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Article Title	Preliminary Evaluation of Carbon and Nitrogen Stable Isotopes for Discriminating Korean Native Black Goat Meat from Australian Boer Goat Meat
Running Title (within 10 words)	Carbon and Nitrogen Isotopes for Goat Meat Discrimination
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research work was carried out with the support of the “Cooperative Research Program for Agriculture Science and Technology Development (Project No. RS-2025-02216992),” Rural Development Administration, Republic of Korea.
Author contributions (This field may be published.)	Conceptualization: Km GD, Baek J; Data curation: Baek J, Park J, Song S; Formal analysis: Baek J, Park J, Song S; Methodology: Kim GD, Baek J; Writing – original draft: Baek J; Writing – review & editing: Baek J, Park J, Song S, Kim HJ, Cheng H, Kim GD.
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

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**Preliminary Evaluation of Carbon and Nitrogen Stable Isotopes for Discriminating
Korean Native Black Goat Meat from Australian Boer Goat Meat**

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Running Title: Carbon and Nitrogen Isotopes for Goat Meat Discrimination

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Abstract

This study evaluated the potential of carbon and nitrogen stable isotope profiles for discriminating Korean native black goat (KNBG) meat from Australian Boer goat (AG) meat. *Longissimus thoracis et lumborum* muscles from KNBG and AG (n = 5 per group) were analyzed for Total carbon (C), $\delta^{13}\text{C}$, Total nitrogen (N), and $\delta^{15}\text{N}$ using an elemental analyzer–isotope ratio mass spectrometry. KNBG showed significantly higher Total C and $\delta^{13}\text{C}$ values but lower Total N and $\delta^{15}\text{N}$ values than AG (adjusted $p < 0.05$). Principal component analysis clearly separated the two groups along PC1, which explained 82.8% of the total variance. Within this preliminary dataset (n = 5 per group), linear discriminant analysis using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ correctly classified all samples under leave-one-out cross-validation. These results provide preliminary evidence that carbon and nitrogen stable isotope profiles may be useful for discriminating KNBG from AG meat. However, larger and more diverse sample sets and independent validation are required to establish a robust origin authentication approach.

Keywords: authentication, goat meat, geographical origin, stable isotope ratio, multivariate analysis

1. Introduction

Food authentication is important for verifying whether meat products comply with their declared geographical origin and production-related labeling information. Meat provenance encompasses not only geographical origin but also production systems and dietary background. Consequently, analytical approaches based on intrinsic product characteristics have been increasingly investigated as complementary tools to conventional traceability systems (Camin et al., 2016; Monahan et al., 2018). Korean native black goat (KNBG; *Capra hircus coreanae*) is an indigenous goat breed of Korea, and its meat has received increasing attention as a food source (Anneke et al., 2025). Therefore, analytical methods that provide objective information on the provenance of goat meat may contribute to strengthening the authenticity and traceability of goat meat products.

Stable isotope ratio analysis (SIRA) has been widely applied to the authentication of foods of animal origin because isotopic signatures in animal tissues reflect information associated with diet, drinking water, and the production environment (Camin et al., 2016; Monahan et al., 2018). Among the isotope systems used for meat authentication, carbon and nitrogen stable isotope ratios provide useful information regarding dietary and production backgrounds. The $\delta^{13}\text{C}$ values of animal tissues are strongly influenced by dietary composition, particularly the relative contributions of C3- and C4-derived feed resources (Camin et al., 2016). Nitrogen isotope ratios can also vary according to dietary and production-related factors. Consequently, geographically associated differences in feeding systems and production environments may generate distinguishable carbon and nitrogen isotopic profiles in animal tissues. However, geographical differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are generally interpreted through region-associated feeding and production conditions rather than as signals generated solely by geographical location (Monahan et al., 2018). Therefore, the

objective of this preliminary study was to characterize and compare the carbon and nitrogen isotope-related profiles of Korean native black goat and Australian Boer goat meat. In addition, we evaluated whether $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ could provide preliminary discriminatory information between the two sampled production groups using multivariate analysis and cross-validated linear discriminant analysis.

