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**Literature Review on Establishment of Japanese Black Cattle (Wagyu)
and Future Prospects of Its Industry**

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1. Introduction

In this paper, Wagyu refers to Japanese Black since it takes over majority of the market and most of the studies have been focused on this breed. It is considered that Japanese black beef palatability is consist of its juiciness, extreme tenderness, and sweet/fatty aroma known as “wagyuko”, the beef aroma. It is often described as an aroma that is likened to coconut or fruit. It is generated remarkably in highly marbled Wagyu beef stored in the presence of oxygen for several days. Its optimal cooking temperature to generate the aroma was found to be 80°C, and the aroma was almost absent in the beef immediately after slaughtering (Matsuishi et al., 2001). Japanese lifestyle has been changing a lot over the past years. Agricultural industry always must follow up and adjust to the market demands and trends. Wagyu is no exception to it. The purpose of Wagyu has changed dramatically, and meat quality has improved a lot since it became a breed for beef production. Many breeding strategies has been applied in order to create the highest marbling cattle since it directly effects on the market in Japan: in general, the higher the marbling, the higher the value. Introducing of BLUP (best linear unbiased prediction) methods was the one of the biggest contributions to achieve the high marbling intramuscular fat content. Modern biotechnology that include genome editing expands the potential in breeding industry in a lot of aspect, however, it is necessary to consider the consequence and possibility in the future in terms of genetic diversity.

2. Wagyu as a Breed

2.1 History

Modern domesticated cattle are categorized into two subspecies, *Bos Taurus* and *Bos indicus*. All of four Japanese Cattle breeds; Black, Brown, Shorthorn, and Polled, belong to *Bos tauru* (Yonesaka et al., 2016), which is one of the ancestry breeds of domesticated cattle which lacks a hump. Throughout the history of Japan, Wagyu were significantly important as a labor source mainly in farming; drafting and fertilizing rice field, also with transportation and foresting. Around years in 1100's to 1600's, Japanese style body armor was made of Wagyu leather. Compare to European countries, history of raising beef cattle in Japan is very short. Up until Meiji Restoration in 1868, Wagyu had no purpose as a meat production since consumption of beef was forbidden due to the religious influence of Buddhism. In 1872, the ban was removed, and meat-eating culture had started. Meat had become one of the important nutrient sources and the demand and popularity continued to grow, however, demands of Wagyu as agriculture use remained strong especially for rice producers. Wagyu were valuable property for them. Crossing with European breeds such as Simmental, Brown Swiss, Aberdeen Angus, Devon, Shorthorn, and Ayrshire, had started in order to increase the body size and milk yield so that Wagyu would have been suited for 'triple purpose' cattle; work, milk and meat. This breeding project was aimed to produce Wagyu, which is close to European breeds, and still have the same type of meat quality of authentic Wagyu breed. During 1990 to 1912, the crossbreeding agenda had gone most intensively, however, the outcome was not what it was expected. Both meat and work quality became poor and unfavorable result. There was also a gap between the government and producer regarding to the expectation of

crossed bred Wagyu. Milk production was the least concern for the farmer, but the stamina and the body size and temperament were the most important characteristics which had to be improved. Soon after, the project got switched from crossbreeding to intra-breeding system to produce Wagyu which is suitable to lifestyle in Japan and culture as well as the climate. In 1944, the 4 breeds of Wagyu were established:

Japanese Black (**Figure 1-A**); Adult bull weighs approximately 480kg. Because of its high performance in marbling throughout the muscle fibers giving the characteristic feature of melt-in-mouth quality. More than 90% of Wagyu raised in Japan is of this breed. Because of this reason, the term ‘Wagyu’ today commonly refer to Japanese Black cattle exclusively. There are several strains of this breed, particularly, in Hyogo prefecture (Tajima-strain: superior meat quality famous under the name of “Kobe-beef”), in Tottori prefecture (Ketaka-strain), and in Shimane prefecture (Itozakura-strain) are the used in breeding. Currently, almost all the Japanese Black in Japan have a pedigree comprising a combination of these three strains.

Japanese Brown (**Figure 1-B**); Adult bull weighs approximately 560kg. This breed has characteristic reddish-brown color, hence also known as Red Wagyu, which was by cross bred with Simmental, Korean Hanwoo, and Devon. As of breed characteristic, moderate marbling and contains lean meat. Mainly raised in Kumamoto prefecture (Higo-strain), southern part of Japan, also in Kochi prefecture (Tosa-strain) in Shikoku Island.

Japanese Shorthorn (**Figure 1-C**); Adult bull weighs approximately 560kg. Cross breeding with European and American Shorthorn led to have lean meat and low-fat content in addition to its dark red color. It also contains high inosinic and glutamate acids. It is bred commonly in northern region of Japan such as Iwate, Aomori, Akita, and Hokkaido prefectures.

Japanese Polled (**Figure 1-D**); It has darker black coat and lower degree of marbling compare to the Japanese Black. It has high in lean meat content and rich in amino acids. They were cross bred mainly with Aberdeen-Angus, and have been maintained in a limited region of Yamaguchi prefecture where they are originated from. The number of cattle is quite low as only around 200 heads that is the smallest population of Wagyu in Japan.



In addition to four Wagyu breeds, there are two other native Japanese Cattle ‘Mishima cattle’ and ‘Kuchinoshima feral cattle’ both of which were isolated from cross breeding agenda with European breeds due to geological restriction, hence their breeds’ characteristics remains as hundreds of years ago that it is considered to be maintained without any genetic influences from European or American breeds (Mannen, 2017). Only a few Mishima cattle and Kuchinosima cattle are living in isolated small island belongs to Yamaguchi prefecture and Kagoshima prefecture respectively.

After mechanization of work force and introducing chemical fertilizer in agriculture, in 1960’s, demand of Wagyu as agricultural use declined significantly. Two purpose out of three had dropped entirely, and history of Wagyu exclusively as a meat production have begun. Among four Wagyu breeds, Japanese Black cattle yield in the highest percentage of marbling meant quality. Today, Japanese Black cattle contributes more than 95% of Wagyu beef production in Japan.

2.2 Registration System

Wagyu Registry Association was first formed in 1948 to ensure that nationally marketed Wagyu cattle are certainly qualified as Wagyu breed. Their major roles are registration and issuing a certificate of the breed. Since the Japanese Black is the most marketed in the industry, description of the registration system below is focused on this breed.

