

宮崎大学大学院
博士学位論文

**A comprehensive study on the selection of meat
production ability of Japanese Black sire**

**黒毛和種種雄牛の産肉能力選抜に関する
総括的研究**

2016年3月

宮崎大学大学院農学工学総合研究科
資源環境科学 専攻

Jomane Fortune Ntengwa

**UNIVERSITY OF MIYAZAKI
PhD THESIS**

**A comprehensive study on the selection of meat
production ability of Japanese Black sire**

March 2016

**Department of Environment and Resource Science
Interdisciplinary Graduate School of Agriculture and
Engineering
University of Miyazaki**

Jomane Fortune Ntengwa

ACKNOWLEDGEMENTS

I would like to express my appreciation to Assoc. Professor T. ISHIDA and Asst. Professor T. TOKUNAGA for their guidance, support and patience throughout the graduate program. Their outstanding attention to details and close supervision of my research work helped me to improve the quality of my study. I would also like to express my gratitude to Prof. T. MORITA for his unwavering support from the days prior to coming to University of Miyazaki, his role in overseeing my Master and Doctoral courses. Special thanks to Emeritus Prof. H. HARADA for his outstanding guidance, friendship and technical support.

I appreciate the lectures and advice given by Professor K. ASHIZAWA, Professor Y. TSUZUKI, Professor K. KAWAHARA, Emeritus Professor N. HASEGAWA, Professor M. IRIE, and Professor R. AKASHI; their lectures enriched my knowledge and enabled me to undertake my research with a multidisciplinary perspective.

Special thanks to the government of Japan MEXT (Ministry of Education, Culture, Sports, Science, and Technology) for the scholarship award that enabled me to undertake my postgraduate studies. I would also like to thank the Japanese Embassy/Consulate General in Zimbabwe for their support.

My whole-hearted and warm gratitude goes to the personal and staff at the Livestock Improvement Association of Miyazaki for their warm acceptance during data gathering, and for providing additional data that made this study possible.

I want to express my gratitude to the Faculty of Agriculture and the Graduate School of Agriculture for their comprehensive post graduate program and the research/learning facilities they provided. The support rendered by the University's Global Support Office (GSO) is greatly appreciated.

The assistance from the Director (Professor M. OSHIMA) of the International Student Center, Kagoshima University is gratefully acknowledged. The guidance from Japanese language lecturer's in Miyazaki and Kagoshima Universities is cordially appreciated.

The hospitality and guidance given by my personal tutor Mr. Takami TETSUYA is greatly acknowledged. His critical support during my early days in Japan made me fall in love with the country and its people. I would also like to thank my study colleague Mr. M. YAMAMOTO for his companionship that I enjoyed throughout the study program. Also, this work would not have been successful without the cooperation and support of members (past and present) of the Laboratory of Animal Breeding and Genetics, University of Miyazaki.

Mr. David WALLACE and Ms Peta McLACHLAN took their precious time to proofread my manuscripts. I appreciate their diligence, particular attention to details, comments and suggestions. I whole heartedly appreciate the support of my former tutor in Kagoshima University, Dr. Elias THIAM who made my transition to life in Japan smooth and pleasant.

I want to express my deep gratitude to my parents, brothers, friends and relatives for their vital moral support, sacrifice, love and unwavering attention during the long course of my study. Special thanks go to numerous Japanese families and individuals that hosted me

during the course of my stay in Japan. I thank my best friend Ringisai P. CHEKERA, for the unconditional love and support despite the long distance that was between us during my entire study period.

Above all, I thank God, for the strength, courage, wisdom and health during my entire study period.

CONTENTS

ABSTRACT.....	1
GENERAL INTRODUCTION.....	6
CHAPTER 1: Genetic parameters for body measurements, feed consumption and feed efficiency traits in young Japanese Black bulls	
1.1 Introduction.....	20
1.2 Materials and Methods	
1.2.1 Experimental animals.....	21
1.2.2 Body measurement procedures and traits.....	23
1.2.3 Feed management and traits.....	23
1.2.4 Statistical methods.....	23
1.3 Results and Discussion	
1.3.1 Summary of phenotypes.....	25
1.3.2 Analysis of variance of body measurements.....	27
1.3.3 Analysis of variance of feed efficiency.....	28
1.3.4 Heritability of body measurements.....	29
1.3.5 Heritability of feed efficiency.....	31
1.4 Conclusion.....	33
CHAPTER 2: Feasibility of using the ultrasound technique in the genetic improvement of young Japanese Black bulls	
2.1 Introduction.....	34
2.2 Materials and Methods	
2.2.1 Experimental animals.....	35
2.2.2 Scanning procedures and traits.....	35

2.2.3 Statistical methods.....	36
2.3 Results and Discussion	
2.3.1 Summary of phenotypes.....	37
2.3.2 Analysis of variance.....	38
2.3.3 Heritability of ultrasonic traits.....	39
2.4 Conclusion.....	42

CHAPTER 3: Prospects of early slaughter based on serially measured ultrasonic traits in Japanese Black steers

3.1 Introduction.....	44
3.2 Materials and Methods	
3.2.1 Experimental animals.....	45
3.2.2 Scanning procedures and traits.....	45
3.2.3 Statistical methods.....	47
3.3 Results and Discussion	
3.3.1 Summary of phenotypes.....	48
3.3.2 Correlation of serially measured traits.....	50
3.4 Conclusion.....	61

CHAPTER 4: Genetic polymorphisms and their association with growth and carcass traits in Japanese Black steers

4.1 Introduction.....	62
4.2 Materials and Methods	
4.2.1 Experimental animals and traits.....	63
4.2.2 Genotyping	64

4.2.3 Statistical methods.....	65
4.3 Results and Discussion	
4.3.1 Association of <i>GH</i> SNP with growth and carcass traits.....	68
4.3.2 Association of <i>GHRH</i> and <i>GDF8</i> SNP with growth and carcass traits	69
4.3.3 Association of <i>LEP</i> SNP with growth and carcass traits.....	70
4.4 Conclusion.....	74
CHAPTER 5: Variations in genes involved in fat metabolism and their association with ultrasonic and carcass traits in Japanese Black steers	
5.1 Introduction.....	75
5.2 Materials and Methods	
5.2.1 Experimental animals and traits.....	76
5.2.2 Genotyping	76
5.2.3 Statistical methods.....	78
5.3 Results and Discussion	
5.3.1 Association of <i>SCD</i> SNP with ultrasonic and carcass traits	81
5.3.2 Association of <i>SREBP1</i> polymorphism with traits.....	86
5.4 Conclusion.....	91
GENERAL CONCLUSION.....	93
REFERENCES.....	97
LIST OF FIGURES AND TABLES.....	112
APPENDIX.....	115

ABSTRACT

The progress made in the genetic improvement of the Japanese Black breed to date is largely due to the traditional quantitative techniques using pedigree, performance and progeny tests to select cattle. However, developments in technology and molecular techniques together with changes in consumer demands have made it not only necessary but also feasible to improve the current selection program. Improvements in technologies like ultrasound have made it feasible to estimate carcass traits in live animals hence evaluation can be done in breeding stock at early stages. Furthermore, advances in molecular techniques have made it possible to pin point genetic markers that affect economically important traits. The aim of this study was to come up with a comprehensive selection program that incorporates recent technological advances and changes in the meat production industry. Thus, the objectives this study were to: (chapter 1) estimate variance components and heritability of growth, feed consumption, and feed efficiency traits in performance test stock; (chapter 2) evaluate the feasibility of using ultrasound technology in the genetic improvement of young Japanese Black bulls; (chapter 3) evaluate the prospects of early slaughter of steers; (chapter 4) determine the association of polymorphisms in the growth hormone (*GH* NCBI dbSNP rs41923484 and rs134687399), somatostatin (*SST* rs17870997), growth hormone releasing hormone (*GHRH* rs380969504), myostatin (*GDF8* rs383271508 and rs137528458) and leptin (*LEP* rs29004487, rs29004488 and rs29004508) genes with growth and carcass traits; (Chapter 5) assess the association between polymorphisms in fatty acid synthase (*FASN* rs208645216), stearoyl-coenzyme A desaturase (*SCD* rs41255691), sterol regulatory element-binding protein 1 (*SREBP1* rs133958066), diacylglycerol acyltransferase 1 (*DGATI* rs109326954), and nuclear receptor subfamily 1, group H, number 3 (*NR1H3* rs109428603) genes with ultrasonic and carcass traits in Japanese Black steers.

In chapter 1 and 2, ultrasonic scanning was done at about 11 mo of age between the 6th-7th and 12th-13th rib cross section on the left side in performance test stock to obtain ultrasonic carcass traits of *longissimus* muscle area (LMA7/13), *subcutaneous* fat thickness (SFT), intermuscular fat thickness (IMFT), rib thickness (RT), beef marbling score (BMS), *trapezius* muscle thickness (TMT), and *latissimus* muscle thickness (LMT). Only LMA was measured on both scanning sites, all other traits were measured on the 6th-7th rib cross section only. Scanning equipment employed in this study was SEM-500 (FHK Co. Ltd., Japan) and HS-2000 (FHK Co. Ltd., Japan) using a frequency of 2 MHz. All linear and areal measurements were done by ImageJ version 1.46r. BMS was assessed subjectively through visual appraisal on a scale ranging from 0.0 (lowest) to 3.0 with intervals of 0.33, and 4.0, 5.0 (highest). The feed consumption and feed efficiency traits studied were concentrate intake (CONI), roughage intake (ROUI), digestible crude protein intake (DCPI), total digestible nutrient intake (TDNI), rate of roughage intake (RRI), digestible crude protein conversion ratio (DCR) and total digestible nutrient conversion rate (TDNC). Body measurement traits studied were body weight at the start of test (BWS), body weight at the end of test (BWE), daily gain during test period (DG). The following body measurements were taken at the end of the test: wither height (WH), hip height (HH), chest depth (CD), body length (BL), rump length (RL), chest width (CW), hip width (HW), thurl width (TW), pin bone width (PBW), and chest girth (CG). Data were then subjected to analysis of variance (ANOVA) and variance components were estimated through mixed model analysis using JMP[®] 5.0.1 (SAS) program. The model included fixed effects of year and season of testing, as well as the covariate of age at measurement and random additive genetic effect of sire. The averages for ultrasonic traits and their standard deviations in parenthesis were 31.9 cm² (2.1) LMA7, 53.6 cm² (4.3) LMA13, 6.1 mm (1.7) SFT, 14.4 mm (3.6) IMFT, 40.2 mm (3.4) RT, 0.74 (0.23) BMS, 12.9 mm (1.1) TMT and 12.9 mm (1.2) LMT. The averages and standard deviations for feed consumption

and feed efficiency traits were 530.3 kg (40.2) CONI, 413.1 kg (45.3) ROUI, 77.9 kg (5.1) DCPI, 579.4 kg (38.8) TDNI, 43.9 % (2.9) RRI, 0.61 (0.09) DCR, and 4.50 (0.54) TDNC. The averages and standard deviations for body measurement traits were 260.8 kg (26.1) BWS, 390.8 kg (31.8) BWE, 1.16 kg/day (0.12) DG, 125 cm (2.9) WH, 126 cm (3.1) HH, 60 cm (1.6) CD, 140 cm (4.2) BL, 48 cm (1.5) RL, 42 cm (1.8) CW, 41 cm (1.5) HW, 43 cm (1.7) TW, 26 cm (1.4) PBW, and 170 cm (4.5) HG. Estimated direct heritabilities for ultrasonic traits were moderate for REA7 (0.39), REA13 (0.39), TMT (0.16), LMT (0.15), RT (0.16), SFT (0.40) and IMFT (0.24) except for BMS (0.1). For feed consumption and feed efficiency traits, the estimated direct heritabilities were all moderate: CONI (0.21), ROUI (0.39), DCPI (0.32), TDNI (0.25), RRI (0.30), DCR (0.20) and TDNC (0.21), respectively. Direct heritability estimates for body measurements were found to be moderate for most traits, BWS (0.22), BWE (0.26), DG (0.21), WH (0.31), HH (0.24), CD (0.31), BL (0.20), HW (0.47), RL (0.17), and HG (0.23). However, heritabilities for TW and PBW were high (0.52 and 0.73, respectively) on the other hand, a low heritability was estimated for CW (0.1). Most estimates of heritability in this study suggest that considerable additive genetic variation exist in the cattle population in Miyazaki prefecture and further improvement of carcass, growth, feed intake and feed efficiency traits can be done through selection.

In chapter 3, in order to explore the possibility of reducing the slaughter age of steers to increase production efficiency and competitiveness; serial/longitudinal measurements of ultrasonic traits were taken from 14 to 26 mo of age on 300 Japanese Black steers under progeny testing at the Livestock Improvement Association of Miyazaki and carcass traits were recorded after slaughter. Additionally, serial measurements of body weight, withers height, chest girth and abdominal girth were taken at 9, 14, 20 and 28 mo of age. Analysis of sequentially measured traits was done through multivariate analysis (MANOVA) of repeated measures using JMP[®] 10 (SAS Institute Cary, NC, USA). The statistical model included

fixed effects of starting test year, starting test season; a linear covariate of the age of the animal at the beginning of the test and a random effect of sire line. Subsequently, growth curves were estimated using an application for statistical analysis (Stat Proc Version 2). The growth curves were estimated using four equations but ultimately one was chosen based on goodness of fit. The equations used were, Logistic: $y = A/(1+\lambda \exp(\beta t))$; Brody: $y = A/(1-\lambda \exp(\beta t))$; Bertalanffy: $y = A/(1-\lambda \exp(\beta t))^3$ and Gompertz: $y = A \exp(-\lambda \exp(\beta t))$. Ultrasound and body measurements were used to estimate carcass yield estimate (YE) percentage through stepwise multiple regression using the Application for Statistical Analysis (Stat Proc Version 2.0, Kyoto, Japan). Univariate analysis of serially measured traits revealed that there are no significant changes between LMA measures at 26 and 28 mo of age. The LMA also exhibited reduced growth rate after 25 mo of age, so did marbling. On the other hand, IMFT and RT exhibited high growth rates after 20 mo. Steers could attain carcass YE of A grade as early as 18 mo of age and daily gain (DG) started to diminish from 24 mo of age. Considering these results, changing consumer demands and globalization, it may be prudent to reduce the slaughter age to about 25 mo.

In chapter 4, in order to better comprehend the association between the aforementioned polymorphisms and growth, serial measurements of body weight, withers height, chest girth and abdominal girth at 9, 14, 20 and 28 mo of age were taken in Japanese Black steers that were under progeny test (n = 280) at the Livestock Improvement Association of Miyazaki. Carcass measurements were taken and their association with genetic polymorphisms was evaluated. An ANOVA was done and *post hoc* analysis was done using Tukey-Kramer's honestly significant test. Polymorphisms in the *GH*, *GHRH*, *GDF8* (rs137528458) and *LEP* (rs29004508) were significantly associated ($p < 0.05$) with some growth and carcass traits. Thus, these polymorphisms can be useful markers for the improvement of growth and carcass traits in Japanese Black cattle.

In chapter 5, in order to have a comprehensive analysis of the association between the aforementioned genetic polymorphisms and ultrasonic traits, longitudinal measurements of ultrasonic traits were taken from 14 to 26 mo of age on 300 Japanese Black steers under progeny testing at the Livestock Improvement Association of Miyazaki. Furthermore, the association of these genetic polymorphisms and carcass traits was evaluated. The polymorphisms in the *SCD* gene and *SREBP1* were associated ($p < 0.05$) with some ultrasonic traits at multiple stages. To add to that, the polymorphisms were associated ($p < 0.05$) with some carcass traits. These findings suggest that the polymorphisms in the *SCD* and *SREBP1* are functional mutations that can aid in selection to improve some ultrasonic and carcass traits.

Overall, a more comprehensive program that is more efficient is feasible through the use of ultrasonic measures and the use of molecular markers.

GENERAL INTRODUCTION

Introduction

Since the initiation of breeding stock unions in 1962 and the registration of animals based on their pedigree, body condition score and body measurements, significant progress has been made in Japanese Black cattle breeding to date. During the 1950s the concept of progeny testing arose and the final standardised procedures were formulated in 1962. In the year 1968, official performance and progeny testing programs were initiated in various prefectures. In the year 1990 the animal model best linear unbiased prediction (BLUP) was introduced to predict breeding values using field progeny testing records (Namikawa 1992). Considering this background the organised genetic improvement of the Japanese Black breed stretches back to about 50 years ago. Even though the organisation of the breeding program is similar at national level, the genetic makeup varies widely from one prefecture to another and environmental effects cannot be completely controlled. Hence, improvement programs tailor-made for each prefecture are the best way forward. This study focused on the Japanese Black population in Miyazaki prefecture.

Traditionally, the information used to predict carcass merit was collected through structured carcass sire progeny tests. Carcass sire progeny tests are time consuming, expensive, and subject to selection bias. Employing ultrasound technique in breeding programs may improve selection programs in numerous ways. Some of the advantages of using ultrasound for predicting carcass merit is that ultrasound is relatively inexpensive, has a shorter generation interval when compared to carcass sire progeny testing programs, and the data provided via ultrasound may be subject to less selection bias than data collected via carcass sire progeny testing program. The most important advantage of using ultrasound measurements collected from young breeding stock is that the data can be used to calculate

non-parent ultrasound carcass expected progeny differences (EPD), allowing for selection to occur at an earlier age. Studies have shown that ultrasound measurements of fat thickness, ribeye area, intramuscular fat thickness and fat percentage are moderate to high in heritability (Bertrand *et al.* 2001).

At early stages of animal development, body measurements are important traits in evaluating growth performance. Information about genetic relationships between growth and carcass traits is important for designing suitable selection programs. Hirooka *et al.* (1996) indicated that the Japanese Black cattle have lower mature size and growth rate relative to other domestic breeds; therefore, there is need to continue working on improving growth rate in this breed.

Since liberalization of beef imports in 1991, Japanese beef producers have faced increased competition from foreign producers. To make Japanese beef more competitive on a liberalised market, reducing the cost of production is of paramount importance. Providing feed to cattle is the single largest expense in most beef production enterprises, and thus, effort at improving the efficiency of feed utilization of animals will help reduce production costs (Hoque *et al.* 2006b).

Heritability is one important component in the prediction of genetic progress from selection to improve a trait. Furthermore, it shows how important efforts to improve a trait through improved management or environmental conditions may be compared to genetic selection. The first two chapters aimed to estimate variance components and heritability of ultrasonic, body measurement, feed consumption and feed efficiency traits.

Though the beef trade was liberalised in 1991, high imports tariffs protected the local beef producers however globalization is increasing and this year (2015) the Trans-Pacific Partnership (TPP) trade pact was agreed on. This development will lead to the reduction of or even removal of import duty/tariffs that were being imposed on foreign meat and meat

products. This change means producers in Japan need to prepare for stiff competition from abroad. The current average slaughter age is around 30 mo for Japanese Black steers which means animals are fattened longer periods than those from competitors such as U.S. and Australia. Beef imported from U.S. was from cattle slaughtered at less 21 mo of age during the 2005 to 2013 period according to the U.S. Department of Agriculture (USDA 2013) while those coming from Australia are slaughtered at 21 or 26 mo of age on average according to the New South Wales Department of Primary Industries (NSW DPI 2007). Growth curves for body measurements that are provided by the National Wagyu Registry Association (2004) show that growth is drastically reduced after about 25 mo of age; keeping animals post this age can be associated with diminishing returns. The other factor that calls for the revision of the current rearing scheme is changing consumer demands. According to data from the Ministry of Agriculture, Forestry and Fisheries (MAFF 2014), the price gap between carcasses of different quality scores is shrinking. This decline in price differences can be attributed to changing consumer demands as they are increasingly favoring carcasses with lesser marbling among other factors. These factors gave the impetus to analyze the impacts of reducing the slaughter age of steers on carcass yield percentage and partly on quality score in the third chapter of this study.

Economically important traits in cattle are determined by both genetic makeup and environmental factors. Animal breeders exploit the genetic influence to improve these traits. Currently, most beef cattle breeders use phenotypic and pedigree information to calculate estimated breeding values (EBV) and subsequently select breeding stock. However, advances in technology are making it much easier to identify the genetic basis of phenotypic variation. During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers that affect traits of interest in livestock, including those for single gene traits and quantitative trait loci (QTL) or genomic regions that affect quantitative

traits (Dekkers 2004). This has enabled opportunities to enhance genetic improvement programs in livestock by direct selection of genes or genomic regions that affect economic traits through marker assisted selection and gene introgression (Dekkers and Hospital 2002). Furthermore, the dramatic reduction in the cost of sequencing has led to the discovery of numerous single nucleotide polymorphisms (SNP) markers throughout livestock genomes (Hayes *et al.* 2009). The third and fourth chapters in this thesis evaluated the association of polymorphisms in genes related with growth and fat metabolism, and economically important traits.

Review of literature

Ultrasonic Measurements of Carcass Characteristics

Johnson *et al.* (1993) noted that real-time ultrasound technology offers a practical, non-destructive, and relatively inexpensive means of collecting indicators of carcass traits from live animals. In the past two decades researchers have been gathering ultrasound records, potentially for developing ultrasound carcass expected progeny differences and for making breeding and management decisions. The scope in which ultrasound technology can be used in the beef cattle industry is dependent on the heritability of ultrasound measures and their relationship with corresponding carcass traits in fed slaughter cattle. Wilson (1992) reported on the use of ultrasound in livestock species, with an emphasis on the potential for genetic improvement programs in beef cattle. Ultrasonic measurements in meat animals are used to record live animal anatomical measurements that will allow the prediction of genetic differences between individual animals for carcass merit. Ultrasound has been used for more than five decades. However, technological advances in ultrasonics, such as real-time imagery and portable equipment, have renewed interest in the livestock industry for the use of this technology for genetic improvement.

Several studies have noted the importance of equipment and technicians for obtaining accurate measures utilizing real-time ultrasound. Results from a two part study by Smith *et al.* (1992) suggested that ultrasound measures prior to slaughter were useful in estimating carcass fat thickness and imprecise in predicting carcass *longissimus* muscle area for measurements taken with an Aloka 210DX machine. The authors noted that fat thickness was underestimated on fatter cattle, whereas, *longissimus* muscle area was underestimated in heavily muscled cattle. In contrast, Herring *et al.* (1994) found that real-time ultrasound can be used to accurately predict fat thickness and *longissimus* muscle area with qualified trained personnel. The authors also noted that significant differences in accuracy and precision of measurements were made with an Aloka 500V machine compared with the Aloka 210DX machine. On the other hand, little difference in the fat thickness was observed between technicians and Aloka 500V and the Aloka 210DX machines. The authors suggested that before ultrasound measurements are accepted for use in national cattle evaluations, technicians must undergo rigorous training to be tested for proficiency.

Perkins *et al.* (1992) scanned yearling feedlot steers and heifers 24 hours prior to slaughter to evaluate the accuracy of ultrasonic measurements of fat thickness and *longissimus* muscle area for prediction of actual carcass measures. Animals were scanned by two technicians on alternating days using Aloka 210DX machine. The technicians had equal but limited experience using ultrasound technology. The authors found no significant differences to exist between technicians for absolute differences between ultrasonic and carcass measures. Unlike Smith *et al.* (1992), the accuracy of predicting ultrasonic measures did not change as the level of experience increased. Perkins *et al.* (1992) concluded that ultrasound measures of fat thickness and *longissimus* muscle area may accurately predict final carcass fat thickness and *longissimus* muscle area may accurately predict final carcass fat thickness and *longissimus* muscle area in beef cattle. Waldner *et al.* (1992) also investigated real-time ultrasound

equipment and technicians for accuracy, precision, and determined the most accurate age to measure fat thickness and *longissimus* muscle area. Real-time ultrasound measures of *longissimus* muscle area and 12th rib fat thickness on a total of 60 Brangus bulls were taken every 4 mo beginning 4 and 12 mo of age, respectively until 24 mo of age. Bulls were scanned with Aloka 210DX and Equisonics LS-300A machines by four technicians with various levels of experience. Ten bulls were slaughtered at each evaluation period to determine actual *longissimus* muscle area and fat thickness. Results suggested that estimation of ultrasound fat thickness was most accurate when measured at either 12 or 16 mo of age. The authors concluded that as fat thickness increased, accuracy of the measurement decreased, significant differences between the four technicians were observed, an increase in technical skill did not improve the accuracy of estimates for ultrasound fat thickness, and no significant difference was found for the two machines for measurements of fat thickness. The data further suggested that accurate estimation of *longissimus* muscle area only occurs at 12 mo of age, increased levels skill did not improve accuracy of *longissimus* muscle area estimates, no significant differences between the four technicians were present for *longissimus* muscle area measurements, and the Equisonics LS-300A unit was more accurate than the Aloka 210DX unit for measuring *longissimus* muscle area. The authors also found that smaller *longissimus* muscle areas were underestimated, whereas larger *longissimus* muscle areas were overestimated.

Bergen *et al.* (1997) assessed the accuracy and repeatability of ultrasound fat thickness and ultrasound *longissimus* muscle area in young performance tested bulls. At the completion of the performance test, weights and ultrasound measurements were taken on 617 bulls using an Aloka 500V unit prior to slaughter. The authors found high correlations for the ultrasound fat thickness (0.95) and ultrasound *longissimus* muscle area (0.94) between successive measurements on the same animal. Accuracy was determined by comparing ultrasonic

measurements of fat thickness and *longissimus* muscle area to the corresponding carcass measurements. Correlations between ultrasound and carcass fat thickness, ultrasound and carcass *longissimus* muscle area were 0.84 and 0.80, respectively. The authors concluded that experienced ultrasound technicians could obtain repeatable and accurate measures of ultrasound fat thickness and *longissimus* muscle area in yearling performance tested bulls.

The combination of value based marketing system and ultrasound technology has increased the importance of live animal measurements of carcass traits, especially intramuscular fat or marbling. Before live animal measures of intramuscular fat can be used as selection criteria or in national cattle evaluations, there must be a means for accurately predicting intramuscular fat from ultrasound measurements. Whittaker *et al.* (1992) reported on the basic engineering concepts of ultrasound technology with implications for ultrasound technology in beef cattle, specifically for measuring intramuscular fat. Results showed that intramuscular fat may be predicted using an A-mode transducer coupled with frequency analysis. The authors noted that ultrasound has the potential for determining marbling in live animals. The A-mode (amplitude mode) is the simplest type of ultrasound that uses a single transducer to scan a line through the body with the echoes plotted on a screen as a function of depth.

In a study by Hassen *et al.* (2001), 500 steers were scanned prior to slaughter to develop models for predicting intramuscular fat percentage in live beef cattle. Steers averaged 455 days. Each steer was scanned across the 11th and 13th ribs using Aloka 500V and Classic Scanner 200 machines. Four to five images were collected per steer per machine. After slaughter, chemical extraction of across sectional slice of the *longissimus* muscle was used to determine actual carcass intramuscular fat percentage. Four prediction models were developed separately for each of the two machines. The authors found that both the Aloka 500V and the Classic Scanner 200 machines could be used to accurately predict

intramuscular fat percentage in live beef cattle. In comparing the ultrasound equipment, authors found no significant difference in accuracy for predicting intramuscular fat percentage for the Aloka 500V and the Classic Scanner 200 machines, when algorithms developed at Iowa State University were used.

Heritability of Ultrasound Measurements in Breeding Cattle

A study by Johnson *et al.* (1993) analyzed ultrasound measurements of fat thickness and *longissimus* muscle area from 2,101 Brangus calves. Heritability estimates for age-constant (weight-constant in parenthesis) ultrasound fat thickness and ultrasound *longissimus* muscle area were 0.14(0.11) and 0.40 (0.39), respectively. The authors concluded that the moderate heritability for *longissimus* muscle area indicated that selection to change this trait could be effective. However, the low heritability estimates for fat thickness would make it very difficult to change the genetic ability of animals to fatten through selection based on this measurement.

Turner *et al.* (1990) analyzed ultrasound measurements of fat thickness and *longissimus* muscle area from 385 yearling Hereford bulls. Heritability estimates for ultrasound fat thickness and ultrasound *longissimus* muscle area were 0.04 and 0.12, respectively. The authors noted that ultrasound measures of fat thickness and *longissimus* muscle area in yearling Hereford bulls are less heritable than reported carcass fat thickness and carcass *longissimus* muscle area. The authors suggested that ultrasound *longissimus* muscle area measurements should be adjusted for age, weaning weight, and fat thickness effects.