2. Materials and Methods

2.1. Meat samples and sample preparation

Longissimus thoracis et lumborum muscles were obtained from Korean native black goats (KNBG; n = 5) and Australian Boer goats (AG; n = 5). One sample was obtained from the carcass of each castrated male goat and was therefore considered an independent biological replicate. KNBG samples were obtained from commercial slaughterhouses in Korea, whereas AG samples were commercially sourced from Australia. Detailed individual information on age at slaughter, live weight, carcass weight, and farm-level feeding history, including pasture- versus concentrate-based feeding and the relative contribution of C3- and C4-derived feed ingredients, was not available because the samples were obtained through commercial slaughter and distribution channels. Muscle samples were collected at 24 h postmortem and stored at -80°C until analysis. Samples were freeze-dried for 48 h using a freeze dryer (Cooling Trap HC3110, GYROZEN Co., LTD., Korea) and subsequently ground into a homogeneous powder using a mortar and pestle. The powdered samples were stored in a desiccator until stable isotope analysis.

2.2. Stable isotope analysis

The carbon and nitrogen stable isotope compositions of the freeze-dried muscle samples were determined using a continuous-flow elemental analyzer–isotope ratio mass spectrometry (EA-IRMS) system (Vision-EA, Isoprime, UK). Total carbon (Total C, %), carbon isotope ratio ($\delta^{13}\text{C}$, ‰), total nitrogen (Total N, %), and nitrogen isotope ratio ($\delta^{15}\text{N}$, ‰) were simultaneously determined. Total C and Total N were obtained through the elemental composition calculation of the EA-IRMS system and were expressed as weight percentages relative to the freeze-dried sample mass on a dry matter basis. Carbon and nitrogen isotope ratios were expressed in delta notation relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (AIR), respectively. Each sample was analyzed in triplicate, and the mean of the three technical replicates was used as the value for each carcass in subsequent statistical analyses.

2.3. Statistical analysis

Statistical analyses were performed using SAS (ver. 9.4; SAS Institute Inc., Cary, NC, USA). Each carcass was considered an independent biological replicate ($n = 5$ per group), and data are presented as the mean $\pm$ standard deviation. Given the small sample size, normality could not be reliably assessed; therefore, differences in Total C, $\delta^{13}\text{C}$, Total N, and $\delta^{15}\text{N}$ between KNBG and AG were evaluated using two-sided exact permutation tests. With 10 independent carcasses divided equally between the two groups, all $C(10,5)=252$ possible group allocations were exhaustively enumerated, allowing exact calculation of permutation p-values without Monte Carlo approximation. P-values were adjusted for multiple comparisons using the Holm method, with adjusted $p < 0.05$ considered statistically significant.

Principal component analysis (PCA) was performed using z-score-standardized Total

C, $\delta^{13}\text{C}$, Total N, and $\delta^{15}\text{N}$ values to explore multivariate isotopic patterns between groups. Linear discriminant analysis (LDA) using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as predictors was performed to evaluate preliminary origin discrimination. Classification performance was assessed by leave-one-out cross-validation (LOOCV), in which the model was refitted using the remaining nine carcasses before predicting each excluded carcass.

3. Results

3.1. *Stable isotope characteristics of KNBG and AG samples*

The carbon and nitrogen stable isotope characteristics of Korean native black goat (KNBG) and Australian Boer goat (AG) meat are presented in Table 1. Total C was significantly higher in KNBG than in AG ($53.36 \pm 2.33\%$ vs. $50.43 \pm 1.12\%$, respectively; adjusted $p = 0.0317$). The $\delta^{13}\text{C}$ value was also higher in KNBG ($-19.07 \pm 0.77\%$) than in AG ($-21.26 \pm 0.79\%$; adjusted $p = 0.0317$). In contrast, Total N was significantly lower in KNBG than in AG ($19.45 \pm 3.74\%$ vs. $31.96 \pm 4.11\%$, respectively; adjusted $p = 0.0317$). Similarly, $\delta^{15}\text{N}$ was lower in KNBG ($4.98 \pm 0.61\%$) than in AG ($12.94 \pm 0.54\%$; adjusted $p = 0.0317$).

