RAPA Revista Argentina de Producción Animal

COMUNICACIÓN

MENAQUINONE-4 (VITAMIN K2) LEVELS IN BEEF FROM WELL-MANAGED GRAZING AND GRAIN-FINISHED SYSTEMS

Niveles de menaquinona-4 (vitamina K2) en carne bovina de sistemas pastoriles bien manejados y de terminación a grano

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ABSTRACT

Vitamin K2 (menaquinones) has gained increasing scientific interest due to its emerging physiological roles, yet food-composition data remain incomplete. Among menaquinones, menaquinone-4 (MK-4) is the predominant analogue in bovine muscle, but no study to date has directly compared MK-4 concentrations in beef obtained from different production systems. This study quantified MK-4 levels by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) in beef from steers raised either under a well-managed, ecologically aligned grazing system or a grain-finished system. Fatty acid composition was additionally assessed to independently contextualize the novel MK-4 determinations. Beef from the grazing system exhibited markedly higher MK-4 concentrations (15.40 ± 2.54 µg/100 g) than grain-finished beef (2.30 ± 0.61 µg/100 g; $P < 0.001$). Fatty acid profiles followed established patterns, with grazing increasing ALA, EPA, DHA, and total n-3 PUFA while reducing LA and total n-6 PUFA, yielding an n-6/n-3 ratio of 2.79 versus 23.80 in grain-finished beef. Together, the expected fatty acid signature, biological plausibility, and the extremely large effect size observed for MK-4 (Hedges' g = 6.79) support the possibility that production system is a key determinant of MK-4 levels in beef. Therefore, this work strengthens current understanding of how rearing practices shape the micronutrient composition of livestock products and may have implications for sustainable livestock systems and for human nutrition.

Key words. grass-fed, commercial conditions, micronutrients, vitamins, production system.

RESUMEN

La vitamina K2 (menaquinonas) ha recibido creciente interés por sus funciones fisiológicas emergentes, pese a que los datos de composición en alimentos siguen siendo incompletos. Entre las menaquinonas, la menaquinona-4 (MK-4) es el análogo predominante en músculo bovino, pero aún no se habían comparado directamente sus concentraciones en carne proveniente de distintos sistemas productivos. Este estudio cuantificó los niveles de MK-4 mediante cromatografía líquida de alta resolución con detección ultravioleta (HPLC-UV) en carne de novillos criados en un sistema pastoril bien manejado, ecológicamente alineado, o en un sistema de terminación a grano. Además, se evaluó la composición de ácidos grasos para contextualizar de forma independiente las determinaciones de MK-4. La carne del sistema pastoril presentó concentraciones de MK-4 marcadamente superiores (15,40 ± 2,54 µg/100 g) respecto de la de terminación a grano (2,30 ± 0,61 µg/100 g; $P < 0,001$). Los perfiles de ácidos grasos siguieron el patrón esperado, ya que el pastoreo incrementó ALA, EPA, DHA y el total de PUFA n-3, redujo LA y el total de PUFA n-6, y arrojó una relación n-6/n-3 de 2,79 versus 23,80 en carne terminada a grano. En conjunto, el perfil lipídico esperado, la plausibilidad biológica, y el tamaño de efecto extremadamente grande para MK-4 (Hedges' g = 6.79) son consistentes con la posibilidad de que el sistema productivo es un determinante clave en los niveles de MK-4 en carne bovina. Estos hallazgos fortalecen la comprensión de cómo las prácticas de manejo influyen en la composición micronutricional de los productos de origen animal y podrían tener implicancias para sistemas ganaderos sustentables y para la nutrición humana.

Palabras clave. alimentación a pasto, condiciones comerciales, micronutrientes, vitaminas, sistema productivo.

Recibido: Marzo 2026
Aceptado: Abril 2026

Introduction

Vitamin K comprises phylloquinone (K1), mainly found in plant foods and widely known for its role in blood coagulation, and a series of menaquinones (vitamin K2; MK-n). Vitamin K2 is only found in animal-sourced foods and fermented vegetable products, and has important biological functions, including support of skeletal and cardiovascular systems (Bonaldo and Leroy 2024). Evidence from randomized controlled trials (RCTs) of vitamin K2 supports skeletal (Zhang *et al.* 2025) and cardiovascular (Li *et al.* 2023) benefits, while observational studies and RCTs indicate additional potential against other non-communicable diseases (Halder *et al.* 2019). However, despite growing interest, food composition data for vitamin K2 remain incomplete (Jensen *et al.* 2025), and current dietary recommendations are still based on vitamin K1 rather than K2 (Akbulut *et al.* 2020).

