

# Revelation of Wagyu's genetics of marbling by melting subcutaneous lipids and intramuscular temperature

<sup>1</sup>Dr Samia Tariq, <sup>2</sup>Dr Nusrat Shaheen, <sup>3</sup>Dr Shaqiaq Hasrat, <sup>4</sup>Tooba Qaiser, <sup>5</sup>Dr Hassan Waheed, <sup>6</sup>Dr Aqsa Mustqeem, <sup>7</sup>Dr Fahmida Khatoon, <sup>8</sup>Kashif Lodhi

<sup>1</sup>Zaman Medical Complex Trarkhel AJK,

<sup>2</sup>AJK Medical College Muzaffarbad,

<sup>3</sup>Aims Hospital Mzd AJK,

<sup>4</sup>Divisional headquarters Mirpur AJ&K,

<sup>5</sup>Bahawal Victoria hospital Bahawalpur,

<sup>6</sup>Ayub Teaching Hospital Abbtabad,

<sup>7</sup>Associate professor, Department of Biochemistry, College of Medicine University of Hail, KSA,

<sup>8</sup>Department of Agricultural, Food and Environmental Sciences. Università Politécnica delle Marche Via Brecce Bianche 10, 60131 Ancona (AN) Italy,

## Abstract

**Objective:** It is largely assumed as inheritance that intramuscular lipid disposition or extreme marbling are correlated with the Wagyu breeds. This research aimed at the evaluation of intramuscular fat and subcutaneous lipids melting temperature as an alternative to visual marbling scores.

## Materials and Methods

Two cohorts were taken including Wagyu full blood sires, varied feed times for the measurement of fat extraction and melting temperature. Animal carcasses were harvested for postmortem fat and meat samples for food production. Within 100% Wagyu full blood prepared under standard conditions the marbling degree scattering is unpredictable. To measure the difference among the lower marbling breeds who were fed for short periods the melting temperature of the striploin and subcutaneous fat samples was compared. This research shows that the melting temperature among the long fed Wagyu prepared under standard conditions is of considerable range. No ethical approval was required in this case.

## Results

The variation is genetic due to the major impact of the individual sires instead of the random error or environmental error. Melting temperature of 50% Wagyu rapidly decreased from 100<sup>th</sup> to 150<sup>th</sup> day of supplementary feeding; 100% European when compared with the 50% European crosses. It speaks for the useful impact of the genetics and considered as an improvement. It is highly penetrant, predictable and useful. Melting temperature was not affected by the contemporary extraction of DNA. Therefore, substitution can be eliminated and provenance can be traced cost-effectively and simply.

## Conclusion

The research concludes that there is melting temperature difference between progeny of the two sires of full blood Wagyu. Both the sires also share paternal and maternal grand. Lipid composition difference finding is not possible through alone Pedigree analysis. It is observable that the C19 haplotypes of the sire 1 and 2 are different. Lipid profile and marbling degree also differ due to site of sampling which is a

major unresolved issue. The samples collected for the comparison require fixed location extraction as the samples showed variation even within the same muscle group. Here it is also shown that the melting temperature is inheritable and the DNA sampling can also be done on the same fat sample without affecting melting temperature. It is recommended to enhance experience by taking vivo samples to monitor feed, genetics and time.

**Keywords:** Wagyu, Melting Temperature, Sire, Intramuscular Fat, Genetics, Subcutaneous Lipids.

## INTRODUCTION

Marbling refers to the intramuscular fat accumulation. It is crucial for the beef procedures, customers and chefs. There is no standard definition or the agreed upon process of its measurement [1]. Countless attempts have been made to increase the reproducibility of scanning and visual scores for superior taste and health benefits but less success has been achieved [2]. Highly marbled samples of the lipid profiles show increased content of the oleic acid and reduced melting temperature [3, 4]. A high and precise method also exists to measure the melting temperature which has also been used here to explore environmental factors and genetics complex interplay [5]. This process is often used by the producer for the utmost satisfaction of the conscious consumer. There is a faithful inheritance of genetically determined traits in Wagyu breeds due to the presence of high oleic content and marbling [6 – 8]. The qualification of breed as superior has not been defined through identified markers in individual dams and sires. The slow progress reasons include complex interaction of metabolic processes [9], multiple small effect genes contribution [10], supplementary feeding and uncontrolled environmental factors [11], difficult quantification of the marbling reproducibility [12], paddock to plate unreliable meat tracing and dangerous perception of fat. It has also been established that the low fat diets had not improved health [13]. Low melting temperature and increased oleic content are preferable for lipid profiles [14]. Here it is also shown that the melting temperature is inheritable and the DNA sampling can also be done on the same fat sample without affecting melting temperature. It is largely assumed as inheritance that intramuscular lipid disposition or extreme marbling are correlated with the Wagyu breeds. This research aimed at the evaluation of intramuscular fat and subcutaneous lipids melting temperature as an alternative to visual marbling scores.