3.2. *Multivariate stable isotope characteristics*

PCA based on the four stable isotope variables revealed distinct multivariate patterns between KNBG and AG (Fig. 1). PC1 and PC2 explained 82.8% and 9.9% of the total variance, respectively, accounting for 92.7% of the cumulative variance. KNBG and AG were completely separated along PC1, with all KNBG samples having negative PC1 scores and all AG samples having positive scores (Fig. 1A). The loading plot showed that Total N and $\delta^{15}\text{N}$

were positively associated with PC1, whereas Total C and $\delta^{13}\text{C}$ were negatively associated with PC1 (Fig. 1B). Along PC2, Total C and $\delta^{15}\text{N}$ had negative loadings, while $\delta^{13}\text{C}$ and Total N had positive loadings.

3.3. *Preliminary discrimination between KNBG and AG samples using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$*

The combined distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ showed clear separation between KNBG and AG samples (Fig. 2A). KNBG samples ranged from -19.91 to -17.99‰ for $\delta^{13}\text{C}$ and from 4.38 to 5.81‰ for $\delta^{15}\text{N}$, whereas AG samples ranged from -22.00 to -20.03‰ and from 12.22 to 13.61‰ , respectively. No overlap between the two groups was observed for either isotope ratio. Linear discriminant analysis using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as predictors generated a linear decision boundary that separated all KNBG and AG observations in the full dataset (Fig. 2A).

Classification performance was further evaluated using leave-one-out cross-validation (LOOCV), in which each animal was excluded once and classified using an LDA model fitted to the remaining nine animals. All five KNBG and all five AG samples were correctly classified, resulting in 10 correct classifications out of 10 observations and an overall LOOCV accuracy of 100%. The confusion matrix contained no misclassified observations, with sensitivity and specificity of 100% for both groups and a balanced accuracy of 100%.

The cross-validated posterior probabilities also showed complete separation between the two groups (Fig. 2B). All KNBG samples showed posterior probabilities of classification as AG below the 0.5 classification threshold, whereas all AG samples showed probabilities above the threshold. No overlap in cross-validated posterior probabilities was observed between the two groups.

4. Discussion

The present study demonstrated distinct carbon and nitrogen stable isotope characteristics between Korean native black goat (KNBG) and Australian Boer goat (AG) meat. KNBG exhibited higher $\delta^{13}\text{C}$ but markedly lower $\delta^{15}\text{N}$ values than AG, and no overlap was observed between the groups for either isotope ratio. These differences were also reflected in the multivariate analyses: PCA completely separated KNBG and AG along PC1, while LDA using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ correctly classified all samples under LOOCV. Collectively, these findings indicate that the two goat populations examined in this study possessed clearly distinguishable isotopic profiles.

The difference in $\delta^{13}\text{C}$ between KNBG and AG is consistent with the established relationship between carbon isotope composition and dietary carbon sources. Animal tissue $\delta^{13}\text{C}$ is strongly influenced by the relative contribution of C3- and C4-derived feed resources, although other biological and production factors can also contribute to their variations (Camin et al., 2016; Inácio and Chalk, 2017; Monahan et al., 2018). This relationship has been demonstrated in livestock studies. Zhao et al. (2013) observed distinct carbon isotope characteristics between cattle predominantly fed C3 pasture and those receiving maize-based diets, whereas Erasmus et al. (2018) reported differences in lamb $\delta^{13}\text{C}$ associated with regional variation in C3 and C4 vegetation. Carbon isotope ratios have therefore been recognized as particularly informative for characterizing dietary background in small ruminants (Pianezze et al., 2021). Accordingly, the approximately 2.2‰ difference in $\delta^{13}\text{C}$ between KNBG and AG may reflect differences in dietary carbon sources and production environments.