Shepard *et al.* (1996) obtained ultrasound measurements from 1,557 Angus bulls and heifers. Images were taken by one of two trained technicians using an Aloka 500V machine. Animals ranged from 250 to 550 days in age at the time of scanning. Heritability estimates for ultrasound fat thickness and ultrasound *longissimus* muscle area were 0.56 and 0.11,

respectively. The authors noted that both ultrasonic fat thickness and *longissimus* muscle area were under some degree of genetic control, allowing selection to make genetic change in these traits. The authors highlighted that more research was needed to determine the genetic correlation between ultrasound measurements of fat thickness and *longissimus* muscle area and other economically important traits so that producers can avoid potential detrimental overall effects on their herds.

A study conducted by Wilson *et al.* (2000) estimated genetic parameters from ultrasound measurements of fat thickness, rump fat, *longissimus* muscle area, and intramuscular fat percentage in 8,620 developing Angus heifers from 851 sires. Ultrasound measurements were adjusted to a common age of 390 days. Heritability estimates for ultrasound measures of fat thickness, rump fat, *longissimus* muscle area, and intramuscular fat percentage were 0.48, 0.56, 0.40, and 0.42, respectively. Sire expected progeny differences (EPD) based on ultrasound measured traits were compared to EPD based upon carcass measured traits of steer progeny. Of the 851 sires with ultrasound EPD, 309 sires also had carcass EPD. Comparisons were made between EPD from the two sources of data using sire ranking correlations. The rank correlations were 0.72, 0.76, and 0.69 for fat thickness, *longissimus* muscle area, and ultrasound intramuscular fat and marbling score, respectively. The authors concluded that producers could use EPD based upon ultrasound measures from developing heifers to predict the same genetic differences observed from steer progeny carcass measures.

Heritability of growth traits

Munim *et al.* (2012) indicated that at early stages of cattle growth, body measurements of animals are important traits for evaluating growth performance of calves because they can be done repeatedly when an animal is young. Growth is one of the most economically important quantitative traits that affect carcass quality in beef cattle (Chung and Kim 2005). Most

studies have found out that growth traits have moderate heritabilities. Shojo *et al.* (2006) analysed data from calf and carcass markets in Hyogo and Tottori prefectures. In their study they estimated direct and maternal heritabilities for calf market weight to be 0.22 and 0.07 in Hyogo, and 0.37 and 0.15 in Tottori, respectively. A comprehensive study by Oyama (2011) analysed genetic variability of Wagyu cattle estimated by various statistical approaches. The study highlighted that heritability of daily gain for performance test stock ranged from 0.19-0.40 while the mean unweighted and weighted heritabilities were 0.29 and 0.34, respectively. Furthermore, heritability of weight at the end of the test ranged from 0.39-0.80 while the mean unweighted and weighted heritabilities were estimated at 0.59 and 0.61, respectively. Calf market weight heritability estimates in the same study ranged from 0.15-0.55 while the unweighted and weighted mean heritability estimates were 0.30 and 0.29, respectively. Fan *et al.* (1995) estimated genetic parameters for postweaning weight gain in Hereford and Angus bulls. Heritability estimates for weaning weight were 0.46 and 0.16 for Hereford and Angus cattle, respectively. The authors also estimated average daily gain (ADG) heritability to be 0.16 and 0.24, respectively for the two breeds.

Heritability of feed consumption and feed efficiency traits

Feed costs represent a major economic input for beef cattle production. Individual cattle differ in their ability to efficiently utilize feed (Bailey *et al.* 1971; Garret 1971; Freeman 1975). Selecting the most efficient animals may effectively lower production costs. The efficiency of feed utilisation is difficult to quantify than that of growth; consequently, different measures of feed efficiency have been developed over the years (Arthur *et al.* 2001).

Fan *et al.* (1995) estimated heritability for metabolizable energy intake to be 0.19 and 0.31 for Hereford and Angus bulls, respectively. The authors also estimated pooled heritabilities for dry matter feed intake, residual feed consumption, feed efficiency and net feed efficiency

to be 0.24, 0.14, 0.16, and 0.21, respectively. A review by Oyama (2011) highlighted a heritability range of 0.26-0.74 for roughage intake in performance test Wagyu cattle. The same study also indicated that heritability for concentrate intake during performance testing ranged from 0.18-0.53. To add to that, heritability estimate ranges for digestible crude protein conversion and total digestible nutrients conversion were reported to be 0.03-0.21 and 0.11-0.46 for performance test stock, respectively.

Prospects of early slaughter based on serially measured ultrasonic traits

Now more than ever the prospects of early slaughter need to be evaluated because of changes that are happening in animal production industry. Two decades of data on carcass prices of locally produced beef have shown a decline in the price gap of carcasses of different quality score. The data from the Ministry of Agriculture, Forestry and Fisheries (MAFF 2014), has shown decreases in prices between carcasses of different quality scores (e.g. A5, A4, A3, A2). The diminishing gap could be attributed to changing consumer tastes as customers demanding carcasses with less marbling among other factors. Given that one of the main reasons to slaughter late is to produce highly marbled beef, the apparent decrease in demand of the highest quality score justifies a reduction in the slaughter age. The other factor is that there is increased competition from foreign products. Beef coming into Japan is from cattle slaughtered at relatively young ages; the one imported from the U.S between 2005 and 2013 was from cattle less than 21 mo of age (U.S. Department of Agriculture, USDA 2013). This implies that these cattle are fattened for less periods of time hence less costly. Furthermore, the sealing of the Trans-Pacific Partnership (TPP) implies that import duty/tariffs of meat and meat products will be removed or reduced thereby lowering the prices further.

A reduced fattening period of the Japanese Black steers will reduce the production costs and ultimately reduce the price of locally produced beef. To estimate the impact of reduced slaughter age on carcass price determinants like carcass yield estimate percentage and marbling (a component of the quality score) analysis of serial measures of relevant traits was necessary. The statistical analysis of repeated measures of data makes it feasible to estimate growth curves and make regression analysis of economically important traits. The detailed merits and ways to analyze serial measures were discussed by Lindsey (1993); Meyer and Hill (1997); Littell *et al.* (1998).

The role of molecular genetics in livestock breeding

The significant achievements attained in the breeding of the Japanese Black cattle in the past decades can be attributed to the traditional quantitative techniques as described by Namikawa (1992). However, the development of the polymerase chain reaction has given animal breeders to capability to do selection at molecular level (Fore *et al.* 2006; Van Marle-Köster *et al.* 2013). During the past few decades, advances in molecular genetics have led to identification of multiple genes or genetic markers that affect traits of interest in livestock including those for single gene traits and quantitative trait loci (QTL) or genomic regions that affect quantitative traits (Dekkers 2004). These developments have opened up opportunities to enhance genetic improvement programs in livestock by direct selection of genes or genomic regions that affect economic traits through marker assisted selection (Dekkers and Hospital 2002). To add to that, the dramatic reduction in the cost of sequencing has led to the discovery of numerous single nucleotide polymorphisms (SNP) markers throughout livestock genomes (Hayes *et al.* 2009). The creation of centralized databases of genomes and genetic variation in various species including livestock has accelerated the progress in molecular

breeding; one such database is the Single Nucleotide Database (dbSNP) of the National Center of Biotechnology Information (NCBI).

Particular attention is being paid on the genes that affect economically important traits. Of paramount importance are genes that affect growth and carcass traits. One of the genes explored is the growth hormone (*GH*). In Japanese black cattle, polymorphisms in the *GH* have been reported to affect calf, heifer and cow body weight (Ardiyanti *et al.* 2009; Ishida *et al.* 2010). Ardiyanti *et al.* (2012) showed that *GH* polymorphisms affect lipogenic gene expression levels hence its association with fat related carcass traits was also evaluated in this study. Apart from the *GH*, polymorphisms in the growth hormone releasing hormone (*GHRH*), myostatin (*GDF8*), leptin (*LEP*) have also been evaluated among other genes. Polymorphisms in *GHRH* have been shown to be associated with hucklebone width and body weight in Chinese (Nanyang and Qinchuan) cattle (Zhang *et al.* 2012). Myostatin mutations have proved to be related with muscular hypertrophy and double muscling in sheep (Gan *et al.* 2008). Furthermore, one of the mutations in the *GDF8* is being used in sheep breeding in New Zealand; sheep that have an A allele for this marker are guaranteed to have at least 5 % more muscling in leg and ramp. The *LEP* hormone is associated with control of body weight, feed intake, immune function and reproduction. A study by Kong *et al.* (2006) reported that a SNP in the exon 2 of the *LEP* gene was associated with backfat thickness and *longissimus* muscle area in Korean cattle.

Genes involved in fat metabolism can have economical implications on meat production as they influence carcass traits like marbling and fat thickness. Among the many genes that are involved in fat metabolism are: fatty acid synthase (*FASN*), stearoyl-coenzyme A desaturase (*SCD*), sterol regulatory element-binding protein 1 (*SREBP1*), diacylglycerol acyltransferase 1 (*DGAT1*), and nuclear receptor subfamily 1, group H, number 3 (*NR1H3*). Polymorphisms in these genes were described in previous studies by (Taniguchi *et al.* 2004;

Abe *et al.* 2009; Kawahara-Miki *et al.* 2011; Lee *et al.* 2013; Oppi-Williams *et al.* 2013). Polymorphisms in *FASN* were associated with fatty acid composition in Japanese Black cattle according to a study by Matsushashi *et al.* (2011). Alternative alleles were shown to affect the fatty acid composition of the *longissimus* muscle area, the proportion of shorter and longer fatty acids were different in carcasses from animals carrying different alleles. Matsushashi *et al.* (2011) also reported that an SNP in the *SCD* gene was associated with luster, firmness and texture in Japanese Black cattle. A study in Korean (Hanwoo) cattle by Lee *et al.* (2013) showed that an SNP in the *SREBP1* was associated with marbling score. An SNP in the *DGATI* was indicated to affect back fat thickness in Spanish commercial beef cattle (Avilés *et al.* 2013). Furthermore, *DGATI* has been implicated as a potential candidate gene for intramuscular fat deposition in cattle (Thaller *et al.* 2003). A recent study has showed that *NR1H3* potentially regulates acetyl-coenzyme A carboxylase, *FASN* and *DGAT* (Oppi-Williams *et al.* 2013).

CHAPTER 1

Genetic parameters for body measurements in young Japanese Black bulls

1.1 Introduction

Growth is one of the most economically important quantitative traits that affect carcass quality in beef cattle (Chung and Kim 2005). Growth can be assessed by measuring different linear dimensions in livestock. These include withers height, chest girth, body length and rump length among others. Body weight can also be measured to assess growth. Body measurements are relatively easy to measure. Apart from growth they also indicate an animal's fitness and reproductive ability. Linear body measurements can also be used to predict body weight; this can be very useful as some farmers do not have weighing equipment but can take linear measurements of their livestock. Yahata and Namikawa (1974) outlined simple equations that can be used to estimate body weight through body measurements. Predictions equations are not only useful in estimating weight but also very useful in evaluating the fattening level of beef stock. Other studies have examined the fitting of non-linear growth models for describing growth pattern of body weight and body measurements in foreign breeds (Brown *et al.* 1976; Lopez de Torre and Rankin 1978), in Japanese Black females (Mukai *et al.* 1980; Mukai and Fukushima 1982), in Japanese Black steers (National Wagyu Registry Association 2004) and Japanese Black young calves (Kumazaki *et al.* 1955; Fukuhara *et al.* 1973).

The Japanese beef industry continues to change due to increased competition from foreign products and other protein sources. In 1960, the level of beef consumption was about the same as pork consumption, corresponding to the same price levels. However, in the process of economic development in Japan, the price of beef rose sharply relative to the price of pork, resulting in a drop in consumption of beef relative to pork. The major factor behind the rise of beef prices relative to pork prices was a lag in technological development to exploit economies of scale. Furthermore, beef import was liberalized in April 1991 according to the Beef Market Access Agreement (BMAA) (Kobayashi 1999). Imports dominated for the first time in 1992 Japanese fiscal year (JFY), and the rate of self-sufficiency of beef has remarkably decreased thereafter, falling to 36% in 1997 JFY. Considering this background, cattle breeders and farmers should prioritize production efficiency in their breeding programs.

In the past, genetic improvement has been aimed mainly at output traits such as fertility and live weight, and more recently carcass and meat quality traits, with little emphasis placed on reducing inputs. Feed is the largest cost category in beef production system. Increasing the efficiency of feed utilization will result in reduced costs and may increase competitiveness Japanese beef on a liberal market. Heritability shows how important efforts to improve a trait through improved management or environmental conditions may be compared to genetic selection. The objective of this chapter, therefore, was to estimate variance components and heritability of growth traits, feed consumption, and feed efficiency in performance test stock.

1.2 Materials and Methods

1.2.1 Experimental animals

Data used in this study were collected in Miyazaki on performance test bulls at the Livestock Improvement Association of Miyazaki. For this study, 525 young bulls performance tested from 1990 to 2012 were used. Bull calves collected from designated

farms within an age limit of 6 to 7 mo and a body weight of 200 to 300kg were performance tested for 112 days at the test station. After a three week adjustment period, bulls were housed individually in 2.7 m x 3.6 m pens with approximately 10 m² of paddock for voluntary exercise. After three weeks of being introduced to the feed, the bulls were given free access to roughage; however, feeding of concentrate was restricted to twice a day, morning and evening. In addition to roughage and concentrate, water was supplied *ad libitum*. Sufficient feed was given to each animal according to previous consumption. Records of roughage and concentrate intake were maintained on a dry matter basis throughout the test. Body weight was measured regularly during the test period and feed intake was measured daily by the difference between supplied and left over feed.

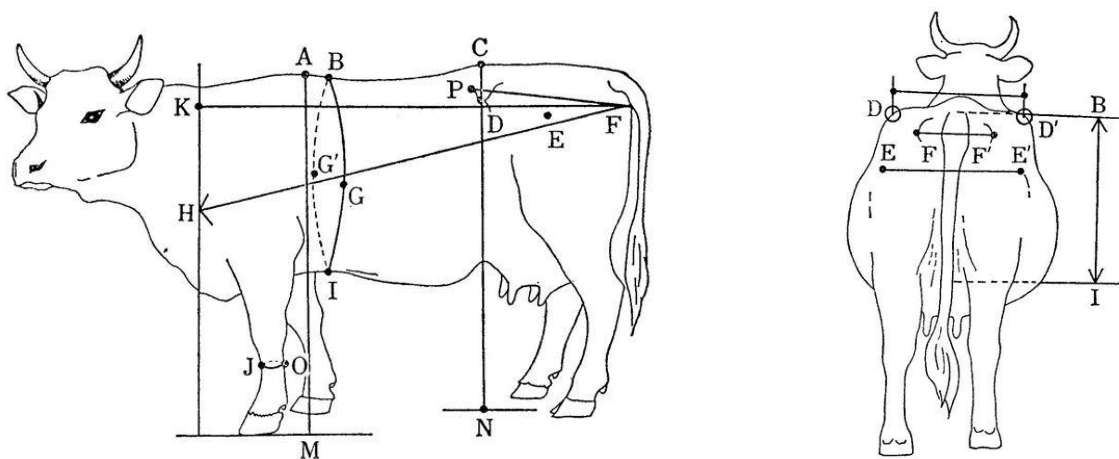


Figure 1. The positions for body measurements

Wither height (WH): A to M

Hip height (HH): C to N

Chest depth (CD): B to I

Body length (BL): K to F

Rump length (RL): P to F

Chest girth (CG): BGIG'B

Chest width (CW): G to G'

Hip width (HW): D to D'

Thurl width (TW): E to E'

Pin bone width (PBW): F to F'

1.2.2 Body measurement procedures and traits

Body measurements were taken on 525 young bulls at about 11 mo of age using a steel rod, caliper and measuring tape calibrated in centimeters. The body measurements positions are illustrated in Figure 1. Body measurement traits studied included body weight at the start of test (BWS), body weight at the end of test (BWE), and daily gain during the test period (DG). The following body measurements were taken at the end of the test: wither height (WH), hip height (HH), chest depth (CD), body length (BL), rump length (RL), chest width (CW), hip width (HW), thurl width (TW), pin bone width (PBW), and chest girth (CG).

1.2.3 Feed management and traits

The feed consumption and feed efficiency traits studied were concentrate intake (CONI), roughage intake (ROUI), digestible crude protein intake (DCPI), total digestible nutrient intake (TDNI), rate of roughage intake (RRI), digestible crude protein conversion ratio (DCR) and total digestible nutrient conversion ratio (TDNC). The RRI was calculated as ROUI divided by total feed intake multiplied by 100. Digestible crude protein conversion ratio was calculated as DCPI divided by weight gain. Total digestible nutrient conversion ratio was calculated as TDNI divided by weight gain.

1.2.4 Statistical methods

Analysis of variance (ANOVA) was done using JMP[®] 5.0.1 (SAS) program. Furthermore, single trait analysis was done to estimate variance components and heritability. The mixed model fitted included fixed effects of testing year, starting season, as well as the covariate age at the start of the test and the random additive genetic effect of the sire. The mixed model equation was as follows:

$$Y_{ijklm} = \mu + Y_i + S_j + a_k(A_{ijkl}-\bar{A}) + s_l + e_{ijklm}$$

Where: Y_{ijklm} = observation, μ = overall mean, Y_i = fixed effect of the i^{th} year, S_j = fixed effect of the j^{th} season, a_k = coefficient of linear regression of age at the start of the test, s_l = random effect of the l^{th} sire, e_{ijklm} = random residual effect of each observation. Standard errors (SE) of estimates of heritability were calculated by formulas of Falconer (1981) as implemented by Carnier *et al.* (2000). The formula was as follows:

$$SE_{h^2} = \sqrt[4]{\frac{2(1-t)^2 (1+(k-1)t)^2}{k(k-1)(s-1)}}$$

Where: t = heritability divided by four, k = number of offspring per sire, and s = number of sires.

1.3 Results and Discussion

1.3.1 Summary of phenotypes

Table 1.1 Data summary including description of body measurement traits, units of measurements, number of records, means, standard deviations (SD), minimum (Min) and maximum values (Max)

Trait	Description	Units	Records	Mean	SD	Min	Max
BWS	Body weight at the start of the test	kg	525	260.8	26.1	147.0	377.0
BWE	Body weight at the end of the test	kg	525	390.8	31.8	300.0	532.0
DG	Daily gain during the test	kg/day	525	1.2	0.1	0.7	1.6
BL	Body length	cm	525	139.9	4.2	128.6	154.8
CD	Chest depth	cm	525	60.4	1.6	55.0	67.0
CW	Chest width	cm	450	41.7	1.8	36.0	48.5
CG	Chest girth	cm	525	169.8	4.5	152.0	188.0
HH	Hip height	cm	525	126.4	3.1	114.6	135.2
HW	Hip width	cm	525	40.8	1.5	36.0	50.0
PBW	Pin bone width	cm	525	26.1	1.4	21.0	31.0
RL	Rump length	cm	525	47.7	1.5	42.0	53.0
TW	Thurl width	cm	525	43.1	1.7	38.0	49.0
WH	Wither height	cm	525	125.0	2.9	111.2	135.0

Mean, standard deviation (SD), minimum and maximum values for each variable are presented in Table 1.1. Mean values for BWS and at BWE were 260.8 and 390.8 kg, respectively. BL, CD, CW, HG, and HH had average values of 139.9 cm, 60.4 cm, 41.7 cm, 169.8 cm, and 126.4 cm. Furthermore, values for HW, PBW, RL, TW, and WH were 40.8 cm, 26.1 cm, 47.7 cm, 43.1 cm, and 125.0 cm, respectively. The results are almost similar to

those reported by Sri Rachma *et al.* (1999b) on performance test stock in Kagoshima prefecture. They reported average values of 138.9 cm, 62.1 cm, 43.3 cm, 172.8 cm, 124.6 cm, 41.5 cm, 29.8 cm, 48.7 cm, 43.1 cm and 123.4 cm for BL, CD, CW, HG, HH, HW, PBW, RL, TW, and WH, respectively. Though the study by Sri Rachma *et al.* (1999b) was done more than decade ago, the body dimension measurements in the two studies are similar. This is because breeding institutions of the Japanese Black breed have a body control system in their selection criteria for livestock management purposes.

Table 1.2 Data summary including description of feed consumption and feed efficiency traits, units of measurements, number of records, means, standard deviations (SD), minimum (Min) and maximum values (Max)

Trait	Description	Units	Records	Mean	SD	Min	Max
CONI	Concentrate intake	Kg	525	530.3	40.2	271.0	851.0
ROUI	Roughage intake	Kg	525	413.1	45.3	149.0	722.0
DCPI	Digestible crude protein intake	Kg	525	77.9	5.1	51.0	116.0
TDNI	Total digestible nutrient intake	Kg	525	579.4	38.8	438.0	824.0
RRI	Rate of roughage intake	%	525	43.9	2.9	20.0	69.0
DCR	Digestible crude protein conversion ratio	-	525	0.6	0.1	0.4	1.9
TDNC	Total digestible nutrient conversion ratio	-	525	4.5	0.6	0.3	11.1

A summary describing feed consumption and feed efficiency traits is given in Table 1.2. Concentrate intake (CONI) and ROUI had average values of 530.3 and 413.1 kg, respectively. Performance test livestock in Miyagi prefecture had average values of 618.7 and 323.2 kg, respectively (Oikawa *et al.* 2006). This highlights variation in feed consumption patterns in

different populations. DCPI, TDNI and RRI had mean values of 77.9 kg, 579.4 kg and 43.9 %, respectively. Hoque *et al.* (2009) reported values of 0.73 kg/day, 5.38 kg/day and 34.78 %, sequentially in performance test stock in Miyagi prefecture. DCR and TDNC mean values in this study were 0.6 and 4.5, in that order. Values reported by Hoque *et al.* (2009) were 0.62 and 4.58 for DCR and TDNC, respectively.

1.3.2 Analysis of variance of body measurements

Table 1.3 Analysis of variance (ANOVA) for body measurement traits

Trait	Effect				Year	Age
	Season					
	Spring	Summer	Autumn	Winter		
BWS (kg)	** 256.27 ± 3.06 ^a	271.68 ± 2.59 ^b	268.47 ± 3.14 ^b	251.86 ± 2.18 ^a	**	**
BWE (kg)	** 383.36 ± 3.77 ^a	399.97 ± 3.19 ^b	386.93 ± 3.87 ^a	387.17 ± 2.71 ^a	*	**
DG (kg/day)	** 1.14 ± 0.01 ^b	1.14 ± 0.01 ^b	1.08 ± 0.01 ^a	1.20 ± 0.01 ^c	**	**
BL (cm)	ns 139.72 ± 0.49	140.33 ± 0.41	139.49 ± 0.51	139.96 ± 0.35	**	**
CD (cm)	ns 60.24 ± 0.19	60.69 ± 0.16	60.29 ± 0.20	60.46 ± 0.13	**	**
CW (cm)	** 41.37 ± 0.23 ^a	42.31 ± 0.20 ^b	41.92 ± 0.24 ^b	41.91 ± 0.15 ^b	**	**
HG (cm)	** 167.79 ± 0.55 ^a	171.45 ± 0.45 ^c	170.61 ± 0.57 ^c	169.03 ± 0.38 ^b	**	**
HH (cm)	ns 126.17 ± 0.36	126.81 ± 0.30	126.14 ± 0.37	126.39 ± 0.26	ns	**
HW (cm)	* 40.57 ± 0.19 ^a	41.24 ± 0.17 ^b	40.74 ± 0.20 ^a	40.76 ± 0.14 ^a	*	**
PBW (cm)	** 25.71 ± 0.18 ^a	26.38 ± 0.16 ^c	26.20 ± 0.19 ^{bc}	25.98 ± 0.14 ^{ab}	*	**
RL (cm)	ns 47.50 ± 0.17	47.89 ± 0.14	47.60 ± 0.18	47.60 ± 0.12	**	**
TW (cm)	ns 42.85 ± 0.21	43.28 ± 0.18	42.83 ± 0.21	43.00 ± 0.16	*	**
WH (cm)	* 124.77 ± 0.35 ^a	125.22 ± 0.30 ^b	124.66 ± 0.36 ^a	125.22 ± 0.26 ^b	*	**

.BWS: body weight at the start of the test, BWE: body weight at the end of the test, DG: daily gain during the test, BL: body length, CD: chest depth, CW: chest width, CG: chest girth, HH: hip height, HW: hip width, PBW: pin bone width, RL: rump length, TW: thurl width, WH: wither height.

* Significant ($p < 0.05$), ** highly significant ($p < 0.001$), ns: not significant. ^{abc} Means bearing the same superscript don't differ significantly.

Table 1.3 shows analysis of variance results for growth traits in performance test stock. Test start season had highly significant effects ($p < 0.001$) on both BWS and BWE. Cattle that started performance testing in summer had higher initial and finishing weights compared to other seasons. Starting season also had highly significant effects ($p < 0.001$) on DG, CW, HG, and PBW. To add to that, season also had significant effects ($p < 0.05$) on HW and WH. However, the effect of season was not evident on BL, CD, HH, RL, and TW. The general trend seems to show that animals that start performance testing in spring and winter have lower dimension measurements. Testing year had highly significant ($p < 0.001$) effects on BWS, DG, BL, CD, CW, HG, and RL. Testing year also had a significant ($p < 0.05$) effect on BWE, HW, PBW, TW, and WH. The effect of testing year was not significant on HH only. Age of animal at the start of test was highly significant ($p < 0.001$) on all growth traits.

1.3.3 Analysis of variance of feed efficiency

Table 1.4 Analysis of variance (ANOVA) for feed consumption and feed efficiency traits

Trait	Effect					
	Season				Year	Age
	Spring	Summer	Autumn	Winter		
CONI (kg)	* 511.25 ± 4.90 ^a	524.49 ± 4.05 ^b	518.17 ± 5.10 ^{ab}	511.47 ± 3.37 ^a	**	**
ROUI (kg)	** 432.96 ± 5.78 ^b	437.86 ± 4.48 ^b	411.60 ± 5.94 ^a	411.39 ± 4.05 ^a	**	**
DCPI (kg)	** 78.76 ± 0.74 ^{bc}	80.28 ± 0.64 ^d	78.69 ± 0.75 ^{ab}	76.65 ± 0.55 ^a	**	**
TDNI (kg)	** 574.29 ± 4.78 ^{ab}	586.41 ± 4.00 ^c	570.80 ± 4.91 ^{ab}	566.34 ± 3.35 ^a	**	**
RRI (%)	* 45.89 ± 0.37 ^b	45.59 ± 0.31 ^b	44.75 ± 0.39 ^a	44.74 ± 0.25 ^a	**	ns
DCR	** 0.64 ± 0.01 ^b	0.64 ± 0.01 ^{bc}	0.66 ± 0.01 ^c	0.59 ± 0.01 ^a	**	**
TDNC	** 4.51 ± 0.07 ^b	4.65 ± 0.06 ^c	4.75 ± 0.07 ^c	4.29 ± 0.05 ^a	**	**

CONI: concentrate intake, ROUI: roughage intake, DCPI: digestible crude protein intake, TDNI: total digestible crude protein intake, RRI: rate of roughage intake, DCR: digestible crude protein conversion ratio, TDNC: total digestible nutrient conversion rate.

* Significant ($p < 0.05$), ** highly significant ($p < 0.001$), ns: not significant. ^{abc}Means bearing the same superscript don't differ significantly.

Analysis of variance results for feed consumption and feed efficiency traits are given in Table 1.4. The effect of starting season was significant ($p < 0.05$) on CONI and RRI. Concentrate intake seems to be low in animals that start performance test in winter and spring season. Starting season was highly significant ($p < 0.001$) on ROUI, DCPI, TDNI, DCR, and TDNC. A study by Mujibi *et al.* (2010) analyzed the effect of season of testing on feed intake and efficiency in growing beef cattle. Their research found out that feed intake correlation with air temperature, relative humidity, solar radiation, and wind speed was different in two seasons they investigated (fall-winter and winter-spring). Factors they highlighted may also be part of the reason of seasonal differences in feed intake and efficiency observed in the current study. Year was highly significant ($p < 0.001$) on all traits. Age at the start of the test was also highly significant ($p < 0.001$) on all traits except RRI.