## Materials and Methods

Two cohorts were taken including Wagyu full blood sires, varied feed times for the measurement of fat extraction and melting temperature. Animal carcasses were harvested for postmortem fat and meat samples for food production. Within 100% Wagyu full blood prepared under standard conditions the marbling degree scattering is unpredictable. To measure the difference among the lower marbling breeds who were fed for short periods the melting temperature of the striploin and subcutaneous fat samples was compared. This research shows that the melting temperature among the long fed Wagyu prepared under standard conditions is of considerable range. No ethical approval was required in this case. A total of 126 Wagyu in two cohorts were fed with proprietary ration for 300 ( $\pm 20$ ) days. This feedlot was commercial. Between the 10<sup>th</sup> and 11<sup>th</sup> rib meat samples (one gram) were taken from longissimus dorsi. Which were assessed for marbling score, progeny and sire comparison. The outcomes have been tabulated. Intramuscular fat was extracted for the measurement of melting temperature from samples by proteinase K digestion. This method allows intramuscular fat simultaneous extraction. Samples were incubated, digested, dissolved and centrifuged. Standard salting method was used for the DNA extraction. For the comparison of melting temperature, the fat was extracted through rendering and digestion methods. Thermocycler method was used for the determination of melting temperature for all fat samples in triplicate.

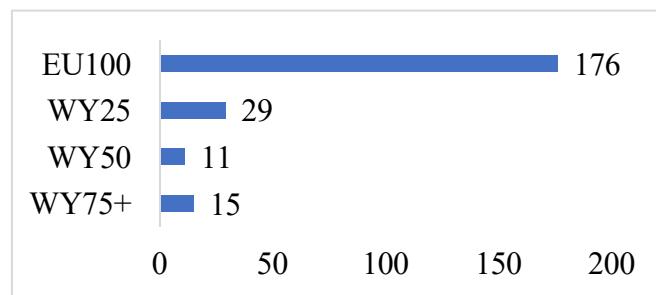
## Results

Sire affects the melting temperature. Cohorts did not significantly differ for initial melting temperature, genetics and feeding duration. Melting temperature of the progeny consistently fell for Sire 1. There was a major environmental effect on the marbling and melting temperature. In terms of quantitative effect of feeding, breed groups were also compared for the analysis of the complex interaction between environment and genetics. Different breeds were also compared in the control group that included EU100

and AK50. The effect of feeding is also clear in the outcomes. The variation is genetic due to the major impact of the individual sires instead of the random error or environmental error. Melting temperature of 50% Wagyu rapidly decreased from 100<sup>th</sup> to 150<sup>th</sup> day of supplementary feeding; 100% European when compared with the 50% European crosses. It speaks for the useful impact of the genetics and considered as an improvement. It is highly penetrant, predictable and useful. Melting temperature was not affected by the contemporary extraction of DNA. Therefore, substitution can be eliminated and provenance can be traced cost-effectively and simply. Detailed outcomes have been tabulated.