First, almost all the calves, which fulfill requirements, are registered in a category of “calf registration” (Figure 2) which is a fundamental to the rest of registration. A calf is evaluated according to individual phenotypic traits and body measurement. The final score will be given as one of the registration records. The black color coat is one of

the fundamental characteristics, so cow with any other color coat, which happens during the breeding process time to time (Figure 3), is not eligible to be registered as the Japanese Black despite of a fact that it was born from certified parents. Within the calf registration, name of the calf, sex, name and address of the owner/producer, date and place of the birth, traceability number, cleanness of undesirable gene, and pedigree; paternal lineage traced back to 3rd generation and maternal lineage back to 2nd generation, must be indicated. The traceability system in Wagyu has been introduced in 2003 due to the first outbreak of BSE in 2001. It allows consumer to look up the information regarding to the meat that they purchased in a store by their devise or computer via homepage of National Livestock Breeding Center.

Second registration step is for breeding cattle under 30 months of age. There are three classes; Basic, High performance, and Breeding stock. For the latter two classes, in addition to the basic qualification as a breed, there are certain criteria that must be fulfilled including progeny test for a bull and reproductive records for a cow. Just like the Thoroughbreds, pedigree is crucial to the Wagyu breeds. According to the registry data of 2018 from the press, Wagyu dayori, published by Wagyu Registry Association (2019), those cattle which were certified in a category of the High performance was only 1.5% among all the registered Japanese Black cattle.

Calf Registration Sex 期待育種価 ACABBA

黒毛和種 子牛登記	此係 Name of calf	育種価資格本原	登記記号番号 2018子島黒 2180504																								
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母 Meternal	Name and Address of Owner		
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育種価CAAAAA			

繁殖者 (管理者) ()			
和牛改良組合認定番号 島 -27			
備考欄	セリ年月日	セリ番号	セリ体重
			価格

母牛初産月齢23カ月 分娩間隔359日 産次 7産

個体識別番号 **traceability number**

Figure 2 Calf Registration sample



Figure 3 Japanese Black with light brown coat, photo taken by Mai Tsujimoto

2.3 Grading system

Evaluation of all meats in Japan is done by the Japan Meat Grading Association (JMGA) according to dressed carcass transaction standards, which having been certified by the Director General of the Agricultural Production Bureau of the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan, serving as a nationwide unified transaction standard. This standard is not for Wagyu exclusively but commonly applied to all bovine breeds in Japan. The standard is a combination of Yield Grade and Quality Grade; Yield Grade is calculated based on measurements from a carcass section, and Quality Grade is evaluated based on scores of Beef Marbling Standard (B.M.S), Beef Color Standard (B.C.S), and Beef Fat Standard (B.F.S). The meat section used for evaluation must be between the sixth to seventh ribs at least one hour after ribbing according to the guideline of the JMGA.

Yield Grade is the ratio of meat to dressed carcass weight which is classified into three grade from A to C (A: the highest yield, score of greater than or equal to 72, B: moderate yield, score from 71 to 69, C: lower yield, score of below 69), and the number is calculated by the measurement of rib eye area, rib thickness, subcutaneous fat thickness, and chilled carcass weight (**Equation 1** and **Figure 4**).

Equation 1 Yield Grade equation, from the Japan Meat Grading Association

$\begin{aligned} \text{Yield Grade \%} &= 67.37 + [0.130 \times \text{Rib eye area (cm}^2\text{)}] \\ &+ [0.667 \times \text{Rib thickness (cm)}] \\ &- [0.025 \times \text{Chilled carcass weight (kg)}] \\ &- [0.896 \times \text{Subcutaneous fat thickness (cm)}] \end{aligned}$ <p>* For Wagyu breed or cross bred among Wagyu, 2.049 must be added as the standard.</p>

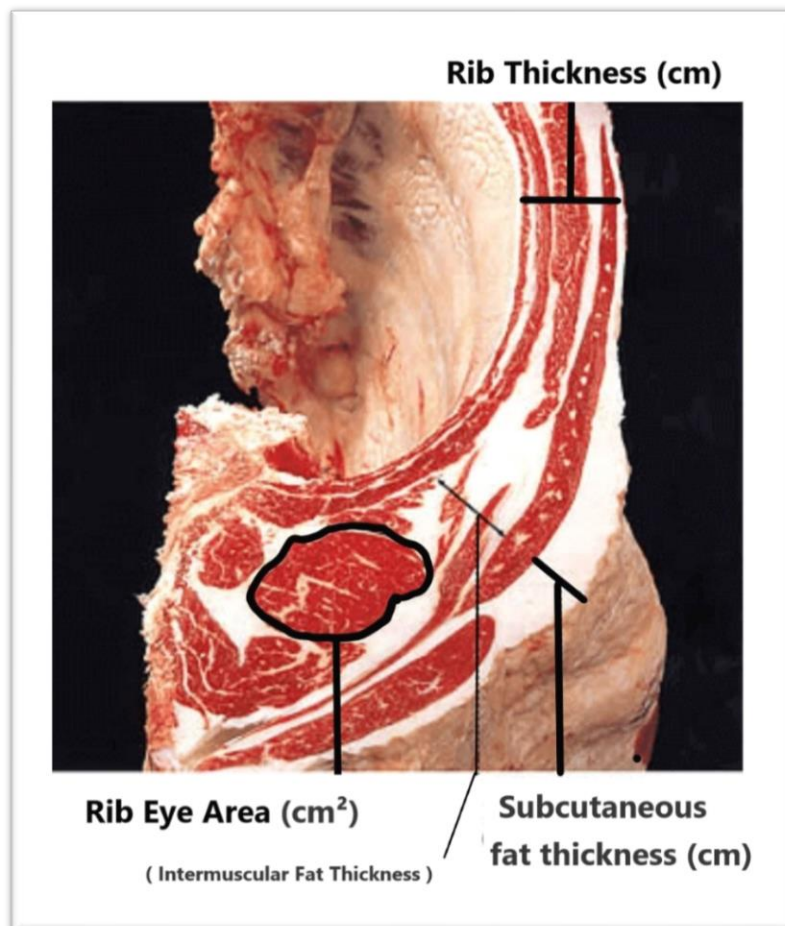


Figure 4 Meat location used for grading carcass, from the Japan Meat Grading Association

Quality Grade consist of grade from 5 to 1, according to the meat marbling, meat color, meat brightness, meat firmness, fat color, fat brightness and fat quality. Beef Marbling Standard (B.M.S) ranges between No.1 to No.12. The Quality Grade 5 (very abundant) correspond to BMS No.8-12, Grade 4 (somewhat abundant) is to BMS No.5-7, Grade 3 (standard) is BMS No.3-4, Grade 2 (somewhat scarce) is BMS No.2, Grade 1 (very scarce) is BMS No.1. (Table 1 and Figure 5). Both Beef Color Standard (B.C.S) and Beef Fat Standard (B.F.S) are classified into scale No. 1 to 5 (Figure 6 and Figure 7), which scores are also taken in consideration to the final Quality Grade.

