AMERICAN SOCIETY OF ANIMAL SCIENCE

Journal of Animal Science, 2021, Vol. 99, No. 6, 1–9

doi:10.1093/jas/skab123
Advance Access publication June 18, 2021
Received: 10 November 2020 and **Accepted:** 21 April 2021
Ruminant Nutrition

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Effects of early high nutrition related to metabolic imprinting events on growth, carcass characteristics, and meat quality of grass-fed Wagyu (Japanese Black cattle)

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Abstract

The study was conducted to clarify how early high plane of nutrition related to metabolic imprinting affected growth, carcass characteristics, and meat quality of grass-fed Wagyu (Japanese Black cattle). Wagyu steers were allocated randomly into 2 dietary groups: (1) steers fed milk replacer (crude protein 26.0%, crude fat 25.5%; maximum intake 0.6 kg/d) until 3 mo of age and then fed roughage (orchard grass hay) ad libitum from 4 to 10 mo of age (roughage group, RG; $n = 11$); (2) steers fed milk replacer (maximum intake of 1.8 kg/d) until 3 mo of age and then fed a high-concentrate diet from 4 to 10 mo of age (early high nutrition, EHN; $n = 12$). After 11 mo of age, all steers were fed roughage ad libitum until 31 mo of age and then slaughtered. Growth performance, carcass traits, longissimus muscle (LM) meat quality and intramuscular fat (IMF) content, plasma insulin-like growth factor I (IGF-I) concentration, and bone mineral density were measured. Body weight was greater in EHN steers (571 kg) than RG steers (520 kg; $P < 0.01$). Plasma IGF-I levels were higher in EHN steers than in RG steers at 3, 10, and 14 mo of age ($P < 0.01$, $P < 0.005$, $P < 0.001$, respectively); however, plasma IGF-I levels were lower in EHN steers compared with RG steers at 30 mo of age ($P < 0.01$). The total weight of the muscles and bones of the left half of the carcass was not different between the 2 groups ($P = 0.065$). Five of the 19 muscles investigated (semimembranosus, $P = 0.036$; infraspinatus, $P = 0.024$; supraspinatus, $P = 0.0019$; serratus ventralis cervicis, $P = 0.032$; serratus ventralis thoracis, $P = 0.027$) were heavier in EHN steers. Total fat weight in the left half of the carcass was 30% greater ($P = 0.025$) in HNE carcasses. Subcutaneous and perirenal fat weights were 53% and 84% greater ($P = 0.008$, $P = 0.002$, respectively) in EHN carcasses. The LM IMF content was greater in EHN loins (13.2%) compared with RG loins (9.4%) at 31 mo of age ($P = 0.038$); however, no differences were found for shear force, tenderness, and cook loss. These results suggested early high-nutrition affected the growth and meat quality of livestock.

Key words: carcass characteristics, grass-fattened cattle, neonatal programming, Wagyu

Abbreviations

ADG	average daily gain
BCS	beef color standard
BFS	beef fat standard
BMS	beef marbling score
BW	body weight
GH	growth hormone
HCW	hot carcass weight
IGF-I	insulin-like growth factor-I
IMF	intramuscular fat
LM	longissimus muscle
TDN	total digestible nutrients

Introduction

Marbled beef with increased intramuscular fat (IMF) is considered valuable in Wagyu (Japanese Black cattle) raised in Japan and requires feeding a large amount of cereal grain to cause its deposition. Similar to Japan, the feedlot system in the United States also utilizes considerable amounts of concentrates during the finishing phase to produce grain-fed beef. The main alternative to grain-fed beef, grass-fed beef, is produced on pastures and minimizes environmental impacts (Provenza et al., 2019). Moreover, grass-fed beef contains a higher proportion of unsaturated fatty acids, which contribute to human health (Provenza et al., 2019). While consumer interest in grass-fed beef is increasing in developed countries, problems associated with grass-fed beef including productivity (final carcass weight), meat quality (marbling), flavor (Mandell et al., 1998; Sitz et al., 2005), and toughness are issues needing to be addressed.

Nutritional stimuli during human fetal and neonatal periods affect later-life metabolism and consequently modify phenotype under the concept of developmental origins of health and disease (Waterland and Gorza, 1999, Barker et al., 2002; Du et al., 2010a, b). In cattle, early life events during the suckling period and months after weaning significantly affect long-term performance and productivity (Heinrichs and Heinrichs, 2010; Khan et al, 2010; Van Amburgh et al., 2013). Diet and feeding strategies in calves may represent a cost-effective means of altering carcass composition in Wagyu beef.

When compared with calves weaned at 8 mo of age and backgrounded, early-weaned calves fed high-concentrate feed for 148 d, followed by grazing and feedlot entry, did not improve feedlot performance; however, this management scheme did improve marbling score and increased hot carcass weight (Scheffler et al., 2014). Wertz et al. (2002) reported early weaning (at 142 d of age), followed by feeding with high concentrates until 9 mo of age, accelerated the accumulation of IMF in crossbred Wagyu compared with those reared by grazing until 22 mo, followed by concentrate feeding. Similarly, feeding Holstein bulls milk replacer over an extended period (to 200 d of age) shortened the fattening period by increasing growth rate per unit time, but did not affect IMF (Abdelsamei et al., 2005). These results suggest quality and quantity of milk replacer fed during the suckling stage and the diet fed during the rearing stage may significantly influence long-term growth. The objective of this study was to evaluate the influence of early and intensive high-plane of nutrient feeding on growth, carcass characteristics, and meat quality of grass-fattened Japanese Black cattle.

