, 1979). Amri et al. (1994) observed that an increase in the intracellular concentration of fatty acids triggers the proliferation of preadipose cells. Hyperplasia, the increase in the number of cells, is emphasized as being more important than hypertrophy, an increase in cell size, during the early stages of cattle growth for marbling formation (Albrecht et al., 2006). The study by Miao et al. (2019) is mentioned to show that inhibition of adipocyte differentiation increases the proliferative potency of preadipocytes. This could further support the idea that RIF supplementation might influence adipocyte proliferation.

The experiment showed that in steers supplemented with RIF, relative mRNA expression of most of the genes, except ATGL and BSCL2 were downregulated in the LM muscle obtained through biopsy at 14 mo. However, in the LT muscle sampled at 30 mo of age, relative mRNA expression of genes was upregulated, except for SREBP1. RIF supplementation during the growing phase caused downregulation of genes encoding transcriptional factors (PPARa, ZFP423, and SREBP1) involved in preadipocyte differentiation. This suggests suppression of differentiation and lipid filling in the intramuscular adipose tissue. $PPAR\alpha$ overexpression and activation in preadipocytes of mice have been shown to increase differentiation to adipocytes (Goto et al., 2011). Therefore, the lower transcript levels of $PPAR\alpha$ in bovine intramuscular adipose tissue due to RIF supplementation may have the opposite effect, inhibiting adipocyte differentiation. ZFP423 is a key transcription factor for adipogenic commitment in cells and differentiation of committed preadipocytes through *PPAR* γ induction (Gupta et al., 2010). However, the relative mRNA expression of $PPAR\gamma$ was not affected by RIF supplementation in this study. The existence of $PPAR\alpha$ binding sites in the FABP4 promoter (Shin et al., 2009) supports the concurrent downregulation of $PPAR\alpha$ and FABP4 transcripts by RIF in the growing phase. The downregulation of SREBP1 transcript level by RIF supplementation could play a role in adipocyte differentiation (Kim and Spiegelman, 1996) and may also be responsible for the subsequent reduction in the relative mRNA expression of FASN and SCD (Ladeira et al., 2018). SREBP1 binds to the sterol regulatory sequences present in cis-acting elements of the FASN and SCD genes (Moldes et al., 1999; Miyazaki et al., 2004). In this respect, supplementing with RIF high in unsaturated bypass fatty acids during the growing phase may serve as a mechanism to depress adipose tissue development during the growing phase. The same results were also obtained by Oliveira et al. (2014) in a study of bulls, in which they reported negative correlations of RPF with the expression levels of $PPAR\alpha$, SCD, FABP4, and LPL. The suppression of relative mRNA expression of SCD by RIF supplementation in this study may also explain the decrease in proportions of 18:1n - 9 in the growing phase; SCD catalyzes $\triangle 9$ -cis desaturation of fatty acyl-CoAs, i.e., 18:0 to 18:1*n* – 9, respectively (Enoch et al., 1976). An evaluation of the associations of five candidate genes with the fatty acid composition of Hanwoo steers (Maharani et al., 2012) supported downregulation of the intramuscular relative transcript level of FABP4, and decreased levels of 16:0 in biopsied LM muscle from RIF-supplemented steers during the growing phase in this experiment. Similarly, a relationship between FABP4 transcript level and 16:0 transport from the membrane to the cytoplasm, has been observed in Japanese Black cattle (Hoashi et al., 2008) and Holstein steers.

The *FABP4* gene encoding protein that involved in fatty acids transport was downregulated by RIF supplementation during the growing stage of Hanwoo. Earlier studies revealed the existence of *PPARa* binding sites in *FABP4* promoter (Shin et al., 2009), thus the fact behind the downregulation of *FABP4* in steers fed test diet is associated with the expression