Table 1 BMS score corresponding to the Quality Grade

B.M.S	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10	No.12
Grade	1	2	3		4			5			

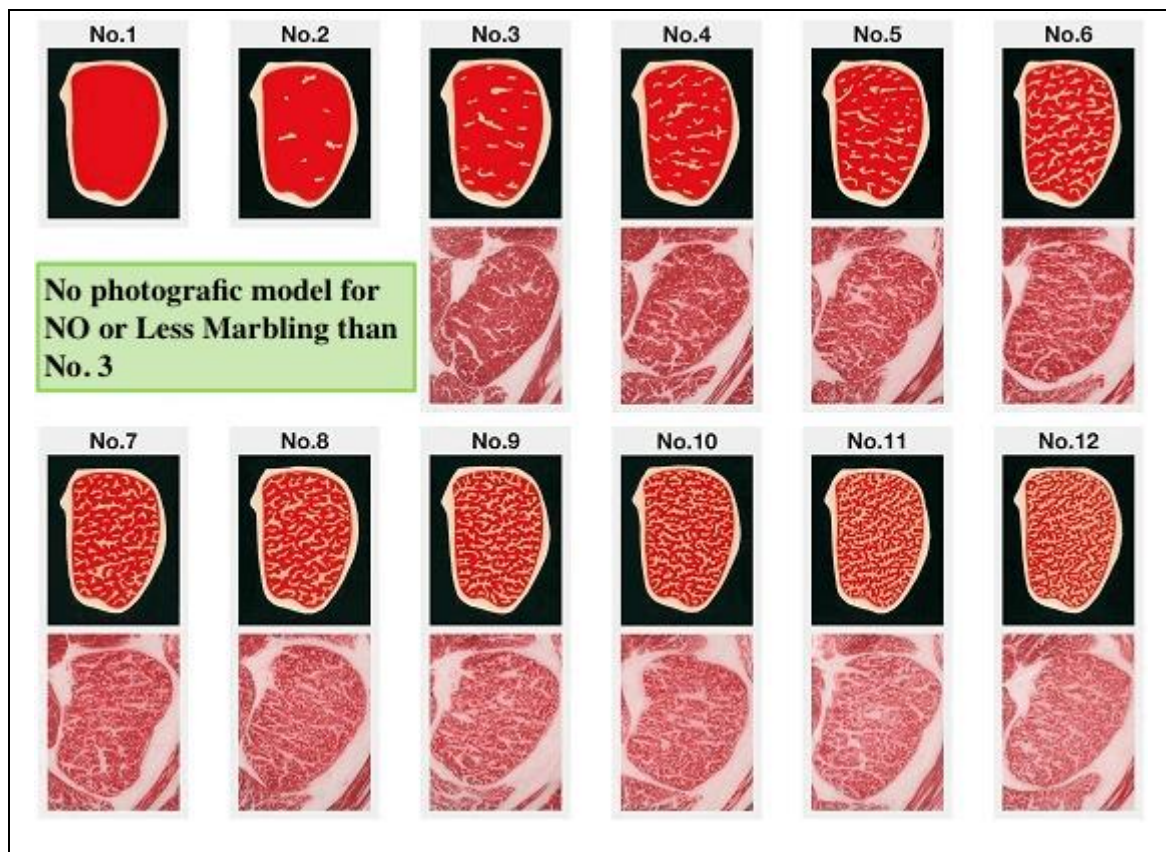


Figure 5 Beef Marbling Standard, silicon model and photographic standard

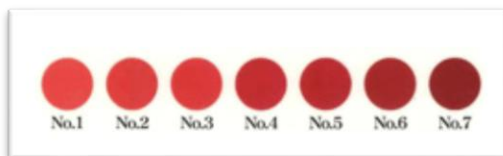


Figure 6 Beef Color Standard

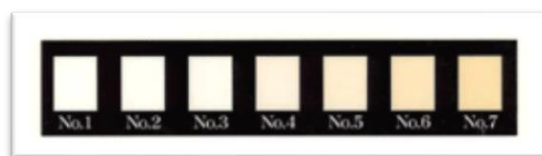


Figure 7 Beef Fat Standard

(Figures from the Japan Meat Grading Association)

After thorough evaluation of the carcasses, the final Grade is given as A5 being the highest quality and C1 the lowest (Table 2). Any pathological condition must be indicated together with the final grade such as hemorrhage, edema, or myelitis. Depends on the grading, the meat value varies in great degree in marketplace.

Table 2 Standard Grading Indication

The Yield Grade	The Quality Grade				
	5	4	3	2	1
A	A5	A4	A3	A2	A1
B	B5	B4	B3	B2	B1
C	C5	C4	C3	C2	C1

2.4 Difference between Wagyu and Kobe beef

Authentic Wagyu products are sealed with “Universal Wagyu Mark” (Figure 8) across the world for the purpose of unified recognition of accredited Wagyu beef and also to avoid misleading of an unreliable beef products labeled as Wagyu to consumers not only in overseas but also in the market in Japan. After the Uruguay Round in 1991, which allowed Japan to import foreign product such as rice, beef, and oranges, created a highly competitive market in Japan. Any product including cross bred with a lineage of Wagyu even in a small degree were labeled as Wagyu lined up on a supermarket shelf where one of the criteria in order to be certified as Wagyu in Japan must be pure bred. According to the Japanese Beef Product Guidebook published by MAFF, it is stated that the Universal Wagyu Mark is given to those products satisfies the following condition; Breed is 1)

Japanese Black, 2) Japanese Brown, 3) Japanese Shorthorn, 4) Japanese Polled, 5) Crossbred resulting from interbreeding of those four breed, or 6) Cross breeds from interbreeding between 1) and varieties 1) – 5) must be issued with proof by means of a registration system in accordance with the Improvement and Increased Production of Livestock Act and through the cattle traceability system. Also breeding condition must be 1) The cattle must be born and bred in Japan, or 2) The ancestry of the cattle must be able to be confirmed by a traceability system.

