



## Amino acid and hematological alterations in beef from BLV-infected cattle: Implications for functional food safety

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### ABSTRACT

**Background:** Beef is a vital source of protein, rich in B vitamins (B12, B6, B3, and B2) and minerals such as iron, zinc, magnesium, and phosphorus. However, bovine viral leukemia (BLV) in cattle poses a biological and public health concern, as it compromises meat quality and safety. Meat from BLV-infected cattle, especially during the hematological stage, exhibits significant nutritional degradation and poses potential health risks.

**Objectives:** This study assessed the nutritional quality of beef from healthy, BLV-infected (asymptomatic), and clinically sick cattle, focusing on caloric content, protein, fat, ash, and moisture. It aimed to evaluate how viral infection affects the functional and bioactive value of meat.

**Methods:** Laboratory tests were conducted at the National Agrarian University of Armenia. BLV diagnosis was based on clinical, serological, and hematological data. Blood analyses were performed using a Micro CC 20 Plus analyzer.

**Results:** Meat from BLV-infected cattle exhibited increased moisture content (up to 2.72%) and reduced protein and fat levels, resulting in a lower caloric value. Cold cuts from infected animals contained tryptophan and lysine degradation products, including heat-stable, potentially carcinogenic compounds, making the meat unsuitable for human consumption.

**Novelty:** This study uniquely compares the chemical composition of beef from healthy and BLV-infected cattle, detailing specific nutritional losses and identifying persistent toxic metabolites that raise concerns about food safety. It was found that the meat obtained from cattle infected with leukemia does not meet the requirements of functional food in terms of its chemical and biochemical composition and nutritional value, and therefore cannot be considered a complete food in the human diet.

**Conclusion:** BLV remains a challenge for livestock. Meat from infected animals exhibits diminished nutritional and biological quality, posing safety risks due to heat-resistant, carcinogenic byproducts.

**Keywords:** Amino acids, BLV-infected cattle, leukemia, cattle, beef, nutritional value of meat, bioactive compounds, functional foods, food safety

**Beef Quality Comparison: Healthy vs. BLV-Infected vs. Clinically Sick Cattle**

Characteristic	Healthy Cattle	BLV-Infected Cattle	Clinically Sick Cattle
Moisture	Normal	Increased	High
Protein	Normal	Reduced	Low
Fat	Normal	Reduced	Low
Caloric Value	Normal	Lower	Low
Tryptophan and Lysine	Normal	Degradation Products	Degradation Products
Nutritional and Biological Quality	High	Diminished	Very Low
Food Safety	Safe	Unsafe	Unsafe
Potential Source for Functional Food Product	Potential Candidate	Does Not Qualify	Does Not Qualify

**Graphical Abstract:** The Effect of Viral Leukemia of Beef on the Quality Characteristics of Meat Introduction

## INTRODUCTION

Despite ongoing discussions about the health implications of red meat, functional meat products are gaining recognition as a key modern food trend [1]. When enriched with bioactive components, meat products can offer enhanced nutritional profiles tailored to consumer needs [2].

Beef is a rich source of protein, providing essential nutrients critical for muscle function, recovery, and metabolism, such as B vitamins (B12, B6, B3, and B2), iron, zinc, magnesium, and phosphorus [3-6]. Despite concerns over fat and cholesterol, beef also delivers high-quality protein and unique bioactive compounds (e.g., carnosine, taurine, creatine), which are beneficial when consumed as part of a balanced diet [7-8].

The demand for red meat continues to rise, especially in developing regions. Improvements in animal breeding and nutrition may enhance the value of red meat in addressing global health concerns, supporting child development, and promoting healthy aging [9-11]. Still, concerns linking red meat to obesity, type 2 diabetes, and cardiovascular disease continue to drive research [12].

Functional foods (FF) enriched with bioactive ingredients are increasingly valued for their role in preventing disease. Both animal- and plant-based FF offer potential benefits, supported by clinical studies showing reduced risks for cancer, cardiovascular, gastrointestinal, and neurological disorders [13]. The Functional Food Center (FFC) emphasizes the importance of understanding food bioactive compounds to substantiate health claims [14].