1.3.4 Heritability of body measurements

Heritabilities and variance components of growth traits are outlined in Table 1.5. Heritability estimates of 0.22 and 0.26 for BWS and BWE are significantly lower than estimates of 0.50 and 0.63 reported by Oikawa *et al.* (2006). These authors analyzed performance test data on 409 bulls in Miyagi prefecture. However, estimates done by Oikawa *et al.* (2000) in Okayama prefecture were 0.31 and 0.36 for BWS and BWE, respectively. In a review, Oyama (2011) penned a range of 0.39-0.80 for weight at the end of performance.

The current study had a heritability estimate of 0.21 for DG. This value is similar to estimates of 0.23 by Oikawa *et al.* (2000, 2006). In a study by Hoque *et al.* (2006a) data from 740 performance test bulls on performance testing in Okayama prefecture was analyzed; the estimated heritability was 0.21.

Table 1.5 Estimates of variance components and heritability for body measurement traits

Trait	Parameter		
	σ_a^2	σ_p^2	h_a^2
BWS	160.421	720.951	0.22 ± 0.17
BWE	276.484	1081.083	0.26 ± 0.18
DG	0.003	0.015	0.21 ± 0.17
BL	3.585	18.774	0.20 ± 0.17
CD	0.808	2.610	0.31 ± 0.18
CW	0.006	0.059	0.10 ± 0.16
HG	4.810	21.274	0.23 ± 0.18
HH	2.340	9.956	0.24 ± 0.18
HW	1.264	2.685	0.47±0.19
PBW	1.797	2.473	0.73 ± 0.21
RL	0.383	2.297	0.17 ± 0.17
TW	1.695	3.167	0.52 ± 0.20
WH	2.954	9.318	0.31 ± 0.18

BWS: body weight at the start of the test, BWE: body weight at the end of the test, DG: daily gain during the test, BL: body length, CD: chest depth, CW: chest width, CG: chest girth, HH: hip height, HW: hip width, PBW: pin bone width, RL: rump length, TW: thurl width, WH: wither height.

σ_a^2 : additive genetic variance, σ_p^2 : phenotypic variance, h_a^2 : direct heritability ± standard error.

Heritability estimates of 0.20 and 0.31 were obtained for BL and CD, respectively. Munim *et al.* (2012) reported estimates of 0.29 and 0.26 for BL in four and eight mo old calves, respectively in Okayama prefecture. The same study also had estimates of 0.37 and 0.18 for CD in calves at four and eight mo of age, in the same order.

Chest width (CW), HG, and HH had estimates of 0.10, 0.23, and 0.24, respectively. Higher estimates were obtained in four mo old calves by Munim *et al.* (2012). They reported values of 0.19, 0.47, and 0.55 for CW, HG, and HH. The same authors had estimates of 0.35

and 0.47 for HG and HH, respectively; in the same population of calves at eight mo of age. On the contrary, their heritability estimate of CW at 8 mo of age was significantly low (0.06); this highlights the volatility of heritability with age.

This study had heritability estimates of 0.47, 0.73, and 0.17 for HW, PBW, and RL, sequentially. In a recent study by Munim *et al.* (2012); serially measured HW had estimates of 0.28 and 0.36, respectively. The same study had heritability estimates of 0.35 and 0.41 for PBW at four and eight mo of age, respectively. Furthermore, the authors estimated heritability for RL to be 0.31 and 0.27 at four and eight mo of age in the same population of Japanese Black calves. Though these estimates differ significantly with the ones in the current study, the pattern of heritability for the three traits was similar in the two studies. PBW had the highest heritability while RL showed the lowest heritability.

Thurl width (TW) and WH had heritability estimates of 0.52 and 0.31, in the same order. Mukai (1994) reported a lower heritability estimate of 0.30 for TW; while Yang *et al.* (1985) reported a heritability estimate of 0.76 in Japanese Black cattle. A study conducted on Korean cattle at 12 mo of age reported estimates of 0.33 and 0.24 for WH at 12 and 27 mo of age, respectively (Han *et al.* (1996). Munim *et al.* (2012) had estimates of 0.42 and 0.23 for sequentially measured TW. The same authors reported estimates of 0.36 and 0.49 for sequentially measured WH in the same population.

1.3.5 Heritability of feed efficiency

Estimates of variance components and heritability for feed consumption and feed efficiency traits are given in Table 1.6. Concentrate intake (CONI) and ROUI had heritability estimates of 0.21 and 0.39, sequentially. Hoque *et al.* (2009) reported a heritability range of 0.27-0.41 for CONI using data on 514 performance tested bulls. They also reported a heritability range of 0.28-0.41 for feed intake; the estimate is close to estimates in the current

study. An estimate of 0.39 was reported by Arthur *et al.* (2001) in young Angus bulls. Furthermore, Hoque *et al.* (2006a) estimated heritability for daily feed intake to be 0.34 in performance stock in Okayama prefecture. On the other hand, a study by Oikawa *et al.* (2006) in Miyagi prefecture estimated heritability for CONI and ROUI to be 0.48 and 0.21, respectively.

Table 1.6 Estimates of variance components and heritability for feed consumption and feed efficiency traits

Trait	Parameter		
	σ_a^2	σ_p^2	h_a^2
CONI	359.656	1703.282	0.21 ± 0.17
ROUI	887.609	2272.968	0.39 ± 0.19
DCPI	8.998	27.848	0.32 ± 0.18
TDNI	405.791	1607.680	0.25 ± 0.18
RRI	2.796	9.322	0.30 ± 0.18
DCR	0.002	0.009	0.20 ± 0.17
TDNC	0.065	0.314	0.21 ± 0.17

CONI: concentrate intake, ROUI: roughage intake, DCPI: digestible crude protein intake, TDNI: total digestible crude protein intake, RRI: rate of roughage intake, DCR: digestible crude protein conversion ratio, TDNC: total digestible nutrient conversion rate.

σ_a^2 : additive genetic variance, σ_p^2 : phenotypic variance, h_a^2 : direct heritability ± standard error.

Digestible crude protein intake (DCPI), TDNI, and RRI had heritability estimates of 0.32, 0.25, and 0.30, sequentially. A comprehensive review by Oyama (2011) had a very wide range for heritability estimates of roughage intake during performance test. The study reported a range of 0.26-0.74 while unweighted and weighted mean heritabilities were estimated to be 0.49 and 0.44, respectively. Hoque *et al.* (2009) reported heritability values ranging from 0.32 to 0.38 for DCPI using three different models in the same population.

Furthermore, their study had a heritability range of 0.26-0.38 for TNDI while heritability for RRI had a range of 0.43-0.50. The heritability for RRI in the current study was outside the range given by Hoque *et al.* (2009); highlighting differences in the two populations.

This study estimated heritabilities for DCR and TDNC to be 0.20 and 0.21, respectively. A similar value was estimated in young Angus bulls by Arthur *et al.* (2001); they reported a heritability estimate of 0.28. Heritability estimate for DCR in this study falls within the upper limit of a range reported by Oyama (2011). The author reported a heritability range of 0.03-0.21 and unweighted and weighted mean values of 0.14 and 0.15, respectively. To add to that, the review reported a range of 0.11-0.46 and an unweighted and weighted mean heritability value of 0.22 for TDNC.

1.4 Conclusion

All growth traits except CW had heritability values in the moderate to high category. These results indicate that genetic improvement for growth can be achieved through selection. Phenotypic values for most body measurements are moderate or good indicators of an animal's genetic merit or breeding value. The analysis of variance indicated that environmental effects significantly affect growth of animals. Seasonal changes in both feed consumption and efficiency of feed utilization were evident. This highlights the importance of considering seasonal differences when evaluating cattle. There was considerable genetic variation in feed consumption and efficiency of feed utilization. This implies that feed efficiency can be improved through selection.

The selection based on body measurements and feed efficiency traits in young bulls will result in a population with not only increased growth rates but also impressive feed utilization. This will result in an efficient selection program and increased productivity in the field.

CHAPTER 2

Feasibility of using the ultrasound technique in the genetic improvement of young Japanese Black bulls

2.1 Introduction

Growth and carcass traits constitute some of the most important traits in performance test programs. Growth is relatively easy to assess through measurement of various body dimensions. However, assessment of carcass quality and quantity in live animals requires much sophisticated technology and can be time consuming; consequently, a number of methods have been devised to assess these traits in livestock. A number of ways have been innovated to assess live animal carcass traits, these include: ultrasound (US), mechanical and optical probes, electromagnetic scanning, electrical impedance, computer tomography (CT) and nuclear magnetic resonance (Wilson 1992). Computer Tomography (CT) has been highlighted to be more accurate in predicting the body composition, however, US is much cheaper, quicker, easier to operate and has a tremendous advantage of mobility (Junkuszew and Ringdorfer 2005). The ultrasound technique has been demonstrated to be satisfactory in predicting rib eye area (REA), rib thickness (RT), *subcutaneous* fat thickness (SFT) and intermuscular fat thickness (IMFT) (Stouffer *et al.* 1961; Davis *et al.* 1966; Forrest *et al.* 1989).

Beef cattle breeders and farmers select breeding stock according to their phenotypic values; their success in changing the characteristics of the population can be predicted only from the knowledge of the degree of correspondence between phenotypic values and

breeding values. This degree of correspondence is measured by heritability. Genetic parameters for ultrasonic traits in the Japanese Black population are very scarce; therefore, this chapter aimed to estimate genetic parameters for ultrasonic traits in young performance test bulls in Miyazaki prefecture.

2.2 Materials and Method

2.2.1 Experimental animals

Data used in this chapter were collected in Miyazaki on performance test bulls (525) at the Livestock Improvement Association of Miyazaki. Details on animal management are given in Chapter 1.

2.2.2 Scanning procedures and traits

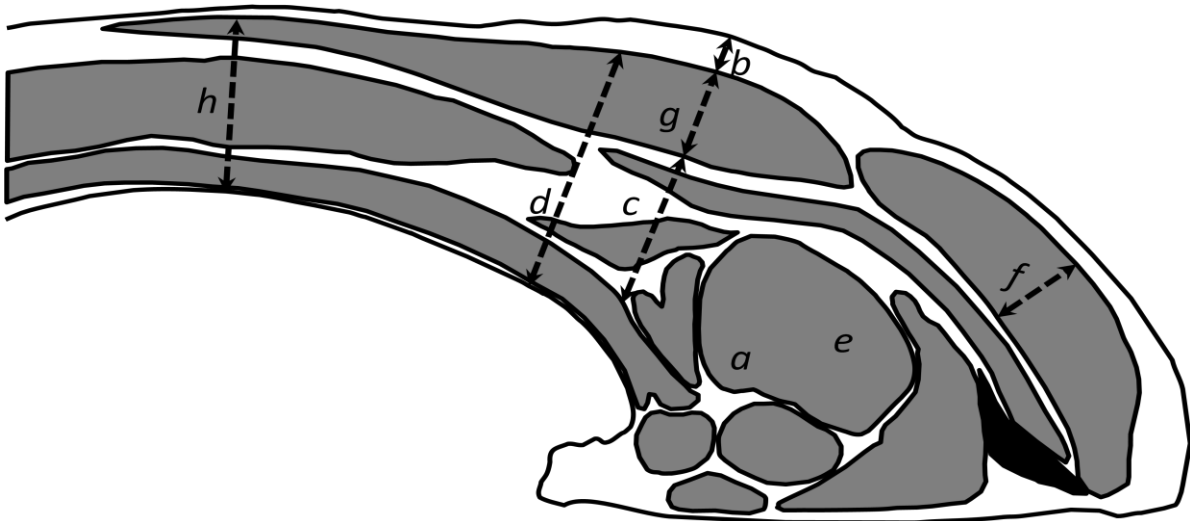


Figure 2 Schema showing positions of the ultrasonic trait measurements between the 6th and 7th thoracic vertebra area. *a*: longissimus muscle area (ULMA, cm²); *b*: subcutaneous fat thickness (USFT, mm); *c*: intermuscular fat thickness (UIMFT, mm); *d*: rib thickness (URT, mm); *e*: beef marbling score (UBMS); *f*: trapezius muscle thickness (UTMT, mm), *g*: latissimus muscle thickness (ULMT, mm) and *h*: is the actual position of rib thickness measurement (RT).

Ultrasonic scanning was done at about 11 mo of age between the 6th-7th and 12th-13th rib on the left side in performance test stock to obtain ultrasonic carcass traits of *longissimus* muscle area (ULMA) which is also referred to as rib eye area, *subcutaneous* fat thickness (USFT), intermuscular fat thickness (UIMFT), rib thickness (URT), beef marbling score (UBMS), *trapezius* muscle thickness (UTMT), and *latissimus* muscle thickness (ULMT); positions of measurements are shown in Figure 2. Only ULMA was measured on both scanning sites, all other traits were measured on the 6th-7th rib section only. Scanning equipment employed was SEM-500 (FHK Co. Ltd., Japan) and HS-2000 (FHK Co. Ltd., Japan) using a frequency of 2 MHz. All linear and areal measurements were done by ImageJ version 1.46r, UBMS was assessed subjectively through visual appraisal on a scale ranging from 0.0 (lowest) to 3.0 with intervals of 0.33, and 4.0, 5.0 (Nishimura *et al.* 1995). Ultrasonic estimates for RT were measured at 3.45 cm from the *musculus iliocostalis* (the point for measuring IMFT, SFT, and LMT). The UTMT was measured at the midpoint of the *trapezius* muscle.

2.2.3 Statistical methods

Analysis of variance (ANOVA) was done using JMP[®] 5.0.1 (SAS) program. Furthermore, single trait analysis was done to estimate variance components and heritability. The mixed model fitted included fixed effects of testing year, starting season, as well as the covariate age at the start of the test and the additive random genetic effect of the sire. The mixed model equation was as follows:

$$Y_{ijklm} = \mu + Y_i + S_j + a_k(A_{ijkl} - \bar{A}) + s_l + e_{ijklm}$$

Where: Y_{ijklm} = observation, μ = overall mean, Y_i = fixed effect of the i^{th} year, S_j = fixed effect of the j^{th} season, a_k = coefficient of linear regression of age at the start of the test, s_l = random effect of the l^{th} sire, e_{ijklm} = random residual effect of each observation.

Standard errors (SE) of estimates of heritability were calculated by formulas of Falconer (1981) as implemented by Carnier *et al.* (2000). The formula was as follows:

$$SE_{h^2} = \sqrt[4]{\frac{2(1-t)^2 (1+(k-1)t)^2}{k(k-1)(s-1)}}$$

Where: t = heritability divided by four, k = number of offspring per sire, and s = number of sires.

2.3 Results and Discussion

2.3.1 Summary of phenotypes

Table 2.1 Data summary including description of ultrasonic traits, units of measurements, number of records, means, standard deviations (SD), minimum (Min) and maximum values (Max)

Trait	Description	Units	Records	Mean	SD	Min	Max
ULMA7	<i>Longissimus</i> muscle area at the 6 th -7 th rib cross section	cm ²	525	31.90	2.10	22.30	37.9
ULMA13	<i>Longissimus</i> muscle area at the 12 th -13 th rib cross section	cm ²	525	53.60	4.30	37.70	73.00
UTMT	<i>Trapezius</i> muscle thickness	mm	525	12.90	1.10	10.20	15.30
ULMT	<i>Latissimus</i> muscle thickness	mm	525	12.90	1.20	10.10	19.30
URT	Rib thickness	mm	525	40.20	3.40	25.40	64.90
USFT	<i>Subcutaneous</i> fat thickness	mm	525	6.10	1.70	1.90	15.30
UIMFT	Intermuscular fat thickness	mm	525	14.40	3.60	4.80	28.10
UBMS	Beef marbling score	-	525	0.74	0.24	0.33	1.67

Mean, standard deviation (SD), minimum and maximum values for each variable are presented in Table 2.1. The mean for ULMA at the 12th rib position in the current study was smaller than one reported by Reverter *et al.* (2000); they reported values of 72.0 cm² and 68.7 cm² in Australian Angus and Hereford yearling bulls, respectively. A study involving multiple breeds by Perking *et al.* (1992) showed mean values of 76.3 cm², 67.1 cm², and 64.0 cm² in Brown Swiss steers, Zebu-cross Mexican steers, and Corriente Mexican steers, respectively. However, Sri Rachma *et al.* (1999b) reported similar results; 32.6 cm² for LMA7 and 55.9 cm² for LMA13 in Japanese Black bulls in Kagoshima prefecture. Their study also reported a slightly lower UBMS of 0.69 than the one reported in this study. The USFT reported in this study was similar to reports on most foreign breeds (De Rose *et al.* 1988; Lamb *et al.* 1990; Shepard *et al.* 1996).

2.3.2 Analysis of variance

The least squares means and standard errors of ultrasonic estimates of carcass traits are presented in Table 2.2. Starting season did not significantly affect ULMA, UTMT, ULMT, URT, and UBMS; however, it significantly ($p < 0.05$) affected both USFT and UIMFT. The cattle that were performance tested beginning spring season exhibited significantly lower levels of *subcutaneous* and intermuscular fat thickness; this could be attributed to the hot season during which they were tested. Cattle that start the test in spring are performance tested through most of the hot summer season hence; animals have reduced feed intake and fat deposition consequently. Except fat thickness seasonal effects did not affect other traits, this is in agreement with reports by Chistison *et al.* (1990) and Bergen *et al.* (1997). The testing year had a highly significant effect ($p < 0.001$) on all ultrasonic carcass traits. Age of cattle at the start of the test did not significantly affect ULMA, URT, and UIMFT, however, it significantly ($p < 0.05$) affected UTMT, ULMT, USFT and UBMS.

Table 2.2 Analysis of variance (ANOVA) for ultrasonic traits

Trait	Effect						
		Season				Year	Age
		Spring	Summer	Autumn	Winter		
ULMA7 (cm ²)	ns	31.62 ± 0.25	31.63 ± 0.21	32.17 ± 0.25	32.04 ± 0.19	**	ns
ULMA13(cm ²)	ns	53.16 ± 0.52	53.27 ± 0.44	53.71 ± 0.53	53.25 ± 0.39	**	ns
UTMT (mm)	ns	12.70 ± 0.13	12.94 ± 0.11	12.92 ± 0.13	12.88 ± 0.09	**	*
ULMT (mm)	ns	12.62 ± 0.14	12.91 ± 0.11	12.99 ± 0.14	12.85 ± 0.10	**	*
URT (mm)	ns	39.09 ± 0.47	39.76 ± 0.39	39.60 ± 0.50	40.35 ± 0.32	**	ns
USFT (mm)	*	5.30 ± 0.21 ^a	6.03 ± 0.18 ^b	6.21 ± 0.21 ^b	6.05 ± 0.16 ^b	**	*
UIMFT (mm)	*	13.60 ± 0.43 ^a	14.88 ± 0.35 ^b	14.11 ± 0.41 ^b	14.82 ± 0.31 ^b	**	ns
UBMS	ns	0.70 ± 0.30	0.75 ± 0.02	0.78 ± 0.03	0.73 ± 0.02	**	*

ULMA7: *longissimus* muscle area at the 6th-7th rib cross section, ULMA13: *longissimus* muscle area at the 12th-13th rib cross section, UTMT: *trapezius* muscle thickness, ULMT: *latissimus* muscle thickness, URT: rib thickness, USFT: *subcutaneous* fat thickness, UIMFT: intermuscular fat thickness, UBMS: beef marbling score.

* Significant ($p < 0.05$), ** highly significant ($p < 0.001$), ns: not significant. ^{ab}Means bearing the same superscript don't differ significantly.

2.3.3 Heritability of ultrasonic traits

Heritability informs the breeder how much confidence to place in the phenotypic performance of an animal when selecting parent stock. For highly heritable traits where heritability (h^2) exceeds 0.40, the animal's phenotype is a good indicator of genetic merit of breeding value. For lowly heritable traits, where heritability is below 0.15, an animal's performance is much less useful in identifying the individuals with the best genes for the trait Cassell (2009). Variance components and heritability estimates for ultrasonic traits are presented in Table 2.3.

Heritability for ULMA (0.39) was within the range of estimates by Fukuhara *et al.* (1989; 0.32) in Japanese Black steers, Hirooka *et al.* (1996) in Japanese Brown steers. A comprehensive review by Oyama (2011) indicated a heritability range of 0.28-0.61 in Japanese Black cattle. The study also reported a heritability range of 0.29-0.44 in Japanese

Brown cattle. In a study by Kim *et al.* (2006) four different models were employed to estimate genetic parameters for carcass traits, the study reported estimates ranging from 0.19 to 0.31 for carcass LMA. On the contrary, Kuchida *et al.* (1990), Arnold *et al.* (1991), Mukai *et al.* (1993), Mukai (1994) obtained higher estimates of heritability values than the present study, (0.65, 0.46, 0.54 and 45) for carcass traits of Japanese Black steers and from field carcass traits of Japanese Black cattle, respectively. These differences could be attributed to differences in breed, age, analysis model, management and gene frequencies in the populations reported on by these authors.

Table 2.3 Estimates of variance components and heritability for ultrasonic traits

Trait	Parameter		
	σ_a^2	σ_p^2	h_a^2
ULMA7	1.833	4.745	0.39 ± 0.19
ULMA13	8.001	20.526	0.39 ± 0.19
UTMT	0.211	1.345	0.16 ± 0.17
ULMT	0.184	1.548	0.15 ± 0.17
URT	1.858	11.720	0.16 ± 0.17
USFT	1.277	3.219	0.40 ± 0.19
UIMFT	3.259	13.812	0.24 ± 0.18
UBMS	0.006	0.058	0.10 ± 0.16

ULMA7: *longissimus* muscle area at the 6th-7th rib cross section, ULMA13: *longissimus* muscle area at the 12th-13th rib cross section, UTMT: *trapezius* muscle thickness, ULMT: *latissimus* muscle thickness, URT: rib thickness, USFT: subcutaneous fat thickness, UIMFT: intermuscular fat thickness, UBMS: beef marbling score.

σ_a^2 : additive genetic variance, σ_p^2 : phenotypic variance, h_a^2 : direct heritability ± standard error.

The heritability estimate of ULMA13 (0.39) obtained in this study was higher than some previous estimates. Turner *et al.* (1990) estimated the heritability values of LMA13 to be 0.21

while Arnold *et al.* (1991) reported an estimate 0.28. Robinson *et al.* (1993) and Shepard *et al.* (1996) estimated heritability of LMA13 to be 0.24 and 0.11 in Angus cattle, respectively. In yearling Brangus cattle, Moser *et al.* (1998) reported an estimate of 0.29. Estimates similar to this study were reported by Wilson *et al.* (1993) in Angus cattle (0.40). Furthermore, an estimate of 0.40 was reported by Johnson *et al.* (1993).

Heritabilities estimates of UTMT and ULMT in this study are in the lower limit of the intermediate heritability range. To our knowledge they are no reports on the heritability of ultrasonic TMT and LMT; however image analysis estimates of *trapezius* and *latissimus* muscle area heritability are considerably higher than the current study. Osawa *et al.* (2004) found heritability values of 0.55 and 0.67 for *trapezius* and *latissimus* muscle area in Japanese Black cattle. In a similar study involving Japanese Black steers Osawa *et al.* (2007) reported a heritability of 0.47 for *trapezius* muscle area. There is need to research on the phenotypic and genetic relationship between ultrasonic measures of TMT, LMT and their corresponding areal measurements in carcasses.

The heritability estimate for URT (0.16) was slightly lower than most estimates in literature. Fukuhara *et al.* (1989) reported an estimated of 0.23 while Mukai *et al.* (1993) and Hirooka *et al.* (1996) had estimates of 0.29 and 0.26, respectively. The ultrasonic rib thickness considered in this study is measured on a different position from that in slaughter houses; this might therefore, lead to different estimates.

The estimated heritability for USFT (0.40) was in the same range with estimates by Koch *et al.* (1982). Their heritability estimate was from data pooled across 16 different sire breeds. DeRose *et al.* (1988) reported a heritability estimate of 0.49 in Angus cattle. On the contrary, lower estimates were reported by a number of authors, Lamb *et al.* (1990) reported an estimate of 0.24 while Arnold *et al.* (1991) reported a similar estimate of 0.26. Some

estimates are higher than the one estimated in this study. Kuchida *et al.* (1990) and Shepard *et al.* (1996) reported estimates of 0.62 and 0.56, respectively.

In the current study a considerably low heritability, 0.1 was obtained for UBMS. The current estimate falls out of range of values stated in a review by Oyama (2011). He indicated a range of 0.32-0.63 and unweighted and weighted heritability means of 0.49 and 0.61, respectively in Japanese Black cattle. In the current study UBMS exhibited very little variation; consequently genetic variation and heritability. It has been long thought that marbling is a late developing tissue. This philosophy is based on the body's prioritization of fuel use. Calories are first used to meet bone growth, then muscle growth followed by development of fat. The results of this study are in agreement with this theory; considering the tender age (11 mo) of cattle sampled in this study. Furthermore, the cattle sampled in this study were also been reared as breeding stock on restricted concentrate feed intake.

2.4 Conclusion

Heritability estimates for rib eye area, muscle and fat thickness, and rib thickness ranged from 0.15-0.40. For highly heritable traits where heritability exceeds 0.40, the animal's phenotype is a good indicator of genetic merit or breeding value. Traits that are moderately heritable where heritability ranges from 0.15 to 0.40, the animals phenotypic value is a moderate indicator of genetic merit or breeding value. For lowly heritable traits, where heritability is below 0.15, an animal's performance is much less useful in identifying the individuals with best genes for the traits. This implies that most ultrasonic traits can be improved genetically through selection. However, the heritability for marbling score was low indicating low genetic variation at this stage nevertheless; taking ultrasonic measurements at an older age might reveal more genetic variation. It would therefore be possible to select the

bulls with the higher values despite the low heritability at this stage if their marbling score becomes considerably higher than their counterparts with age.

CHAPTER 3

Prospects of early slaughter based on serially measured ultrasonic traits in Japanese Black steers

3.1 Introduction

The first two chapters of this study focused on the feasibility of early selection of breeding stock in Japanese Black cattle. This chapter however, focuses on improvements that can be done to the current fattening program with emphasis on the prospect of reducing the slaughter age. Among the factors that necessitate the shortening of the fattening period are, changing consumer demands and globalization including the recent Trans-Pacific Partnership (TPP) trade pact. According to data from the Ministry of Agriculture, Forestry and Fisheries (MAFF 2014), the price gap between carcasses of different quality scores is shrinking. This decline in price differences can be attributed to changing consumer demands as they are increasingly favoring carcasses with lower marbling among other factors. Given that one of the reasons for prolonged fattening periods is to develop marbling, the growing demand for less marbled beef warrant for a reduced fattening period. To add to that, the increased competition from foreign meat and meat products which are slaughtered earlier in most cases or fattened for shorter periods calls for the revision of the current fattening regime to increase competitiveness.

Serial measurements of carcass related traits were taken to determine the impact of shortened fattening period on carcass yield estimate percentage (YE) which is one of the factors that determine carcass price together with marbling which is one of the traits used to

determine carcass quality score. Serial measurements are also referred to as repeated or longitudinal measures. In this chapter, the term serial measures is mostly used and refers to multiple measures of the same traits taken on the same animal over time. Detailed statistical approaches of analyzing serial measures were reported in previous studies (Meyer and Hill 1997; Littell *et al.* 1998). Serial measures facilitate the analysis of growth curves and regression analysis of traits at different stages. In this chapter, the growth curves of various traits associated with carcass YE and partly with quality score were analyzed. The impact of reduced slaughter age on YE was estimated through linear regression.

3.2 Materials and Methods

3.2.1 Experimental animals

Data were collected from 300 Japanese Black steers that were under progeny testing at the Livestock Improvement Association of Miyazaki from June 2005 to July 2013. The steers were progeny of 35 sires from 4 lines of descent. The steers were given *ad libitum* access to roughage, concentrate and water. Feed was formulated to meet the recommended diet nutrient content for growing and finishing beef steers by the Japan Livestock Industry Association (JLIA 2000). The nutrient content of the initial diet was 13.3% crude protein, 69% total digestible nutrients, 3.04 Mcal/kg digestible energy and 2.49 Mcal/kg metabolizable energy; the nutrient content was adjusted with age. Steer calves were housed in groups of 8 animals at 9 mo of age and group fed. The cattle pens had a feeding area of 50 m² with an adjacent exercise paddock of 40 m².