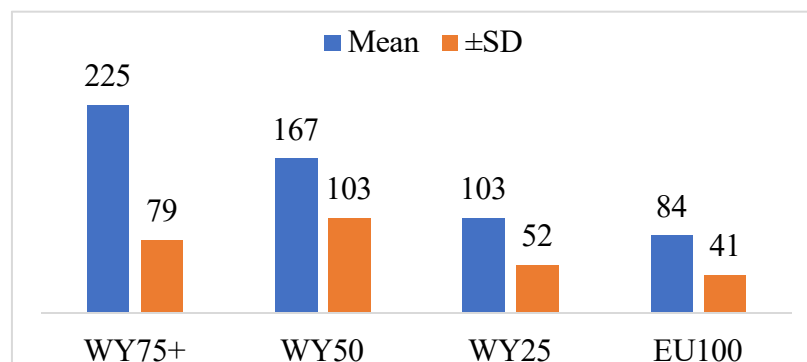
**Table – I:** Comparison of Number

Breed	WY75+	WY50	WY25	EU100
Number	15	11	29	176



**Table – II:** Mean and Standard Deviation

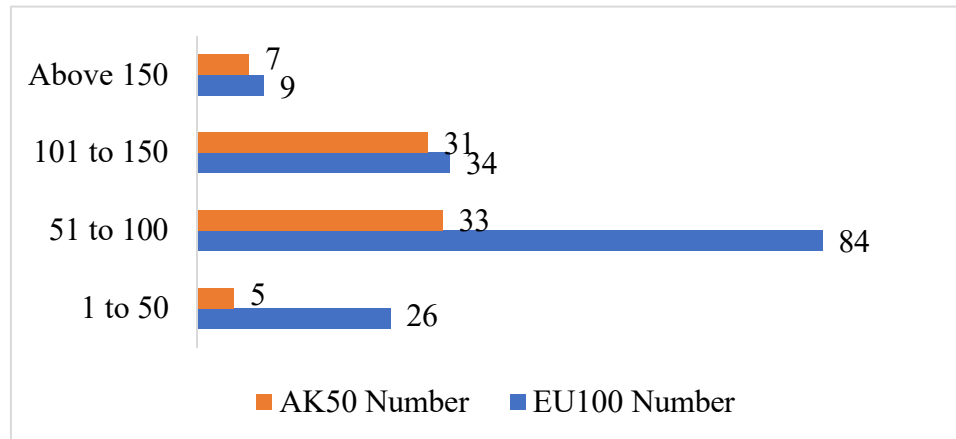
DOF	Mean	±SD
WY75+	225	79
WY50	167	103
WY25	103	52
EU100	84	41



**Table – III:** Comparison of Feed Days

Feed Days	EU100 Number	AK50 Number
1 to 50	26	5
51 to 100	84	33

101 to 150	34	31
Above 150	9	7



## Discussion

The aim of this research was to resolve issues concerning confusion manifested in the mind of the producer and the consumer about the healthy beef. The superiority of the Wagyu beef is clear which is reflected in the form of commercial returns. It is highly marbled beef but the increased use of this beef is also prone to be misused. The lack of the reproducible measurement of the marbling degree is a major issue in this regard. For the advantage of some sectors multiple scoring systems have been retained which have also proved incompatible. It is possible at the successive production stages to measure melting temperature for the confirmation of the quality. DNA can also be extracted for the confirmation of the provenance. It is also important to give due consideration to the difficulty faced by the breeder. The non-reproducible measurement also complicates the identification of the breed values which is also a confound selection. The issue remained more relevant for the upgrade of the first crosses. The outcomes show that even WY25 can have reduced melting temperature but the scatter shows considerable consistency. Further research work may also identify the sires which suit for the cross breeding. In this research the reduced number of variables were sampled AK50 on various days of feeding. The progressive decline in the melting temperature observed as initial outcomes is promising. More research work may also define the preferred duration and type of supplementary feed. The controversies of grass versus grain are also important for potential novel examinations in research studies. Consumer demand is growing for the less intensive feeding strategies specially for grass feed. It is also perceived that the use of grains and corns is crucial for extreme marbling. On the basis of reproducible measurements, the definition of supplement acceptability depends on the healthiness and tastiness.

## Conclusion

The research concludes that there is melting temperature difference between progeny of the two sires of full blood Wagyu. Both the sires also share paternal and maternal grand. Lipid composition difference finding is not possible through alone Pedigree analysis. It is observable that the C19 haplotypes of the sire 1 and 2 are different. Lipid profile and marbling degree also differ due to site of sampling which is a major unresolved issue. The samples collected for the comparison require fixed location extraction as the samples showed variation even within the same muscle group. It is recommended to enhance experience by taking vivo samples to monitor feed, genetics and time.

