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POLSKA AKADEMIA NAUK

Polish Journal of Veterinary Sciences Vol. 28, No. 2 (2025), 303–309

DOI 10.24425/pjvs.2025.154951

Original article

Investigation of A2 allele frequency in Taiwanese Holstein cattle using genetic testing

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Abstract

β-Casein accounts for approximately 30% of total milk protein, with the A1 and A2 variants being the most common. A1 β-casein may release β-casomorphin-7 (BCM-7) during digestion, which is associated with adverse health effects, whereas A2 β-casein does not. This study investigated the A2 allele frequency and its potential influence on milk performance in Holstein cattle in Taiwan. A total of 1,050 cows from five herds were genotyped using the GeneSeek 50K SNP chip. The A2 allele frequency ranged from 0.58 to 0.75 among herds, with an average of 0.66. Hardy-Weinberg equilibrium tests indicated no significant deviation within herds. Pedigree validation confirmed Mendelian inheritance of A1 and A2 alleles. Furthermore, 348 cows with complete lactation records were evaluated for 305-day mature equivalent milk and fat yields across genotypes. Although cows with the A1/A1 genotype showed numerically higher milk and fat yields, one-way ANOVA and Tukey's HSD tests revealed no statistically significant differences. These results suggest that while A2 allele selection is increasing in Taiwan, β-casein genotype does not significantly influence milk or fat production in the studied population.

Keywords: Holstein cattle, genetic testing, A2 β-casein

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Introduction

In recent years, the rising demand for A2 milk, valued for its potential digestive benefits and associated health advantages, has significantly influenced consumer preferences and reshaped market dynamics (Ho et al. 2014, He et al. 2017, Choi et al. 2024, Jeong et al. 2024). A2 milk is produced by cows carrying the A2 allele for beta-casein, a primary milk protein. Studies suggest that A2 beta-casein may be less likely to cause gastrointestinal discomfort compared to A1 beta-casein, making it a promising option for individuals sensitive to traditional milk (He et al. 2017, Ramakrishnan et al. 2020, Summer et al. 2020, Park and Haenlein, 2021, Ramakrishnan et al. 2024). This increasing consumer interest has driven the dairy industry to adopt strategies aimed at enhancing A2 milk production, particularly through selective breeding programs that prioritize the A2 allele in dairy herds (Fernández-Rico et al. 2022, Żbik et al. 2024).

Holstein cows, renowned globally as a premier dairy breed, demonstrate genetic variability in the A1 and A2 beta-casein variants (Kamiński et al. 2007, Caroli et al. 2009). Research conducted in various regions has highlighted differences in the distribution of A1 and A2 alleles, shaped by historical breeding practices and genetic drift (Sebastiani et al. 2020, Miluchová et al. 2023, Bors et al. 2024). While these international studies offer valuable perspectives, the genetic profile of Holstein cows in Taiwan – particularly the prevalence of A2 carriers – remains largely unexplored. Understanding this genetic composition is essential for breeders seeking to meet the growing demand for A2 milk while simultaneously maintaining herd productivity and preserving genetic diversity (Caroli et al. 2009, Sebastiani et al. 2020).

Projections suggest that, by 2029, the A2 milk market in North America will expand, largely driven by consumer preference. This growth aligns with the broader shift toward health-conscious diets, in which A2 milk is regarded as a potentially more digestible alternative (Dantas et al. 2023). Recent studies indicate that β-casein polymorphisms may also influence milk processing characteristics, with A2 milk demonstrating distinct renneting properties relevant for dairy production (Faggion et al. 2024). Breeding for A2 milk production presents a unique opportunity to align consumer-driven goals with sustainable and health-oriented practices in the dairy industry. By prioritizing A2 allele frequency, producers can cater to niche market demands, enhance milk quality, expand product offerings, and increase the value of local dairy markets (Fernández-Rico et al. 2022). As Taiwan's dairy sector evolves to address these trends, a thorough understanding of the genetic composition of its Holstein cattle is essential. Such insights will enable the development of targeted breeding strategies and production systems that efficiently meet the rising demand for A2 milk while maintaining herd productivity and genetic diversity (Caroli et al. 2009).