3.2.2 Scanning procedures and traits

Ultrasonic scanning was done at 14, 16, 20, and 26 mo of age at the left side, between the 6th and 7th rib cross section area: the same position at which carcasses are graded by the Japan

Meat Grading Association (JMGA 1988). The traits measured were ultrasonic *longissimus* muscle area (ULMA), ultrasonic *subcutaneous* fat thickness (USFT), ultrasonic intermuscular fat thickness (UIMFT), ultrasonic rib thickness (URT) and ultrasonic beef marbling score (UBMS); positions of measurements are shown in Figure 1 (Chapter 2). The URT was defined as the distance between the *latissimus* muscle and pleural membrane measured 3.4 cm (laterally) from the edge of the *iliocostalis* muscle (Sri Rachma *et al.* 1999a). The ultrasonic beef marbling score was assessed subjectively through visual appraisal on a 12 category scale ranging from 0.0 (lowest) to 3.0 with intervals of 0.33, and 4.0, 5.0 (Nishimura *et al.* 1995). The URT was measured at a different position from carcass RT measurement to facilitate its evaluation together with USFT and UIMFT from the same ultrasound image reducing the number of images needed for evaluation. Carcass RT is measured further below at the mid-point of the 6th and 7th rib, nevertheless URT and carcass RT are highly correlated (Tokunaga *et al.* 2013). The scanning equipment utilized was the HS-2000 (FHK Co. Ltd. Japan) using a frequency of 2 MHz. All linear and areal measurements were conducted using image analysis software (ImageJ version 1.46r). Body measurements were taken at 9, 14, 20 and 28 mo of age. The serial measurements were: body weight (BW), withers height (WH), chest girth (CG) and abdominal girth (AG).

After slaughter (at 28 mo of age), each carcass was graded between the 6th and 7th rib cross section area by official graders in accordance with the Japanese grading standards (JMGA 1988). The carcass traits evaluated were: carcass weight (CW), *longissimus* muscle area (LMA), *subcutaneous* fat thickness (SFT), rib thickness (RT), carcass yield estimate (YE), quality score (QS), beef marbling score (BMS), beef color standard (BCS, ranging from 1 (inferior) to 5 (very good)), firmness and texture (FNT, ranging from 1 (inferior) to 5 (very good)), and fat luster and color standard (FCS, 1 (inferior) to 5 (excellent)). YE is calculated using the following equation:

$YE \text{ percentage} = 67.37 + (0.130 \times LMA, \text{ cm}^2) + (0.667 \times RT, \text{ cm}) - (0.025 \times \text{Cold left side weight, kg}) - (0.896 \times SFT, \text{ cm}) + 2.049$. QS is the overall meat quality computed using BMS, BCS, FNT and FCS.

3.2.3 Statistical methods

Analysis of sequentially measured traits was done through multivariate analysis (MANOVA) of repeated measures using JMP[®] 10 (SAS Institute Cary, NC, USA). The statistical model included fixed starting test year, starting test season; a linear covariate of the age of the animal at the beginning of the test and a random effect of sire line. Carcass measures of IMFT and RT were excluded from MANOVA because IMFT is not measured in carcasses and RT is measured on a different position to URT. Subsequently, growth curves were estimated using an application for statistical analysis (Stat Proc Version 2). The growth curves were estimated using four equations but ultimately one was chosen based on goodness of fit. The equations used were:

Logistic: $y = A/(1+\lambda \exp(\beta t))$;

Brody: $y = A/(1-\lambda \exp(\beta t))$;

Bertalanffy: $y = A/(1-\lambda \exp(\beta t))^3$ and

Gompertz: $y = A \exp(-\lambda \exp(\beta t))$.

Ultrasound and body measurements were used to estimate carcass YE percentage through stepwise multiple regression using the Application for Statistical Analysis (Stat Proc Version 2.0, Kyoto, Japan).

3.3 Results and Discussion

3.3.1 Summary of phenotypes

Table 3.1 shows the data summary of serially measured traits. All traits included ultrasonic measurements from 14 mo to 26 mo of age; to add to that, carcass measurements for LMA, SFT and BMS at 28 mo of age were also included in the multivariate analysis. Some stages have fewer records because measurements could not be taken due to bio security concerns.

Table 3.1 Data summary including description of traits, units of measurements, number of records, means, standard deviations (SD), minimum (Min) and maximum values (Max) (*part 1*)

Age	Trait	Description	Units	Records	Mean	SD	Min	Max
14 mo	ULMA	Ultrasonic <i>longissimus</i> muscle area	cm ²	282	33.83	3.75	25.90	44.60
	USFT	Ultrasonic <i>subcutaneous</i> fat thickness	mm	282	8.13	2.32	2.90	16.70
	UIMFT	Ultrasonic intermuscular fat thickness	mm	282	16.46	5.08	2.90	34.90
	URT	Ultrasonic rib thickness	mm	282	40.16	5.98	24.10	62.90
	UBMS	Ultrasonic beef marbling score		282	0.26	0.14	0.00	0.67
16 mo	ULMA	Ultrasonic <i>longissimus</i> muscle area	cm ²	282	41.36	4.46	30.00	55.30
	USFT	Ultrasonic <i>subcutaneous</i> fat thickness	mm	282	10.32	2.97	4.40	23.80
	UIMFT	Ultrasonic intermuscular fat thickness	mm	282	23.79	7.37	9.70	48.80
	URT	Ultrasonic rib thickness	mm	282	50.97	9.14	30.30	82.70
	UBMS	Ultrasonic beef marbling score		282	0.47	0.20	0.00	1.33

Table 3.1 Data summary including description of traits, units of measurements, number of records, means, standard deviations (SD), minimum (Min) and maximum values (Max) (*part 2*)

Age	Trait	Description	Units	Records	Mean	SD	Min	Max
20 mo	ULMA	Ultrasonic <i>longissimus</i> muscle area	cm ²	283	51.34	5.67	39.7	69.20
	USFT	Ultrasonic <i>subcutaneous</i> fat thickness	mm	283	13.34	3.43	5.90	27.20
	UIMFT	Ultrasonic intermuscular fat thickness	mm	283	38.41	10.81	13.70	76.00
	URT	Ultrasonic rib thickness	mm	283	69.55	12.03	41.80	100.90
	UBMS	Ultrasonic beef marbling score		283	0.91	0.28	0.33	2.00
26 mo	ULMA	Ultrasonic <i>longissimus</i> muscle area	cm ²	294	61.43	7.78	42.00	84.80
	USFT	Ultrasonic <i>subcutaneous</i> fat thickness	mm	294	17.34	4.70	8.30	43.80
	UIMFT	Ultrasonic intermuscular fat thickness	mm	294	59.79	13.66	30.20	91.40
	URT	Ultrasonic rib thickness	mm	294	89.57	11.50	55.10	125.40
	UBMS	Ultrasonic beef marbling score	cm	294	1.57	0.57	0.67	4.00
28 mo	LMA	<i>Longissimus</i> muscle area	cm ²	300	64.91	9.87	43.00	90.00
	SFT	<i>Subcutaneous</i> fat thickness	mm	300	26.03	6.47	13.00	5.00
	BMS	Beef marbling score		300	1.77	0.77	0.67	5.00

The LMA showed highest growth rate (about 3.8 cm²/mo) from 14 mo to 16 mo of age but it declined to about 1.7 cm²/mo from 26 to 28 mo of age. *Subcutaneous* fat thickness increased dramatically (about 4mm/mo) from 26 to 28 mo compared to about 1 mm/mo in previous periods. Intermuscular fat thickness had a steady rate of increase, about 3.7 mm/mo

at all stages measured. Similarly, BMS showed a steady rate of increase during the entire period.

3.3.2 Correlation of serially measured traits

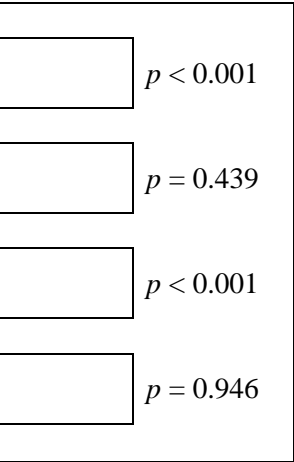
Table 3.2 Phenotypic correlations of serially measured *longissimus* muscle area

Trait [†]	ULMA (14 mo)	ULMA (16 mo)	ULMA (20 mo)	ULMA (26 mo)	LMA (28 mo)
ULMA (14 mo)	1.00				
ULMA (16 mo)	0.72	1.00			
ULMA (20 mo)	0.47	0.71	1.00		
ULMA (26 mo)	0.41	0.57	0.84	1.00	
LMA (28 mo)	0.39	0.51	0.78	0.93	1.00

ULMA, ultrasonic *longissimus* muscle area; LMA, carcass *longissimus* muscle area.

[†]The age in months is indicated in parenthesis.

Table 3.3 Results of multivariate analysis of serially measures *longissimus* muscle area

Trait [†]	Mean [‡] (cm ²)	<i>P</i> Values
ULMA (14 mo)	33.87 ^c	
ULMA (16 mo)	41.42 ^b	
ULMA (20 mo)	51.77 ^b	
ULMA (26 mo)	61.85 ^a	
LMA (28 mo)	64.81 ^a	

ULMA: ultrasonic *longissimus* muscle area, LMA: carcass *longissimus* muscle area.

[‡]Least squares means, ^{ab} Values bearing the same superscripts don't differ significantly. [†]The age in months is indicated in parenthesis.

Table 3.2 shows phenotypic correlations of serially measured *longissimus* muscle area. A high correlation coefficient was observed between ultrasonic measures of LMA at 20 mo of age and carcass LMA. An even higher correlation coefficient was obtained between ultrasonic LMA measured at 26 mo of age and carcass LMA. As highlighted in the summary of phenotypes, there is reduced growth rate of LMA from 20 mo of age resulting in high correlations being observed in latter stages of growth.

The results of multivariate analysis of serially measured LMA are shown in Table 3.3. Overall, there was a significant difference in serial measurements ($p < 0.001$); however, no differences were observed between ULMA at 16 mo and the same at 20 mo. Furthermore, there was no significant difference between ULMA at 26 mo and carcass LMA.

Figure 3.1 shows the growth curve of LMA estimated by the Gompertz equation. The curve indicates that LMA has high growth rate from 5 mo to about 20 mo; thereafter, the decreased slope of the curve indicates slower growth rate of LMA. The reduced growth rate beyond 25 mo of age can result in diminishing returns from an economic point of view.

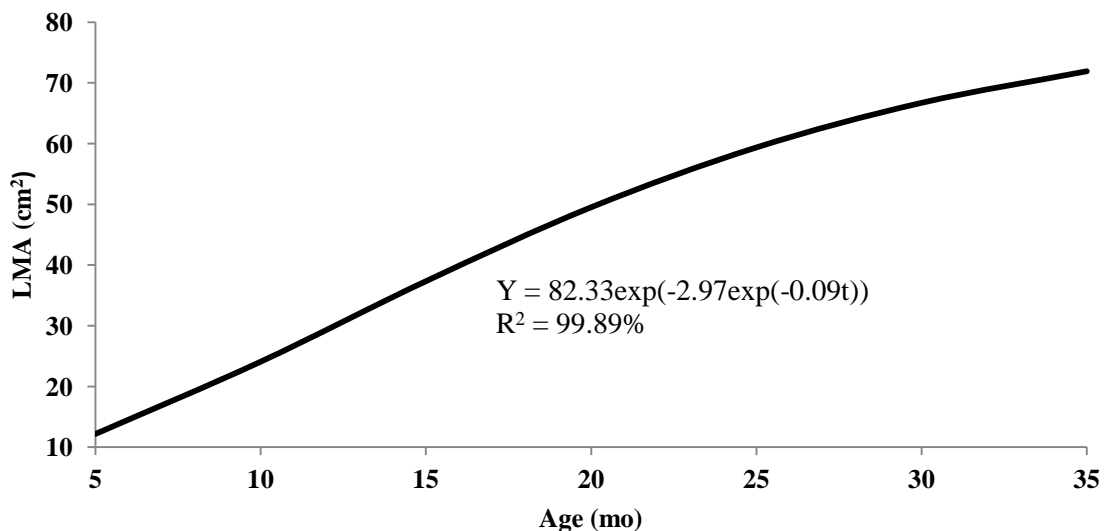


Figure 3.1 *Longissimus* muscle area (LMA) growth curve estimated by the Gompertz equation

Phenotypic correlations of serially measured SFT are shown in Table 3.4. A moderate correlation was observed between USFT measured at 20 mo and carcass SFT. On the other hand, a correlation of 0.75 was observed between USFT at 26 mo and carcass SFT. This was lower than the 0.93 observed in LMA during the same period. The possible explanation is that carcass SFT may be affected more by processes such as skinning and chilling resulting relatively lower correlations being observed.

Table 3.4 Phenotypic correlations of serially measured *subcutaneous* fat thickness

Trait [†]	USFT (14 mo)	USFT (16 mo)	USFT (20 mo)	USFT (26 mo)	SFT (28 mo)
USFT (14 mo)	1.00				
USFT (16 mo)	0.78	1.00			
USFT (20 mo)	0.57	0.71	1.00		
USFT (26 mo)	0.47	0.57	0.77	1.00	
SFT (28 mo)	0.48	0.53	0.67	0.75	1.00

USFT: ultrasonic *subcutaneous* fat thickness, SFT: carcass *subcutaneous* fat thickness.

[†]The age in months is indicated in parenthesis.

Table 3.5 shows the results of multivariate analysis of serial measured SFT. There was no significant difference among serial measures of SFT. Though the least squares means at 26 and 28 mo differ by almost 9 mm the difference was statistically insignificant.

Table 3.5 Results of multivariate analysis of serially measures *subcutaneous* fat thickness

Trait [†]	Mean [‡] (mm)	P Values
USFT (14 mo)	8.04	
USFT (16 mo)	10.23	
USFT (20 mo)	13.31	
USFT (26 mo)	17.20	
SFT (28 mo)	26.19	

USFT: ultrasonic *subcutaneous* fat thickness, SFT: carcass *subcutaneous* fat thickness.

[‡]Least squares mean. [†]The age in months is indicated in parenthesis.

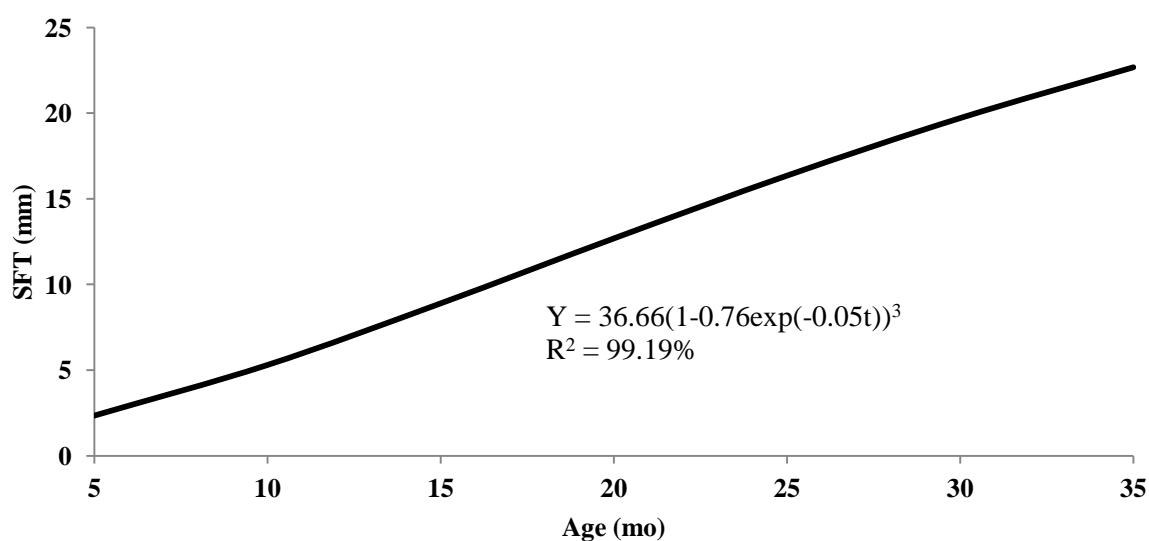


Figure 3.2 *Subcutaneous* fat thickness (SFT) growth curve estimated using the Bertalanffy equation

The SFT growth curve shown in Fig 3.2 was estimated using the Bertalanffy equation. *Subcutaneous* fat thickness still showed high growth rate even after 20 mo of age. Keeping livestock for long will increase their SFT which may adversely affect yield estimate since it is negatively related with carcass yield.

Table 3.6 Phenotypic correlations of serially measured intermuscular fat thickness

Trait [†]	UIMFT (14 mo)	UIMFT (16 mo)	UIMFT (20 mo)	UIMFT (26 mo)
UIMFT (14 mo)	1.00			
UIMFT (16 mo)	0.60	1.00		
UIMFT (20 mo)	0.45	0.61	1.00	
UIMFT (26 mo)	0.21	0.34	0.56	1.00

UIMFT: ultrasonic intermuscular fat thickness. [†]The age in months is indicated in parenthesis.

Phenotypic correlations of serially measured IMFT are indicated in Table 3.6. The correlation coefficient at 20 mo and 26 mo of age was moderate (0.56). The moderate correlation could be attributed to increased growth rate at stages beyond 20 mo. This can be confirmed by results of multivariate analysis in Table 3.7 where significant differences were observed between measurements at 20 and 26 mo of age. Furthermore, the growth curve in

Table 3.7 Results of multivariate analysis of serially measures intermuscular fat thickness

Trait [†]	Mean [‡] (mm)	<i>P</i> Values
UIMFT (14 mo)	15.93 ^b	
UIMFT (16 mo)	23.38 ^b	
UIMFT (20 mo)	38.02 ^b	
UIMFT (26 mo)	57.40 ^a	

UIMFT: ultrasonic intermuscular fat thickness.

[†]The age in months is indicated in parenthesis. [‡]Least squares mean.

Figure 3.3 Shows continued growth in IMFT well beyond 20 mo. Though IMFT is not used in calculating carcass YE percentage, consumers may dislike carcasses with thick IMFT.

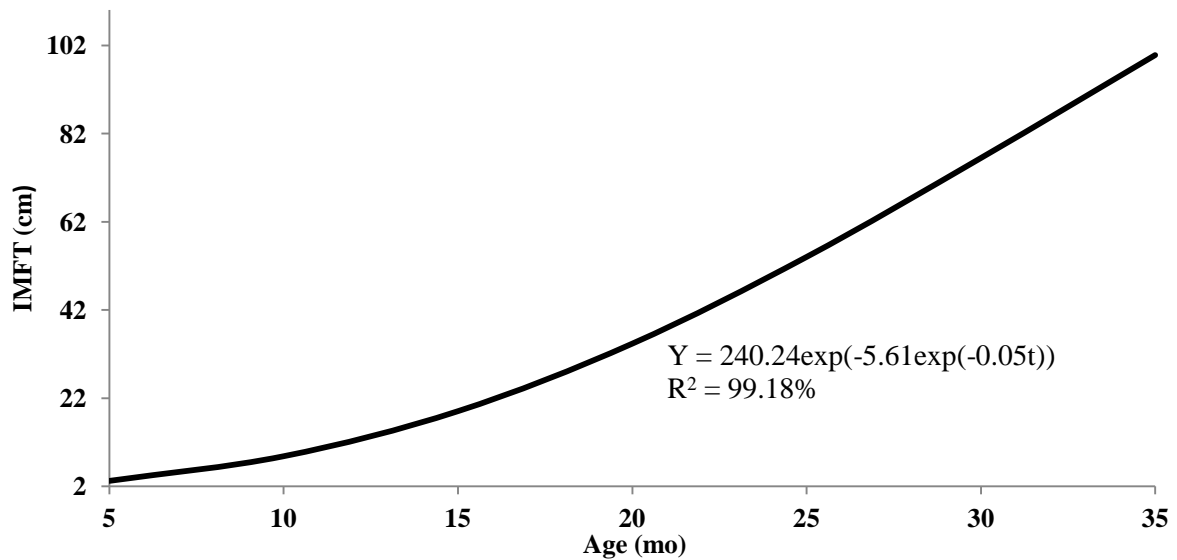


Figure 3.3 Intermuscular fat thickness (IMFT) growth curve using the Gompertz equation

Table 3.8 Phenotypic correlations of serially measured rib thickness

Trait [†]	URT (14 mo)	URT (16 mo)	URT (20 mo)	URT (26 mo)
URT (14 mo)	1.00			
URT (16 mo)	0.65	1.00		
URT (20 mo)	0.41	0.47	1.00	
URT (26 mo)	0.28	0.31	0.60	1.00

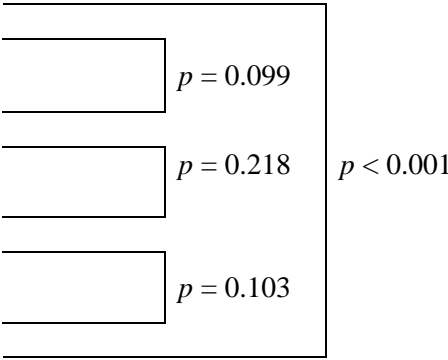
URT: ultrasonic rib thickness.

[†]The age in months is indicated in parenthesis.

Table 3.8 shows the phenotypic correlations of serially measured RT. The ultrasonic RT showed a medium correlation between 20 and 26 mo of age. Results of multivariate analysis of serially measured RT are shown in Table 3.9. The overall measurements were significantly different ($p < 0.001$). Among the significantly different measurements were those at 16 and

26 mo of age; however, measurements at 20 and 26 mo of age were not significant. The growth curve (Figure 3.4) of RT also shows a gradually decreasing growth rate after 20 mo of age.

Table 3.9 Results of multivariate analysis of serially measured rib thickness

Trait [†]	Mean [‡] (mm)	P Values
URT (14 mo)	39.87 ^c	
URT (16 mo)	50.66 ^{bc}	
URT (20 mo)	70.29 ^{ab}	
URT (26 mo)	89.27 ^a	

URT, ultrasonic rib thickness. [†]The age in months is indicated in parenthesis.

[‡]Least squares mean, ^{abc} Values bearing same superscripts don't differ significantly.

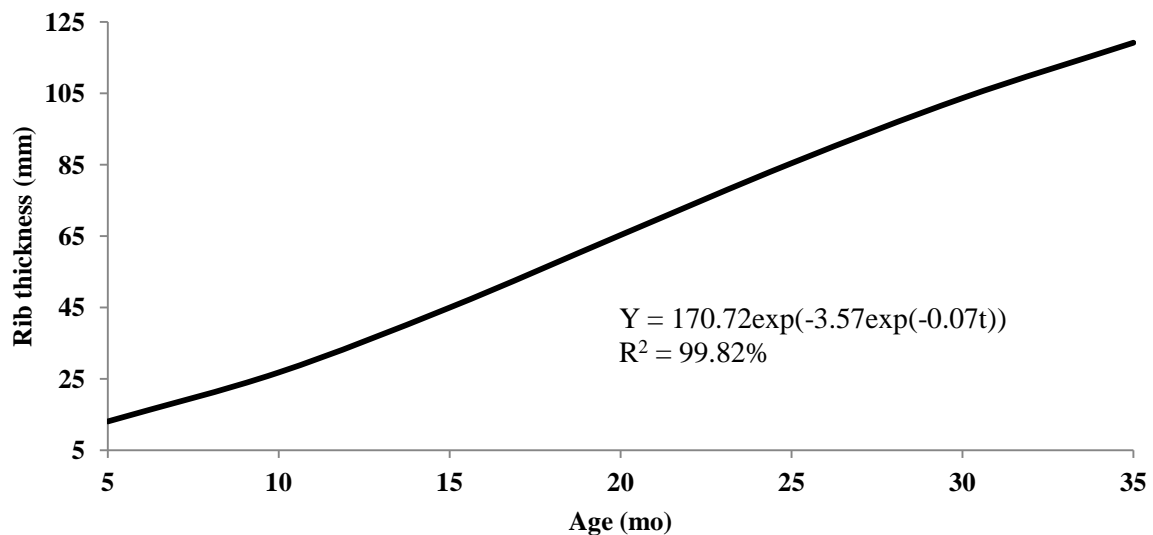


Figure 3.4 Intermuscular rib thickness (RT) growth curve estimated using the Gompertz equation

Phenotypic correlations of serially measured BMS are shown in Table 3.10. Though the correlation between BMS and measurements at 26 and 28 mo was high; a moderate

Table 3.10 Phenotypic correlations of serially measured beef marbling score

Trait [†]	UBMS (14 mo)	UBMS (16 mo)	UBMS (20 mo)	UBMS (26 mo)	BMS (28 mo)
UBMS (14 mo)	1.00				
UBMS (16 mo)	0.48	1.00			
UBMS (20 mo)	0.36	0.48	1.00		
UBMS (26 mo)	0.23	0.37	0.70	1.00	
BMS (28 mo)	0.17	0.32	0.54	0.85	1.00

UBMS: ultrasonic beef marbling score; BMS, carcass beef marbling score.

[†]The age in months is indicated in parenthesis.

Table 3.11 Results of multivariate analysis of serially measured marbling score

Trait [†]	Mean [‡]	<i>P</i> Values
UBMS (14 mo)	0.25 ^b	
UBMS (16 mo)	0.45 ^a	
UBMS (20 mo)	0.90 ^a	
UBMS (26 mo)	1.57 ^a	
BMS (28 mo)	1.77 ^a	

UBMS: ultrasonic beef marbling score; SFT: carcass beef marbling score. [†]The age in months is indicated in parenthesis. [‡]Least squares means, ^{ab} Values bearing same superscripts don't differ significantly.

correlation was obtained for BMS between 20 and 26 mo of age. Table 3.11 shows results of multivariate analysis of serially measured BMS. Though the overall measurements were significantly different ($p < 0.001$); there was no significant difference among measurements

from 16 mo onwards. The marbling growth curve is shown in Figure 3.5. The curve indicates that marbling starts to increase from about 14 mo of age.

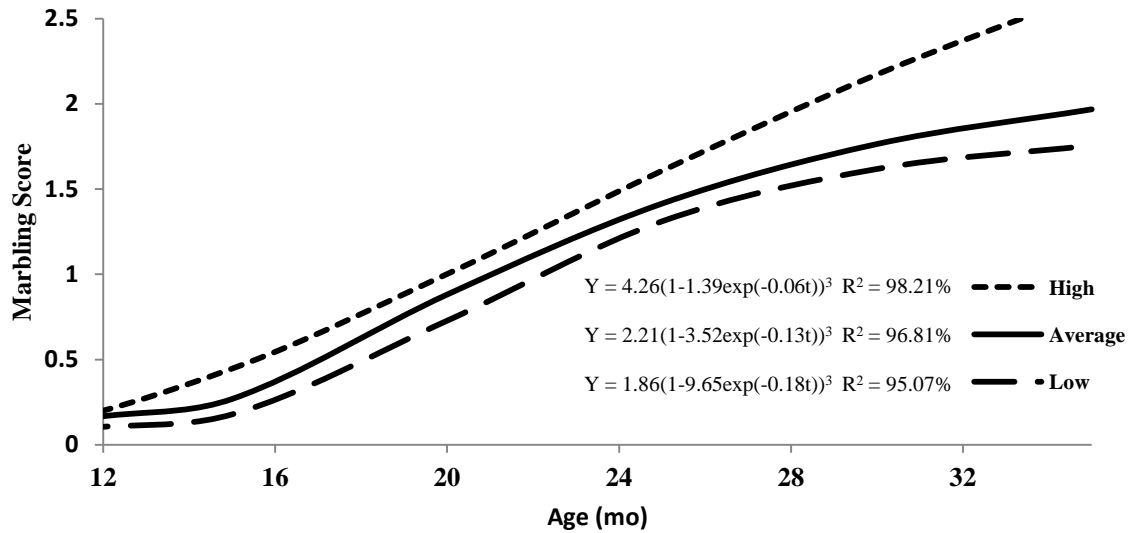


Figure 3.5 Beef marbling score (BMS) growth curve of animals with high, low, and average initial marbling estimated using Bertalanffy equation

Table 3.12 Estimated yield percentage (YE) and daily gain (DG) at different ages and corresponding values of traits used in multiple regression

Age (mo)	YE (%)	DG (kg/day)	LMA (cm ²)	SFT (mm)	MS	BW (kg)	RT (mm)
10	70.27	0.90	24.06	5.30	0.00	305.98	26.79
15	71.43	0.95	37.33	8.90	0.27	450.94	44.95
20	73.03	0.51	49.50	12.69	0.88	528.27	65.28
22	73.14	1.64	53.75	14.18	1.12	628.18	73.46
24	73.54	0.69	57.59	15.64	1.32	669.86	81.49
26	73.91	0.62	61.02	17.06	1.50	707.29	89.24
28	74.22	0.55	64.05	18.42	1.64	740.60	96.64
30	74.49	0.49	66.71	19.72	1.74	770.01	103.64

YE %: estimated carcass yield percentage, DG: daily gain, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness, MS: marbling score; BW: body weight; RT: rib thickness.