Materials and Methods

Animals and sampling

Animals used in this study were treated in accordance with the Guidelines for Animal Experiments of the Faculty of Agriculture of Kyushu University (Fukuoka, Japan) and with Japanese law (Law No. 105, notification No. 6).

Twenty-three male Japanese Black steers were obtained by crossing a Yasushigekatsu bull (a sire from the Livestock Improvement Association of Japan, Inc.) with cows from the National Cattle Breeding Center of Japan. The bull was from one of the popular Japanese Black cattle lines, "Kedaka," and was a good representative of the Japanese Black breed. Steers were castrated at ~4 mo of age. Calves were randomly allocated into 2 dietary groups: (1) early high nutrition (EHN; *n* = 12) and (2) roughage-fed (RG; *n* = 11) group (Figure 1). Until 14 d of age, calves were kept in conditions similar to those of calves held in a free-stall barn. After 14 d of age, calves in both groups were fed the same quality of milk replacer (ME 4.6 M cal/kg, crude protein 26.0%, crude fat 25.5%) by an automaticfeeder (Calf-Feeders TAP5-SA2-30-P, KFA3-MA3; Forster Technik, Engen, Germany).

As a control, the RG group was given milk replacer (Calftop ET; Zenrakuren, Tokyo, Japan) at the standard Japanese level

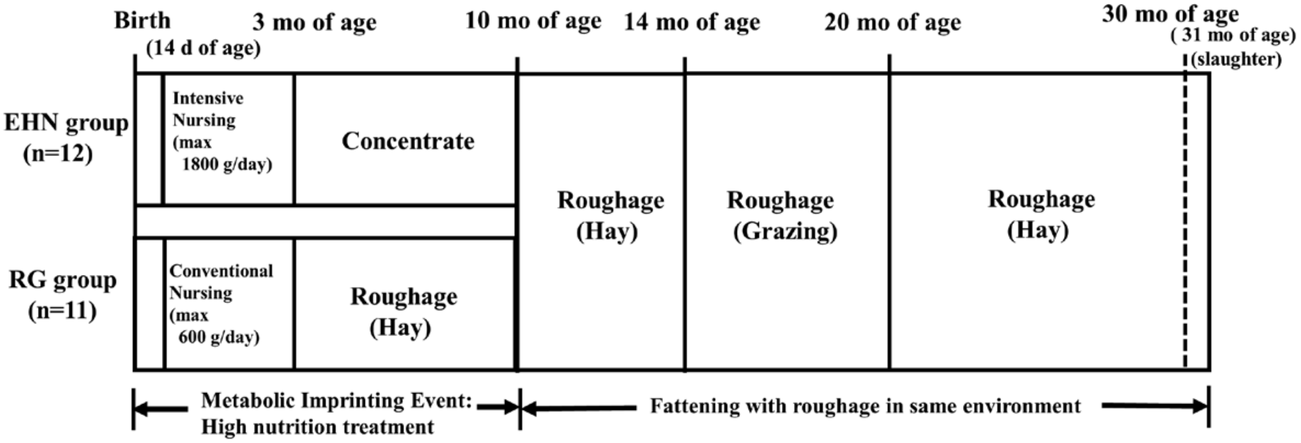

Figure 1. Experimental design. Two different diets were fed during the early growth stage. The high-energy (metabolic imprinting, EHN) group was intensively fed milk replacer (maximum intake of 1.8 kg (fresh matter) per day) with calf starter until 3 mo of age and then fed concentrate (with roughage available ad libitum). The roughage (RG) group was given milk replacer at the Japanese standard level (maximum intake of 0.6 kg (fresh matter) per day) with calf starter until 3 mo of age and then fed with only hay ad libitum until 10 mo of age. Thereafter, the 2 groups were fed the same roughage diets during the fattening period (11 to 31 mo of age).

[maximum intake of 0.6 kg (fresh matter) per day] from 14 d until 3 mo of age and fed a constant amount of dry calf starter (1.2% of body weight, ME 3.2 M cal/kg, crude protein 25.6%, crude fat 4.3%; Table 1) and hay (ME 1.2 to 1.6 Mcal/kg, crude protein 4.0% to 8.4%). The RG steers were then fed only roughage (orchard grass hay) ad libitum from 4 to 10 mo of age. The EHN group was intensively fed milk replacer [maximum intake of 1.8 kg (fresh matter) per day] from 14 d until 3 mo of age followed by a high-concentrate diet from 4 to 10 mo of age (Table 1). All calves were allowed free access to water and were provided with a mineralized salt block that contained minerals, salt, and a diuretic (Cowstone A; Nippon Zenyaku Kogyo, Japan) from the weaning stage onwards. After 11 mo of age, both groups were fed only roughage [orchard grass (*Dactylis glomerata*) hay, fresh matter ME 1.3 Mcal/kg, crude protein 9.8%] ad libitum in the paddock until 14 mo of age. All animals were then put onto the same pasture [perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)] and grazed until 20 mo of age, fed only roughage (orchard grass hay) ad libitum in the paddock from 21 to 31 mo, and slaughtered at 31 mo of age at Fukushima Meat Factory Co.,Ltd. under the government oversight (Figure 1).