It is well established that a link exists between red meat consumption and an increased risk of cardiovascular diseases [15-18]. Red meat's potential role in cancer remains a concern [19-21], particularly due

to compounds like nitrosamines and heterocyclic amines [22]. Although less studied, a possible link between red meat and leukemia has emerged, prompting further investigation into the relationship between red meat and leukemia [23-24].

BLV is a chronic infectious disease that alters the genetics of lymphatic cells and is prevalent in livestock. It results in economic losses and trade limitations [25-27]. Meat from BLV-infected animals often lacks the quality needed for FF classification and may pose health risks, reinforcing the importance of strict sanitary inspection [28].

In Armenia, research on the quality of BLV-infected meat is limited. Current available studies indicate lower organoleptic quality and higher microbial contamination than meat from healthy animals, stressing the need for proper veterinary control and quality monitoring in FF development.

## MATERIALS AND METHODS

**Study Location and Sampling:** Laboratory studies were conducted at the Research Center for Veterinary and Veterinary Sanitary Examination of the Armenian National Agrarian University. Samples were collected from slaughterhouses during sanitary slaughter in the Republic of Armenia.

**Animal Grouping and Diagnosis:** Animals were divided into three groups, each consisting of five animals:

- First group (control): Meat obtained from healthy animals.
- Second group (infected): Meat obtained from leukemia-infected animals.
- Third group (sick): Meat obtained from leukemia-sick animals.

In the healthy group, the animals exhibited active behavior, demonstrated normal mobility and reflexes, and had adequate nutrition. Body temperature, pulse, and respiratory movements were within normal limits. The live weight ranged from 380 to 470 kg, and the slaughter yield was 53.4%.

The animal groups were formed based on pre-slaughter clinical examination data as well as results of laboratory blood tests and diagnostic screenings.

In leukemia-infected animals, the live weight ranged from 320 to 367 kg, and the slaughter yield was 43.3%.

Compared to healthy animals, those with leukemia showed a rise in temperature by 0.2–0.8°C, enlarged superficial lymph nodes, weakness, indifference to their surroundings, and poor appetite.

Hematological analysis was also conducted using the Micro CC 20 analyzer, along with preliminary laboratory testing, and the results served as the basis for group classification.

The diagnosis of bovine leukemia was established using commonly applied methods, incorporating epidemiological data, clinical signs, gross pathological changes in organ systems, and serological and hematological data.

**Analytical Procedures:** Hematological studies on blood samples from healthy and diseased animals were performed using a Micro CC 20 Plus analyzer.

Biochemical analysis of meat from leukemia-infected and sick animals was conducted to determine quantitative changes in essential and non-essential amino acids.

Microbiological contamination of beef and internal organ samples was assessed by GOST 21375-75. Smears were prepared, air-dried, Gram-stained, and examined microscopically for microbial analysis. The arithmetic mean of detected microorganisms was calculated.

Veterinary and sanitary examination of meat involves veterinary inspection of slaughtered animals, as well as subsequent veterinary and hygienic evaluation of the meat products.

**Caloric Value Calculation:** The caloric value (K) of meat, expressed in kcal, was calculated using the following formula:

$$K=[C-(J+Z)]\times 4.1+(J\times 9.3)$$

Where:

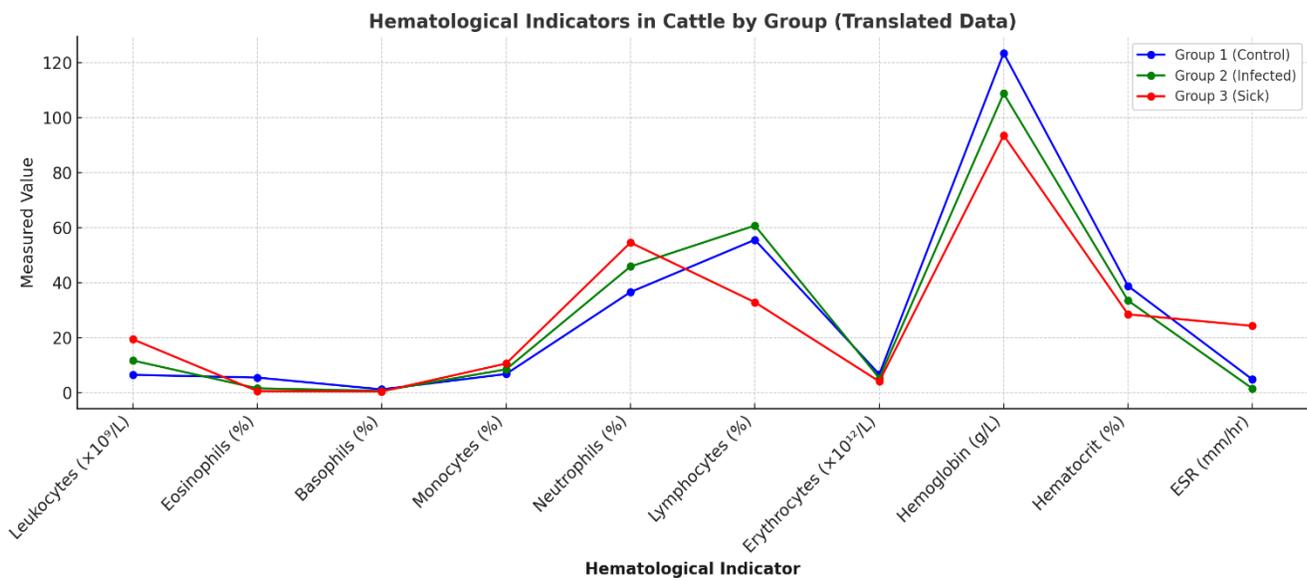
- C – Amount of dry matter (g)
- J – Fat content (g)
- Z – Ash content (g)

**Statistical Analysis:** The data were analyzed using Student's t-test in Microsoft Excel (version 2003)

## RESULTS AND DISCUSSION

A comprehensive hematological analysis of the blood from healthy, leukemia-infected, and leukemia-affected animals was conducted, with a particular focus on the leukemia-infected animals.

Figure 1 illustrates that leukemia-infected and particularly ill animals exhibit significant changes in blood composition. An increased leukocyte count reflects immune activation, while a reduction in eosinophils and basophils may indicate immune suppression. Lymphocytes initially rise but later drop sharply due to impaired hematopoiesis. Decreased erythrocytes, hemoglobin, and hematocrit levels indicate anemia, while an elevated erythrocyte sedimentation rate (ESR) suggests chronic inflammation.



**Figure 1.** Hematological Indicators of Leukemia-Sick Animals (n=5).

Studies confirm that anemia can develop alongside leukemia. Rising ESR, neutrophils, and monocytes suggest ongoing inflammation and possible secondary complications. A comparative analysis of meat from healthy, infected, and sick cattle revealed that hematological abnormalities correspond to disease severity.

Early infection exhibits lymphocyte proliferation, whereas advanced stages are characterized by a sharp decline in lymphocytes, reflecting BLV’s impact on hematopoietic function. Significant reductions in red blood cells, hemoglobin, and hematocrit reflect anemia caused by chronic infection. Increased neutrophils and monocytes suggest acute-phase immune responses. These changes compromise the nutritional and bioactive qualities of meat due to tissue degradation and metabolic imbalance.

While this study sheds light on the effects of BLV on blood and meat quality, further research is needed. Future studies should track hematological trends over time, with a focus on immune cell suppression and bone

marrow function. Suppressed eosinophils and basophils may indicate immune exhaustion and merit deeper investigation. Elevated ESR and arginine could be biomarkers for subclinical disease or meat unsuitability. Including oxidative stress, immune markers, and molecular diagnostics would clarify how systemic inflammation affects meat quality. Larger sample sizes and diverse animal populations are necessary for broader relevance to food safety and public health.