of PPAR α . Whereas, the expression of FAPB4 in LT muscle at slaughter was upregulated in RIF-supplemented group. In addition to intramuscular fat, the levels of adipose FABP positively correlated to adipocyte number and lipid content was demonstrated as an indicator of marbling score in a previous study (Damon et al., 2006). Furthermore, a positive correlation of *PPARa* with marbling score and quality grade score in that study demonstrated that it was mediated by $PPAR\alpha$ regulated FABP4 expression. Similarly, the relative mRNA expression of intramuscular SCD was downregulated in the RPF supplementation group. The positive correlation of SCD with FABP4 indicates the association of the preceding mechanisms such as FABP4-mediated fatty acid transport is required for SCD expression (Yang et al., 2017). Further, the SREBP binds to sterol regulatory sequences present in the *cis*-acting elements of FASN and SCD gene (Shimano, 2001). Thus, downregulation of SREBP in the current study is responsible for the subsequent reduction in the expression of FASN and SCD in RIF supplemented group. Supplementation of RIF during the growing stage up regulated the expression of ACACA without affecting the SCD in LT muscle at slaughter in our study. Chakrabarty and Romans (1972) noticed that the activity of acetyl CoA carboxylase was correlated to the levels of marbling in beef cattle. Among the enzymes involved in lipogenesis, the expression of ACACA and FASN but SCD is important for IMF deposition and subsequent development of marbling in beef cattle (Ward et al., 2010).

Supplementation with RIF suppressed the intramuscular relative mRNA expression of GPAT1 and DGAT2 in the growing phase, which would decrease triacylglycerol accumulation. The depression of preadipocyte differentiation in the early stage by supplementation of RIF may be also related to an increase in intramuscular 18:3n - 3 and 18:3n - 6 in biopsied LM muscle samples, and to the downregulation of relative mRNA expression of GPAT1 and DGAT2 (Petersen et al., 2003). Earlier studies demonstrated that preadipocyte proliferation could be increased by inhibiting its differentiation to mature adipocyte and DGAT expression (Zhu et al., 2015). In support of the above statement, the present study revealed that supplementation of RPF during the growing stage downregulated the expression of DGAT2 and other adipocyte differentiation-responsive genes (Cao et al., 2006). In addition, a positive correlation was noticed between relative mRNA expression of genes GPAT1 and SREBP in intramuscular adipose tissue at 14 mo of age. The fact behind this positive correlation could be explained by the presence of three sterol regulatory elements (SREs) in the proximal promoters region of GPAT1 for SREBP1-mediated transactivation (Ericsson et al., 1997). Thus, downregulation of SREBP has mediated the repression of intramuscular GPAT1 expression during the early growth phase of RIF-supplemented beef cattle. The present study also revealed that at 14 mo of age, the 16:0 and 18:1n - 9 contents were noticed to be decreased with RIF supplementation. Both, 16:0 and 18:1n - 9 was reported to stimulate adipocyte differentiation (Madsen et al., 2005). 16:0, the main product of FASN, led to a dose-dependent increase in expression of terminal differentiation-related genes and mediated preadipocyte differentiation (Amri et al., 1994). Our study revealed RIF supplementation during the growing stage of beef cattle downregulated the intramuscular expression of FASN and the levels of 16:0. It was also demonstrated that FASN does not only provide 16:0 as a substrate for triacylglycerol synthesis but 16:0 also helps to maintain and sustain differentiation signaling.

The enzyme catalyzing the first step in the lipolysis of triacylglycerol is ATGL, whereas VLCAD catalyzes the initial step in the mitochondrial β-oxidation of long-chain fatty acids. The relative mRNA expression of ATGL and VLCAD were up-regulated by RIF supplementation during the fattening phases in this study. This indicates that the lipid turnover rate, i.e., the balance between the synthesis and degradation of triacylglycerol, was higher for steers in the fattening phase than the growing stage. The greater relative mRNA expression of LPL during the fattening phase would promote the uptake of fatty acids from chylomicrons and VLDL, and increased FABP4 transcript level would enhance the uptake of fatty acids released by the action of LPL. Damon et al. (2006) suggested that the FABP4 gene may be a valuable marker of lipid accretion in the longissimus muscle. Damon et al. (2006) also found a positive correlation between FABP4 expression and adipocyte number.

Kim et al. (2007) reported that DM intake of Hanwoo steers was increased at the age of 18 mo, and the daily BW gain then peaked over 2 to 3 mo. In addition, the concentration of net energy in the daily BW gain was maintained at a relatively constant level due to preferential protein accretion and a small change in the lean-to-fat ratio within this period. A switch occurred at around 21 mo of age for steers fed a high-energy diet. Consequently, the steers in our test group might have less lipid turnover, leading to a high prevalence of 1++ grade and increase in intramuscular adipose tissue deposition, total fatty acids, and 18:1n - 9 by 118%, 125%, and 122%, respectively, compared to the control group. These results were supported by an earlier study reporting that less mature and more proliferative active adipocytes were responsible for the higher intramuscular adipose tissue in Wagyu than in Angus beef cattle (May et al., 1994).