## References

1. Picard, B., & Gagaoua, M. (2020). Muscle fiber properties in cattle and their relationships with meat qualities: An overview. *Journal of Agricultural and Food Chemistry*, 68(22), 6021-6039.
2. Chen, Q., Shen, J., Hanif, Q., Chen, N., Huang, Y., Dang, R., ... & Lei, C. (2021). Whole genome analyses revealed genomic difference between European taurine and East Asian taurine. *Journal of Animal Breeding and Genetics*, 138(1), 56-68.
3. Martins, T. D. S. (2021). *Diferença esperada na progênie (DEP) para precocidade de tourinhos Nelore e sua relação entre adipogênese, fibrogênese, lipogênese e metabolismo lipídico* (Doctoral dissertation, Universidade de São Paulo).
4. Muniz, M. M. M., Fonseca, L. F. S., dos Santos Silva, D. B., Magalhães, A. F. B., Ferro, J. A., Chardulo, L. A. L., ... & de Albuquerque, L. G. (2022). Small genetic variation affecting mRNA isoforms associated with marbling and meat color in beef cattle. *Functional & Integrative Genomics*, 1-16.
5. Oswalt, H., Smith, S., Williams, B., Ferdous, F., Burns, M., Bridges, W., ... & Dunn, H. W. (2021). Methodology to identify candidate genes from beef carcass traits at weaning: A pilot study. *Animal Gene*, 20, 200113.
6. Alam, M., Lee, S. H., Lee, D. H., Cho, C., & Park, M. N. (2021). Genetic analysis of major carcass traits of Korean Hanwoo males raised for thirty months. *Animals*, 11(6), 1792.
7. Beak, S. H., & Baik, M. (2022). Comparison of transcriptome between high-and low-marbling fineness in longissimus thoracis muscle of Korean cattle. *Animal Bioscience*, 35(2), 196.
8. Tyulebaev, S. D., & Kadysheva, M. D. (2021, September). Polymorphism of genes among heifers with different types of constitution. In *IOP Conference Series: Earth and Environmental Science* (Vol. 848, No. 1, p. 012213). IOP Publishing.
9. Liu, Q., Long, Y., Zhang, Y. F., Zhang, Z. Y., Yang, B., Chen, C. Y., ... & Su, Y. (2021). Phenotypic and genetic correlations of pork myoglobin content with meat colour and other traits in an eight breed-crossed heterogeneous population. *Animal*, 15(11), 100364.
10. Duff, C. J., Van Der Werf, J. H. J., Parnell, P. F., & Clark, S. A. (2021). Redefining residual feed intake to account for marbling fat in beef breeding programs. *Animal Production Science*.
11. Martín, N. P., Schreurs, N. M., Morris, S. T., López-Villalobos, N., McDade, J., & Hickson, R. E. (2022). Meat quality of beef-cross-dairy cattle from Angus or Hereford sires: A case study in a pasture-based system in New Zealand. *Meat Science*, 190, 108840.
12. Connolly, S. (2022). *Metabolomics of marbling and residual feed intake in crossbred Wagyu steers* (Doctoral dissertation).
13. Zoda, A., Kagawa, R., Obinata, R., Urakawa, M., Oono, Y., Ogawa, S., ... & Satoh, M. (2022). Genetic relationship between superovulatory response traits and carcass traits in Japanese Black cattle. *Animal Science Journal*, 93(1), e13731.
14. Ogawa, S., Matsuda, H., Taniguchi, Y., Watanabe, T., Sugimoto, Y., & Iwaisaki, H. (2022). Estimation of the autosomal contribution to total additive genetic variability of carcass traits in Japanese Black cattle. *Animal Science Journal*, 93(1), e13710.
15. Duckett, S. K., & Greene, M. A. (2022). Identification of microRNA Transcriptome Involved in Bovine Intramuscular Fat Deposition. *Frontiers in veterinary science*, 9.
16. Shi, B., Shi, X., Zuo, Z., Zhao, S., Zhao, Z., Wang, J., ... & Hickford, J. G. (2022). Identification of differentially expressed genes at different post-natal development stages of longissimus dorsi muscle in Tianzhu white yak. *Gene*, 823, 146356.