Moreover, milk protein polymorphisms – including β-casein variants – have been shown to influence both breeding outcomes and human nutrition (Caroli et al. 2009, Smolenski et al. 2025). Comparative studies of A1 versus A2 beta-casein further suggest that switching to A2 milk could alleviate gastrointestinal issues in some individuals, highlighting its potential market advantage and health implications (Ho et al. 2014, Hohmann et al. 2021, Jeong et al. 2024). Additionally, research in dairy calves suggests that A2 milk does not adversely affect growth performance, further supporting its viability as a mainstream alternative (Kappes et al. 2024).

This study aims to bridge the knowledge gap by analyzing the frequency of A2 carriers in five Holstein herds in Taiwan. The results are intended to inform local breeding strategies, facilitate the development of an A2 milk production pipeline, and demonstrate how genetic resources can be optimized to align with evolving consumer demands. By establishing a comprehensive baseline for A2 allele frequency in Taiwan's Holstein population, this research provides a foundation for evidence-based decision-making in the dairy sector, paving the way for innovation and sustainable growth in response to both global and regional market trends (Dantas et al. 2023, Ariton et al. 2023, Bors et al. 2024).

Materials and Methods

Ethical statement and sample collection

This study collected blood and hair follicle samples from a total of 1,050 Holstein cows across five dairy farms in Taiwan. The number of genotyped cows from each farm was as follows: 412 cows from Herd 1, 240 cows from Herd 2, 50 cows from Herd 3, 48 cows from Herd 4, and 300 cows from Herd 5. The sampling protocol adhered to the methodology described in previous studies (Chao et al. 2022). In compliance with animal welfare and ethical standards, the study provided a clear justification for the experimental design and the necessity of using the selected animal species. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Taiwan Livestock Research Institute (LRI-IACUC107-5, LRI-IACUC108-1, LRI-IACUC109-1). The principles followed in this study align with international ethical guidelines, inclu-

Table 1. A2 carrier frequencies among 5 herds of Holstein cows in Taiwan.

Herd	No. of genotyped cattle	A1A2 genotype			Frequency of A2 carrier (%)
		A1A1	A1A2	A2A2	
1	412	30	180	202	0.71
2	240	38	118	84	0.60
3	50	8	26	16	0.58
4	48	3	18	27	0.75
5	300	38	140	122	0.64
Total	1050	117	482	451	0.66

ding those outlined by the NIH OLAW IACUC resources (https://olaw.nih.gov/resources/tutorial/iacuc.htm). Sample collection was carried out by licensed veterinarians or trained personnel. Each participating farm provided informed consent for sampling and genotyping as part of a breed improvement program.

Genotyping procedure

DNA was extracted from the collected blood and hair follicle samples following a standardized extraction protocol outlined in a previous study (Chao et al. 2022). Genotyping was performed using the GeneSeek Prime 50K SNP chip, which utilizes Illumina Infinium technology. The cows were genotyped for their beta-casein gene variants (A1 and A2), and the A2 carrier frequencies were evaluated across all five herds. Genotyping was performed by a CDCB-certified Neogen Genomics laboratory (Lincoln, NE, USA), which provided high-quality genotyping results based on Illumina Infinium XT genotyping assay platforms (Illumina, 2017). From the SNP chip data, β-casein genotypes (A1A1, A1A2, and A2A2) were identified using the specific markers that discriminate the A1 and A2 alleles. Quality control procedures involved excluding samples or loci with call rates below a predefined threshold (< 90%), as recommended by the SNP chip provider.

Hardy-Weinberg equilibrium (HWE) testing

HWE was assessed within each herd by comparing observed genotype counts (A1A1, A1A2, A2A2) with those expected under HWE using a Chi-square (χ^2) test.

Pedigree analysis

Pedigree analysis was conducted to evaluate the inheritance patterns of the A1 and A2 alleles. For example, two sires 200HO05592 (A1A1) and 007HO12788 (A2A2) were crossed with a dam, 42104M1287 (A2A2), to examine the genetic transmission of the A1A2 genotype. The resulting offspring from these matings were genotyped to determine their allelic compositions. This allowed for the analysis of the inheritance patterns of the A1 and A2 alleles and the confirmation of how these alleles were passed down from the sires and dam to the offspring.