Table 3.12 shows the results of stepwise multiple linear regressions used to estimate YE percentage and the corresponding values of traits used. Though ultrasonic traits at different ages were used initially, the best estimates were obtained using measurements obtained at 26 mo and BW at 20 mo. The regression equation used to estimate YE percentage is shown below:

$$YE\% = 68.66 + (0.145 \times LMA, cm^2) - (0.084 \times SFT, mm) + (0.386 \times BMS) - (0.006 \times BW, kg) + (0.020 \times RT, mm).$$

The coefficient of determination (R-squared, R²) value was 0.79.

The growth curves are in line with the theory of pattern of growth reported by Hammond *et al.* (1971). Hammond *et al.* (1971) reported that development of animals starts with the development of the skeleton, followed by muscle and finally fat. Similarly, the LMA curve in this chapter showed fast growth in the early stages and reduced growth after 20 mo. On the other hand, growth rate of fat related traits seems to increase well beyond 20 mo of age. Marbling development was delayed until 14 mo of age, confirming the theory that it's a late developing tissue. In general, fat is the last developing tissue as it serves as an energy reserves and the increase in the proportion ultrasound predicted intramuscular fat was also observed in Angus cattle (Hassen *et al.* 2003). Early studies that employed dissection instead of ultrasound also showed that fat tissue development is delayed compared to muscle and bone in both cattle and pigs (Callow 1944; McMeekan 1940). As animals grow at high rates at young stages there is little residual energy however, due to reduced growth at older ages there is more residual energy that is stored as fat. Different fat tissues also develop at different rates; in this study, SFT seems to develop at faster rates than IMFT at early stages however, IMFT shows faster rates of development at latter stages of growth.

The estimated yield percentage (YE) exhibited an increasing trend from 10 mo to 30 mo of age. The increase in the YE with age can be attributed to the increase in LMA, BMS and RT values. The corresponding increase in SFT and BW (both negatively related with YE) did not

offset the increase in values of traits that have a positive relationship with YE. The JMGA (1988) divides carcasses into three yield grades: A (over 72 % yield estimate), B (69 to 72%), and C (less than 69 %). The YE is one of the determinants of carcass price the other factor is the quality score. Results from this study suggest that yield grade A (over 72 %) is attained at about 18 mo of age. Considering that the highest DG is observed around 20 mo of age and starts diminishing from 24 mo of age it might be more economic to slaughter cattle at about 24 mo. The DG observed after 24 mo might be attributed to more fat deposition than muscle. The advantage of keeping cattle longer is that they will develop more marbling however, they will be deposition of unwanted fat too (e.g. thick SFT and IMFT) and the cost of maintaining large animals in terms of feed is very high. Some of the reasons that warrant the reduction of slaughter age are increased globalization particularly the implementation of the Trans-Pacific Partnership (TPP), demands of foreign consumers and the changing demands of domestic markets. The TPP and globalization in general bring stiff competition to the local Japanese beef producers due to reduced import tariffs on meat; nevertheless, it also brings opportunities by making it easier to export. Considering the current Japanese national average slaughter age of 30 mo, cattle are raised for very long periods compared to the ones imported from U.S. which were slaughtered at less 21 mo during the 2005 to 2013 period according to the U.S. Department of Agriculture (USDA 2013). Beef coming from Australia is from cattle slaughtered at 21 or 26 mo of age on average after being fattened for 150 days (short fed) or up to 350 days (long fed), respectively according to the New South Wales Department of Primary Industries (NSW DPI 2007).

The issue of changing consumer demands is also a factor necessitates early slaughter at early stages. According to official statistics from the Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF 2014), the differences in carcass price per kg between carcass of different quality score (e.g. A5, A4, A3 and A2) has been shrinking gradually. For example:

the carcass prices for Wagyu steer carcasses ranked A5, A4, A3, and A2, in 1996 were 2,331 ¥/kg, 1,796 ¥/kg, 1,591 ¥/kg, and 1,181 ¥/kg, respectively. In 2014 however, the carcass prices were 2,256 ¥/kg, 1,987 ¥/kg, 1,791 ¥/kg, and 1,627 ¥/kg for the respective carcass ranks. The change in price differences can be attributed to consumers demanding carcass with modest quality score which can be attributed in part to marbling score. Since one of the reasons cattle are slaughtered late in Japan is to attain high marbling, producers can shorten the fattening period given that the demand for carcass with modest quality score is increasing. Though the current study managed to estimate YE at different ages, the change in quality score which is determined by 1) beef marbling, 2) color and brightness, 3) firmness and texture, and 4) fat color, luster and quality is beyond the scope of this study.

3.4 Conclusion

Multivariate analysis of serially measured traits revealed that there are no significant changes between LMA measures at 26 and 28 mo of age. The LMA also exhibited reduced growth rate after 25 mo of age, so did overall marbling. On the other hand, IMFT and RT exhibited high growth rates after 20 mo. Steers could attain carcass YE of A grade as early as 18 mo of age and DG started to diminish from 24 mo of age. Considering these results, changing consumer demands, and globalization, it may be prudent to reduce the slaughter age to about 25 mo.

CHAPTER 4

Genetic polymorphisms and their association with growth and carcass traits in Japanese Black steers

4.1 Introduction

Economically important traits in cattle are determined by both genetic makeup and environmental factors. Animal breeders exploit the genetic influence to improve these traits. Currently, most beef cattle breeders use phenotypic and pedigree information to calculate estimated breeding values (EBV) and subsequently select breeding stock. However, advances in technology are making it much easier to identify the genetic basis of phenotypic variation. During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers that affect traits of interest in livestock, including those for single gene traits and quantitative trait loci (QTL) or genomic regions that affect quantitative traits (Dekkers 2004). This has enabled opportunities to enhance genetic improvement programs in livestock by direct selection of genes or genomic regions that affect economic traits through marker assisted selection and gene introgression (Dekkers and Hospital 2002). Furthermore, the dramatic reduction in the cost of genetic sequencing has led to the discovery of numerous single nucleotide polymorphisms (SNP) markers throughout livestock genomes (Hayes *et al.* 2009).

Though the effects of the growth hormone (*GH*) polymorphisms on calf, heifer and cow body weight have been reported in Japanese Black cattle (Ardiyanti *et al.* 2009; Ishida *et al.* 2010); studies that have evaluated the association of the *GH* polymorphisms and

longitudinally measured growth traits are scant. To add to that, effects of genes on quantitative traits have been shown to vary with age (Meyer and Hill 1997; Hassen *et al.* 2003). In this regard, the current chapter evaluated the association of SNP with longitudinally measured growth traits and subsequently analyzed their affiliation with carcass traits. Ardiyanti *et al.* (2012) showed that *GH* polymorphisms affect lipogenic gene expression levels hence; this chapter evaluated the association of growth related SNP with fat linked carcass traits as well.

In this chapter, the association of SNP in growth related genes with growth and carcass traits in Japanese Black steers was appraised. A total of nine SNP in five genes were evaluated. To have a more holistic analysis, longitudinal measurements of growth traits were taken and their association with the genetic polymorphisms was analyzed. Furthermore, the affiliation of the polymorphisms with carcass traits was evaluated.

4.2 Materials and Methods

4.2.1 Experimental animals and traits

The data were collected from 280 Japanese Black steers that were under progeny testing at the Livestock Improvement Association of Miyazaki from 2005 to 2013. Details on animal management are given in Chapter 3.

Body measurements were taken at 9, 14, 20 and 28 mo of age. The serial measurements were: body weight (BW), withers height (WH), chest girth (CG) and abdominal girth (AG). The steers were slaughtered at the end of the test; each carcass was graded at the 6th to 7th rib cross-section area by official graders following Japanese Grading Standards (JMGA 1988). Carcass traits recorded were: carcass weight (CW), *longissimus* muscle area (LMA), rib thickness (RT), *subcutaneous* fat thickness (SFT), carcass yield estimate (YE), quality score

(QS), beef marbling score (BMS), beef color standard (BCS), fat color standard (FCS), firmness and texture (FNT).

4.2.2 Genotyping

All the SNPs evaluated in this chapter are registered in the Single Nucleotide Polymorphism Database (dbSNP) of the National Center of Biotechnology Information (NCBI 2010). The SNP (with their reference cluster ID numbers) were in the growth hormone (*GH*, rs41923484 and rs134687399), somatostatin (*SST*, rs17870997), growth hormone releasing hormone (*GHRH*, rs380969504), myostatin (*GDF8*, rs383271508 and rs137528458) and leptin (*LEP*, rs29004487, rs29004488 and rs29004508) genes. The mutations in the *GH*, *SST* and *LEP* genes result in amino acid changes. The locations of the genes are 19q22, 1q23-q25, 13q, 2q14-q15 and 4q32 for *GH*, *SST*, *GHRH*, *GDF8* and *LEP*, respectively. For *GH* three haplotypes were present, *A* (nucleotide C at rs41923484 and rs134687399), *B* (G at rs41923484 and C at rs134687399) and *C* (G at rs41923484 and T at rs134687399). These *GH* variants were previously reported by Chikuni *et al.* (1994).

Approximately 10 ml of whole blood was obtained from each steer and placed in heparinized vacuum tubes. Genomic DNA extraction was undertaken based on the phenol-chloroform extraction technique. After extraction, the DNA was amplified using either TaKaRa Ex Taq HS polymerase or MightyAmp polymerase (Takara Bio, Shiga, Japan). The PCR reaction mixture was composed of either 20 ng genome DNA, 10 × TaqBuffer and dNTP mixture each 4 nmol, or 20 ng genome DNA, 2 × MightyAmp Buffer Ver. 2, sense and antisense primer (each 0.01 pmol), and TaKaRa Ex TaqHS 0.25 U or MightyAmp 0.25 U. Oligonucleotide primer base sequences, amplicon sizes and corresponding restriction enzymes are indicated in Table 4.1. The Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) was used under conditions suitable for each primer set.

The PCR conditions for *GH* (rs41923484), *SST*, *GDF8* (rs137528458) and *LEP* were: denaturation at 94 °C for 2 min followed by 35 cycles of denaturation, annealing, and extension at 94 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min, respectively. Finally, extension was done at 72 °C for 7 min. The same conditions were applied for *GH* (rs134687399) and *GDF8* (rs383271508) except that the annealing temperature was 63 °C and 60°C, respectively. For *GHRH* the initial denaturation and final extension conditions were the same with other primer sets. However, there were three 5 cycle phases and a single 20 cycle phase. Except the annealing temperature, all conditions were the same as other primer sets. The annealing temperatures were: 60 °C, 58 °C, 56 °C and 54 °C for the respective four phases of cycles. Subsequently, PCR products were digested at 37 °C using restriction enzymes: *Alu I*, *Msp I*, *Hif I*, *Apek I*, *Cla I*, *Aci I* and *Nru I* (New England Biolabs, Ipswich, MA, USA). For the two *GH*, and the *SST* SNP, the reagents for DNA digestion were: 3.85µL super pure water (SPW), 1µL 10×NE buffer, 0.15 µL restriction enzyme and 5 µL PCR products. Regarding the *GHRH*, *GDF8* (rs383271508), *LEP* (rs29004488 and rs29004508) SNP, the reagents for DNA digestion were: 3.7 µL SPW, 1 µL 10×NE buffer, 0.3 µL restriction enzyme and 5 µL PCR products. For *GDF8* (rs137528458) SNP, the reagents for DNA digestion were: 3.25 µL SPW, 1 µL 10×NE buffer, 0.75 µL restriction enzyme and 5 µL PCR products. As for the *LEP* (rs29004487) SNP, the reagents for DNA digestion were: 3.3 µL SPW, 0.1 µL BSA, 1 µL 10×NE buffer, 0.6 µL restriction enzyme and 5 µL PCR products. Genotypes were determined through electrophoresis using MultiNA (Shimadzu, Kyoto, Japan) as outlined in the MultiNA operation manual.

4.2.3 Statistical methods

Association between the SNP genotypes and traits was analyzed using analysis of variance (ANOVA) in JMP[®] 5.0.1 (SAS Institute Cary, NC, USA). The statistical model included

fixed effects of SNP genotype, starting test year, starting test season; a linear covariate of the age of the animal and a random effect of sire line. Subsequently, Tukey-Kramer's honestly significant difference (HSD) test was conducted. Genotypes that constituted less than 1 % of the sample population were excluded from the analysis.

Table 4.1 Oligonucleotide primer base sequences, amplicon sizes and corresponding restriction enzymes for PCR-RFLP in Japanese Black steers under progeny testing

Primer set [†]	Base sequence [‡]	Size (bp)	Enzyme
A	GH-F 5'-GCTGCTCCTGAGGGCCCTTC-3'	276	<i>Alu I</i>
	GH-R 5'-AGGGGCAAACAACAGATGGCT-3'		
B	GH-F 5'-TCCGGAAGGACCTGCATAAGc-3'	224	<i>Msp I</i>
	GH-R 5'-ACCCCACCCCCCAGAATAGA-3'		
C	SST-F 5'-CTTCCTTCCACCCCATGcG-3'	135	<i>Msp I</i>
	SST-R 5'-CCAGCCTCATTTTCATCCTGCTC-3'		
D	GHRH-F 5'-TCATAGCTCTCACGGACCAGGC-3'	243	<i>Hif I</i>
	GHRH-R 5'-CCTCAAAGGGCCTCTCTTGCTT-3'		
E	GDF8-F 5'-GCATGTTTGTGGAGGGAAAA-3'	296	<i>Alu I</i>
	GDF8-R 5'-GCAGTCAGCAGAGTCGTTGCTC-3'		
F	GDF8-F 5'-CAAGTGGAAGGAAAACCCAAATGcTG-3'	232	<i>ApeK I</i>
	GDF8-R 5'-CAATGCTCTGCCAAATACCAAGTGCC-3'		
G; H	LEP-F 5'-CTGAAGACCTGGATGCGGGTGGTAACGGA-3'	281	<i>Cla I; Aci I</i>
	LEP-R 5'-TCGTCTCCAGTCCCTCCCTACCGTGTGT-3'		
I	LEP-F 5'-TGAGTTTGTCCAAGATGGACCAGACATTcG-3'	191	<i>Nru I</i>
	LEP-R 5'-AACGCCCAAGCTCTCCAAGCTCTCC-3'		

[†]A to I: primer sets for *GH* (rs41923484 and rs134687399), *SST* (rs17870997), *GHRH* (rs380969504), *GDF8* (rs383271508 and rs137528458) and *LEP* (rs29004487, rs29004488 and rs29004508), respectively. [‡]Lower case letters indicate mismatched bases to create restriction enzyme recognition sites.

4.3 Results and Discussion

The number of records and genotypic frequencies for each marker are indicated in Table 4.2. The *SST*, *GDF8* (rs383271508) and *LEP* (rs29004488) SNP were not associated with any growth or carcass traits hence their results are not discussed hereafter but are shown in the

Table 4.2 Genotypic frequencies and number of records for markers in the Japanese Black steer study population

Marker	RS Number	Records	Genotypes	Frequency
<i>GH</i>	rs41923484, rs134687399	279	AA	0.20
			AB	0.38
			AC	0.11
			BB	0.18
			BC	0.11
			CC	0.01
<i>SST</i>	rs17870997	263	AA	0.08
			AG	0.28
			GG	0.64
<i>GHRH</i>	rs380969504	280	CC	0.95
			CT	0.05
<i>GDF8</i>	rs383271508	273	AA	0.30
			AG	0.51
			GG	0.19
<i>GDF8</i>	rs137528458	262	TT	0.01
			TC	0.17
			CC	0.82
<i>LEP</i>	rs29004487	280	AA	0.83
			AT	0.16
			TT	0.01
<i>LEP</i>	rs29004488	279	CC	0.60
			TC	0.35
			TT	0.05
<i>LEP</i>	rs29004508		CC	0.85
			CT	0.14
			TT	0.01

appendix, supplementary tables (TS1 to TS6). However, *SST* had two alleles, A and G with frequencies of 0.22 and 0.78, respectively. For *GDF8*, alleles A and G were present with

respective frequencies of 0.55 and 0.45. For *LEP*, alleles C and T were present with respective frequencies of 0.77 and 0.23.

4.3.1 Association of *GH* SNP with growth and carcass traits

Results of the association analysis for *GH* SNP with growth and carcass traits are shown in Table 4.3. The *GH* polymorphisms had a consistent association with WH at all stages evaluated. Furthermore, the SNP were associated with BW and AG at 28 mo of age. To add to that the *GH* polymorphisms were associated with CW and RT. The AA genotype steers had significantly higher values for WH than the BB type from 9 to 20 mo of age. On top of that, the AA type cattle had heavier carcasses than BB type. However, CC genotype steers had heavier weight and larger AG at 28 mo of age than the AA and BB groups. Moreover, the CC group had heavier carcasses than the AA and BB groups. On average, CC genotype steers had carcasses that weighed about 30 and 40 kg more than the AA and BB type, respectively. A study that examined *GH* polymorphisms and their association with calf weight in Japanese Black cattle revealed that AA genotype calves were heavier at birth and after 30 days than BB type calves (Ishida *et al.* 2010). These results are similar to trends observed in this chapter. To add to that, Oka *et al.* (2007) and Tatsuda *et al.* (2008) found out that the B haplotype negatively affected carcass weight in Japanese Black cattle. In dairy cattle, Dario *et al.* (2008) reported that cows having the A haplotype produced more milk than those with the B haplotype. This report highlights that apart from growth, the *GH* polymorphisms are also associated with milk production. In both growth and milk production traits animals with the AA genotype had higher values than their BB genotype counterparts. However, there is a paucity of reports on the C haplotype because this variation has only been identified in Japanese Black and Japanese Brown cattle to date (Bahrami *et al.* 2013). It is also noteworthy that the CC genotype steers were few in the sample population resulting in relatively large

standard errors for the group. Though, Ardiyanti *et al.* (2012) showed that *GH* polymorphisms affect lipogenic gene expression levels, this study did not reveal any association between *GH* SNP and fat related carcass traits such as SFT and BMS.

4.3.2 Association of *GHRH* and *GDF8* SNP with growth and carcass traits

Table 4.4 shows the association of *GHRH* and *GDF8* polymorphisms with growth and carcass traits. The *GHRH* SNP was associated with WH at 9 and 28 mo of age. The CT type steers had higher values than their CC counterparts. Moreover, the SNP was associated with LMA. Similarly, CT type steers had bigger muscle area than the CC type. Though reports on this particular SNP are scant, a novel mutation in intron 1 of the *GHRH* gene was reported to be associated with growth traits in Chinese (Nanyang and Qinchuan) cattle (Zhang *et al.* 2012). The study showed that the SNP was associated with longitudinal measurements of hucklebone width and body weight taken from 6 to 24 mo of age. A study in Korean native (Hanwoo) cattle showed that an SNP in the 5' untranslated region (UTR) of the *GHRH* gene had significant association with cold carcass weight and *longissimus* muscle area (Cheong *et al.* 2006). These reports suggest that polymorphisms in the *GHRH* can be important factors that affect both growth and carcass traits in cattle.

The *GDF8* (rs137528458) SNP was associated with both BW and WH at 20 and 28 mo of age. The SNP was also associated with CG at 28 mo. Additionally, the polymorphism was associated with CW and LMA. The CC type steers had significantly higher values for both growth and carcass traits than the TC type steers. It is of paramount importance to note that TT animals were relatively few, resulting in large standard errors for this genotype. Though this study is probably the only report on the association of this particular SNP with longitudinal measures of growth, there are reports on other myostatin mutations. A different mutation under the brand name MyoMAX[®] (Pfizer Animal Health, Dunedin, New Zealand) is

now used as a gene-marker for sheep breeding in New Zealand and elsewhere (Han *et al.* 2010). Sheep that have an A allele for this marker are asserted to have at least 5 % increase in muscling in leg and rump. A study by Gan *et al.* (2008) analyzed the association of SNP haplotypes of the myostatin gene with muscular hypertrophy in sheep. The study revealed that some mutations had strong association with double muscling.

4.3.2 Association of *LEP* SNP with growth and carcass traits

The results for the association analysis for *LEP* polymorphisms are shown in Table 4.5. The *LEP* (29004487) was associated with AG at 9 and 28 mo of age however, the association was inconsistent. At nine mo, the AT genotype steers had larger AG than the AA type steers but the opposite was observed at 28 mo. Though the SNP was not associated with any carcass traits, there was a tendency ($p < 0.1$) of association with LMA. The *LEP* (rs29004508) was affiliated with WH at 9 mo and FNT. Additionally, there was a tendency ($p < 0.1$) of association with QS and BCS. Though the *LEP* polymorphisms were dissociated with body weight, the leptin hormone is involved in the control of body weight, feed intake, immune function and reproduction (Kadokawa *et al.* 2000 ; Block *et al.* 2001). Despite the association of the *LEP* (rs29004508) SNP with FNT, the polymorphism was not associated with fat thickness and marbling score as anticipated. Buchanan *et al.* (2002) reported significant effects of polymorphisms in exon 2 of the leptin gene on backfat thickness and fat grade. Though the *LEP* SNP evaluated in this study were not associated with muscle area, a study by Kong *et al.* (2006) reported that an SNP in Exon 2 of the leptin gene was associated with backfat thickness and *longissimus* muscle area in Korean cattle.

Table 4.3 Association of *GH* polymorphisms determined through PCR-RFLP with growth and carcass traits in Japanese Black steers under progeny testing

Age	Trait	<i>GH</i> Genotype [†]						P value
		AA (n = 55)	AB (n = 105)	AC (n = 32)	BB (n = 51)	BC (n = 32)	CC (n = 4)	
Growth								
9 mo	Withers height (WH, cm)	117.65 ^a ±0.42	116.14 ^b ±0.34	116.66 ^{ab} ±0.54	115.76 ^b ±0.45	116.29 ^{ab} ±0.56	115.51 ^{ab} ±1.44	0.02
14 mo	Withers height (WH, cm)	128.85 ^a ±0.63	126.81 ^b ±0.56	127.08 ^{ab} ±0.74	126.61 ^b ±0.70	126.42 ^{ab} ±0.84	124.08 ^{ab} ±1.96	0.00
20 mo	Withers height (WH, cm)	137.37 ^a ±0.57	135.48 ^b ±0.47	136.59 ^{ab} ±0.69	135.15 ^b ±0.59	135.56 ^{ab} ±0.75	135.12 ^{ab} ±2.01	0.02
28 mo	Body weight (BW, kg)	752.14 ^b ±7.83	728.58 ^c ±6.16	727.00 ^c ±10.47	734.61 ^c ±8.43	730.15 ^c ±10.70	801.71 ^a ±28.90	0.03
	Withers height (WH, cm)	143.72 ^a ±0.63	141.89 ^b ±0.85	142.60 ^{ab} ±0.77	141.59 ^{ab} ±0.67	142.52 ^{ab} ±0.79	140.96 ^{ab} ±1.88	0.04
	Abdominal girth (AG, cm)	262.06 ^{ab} ±1.22	261.08 ^b ±0.96	261.43 ^{ab} ±1.63	262.04 ^{ab} ±1.31	260.11 ^b ±1.66	274.72 ^a ±4.50	0.04
Carcass								
	Carcass weight (CW, kg)	480.60 ^b ±5.77	463.54 ^c ±4.60	461.22 ^c ±7.59	469.53 ^c ±6.21	464.20 ^c ±7.77	509.99 ^a ±20.68	0.04
	Rib thickness (RT, cm)	7.74 ^{ab} ±0.14	7.68 ^b ±0.12	7.63 ^b ±0.17	7.83 ^{ab} ±0.14	7.65 ^b ±0.17	8.91 ^a ±0.41	0.04

[†]*GH* genotype refers to the following haplotype combinations: *A* (nucleotide C at rs41923484 and rs134687399), *B* (G at rs41923484 and C at rs134687399) and *C* (G at rs41923484 and T at rs134687399). Values are expressed in least square means. n = number of cattle having the specified genotype. ^{abc}Values in the same row bearing the same superscripts don't differ significantly ($p < 0.05$).

Table 4.4 Association of *GHRH* and *GDF8* polymorphisms determined through PCR-RFLP with growth and carcass traits in Japanese Black steers under progeny testing

Age	Trait	<i>GHRH</i> Genotype [†]		P value	<i>GDF8</i> Genotype [‡]			P value
		CC (n = 266)	CT (n = 14)		TT (n = 3)	TC (n = 44)	CC(n = 215)	
Growth								
9 mo	Withers height (WH, cm)	116.38 ^b ±0.23	118.18 ^a ±0.83	0.03	114.31±1.69	116.08±0.51	116.66±0.35	0.22
20 mo	Body weight (BW, kg)	587.59±3.70	598.56±12.48	0.38	543.25 ^{ab} ±25.84	572.73 ^b ±7.54	593.28 ^a ±4.35	0.01
	Withers height (WH, cm)	135.91±0.39	136.95±1.02	0.30	133.63 ^{ab} ±2.02	134.77 ^b ±0.64	136.40 ^a ±0.43	0.02
28 mo	Body weight (BW, kg)	734.53±4.16	756.47±15.78	0.17	726.72 ^{ab} ±33.63	716.15 ^b ±8.97	740.69 ^a ±4.67	0.04
	Withers height (WH, cm)	142.27 ^b ±0.51	145.13 ^a ±1.12	0.01	139.63 ^{ab} ±2.17	140.95 ^b ±0.73	142.80 ^a ±0.56	0.01
	Chest girth (CG, cm)	233.91±0.54	233.44±2.06	0.82	234.85 ^{ab} ±4.27	231.42 ^b ±1.14	234.53 ^a ±0.60	0.04
Carcass								
	Carcass weight (CW, kg)	468.01±3.17	480.79±11.40	0.27	464.69 ^{ab} ±24.01	454.51 ^b ±6.47	471.80 ^a ±3.48	0.04
	<i>Longissimus</i> muscle area (LMA, cm ²)	63.08 ^b ±0.70	68.94 ^a ±2.53	0.02	64.13 ^{ab} ±5.20	59.77 ^b ±1.44	64.15 ^a ±0.83	0.02

[†]*GHRH* (rs380969504), there were no TT genotype cattle. Values are expressed in least square means. n = number of cattle having the specified genotype.

[‡]*GDF8* (137528458). Values are expressed in least square means. n = number of cattle having the specified genotype. ^{ab}Values of the same gene in the same row bearing the same superscripts don't differ significantly ($p < 0.05$).

Table 4.5 Association of *LEP* polymorphisms determined through PCR-RFLP with growth and carcass traits in Japanese Black steers under progeny testing

Age	Trait	<i>LEP</i> Genotype [†]		P value	<i>LEP</i> Genotype [‡]		P value
		AA (n = 233)	AT (n = 46)		CC (n = 237)	CT (n = 41)	
Growth							
9 mo	Withers height (WH, cm)	116.51±0.33	116.28±0.53	0.64	116.68 ^a ±0.38	115.46 ^b ±0.55	0.02
	Chest girth (CG, cm)	152.06±0.59	153.76±0.97	0.07	152.42±0.61	151.95±0.98	0.62
	Abdominal girth (AG, cm)	181.18 ^b ±0.54	184.11 ^a ±1.14	0.02	181.69±0.56	181.38±1.17	0.81
28 mo	Body weight (BW, kg)	738.26±4.34	721.80±9.28	0.09	736.52±4.40	730.86±9.39	0.57
	Abdominal girth (AG, cm)	262.07 ^a ±0.67	258.60 ^b ±1.43	0.02	261.38±0.68	262.47±1.45	0.48
Carcass							
	<i>Longissimus</i> muscle area (LMA, cm ²)	63.84±0.69	61.10±1.48	0.08	62.98±0.90	64.86±1.60	0.24
	Quality score (QS)	3.98±0.09	3.92±0.13	0.57	3.93±0.07	4.14±0.12	0.08
	Beef color score (BSC)	4.09±0.07	3.99±0.13	0.45	4.03±0.06	4.25±0.12	0.08
	Firmness and texture (FNT)	3.98±0.09	3.89±0.14	0.52	3.91 ^b ±0.07	4.19 ^a ±0.13	0.04

[†]*LEP* (rs29004487), a lone TT genotype steer was excluded from the analysis. Values are expressed in least square means. n = number of cattle having the specified genotype. [‡]*LEP* (rs29004508), a lone TT genotype steer was excluded from the analysis. Values are expressed in least square means. n = number of cattle having the specified genotype. ^{ab}Values of the same gene in the same row bearing the same superscripts don't differ significantly ($p < 0.05$).