An even greater difference was observed for $\delta^{15}\text{N}$, with mean values of 4.98‰ in KNBG and 12.94‰ in AG. Nitrogen isotope composition in livestock tissues is closely associated with dietary nitrogen isotope signatures and may also vary among farms and production environments (Inácio and Chalk, 2017; Pianezze et al., 2021). Previous studies have similarly reported differences in $\delta^{15}\text{N}$ among lambs from different countries, farms, and production regions (Piasentier et al., 2003; Erasmus et al., 2016, 2018). Thus, the pronounced $\delta^{15}\text{N}$ difference in the present study indicates substantially different nitrogen isotopic backgrounds between the two groups. However, neither the $\delta^{13}\text{C}$ nor $\delta^{15}\text{N}$ difference should be interpreted as a direct effect of geographical location. Accordingly, the geographical relevance of carbon and nitrogen isotope ratios arises largely from regional differences in feeding and production practices rather than from location itself (Monahan et al., 2018). Because detailed feeding information, including pasture-to-concentrate ratios and the relative contributions of C3- and C4-derived feed sources, was unavailable, the specific dietary factors underlying the observed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differences could not be determined.

The differences observed for the individual variables were consistent with the PCA results. PC1 and PC2 explained 82.8% and 9.9% of the total variance, respectively, and the two groups showed complete separation along PC1. Total N and $\delta^{15}\text{N}$ loaded positively on PC1, whereas Total C and $\delta^{13}\text{C}$ loaded in the opposite direction, consistent with the univariate differences between KNBG and AG. Because PCA was performed without using group information, this separation demonstrates a systematic difference in the overall isotopic characteristics of the sampled populations. Nevertheless, PCA should be interpreted as an exploratory visualization of multivariate structure rather than as evidence of predictive classification.

More directly, LDA using only $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ correctly classified all 10 animals

under LOOCV. Stable isotope-based multivariate classification has previously demonstrated substantial potential for meat provenance authentication. Piasentier et al. (2003) demonstrated discrimination of lamb according to feeding regime and country of origin, while Osorio et al. (2011) achieved 82.2% cross-validated classification of beef from nine countries using multiple stable isotope ratios. Distinct $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiles have also been reported among Korean, American, and European pork (Kim et al., 2013). In South African lamb, Erasmus et al. (2018) achieved 90% validation accuracy when distinguishing two broad production origins. Together with the present findings, these studies support the potential of stable isotope profiles as intrinsic indicators for meat provenance assessment.

However, the 100% LOOCV accuracy obtained in the present study should be interpreted cautiously. Only five animals were examined per group, and LOOCV assesses classification performance within the same limited sample population rather than providing independent external validation. Moreover, classification performance may decline as greater production heterogeneity is introduced. Erasmus et al. (2018), for example, reported considerably lower classification performance when lambs were divided among nine individual production regions rather than two broader origins. Bong et al. (2010) also found that although isotope characteristics differed among beef from Korea, Australia, the USA, Mexico, and New Zealand, some overlap occurred among origins and discrimination improved when multiple isotope systems were combined. Therefore, the complete separation observed here should be regarded as preliminary evidence of discriminatory potential rather than as validation of a universally applicable classification model.

A further limitation is that geographical origin, breed, and production background were confounded in the present study. Consequently, the observed isotope differences cannot be independently attributed to country of origin, breed, diet, or production environment. This

limitation is particularly relevant because $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can vary with multiple dietary and production factors (Inácio and Chalk, 2017; Monahan et al., 2018). The isotope profiles identified in this study should therefore be considered characteristics of the sampled production groups rather than definitive geographical signatures of all KNBG or AG.