Statistical analysis

Genotypic data were analyzed to determine the distribution and frequency of A2 allele carriers in each herd. The data were subjected to statistical analysis to evaluate the significance of any differences in A2 allele frequency between the farms. Results were expressed as frequencies, and the overall prevalence of A2 carriers in the Taiwan Holstein population was calculated. Phenotypic records from 348 cows with complete lactation data were analyzed to evaluate the effect of β-casein genotypes (A1/A1, A1/A2, A2/A2) on 305-day mature equivalent milk yield (milk 305ME) and fat yield (fat 305ME). One-way ANOVA was used to test for statistical differences among genotype groups, followed by Tukey's HSD post-hoc test for pairwise comparisons. All analyses were conducted using R software, and significance was set at $p<0.05$.

Results

The frequency of A2 carriers was assessed among five Holstein herds in Taiwan, as summarized in Table 1. The number of genotyped cattle in each herd ranged from 48 to 412, totaling 1,050 animals. Across herds, A1A2 was the most prevalent genotype, while A2 carrier frequencies (A1A2 + A2A2) varied from 0.58 to 0.75, with an overall average of 0.66. Herd 4 exhibited the highest A2 carrier frequency at 0.75, followed by Herd 1 at 0.71, whereas Herd 3 displayed the lowest at 0.58. In total, 482 cows were A1A2 and 451 were A2A2, indicating a considerable proportion of A2 genotypes within the Taiwan Holstein population. These findings partially align with other regional data showing significant A2 gene distributions (Sebastiani et al. 2020, Yamada et al. 2021).

To verify whether each herd's genotype frequencies followed expected distributions, HWE analysis was

Table 2. Hardy-Weinberg equilibrium analysis among 5 herds of Holstein cows in Taiwan.

Herd	χ^2	p-value
1	1.36	0.24
2	0.10	0.75
3	0.23	0.63
4	0	1.00
5	0.05	0.82

Table 3. Comparison of 305-day mature equivalent milk yield (Milk 305ME) and fat yield (Fat 305ME) among different A2 β-casein genotypes of Holstein cows in Taiwan.

Genotype	N	Milk 305ME (mean ± SD)	Fat 305ME (mean ± SD)
A1/A1	46	8889.26 ± 1695.5	409.04 ± 69.15
A1/A2	166	8623.6 ± 1537.99	385.99 ± 74.29
A2/A2	136	8817.9 ± 1627.39	392.38 ± 75.18

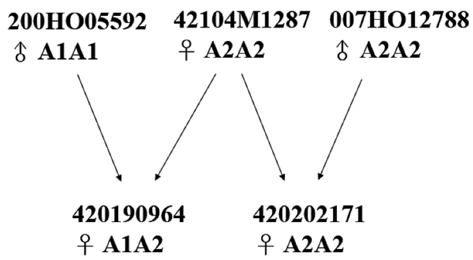

Fig. 1. The transmission analysis of A1A2 genotype.

performed (Table 2). None of the herds showed a significant deviation from HWE (p>0.05), implying minimal inbreeding or strong selection at the A1/A2 locus. Despite targeted efforts to increase A2 frequency – such as employing homozygous A2 sires – the overall genetic structure in each herd remains balanced.

A complementary pedigree analysis further confirmed the inheritance patterns of A1 and A2 alleles (Fig. 1). Two sires – 200HO05592 (A1A1) and 007HO12788 (A2A2) – were crossed with the same dam (42104M1287, A2A2). The offspring of the A1A1 sire and the A2A2 dam (ID: 420190964) exhibited the A1A2 genotype, whereas the offspring of the A2A2 sire and the same A2A2 dam (ID: 420202171) was A2A2. This demonstrates that mating an A1A1 sire with an A2A2 dam reliably produces A1A2 offspring, and that crossing two A2A2 parents yields all A2A2 progeny (Kamiński et al. 2007).