Earlier studies from our research group have found that beef from grass-fed animals contains higher levels of antioxidant vitamins (ascorbic acid, beta-carotene, alpha-tocopherol) than beef from grain-fed animals (Descalzo and Sancho 2008), as well as increased amounts of desirable fatty acids and a lower omega-6/omega-3 ratio (Descalzo *et al.* 2005). These findings were confirmed by several authors (Bronkema *et al.* 2019; Daley *et al.* 2010) and expanded by more recent multi-omics experiments, indicating that grass-fed beef contains higher levels of a wide array of bioactive compounds, with potential health benefits, than grain-fed beef (Krusinski *et al.* 2025). However, although it is well established that the production system directly impacts the amount of several micronutrients that livestock accumulate in their tissues, no experimental evidence exists regarding how different rearing practices influence vitamin K2 levels in meat products. In addition, current evidence indicates that well-managed grazing systems can provide potential environmental benefits across multiple sustainability indicators, including mitigation of greenhouse gas (GHG) emissions through soil carbon sequestration (Maree *et al.* 2025).

Long-chain menaquinones (MK-7, MK-8, MK-9) can be found in vegetable foods -especially in fermented products- since they are a result of bacterial metabolism. Conversely, menaquinone-4 (MK-4) is present exclusively in animal-sourced foods (Bonaldo and Leroy 2024). In fact, experiments performed in mice indicate that dietary vitamin K1 can be effectively converted to MK-4 in several tissues (Ellis *et al.* 2022). Accordingly, despite the limited availability of food composition data (Jensen *et al.* 2025), MK-4 is considered the predominant vitamin K2 analogue in bovine muscle (Koivu-Tikkanen *et al.* 2000; Rødbotten *et al.* 2014). In addition, pasture intake has been reported to increase MK 4 levels in dairy relative to grain intake (Zhou *et al.* 2025), which, to the best of our knowledge, represents the only study to date comparing different feeding regimens in relation to menaquinone concentrations in livestock products.

Considering all the above, the aim of the present study was to quantify MK-4 (vitamin K2) in beef obtained from a well-managed grazing system compared with a grain-finished system, under real-world production settings in commercial operations where beef enters consumer channels. In addition, the fatty acid profile was included as a well-established indicator of production-system differences and as an independent verification of their expected functioning, thereby contextualizing and supporting interpretation of the novel MK-4 assessment.

Materials and Methods

Production systems, animals and sampling

This study compared beef obtained from two production systems: a well-managed grazing system and a grain-finished (feedlot) system. Both systems were located in Marcos Juárez, Córdoba, in the geographic center of Argentina (32°41'52"S, 62°06'11"W) and involved Aberdeen Angus steers within the same agroecological region and seasons. The animals in the grazing system were raised on high-quality perennial pastures throughout the entire production cycle under rotational management, taking into account forage quality and availability, and providing adequate recovery periods in the pasture paddocks before the plants are grazed again; pastures were managed without irrigation, mineral fertilizers, or agrochemicals since 1983, and the system operated under a Grass-Fed certification protocol (LIAF Control, Argentina) that verifies the type and management of the grazing system. In the grain-finished system, animals were pasture-reared and then finished for 90 days on a concentrate-based ration containing (dry-matter basis) 73% corn grain, 15% soybean expeller, 7% alfalfa hay (effective fiber), and 5% vitamin-mineral premix. Animals from both systems were transported to the same commercial abattoir and slaughtered in spring (September 2024), in accordance with Argentine animal-welfare regulations of the Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) under identical lairage and slaughter procedures. Twenty animals (n=10 from each production system) were used for the experiments; mean live weight at slaughter was 380 ± 18 kg (grazing) and 370 ± 12 kg (grain-finished). Using a standardized protocol and the same personnel, internal and external intercostal muscles adjacent to the *longissimus dorsi* (ribs 6–12) were sampled from each carcass (160 g of meat), placed in labeled containers, and kept refrigerated during transport; these muscles are routinely directed to minced-meat and burger-manufacturing and therefore represent tissue destined for human consumption. Samples arrived at our laboratory within 1 h, were sub-aliquoted for analyses, and stored at −80 °C until assays described below.