Frequently, the term Wagyu and Kobe beef are confused amongst consumers not only in overseas but also in Japan. Quite often both terms are mistakenly considered essentially the same. Major difference is that the term Wagyu indicates the breed which includes all of four Japanese Breed Cattle (Black, Brown, Shorthorn, Polled), while the Kobe beef is a brand name of the Japanese Black breed product specifically of Tajima strain which must be born and raised in Hyogo prefecture (Kobe is the capital city) and also must be certified by the Kobe Beef Marketing & Distribution Promotion Association. It was established in 1983 to achieve the in protection and promotion of the Kobe beef. Main tasks are to issue “Kobe Beef Certificate” for those of which qualified its authenticity, to designate retail stores and producers, and to install a bronze Kobe beef statuette (Figure 9-A) to designated retail stores for the proof of the meat qualification. The retail stores and restaurant in Japan as well as in overseas can be checked via website of the Kobe Beef Marketing & Distribution Promotion Association. Customer are also able to ensure its safety and reliability by using 10 digits traceability number. In order to be certified as Kobe beef, the carcass must meet stringent criteria, in addition to being Tajima strain, born and raised, also slaughtered and processed in Hyogo prefecture. It had to be the age between 28 and 60 months of bullock or uncalved cow. Furthermore, carcass

must be graded A or B of the meat Quality Grade (but B.M.S must be No.6 or higher), and 4 or 5 of the Yield Grade after the grading process. At last, gross carcass weight must be 470kg or less. Certified carcasses receive an official purple stamp (Figure 9-B), designed from Chrysanthemum which is a floral emblem for Hyogo prefecture, directly on to the carcasses. Both of Tajima-gyu (beef) and Kobe beef have been registered as Geographical Indications (GI) on the market since the Act on Protection of the Names of Specific Agricultural, Forestry and Fisher Products and Foodstuffs (Geographical Indication (GI) Act) entered in to force in June 2015.



Figure 8 Universal Wagyu Mark, from Ministry of Agriculture, Forestry and Fisheries



A



B

Figure 9 Bronze Kobe Beef Statuette (A) and Kobe Beef trademark (B),

Figures from the Kobe Beef Marketing & Distribution Promotion Association

3. Breeding method

3.1 Traditional breeding

In the early history of selective breeding, it was based on topographic and conformational characteristics. Those offspring that had the closest characteristics to ideal cattle, which is suitable as work force (fur coat, color, skin, horn, hoof, muscle tension) were selected out for the next breeding, while others were eliminated from the pool. As it is mentioned earlier, the purpose of Wagyu breed had made a huge turn since the introduction of agricultural machineries and chemical fertilizer, and meat quality has been focused on the beef industry. Therefore, phenotypic assessment of performance became significantly important. Traits to be analyzed are such as final weight, average daily gain, concentrate intake, roughage intake, ratio of roughage to total feed intake, feed conversion ratio of digestible crude protein, feed conversion ratio of total digestible nutrients, measurement of rib eye area, marbling percentage, milk yield, weaning weight, etc.

3.2 On-station beef bull testing program

Earlier breeding program was explained in (Obata, 1996); Two-stage selection based on the performance and the progeny testing has been practiced on a station testing system since 1968. The first stage is the On-station beef bull testing program. Male progenies from the planned mating are chosen at weaning and transferred to the breeding station in within each prefecture and/or Livestock Improvement Association where they receive controlled feed and unified environment. The selection criteria include growth rate, 365-day body weight, feed efficiency, semen quality, etc. Those initially selected growing bulls can only proceed to the progeny testing. During the program, particular bulls are fattened and evaluated according to the selection criteria including marbling

score at the rib eye area at the level of 6th to 7th rib section, fat thickness, growth rate, etc.; marbling score at the rib eye muscle is given priority in the assessment of performance. These testing program have played a major role in beef cattle breeding, however, there were defects in these programs: 1) The performance of carcass quality traits, especially marbling score was not evaluated in the test in the initial selection at the station, 2) The performance and progeny testing in a station was costly, and 3) Only males were tested. Sasaki (2007) also stated that this evaluation system was no set for comparison between generation, and most of improvement on meat quality in general was due to environmental effect, and genetical change took a part in only a small amount. However, four major factors led to the change of the condition of the testing program: a) new reproductive technologies such as Artificial Insemination (AI), b) innovation in computer technology, c) standardization of carcass grading system, and d) optimum utilization of Japanese beef cattle. As the BLUP (best linear unbiased prediction) procedure has become widely used for estimating breeding value, calculation of large equation on a computer is possible due to the innovation in technology. Advanced in computer technology have contributed significantly to the increase of the power of a personal computer and equally reduced the cost. It is now possible to carry out multi-focal calculations that provide a more accurate prediction of the breeding value throughout the use of cattle pedigree data and that enable to make suitable corrections for environmental factors.

3.3 On-farm progeny testing program

The second stage is the On-farm progeny testing program. All feeder calves that participated in this scheme were ear-tagged with an individual code at the weaning age. When they were slaughtered at the meat market, the date on identified field carcasses

were collected and processed in the central database center (Sasaki, 2007) used to estimate the breeding value for both the sire and the dam by matching pedigree information. There are four major advantages in this program: a) more cattle can be tested, and higher selection pressure and faster improvement achieved. b) the cost of testing is reduced, c) before the performance testing, it is possible to evaluate the performance of young bulls, and d) by planned mating between sire and cow with a high breeding value, it is possible to produce elite breeding stocks. Application of BLUP method by using field data result in great progress in terms of increasing in intramuscular fat content, measurement of rib eye area, rib thickness, and subcutaneous fat thickness.

4. Genome Analysis

4.1 Economical Traits

For long period of time, BLUP was the fundamental method of the improvement of a breed quality. Contemporary breeding method has been shifting the focus on genome analysis along with development of molecular genetics, especially from mid-1980's to 1990's. Meuwissen et al. (2001) introduced the theory of genomic selection. Quantitative Trait Locus (QTL) analysis and Marker Assisted Selection (MAS) widened the possibility of identifying the favorable traits. The success of a MAS scheme depended on how close the marker was to the actual gene locus of interest. The term linkage disequilibrium (LD) was used to indicate the usefulness of a marker. High LD meant that the marker was close to the gene. To be useful, markers needed an LD of 30% or more. High LD means that an allele of the marker is on the same stretch of DNA as the favorable allele of the gene. These markers can be DNA based type such as single nucleotide polymorphisms (SNPs). Determination of the genome responsible for the marbling enables to differentiate between individuals that has higher potential to produce marbling and the one less likely to produce marbling. In the previous studies, following SNPs that are responsible for marbling has been identified.