Among the A2 β-casein genotyped cows, a total of 348 individuals had complete records for both 305-day mature equivalent milk yield (milk 305ME) and fat yield (fat 305ME), and were classified into three genotype groups: A1/A1 (n=46), A1/A2 (n=166), and A2/A2 (n=136) (Table 3). The average milk and fat yields were evaluated across these genotypes. For milk 305ME, the highest mean yield was observed in the A1/A1 group (8,889.26 ± 1,695.50 kg), followed by A2/A2 (8,817.90 ± 1,627.39 kg), and A1/A2 (8,623.60 ± 1,537.99 kg). Although A1/A1 cows had a numerically higher yield, one-way ANOVA indicated that the differences among genotypes were not statistically significant (p=0.448). Tukey's HSD post-hoc analysis also confirmed the absence of significant pairwise differences. Similarly, fat 305ME was highest in A1/A1 cows (409.04 ± 69.15 kg), followed by A2/A2 (392.38 ± 75.18 kg) and A1/A2 (385.99 ± 74.29 kg). ANOVA revealed no significant difference among genotypes (p=0.173), and Tukey's test showed no significant pairwise comparisons. These findings suggest that the A2 β-casein genotype (A1/A1, A1/A2, A2/A2) has no statistically significant effect on either milk or fat yield in this population of Taiwanese Holstein cows.

Discussion

The present findings provide crucial insights into the genetic makeup of Taiwanese Holstein herds, indicating a strong potential for enhancing A2 milk production. Herds with higher A2 carrier frequencies may be particularly well positioned to supply the emerging A2 milk market, given that studies have demonstrated the benefits of A2 β-casein for certain populations (Sheng et al. 2019, Jeong et al. 2024). Clinical trials show improved gastrointestinal tolerance in children consuming exclusively A2 milk (Sheng et al. 2019), while mechanistic in vitro research points to a lower release of bioactive peptides (β-casomorphins) from A2 variants (Ul Haq et al. 2015, Cattaneo et al. 2023), potentially explaining reduced digestive discomfort. Furthermore, the European Food Safety Authority's 2009 report notes that BCM-7 is generated specifically during A1 β-casein digestion, yet not from A2 β-casein (European Food Safety Authority, 2009). As a result, consumer perception of A2 milk as a healthier or more digestible option may drive broader adoption and premium pricing (Ho et al. 2014, Ariton et al. 2023).

Recent bibliometric studies highlight a global surge in A2 milk research, particularly in the fields of food science, dairy technology, and biochemistry (Jiménez-Montenegro et al. 2022). The United States, New Zealand, and Australia are leading in A2 β-casein research, with significant contributions from India, France, and Germany. This international research expansion reflects the growing commercial and scientific interest in A2 milk production and its potential health advantages. Taiwan, where A2 milk research is still in its early stages, can benefit from lessons learned in these regions, particularly regarding breeding programs and market development.

Recent human trials further support these benefits. Choi et al. (2024) demonstrated that A2 milk consumption significantly reduced symptoms such as abdominal pain, fecal urgency, and borborygmus compared to A1/A2 milk in lactose-sensitive individuals. Ramakrishnan et al. (2024) confirmed that A2 milk reduced gastrointestinal inflammation, as measured by biomarkers like calprotectin. However, despite the growing number of studies on A2 milk, a bibliometric analysis by Jiménez-Montenegro et al. (2022) noted that clinical trials in humans remain limited. While mechanistic research suggests reduced BCM-7 release and improved digestion with A2 milk, further controlled human trials are necessary to establish definitive health benefits and to support public health recommendations.

In calves, Kappes et al. (2024) reported no adverse effects on body composition or growth in Holstein, Simmental, and crossbred calves fed A2 milk, though minor differences in visceral adipose tissue accumulation suggest potential metabolic effects. Hohmann et al. (2021) further found that A1 milk-fed calves had nearly five times higher plasma β-BCM-7 levels than those fed A2 milk, while A2 milk-fed calves had a higher prevalence of diarrhea. These findings underscore the physiological impact of β-casein variants and the need for further research on early-life digestion and metabolism.

Studies also explored implications for milk processing. Smolenski et al. (2025) examined the release of BCM-7 from various processed dairy products and confirmed that A2-only dairy products produced negligible BCM-7 yields compared to A1-containing counterparts. This reinforces the idea that BCM-7-associated digestive discomfort is largely absent in A2 milk and its derivatives, providing a biochemical basis for its proposed health benefits. Additionally, Faggion et al. (2025) showed that A2 milk had lower curd firmness and cheese yield, suggesting a tradeoff in processing properties that should be considered in breeding decisions.