Menaquinone-4 (MK-4) quantification

MK-4 was quantified by high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV), using a Thermo Scientific Dionex UltiMate 3000 RS. We optimized the approach of Koivu-Tikkanen *et al.* (2000) used for quantification of vitamin K2, with specific focus on MK-4 given its relevance in bovine muscle (as stated in the Introduction). For each animal (n=20; 10 per system), 1.5 g of meat were processed in triplicate. Each replicate was placed in a wide amber glass vial, mixed with 5 mL 2-propanol, and heated 5 min in a boiling-water bath. Vials were chilled on ice, 5 mL 2-propanol were added, and the sample was homogenized with an Ultra-Turrax T25 (IKA, Darmstadt,

Germany) for ≤ 2 min. Keeping the vials on ice, 5 mL n-hexane were added and the mixture was gently homogenized for 1 min; 5 mL ultrapure water were then added and the sample was again gently homogenized for 1 min. The upper hexane phase (supernatant) was transferred to a clean glass tube, the residual phase was re-extracted on ice with 5 mL n-hexane and gently homogenized for 1 min, and the organic layers were combined. To aid phase clarification before solvent removal, the combined extract was centrifuged at 1,500 × g for 5 min; the hexane phase was then evaporated to dryness. The residue was reconstituted in 500 µL ethanol for HPLC analysis. Calibration curves were prepared using MK-4 standard (Sigma-Aldrich) and detection was performed with a diode array detector set at 249 nm.

Fatty-acid profile analysis

The fatty-acid profile of beef was determined by GC-FID following an in-situ transesterification workflow, as previously reported by our group (Merayo *et al.* 2023). For each animal (n = 10; 5 per system), samples were processed in duplicate. Briefly, 0.1 g of lyophilized meat were incubated with dichloromethane-NaOH/methanol at 90 °C for 10 min to generate fatty-acid methyl esters (FAMEs). The transesterification step was then repeated using boron trifluoride, after which FAMEs were partitioned into n-hexane using 0.9% NaCl. The hexane extract was injected on a Nexis GC-2030 (Shimadzu, Kyoto, Japan) equipped with a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d., 0.20 µm film; Varian CP7489). Fatty acids were identified and quantified from the chromatograms, and results are expressed as percentage of total fatty acids detected.

Statistical analyses

Data were analyzed in InfoStat v2020 (UNC, Argentina) and animals were treated as independent experimental units, while the production system was considered an explanatory factor rather than a replicated experimental treatment, consistent with real-world comparative studies of commercial livestock systems. MK-4 concentrations and fatty acids are reported as mean ± standard deviation (SD). For each variable, normality within groups was assessed using the Shapiro–Wilk test, and homogeneity of variances with Levene's test (median-centered). When both groups met normality and variances were homogeneous, comparisons between production systems (well-managed grazing vs grain-finished) were performed using Student's t-test (equal variances); when variances were unequal, Student's t-test assuming unequal variances (Welch's correction) was applied. If normality was not met in either group, the two-sided Mann-Whitney U test was used instead. All tests were two-tailed with α = 0.05. For MK-4, effect size (Hedges' g) was calculated from group means, SDs and sample sizes using an online calculator (Wilson, D. B. v 2023.11.27).

Results and Discussion

This comparative field study evaluated MK-4 content in beef from well-managed grazing and grain-finished systems under routine commercial conditions, where, unlike controlled experimental designs, the meat effectively reaches human consumption channels. Therefore, our approach was not intended to replace a randomized trial -the gold standard for minimizing confounders- which is logistically unfeasible in this context and incompatible with the aim of the study. In other words, our rationale was to examine two real-world, well-characterized systems using animals of the same breed and sex, managed and sampled with harmonized protocols in the same region and during the same seasons, differing primarily in production management.

Menaquinone-4 (MK-4) concentrations differed markedly between the two production systems (Figure 1). Beef from steers raised under well-managed grazing showed substantially higher MK-4 levels (15.40 ± 2.54 µg/100 g) than beef from grain-finished steers (2.30 ± 0.61 µg/100 g). A significant difference between systems was detected ($p < 0.001$), with an extremely large effect size (Hedges' g = 6.79). Although the grazing group exhibited a higher absolute standard deviation (SD) due to its larger mean,