In the study of (Yamada et al., 2008), following was determined; The *endothelial differentiation sphingolipid G-protein-coupled receptor 1 (EDG1)* gene, involved in blood vessel formation, has been previously shown to be expressed at different levels in musculus longissimus muscle between low-marbled and high-marbled steer groups. It is located within the genomic region of a quantitative trait locus for marbling, and thus was considered as a positionally functional candidate for

the gene responsible for marbling. Two single nucleotide polymorphisms (SNPs) in the 5' untranslated region (UTR) and the 3' UTR of *EDG1*, referred to as c.-312A>G and c.*446G>A, respectively, were detected between the two steer groups. The two SNPs were associated with the predicted breeding value for beef marbling. The effect of genotypes at each of the SNPs on the predicted breeding value for subcutaneous fat thickness was not statistically significant ($P > 0.05$). Reporter gene assays revealed no significant differences in gene expression between alleles at each of the SNPs. These findings suggest that *EDG1* SNPs, although they may not be regarded as a causal mutation, may be useful for effective marker-assisted selection to increase the levels of marbling in Japanese Black beef cattle. Another study by (Yamada et al., 2009a) state that the *titin* (TTN) gene, involved in myofibrillogenesis, referred to as *g.231054C>T*, is associated with marbling and may be useful for effective marker-associated selection to increase the level of marbling. Moreover, by (Yamada et al., 2009b) the *akirin 2* (AKIRIN2) gene containing the c17-25 EST sequence was considered as positional functional candidate for the gene responsible for marbling. A SNP in the 3' untranslated region of the AKIRIN2, referred as c.*188G>A, was the only difference detected between high- and low-marbled steer group. The SNP was associated with marbling in 3 experiments. The effect of genotypes of the SNP on subcutaneous fat thickness was not statically significant ($P>0.05$). These findings suggest that the *AKIRIN 2* SNP polymorphism is associated with marbling and may be useful for effective marker-assisted selection to increase the levels of marbling. At last, following was also determined by (Yamada et al., 2009c); the c2-11#2 expressed sequence tag (EST) has been previously shown to possess

expression difference in musculus longissimus muscle between low-marbled and high-marbled steer groups, and to be located within genomic region of a quantitative trait locus for marbling. Thus, the *ribosomal protein L27a (RPL27A)* gene containing the c2-11#2EST sequence was considered as a positional candidate for the gene responsible for marbling. In the present study, a single nucleotide polymorphism (SNP) in the promoter region of the RPL27A, referred to as g.3109537C>T, was detected between the 2 steer groups. The SNP was associated with the predicted breeding value for beef marbling standard number by the analyses using Japanese Black beef cattle population. The effect of genotypes of the SNP on the predicted breeding value for subcutaneous fat thickness was not statistically significant. These findings suggest that the RPL27A SNP may be useful for effective marker-assisted selection to increase the levels of marbling.

4.2 Defective Hereditary Traits

Genome analysis enable to determine not only the favorable traits but also unfavorable defective traits which also effects on economic value on a market. According to the list by MAFF, followings are the nationally monitored defective traits of Japanese Black cattle;

- 1) Band 3 deficiency (B3): B3 deficiency is a hereditary disease results in hemolytic anemia, spherocytosis, splenomegaly, retarded growth, acidosis. As B3 is the transmembrane glycoprotein in mammalian red blood cells, it contributes to many physiological activities, therefore it is essential to the survival of animals (Inaba et al., 1996).

- 2) Bovine Blood Coagulation Factor XIII Deficiency (F13): This disorder is where coagulation factor XIII is missing or reduced, hence the symptoms include severely prolonged bleeding time, and calves usually die in several days after the parturition due to hemorrhages and hematoma.
- 3) Claudin-16 Defect (CL16): A homozygous deletion of gene is transmitted by autosomal recessive inheritance causing severe renal failure, growth retardation, and overgrowth of hooves in young animal. This condition in Japanese Black is also called renal tubular dysplasia or bovine chronic interstitial nephritis with diffuse zonal fibrosis. It occurs particularly in bulls with high meat quality therefore it leads to a large economical loss (Okada et al., 2005). Most of the time death occurs within several months of age. There are Type 1 and Type 2, and both show the same symptoms.
- 4) Isoleucyl-tRNA synthetase (IARS): Substitution of IARS c.235G>C (p.Val79Leu) is the causative mutation for neonatal weakness with intrauterine growth retardation known as perinatal weak calf syndrome (Hirano et al., 2013). Calves with this syndrome exhibit such as anemia, depression, weakness, variable body temperature, ataxia, difficulty nursing, growth retardation, and increased susceptibility to infection, and the pathological features of perinatal weak calf syndrome are anaemia with bone marrow dysfunction and foeto-placental dysfunction.
- 5) Bovine Bartter syndrome type 1 (BAS1): Mutation in solute carrier family 12, member 1 (SLC12A1) causes hydrallantois, which is the excessive accumulation of fluid within the allantoic cavity in pregnant animals and is associated with fetal mortality. It is indicated that (Sasaki et al., 2016) in kidneys of

hydrallantois-affected fetuses, the mutation in SLC12A1 impaired the apical membrane localization of SLC12A1 and reabsorption of $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ in the thick ascending limb of the loop of Henle, leading to a defect in the concentration of urine via the countercurrent mechanism. Consequently, the affected fetuses exhibited polyuria that accumulated in the allantoic cavity. It often causes a death of both dam and fetus.

- 6) Forelimb-gridle muscular anomaly (FMA): It is due to nonsense mutation of GFRA1 gene (GDNF family receptor alpha) which located at the center of bovine chromosome 26, and it is an autosomal recessive disorder of Japanese black cattle characterized by tremor, astasia, suckling difficulty, and abnormal shape of the shoulders with swelling of scapula. Pathological examination of affected animals reveals hypoplasia of forelimb (Watanabe, 2017).
- 7) Molybdenum cofactor sulfurases deficiency (MCSU): It is a xanthine metabolic disorder controlled by autosomal recessive gene in the Japanese Black cattle (Fujita et al., 2004). Xanthine is not metabolized and accumulated in the body resulting urolithiasis causing renal failure. No abnormality is detected at the birth, but around two months of age retarded growth and overgrowing of hooves are observed due to renal failure. Most of the calves showing the symptom die by the age of 8 months.
- 8) Multiple ocular defect (MOD): It is an autosomal recessive hereditary disorder caused by one-nucleotide insertion in the WFDC1 gene at the proximal region of bovine chromosome 18 that is characterized by dysplasia of the lens, retinal detachment, persistence of the hyoid artery, and microphthalmia (Abbasi et al., 2009).

- 9) Chediak-Higashi syndrome (CHS): It is an autosomal recessive hereditary disorder in caused by a mutation of the Lyst gene, which the mutation can be detected by real-time PCR with TaqMan minor groove binder probes (Abdeen et al., 2013). It is related to abnormality of platelet causing slight coagulation problem, and affected cattle have a deficiency in cells that make up a functional immune system. These cattle may also have a light coat color and red eye has been reported. This disorder is usually not lethal.

As these traits are nationally monitored defective traits, it is highly recommended for the owners to take a sample of own cows for the DNA examination also for carrying our more efficient breeding plan and achieving in high productivity. Owners can apply to this DNA analysis through the Division of Animal Genetics, Maebashi Institute of Animal Science, Livestock Improvement Association of Japan, Inc..