Table 1. Diet compositions and dietary and nutrient intake during the intervention period from 0 to 10 mo of age[1]

	EHN	RG	*P*-value
Composition of diet			
Milk replacer, 0 to 3 mo of age			
Dry matter, %	97.0	97.0	
Metabolizable energy, Mcal/kg	4.6	4.6	
Crude protein, %	26.0	26.0	
Crude fat, %	25.5	25.5	
Dry calf starter, 0 to 3 mo of age			
Dry matter, %	87.0	87.0	
Metabolizable energy, Mcal/kg	3.2	3.2	
Crude protein, %	25.6	25.6	
Crude fat, %	4.3	4.3	
Concentrate of diet, 4 to 10 mo of age			
Dry matter, %	87.3	87.3	
Metabolizable energy, Mcal/kg	2.7	2.7	
Crude protein, %	16.0	16.0	
Crude fat, %	2.0	2.0	
Dietary intake (fresh matter, kg/head), 0 to 3 mo of age			
Replacer milk	101.5 ± 5.4	42.4 ± 0.3	<0.01
Dry calf starter	23.8 ± 11.2	42.7 ± 9.0	<0.01
Roughage	24.9 ± 5.2	32.4 ± 15.9	
Total	150.1 ± 19.2	117.6 ± 24.5	
Nutritional intake (fresh matter, kg/head), 0 to 3 mo of age			
Metabolizable energy, Mcal/kg	5.8 ± 0.3	3.6 ± 0.2	<0.01
Crude protein	32.8 ± 3.4	21.2 ± 2.6	<0.01
Crude fat	26.8 ± 1.6	12.1 ± 0.5	<0.01
Dietary intake (fresh matter, kg/head), 4 to 10 mo of age			
Concentrate	1175.7 ± 110.7		
Roughage	437.5 ± 13.7	923.4 ± 61.4	<0.01
Nutritient intake (fresh matter, kg/head), 4 to 10 mo of age			
Metabolizable energy, Mcal/kg	43.6 ± 3.0	16.8 ± 1.0	<0.01
Crude protein	224.9 ± 17	77.0 ± 6.5	<0.01
Crude fat	31.4 ± 2.1	16.2 ± 1.4	<0.01

[1] EHN, metabolic imprinting group, *n* = 12; RG, roughage group, *n* = 11. Values are means ± SD.

Carcass traits and grade

Body weight was recorded just before slaughter after fasting for 24 hr. Hot carcass, muscles, bones, and adipose tissue weights were measured as basic carcass traits. The Japan Meat Grading Association (JMGA, 1988) measured meat yield, ribeye area, rib thickness, and subcutaneous fat thickness and evaluated the carcass grade, beef marbling score (BMS), beef color standard (BCS), and beef fat standard (BFS) of all carcasses. Subcutaneous adipose tissue was dissected from the surface of the carcass and the inside of the skin. Total weights of the muscles, bones, adipose tissues of the thoracic and visceral cavities, and perirenal fat were measured after separating them by dissection from the cold carcass 24 hr after slaughter. Five randomly chosen carcass left sides from each group were dissected and weights of muscles, fat, and bones were measured.

Physical and chemical properties of the longissimus muscle (LM)

Water holding capacity was measured by drip, thawing, cooking, and grilling loss. Drip loss was quantified as described by Honikel (1987). Thawing and cooking losses were determined in 2.5-cm-thick longissimus steaks frozen in polyethylene bags at −20 °C. The percentage of thawing loss was evaluated based on the weight difference between before and after thawing at 4 °C for 24 hr. Before cooking, meat pH was determined in the LM by a pH meter (iQ170; IQ Scientific Instruments). Samples were cooked in plastic bags in a water bath at 80 °C until a meat core temperature of 70 °C was reached. The temperature was monitored by a thermocoupler. Samples were cooled to ambient temperature and weighed after their surfaces had been dried with tissue paper. For determination of the grilling loss, 2.5-cm-thick steaks were grilled in a convection oven at 150 °C until a meat core temperature of 70 °C was reached.

Broiled samples were used to determine shear force after cooling and drying with soft paper. Six 1.27-cm cores were collected parallel to the muscle fiber orientation and sheared perpendicular to the fiber direction using Instron model 5542 (Instron Co., Norwood, MA). Calibration distances were taken and the mean of the maximum forces was used for data analysis.

The IMF content of the LM was measured in triplicate via the Soxhlet extraction method. Petroleum ether was used as the solvent and fat content was determined gravimetrically after evaporating the extracted solvent (AOAC, 2000).

Muscle and bone sample collection and analysis of bone mineral density

Eighteen muscles were then removed and weighed from 5 carcasses per group: longissimus thoracis, psoas major, trapezius, latissimus dorsi, spinalis and semispinalis thoracis and cervices, infraspinatus, supraspinatus, serratus ventralis cervicis, serratus ventralis thoracis, semispinalis capitis, tensor fascia latae, biceps femoris, gluteus medius, vastus lateralis, rectus femoris, semitendinosus, semimembranosus, and adductor muscles.

Bones including the cervical vertebrae, thoracic vertebrae, lumbar vertebrae, ribs, scapula, humerus, radius, carpal bones, femur, tibia, patella, tuber coxae, and other small bones were collected after muscle removal. After the adhering connective tissues and muscles had been carefully removed, bones were weighed and mineral density of the left femur of each animal was measured using dual-energy X-ray absorptiometry (QDR-2000; Hologic, Waltham, MA).