4.4 Conclusion

In conclusion, of the nine SNP evaluated in this chapter, five were found to be functional mutations that had significant effect on both growth and carcass traits. The SNP in the *GH*, *GHRH*, *GDF8* and *LEP* genes were associated with growth and carcass traits. These polymorphisms might be able to aid in the selection of Japanese Black cattle to improve growth and meat quantity or quality. In this sample population there were no cattle possessing all the favorable alleles for the *GH*, *GHRH*, *GDF8* and *LEP* genes however, the inclusion of these genetic markers in the current selection program may facilitate the making of selection decisions early as animals with favorable genotypes can be identified at a young age.

CHAPTER 5

Variations in genes involved in fat metabolism and their association with ultrasonic and carcass traits in Japanese Black steers

5.1 Introduction

The organized genetic improvement of the Japanese Black cattle as a beef breed was initiated in the 1950s. In the year 1968, official performance and progeny testing programs were initiated in various prefectures. The BLUP using an animal model was later introduced to predict breeding values using field progeny testing records (Namikawa 1992). Since the development of the polymerase chain reaction technology (Fore *et al.* 2006), animal breeders have aimed to identify specific regions of interest that affect quantitative traits and incorporate them into breeding programs by using marker assisted selection (Van Marle-Köster *et al.* 2013). Among genetic markers of interest are single nucleotide polymorphisms (SNP) in genes involved in fat metabolism.

Among the genes involved in fat metabolism are fatty acid synthase (*FASN*), stearoyl-coenzyme A desaturase (*SCD*), sterol regulatory element-binding protein 1 (*SREBP1*), diacylglycerol acyltransferase 1 (*DGAT1*) and nuclear receptor subfamily 1 group H, number 3 (*NR1H3*). Fatty acid synthase is a complex homodimeric enzyme that regulates de novo biosynthesis of long chain fatty acids. It catalyses the formation of palmitate from acetyl-coenzyme A and malonyl-coenzyme A in the presence of NADPH (Roy *et al.* 2006). Stearoyl-coenzyme A desaturase is an enzyme that catalyses the $\Delta 9$ desaturation of saturated fatty acids (SFA) to mono unsaturated fatty acids (MUFA) (Ohsaki *et al.* 2009). Sterol regulatory element binding protein is considered the main regulator of *SCD* expression

although *SCD* mRNA expression is affected by environmental factors (Martin *et al.* 1999). The *DGATI* enzyme plays a key role in triacylglycerol synthesis; it catalyses the esterification of a fatty acyl-coenzyme A to the sn-3 position of a diacylglycerol (Schennink *et al.* 2008). A study by Oppi-Williams *et al.* (2013) suggested that *NR1H3* regulates acetyl coenzyme A carboxylase, *FASN* and *DGAT*. Given the role of these genes in fat metabolism, mutations in them might have effects on economically important traits such as carcass traits particularly those related to fat composition.

Polymorphisms in the *SCD* and *FASN* genes have been reported to be associated with fatty acid composition in Japanese Black cattle (Mannen 2011; Matsushashi *et al.* 2011) however; the relationship of these polymorphisms with pre-mortem ultrasonic traits is unknown. The objective of this chapter, therefore, was to assess the association between polymorphisms in genes involved in fat metabolism and ultrasonic traits in Japanese Black steers at various stages of growth. Furthermore, the association of the polymorphisms and carcass traits was assessed.

5.2 Materials and Methods

5.2.1 Experimental animals and traits

Data were collected from 300 Japanese Black steers that were under progeny testing at the Livestock Improvement Association of Miyazaki from June 2005 to July 2013. Animal management measurements of traits are given in detail in Chapter 3.

5.2.2 Genotyping

All the polymorphisms evaluated in this study are registered in the Single Nucleotide Database of the National Center of Biotechnology Information (NCBI 2010). The polymorphisms with their reference cluster ID numbers in parenthesis were in the fatty acid

synthase (*FASN*, rs208645216), stearoyl-coenzyme A desaturase (*SCD*, rs41255691), sterol regulatory element-binding protein 1 (*SREBP1*, rs133958066), diacylglycerol acyltransferase 1 (*DGATI*, rs109326954), and nuclear receptor subfamily 1, group H, number 3 (*NR1H3*, rs109428603). The polymorphisms in the *FASN*, *SCD*, *DGATI*, and *NR1H3* genes were SNP, whereas the polymorphism in the *SREBP1* gene was an 84 base pair (bp) insertion/deletion (in/del) variation. These polymorphisms were described in previous studies by (Taniguchi *et al.* 2004; Abe *et al.* 2009; Kawahara-Miki *et al.* 2011; Lee *et al.* 2013; Oppi-Williams *et al.* 2013). The polymorphisms in the *FASN*, *DGATI* and *NR1H3* result in amino acid changes.

Approximately 10 ml of whole blood were obtained from each steer and placed in heparinised vacuum tubes. Genomic DNA extraction and amplification was done as previously described in chapter 4. Oligonucleotide primer base sequences, amplicon sizes and corresponding restriction enzymes are indicated in Table 5.1. The PCR conditions for *FASN*, *SCD*, and *NR1H3* were: denaturation at 94°C for 2 min followed by 35 cycles of denaturation, annealing, and extension at 94°C for 30 s, 65°C for 30 s and 72°C for 1 min, respectively. Finally, extension was done at 72°C for 7 min. The same conditions were applied for *SREBP1* except that the annealing temperature was 60°C. For *DGATI* the initial denaturation were at 98°C for 2 min followed by three 5 cycle phases and a single 20 cycle phase with denaturation, annealing, and extension at 98°C for 10 s, 72 to 66°C touchdown for 15 s and 68°C for 1 min, respectively.

After PCR amplification, 3 units of restriction enzymes (New England Biolabs, Ipswich, MA, USA) were utilized to cleave the 5 µL of amplified DNA. The reaction temperature was 37 °C with duration of 14 hours for all the restriction enzymes. Subsequently, genotypes were determined through electrophoresis using MultiNA (Shimadzu, Kyoto, Japan). The procedure was carried out as outlined in the MultiNA operation manual.

5.2.3 Statistical methods

Association between genetic polymorphisms and traits was analyzed using ANOVA in JMP[®] 5.0.1 (SAS Inst. Inc., Cary NC, USA). The statistical model included fixed effect of genotype, starting test year, starting test season, a linear covariate of age of the animal at the time of measurement and a random effect of sire line (4 categories). *Post hoc* analysis was done using Tukey-Kramer's honestly significant difference test. Probability levels of $p < 0.05$ were considered to be significant while $p < 0.1$ were considered tendencies. The proportion of dominance variance to total genetic variance (d_g^2) was calculated using a formula given by Falconer (1981) as follows:

$$d_g^2 = \frac{(2pqd)^2}{(2pqd)^2 + [a + d(q - p)]^2}$$

where p and q stand for frequencies of the alleles that increase and decrease value, respectively; a stands for the additive effect that increases value; whereas d is the dominance effect of the heterozygote.

Table 5.1 Oligonucleotide primer base sequences, amplicon sizes and corresponding restriction enzymes

Primer [†]	Base sequence	Size(bp)	Enzyme
A	FASN-F 5'-GCCAATCCTCTCTCACTGTCTGTCCC-3'	371	Hha I
	FASN-R 5'-GGGTGCCATTGTACTTGGGCTTGTT-3'		
B	SCD-F 5'-GAGAGTGGAAAATCAGGTAGGTCTC-3'	364	Nco I
	SCD-R 5'-GTAACCTAATACCCTAAGCAGCAGAC-3'		
C	SREBP1-F 5'-CCACAACGCCATCGAGAAACGCTAC-3'	432	-
	SREBP1-R 5'-GGCCTTCCCTGACCACCCAAGTTAG-3'		
D	DGAT1-F 5'-CTCGTAGCTTTGGCAGGTAAG-3'	201	Eae I
	DGAT1-R 5'-AAGTTGAGCTCGTAGCACAGG-3'		
E	NR1H3-F 5'-GGACAAGGCCTCCGGCTTCCACTACAAC-3'	241	HpyCH4IV
	NR1H3-R 5'-TCCGGTGTCCAGACACTCACACTCCTCC-3'		

FASN: fatty acid synthase, *SCD*: stearoyl-coenzyme A desaturase, *SREBP1*: sterol regulatory element-binding protein 1, *DGAT1*: diacylglycerol acyltransferase 1, *NR1H3*: nuclear receptor subfamily 1, group H, number 3.

[†]A to E, primer sets for *FASN*, *SCD*, *SREBP1*, *DGAT1*, and *NR1H3* respectively.

5.3 Results and Discussion

The number of records and genotypic frequencies for each marker are indicated in Table 5.2. It is noteworthy that GG and AA genotypes of *FASN* and *NR1H3*, respectively were excluded from the analysis because they were found in less than 5 percent of the sample population. Given that the standard error of small samples tends to systematically underestimate the population standard error, a threshold of 5% was chosen based on Gurland and Tripathi (1971). Results for *FASN*, *DGAT1* and *NR1H3* are not discussed because these genes exhibited limited association with ultrasonic traits, *i.e.*, less than two ultrasonic traits at 20 and 26 mo of age. The 20 mo threshold was chosen because cattle are likely to be

slaughtered from this age onwards. However, their association results are shown in the appendix, supplementary tables (TS7 to TS12).

Table 5.2 Genotypic frequencies and number of records for markers in the Japanese Black steer study population

Marker	RS Number	Records	Genotypes	Frequency
<i>FASN</i> [†]	rs208645216	299	AA	0.76
			AG	0.23
			GG	0.01
<i>SCD</i>	rs41255691	300	AA	0.50
			VA	0.42
			VV	0.07
<i>SREBP1</i>	rs133958066	299	LL	0.53
			LS	0.38
			SS	0.09
<i>DGATI</i>	rs109326954	293	AA	0.46
			CA	0.47
			CC	0.07
<i>NR1H3</i> [‡]	rs109428603	239	GG	0.84
			GA	0.15
			AA	0.01

FASN: fatty acid synthase; *SCD*: stearyl-coenzyme A desaturase; *SREBP1*: sterol regulatory element-binding protein 1; *DGATI*: diacylglycerol acyltransferase 1; *NR1H3*: nuclear receptor subfamily 1, group H, number 3.

[†]GG genotype animals for *FASN* were excluded from the analysis because they were found in less than 5 percent of the sample population.

[‡]AA genotype animals for *NR1H3* were excluded from the analysis because they were found in less than 5 percent of the sample population.

5.3.1 Association of *SCD* SNP with ultrasonic and carcass traits

Table 5.3 Association of *SCD* polymorphism with ultrasonic traits in Japanese Black steers determined through ANOVA

Age	Trait	<i>SCD</i> Genotype			<i>P</i> value
		AA	VA	VV	
14 mo	ULMA (cm ²)	33.81 ± 0.30	33.47 ± 0.34	32.89 ± 0.80	0.458
	USFT (mm)	8.18 ± 0.34	8.33 ± 0.36	7.77 ± 0.61	0.594
	UIMFT (mm)	15.94 ± 0.99 ^b	17.71 ± 1.02 ^a	17.84 ± 1.45 ^{ab}	0.008
	URT (mm)	39.81 ± 1.08	41.01 ± 1.12	39.85 ± 1.70	0.239
	UBMS	0.22 ± 0.02	0.25 ± 0.02	0.19 ± 0.03	0.072
16 mo	ULMA (cm ²)	41.17 ± 0.37	41.37 ± 0.40	39.65 ± 0.92	0.205
	USFT (mm)	10.63 ± 0.55	10.67 ± 0.56	9.66 ± 0.80	0.304
	UIMFT (mm)	23.53 ± 1.38	24.87 ± 1.40	23.38 ± 1.98	0.250
	URT (mm)	50.64 ± 1.36	51.86 ± 1.40	48.64 ± 2.23	0.209
	UBMS	0.48 ± 0.02	0.51 ± 0.03	0.45 ± 0.05	0.262
20 mo	ULMA (cm ²)	50.39 ± 0.57 ^{ab}	51.27 ± 0.61 ^a	48.05 ± 1.34 ^b	0.043
	USFT (mm)	13.55 ± 0.60	13.58 ± 0.62	13.05 ± 0.98	0.827
	UIMFT (mm)	37.64 ± 1.87 ^b	39.83 ± 1.92 ^a	34.18 ± 2.92 ^b	0.033
	URT (mm)	68.13 ± 1.01	69.56 ± 1.11	64.84 ± 2.76	0.212
	UBMS	0.85 ± 0.02 ^b	0.95 ± 0.03 ^a	0.75 ± 0.07 ^b	0.001
26 mo	ULMA (cm ²)	59.54 ± 0.64	61.12 ± 0.72	59.32 ± 1.62	0.176
	USFT (mm)	17.38 ± 0.90	16.94 ± 0.93	15.93 ± 1.30	0.361
	UIMFT (mm)	56.96 ± 2.05 ^b	61.64 ± 2.11 ^a	54.34 ± 3.10 ^b	0.001
	URT (mm)	86.88 ± 1.39 ^b	90.41 ± 1.47 ^a	82.49 ± 2.60 ^b	0.001
	UBMS	1.44 ± 0.05 ^b	1.63 ± 0.06 ^a	1.30 ± 0.12 ^b	0.003

SCD: stearoyl-coenzyme A desaturase, ANOVA: analysis of variance, ULMA: ultrasonic *longissimus* muscle area, USFT: ultrasonic *subcutaneous* fat thickness, UIMFT: ultrasonic intermuscular fat thickness, URT: ultrasonic rib thickness, UBMS: ultrasonic beef marbling score. Values are expressed in least squares mean ± standard error.

^{a,b}Least square mean values within a row bearing the same superscript don't significantly ($p < 0.05$).

The association of the *SCD* SNP with ultrasonic traits is shown in Table 5.3. At 14 mo of age the polymorphism was associated with UIMFT. Furthermore, it was associated with the same trait at 20 and 26 mo. The polymorphism was associated with ULMA at one stage (20 mo) only. Nonetheless, the SNP was affiliated with UBMS at 20 and 26 mo of age. Apart from UIMFT at 14 mo, the polymorphism seems to be associated with ultrasonic traits at late stages of growth (20 and 26 mo). To add to ultrasonic traits, the *SCD* polymorphism was associated with numerous carcass traits as shown in Table 5.4. These traits were RT, YE, QS, BMS, BCS, and FNT. It is of paramount importance to note that the association of the *SCD* SNP with YE and QS has economic consequences because carcass price is ultimately determined by these traits. Since the SNP is associated with RT which is one of the traits used to compute YE, it subsequently resulted in an association with YE. Similarly, the association of the SNP with BMS, BCS and FNT eventually affected QS as it is determined in part by these traits.

It was fascinating to note that the steers with the heterozygous genotype (VA) had higher values for ultrasound and carcass measurements than their homozygous counterparts. There are three possible explanations for this observation: over dominance, epigenetic factors, and epistatic interactions. The over dominance hypothesis, developed independently by East (1908) and Shull (1908) attributes heterozygote advantage (improved or increased function) to over expression of genes in heterozygous individuals compared to homozygous ones. It is plausible to surmise that heterozygosity in the *SCD* gene results in the two alleles interacting more productively affecting the expression of the gene and some quantitative traits subsequently. On the other hand, epigenetic factors could be responsible for the observed phenomenon. A possible epigenetic mechanism is the involvement of micro RNA (miRNA). It has been reported that in hybrid plants most miRNA have non additive expression (Baranwal *et al.* 2012), they have been shown to repress translation of messenger RNA

(mRNA) or degrade it in mammalian cells (Zhou *et al.* 2007). It is possible that heterozygous animals might have less expression of miRNA resulting in more expression of genes in heterozygous individuals compared to homozygous ones. Additionally, epistatic interactions could be responsible for the high values in heterozygous steers.

As shown in Tables 5.5 and 5.6, the proportion of dominance variance (d_g^2) is high in most ultrasonic traits that were significantly associated with the *SCD* polymorphism and it ranged from 0 to 0.66 in carcass traits. Considering that dominance variance is any genetic variation that cannot be explained by average allelic differences or additive genetic variance, effects of other loci on a trait will appear as variance not associated with additive genetic variance *i.e.*, epistatic effects appear as dominance variance. However, there is need to clarify the probable involvement of over dominance and epigenetic factors through expression studies employing techniques such as biopsy. Though there are no reports on the association of the *SCD* SNP with longitudinally measured ultrasonic traits, previous studies have evaluated its association with carcass traits. Matsushashi *et al.* (2011) reported that the SNP had significant association with luster, firmness and texture in Japanese Black cattle. Contrary to the current study they did not observe any association with other carcass traits. Furthermore, a study by Ohsaki *et al.* (2009) did not find any association between the *SCD* SNP and carcass traits. However, the current study showed an association with some ultrasonic traits at multiple stages, to add to that, there was an association the numerous carcass traits. Most ultrasonic and carcass traits have fat as an integral part hence the mutation in the *SCD* gene affected them.

Table 5.4 Association of *SCD* polymorphism with carcass traits in Japanese Black steers determined through ANOVA

Trait	<i>SCD</i> Genotype			<i>P</i> value
	AA	VA	VV	
CW (kg)	468.09 ± 4.31	470.85 ± 4.62	463.82 ± 9.59	0.717
LMA (cm ²)	62.35 ± 0.88	64.57 ± 0.96	61.94 ± 2.14	0.121
SFT (mm)	27.08 ± 0.12	26.57 ± 0.12	25.73 ± 0.18	0.607
RT (mm)	76.14 ± 0.12 ^b	78.76 ± 0.12 ^a	76.21 ± 0.20 ^{ab}	0.023
YE (%)	74.31 ± 0.21 ^b	74.78 ± 0.21 ^a	74.43 ± 0.35 ^{ab}	0.023
QS	3.89 ± 0.10 ^b	4.10 ± 0.11 ^a	3.91 ± 0.17 ^{ab}	0.029
BMS	1.62 ± 0.09 ^b	1.84 ± 0.10 ^a	1.64 ± 0.18 ^{ab}	0.043
BCS	3.96 ± 0.09 ^b	4.21 ± 0.09 ^a	4.06 ± 0.17 ^{ab}	0.020
FNT	3.83 ± 0.11 ^b	4.12 ± 0.11 ^a	4.05 ± 0.19 ^{ab}	0.007
FCS	4.99 ± 0.00	4.99 ± 0.00	5.01 ± 0.01	0.539

SCD: stearoyl-coenzyme A desaturase, ANOVA: analysis of variance, CW: carcass weight, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness, RT: rib thickness, YE: yield estimate, QS: quality score, BMS: beef marbling score, BCS: beef color standard, FNT: firmness and texture, FCS: fat luster and color standard.

^{a,b}Least square mean values within a row bearing the same superscript don't differ significantly ($p < 0.05$). Values are expressed in least squares mean ± standard error.

Table 5.5 Additive and dominance effect, and proportion of dominance variance in ultrasonic traits for *SCD* polymorphism in Japanese Black steers

Age	Trait	<i>SCD</i>		
		<i>a</i>	<i>d</i>	d_g^2
14 mo	ULMA (cm ²)	0.17	-0.11	0.11
	USFT (mm)	0.02	0.17	0.12
	UIMFT (mm)	1.22	0.25	0.01
	URT (mm)	0.33	0.78	0.17
	UBMS	0.01	0.03	0.93
16 mo	ULMA (cm ²)	0.28	0.68	0.99
	USFT (mm)	0.23	0.24	0.36
	UIMFT (mm)	0.71	0.92	0.11
	URT (mm)	0.02	1.46	0.52
	UBMS	0.00	0.02	0.62
20 mo	ULMA (cm ²)	0.86	2.23	0.98
	USFT (mm)	0.11	0.21	0.97
	UIMFT (mm)	1.52	4.15	0.97
	URT (mm)	1.21	3.50	0.95
	UBMS	0.06	0.16	0.95
26 mo	ULMA (cm ²)	0.03	2.24	0.49
	USFT (mm)	0.56	0.37	0.12
	UIMFT (mm)	0.86	5.31	0.69
	URT (mm)	2.65	6.22	0.99
	UBMS	0.12	0.30	0.99

SCD: stearoyl-coenzyme A desaturase, *SREBP1*: sterol regulatory element-binding protein, ULMA: ultrasonic *longissimus* muscle area, USFT: ultrasonic *subcutaneous* fat thickness, UIMFT: ultrasonic intermuscular fat thickness, URT: ultrasonic rib thickness, UBMS: ultrasonic beef marbling score.

a: additive effect, *d*: dominance effect, d_g^2 : proportion of dominance variance to total genetic variance.

Table 5.6 Additive and dominance effect, and proportion of dominance variance in carcass traits for *SCD* polymorphisms in Japanese Black steers

Trait	<i>SCD</i>		
	<i>a</i>	<i>d</i>	d_g^2
CW (kg)	1.85	4.65	0.20
LMA (cm ²)	0.29	2.57	0.36
SFT (mm)	0.04	-0.01	0.00
RT (mm)	0.03	0.19	0.32
YE (%)	0.06	0.42	0.33
QS	0.01	0.23	0.42
BMS	0.03	0.24	0.34
BCS	0.08	0.21	0.20
FNT	0.12	0.21	0.14
FCS	0.00	-0.00	0.66

SCD: stearyl-coenzyme A desaturase, *SREBP1*: sterol regulatory element-binding protein, CW: carcass weight, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness, RT: rib thickness, YE: yield estimate, QS: quality score, BMS: beef marbling score, BCS: beef color standard, FNT: firmness and texture, FCS: fat luster and color standard.

a: additive effect, *d*: dominance effect, d_g^2 : proportion of dominance variance to total genetic variance.

5.3.2 Association of *SREBP1* polymorphisms with traits

Table 5.7 and 5.8 show the association of *SREBP1* in/del polymorphism with ultrasonic and carcass traits, respectively. The polymorphism had consistent association with URT from 16 to 26 mo of age. To add to that, it was also associated with USFT at 20 and 26 mo. Furthermore, the polymorphism was also associated with ULMA and UIMFT at 16 and 26 mo, respectively. An association analysis of the *SREBP1* polymorphism and carcass traits revealed that it was affiliated with RT. The association with RT was consistent in both live animals (from 16 mo) and in carcasses. On the other hand, an association with SFT was observed in live animals but not in carcasses. Even though the polymorphism was associated

with carcass RT and a number of ultrasonic traits, it was neither associated with YE or QS which are traits of paramount importance in determining carcass prices. Nevertheless, it should be highlighted that if slaughter age is reduced to between 20 and 26 mo the polymorphism could have significant effects on carcass SFT apart from RT. Steers with the SS genotype had significantly higher values for ultrasonic and carcass traits than the LL and LS ones.

The *SREBP1* polymorphism evaluated in this study is an 84 base pair insertion (L)/deletion (S) found in intron 5 (Ohsaki *et al.* 2009). The *SREBP1* is a transcription factor that regulates the expression of several fatty acid synthesis genes including *SCD* and *FASN* (Horton *et al.* 2003; Matsuzaka *et al.* 2007). Furthermore, *SREBP1* has been shown to regulate acetyl-coenzyme A carboxylase, *FASN*, *DGAT1* and *SCD* (Oppi-Williams *et al.* 2013). The association of the *SREBP1* polymorphism with ultrasonic and carcass traits can be attributed to the effects of the gene on some genes involved in fat metabolism. In spite of the fact that previous reports did not find an association between the *SREBP1* polymorphism with carcass traits (Matsushashi *et al.* 2011), a different SNP in the gene was associated with marbling score in Korean (Hanwoo) cattle (Lee *et al.* 2013). In addition, a study by Ohsaki *et al.* (2009) showed an association of the *SREBP1* in/del polymorphism with carcass weight in one group of Japanese cattle, however it was insignificant in another. Despite the fact that the *SREBP1* mutation in the current study was associated with a lone carcass trait (RT), its association with multiple ultrasonic traits at young age (20 to 26 mo) could be important if animals are slaughtered earlier. Currently there are efforts to reduce the slaughter age to increase production efficiency and increase competitiveness on international markets (personal communication).

Tables 5.9 and 5.10 show the proportion of dominance variance (d_g^2) in ultrasonic and carcass traits, respectively. The proportion of dominance variance ranged from 0 to 0.94 in

ultrasonic traits; while in carcass traits it ranged from 0.07 to 0.91. These values indicate a varying degree of dominance effects on expression of both ultrasonic and carcass traits.

Table 5.7 Association of *SREBP1* polymorphism with ultrasonic traits in Japanese Black steers determined through ANOVA

Age	Trait	<i>SREBP1</i> Genotype			P value
		LL	LS	SS	
14 mo	ULMA (cm ²)	33.56 ± 0.31	33.38 ± 0.34	35.01 ± 0.66	0.070
	USFT (mm)	8.21 ± 0.34	8.28 ± 0.35	8.01 ± 0.52	0.855
	UIMFT (mm)	16.91 ± 1.01	16.67 ± 1.03	16.00 ± 1.32	0.677
	URT (mm)	40.44 ± 1.10	39.75 ± 1.13	41.76 ± 1.51	0.252
	UBMS	0.23 ± 0.02	0.23 ± 0.02	0.24 ± 0.03	0.967
16 mo	ULMA (cm ²)	41.13 ± 0.39 ^b	40.63 ± 0.42 ^b	43.39 ± 0.82 ^a	0.009
	USFT (mm)	10.51 ± 0.55	10.52 ± 0.56	11.14 ± 0.75	0.572
	UIMFT (mm)	23.69 ± 1.41	23.93 ± 1.42	26.66 ± 1.87	0.145
	URT (mm)	50.18 ± 1.39 ^b	50.93 ± 1.41 ^b	55.46 ± 2.05 ^a	0.021
	UBMS	0.48 ± 0.02	0.49 ± 0.02	0.54 ± 0.04	0.290
20 mo	ULMA (cm ²)	50.94 ± 0.60	49.84 ± 0.64	52.15 ± 1.15	0.086
	USFT (mm)	13.43 ± 0.63 ^b	13.25 ± 0.65 ^b	15.24 ± 0.88 ^a	0.028
	UIMFT (mm)	38.44 ± 1.96	37.35 ± 2.00	42.09 ± 2.67	0.099
	URT (mm)	67.99 ± 1.02 ^b	67.13 ± 1.14 ^b	76.74 ± 2.27 ^a	0.001
	UBMS	0.88 ± 0.03	0.88 ± 0.03	0.93 ± 0.06	0.740
26 mo	ULMA (cm ²)	60.58 ± 0.67	59.34 ± 0.73	61.61 ± 1.43	0.218
	USFT (mm)	16.56 ± 0.89 ^b	17.30 ± 0.90 ^{ab}	19.15 ± 1.19 ^a	0.028
	UIMFT (mm)	58.32 ± 2.14 ^{ab}	57.83 ± 2.18 ^b	63.94 ± 2.94 ^a	0.044
	URT (mm)	87.80 ± 1.33 ^{ab}	87.19 ± 1.40 ^b	92.76 ± 2.31 ^a	0.045
	UBMS	1.48 ± 0.05	1.55 ± 0.06	1.49 ± 0.11	0.592

SREBP1: sterol regulatory element-binding protein, ANOVA: analysis of variance, ULMA: ultrasonic *longissimus* muscle area, USFT: ultrasonic *subcutaneous* fat thickness, UIMFT: ultrasonic intermuscular fat thickness, URT: ultrasonic rib thickness, UBMS: ultrasonic beef marbling score.