Variation in A2 frequencies (0.58 to 0.75) among the herds suggests that factors such as breeding policies, herd management, and initial genetic backgrounds could play a role (Kamiński et al. 2007, Caroli et al. 2009). Farms displaying elevated A2 rates may already be conducting selective breeding favoring A2 alleles, whereas Herd 3 (0.58) might benefit from intensifying such efforts. Strategic pairing of A2A2 cows is particularly beneficial, as two A2A2 parents produce 100% A2A2 offspring (Kamiński et al. 2007). However, while herd-level selection can contribute to increasing A2 frequency, a national-scale selection index that integrates multiple traits – such as milk yield, fertility, and overall cow health – is essential for achieving sustainable genetic improvement. Currently, Taiwan lacks a nationwide selection index for Holstein cattle, which may limit the effectiveness of coordinated breeding strategies. Bibliometric research has shown that countries leading A2 milk research have benefited from structured national breeding programs to enhance A2 allele frequency while maintaining dairy performance (Jiménez-Montenegro et al. 2022). Establishing such an index would provide a systematic approach to balancing economic and performance traits while optimizing A2 milk production efficiency. Recent studies also highlight the importance of refining processing methods and monitoring genetic traits to further optimize A2 milk production (Cattaneo et al. 2023, Dantas et al. 2023, Bors et al. 2024, Żbik et al. 2024).

While the five selected herds in this study represent a diverse range of management practices and breeding strategies, we acknowledge that the geographic scope of our dataset is relatively narrow, potentially limiting the generalizability of our findings to the broader Taiwanese dairy population. To address this limitation, future research should expand the sample size across more geographically distinct herds to capture a more representative genetic and environmental variation. This would allow for a more comprehensive assessment of A2 allele distribution and its association with dairy productivity traits in diverse farming conditions.

The inheritance patterns observed here reinforce the idea that targeted selection can steadily shift allele frequencies toward A2 dominance. Although the proportion of A2A2 offspring remains moderate in this dataset, systematic breeding could increase that percentage over time. This aligns with literature advocating the use of A2A2 sires and dams to expedite genetic gains (Kamiński et al. 2007, Caroli et al. 2009, Sebastiani et al. 2020, Yamada et al. 2021). Future investigations might examine the influence of A2A2 genotypes on milk composition, productivity traits, and herd health across multiple lactations, ensuring both economic viability and consumer satisfaction (Sebastiani et al. 2020, Dantas et al. 2023, Bors et al. 2024).

In addition to genotypic distribution, this study evaluated the association between β-casein genotype and milk production traits. Among 348 cows with complete lactation data, the A1/A1 group exhibited numerically higher average milk and fat yields compared to the A1/A2 and A2/A2 groups. However, statistical analysis revealed no significant differences in either milk yield or fat yield among genotypes. These findings are consistent with previous studies (Ardicli et al. 2023, Miluchová et al. 2023) that also reported no significant associations between CSN2 genotypes and milk production traits. This suggests that selecting for the A2 allele does not compromise basic performance traits, supporting the feasibility of integrating A2-based selection into breeding programs.

Overall, the relatively high A2 allele prevalence (0.66) found in Taiwanese Holstein herds presents a promising basis for developing A2-focused breeding strategies to satisfy rising market demand and potential health interests. These results reinforce previous evidence linking the A2 genotype with digestive benefits and underscore the value of genotype-based selection for herd-level advancements (Ho et al. 2014, He et al. 2017, Jeong et al. 2024). Ongoing efforts to monitor the long-term performance and health outcomes of A2A2 cattle will be critical for establishing a robust and profitable A2 dairy sector in Taiwan.

Conclusion

This study investigated β-casein genotypes and their production relevance in Taiwanese Holsteins. The overall A2 carrier frequency was 0.66, with herd-level variation from 0.58 to 0.75. Genotype frequencies followed Hardy-Weinberg equilibrium, and pedigree analysis confirmed expected inheritance patterns. Among 348 cows with complete lactation data, no significant differences in milk or fat yield were found among A1A1, A1A2, and A2A2 genotypes, suggesting that A2-based selection does not negatively affect production. These findings support the feasibility of integrating A2 genotype selection into breeding programs to meet rising consumer demand for A2 milk, without compromising herd performance. Further research is recommended to evaluate long-term impacts on productivity and health.

Acknowledgements

This work was funded by the Ministry of Agriculture, Taiwan, Republic of China (grant number 108AS-2.1.2-L1(1) and 109AS-2.1.2-L1(1).

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