Measurements of plasma insulin-like growth factor-I (IGF-I) levels

To establish the influence of intensified milk replacer intake on the secretion of growth factors in Japanese Black calves, change in plasma IGF-I over the study period was investigated. Before morning feeding, blood samples were drawn from the jugular vein into heparin-sodium tubes at 3, 10, 14, 20, and 30 mo of age. Plasma was separated by centrifugation at 1,752 × g for 20 min at a temperature of 4 °C and stored at –40 °C until measurement. After the removal of binding proteins, the level of IGF-I in the plasma was determined by radioimmunoassay (IGF-I IRMA, SRL, Inc., Tokyo, Japan). IGF-I intra- and interassay CV were 1.8% and 2.4 %, respectively

Statistical analysis

Statistical analyses were performed using SAS statistical software (SAS University Edition; SAS Inst. Inc. 2014, Cary, NC). Between-group comparisons were made by ANOVA using the Mixed procedure with a group as a fixed effect. Data are presented as the means ± SD for all animals in each experimental group (EHN, $n = 12$; and RG, $n = 11$), while data on carcass composition and the weights of dissected muscles, fat depots, and bones are from subsets of animals (EHN, $n = 5$; and RG, $n = 5$). The analyses of change of IGF-I concentration during growth were performed using repeated measures ANOVA of EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics (Kanda, 2013). Differences were considered statistically significant at $P \leq 0.05$. Different superscript capital letters (A, B) indicate differences between treatments ($P < 0.05$ and $P < 0.01$, respectively).

Results

Feed efficiency and growth performance

Steers in the EHN group consumed 1.5 times more crude protein ($P < 0.01$) and 2.2 times more crude fat ($P < 0.01$) than those in the RG group from week 2 until 3 mo of age. Afterward, EHN steers consumed 2.9 times more crude protein ($P < 0.01$) and 1.9 times more crude fat ($P < 0.01$) until 10 mo of age (Table 1). Intensive feeding of milk replacer and concentrate increased ($P < 0.001$) EHN average daily gain (ADG) during the metabolic imprinting intervention (0 to 10 mo of age; Table 2).

Upon entering the roughage fattening phase, EHN steers were 165-kg heavier ($P < 0.01$) than those in the RG group; however, EHN steers did not gain any additional body weight for the first 5 mo after the switch from a concentrate- to a hay-based diet (Figure 2).

From 11 to 14 mo of age, RG steers had greater ($P < 0.001$) ADG than EHN steers (Table 2). Nonetheless, EHN steers remained heavier ($P < 0.001$) at 15 to 20 mo of age, when they entered the next grazing phase. Steers in the EHN group began gaining body weight after 16 mo of age, while in the transition-to-grazing phase.

Table 2. Growth performance of cattle in EHN and RG groups[1]

	EHN		RG		
	Mean	SD	Mean	SD	P-value
Growth performance					
Birth weight, kg	34	5.3	35	4.2	0.703
ADG from 0 to 90 d[2], kg/d	**1.02**	0.09	0.75	0.12	<0.001
BW at 90 d, kg	**126**	11.2	103	11.2	<0.001
ADG from 90 to 300 d[3], kg/d	**1.06**	0.07	0.45	0.08	<0.001
BW at 300 d, kg	**370**	23.7	205	23.6	<0.001
ADG from 300 to 450 d[4], kg/d	0.16	0.08	**0.38**	0.10	<0.001
BW at 450 d, kg	**394**	26.9	262	36.3	<0.001
ADG from 450 to 600 d[5], kg/d	0.50	0.19	**0.91**	0.13	<0.001
BW at 600 d, kg	**470**	23.7	399	33	<0.001
ADG from 600 to 900 d[6], kg/d	0.21	0.05	**0.28**	0.07	<0.001
Final BW, kg	**571**	36.3	520	39.2	0.0049

[1] EHN, metabolic imprinting group, $n = 12$; RG, roughage group, $n = 11$.

[2] The EHN group was intensively fed high-protein and high-fat milk replacer (maximum 1.8 kg/d), and the RG group was received conventional feeding during the suckling period (maximum 0.6 kg/d).

[3] The EHN group was fed concentrates (2.5% of body weight) and hay, and the RG group was fed only hay ad libitum.

[4] The 2 groups were fed the same hay during fattening.

[5] The 2 groups grazed on the same pasture.

[6] The 2 groups were fed the same hay during finishing.

Growth performance and serum IGF-I level

Serum IGF-I levels were greater in EHN steers at 3, 10, and 14 mo of age ($P < 0.01$, $P < 0.005$, and $P < 0.01$, respectively; Figure3). Serum IGF-I levels were less in EHN steers at 30 mo of age ($P < 0.05$). The change in IGF-I levels with growth seen in this study was consistent with the change in ADG during the study. Body weight was always higher in EHN steers ($P < 0.01$, Figure 2); however, its relationship with ADG changed after 10 mo of age (Table 2). The IGF-I levels in EHN steers decreased after the switch from a concentrate based to a roughage-based diet at 11 mo of age.