The research revealed notable disruptions in the chemical composition of meat from cattle infected with leukemia and those clinically sick, particularly during the hematological stage of the disease. Table 1 shows the chemical composition of meat obtained from animals with leukemia. Compared to healthy animals, meat from infected cattle showed a 1.95% increase in moisture, while samples from sick animals demonstrated a 2.72% rise. Higher moisture levels negatively affect meat quality by reducing flavor, processing efficiency, and shelf life, all of which are critical for consumer acceptance and qualifying as a functional food.

**Table 1.** Chemical composition of meat obtained from animals with leukemia (n=5).

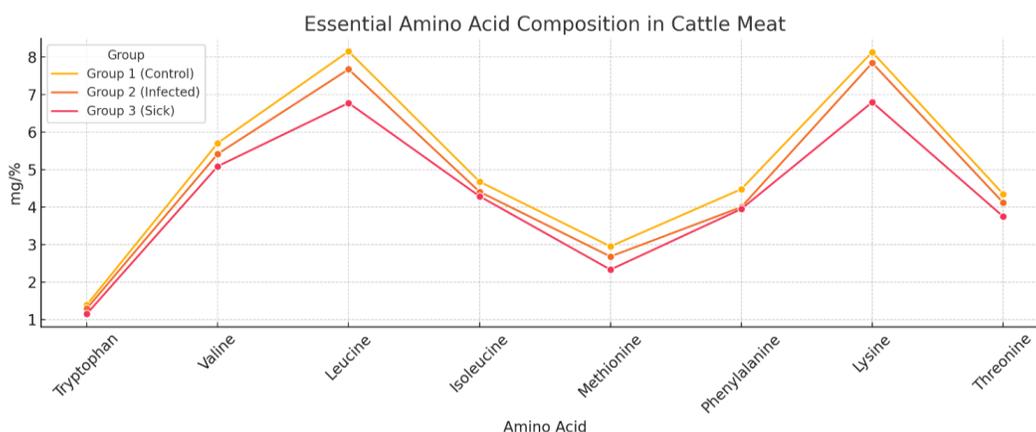
Indicators	Animal groups		
	Group 1 /tested/	group 2 /infected/	group 3 /sick/
Caloric content (kcal)	135.2 ± 1.4	115.3 ± 1.6 (-12.5%)	99.6 ± 1.8 (-27.74%)
Proteins, (%)	20.5 ± 0.15	20.3 ± 0.05 (-1.0%)	20.2 ± 0.08 (-1.5%)
Fats,(%)	3.51 ± 0.03	3.48 ± 0.02 (-0.85%)	3.46 ± 0.01 (-1.4%)
Ash, (%)	1.00 ± 0.01	1.1 ± 0.022 (+10%)	1.17 ± 0.015 (+17%)
Humidity, (%)	74.92 ± 0.05	76.38 ± 0.2 (+1.95%)	76.96 ± 0.09 (+2.72%)

In addition to elevated moisture, the study found significant reductions in fat and protein content, two macronutrients essential to the meat’s nutritional value. Consequently, the caloric value dropped by 12.5% in infected animals and by a substantial 26.3% in those clinically sick, severely limiting the energy provided by the meat. An increased ash content of up to 17% in sick animals likely reflects disturbances in electrolyte balance and mineral distribution, signaling broader metabolic dysfunction.