Despite these limitations, stable isotope profiling has potential value as a complementary tool for goat meat authentication. Existing meat discrimination methods differ in their analytical targets, accuracy, cost, and practical applicability (Song et al., 2024). Unlike genetic methods used primarily to identify biological characteristics such as species or breed, stable isotope profiles provide information associated with dietary and production backgrounds. This complementary information may be particularly relevant as the Korean native black goat industry becomes increasingly oriented toward commercial meat production (Son et al., 2026).

Future studies should therefore expand the reference population to include Korean native and imported goats from multiple farms, regions, seasons, breeds, and feeding systems, together with detailed production metadata and independent external validation. Additional isotope systems may further improve geographical resolution. Hydrogen and oxygen isotope ratios, for example, can provide information complementary to carbon and nitrogen isotope ratios (Camin et al., 2016; Monahan et al., 2018; Pianezze et al., 2021), and improved origin discrimination through the combination of multiple isotope systems has been demonstrated in beef (Nakashita et al., 2008; Bong et al., 2010). Such expanded datasets will be necessary to determine whether the clear separation observed in the present study can be maintained under heterogeneous commercial production conditions.

5. Conclusion

Korean native black goat (KNBG) meat and Australian Boer goat (AG) meat exhibited distinct carbon and nitrogen stable isotope characteristics in the present sample set. In particular, differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ contributed to clear multivariate separation between the two production groups, and their combination correctly classified all samples under LOOCV. These findings provide evidence that carbon and nitrogen stable isotope profiles have potential as analytical markers for discriminating KNBG meat from AG meat and highlight the feasibility of a stable isotope-based approach for goat meat origin and production authentication. The isotope profiles observed in this study should, however, be interpreted as characteristics of the sampled production groups rather than universally applicable geographical signatures. Further studies involving broader sampling across farms, regions, seasons, and production systems, together with independent external validation, will be important to confirm the reproducibility and broader applicability of the observed isotope patterns. Nevertheless, the clear separation and complete classification achieved using the combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the present sample set represent an important finding and provide a valuable foundation for further development of stable isotope-based authentication methods for goat meat. With expanded reference databases and independent validation, this approach may contribute to the development of a practical analytical tool for verifying the origin and production background of commercially available goat meat.

273 **Acknowledgements**

274 This research work was carried out with the support of the “Cooperative Research Program
275 for Agriculture Science and Technology Development (Project No. RS-2025-02216992),”
276 Rural Development Administration, Republic of Korea.

277

278 **Conflicts of interest**

279 The authors declare no potential conflict of interest.

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Accepted

Table 1. Carbon and nitrogen stable isotope characteristics of Korean native black goat and Australian Boer goat meat.

Variable	KNBG	AG	P-value	Adjusted P-value
Total C (%)	53.36 ± 2.33	50.43 ± 1.12	0.0238	0.0317
$\delta^{13}\text{C}$ (‰)	-19.07 ± 0.77	-21.26 ± 0.79	0.0079	0.0317
Total N (%)	19.45 ± 3.74	31.96 ± 4.11	0.0079	0.0317
$\delta^{15}\text{N}$ (‰)	4.98 ± 0.61	12.94 ± 0.54	0.0079	0.0317

Data are presented as mean ± standard deviation. KNBG, Korean native black goat; AG, Australian Boer goat; Total C, total carbon content; Total N, total nitrogen content. **Total N (%) represents the weight percentage of elemental nitrogen relative to the freeze-dried sample mass on a dry matter basis.** $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expressed relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (AIR), respectively. P-values were obtained using two-sided exact permutation tests. Adjusted p-values were calculated using the Holm method for multiple comparisons. Differences were considered statistically significant at adjusted $p < 0.05$.