Carcass characteristics

At 30 mo of age, after the steers had been fed hay from 21 to 30 mo, differences ($P < 0.001$) in body weight were found between the groups (Table 2). Steers in the EHN group finished an average of 51 kg heavier ($P < 0.005$) than RG steers (Table 2), with a corresponding increase in hot carcass weight ($P = 0.0001$; Table 3). When the ratio of each fat depot to hot carcass weight was calculated, EHN steers had greater subcutaneous fat (9.8% vs. 7.2%, $P = 0.018$) and perirenal fat (3.1% vs. 1.8%, $P = 0.003$) ratios. The hot carcass weight was greater at different subcutaneous fat and rib thicknesses for EHN steers, which suggests that they deposited subcutaneous fat as readily as the steers in the RG group relative to their changes in body weight.

The beef marbling standard (BMS, JMGA, 2014; 1 = the least marbled, 12 = the most marbled beef), beef color standards (BCS, JMGA, 1988; 1 = pale red, 7 = dark red), and beef fat standard (BFS, JMGA, 1988; 1 = white, 7 = yellow.) were measured at 6th and 7th thoracic vertebrae junction by professional graders. Results indicated beef from EHN carcasses were better marbled, more pale-red in lean meat color, and whiter in fat color ($P = 0.0022$, $P = 0.0257$, and $P = 0.0007$, respectively; Table 3).

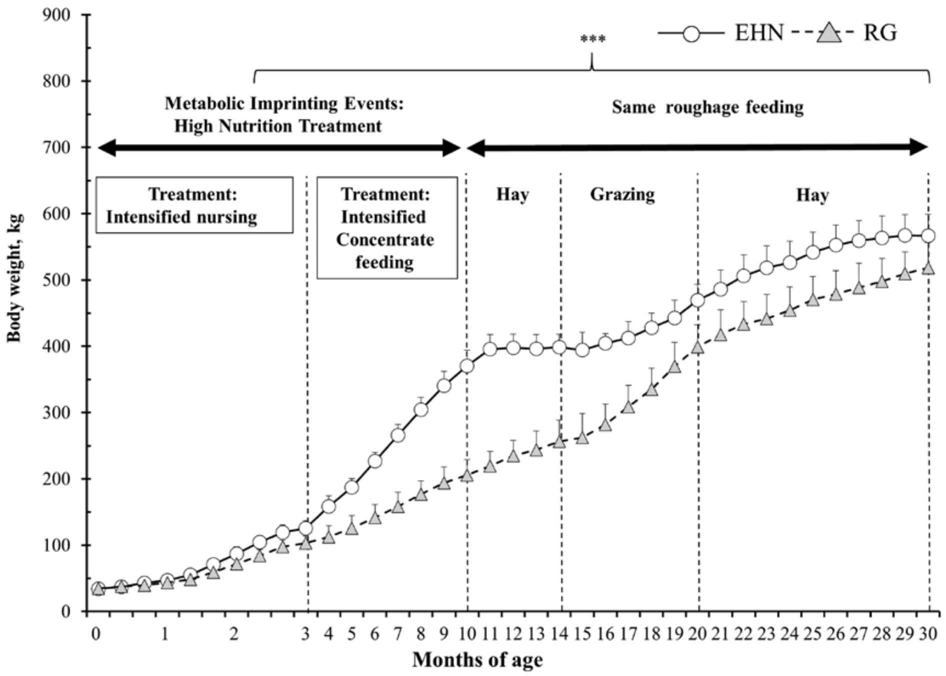

Figure 2. Changes in mean body weight over the study period in EHN and RG groups. EHN metabolic imprinting group, *n* = 12. RG: roughage group, *n* = 11. ***: P < 0.01.

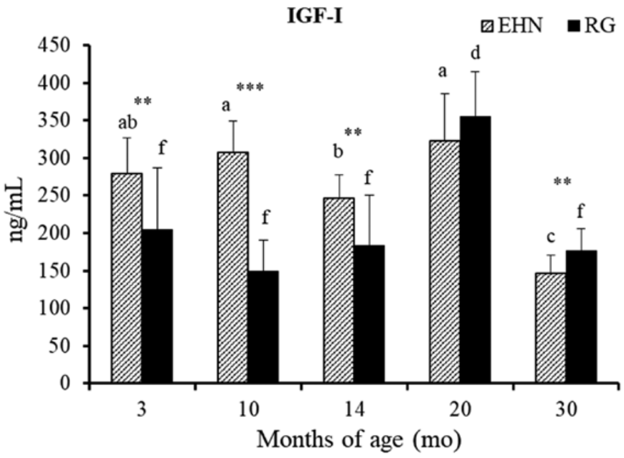

Figure 3. Comparison of serum IGF-I level during growth between EHN and RG groups. EHN: metabolic imprinting group, *n* = 12. RG: roughage group, *n* = 11. Values are means ± SD. ** $P < 0.01$. *** $P < 0.005$. ^{a,b,c}Significant differences among months of age in EHN group ($P < 0.05$). ^{e,f}Significant differences among months of age in RG group ($P < 0.05$).

No between-group differences were found in shear force, tenderness, and cooking loss in the LM (Table 3). However, the LM from EHN cattle had a higher lipid percentage and lower moisture percentage ($P = 0.0388$ and $P = 0.0279$, respectively; Table 3), although LM weight did not differ between the groups (Table 4). The pH of the EHN LM was lower ($P = 0.0327$).