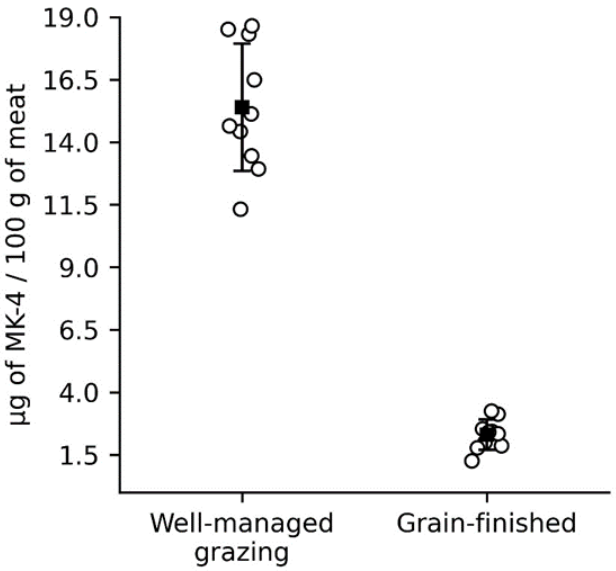

Figure 1. Menaquinone-4 (MK-4) levels in beef from steers raised under well-managed grazing and grain-finished systems. Each white circle with a black outline represents one animal (n=10 animals per system). Black squares indicate the group means, and the error bars represent the standard deviation (±SD).

Figura 1. *Menaquinona-4 (MK-4) en carne de novillos criados bajo sistemas de pastoreo bien manejado y de terminación a grano. Cada círculo blanco con borde negro representa un animal (n=10 animales por sistema). Los cuadrados negros indican las medias grupales, y las barras corresponden al desvío estándar (±SD).*

relative variability was lower compared with the grain-finished group since coefficient of variations (CV) were 16.5% and 26.4%, respectively. Importantly, the values observed here fall within the general magnitude reported in the few available studies on MK-4 concentrations in bovine muscles (Koivu-Tikkanen *et al.* 2000; Rødbotten *et al.* 2014). Koivu-Tikkanen *et al.* (2000) reported ~2.8 µg MK-4/100 g of beef, although the authors did not provide further details on the type of meat used. Rødbotten *et al.* (2014) evaluated MK-4 in beef (*biceps femoris*, *psoas major*, and *longissimus dorsi*) from Norwegian Red and Jersey steers finished on grain and found similar values; overall, MK-4 ranged from ~1.82 to ~4.85 µg/100 g. Interestingly, these levels align with our

grain-finished group (~2.30 µg/100 g) and are markedly lower than those observed in our well-managed grazing group (~15.40 µg/100 g). Although it may be tempting to interpret such differences as a direct effect of higher forage intake in our grazing system compared with the grain-finishing regimen in Rødbotten *et al.* (2014), cross-study comparisons of absolute values are limited by differences in HPLC methodologies and other potential confounders, and therefore should not be used as supportive evidence for our findings.

In this context, our results are in accordance with a recent study by Zhou *et al.* (2025) reporting that pasture intake in dairy cows can increase vitamin K1 and MK-4 concentrations in dairy compared with grain-based regimens. Moreover, the hypothesis that grass-fed beef contains more MK-4 than grain-fed beef is biologically plausible, as forage contains higher K1 concentrations than grains (Bonaldo and Leroy 2024), and mechanistic evidence demonstrates that dietary K1 is readily converted to MK-4 *in vivo*: isotopic-labelling experiments in mice showed efficient conversion of both dietary K1 and other menaquinones into MK-4 across several tissues (Ellis *et al.* 2022). However, direct, side-by-side measurements of MK-4 levels in beef from different production systems had not yet been documented in the peer-reviewed literature. Notably, our findings fill this knowledge gap and are consistent with current understanding of the biological pathways involved in menaquinone metabolism. In addition, the novel nutritional information regarding MK-4 in beef could further enhance the value of well-managed, pasture-based systems within sustainable livestock production (Maree *et al.* 2025).

As shown in Table 1, significant differences were detected between production systems for ALA, EPA, DHA, and consequently for total n-3 PUFA. Among n-6 fatty acids, LA differed between systems, as did total n-6. Reflecting these shifts across families, the n-6/n-3 ratio also differed markedly. In contrast, no statistically significant differences were observed for CLA, SFA, MUFA, or PUFA. Although inter-study variability in individual fatty acids is expected (Bronkema *et al.* 2019), grass-fed beef consistently exhibits higher n-3 PUFA and lower n-6 PUFA compared with grain-fed beef -a pattern considered potentially beneficial for human health (Bronkema *et al.* 2019; Daley *et al.* 2010; Krusinski *et al.* 2025). Therefore, our findings align with the well-established understanding of how forage- versus grain-based feeding shapes bovine fatty acid composition and reinforce its continued use as a reliable marker of production-system differences.