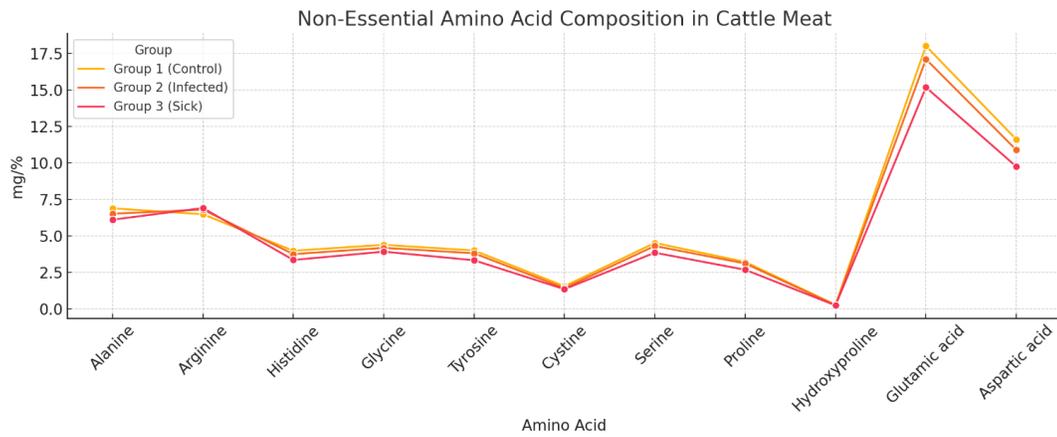
From a functional food science (FFS) standpoint, these compositional changes are highly significant. Functional foods must deliver nutritional adequacy, metabolic support, and bioactive functionality. Protein and fat are core nutrients and vital carriers of essential

amino acids and fat-soluble vitamins. Their depletion, combined with increased moisture and altered mineral balance, compromises the meat’s health-supporting properties. Furthermore, disease-driven metabolic disruptions, such as reduced fat deposition and tissue integrity, underscore the impact of animal health on meat quality. These findings underscore the importance of rigorous veterinary oversight to ensure that meat products meet the safety and nutritional standards required in the functional food sector.

In addition, the study assessed the amino acid composition of meat samples obtained from healthy, leukemia-infected, and clinically sick cattle, as presented in Figures 2 and 3.



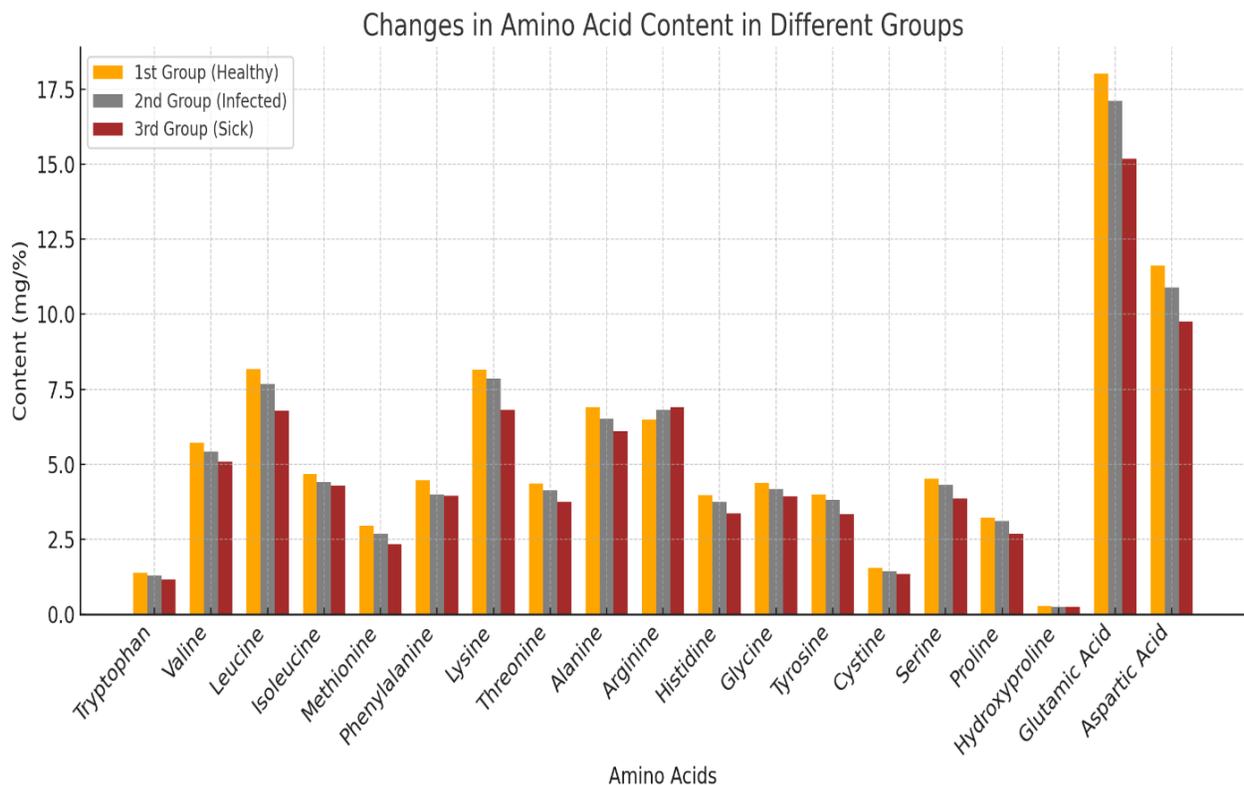
**Figure 2.** Changes in Amino Acid Content in Biological Samples