Comparison of individual muscle, fat depots, and bone weight between EHN and RG groups

The total weight of the muscles of the left half of the carcass did not differ between EHN and RG carcasses ($P = 0.065$, Table 4). Only 5 of the 19 muscles investigated (semimembranosus, $P = 0.036$; infraspinatus, $P = 0.024$; supraspinatus, $P = 0.0019$; serratus ventralis cervicis, $P = 0.032$; serratus ventralis thoracis, $P = 0.027$) were heavier in EHN carcasses (Table 4). When individual muscle weight was normalized to total muscle weight, just 2 muscles (semitendinosus and serratus ventralis thoracis) were heavier in the EHN carcasses ($P = 0.019$ and $P = 0.041$, respectively; Table 4).

Total fat weight in the left half of the carcass was 30% greater in the EHN group ($P = 0.025$, Table 4). The weights of the subcutaneous and perirenal fat were 53% ($P = 0.008$) and 84% ($P = 0.002$) greater, respectively, in EHN carcasses. Calculated subcutaneous:total fat and perirenal:total fat weights were greater in EHN carcasses ($P = 0.025$ and $P = 0.006$, respectively) and lower for intermuscular: total fat and visceral cavity:total fat ($P = 0.014$ and $P = 0.003$, respectively). By contrast, no differences in bone weight ($P = 0.244$) or bone mineral density ($P = 0.830$) were found (Table 4). Thus, there was a higher fat percentage in subcutaneous and perirenal fat of EHN carcasses with the tendency of a smaller percentage of the visceral cavity ($P = 0.092$) in EHN carcasses.

General carcass composition

Regarding general carcass composition (Figure 4), no significant differences in the percentages of muscle, fat, and bone were observed; however, the absolute amount of fat in the carcass (Table 4) was larger ($P = 0.025$) in EHN carcasses.

Discussion

In this study, we compared the performance of calves that were intensively fed milk replacer for 3 mo and then fed a high-concentrate diet for 7 mo with that of calves raised in a grass-based feeding system. This schedule was selected because the effects of neonatal metabolic imprinting events are thought to continue until weaning (Vickers et al., 2008). Metabolic programming (or imprinting) is characterized by (1) a susceptibility limited to a critical ontogenic window early in development; (2) a persistent effect lasting through to adulthood; (3) a specific and measurable phenotypic outcome

Table 3. Carcass characteristics and physical and chemical properties of the LM in EHN and RG groups at slaughter[1]

	EHN		RG		
	Mean	SD	Mean	SD	P-value
Carcass characteristics and meat quality					
HCW, kg	**293.63**	17.27	256	20.57	0.0001
Meat yield,[2]%	71.5	0.4	71.7	0.7	0.4900
Rib eye area,[3] cm^2	32.67	2.5	29.36	6.7	0.1483
Rib thickness,[4] cm	**4.28**	0.32	3.64	0.22	<0.0001
Subcutaneous fat thickness,[5] cm	**1.45**	0.3	0.83	0.4	0.0004
Carcass grade[6]	**2**	0.0	1.36	0.5	<0.0001
BMS No[7]	**2.41**	0.66	1.54	0.52	0.0022
BCS No[8]	4.91	0.67	**5.73**	0.90	0.0257
BFS No[9]	4.25	0.87	**5.55**	0.69	0.0007
Physical and chemical properties of LM					
Shear force after CL[10]	3.13	0.61	3.26	0.82	0.6734
Tenderness,[10] kgw/cm^2	63	21.2	67	30.3	0.7223
Moisture, %	66.54	2.69	**69.66**	2.19	0.0279
Lipid, %	**13.24**	3.68	9.35	2.80	0.0388
Protein, %	19.26	1.46	19.95	0.89	0.1869
pH	5.58	0.09	**5.7**	0.10	0.0327
Cookinloss, %	25.1	1.9	25.7	2.2	0.5100
Water holding capacity of LM					
Press method	80.22	2.08	80.31	1.48	0.9049
Centrifuge method	70.84	1.79	71.78	2.10	0.2667

[1] EHN, metabolic imprinting, n = 12; RG, roughage, n = 11.

[2] Meat yield percentage was calculated using the following equation: dressing percentage value = 67.37+[0.130 × cross-sectional area of longissimus thoracis muscle at thoracic vertebrae 6 to 7 (cm^2)] + [0.667 × thickness of ribs including meat (cm)]−[0.025 × half-carcass weight (kg)] − [0.896 × subcutaneous fat thickness (cm)] (Japan Meat Grading Association (JMGA), 1988; Gotoh et al., 2014).

[3] Cross-sectional area of longissimus thoracis at thoracic vertebrae 6 to 7 (JMGA, 1988).

[4] Thickness of specific region of the ribs including meat, measured by the Japan Beef Grading Standard (JMGA, 1988; Gotoh et al., 2014).

[5] Subcutaneous fat thickness of the specific region indicated by the Japan Beef Grading Standard (JMGA, 1988; Gotoh et al., 2014).

[6] 1 = the lowest grade, 5 = the highest grade.

[7] Beef Marbling Standard No. evaluated by Graders based on the revised Japan Beef Grading Standard (JMGA, 2014) at thoracic vertebrae 6 to 7 of the LM. 1 = the least marbled, 12 = the most marbled beef.

[8] Beef Color Standard No. evaluated by Graders based on the Japan Beef Grading Standard (JMGA, 1988). 1 = pale red, 7 = dark red.

[9] Beef fat (Color) Standard No. evaluated by Graders based on the Japan Beef Grading Standard (JMGA, 1988). 1 = white, 7 = yellow.