5. Genome-editing technology

Current breeding method using genome editing technology is evolving with remarkable speed. Genome modification tools including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas system (RNA-guided engineered nucleases), which act as so called “genetic scissors” enables targeted genetic modifications which correct and edit genetic defects (Ain et al., 2014; Kim et al., 2014).

CRISPR/Cas system is found in bacteria and archaea of their adaptive immune defense against foreign DNA through the RNA guided DNA cleavage system. CRISPR/Cas system is known that it is much easier in generating the endonuclease compare to other nucleases, ZFN and TALEN (Ding et al., 2013; Ain et al., 2014), in other words, ZFNs and TALENs are artificial DNA cutting enzymes (nucleases) with a DNA–protein binding domain that directs the nucleases to a target sequence in the genome. CRISPR/Cas9 adopts a separate type of DNA-RNA binding system that can be readily prepared in most laboratories. Thus, the use of CRISPR/Cas9 has been particularly spreading worldwide (Ishii, 2017). One of the defective traits mentioned above, IARS, which is a recessive disease of Japanese Black cattle caused by a single nucleotide substitution resulting in calf with low birth body weight, anorexia, unable to stand, delay in growth; also known as Week Calf Syndrome, was successfully repaired (Ikeda et al., 2017) by using CRISPR/Cas9-assisted genome editing, and somatic cell cloning technology. Another example of gene-editing is the production of hornless bull (Young et al., 2019). In the study, genome editing followed by reproductive cloning was previously used to produce two hornless dairy bulls. The background of this is that in the current US

dairy cattle industry, destruction of horn-producing cells before they grow and attach to the skull (disbudding) is a routine practice to prevent horn growth because animals that do not have horns do not injure other animals, require less feeding trough space, are less dangerous to handle and transport than horned animals and have fewer aggressive behaviors. Disbudding is an unpleasant process that has important implications for animal welfare, and many stakeholder groups have campaigned for alternative, humane solutions. One option is to select and breed animals that do not have horns, a phenotype referred to as polled. However, in order to use these biotechnology, further investigation has to be done to avoid any problems such as off-target alterations, unanticipated insertions, substitutions or deletions.

Recent study discovered so called “fetal and neonatal programming,” which is based on the developmental origins of health and disease that have shown alterations to the fetal and early postnatal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology, and metabolism of the affected animals during adult life. What has been trying currently in Japan (Gotoh et al., 2018) is that a metabolic programming and Internet of Things (IoT) management system for Wagyu beef production in order to produce safe, high-quality Wagyu beef using domestic grass and pasture resources.

6. Consequences continues breeding selection and gene-editing

After the successful utilization of BLUP method, meat quality increased significantly. Artificial Insemination also enhanced the speed of spreading of a selected superior genome across the entire country. Same genome type is used quite frequent in Wagyu industry, consequently, it has been losing the genetic diversity with increasing inbreeding coefficient. It must be considered that losing genetic diversity is a risky fate for the breed. A good example is the Panama disease over the “Gros Michel” banana plantation crops (Ordóñez et al., 2015) that developed major epidemics in 1900’s. The breeding method had changed from asexually through rhizomes to tissue culture that has enabled mass production of cultivars, which rollout the genetically identical plants around the world. However, the vulnerability of monocultures to the disease has result in chaos on banana production over the last century. Another example is reduced genetic diversity among Holsteins in North America (Yue et al., 2015). The study suggested minimal genetic diversity on the Y chromosome which indicates that intensive selection for improving milk production and conformation led to a reduction of genetic diversity.

As biotechnology develops remarkably, expectation also increases such as cure of disease like HIV (Dash et al., 2019) or elimination of the defective traits. However, there are several issues that need to be considered, for example, accuracy and safety level of the technology, ethical aspect, and regulations. Rodriguez (2016) stated that the efficacy of CRISPR/Cas9 technique to obtain precise genetic modifications makes more difficult to identify a genetically modified organism once outside the lab and also to regulate this organism in the market.

7. Discussion

Wagyu as a breed had adjusted its phenotype characteristics according to economic changes and what people demand. Prior to the Meiji Restoration in 1868, Japan as a country was closed to foreign trade. Eating beef was not part of culture due to the religious influence of Buddhism, and it was forbidden for 1200 years except for medicinal use. Wagyu had a significant role, not for the beef production, but in agriculture as a labor source. During Meiji era, Japanese culture fundamentally changed under the influence of American and European cultural influence. Especially after World War II, westernization of eating habits proceeded intensively that the demand for meat production continuously grew. A period of inflated economic growth starting from 1987 established a societal trend which selected Wagyu as a luxury food. People preferred the marbled beef like never before. At the same time, breeding technology was improved in great degree. Early breeding method which predicted the meat quality based on topographical characteristics changed to on-station bull testing program because it had disadvantage that the quality of the meat is only evaluated only after slaughtering therefore further breeding is not possible even though it produced good meat quality. With on-station bull testing program, the method still requires evaluation in order to produce elite cattle efficiently; therefore, nationwide analysis and testing for the potential superior bull sample was gathered under the governmental project. It enabled to predict the breeding value over a massive scale of data, which led to the best semen distributed across the country for AI using frozen semen. Innovation in computer technology enhanced the development of this technology, led to more accurate estimated breeding value estimates. Although applying the BLUP method using the field data resulted in progress within the Wagyu beef sector, the lengthy waiting

period to get the result with the enormous cost had to be resolved in order to be economically efficient. By the 1980's, modern technology of molecular genetics had developed to the point that it made possible to determine the responsible gene for the marbling based on QTL analysis and utilization of SNPs. Identifying the economical traits leads to the beneficial outcome in terms of selecting the bull to be used for the breeding plan with better efficiency and quality. Elimination of defective hereditary traits during the selection based on DNA analysis will further provide financial benefit to the producer since it reduces production loss. It is also beneficial for the pedigree. However, it must be taken into a consideration that there are also risks requiring solving in future studies. Continuous selection of the elite gene leads to the loss of genome diversity, which threatens the long-term viability of the breed, as it was mentioned earlier, the example of reduced genetic diversity among Holsteins in North America and the epidemic case of the Panama disease against "Gros Michel" banana plantation crops. The major shift from working type to beef production in the past 75 years was successful due to the new alleles introduced into the breed during cross breeding with imported western cattle breeds in the past.