**Figure 3.** Changes in Non-Essential Amino Acid Content in Biological Samples.

Figure 4 illustrates the changes in amino acid content among different groups: healthy, infected, and leukemia-affected animals. The study revealed significant differences in the amino acid composition of meat from leukemia-infected and leukemia-affected cattle compared to healthy controls, with the most pronounced alterations found in clinically sick animals. In leukemia-infected cattle, amino acid levels declined by an average

of 5–10%, except for arginine, which increased by 4.9%. The most significant reductions among essential amino acids were observed in phenylalanine (10.7%), methionine (9.2%), tryptophan (7.2%), leucine (5.9%), and threonine (5.3%). Non-essential amino acids also declined, most notably cystine (8.4%), hydroxyproline (7.4%), and aspartic acid (6.2%).



**Figure 4.** The changes in the amino acid content in different groups: Healthy, infected, and leukemia sick animals (n=5)

In clinically sick animals, the decreases were more substantial. Essential amino acids declined by 15–25%, with methionine (21%), tryptophan (17.3%), leucine (16.9%), and lysine (16.4%) showing the most significant reductions. Non-essential amino acids decreased by 10–20%, particularly tyrosine and proline (both 16.8%), aspartic acid (16.0%), and glutamic acid (15.8%). Arginine levels again increased, this time by 6.6%, potentially due to immune activation associated with disease progression.

Essential amino acids are vital for immune function, protein synthesis, tissue repair, and neurological health. Their depletion diminishes the nutritional and functional value of meat. Tryptophan and methionine, for example, are precursors to serotonin and methyl donors,

respectively—both crucial for cognitive and cellular processes.

Functional foods are expected to provide not only basic nutrition but also support health through bioactive efficacy. The reduced amino acid profile seen here compromises the potential of this meat to meet functional food standards. The observed increase in arginine may reflect compensatory metabolic activity in response to inflammation, but it does not offset the broader nutritional losses.

To further understand the health implications, microbiological studies were also performed on meat, offal, and lymph node samples from animals at various stages of the disease.

**Table 2.** Bacterial contamination of the meat and offal of animals with leukemia (n=5).

Detected microorganisms	Animal groups		
	Group 1 (healthy)	Group 2 (infected)	Group 3 (sick)
<i>E. coli</i>	0	25.1 ± 0.2	42.8 ± 3.3
<i>Salmonella spp.</i>	0	7.5 ± 0.08	15.6 ± 1.02
<i>Proteus spp.</i>	0	3.5 ± 0.05	11.2 ± 0.9
<i>Staphylococcus</i>	1.3 ± 0.07	5.4 ± 0.06	22.3 ± 1.3

Microbial contamination was significantly higher in meat from cattle infected with leukemia, especially in clinically sick animals, compared to healthy controls. Smears from healthy animals showed only *Staphylococci*, while those from sick animals revealed intestinal bacilli, such as *E. coli*, *Salmonella*, and *Proteus* species, in addition to *Staphylococci*, with bacterial counts 2–4 times higher than in healthy animals. This indicates a substantial rise in tissue contamination in diseased cattle.