[10] Measurements using a tensipresser before cooking loss (CL) analysis (Tsuji, 1982).

that may differ quantitatively among individuals; and (4) a dose–response or threshold relationship between a specific exposure and outcome (Waterland and Garza 1999). In this study, a different plane of nutrition from birth to weaning affected body weight and IMF of the LM in Wagyu steers fattened with only grass. An absence of EHN growth, as measured by body weight, occurred from 11 mo to 14 mo of age revealing a shortcoming with the management system. It is likely that the EHN steers did not adapt readily to their change in diet, which was more extreme than for the steers in the RG group. Depressed growth in the EHN steers likely reflects a change in the ruminal microbe populations that allows for digestion of the roughage-based diet (Brown et al., 2006; Chen et al., 2011). Potentially, this could be alleviated by gradually decreasing the level of concentrate being fed during the transition period back to a hay-based diet.

In this study, serum IGF-I levels were greater in the EHN group than in the RG group until 14 mo of age followed by a decline in serum content. IGF-I is the primary mediator of many of the responses regulated by growth hormone (GH) in tissues throughout the body, and is generally secreted by the liver as a result of stimulation by GH from the pituitary. It affects most cells, but functions especially in the muscle and bone (Cohen et al., 1991). Matsuzaki et al. (2001) reported that the crude protein intake from an experimental diet influenced the plasma IGF-I level in Holstein cattle. In this study, the lower protein level of roughage compared with that of milk replacer and concentrate during the suckling period may have resulted in the reduced level of IGF-I during the fattening period.

Adipose tissue is not only an energy storage site but also an endocrine organ. Adipocytes secrete adipokines (Avram et al., 2007) that control energy homeostasis (Trujillo and Scherer, 2006). Japanese Black cattle have a greater ability to accumulate intramuscular adipocytes than European cattle (Gotoh et al., 2009). Most adipocytes form during the fetal and early postnatal stages of development, and adipocyte hyperplasia in perirenal fat is complete at birth (Du et al., 2010b). Du et al. (2013) suggested that the marbling process occurs from late gestation until about 250 d of age in beef cattle. Goessling et al. (2009) suggested that the total number of adipocytes is set by adolescence. However, a visceral depot of adipocytes forms from mid-gestation to the early postnatal period (Robelin, 1981). Several studies have identified the time from early weaning until approximately 250 d of age as the “marbling window.” In this study, early higher fat and protein nutrition with milk replacer strongly affected the accretion of IMF in the longissimus, along with higher levels of accumulation of subcutaneous and perirenal fat.

In cattle, early nutrition influences carcass composition and meat quality. The growth performance and carcass quality of Holstein bulls fed varying amounts of milk replacer over an extended period (to 200 d of age) were investigated by Abdelsamei et al. (2005), which reported that feeding increased amounts of

Table 4. Body composition of cattle in EHN and RG groups[1]

	Weight			Ratio, %		
	EHN	RG	P-value	EHN	RG	P-value
Half-carcass weight[2], g	147,240	130,336	0.028	—	—	—
Muscle[3], g						
Total muscle (18 muscles with all other muscles)	86,795	79,574	0.065	—	—	—
Tensor fasciae latae	1,019	1,007	0.914	1.17	1.26	0.384
Biceps femoris	6,047	5,619	0.186	6.96	7.06	0.445
Gluteus medius	3,163	2,914	0.148	3.64	3.66	0.877
Vastus lateralis	2,133	1,918	0.147	2.46	2.41	0.730
Rectus femoris	1,828	1,677	0.221	2.11	2.10	0.987
Semitendinosus	1,799	1,844	0.691	2.07	2.32	0.019
Semimembranosus	4,140	3,685	0.036	4.77	4.63	0.170
Adductor	1,484	1,363	0.157	1.71	1.71	0.988
Psoas major	1,599	1,503	0.161	1.85	1.89	0.634
Latissmus dorsi	1,614	1,569	0.634	1.86	1.97	0.118
Trapezius	7,97	800	0.980	0.93	1.00	0.635
Longissimus thoracis	5,575	5,095	0.178	6.43	6.39	0.899
Spinalis et semispinalis thoracis et cervicis	874	708	0.055	1.01	0.89	0.196
Infraspinatus	2,081	1,798	0.024	2.39	2.26	0.125
Supuraspinatus	1,424	1,254	0.019	1.64	1.58	0.158
Serratus ventralis cervicis	2,967	2,642	0.032	3.42	3.32	0.209
Serratus ventralis thoracis	901	674	0.027	1.04	0.84	0.041
Semispinalis capitis	1,401	1,374	0.797	1.62	1.72	0.352
Fat, g						
Total fat	34,715	26,385	0.025	—	—	—
Subcutaneous fat	14,464	9,473	0.008	41.6	35.8	0.025
Intermuscular fat	13,872	12,109	0.256	40.0	45.9	0.014
Thoracic cavity fat	615	554	0.805	1.75	2.12	0.598
Visceral cavity fat	1,309	1,830	0.092	3.78	6.94	0.003
Perirenal fat	4,455	2,419	0.002	12.82	9.23	0.006
Bone, g						
Total bone	22,975	21,675	0.244	—	—	—
Vertebrae cervicales	1,517	1,494	0.813	6.60	6.93	0.191
Vertebrae thoracicae	2,173	2,063	0.643	9.43	9.59	0.859
Vertebrae lumbales	1,194	1,039	0.109	5.20	4.81	0.184
Coastae	4,345	4,004	0.134	18.94	19.02	0.852
Scapula	1,259	1198	0.303	5.48	5.67	0.209
Humerus	2,121	1,971	0.137	9.24	9.30	0.769
Radius	1,535	1,512	0.785	6.69	6.80	0.358
Ossa carpi	299	279	0.390	1.30	1.29	0.925
Os femoris	2,820	2,637	0.381	12.25	12.63	0.403
Tibia	1,752	1,671	0.375	7.62	7.88	0.079
Patella	186	300	0.399	0.81	1.42	0.356
Os coxae	2,956	2,718	0.105	12.89	12.93	0.925
Others	818	504	0.083	3.56	1.73	0.066
Bone mineral density[4],**g/cm^2**	0.701	0.705	0.830	-	-	-