The results from the present study also revealed that supplementation of RIF during the growing phase upregulated the expression of mRNA-encoding proteins involved in fatty acid transport and esterification (CD36, LPL, ATGL, and DGAT2) rather than the proteins involved in fatty acids synthesis (ACACA, FASN, and SCD) in LT muscle at slaughter stage. This indicates that the increase in total fatty acid content in intramuscular adipocytes is not a result of increased fatty acid synthesis within those cells. Instead, it is due to an increase in the transport of fatty acids from the bloodstream into the adipocytes. The underlying cause of this phenomenon would depend on various factors, including diet, hormonal regulation, and metabolic status. Through the downregulation of FASN and SCD mRNA, C18:3 supplementation did not enhance de novo lipogenesis (Ladeira et al., 2018). Additionally, Choi et al. (2015) showed that SCD expression was downregulated in bovine subcutaneous and intramuscular preadipocytes by 18:1n - 9 and 18:2n - 6. Likewise, it has been noted that feeding growing cattle oil (rich in 18:1n - 9and PUFA) results in a downregulation of SCD mRNA expression in subcutaneous adipose tissue (Choi et al., 2015, 2016). As a result, MUFA and PUFA in general seem to be connected to the expression of SCD by fatty acids. Additionally, there were no changes in the expression of SREBP mRNA, which controls lipid synthesis in animal cells (Matsuda et al., 2001).

In spite of the high omega-6 fatty acid level in the concentrate, the total intramuscular omega-6 and omega-3 fatty acid percentage in LT muscle was not significantly altered, also the steers finished on the RIF supplemented diet had greater percentages of 18:3n - 3 and decreased percentages

in 18:3*n* – 6. In addition, RIF-mediated upregulation of *VLCAD* and its associated lipid turnover mechanism might play a key role in maintaining the omega-6/omega-3 fatty acid levels. The indispensable role of *VLCAD* in maintaining the level of omega-3 fatty acids has been confirmed using *VLCAD* null mice model (Gélinas et al., 2011). Typically, in ruminants, a significant proportion of 18:0 is converted to 18:1*n* – 9 by *SCD* in adipose tissues (Kellerman and Jollow, 1964). However, unlike the biopsied LM tissue, the RIF supplementation didn't alter the expression of *SCD* mRNA in LT muscle at the slaughter stage, which is responsible for unchanged levels of 18:1*n* – 9. The relative mRNA expression of *SCD* has been reported to be inhibited by dietary unsaturated fatty acid and more specifically by 16:1*n* – 7 (Angulo et al., 2012; de Souza et al., 2018).

Kociucka et al. (2016a, 2016b) reported that adipocyte size in pigs, as well as porcine mesenchymal cells isolated from bone marrow, was positively correlated with the expression of SNAP23, which increased the rate of fusion and size of lipid droplets (Boström et al., 2007). In this study, RIF supplementation during the growth stage downregulated the intramuscular relative mRNA expression of SNAP23, encoded SNARE proteins, and may have contributed to the reduced adipocyte size. The relative mRNA expression of BSCL2 was up-regulated in this experiment; this is considered to be important for normal adipogenesis and expression of the key genes mediating lipogenesis (Payne et al., 2008). The BSCL2 gene induces the expression of SREBP1 and the lipogenic enzymes GPAT1 and DGAT2 (Payne et al., 2008). Therefore, RIF supplementation during the growing stage of cattle could retain intramuscular adipogenic potential by upregulating the relative mRNA expression of BSCL2. These findings collectively suggest that fatty acid transporters may be crucial for the absorption of fatty acids into cells for the formation of intramuscular fat. These fatty acids are raised in the intramuscular adipocytes when we supplement oils rich in particular types of fatty acids; depending on the fatty acid type, these fatty acids may change the metabolism.

Conclusion

Supplementation with RIF during the growing phase (9 to 13 mo of age) reduced the differentiation and lipid filling of intramuscular preadipocytes. The differentiation of preadipocytes to adipocytes after the growing phase might have increased the intramuscular lipid content during the fattening phase. Therefore, RIF supplementation and its consequent suppression of lipid synthesis in the growing phase can positively alter the gene networks associated with lipogenesis in intramuscular adipose tissue and lead to long-term increases in intramuscular adipose tissue development.

Acknowledgment

This study was supported by the National Institute of Animal Sciences, Rural Development Administration, Republic of Korea (research project PJ014234012021). Equal contributions to this manuscript were made by R.B. and K.T.

Conflict of Interest statement

The authors declare no conflicts of interest.

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