Favorable traits and unfavorable traits have been determined, also tools to edit these genome have already introduced with current highly sophisticated biotechnology, even though these tools still requires further investigation to be used in the industry, it is a matter of time that we see beef packages from a same strain of cattle with the best carcass yielding, the shortest feeding period, and A5 class marbling quality with no genetic defect which was produced by gene-editing. One disease that no one have even thought that could kill an animal may end up wiping off the entire herd, even in nationwide level if there is no other gene that can survive during the outbreak. In

epidemiological aspect, we are already facing an outbreak threat in such case of Foot and Mouth Disease (FMD) in Miyazaki prefecture in 2010, which ended up culling about 290,000 animals in two months (Muroga et al., 2012). Furthermore, favorable traits can change over the time. As a matter of fact, people today care more on healthy diet, food safety, and eco-friendly industry products compare to the past. According to the study on eating preference for marbling (Sasaki et al., 2017) it concluded “In particular, moderately marbled beef, which could be called ‘leaner beef’ compared with Wagyu beef, is a potential new category in the Japanese beef market.” As the average lifespan increases, people care more on the quality of daily nutrition that especially associate with a lifestyle-related disease such as arteriosclerosis and diabetes mellitus. As it was mentioned above, the fate of Wagyu has been always depending on the demands and trends on the marketplace over the years as well as in any other industry. Wagyu must be ready for a next turning point with genetic flexibility. Current gene-editing technology is developing in remarkable speed. However, establishment of new regulations and official examinations for detecting edited gene are not following up. I think it is hard enough to establish a new regulation in a country, and establishing an international standards or regulation is even more difficult, especially when it is based on ethical problem because each country has different culture and background. As long as there is an economical interest, research will keep going without slowing down, so it is urgent to develop the regulations and their associated formal examinations. I would also add another agenda to it which is to apply the gene-editing technology to widen the genetic diversity in order to maintain diverse DNA database for the future.

8. Summary

A term “Wagyu” can be divided into two words “Wa” and “Gyu”; “Wa” indicates Japan or Japanese culture and “Gyu” means Cattle in Japanese. Wagyu literally means “Japanese cattle.” Wagyu consist of four breeds; Japanese Black, Japanese Brown, Japanese Shorthorn, and Japanese Polled. Today, Wagyu often referred to the Japanese Black cattle, which are known for their high marbling intramuscular fat content, because they contribute to more than 95% of the beef products in Japan. Essentially, certain cattle that fulfill requirements such as purebred, can only be labeled as Wagyu, but it is not necessary the same standard outside of Japan. This paper is a review of developmental history of the Japanese Black Cattle and their breeding methods over the past years, also discussion regarding to future prospect of their breeding technology and industry.

Bibliography

Abbasi A., Khalaj M., Tsuji T., Tanahara M., Uchida K., Sugimoto Y. and Kunieda T., 2009: A mutation of the WFDC1 gene is responsible for multiple ocular defects in cattle. *Genomics*, 94(1), pp.55-62.

Abdeen A., Sonoda H., Kobayashi I., Kitagawa G. and Ikeda M., 2013: A New Method for Rapid Detection of the Mutant Allele for Chediak-Higashi Syndrome in Japanese Black Cattle. *Journal of Veterinary Medical Science*, 75(9), pp.1237-1239.

Ain Q., Chung J. and Kim Y., 2015: Current and future delivery systems for engineered nucleases: ZFN, TALEN and RGEN. *Journal of Controlled Release*, 205, pp.120-127.

Dash P., Kaminski R., Bella R., Su H., Mathews S., Ahooyi T., Chen C., Mancuso P., Sariyer R., Ferrante P., Donadoni M., Robinson J., Sillman B., Lin Z., Hilaire J., Banoub M., Elango M., Gautam N., Mosley R., Poluektova L., McMillan J., Bade A., Gorantla S., Sariyer I., Burdo T., Young W., Amini S., Gordon J., Jacobson J., Edagwa, B., Khalili K. and Gendelman H. 2019: Sequential LASER ART and CRISPR Treatments Eliminate HIV-1 in a Subset of Infected Humanized Mice. *Nature Communications*, 10(1).

Ding Q., Regan S., Xia Y., Oostrom L., Cowan C., and Musunuru K., 2013: Enhanced Efficiency of Human Pluripotent Stem Cell Genome Editing through Replacing TALENs with CRISPRs. *Cell Stem Cell*, 12(4), pp.393-394.

Fujita T., Ito M., Sato W., Kurahara T., Miyake T., Shiga K., and Sasaki Y., 2004: Association between the deletion mutant allele of MCSU deficiency and carcass traits in Japanese Black cattle. *The journal of animal genetics*, 32(1), pp.11-16. (In Japanese)

Gotoh T., Nishimura T., Kuchida K., and Mannen H., 2018: The Japanese Wagyu beef industry: current situation and future prospects — A review. *Asian-Australasian Journal of Animal Sciences*, 31(7), pp.933-950.

Hirano T, Kobayashi N, Matsushashi T, Watanabe D, Watanabe T, Takasuga A, et al. (2013) Mapping and Exome Sequencing Identifies a Mutation in the *IARS* Gene as the Cause of Hereditary Perinatal Weak Calf Syndrome. *PLoS ONE* 8(5). [online] Available at: <https://doi.org/10.1371/journal.pone.0064036> [Accessed 15 Nov. 2019]

Hirooka H., 2010: Evolution of breeding value estimation -From index selection to genomic selection-. *The Journal of Animal Genetics*, 38,pp.93-98. (in Japanese)

Ikeda M., Matsuyama S., Akagi S., Ohkoshi K., Nakamura S., Minabe S., Kimura K., and Hosoe M., 2017: Correction of a Disease Mutation using CRISPR/Cas9-assisted Genome Editing in Japanese Black Cattle. *Scientific Reports*, 8(1). [online] Available at: <https://www.nature.com/articles/s41598-017-17968-w> [Accessed 10 Nov. 2019].

Inaba M., Yawata A., Koshino I., Sato K., Takeuchi M., Takakuwa Y., Manno S., Yawata Y., Kanzaki A., Sakai J., Ban A., Ono K., and Maede Y., 1996: Defective anion transport and marked spherocytosis with membrane instability caused by hereditary total deficiency of red cell band 3 in cattle due to a nonsense mutation. *Journal of Clinical Investigation*, 97(8), pp.1804-1817. (in Japanese)

Ishii T., 2017: Genome-edited livestock: Ethics and social acceptance. *Animal Frontiers*, 7(2), pp.24-32.

Japan Meat Grading Association (JMGA): Equation and meat grading standards. [online] Available at: <http://www.jmga.or.jp/standard/beef/> [Accessed 10 Nov. 2019]. (In Japanese)

Japan Meat Information Service Center (JMISC): Pictures of Japanese Cattle. [online] Available at: <http://www.jmi.or.jp/en/info/index1.html> [Accessed 10 Nov. 2019].

Kim H., and Kim J., 2014: A guide to genome engineering with programmable nucleases. *Nature Reviews Genetics*, 15(5), pp.321-334.