^{a,b} Least square mean values within a row bearing the same superscripts don't differ significantly ($p < 0.05$). Values are expressed in least squares mean ± standard error.

Table 5.8 Association of *SREBP1* polymorphism with carcass traits in Japanese Black steers determined through ANOVA

Trait	<i>SREBP1</i> Genotype			<i>P</i> value
	LL	LS	SS	
CW (kg)	467.50 ± 4.30	467.79 ± 4.66	481.94 ± 8.44	0.244
LMA (cm ²)	63.91 ± 0.90	62.09 ± 0.99	64.62 ± 1.89	0.216
SFT (mm)	26.48 ± 0.12	26.57 ± 0.13	29.26 ± 0.17	0.107
RT (mm)	76.80 ± 0.11 ^b	76.77 ± 0.12 ^b	81.23 ± 0.18 ^a	0.028
YE (%)	74.61 ± 0.21	74.38 ± 0.21	74.57 ± 0.32	0.426
QS	3.93 ± 0.10	4.04 ± 0.10	4.01 ± 0.15	0.404
BMS	1.66 ± 0.09	1.77 ± 0.10	1.77 ± 0.16	0.494
BCS	4.02 ± 0.09	4.15 ± 0.09	4.04 ± 0.15	0.385
FNT	3.91 ± 0.10	4.03 ± 0.10	3.98 ± 0.17	0.427
FCS	4.99 ± 0.01	5.00 ± 0.01	4.99 ± 0.02	0.614

SREBP1: sterol regulatory element-binding protein, ANOVA: analysis of variance, CW: carcass weight, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness, RT: rib thickness, YE: yield estimate, QS: quality score, BMS: beef marbling score, BCS: beef color standard, FNT: firmness and texture, FCS: fat luster and color standard.

^{a,b} Least square mean values within a row bearing the same superscripts don't differ significantly ($p < 0.05$). Values are expressed in least squares mean ± standard error.

Table 5.9 Additive and dominance effect, and proportion of dominance variance in ultrasonic traits for *SREBP1* polymorphism in Japanese Black steers

Age	Trait	<i>SREBP1</i>		
		<i>a</i>	<i>d</i>	d_g^2
14 mo	ULMA (cm ²)	0.61	-0.60	0.33
	USFT (mm)	0.04	0.29	0.36
	UIMFT (mm)	0.33	0.64	0.94
	URT (mm)	0.86	-1.03	0.49
	UBMS	0.03	-0.01	0.02
16 mo	ULMA (cm ²)	1.11	-1.31	0.50
	USFT (mm)	0.38	0.03	0.00
	UIMFT (mm)	0.86	-0.42	0.06
	URT (mm)	1.67	-0.75	0.05
	UBMS	0.05	-0.01	0.00
20 mo	ULMA (cm ²)	0.52	-1.68	0.88
	USFT (mm)	0.88	-0.87	0.34
	UIMFT (mm)	0.74	-2.11	0.94
	URT (mm)	3.21	-4.67	0.74
	UBMS	0.02	-0.02	0.25
26 mo	ULMA (cm ²)	0.57	-1.85	0.92
	USFT (mm)	1.34	-0.51	0.03
	UIMFT (mm)	2.16	-2.85	0.60
	URT (mm)	2.38	-3.80	0.81
	UBMS	0.02	0.04	0.19

SCD: stearoyl-coenzyme A desaturase, *SREBP1*: sterol regulatory element-binding protein, ULMA: ultrasonic *longissimus* muscle area, USFT: ultrasonic *subcutaneous* fat thickness, UIMFT: ultrasonic intermuscular fat thickness, URT: ultrasonic rib thickness, UBMS: ultrasonic beef marbling score.

a: additive effect, *d*: dominance effect, d_g^2 : proportion of dominance variance to total genetic variance.

Table 5.10 Additive and dominance effect, and proportion of dominance variance in carcass traits *SREBP1* polymorphism in Japanese Black steers

Trait	<i>SREBP1</i>		
	<i>a</i>	<i>d</i>	d_g^2
CW (kg)	4.31	-5.06	0.49
LMA (cm ²)	0.64	-2.10	0.91
SFT (mm)	0.16	-0.09	0.08
RT (mm)	0.26	-0.21	0.21
YE (%)	0.07	-0.27	0.85
QS	0.02	0.08	0.27
BMS	0.01	0.06	0.28
BCS	0.02	0.13	0.68
FNT	0.01	0.11	0.53
FCS	0.01	0.01	0.07

SCD: stearoyl-coenzyme A desaturase, *SREBP1*: sterol regulatory element-binding protein, CW: carcass weight, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness, RT: rib thickness, YE: yield estimate, QS: quality score, BMS: beef marbling score, BCS: beef color standard, FNT: firmness and texture, FCS: fat luster and color standard.

a: additive effect, *d*: dominance effect, d_g^2 : proportion of dominance variance to total genetic variance.

5.4 Conclusion

The *SCD* SNP evaluated in this study is a functional mutation that had substantial effects not only on live animal ultrasonic traits but also numerous carcass traits. Worth noting is that the SNP affects both YE and QS which are the traits that dictate carcass price. The *SCD* SNP could aid in the selection of Japanese Black cattle to improve carcass yield and quality. Though the *SREBP1* in/del polymorphism evaluated in this study was associated with a lone carcass trait (RT), it was also associated with USFT, UIMFT and URT at 26 mo of age. The *SREBP1* polymorphism might therefore be important if slaughter age of animals is reduced.

In both *SCD* and *SREBP1* polymorphisms the proportion of dominance variance was high in some traits indicating the possibility that multiple loci affect the traits.

GENERAL CONCLUSION

To evaluate the feasibility of early selection of bulls, genetic parameters for ultrasonic carcass traits, growth, and feed intake and efficiency traits of 525 eleven month old performance test bulls were estimated using JMP[®] 5.0.1 (SAS) program. Heritability estimates for rib eye area, muscle and fat thickness, and rib thickness ranged from 0.15-0.40. For highly heritable traits where heritability exceeds 0.40, the animal's phenotype is a good indicator of genetic merit or breeding value. Traits that are moderately heritable where heritability ranges from 0.15 to 0.40, the animal's phenotypic value is a moderate indicator of genetic merit or breeding value. For lowly heritable traits, where heritability is below 0.15, an animal's performance is much less useful in identifying the individuals with best genes for the traits. All growth traits except CW had heritability values in the moderate to high category. These results indicate that genetic improvement for growth can be achieved through selection. Phenotypic values for most body measurements are moderate or good indicators of an animal's genetic merit or breeding value. The analysis of variance indicated that environmental effects significantly affect growth of animals. Seasonal changes in both feed consumption and efficiency of feed utilization were evident. This highlights the importance of considering seasonal differences when evaluating cattle. There was considerable genetic variation in feed consumption and efficiency of feed utilization. This implies that feed efficiency can be improved through selection. The range of heritability values for most ultrasonic traits implies that they can be improved genetically through selection. However, the heritability for marbling score was low; taking ultrasonic measurements at an older age results in increased variation between cattle as indicated in chapter 3 marbling growth curve. Despite the low heritability exhibited, animals with higher marbling score at younger age have significantly higher marbling values as they grow older. Hence, selection of these animals is likely to be effective as well.

To combat increased competition from foreign products and to address the changing consumer demands while increasing competitiveness the possibility of early slaughter was evaluated. Serial measurements of body and ultrasonic traits were collected from 300 Japanese Black steers. Carcass measurements were taken after slaughter. Growth curves of ultrasonic traits were estimated and carcass YE at different ages was estimated using stepwise multiple regression. Multivariate analysis of the serial measures of LMA, RT and BMS revealed that there are no significant changes between measures at 26 and 28 mo of age however, intermuscular fat increased significantly during the same period. Linear regression analysis revealed that steers could attain carcass YE of grade A *i.e.*, above 72 % as early as 18 mo of age. Daily weight gain started to decline from 20 mo of age. These results suggest that a reduction of slaughter age to about 25 mo will result in a more efficient production system moreover; the results of carcass data will be available early facilitating early selection. It is noteworthy that the impact of early slaughter on carcass QS which is computed using BMS, BCS, FNT and FCS is beyond the scope of this study. Nevertheless, this study revealed that there is no significant difference in BMS measurements taken at 26 and 28 mo of age.

The current selection program is based on the phenotypic and pedigree information. However, advances in technology have made sequencing possible and gradually cheaper; this has made it possible to identify genomic regions that affect economically important traits. To improve the current program polymorphisms in genes that affect growth and carcass traits were appraised. The PCR-RFLP method was used to determine genotypes of a total of 300 Japanese Black steers. Association analysis was done using ANOVA and post hoc analysis using HSD test. Among the growth related polymorphisms evaluated, SNP in the *GH*, *GHRH*, *GDF8* (rs137528458) and *LEP* (rs29004487; rs29004508) genes were associated with growth and carcass traits. These polymorphisms might be able to aid in the selection of cattle to improve growth and meat quality or quantity. Evaluation of fat related genes revealed that

SCD SNP was associated with ultrasonic traits and numerous carcass traits. It is of paramount importance to note that the SNP was associated with carcass YE and QS which dictate carcass price. The *SREBP1* in/del polymorphism evaluated in this study was associated with USFT, UIMFT and URT at 26 mo of age however it was associated with a carcass RT only. The *SREBP1* polymorphism might play an important role if slaughter age of animals is reduced. The polymorphisms with economic impact were those in the *GH*, *GDF8*, because they affect the CW. Furthermore, the *SCD* polymorphism affects both YE and QS which are price determinants. These are functional mutations that can be included in the selection program to improve meat quality and quantity. Though this study focused on growth and carcass traits it is of paramount importance to mention that reproductive traits should not be ignored in order to have a prosperous beef production industry. Immense selection is being done to improve meat production traits but there is a trend of increased calving interval which is counterproductive. Reproductive traits are beyond the scope of this study but their importance in having a sustainable and competitive beef industry should not be overlooked.

Overall, a comprehensive selection program that utilizes ultrasound to facilitate selection of bulls at an early stage, estimates carcass traits in progeny early, and includes genetic markers is recommended. The early slaughter of progeny will not only improve production efficiency but also reduce the time for breeders to receive and evaluate carcass data for selection purposes. Inclusion of the favorable CC genotype of *GH* can result in as much as 30 or 40kg/head increase in carcass weight. The outstanding merit of molecular markers is that some selection decisions can be made even before birth of an animal based on parental genotypes or just after birth of the animal resulting in reduced costs since animals that don't meet the requirements can be raised as meat stock without using expensive breeding stock testing facilities and resources. Furthermore, animals that possess unfavorable markers on

important traits can be managed differently from their superior counterparts in order to boost their production potential through environmental approaches such as feeding.

REFERENCES

- Abe T, Saburi J, Hasebe H, Nakagawa T, Misumi S, Nade T, Nakajima H, Shoji N, Kobayashi M, Kobayashi E. 2009. Novel mutations of the *FASN* gene and their effect on fatty acid composition in Japanese Black beef. *Biochemical Genetics*, 47: 397-411.
- Ardiyanti A, Abe F, Kobashikawa H, Hirayama T, Sugino T, Suzuki K, Katoh K. 2009. Plasma hormone and metabolite concentration involved in the somatotrophic axis of Japanese Black heifers in association with growth hormone gene polymorphism. *Domestic Animal Endocrinology*, 37: 243-249.
- Ardiyanti A, Abe T, Tameoka N, Kobayashi E, Shoji N, Ohtani Y, Suzuki K, Roh S, Katoh K. 2012. Effects of growth hormone gene polymorphism on lipogenic gene expression levels in diaphragm tissues of Japanese Black heifers. *Asian-Australasian Journal of Animal Science*, 20: 1055-1062.
- Arnold JW, Bertrand JK, Benyshek LL, Ludwig C. 1991. Estimates of genetic parameters for live animal ultrasound, actual carcass data and growth traits in beef cattle. *Journal of Animal Science*, 69: 985-992.
- Arthur PF, Renand G, Krauss D. 2001. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. *Livestock Production Science*, 68:131-139.
- Avilés C, Polvillo O, Peña F, Juárez M, Martínez AL, Molina A. 2013. Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef. *Journal of Animal Science*, 91: 4571-4577.
- Bahrami A, Ardiyanti A, Tanosaki K, Suzuki K, Tanida N, Hirayama T, Roh SG, Nishio T, Katoh K. 2013. Haplotype C of growth hormone (*GH*) gene in Japanese Black cattle: structure of GH protein and a novel method for detection of the gene. *Animal Science*

Journal, 84: 802-804.

Bailey CM, Harvey WR, Hunter JE, Torell CR. 1971. Estimated direct and correlated response to selection for performance traits in closed Hereford lines under different types of environments. *Journal of Animal Science*, 33: 541.

Baranwal VK, Mikkilineni V, Zehr UB, Tyagi AK, Kapoor S. 2012. Heterosis: emerging ideas about hybrid vigour. *Journal of Experimental Botany*, 63: 6309-6314.

Bergen RD, McKinnon JJ, Christensen DA, Kohle N, Belanger A. 1997. Use of real time ultrasound to evaluate live animal carcass traits in young performance tested beef bulls. *Journal of Animal Science*, 75: 2300-2307.

Bertrand JK, Green RD, Herring WO, Moser DW. 2001. Genetic evaluation for beef carcass traits. *Journal of Animal Science*, 79: (E-Suppl.) E190-E200.

Block SS, Butler WR, Ehrhardt RA, Bell AW, Van-Amburgh ME, Boisclair YR. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. *Journal of Endocrinology*, 171: 339-348.

Brown JE, Fitzhugh HA Jr., Cartwright TC. 1976. A comparison of nonlinear models for describing weight-age relationships in cattle. *Journal of Animal Science*, 42: 810-818.

Buchanan FC, Fitzsimmons CF, Van Kessel AG, Thue TD, Winkelman-Sim DC, Schmutz SM. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Genetics Selection Evolution*, 34: 105-116.

Callow EH. 1944. The food value of beef from steers and heifers, and its relation to dressing-out percentage. *Journal of Agricultural Science, Cambridge*, 34: 177.

Carnier P, Albera A, Dal Zotto R, Groen AF, Bona M, Bittante G. 2000. Genetic parameters for direct and maternal calving ability over parities in Piedmontese cattle. *Journal of Animal Science*, 78: 2532-2539.

- Cassell B. 2009. Using heritability for genetic improvement. Virginia Cooperative Extension. Publication Number 404-084: 1-4.
- Cheong HS, Yoon D, Kim LH, Park BL, Choi YH, Chung ER, Cho YM, Park EW, Cheong I, Oh S, Yi S, Park T, Shin HD. 2006. Growth hormone-releasing hormone (*GHRH*) polymorphisms associated with carcass traits of meat in Korean cattle. *BMC Genetics*, 7: 35. <http://dx.doi.org/10.1186/1471-2156-7-35>
- Chikuni K, Nagatsuma T, Tabata T, Monma M, Saito M, Ozawa S, Ozutsumi K. 1994. Genetic variants of the growth hormone gene in Japanese cattle. *Animal Science Technology (Japan)*, 65: 340-346.
- Christison GI, Cymbaluk NF, Nicholson HH. 1990. Feed intake of growing bulls during severe winters. *Canadian Journal of Animal Science*, 70: 441-450.
- Chung ER, Kim WT. 2005. Association of SNP marker in IGF-1 and MYF5 candidate genes with growth traits in Korean cattle. *Asian-Aust. Journal of Animal Science*, 18: 1061-1065.
- Dario C, Carnicella D, Ciotola F, Peretti V, Buffano G. 2008. Polymorphism of growth hormone GH1-AluI in Jersey cows and its effect on milk yield and composition. *Asian-Australasian Journal of Animal Science*, 21: 1-5.
- Davis JK, Temple RS, McCormick WC. 1966. A comparison of ultrasonic estimates of rib eye area and fat thickness in cattle. *Journal of Animal Science*, 25: 1087-1090.
- Dekkers JCM, Hospital F. 2002. The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews Genetics*, 3: 22-32.
- Dekkers JCM. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. *American Society of Animal Science*, 82(Supplement): E313-E328.

- DeRose EP, Wilton JW, Schaffer LR. 1988. Estimation of variance components for traits measured on station-tested beef bulls. *Journal of Animal Science*, 66: 626-634.
- East EM. 1908. Inbreeding in corn. Reports of the Connecticut Agricultural Experiments Station for 1907, 419-428.
- Falconer DS. 1981. *Introduction to Quantitative Genetics*, 2nd edn, pp. 115-116. Longman, New York.
- Fan LQ, Bailey DRC, Shannon NH. 1995. Genetic parameter estimation of postweaning gain, feed intake, and feed efficiency for Hereford and Angus Bulls fed two different diets. *Journal of Animal Science*, 73: 365-372.
- Fore Jr. J, Wiechers R, Cook-Deegan R. 2006. The effects of business practices, licensing and intellectual property on development and dissemination of the polymerase chain reaction: Case study. *Journal of Biomedical Discovery and Collaboration*, 1: 7.
- Forrest JC, Kuei CH, Orcutt MW, Schinckel AP, Stouffer JR, Judge MD. 1989. A review of potential new methods of on-line pork carcass evaluation. *Journal of Animal Science*, 67: 2164-2171.
- Freeman AE. 1975. Genetic variation in nutrition of dairy cattle. The effect of genetic variance on nutritional requirements of animals. *National Academy of Science*, Washington DC, page 19.
- Fukuhara R, Obata T, Kihara Y. 1973. Growth and growth patterns of Japanese cattle on pasture 1. Estimation of the growth curves and determination of the size ranges of normal growth. *Bulletin of the Chugoku National Agricultural Experimental Station* B20: 1-50.
- Fukuhara R, Moriya K, Harada H. 1989. Estimation of genetic parameters and sire evaluation for carcass characteristics with special reference to yield grade of new carcass grading

- standards. Japanese Journal of Zootechnology Science, 60: 1128-1134 (in Japanese with English summary).
- Gan SQ, Du Z, Liu SR, Yang YL, Shen M, Wang XH, Yin JL, Hu XX, Fei J, Fan JJ, Wang JH, He QH, Zhang YS, Li N. 2008. Association of SNP haplotypes at the myostatin gene with muscular hypertrophy in sheep. Asian-Australasian Journal of Animal Science, 21: 928-935.
- Garret WN. 1971. Energetic efficiency of beef and dairy steers. Journal of Animal Science, 32: 451-456.
- Gurland J, Tripathi RC. 1971. A simple approximation for unbiased estimation of the standard deviation. American Statistician (American Statistical Association), 25: 30-32.
- Hammond Jr. J, Mason IL, Robinson TJ. 1971. Hammond's Farm Animals (4th edition). Edward Arnold Limited, London, UK.
- Han J, Zhou H, Forrest RH, Sedcole JR, Frampton CM, Hickford JGH. 2010. Effects of myostatin (*MSTN*) g+6223G>A on production and carcass traits in New Zealand Romney sheep. Asian-Australasian Journal of Animal Science, 23: 863-866.
- Han KJ, Lee JK, Nam IS, YS Won, Kim JB, Park YI. 1996. Estimation of genetic parameters for body weight and body measurements of Korean cattle. Proceedings of the 8th AAAP Animal Science Congress held in Tokyo, Japan. 2: 362-363.
- Hassen A, Wilson DE, Rouse GH. 2003. Estimation of genetic parameters for ultrasound-predicted percentage of intramuscular fat in Angus cattle using random regression models. Journal of Animal Science, 81: 35-45.
- Hayes BJ, Bowman PJ, Chamberlain AJ, Goddard ME. 2009. Invited review: Genomic selection in dairy cattle: Progress and challenges. Journal of Dairy Science, 92: 433-443.

- Herring WO, Miller DC, Bertrand JK, Benyshek LL. 1994. Evaluation of machine, technician, and interpreter effects on ultrasonic measures of backfat and *longissimus* muscle area in beef cattle. *Journal of Animal Science*, 72: 2216-2226.
- Hassen A, Wilson DE, Amin VR, Rouse GH, Hays CL. 2001. Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. *Journal of Animal Science*, 79: 11-18
- Hirooka H, Groen AF, Matsumoto M. 1996. Genetic parameters for growth and carcass traits in Japanese Brown cattle estimated from Field records. *Journal of Animal Science*, 74: 2112-2116.
- Hoque MA, Arthur PF, Hiramoto K, Oikawa T. 2006a. Genetic relationship between different measures of feed efficiency and its component traits in Japanese Black (Wagyu) bulls. *Livestock Science*, 99: 111-118.
- Hoque MA, Arthur PF, Hiramoto K, Oikawa T. 2006b. Genetic parameters for carcass traits of field progeny and their relationships with feed efficiency traits of their sire population for Japanese Black cattle. *Livestock Science*, 100: 251-260.
- Hoque MA, Hosono M, Suzuki K. 2009. Direct and maternal genetic parameters for measures of feed consumption and feed efficiency in young male Japanese Black cattle. *Livestock Science*, 122: 333 - 338.
- Horton JD, Shah NA, Warrington JA, Underson NN, Park SW, Brown MS, *et al.* 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct *SREBP* target genes. *Proceedings of the National Academy of Sciences USA*, 100: 12027-12032.
- Ishida T, Umebayashi A, Tsurata S, Akashi R, Harada H. 2010. Polymorphisms in growth hormone gene and their association with calf weight in Japanese Black cattle. *Animal*

- Science Journal, 81: 623-629.
- Japan Livestock Industry Association (JLIA). 2000. Japanese feeding standard for beef cattle. Japan Livestock Industry Association, Tokyo, Japan.
- Japan Meat Grading Association (JMGA). 1988. New Beef Carcass Grading Standards. Japan Meat Grading Association, Tokyo, Japan.
- John SWM, Weitzner G, Rozen R, Scriver CR. 1991. A rapid procedure for extracting genomic DNA from leukocytes. *Nucleic Acids Research*, 19: 408.
- Johnson MZ, Schalles RR, Dikeman RR, Golden BL. 1993. Genetic parameter estimates of ultrasound-measured *longissimus* muscle area and 12th rib fat thickness in Brangus cattle. *Journal of Animal Science*, 71: 2623-2630.
- Junkuszew A, Ringdorfer F. 2005. Computer tomography and ultrasound measurement as methods for the prediction of the body composition of lambs. *Small ruminant Research*, 56: 121-125.
- Kawahara-Miki R, Tsuda K, Shiwa Y, Arai-Kichise Y, Matsumoto T, Kanasaki Y, *et al.* 2011. Whole-genome resequencing shows numerous genes with nonsynonymous SNPs in the Japanese native Kuchinoshima–Ushi. *BMC Genomics*, 12: 103.
- Kim J, Lee C, Tsuyuki T, Shimogiri T, Okamoto S, Maeda Y. 2006. Sire-maternal grandsire Model and Sire model in estimation of genetic parameters for average daily gain and carcass traits of Japanese Black cattle. *Asian-Australasian Journal of Animal Science*, 19: 1678-1684.
- Kobayashi H. 1999. Effects of trade liberalization on agriculture in Japan: commodity aspects. CGPRT Centre Working Paper Series, November.

- Koch RM, Cundiff LV, Gregory KE. 1982. Heritabilities and genetic, environmental and phenotypic correlations of carcass traits in a population of diverse biological types and their implications in selection programs. *Journal of Animal Science*, 55: 1319-1329.
- Kadokawa H, Blache D, Yamada Y, Martin GB. 2000. Relationships between changes in plasma concentration of leptin before and after parturition and the timing of first post-partum ovulation in high producing Holstein dairy cows. *Reproduction and Fertility*, 12: 405-411.
- Kong HS, Oh JD, Lee SG, Hong YS, Song WI, Lee SJ, Kim HC, Yoo BH, Lee HK, Jeon GJ. 2006. Association of polymorphisms in the bovine leptin gene with ultrasound measurements for improving in Korean cattle. *Asian-Australasian Journal of Animal Science*, 19: 1691-1695.
- Kuchida K, Yamagishi T, Yamaki K, Mizuma Y. 1990. The estimation of genetic parameters for growth and carcass traits of Japanese Shorthorn. *Japanese Journal of Zootechnology Science*, 61: 401-405.
- Kumazaki K, Tanaka H, Kihara Y. 1955. Studies on the Growth of Japanese Black cattle. 1. Functional study of the normal growth of Japanese Black breed. *Bulletin of the Chugoku National Agricultural Experimental Station* B4.73-108.
- Lamb MS, Robinson OW, Tess MW. 1990. Genetic parameters for carcass traits in Hereford bulls. *Journal of Animal Science*, 8: 64-69.
- Lee Y, Oh D, Lee J, La B, Yeo J. 2013. Novel single nucleotide polymorphisms of bovine *SREBP1* gene are associated with fatty acid composition and marbling score in commercial Korean cattle (Hanwoo). *Molecular Biology Reports*, 40: 247-254.
- Lindsey JK. 1993. Models for repeated measurements. *Oxford Statistical Science Series*, Clarendon Press, Oxford.

- Littell RC, Henry PR, Ammerman CB. 1998. Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science*, 76: 1216-1231.
- Lopez de Torre G, Rankin BJ. 1978. Factors affecting growth curve parameters of Hereford and Brangus cows. *Journal of Animal Science*, 46: 604-613.
- Mannen H. 2011. Identification and utilization of genes associated with beef qualities. *Animal Science Journal*, 82: 1-7.
- Martin GS, Lunt DK, Britain KG, Smith SB. 1999. Postnatal development of stearoyl coenzyme A desaturase gene expression and adiposity in bovine *subcutaneous* adipose tissue. *Journal of Animal Science*, 77: 630-636.
- Matsuhashi T, Maruyama S, Uemoto Y, Kobayashi N, Mannen H, Abe T, *et al.* 2011. Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle. *Journal of Animal Science*, 89: 12-22.
- Matsuzaka T, Shimano H, Yahagi N, Kato T, Atsumi A, Yamamoto T, *et al.* 2007. Crucial role of long-chain fatty acid elongase, Elov16, in obesity-induced insulin resistance. *Nature Medicine*, 13: 1193-1202.
- McMeekan CP. 1940. Growth and development in the pig with special reference to carcass quality characters. *Journal of Agricultural Science, Cambridge*, 30: 276.
- Meyer K, Hill WG. 1997. Estimation of genetic and phenotypic covariance functions for longitudinal or “repeated” records by restricted maximum likelihood. *Livestock Production Science*, 47: 185-200.
- Ministry of Agriculture, Forestry and Fisheries (MAFF). 2014. Animal products distribution statistics. Tokyo, Japan.

- Moser DW, Bertrand JK, Misztal I, Kriese LA, Benyshek LL. 1998. Genetic parameter estimates for carcass and yearling ultrasound measurements in Brangus cattle. *Journal of Animal Science*, 76: 2542-2548.
- Mujibi FDN, Moore SS, Nkrumah DJ, Wang Z, Basarab JA. 2010. Season of testing and its effect on feed intake and efficiency in growing beef cattle. *Journal of Animal Science*, 88: 3789-3799.
- Mukai F, Wada Y, Namikawa K, Tanase K. 1980. The fitting of the non linear growth models for describing growth pattern of body weight and measurements in Japanese Black Females. *Animal Science and Technology (Japan)*, 51: 247-255. (in Japanese)
- Mukai F, Fukushima T. 1982. Application of logistic function with adjustment for gestation to size-age data and relationships between the growth curve parameters and reproductive performance in Japanese Black cows. *Animal Science and Technology (Japan)*, 53: 251-259. (in Japanese).
- Mukai F, Okanishi T, Yoshimura T. 1993. Genetic relationships between body measurements of heifers and carcass traits of fattening cattle in Japanese Black. *Animal Science and Technology (Japan)*, 64: 865-872.
- Mukai F. 1994. Genetic variation and selection methods for carcass traits in Japanese Black cattle. *Animal Science and Technology (Japan)*, 65: 890-905. (in Japanese).
- Munim T, Oikawa T, Ibi T, Kunieda T. (2012), Genetic relationship of body measurement traits at early age with carcass traits in Japanese black cattle. *Animal Science Journal*. doi: 10.1111/asj.12005
- Namikawa K. 1992. Breeding History of Japanese Beef Cattle and preservation of genetic resources as economic farm animals. *Wagyu (2nd ed)*. Wagyu Registry Association, Kyoto, Japan.