The study compared meat from healthy, leukemia-infected, and sick animals to assess quality under functional food standards. Infected cattle showed moderate declines in nutritional and microbiological quality, suggesting limited industrial use. Meat from sick animals exhibited severe degradation, characterized by

reduced essential amino acids and high microbial loads, rendering it unfit for human consumption.

Additionally, meat from both infected and sick animals contained heat-resistant carcinogenic metabolites from amino acids such as tryptophan and lysine. These findings confirm that meat from BLV-affected cattle is unsafe and unsuitable for human dietary use [29].

**Phytogetic feed additives (PFAs):** Among the three types of meat evaluated in this study, sourced from healthy, leukemia-infected, and clinically sick cattle, only the meat from healthy animals meets the criteria for potential use in functional food development. This meat maintained higher nutritional integrity and was particularly rich in bioactive compounds, including essential amino acids

such as leucine, lysine, and methionine, which play key roles in immune function, tissue regeneration, and metabolic balance. In contrast, meat from infected and sick animals exhibited substantial losses in amino acid content and overall nutritional value, rendering it unsuitable for classification as functional food.

To address such nutritional degradation, current strategies in animal nutrition are increasingly focusing on PFAs, as well as plant-derived substances, including herbs, spices, essential oils, and botanical extracts. Common examples include oregano, thyme, rosemary, ginger, turmeric, aloe vera, and green tea polyphenols. These compounds offer notable antimicrobial, antioxidant, and digestive benefits [30].

Poultry and livestock systems often use PFAs as natural alternatives to antibiotic growth promoters, particularly in regions where restrictions on synthetic additives are enforced. Research indicates that PFAs improve growth performance and feed efficiency, enhancing blood profiles and carcass characteristics [31], ultimately leading to improved nutritional and functional properties.

Integrating PFAs in beef cattle diets could offer a promising approach to improve meat quality, particularly in mitigating the biochemical deficits observed in animals affected by viral infections. Future research should investigate the potential of phytogetic supplementation to enhance the amino acid profiles, antioxidant capacity, and overall bioactive content of beef, thereby supporting the broader goal of producing safe, nutrient-dense, and health-promoting meat products.

**FFC's Evaluation of Beef from Healthy, Leukemia-Infected, and Clinically Sick Cattle:** The FFC has established a science-based framework for evaluating functional foods, emphasizing standardized criteria, clinical relevance, and health benefits [32]. Led by Dr. Martirosyan, the FFC introduced a comprehensive definition and development model for functional foods

[33]. According to the FFC, functional foods are “natural or processed foods containing biologically active compounds that, in defined, effective, and non-toxic amounts, provide a clinically proven health benefit using specific biomarkers to promote optimal health and reduce the risk of chronic and viral diseases” [34].

This definition underpins the FFC's evaluation process, which reviews bioactive content, safety, mechanisms of action, and clinical efficacy. Within this model, beef from BLV-infected cattle raises serious concerns and does not qualify as a functional food source. The FFC requires rigorous preclinical and clinical testing to confirm safety, dosage, and efficacy [35]. Due to poor nutritional quality, microbial contamination, and the presence of harmful metabolites, meat from infected or sick cattle fails to meet FFC standards.

Of the three beef categories analyzed—healthy, BLV-infected, and clinically sick—only meat from healthy animals qualifies as a potential candidate for functional food development. Even then, nutritional value may be improved through targeted dietary interventions, such as phytogetic feed additives.

**Connection to Functional Food Safety:** The integrity of functional food safety—especially animal-derived products such as meat—is closely tied to the health status of livestock. For instance, BLV proviral DNA has been detected in fresh milk and raw beef for human consumption, raising concerns about nutritional degradation and potential carcinogenic risks from consuming infected meat [36]. Ensuring the safety of value-added or functional meat products requires rigorous oversight of animal health, processing methods, and contaminant testing to protect consumers [37]. This aligns with broader challenges in meat safety—ranging from microbial pathogens to chemical hazards—underscoring the importance of strong regulatory frameworks and scientific validation to safeguard the food supply [38].