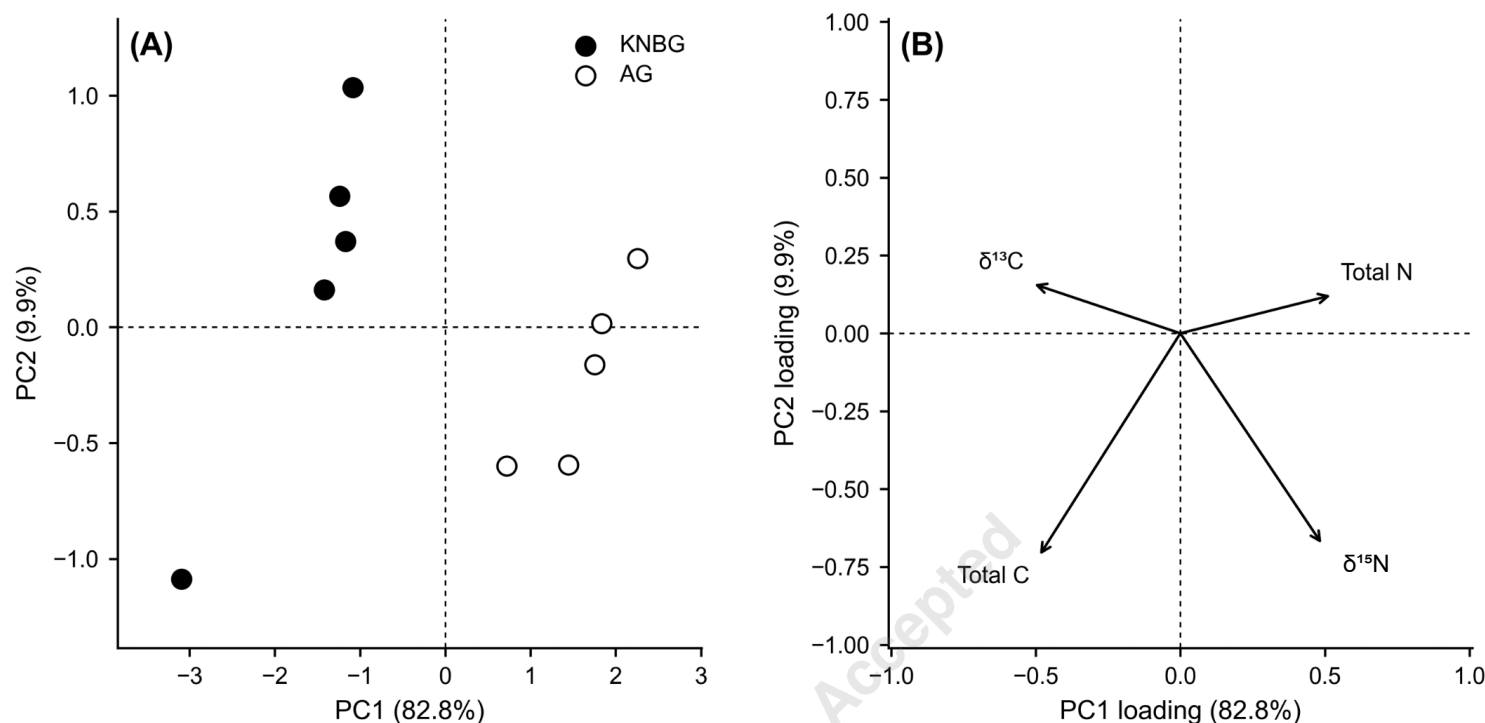


Fig. 1. Principal component analysis (PCA) of carbon and nitrogen stable isotope characteristics in Korean native black goat and Australian Boer goat meat. (A) PCA score plot based on z-score-standardized Total C, $\delta^{13}\text{C}$, Total N, and $\delta^{15}\text{N}$ values. Each point represents an individual carcass ($n = 5$ per group). PC1 and PC2 explained 82.8% and 9.9% of the total variance, respectively. (B) Loading plot showing the contributions of the four variables to PC1 and PC2. KNBG, Korean native black goat; AG, Australian Boer goat; PC, principal component; Total C, total carbon content; Total N, total nitrogen content.