- National Center for Biotechnology Information (NCBI). 2010. The Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation [homepage on the Internet]. National Center for Biotechnology Information, Bethesda, MD; [cited 10 January 2010]. Available from URL: <http://www.ncbi.nlm.nih.gov/SNP/>
- National Wagyu Registry Association. 2004. Japanese Black cattle growth curves. Kyoto, Japan. (in Japanese)
- New South Wales Department of Primary Industries (NSW DPI). 2007. Market specifications for beef cattle. New South Wales, Australia.
- Nishimura T, Moriya K, Sasaki Y. 1995. Investigation of an equation for converting marbling score used in the old beef carcass grading standards into beef marbling standard. *Animal Science Technology (Japan)*, 66: 527-532.
- Ohsaki H, Tanaka A, Hoashi S, Sasazaki S, Oyama K, Taniguchi M, *et al.* 2009. Effect of *SCD* and *SREBP* genotypes on fatty acid composition in adipose tissue of Japanese Black cattle herds. *Animal Science Journal*, 80: 225-232.
- Oikawa T, Sanehira T, Sato K, Mizoguchi Y, Yamamoto H, Baba M. 2000. Genetic parameters for growth and carcass traits of Japanese Black (Wagyu) cattle. *Animal Science*, 71: 56-64.
- Oikawa T, Hoque MA, Hitomi T, Suzuki K, Uahida H. 2006. Genetic parameters for traits in performance and Progeny tests and their genetic relationships in Japanese Black cattle. *Asian-Australasian Journal of Animal Science*, 19: 611-616.
- Oka A, Iwaki F, Iwamoto E, Tatsuda K. 2007. Effects of growth rate during the early fattening period on growth, carcass characteristics and circulation hormones in the different growth hormone genotypes of Japanese Black steers. *Animal Science Journal*, 78: 142-150.

- Oppi-Williams C, Suagee JK, Corl BA. 2013. Regulation of lipid synthesis by liver X receptor α and sterol regulatory element-binding protein 1 in mammary epithelial cells. *Journal of Dairy Science*, 96: 112-121.
- Osawa T, Hasegawa M, Kuchida K, Hidaka S, Sekikawa M, Tsukuda H. 2004. Estimation of genetic parameters for muscle area, subcutaneous fat carcass cross section in Japanese Black cattle. *Nihon Chikusan Gakkaiho*, 75: 521-526.
- Osawa T, Kuchida K, Hidaka S, Kato T. 2007. Genetic parameters for image analysis traits on *M. longissimus thoracis* and *M. trapezius* of carcass cross section in Japanese Black steers. *Journal of Animal Science*, 86(Supl. 1): 40-46.
- Oyama K. 2011. Genetic variability of Wagyu cattle estimated by statistical approaches. *Animal Science Journal*, 82: 367-373.
- Perkins TL, Green RD, Hamlin KE. 1992. Evaluation of ultrasonic estimates of carcass fat thickness and *longissimus* muscle area in beef cattle. *Journal of Animal Science*, 70: 1002-1010.
- Reverter A, Johnson DJ, Graser HU, Wolcott ML, Upton WH. 2000. Genetic analyses of live-animal ultrasound and abattoir carcass traits in Australian Angus and Hereford cattle. *Journal of Animal Science*, 78: 1786-1795.
- Robinson DL, Hammond K, McDonald CA. 1993. Live animal measurement of carcass traits: estimation of genetic parameters for beef cattle. *Journal of Animal Science*, 71: 1128-1135.
- Roy R, Ordovas L, Zaragoza P, Romero A, Moreno C, Altarriba J, *et al.* 2006. Association of polymorphisms in the bovine *FASN* gene with milk-fat content. *Animal Genetics*, 37: 215-218.

- Schennink A, Heck JLM, Bovenhuis H, Visker MHPW, Van Valenberg HJF, Van Arendonk JAM. 2008. Milk fatty acid unsaturation: genetic parameters and effects of stearoyl-CoA desaturase (*SCD1*) and acyl CoA: Diacylglycerol acyltransferase 1 (*DGAT1*). *Journal of Dairy Science*, 91: 2135-2143.
- Shepard H, Green RD, Golden BL, Hamlin KE, Perkins TL, Diles JB. 1996. Genetic parameter estimates of live animal ultrasonic measures of retail yield indicators in yearling breeding cattle. *Journal of Animal Science*, 74: 761-768.
- Shojo M, Okanishi T, Anada K, Oyama K, Mukai F. 2006. Genetic analysis of calf market weight and carcass traits in Japanese Black cattle. *Journal of Animal Science*, 84: 2617-2622.
- Shull GH. 1908. The composition of field maize. *Reports of the American Breeders Association*, 296-301.
- Smith MT, Oltjen JW, Dolezal HG, Gill DR, Behrens BD. 1992. Evaluation of ultrasound for prediction of carcass fat thickness and *longissimus* muscle in feed lot steers. *Journal of Animal Science*, 70: 29-37.
- Sri Rachma ATD, Harada H, Fukuhara R. 1999a. Effects of genetic and environmental factors on ultrasonic estimates of carcass traits of Japanese Brown cows. *Asian-Australasian Journal of Animal Science*, 12: 506-510.
- Sri Rachma ATD, Harada H, Fukuhara R. 1999b. Studies on predicting carcass traits as one criteria for selecting Japanese Black bulls. *Bulletin of the Faculty of Agriculture, Miyazaki University*, 46: 61-67.
- Stouffer JR, Wallentine MV, Wellington GH, Diekman A. 1961. Development and application of ultrasonic methods for measuring fat thickness and rib eye area in cattle and hogs. *Journal of Animal Science*, 20: 759-767.

- Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayashi M, Tanabe Y, *et al.* 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, 14: 142-148.
- Tatsuda K, Oka A, Iwamoto E, Kuroda Y, Takeshita H, Kataoka H, Kouno S. 2008. Relationship of the bovine growth hormone gene to carcass traits in Japanese cattle. *Journal of Animal Breeding and Genetics*, 125: 45-49.
- Thaller G, Kühn C, Winter A, Ewald G, Bellmann O, Wegner J, Zühlke H, Fries R. 2003. *DGATI*, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Animal Genetics*, 34: 354-357.
- Tokunaga T, Ishida T, Takami T, Oyama S, Harada H, Morita T. 2013. Estimation of the growth patterns of carcass composition by use of ultrasound measurements in fattening Japanese Black cattle. *Journal of Society of Beef Cattle Science*, 94: 9-15. (in Japanese)
- Turner JW, Pelton LS, Cross HR. 1990. Using live animal ultrasound measures of rib eye area and fat thickness in yearling Hereford bulls. *Journal of Animal Science*, 68: 3502-3506.
- United States Department of Agriculture (USDA). 2013. Livestock, dairy and poultry outlook: Special article. USDA, Washington, D.C., United States of America.
- Van Marle-Köster E, Visser C, Berry DP. 2013. A review of genomic selection – Implications for the South African beef and dairy cattle industries. *South African Journal of Animal Science*, 43: 1-17.
- Waldner DN, Dikeman ME, Schalles RR, Olson WG, Houghton PL, Unruh JA, Corah LR. 1992. Validation of real-time ultrasound technology for predicting fat thickness, *longissimus* muscle areas, and composition of Brangus bulls from 4 months to 2 years of age. *Journal of Animal Science*, 70: 3044-3054.

- Whittaker AD, Park B, Thane BR, Miller RK, Savell JW. 1992. Principles of ultrasound and measurement of intramuscular fat. *Journal of Animal Science*, 70: 942-952.
- Wilson DE. 1992. Application of ultrasound for genetic improvement. *Journal of Animal Science*, 70: 973-983.
- Wilson DE, Willham RL, Northcutt SL, Rouse GH. 1993. Genetic parameters for carcass traits estimated from Angus field records. *Journal of Animal Science*, 71: 2365-2370.
- Wilson DE, Rouse GH, Hays CL, Hassen A. 2000. Carcass expected progeny differences using real-time ultrasound measures from developing Angus heifers. *Journal of Animal Science*, 78(Supl. 1): 58 (Abstr).
- Yahata S, Namikawa K. 1974. Basic Text of Beef Production. Nonsan-gyoson bunka-kyokai, Tokyo. 106-113.
- Yang M, Mukai F, Sasaki Y. 1985. Estimation of heritability for growth and carcass traits and their genetic and phenotypic correlation in Japanese Black cattle. *Japanese Journal of Zootechnology Science*, 56: 193-198. (in Japanese).
- Zhang B, Zhao G, Lan X, Lei C, Zhang C, Chen H. 2012. Polymorphism in *GHRH* gene and its association with growth traits in Chinese native cattle. *Research in Veterinary Science*, 92: 243-246.
- Zhou Y, Ferguson J, Chang JT, Kluger Y. 2007. Inter- and intra-combinatorial regulation by transcription factors and microRNAs. *BMC Genomics*, 8: 396.

LIST OF FIGURES AND TABLES

Figure	Title	Page
1	The positions for body measurements.....	22
2	Schema showing positions of the ultrasonic trait measurements.....	35
3.1	LMA growth curve.....	51
3.2	SFT growth curve.....	53
3.3	IMFT growth curve.....	55
3.4	RT growth curve.....	56
3.5	BMS growth curve.....	58
Table	Title	Page
1.1	Data summary including description of body measurement traits.....	25
1.2	Data summary including description of feed consumption and feed efficiency traits.....	26
1.3	Analysis of variance (ANOVA) for body measurement traits.....	27
1.4	Analysis of variance (ANOVA) for feed consumption and feed efficiency traits..	28
1.5	Estimates of variance components and heritability for body measurement traits.....	30
1.6	Estimates of variance components and heritability for feed consumption and feed efficiency traits.....	32
2.1	Data summary including description of ultrasonic traits.....	37
2.2	Analysis of variance (ANOVA) for ultrasonic traits.....	39
2.3	Estimates of variance components and heritability for ultrasonic traits.....	40
3.1	Data summary including description traits.....	48
3.2	Phenotypic correlation of serially measured LMA.....	50
3.3	Results of multivariate analysis of serially measured LMA.....	50
3.4	Phenotypic correlation of serially measured SFT.....	52

Table	Title	Page
3.5	Results of multivariate analysis of serially measured SFT.....	53
3.6	Phenotypic correlation of serially measured IMFT.....	54
3.7	Results of multivariate analysis of serially measured IMFT.....	54
3.8	Phenotypic correlation of serially measured RT.....	55
3.9	Results of multivariate analysis of serially measured RT.....	56
3.10	Phenotypic correlation of serially measured BMS.....	57
3.11	Results of multivariate analysis of serially measured BMS.....	57
3.12	YE percentage at different at ages.....	58
4.1	Oligonucleotide primer base sequences, amplicon sizes and corresponding restriction enzymes.....	66
4.2	Genotypic frequencies and number of records for markers in the Japanese Black steer study population.....	67
4.3	Association of <i>GH</i> polymorphisms determined through PCR-RFLP with growth and carcass traits in Japanese Black steers under progeny testing.....	71
4.4	Association of <i>GHRH</i> and <i>GDF8</i> polymorphisms determined through PCR- RFLP with growth and carcass traits in Japanese Black steers under progeny testing.....	72
4.5	Association of <i>LEP</i> polymorphisms determined through PCR-RFLP with growth and carcass traits in Japanese Black steers under progeny testing.....	73
5.1	Oligonucleotide primer base sequences, amplicon sizes and corresponding restriction enzymes.....	79
5.2	Genotypic frequencies and number of records for markers in the Japanese Black steer study population.....	80
5.3	Association of <i>SCD</i> polymorphism with ultrasonic traits in Japanese Black steers determined through ANOVA.....	81

Table	Title	Page
5.4	Association of <i>SCD</i> polymorphism with carcass traits in Japanese Black steers determined through ANOVA.....	84
5.5	Additive and dominance effect, and proportion of dominance variance in ultrasonic traits for <i>SCD</i> polymorphism in Japanese Black steers.....	85
5.6	Additive and dominance effect, and proportion of dominance variance in carcass traits for <i>SCD</i> polymorphism in Japanese Black steers.....	86
5.7	Association of <i>SREBP1</i> polymorphism with ultrasonic traits in Japanese Black steers determined through ANOVA.....	88
5.8	Association of <i>SREBP1</i> polymorphism with carcass traits in Japanese Black steers determined through ANOVA.....	89
5.9	Additive and dominance effect, and proportion of dominance variance in ultrasonic traits for <i>SREBP1</i> polymorphism in Japanese Black steers.....	90
5.10	Additive and dominance effect, and proportion of dominance variance in carcass traits for <i>SREBP1</i> polymorphism in Japanese Black steers.....	91

Appendix

Table S1 Association of *SST* polymorphism with growth traits in Japanese Black steers

Age	Trait	<i>SST</i> Genotype [†]			<i>P</i> Value
		AA (n = 22)	AG (n = 73)	GG (n = 168)	
9 mo	Body weight (BW, kg)	271.33	282.53	277.71	0.15
	Withers height (WH, cm)	116.17	116.66	116.47	0.78
	Chest girth (CG, cm)	151.60	153.17	152.07	0.23
	Abdorminal girth (CG, cm)	179.80	182.10	181.66	0.44
14 mo	Body weight (BW, kg)	427.55	425.13	423.85	0.91
	Withers height (WH, cm)	127.81	127.27	127.13	0.75
	Chest girth (CG, cm)	179.05	177.20	177.78	0.41
	Abdorminal girth (CG, cm)	210.12	209.13	210.02	0.69
20 mo	Body weight (BW, kg)	583.35	588.15	591.13	0.76
	Withers height (WH, cm)	135.90	135.98	135.97	0.99
	Chest girth (CG, cm)	207.43	207.26	208.30	0.64
	Abdorminal girth (CG, cm)	235.17	236.63	237.50	0.51
28 mo	Body weight (BW, kg)	739.66	732.74	737.69	0.81
	Withers height (WH, cm)	142.53	142.37	142.33	0.98
	Chest girth (CG, cm)	235.80	232.67	234.23	0.15
	Abdorminal girth (CG, cm)	261.88	260.62	261.96	0.59

[†]*SST* : values are expressed in least square means. n = number of cattle having the specified genotype.

Table S2 Association of *SST* polymorphism with carcass traits in Japanese Black steers

Trait	<i>SST</i> Genotype [†]			<i>P</i> Value
	AA (n = 22)	AG (n = 73)	GG (n = 168)	
Carcass weight (CW, kg)	468.21	465.35	471.26	0.63
<i>Longissimus</i> muscle area (LMA, cm ²)	63.52	62.62	63.88	0.65
Rib thickness (RT, cm)	7.91	7.64	7.73	0.38
<i>Subcutaneous</i> fat thickness (SFT, cm)	2.59	2.71	2.68	0.75
Yield estimate percentage (YE, %)	74.80	74.38	74.57	0.41
Quality score (QS)	4.09	3.98	3.95	0.68
Beef marbling score (BMS)	1.93	1.67	1.69	0.33
Beef color standard (BCS)	4.26	4.03	4.05	0.42
Fat color standard (FCS)	5.01	5.00	4.98	0.22
Firmness and texture (FNT)	4.13	3.96	3.94	0.58

[†]*SST*: values are expressed in least square means. n = number of cattle having the specified genotype.

Table S3 Association of *GDF8* polymorphism with growth traits in Japanese Black steers

Age	Trait	<i>GDF8</i> Genotype [†]			<i>P</i> Value
		AA (n = 82)	AG (n = 139)	GG (n = 52)	
9 mo	Body weight (BW, kg)	279.43	279.68	276.40	0.77
	Withers height (WH, cm)	116.83	116.41	116.02	0.29
	Chest girth (CG, cm)	152.91	152.32	150.93	0.12
	Abdominal girth (CG, cm)	182.39	181.78	179.73	0.11
14 mo	Body weight (BW, kg)	427.07	423.61	420.31	0.56
	Withers height (WH, cm)	127.74	126.98	126.76	0.24
	Chest girth (CG, cm)	177.69	178.26	176.46	0.17
	Abdominal girth (CG, cm)	209.71	209.50	209.13	0.92
20 mo	Body weight (BW, kg)	590.83	587.31	584.61	0.75
	Withers height (WH, cm)	135.28	135.89	135.58	0.55
	Chest girth (CG, cm)	208.36	207.66	206.62	0.48
	Abdominal girth (CG, cm)	236.99	236.35	237.62	0.62
28 mo	Body weight (BW, kg)	738.76	733.13	737.06	0.77
	Withers height (WH, cm)	142.50	142.35	142.27	0.93
	Chest girth (CG, cm)	234.02	233.95	233.15	0.78
	Abdominal girth (CG, cm)	261.11	261.72	261.61	0.88

[†]*GDF8* (rs383271508). Values are expressed in least square means. n = number of cattle having the specified genotype.

Table S4 Association of *GDF8* polymorphism with carcass traits in Japanese Black steers

Trait	<i>GDF8</i> Genotype [†]			<i>P</i> Value
	AA (n = 82)	AG (n = 139)	GG (n = 52)	
Carcass weight (CW, kg)	470.84	467.40	468.53	0.84
<i>Longissimus</i> muscle area (LMA, cm ²)	62.63	63.48	65.23	0.28
Rib thickness (RT, cm)	7.72	7.73	7.67	0.90
<i>Subcutaneous</i> fat thickness (SFT, cm)	2.74	2.68	2.58	0.37
Yield estimate percentage (YE, %)	74.36	74.57	74.83	0.17
Quality score (QS)	3.97	3.98	3.91	0.80
Beef marbling score (BMS)	1.69	1.74	1.61	0.60
Beef color standard (BCS)	3.99	4.10	4.07	0.56
Fat color standard (FCS)	4.99	5.00	4.97	0.14
Firmness and texture (FNT)	3.90	3.99	3.98	0.70

[†]*GDF8* (rs383271508). Values are expressed in least square means. n = number of cattle having the specified genotype.

Table S5 Association of *LEP* polymorphism with growth traits in Japanese Black steers

Age	Trait	<i>LEP</i> Genotype [†]			<i>P</i> Value
		CC (n = 166)	TC (n = 98)	TT (n = 15)	
9 mo	Body weight (BW, kg)	278.14	278.69	283.26	0.77
	Withers height (WH, cm)	116.37	116.61	116.52	0.83
	Chest girth (CG, cm)	152.19	152.46	152.43	0.93
	Abdominal girth (CG, cm)	181.15	182.22	182.85	0.45
14 mo	Body weight (BW, kg)	423.63	424.07	424.99	0.99
	Withers height (WH, cm)	127.07	127.42	126.75	0.68
	Chest girth (CG, cm)	177.68	177.77	179.33	0.63
	Abdominal girth (CG, cm)	209.45	209.54	208.48	0.89
20 mo	Body weight (BW, kg)	586.64	588.58	593.01	0.88
	Withers height (WH, cm)	135.65	136.31	137.37	0.17
	Chest girth (CG, cm)	207.08	208.20	212.06	0.09
	Abdominal girth (CG, cm)	236.85	236.42	237.93	0.83
28 mo	Body weight (BW, kg)	736.10	733.83	729.65	0.91
	Withers height (WH, cm)	142.10	142.73	143.34	0.30
	Chest girth (CG, cm)	233.84	234.01	232.59	0.79
	Abdominal girth (CG, cm)	262.10	260.67	258.18	0.20

[†]*LEP* (rs29004488). Values are expressed in least square means. n = number of cattle having the specified genotype.

Table S6 Association of *LEP* polymorphism with carcass traits in Japanese Black steers

Trait	<i>LEP</i> Genotype [†]			<i>P</i> Value
	CC (n = 166)	TC (n = 98)	TT (n = 15)	
Carcass weight (CW, kg)	468.69	467.47	468.07	0.98
<i>Longissimus</i> muscle area (LMA, cm ²)	63.82	62.90	58.99	0.16
Rib thickness (RT, cm)	7.71	7.74	7.70	0.97
<i>Subcutaneous</i> fat thickness (SFT, cm)	2.68	2.71	2.86	0.58
Yield estimate percentage (YE, %)	74.58	74.48	73.80	0.13
Quality score (QS)	4.00	3.94	3.81	0.51
Beef marbling score (BMS)	1.72	1.71	1.53	0.65
Beef color standard (BCS)	4.09	4.05	3.88	0.60
Fat color standard (FCS)	4.99	5.00	5.00	0.86
Firmness and texture (FNT)	4.00	3.94	3.69	0.33

[†]*LEP* (rs383271508). Values are expressed in least square means. n = number of cattle having the specified genotype.

Table S7 Association of *FASN* polymorphism with ultrasonic traits in Japanese Black steers

Age	Trait	<i>FASN</i> Genotype [†]		<i>P</i> value
		AA	AG	
14 mo	ULMA, cm ²	33.68	33.44	0.633
	USFT, mm	8.30	8.01	0.403
	UIMFT, mm	16.55	17.23	0.362
	URT, mm	40.20	40.47	0.761
	UBMS	0.22	0.25	0.145
16 mo	ULMA, cm ²	40.97	41.68	0.247
	USFT, mm	10.45	11.10	0.133
	UIMFT, mm	23.81	25.31	0.159
	URT, mm	50.41 ^b	53.01 ^a	0.047
	UBMS	0.48	0.53	0.066
20 mo	ULMA, cm ²	50.88	49.85	0.200
	USFT, mm	13.37	14.07	0.164
	UIMFT, mm	38.32	38.19	0.927
	URT, mm	68.54	68.46	0.963
	UBMS	0.86 ^b	0.96 ^a	0.026
26 mo	ULMA, cm ²	60.52	59.12	0.189
	USFT, mm	16.91	17.53	0.365
	UIMFT, mm	58.51	59.60	0.530
	URT, mm	88.69	86.35	0.147
	UBMS	1.49	1.57	0.361

ULMA: ultrasonic *longissimus* muscle area, USFT: ultrasonic *subcutaneous* fat thickness, UIMFT: ultrasonic intermuscular fat thickness, URT: ultrasonic rib thickness, UBMS: ultrasonic beef marbling score.

[†] GG Genotype steers were excluded from the analysis because they were found in less than 5 percent of the sample population.

^{a,b} Least square mean values within a row bearing the same superscripts don't differ significantly ($p < 0.05$).

Table S8 Association of *FASN* polymorphism with carcass traits in Japanese Black steers

Trait	<i>FASN</i> Genotype [†]		<i>P</i> value
	AA	AG	
CW, kg	470.81	463.83	0.243
LMA, cm ²	63.71	61.78	0.154
SFT, mm	26.58	27.10	0.575
RT, mm	77.51	75.78	0.142
YE, %	74.57	74.29	0.170
QS	3.97	3.99	0.846
BMS	1.70	1.74	0.718
BCS	4.07	4.08	0.918
FNT	3.99	3.89	0.388
FCS	4.99	5.00	0.783

CW: carcass weight, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness, RT: rib thickness, YE: yield estimate, QS: quality score, BMS: beef marbling score, BCS: beef color standard, FNT: firmness and texture, FCS: fat color standard.

[†] GG Genotype steers were excluded from the analysis because they were found in less than 5 percent of the sample population.

Table S9 Association of *DGATI* polymorphism with ultrasonic traits in Japanese Black

steers

Age	Trait	<i>DGATI</i> Genotype			<i>P</i> value
		AA	CA	CC	
14 mo	ULMA, cm ²	33.59	33.63	33.77	0.976
	USFT, mm	8.31	8.14	8.27	0.819
	UIMFT, mm	16.92	16.69	16.27	0.840
	URT, mm	40.40	40.21	40.21	0.966
	UBMS	0.23	0.23	0.20	0.533
16 mo	ULMA, cm ²	40.79	41.41	41.59	0.419
	USFT, mm	10.83	10.36	10.65	0.408
	UIMFT, mm	24.04	24.29	23.21	0.785
	URT, mm	50.59	51.40	50.60	0.736
	UBMS	0.52	0.48	0.42	0.052
20 mo	ULMA, cm ²	49.95	51.14	50.72	0.201
	USFT, mm	13.93	13.12	14.02	0.129
	UIMFT, mm	37.70	38.93	37.97	0.610
	URT, mm	67.55	69.37	69.02	0.437
	UBMS	0.91	0.87	0.90	0.558
26 mo	ULMA, cm ²	59.72	60.48	60.98	0.611
	USFT, mm	17.37	16.89	17.33	0.677
	UIMFT, mm	58.25	59.08	58.55	0.843
	URT, mm	87.15	88.86	87.93	0.442
	UBMS	1.47	1.54	1.46	0.537

ULMA: ultrasonic *longissimus* muscle area, USFT: ultrasonic *subcutaneous* fat thickness, UIMFT: ultrasonic intermuscular fat thickness,

URT: ultrasonic rib thickness, UBMS: ultrasonic beef marbling score.

Table S10 Association of *DGATI* polymorphism with carcass traits in Japanese Black steers

Trait	<i>DGATI</i> Genotype			<i>P</i> value
	AA	CA	CC	
CW, kg	465.69	470.34	480.39	0.280
LMA, cm ²	62.41	63.80	63.62	0.481
SFT, mm	27.53	26.11	27.14	0.173
RT, mm	76.38	77.77	78.38	0.306
YE, %	74.30	74.69	74.47	0.080
QS	3.90	4.04	3.97	0.212
BMS	1.63	1.79	1.67	0.191
BCS	3.97	4.15	4.07	0.122
FNT	3.84 ^b	4.07 ^a	3.96 ^b	0.048
FCS	4.98	5.00	5.01	0.059

CW: carcass weight, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness, RT: rib thickness, YE: yield estimate, QS: quality score, BMS: beef marbling score, BCS: beef color standard, FNT: firmness and texture, FCS: fat color standard.

^{a,b}Least square mean values within a row bearing the same superscripts don't differ significantly ($p < 0.05$).

Table S11 Association of *NR1H3* polymorphism with ultrasonic traits in Japanese Black steers

Age	Trait	<i>NR1H3</i> Genotype [†]		<i>P</i> value
		GG	GA	
14 mo	ULMA, cm ²	33.47	33.42	0.940
	USFT, mm	8.12	8.81	0.075
	UIMFT, mm	16.69	17.27	0.532
	URT, mm	39.95	40.30	0.740
	UBMS	0.23	0.20	0.216
16 mo	ULMA, cm ²	40.91	41.19	0.720
	USFT, mm	10.37	11.21	0.094
	UIMFT, mm	23.43 ^b	27.05 ^a	0.005
	URT, mm	50.36	51.74	0.385
	UBMS	0.49	0.48	0.918
20 mo	ULMA, cm ²	50.47	51.60	0.285
	USFT, mm	13.23	13.99	0.255
	UIMFT, mm	37.51	40.85	0.077
	URT, mm	77.16	77.21	0.977
	UBMS	0.88	0.82	0.258
26 mo	ULMA, cm ²	60.29	58.99	0.349
	USFT, mm	16.53	17.86	0.100
	UIMFT, mm	58.15	62.52	0.052
	URT, mm	87.97	89.49	0.446
	UBMS	1.47	1.46	0.874

ULMA: ultrasonic *longissimus* muscle area, USFT: ultrasonic *subcutaneous* fat thickness, UIMFT: ultrasonic intermuscular fat thickness, URT: ultrasonic rib thickness, UBMS: ultrasonic beef marbling score.

[†] AA Genotype steers were excluded from the analysis because they were found in less than 5 percent of the sample population. ^{a,b} Least square mean values within a row are different if superscripts differ ($p < 0.05$).

Table S12 Association of *NR1H3* polymorphism with carcass traits in Japanese Black steers

Trait	<i>NR1H3</i> Genotype [†]		<i>P</i> value
	GG	GA	
CW, kg	469.11	468.80	0.968
LMA, cm ²	63.20	62.29	0.601
SFT, mm	2.60	2.72	0.264
RT, mm	74.56	77.18	0.086
YE, %	74.57	74.17	0.126
QS	3.96	3.96	0.985
BMS	1.70	1.67	0.840
BCS	4.07	4.02	0.756
FNT	3.97	3.92	0.762
FCS	4.99	4.99	0.969

CW: carcass weight, LMA: *longissimus* muscle area, SFT: *subcutaneous* fat thickness; RT: rib thickness, YE: yield estimate, QS: quality score, BMS: beef marbling score, BCS: beef color standard; FNT: firmness and texture, FCS: fat color standard.

[†]AA Genotype steers were excluded from the analysis because they were found in less than 5 percent of the sample population.