**Future Directions:** This study reveals a significant decline in the nutritional and functional quality of meat from cattle infected with leukemia. Future research should aim to enhance meat quality through innovative dietary strategies and scientific validation. Advancing functional food science in animal-derived products requires a multidisciplinary approach, outlined below:

1. **Feed-Based Nutritional Enhancement:** Improving meat quality via dietary interventions is a promising area. PFAs—including herbs and plant extracts like oregano, thyme, turmeric, and aloe vera—have shown benefits in poultry and swine by enhancing antioxidant status and gut health. Future studies should assess their role in cattle nutrition, particularly during early stages of infection, to preserve amino acids, improve lipid profiles, and reduce harmful metabolites. Such approaches align with consumer demand for cleaner, bioactive-rich meat with health-promoting properties.
2. **Functional Meat for Targeted Health Applications:** Meat enriched with bioactive compounds offers potential for clinical and therapeutic nutrition. Research should evaluate whether diets enhanced with PFAs improve muscle function, immunity, or metabolic resilience. A special focus should be on amino acids like leucine and methionine, given their crucial roles in protein synthesis and cellular repair. Future studies should investigate the interaction of functional meat with human metabolism, inflammation, and gut microbiota, thereby supporting its role in personalized nutrition for elderly and chronically ill populations.

3. **Strengthening Scientific Rigor:** Larger, well-designed trials are needed to validate functional claims and support regulatory approval. The current study's limited sample size (n = 5 per group) necessitates randomized trials with defined protocols. Future research should utilize omics technologies (e.g., metabolomics, proteomics) to track changes in bioactive compounds and assess their bioavailability through in vivo models or human trials. Standardized methods for sensory analysis, shelf-life, consumer acceptance, and labeling compliance are also essential.

Integrating animal science, functional food development, and clinical nutrition will enhance meat quality and link these advancements to tangible health benefits. The ultimate goal is to position animal-derived foods as scientifically validated contributors to public health and well-being.

**Scientific Innovation:** This study presents an integrated diagnostic approach, combining clinical, epidemiological, and hematological evaluations with precise laboratory analysis to assess meat quality in cattle infected with leukemia. The Micro CC 20 Plus analyzer links hematological disease stages with measurable declines in meat composition. It is among the first to directly correlate blood health markers with biochemical meat degradation.

**Practical Implications:** The findings underscore the need for enhanced veterinary oversight, particularly in regions where bovine leukemia is prevalent. Meat from infected cattle, especially those in advanced stages of infection, exhibits reduced nutritional quality and contains harmful metabolites, rendering it unsafe for consumption. These results support stricter slaughterhouse screening and

emphasize the importance of safe sourcing to protect public health.

## CONCLUSION

Bovine leukemia causes muscle degradation, resulting in lowered protein synthesis, energy levels, and antioxidant capacity. This results in significant losses of amino acids, including methionine, leucine, and glutamic acid. Elevated arginine may aid in disease staging.

Decreased caloric, protein, and fat content, along with increased moisture, ash, and microbial load, reflect tissue damage and immune suppression. Despite control efforts, bovine leukemia remains a major threat. The study supports the development of better diagnostics and control measures, confirming that meat from infected animals is unfit for human consumption and poses significant public health risks.

**Abbreviations:** FF: Functional foods; BLV: Bovine Leukemia Virus; PFAs: phytogetic feed additives; FFS: functional food science; ESR: erythrocyte sedimentation rate

**Authors' Contributions:** LG is the principal author and conductor of the research. VG, MS, ZhM, RG, and SY conducted laboratory tests. VA and NH contributed to statistical processing. DM contributed to the writing and editing of the manuscript and summarized the research's scientific innovations and practical implications. HW edited, organized the literature review, and proofread the final version.

**Competing interest:** The authors declared that there is no competing interest.

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