Kobe Beef Marketing & Distribution Promotion Association: Bronze Kobe Beef statuette and Kobe Beef trademark. [online] Available at: <http://www.kobe-niku.jp/contents/certification/index.html> [Accessed 10 Nov. 2019].

Mannen H., 2017: The genetic diversity of Japanese Wagyu using molecular markers. *Journal of Animal Breeding and Genomics*, 1(1), pp.17-22.

Matsuishi M., Fujimori M., and Okitani A., 2001: Wagyu Beef Aroma in Wagyu (Japanese Black Cattle) Beef Preferred by the Japanese over Imported Beef. *Animal Science Journal*, 72(6), pp.498-504.

Meuwissen T.H.E., Hayes B.J., Goddard M.E., 2001: Prediction of Total Genetic Value Using Genome-wide Dense Marker Maps. *Genetics*, 157, pp.1819-1829

Ministry of Agriculture, Forestry and Fisheries (MAFF): List of hereditary defective traits. [online] Available at: http://www.maff.go.jp/j/chikusan/sinko/lin/l_katiku/ [Accessed 10 Nov. 2019]. (In Japanese)

Ministry of Agriculture, Forestry and Fisheries (MAFF): Wagyu mark. [online] Available at: <http://www.maff.go.jp/e/export/wagyu/mark.html> [Accessed 10 Nov. 2019].

Muroga N., Hayama Y., Yamamoto T., Kurogi A., Tsuda T. and Tsutsui T. 2012: The 2010 Foot-and-Mouth Disease Epidemic in Japan. *Journal of Veterinary Medical Science*, 74(4), pp.399-404.

Obata T., Takeda H., Satoh M., and Wada Y., 1996: Progress in Breeding Techniques for Effective Beef Cattle Production in Japan. *Japan Agricultural Research Quarterly*, 30, pp.203-211.

Okada K., Ishikawa N., Fujimori K., Goryo M., Ikeda M., Sasaki J., Watanabe D., Takasuga A., Hitano T., and Sugimoto Y., 2005: Abnormal Development of Nephrons in *Claudin-16*-Defective Japanese Cattle. *The Journal of Veterinary Medicine Science*. 67(2):171-178.

Ordonez N., Seidl M., Waalwijk C., Drenth A., Kilian A., Thomma B., Ploetz R., and Kema G., 2015: Worse Comes to Worst: Bananas and Panama Disease -When Plant and Pathogen Clones Meet. *PLOS Pathogens*, 11(11). [online] Available at: <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1005197> [Accessed 15 Nov. 2019].

Rodriguez E., 2016: Ethical Issues in Genome Editing using Crispr/Cas9 System. *Journal of Clinical Research & Bioethics*, 07(02). [PDF] Available at : <https://www.longdom.org/open-access/ethical-issues-in-genome-editing-using-crisprcas9-system-2155-9627-1000266.pdf> [Accessed 10 Nov. 2019].

Sasaki K., Ooi M., Nagura N., Motoyama M., Narita T., Oe M., Nakajima I., Hagi T., Ojima K., Kobayashi M., Nomura M., Muroya S., Hayashi T., Akama K., Fujikawa A., Hokiyama H., Kobayashi K., and Nishimura T., 2017: Classification and characterization of Japanese consumers' beef preferences by external preference mapping. *Journal of the Science of Food and Agriculture*, 97(10), pp.3453-3462.

Sasaki S., Hasegawa K., Higashi T., Suzuki Y., Sugano S., Yasuda Y., and Sugimoto Y., 2016: A missense mutation in solute carrier family 12, member 1 (SLC12A1) causes hydrallantois in Japanese Black cattle. *BMC Genomics*, 17(1). [online] Available at: <https://bmegenomics.biomedcentral.com/articles/10.1186/s12864-016-3035-1> [Accessed 15 Nov. 2019]

Sasaki Y., 2007: Selection criterion for Wagyu cattle -visual judging to gene diagnosis-. *The Journal of Animal Genetics*, 35(1), pp.33-49. (in Japanese)
Wagyu Registry Association (WRA), 2019: Wagyu dayori, 49 [online] Available at: http://cgi3.zwtk.or.jp/wp/wp-content/uploads/2019/10/wagyudayori_49.pdf [Accessed 10 Nov. 2019].

Watanabe D., 2017: Current topics in nationally designated defective hereditary traits of Japanese Black cattle - isoleucyl-tRNA synthetase deficiency, Bartter syndrome and forelimb-girdle muscular anomaly -. *Japanese Journal of Large Animal Clinics*, 8(1), pp.1-6. (in Japanese)

Yamada T., Itoh, M. Nishimura S., Taniguchi Y., Miyake T., Sasaki S., Yoshioka S., Fujita T., Shiga K., Morita M., and Sasaki Y., 2008 : Association of single nucleotide polymorphisms in the endothelial differentiation sphingolipid G-protein-coupled receptor 1 gene with marbling in Japanese Black beef cattle. *Animal Genetics*, 40, pp.209-216.

Yamada T., Sasaki S., Sukegawa S., Miyake T., Fujita T., Kose H., Morita M., Takahagi Y., Murakami H., Morimatsu F., and Sasaki Y., 2009c: Association of a single nucleotide polymorphism in ribosomal protein L27a gene with marbling in Japanese Black beef cattle. *Animal Science Journal*, 80, pp.631-635.

Yamada T., Sasaki S., Sukegawa S., Miyake T., Fujita T., Morita M., Ohta T., Takahagi Y., Murakami H., Morimatsu F., and Sasaki Y., 2009b: Association of a single nucleotide polymorphism in akirin 2 gene with marbling in Japanese Black beef cattle. *BMC Research Notes*, 2, p.131.

Yamada T., Sasaki S., Sukegawa S., Yoshioka S., Takahagi Y., Morita M., Murakami H., Morimatsu F., Fujita T., Miyake T., and Sasaki Y., 2009a: Association of a single nucleotide polymorphism in titin gene with marbling in Japanese Black beef cattle. *BMC Research Notes*, 2, p.78.

Yonesaka R., Sasazaki S., Yasue H., Niwata S., Inayoshi Y., Mukai F., and Mannen H. 2016 : Genetic structure and relationships of 16 Asian and European cattle populations using DigiTag2 assay. *Animal Science Journal*, 87, pp.190-196.

Young A., Mansour T., McNabb B., Owen J., Trott J., Brown C., and Van Eenennaam A. 2019: Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. *Nature Biotechnology*. [online] Available at: <https://www.nature.com/articles/s41587-019-0266-0> [Accessed 10 Nov. 2019]

Yue X., Dechow, C. and Liu W. 2015: A limited number of Y chromosome lineages is present in North American Holsteins. *Journal of Dairy Science*, 98(4), pp.2738-2745.

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