[1] EHN, metabolic imprinting group, *n* = 5. RG, roughage group: *n* = 5. [2] Left half-carcass.
[3] Eighteen muscles weight in left half-carcass.
[4] Evaluated from Os femoris. Ratio of each weight to the total weight of half-carcass.

milk replacer shortened the fattening period to achieve the same body weight by accelerating growth, and as a final result IMF content did not decrease at slaughter. These results suggest that the quality and quantity of milk replacer during the suckling stage, and the diet during the rearing stage, significantly influence growth. Scheffler et al. (2014) reported that early weaning and high-concentrate feeding for 148 d, followed by grazing and feedlot entry, did not improve feedlot performance. However, this management scheme did improve the marbling score and increased the hot carcass weights compared with those of conventionally weaned (around 253 ± 6 d of age) and backgrounded calves, although no significant differences in lipid content were found between the conventionally weaned (4.5%) and early weaned-imprinted groups (6.0%). In this study, neonatal metabolic imprinting events, including feeding a greater amount of high-protein and -fat milk replacer until 3 mo of age, had strong impacts on body weight, carcass weight, muscle composition of the carcass, adipose tissue weight, and IMF content in Japanese Black cattle fattened on a roughage diet. Our results mimic those reported in mice (Waterland and Graza, 1999; Gluckman et al., 2008), fed a high-fat diet during suckling that subsequently develop obesity as adults.

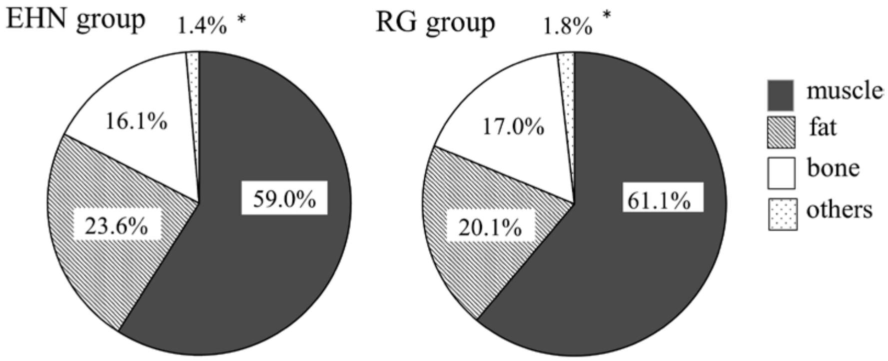

Figure 4. Carcass composition at slaughter. EHN: metabolic imprinting group, *n* = 5. RG: roughage group, *n* = 5. These data were obtained using half-carcasses. *Significant difference of percentage of others (like tendons and larger blood vessels, etc. except for muscle, fat, and bone in half carcass) between among EHN and RG groups ($P < 0.05$).

Daily body weight gain of crossbred steers (Angus × Simmental) fed a 50% grain diet *ad libitum* was significantly higher than that of steers fed a restricted amount of 50% grain diets or a large amount of forage during a specific stage of early development (119–218 d) (Schoonmaker et al., 2003; 2004). Consequently, the former carcasses contained larger subcutaneous fat layers and greater IMF content. In contrast, the steers with restricted feed intake during a specific stage of early development had leaner carcasses with less IMF, as well as poorer growth performance (Myers et al., 1999a,b; Schoonmaker et al., 2002). Although these previous reports suggest that nutritional conditions during early development influence growth performance during fattening and skeletal muscle characteristics at the time of slaughter, our results indicate that nutritional influences during the suckling period may have even greater effects on later growth in beef cattle.

Conclusion

The results of this study suggest that an early high plane of nutrition significantly affects the growth and body composition of Wagyu steers. Additionally, these findings indicate that nutritional conditions during the suckling period can strongly affect growth performance during later development stages. The imprinting phenomenon suggests that there is a unique biological mechanism that allows early-life nutrition to have long-lasting effects on phenotype.

Acknowledgements

This work was supported by Kakenhi (grant nos. 20380150, 25292162, 26310312, and 19KT0013) from Japan Society for the Promotion of Science, Young Researcher Overseas Visit Program for Vitalizing Brain Circulation (grant no. S2305) from Japan Society for the Promotion of Science, and Kyushu University P&P grant. The authors wish to thank Tetsuji Etoh, Yuji Shiotsuka, Shuichi Kaneda, and Hiroyuki Hasebe for their technical assistance. The authors also thank Edanz (https://en-author-services.edanzgroup.com/ac) for editing the English text of a draft of this manuscript.

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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