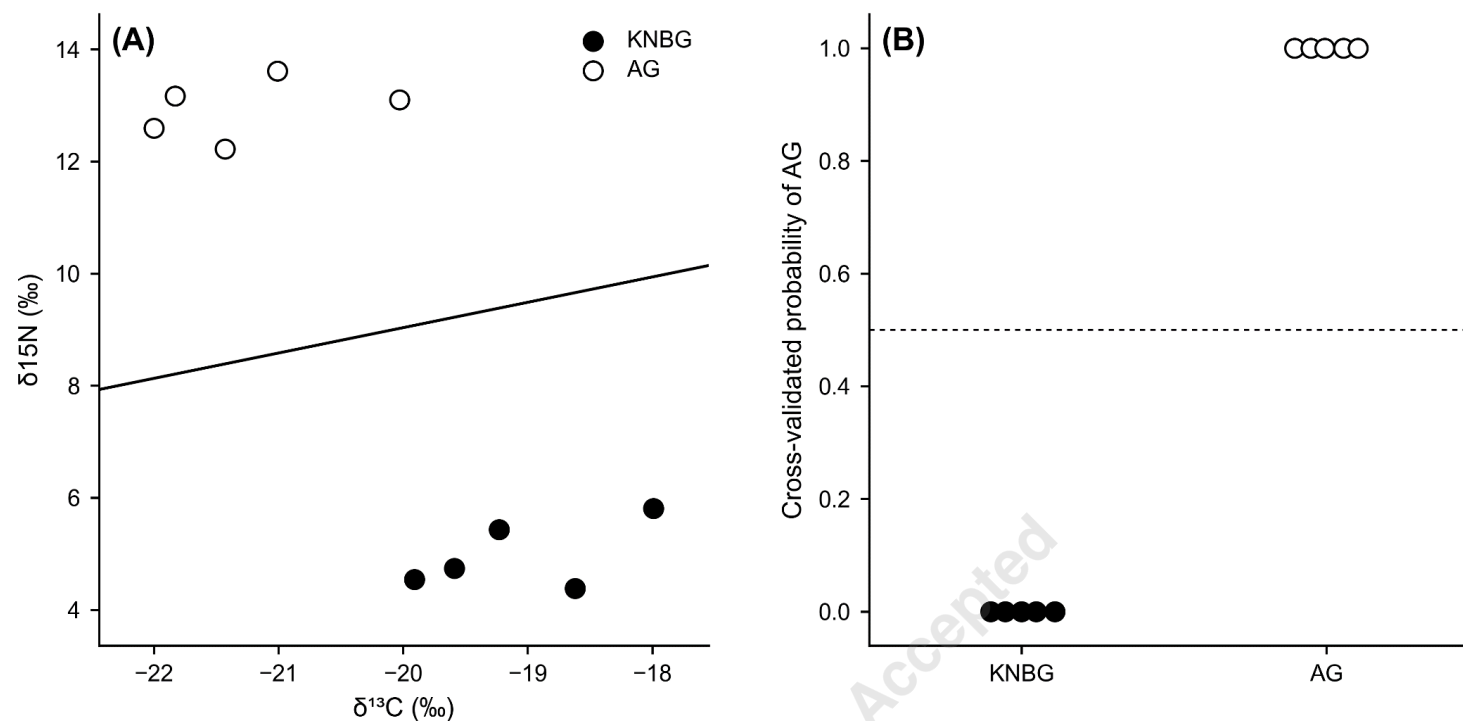


Fig. 2. Preliminary discrimination of Korean native black goat and Australian Boer goat meat using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. (A) Two-dimensional distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in Korean native black goat and Australian Boer goat meat. Each point represents an individual carcass ($n = 5$ per group). The solid line indicates the linear discriminant analysis (LDA) decision boundary fitted to the complete dataset for visualization. Classification performance was evaluated independently using leave-one-out cross-validation (LOOCV). (B) Cross-validated posterior probabilities of classification as Australian Boer goat obtained from LOOCV. The horizontal dashed line at 0.5 indicates the classification threshold. Values below 0.5 were classified as Korean native black goat, whereas values above 0.5 were classified as Australian Boer goat. KNBG, Korean native black goat; AG, Australian Boer goat.