It is important to highlight that, while subtle genetic differences or other unknown confounders potentially influencing our primary outcome cannot be completely ruled out in our study design, the following elements provide convergent evidence: (i) the expected pasture versus grain fatty-acid pattern (internal validity control), (ii) the plausibility of K1-to-MK-4 conversion under greater plant intake -mirroring the behavior of other fat-soluble vitamins such as carotenoids and tocopherols- and (iii) the extremely large effect size observed for MK-4 between the two systems. Together, these elements are consistent with the interpretation that the MK-4 differences are system-related.

From a human nutrition perspective, It should be noted that RCTs demonstrating skeletal and cardiovascular benefits of vitamin K2 have employed supraphysiological doses -such as MK-4 at 45,000 µg/day in fracture-reduction trials or MK-7 at 180-720 µg/day in trials designed to slow the progression of coronary artery calcification (Li *et al.* 2023), as well as MK-7 at 180 µg/day in studies reporting attenuation of age-related bone loss (Zhang *et al.* 2025). However, observational cohorts from the large PREDIMED trial, for example, indicated that increases in dietary menaquinone intake within the range obtainable from foods (18–58 µg/day) were associated with lower cancer and all-cause mortality (Juanola-Falgarona *et al.*

Table 1. Fatty acid composition in beef from well-managed and grain-finished production systems.
Tabla 1. *Composición de ácidos grasos en carne bovina de sistemas pastoriles bien manejados y de terminación a grano.*

Fatty acid	Well-managed grazing	Grain-finished	*P*-value
ALA	0.93 ± 0.19	0.20 ± 0.03	<0.001*
EPA	0.23 ± 0.12	0.03 ± 0.04	0.006*
DHA	0.41 ± 0.23	0.02 ± 0.05	0.011*
n-3 PUFA	1.57 ± 0.52	0.25 ± 0.05	0.005*
LA	3.28 ± 0.70	4.88 ± 0.72	0.016*
n-6 PUFA	4.25 ± 1.01	5.65 ± 0.85	0.045*
CLA	0.75 ± 0.03	0.54 ± 0.31	0.056
n-6/n-3	2.79 ± 0.43	23.80 ± 6.11	<0.001*
SFA	43.52 ± 3.08	42.22 ± 1.50	0.222
MUFA	45.32 ± 2.90	45.76 ± 0.48	0.151
PUFA	5.83 ± 1.51	5.90 ± 0.83	0.927

ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3 PUFA, C18:3+C20:5+C22:5+C22:6; LA, linoleic acid; n-6 PUFA, C18:2+C18:3+C20:4+C22:4; CLA, conjugated linoleic acid SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values are mean ± SD (n = 5 steers per system) and expressed as percentages of total fatty acids detected. P-values are two-sided and * indicates P < 0.05.

2014), supporting the possibility that meaningful health benefits may arise from vitamin K2 intakes achievable through ordinary dietary sources. Therefore, even though beef provides smaller amounts of menaquinones than the supplemental doses used in RCTs, its contribution to overall vitamin K2 intake may still be nutritionally relevant and should not be dismissed without further research. This is particularly important given the recent nature of the evidence in this field and the absence of an established Recommended Daily Intake (RDI) for vitamin K2 (Akbulut *et al.* 2020).

Conclusions

To the best of our knowledge, this study provides the first evidence that forage intake in well-managed grazing systems, compared with grain-finished feeding, is possibly a key determinant of MK-4 accumulation in beef. Although additional studies are necessary to confirm our findings in other experimental settings and in different muscles, breeds, or even species raised under herbivore-type feeding systems, the present work advances our understanding of how the production system influences the nutritional composition of livestock products and may have important implications for the valorization of sustainable livestock systems and for human health.

Acknowledgments

We would like to thank Tatiana Nicola for her valuable technical assistance. This work was funded by Instituto Nacional de Tecnología Agropecuaria (INTA) - 2023-PE-L04-I119 “Abordaje de la calidad y procesos de agregado de valor de productos en sistemas agrobioindustriales sostenibles”.

Contributions

Conceptualization: JP, SR, CM, AD, DP; Methodology: SR, JP, CM, LR, AB, MV, SC; Data curation: SR, JP, LR, AB, MV, SC, GG, DP, AD, CM; Formal analysis: JP, SR, GG, CM, DP, AD; Statistical analysis: JP, SR, SC, AD; Writing (original draft): SR, JP, CM; Writing (review and editing): JP, SR, LR, AB, MV, SC, GG, DP, AD, CM; Funding acquisition: AD, JP, SR, LR, GG

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