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Review Article

Safety of microbial enzyme and *Allium*-probiotic bioproducts in poultry: a review

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Abstract

Microbial enzymes, strain-defined probiotics, and fermented botanical substrates are increasingly developed as poultry bioproducts, but evidence of efficacy does not establish safety itself. This narrative review synthesizes published poultry studies and regulatory/scientific guidance to propose an operational whole-bioproduct framework for microbial enzyme and *Allium*-probiotic preparations. The framework separates product identity and classification, hazard characterization, exposure assessment, target-animal tolerance, worker/consumer/environmental safety, manufacturing controls, and batch-release specifications. It also distinguishes safety and tolerance endpoints from efficacy and mechanistic outcomes, which are often conflated in poultry additive studies. Particular attention is given to strain-level genomic characterization, antimicrobial-resistance assessment, enzyme allergenicity, dose–response design, *Allium*-related compositional variability and contaminants, fermentation-derived metabolites, and environmental persistence. Evidence from fermented chives, purple onions, *Bacillus* and *Lactiplantibacillus* systems is used as an illustrative case base rather than as proof of class-wide safety. The proposed framework is intended for researchers, product developers, and risk assessors and should be adapted to the applicable jurisdiction. A defensible safety dossier therefore evaluates the defined final product under its intended conditions of use, not a single active component in isolation.

Keywords: microbial enzyme; biosafety; risk assessment; *Allium*; probiotic; tolerance; antimicrobial resistance; quality control; poultry; narrative review

1. Introduction

Reducing unnecessary antimicrobial exposure is a major goal in modern poultry production, which has increased interest in microbial enzymes, probiotics, phytogenic additives, and fermented plant products [1–7]. Feed and drinking-water products based on *Allium cepa*, *Allium schoenoprasum*, *Lactiplantibacillus plantarum*, and *Bacillus subtilis* are being studied for effects on gut health, pathogen control, and production resilience [8–16]. Recent studies from Vietnam specifically evaluated *L. plantarum*-fermented chive in *Escherichia coli*- and *Eimeria acervulina*-challenged broilers, fermented purple onion and chive extracts against toxin-associated enteric bacteria, and chive extract in laying hens [12–15]. These studies provide product-specific efficacy or mechanistic evidence; they do not establish class-wide safety, fermented-shallot efficacy, or heat-stress mitigation in laying hens.

The safety problem is increasingly complex because poultry bioproducts are rarely single molecules. A final preparation may contain an enzyme, viable vegetative cells or spores, botanical substrates, organic acids, antimicrobial peptides, transformed phytochemicals, residual culture components, and inanimate microbial fractions. Accordingly, the same product may show efficacy signals through several mechanisms while also presenting distinct manufacturing, exposure, and biosafety questions. This review is written primarily for poultry researchers, bioproduct developers, and risk assessors who need to distinguish these evidence domains.

A central principle is that "natural", "botanical", or "probiotic" origin is not a safety conclusion. Enzyme proteins can cause occupational sensitization; production or probiotic strains may contain acquired antimicrobial-resistance (AMR) or virulence determinants; botanical composition can vary by cultivar, plant part, harvest, and contaminants; and fermentation can generate or transform metabolites. Safety assessment should therefore integrate product definition, hazard identification, exposure, target-animal tolerance, microbial genomics, toxicology, manufacturing quality, and post-use environmental considerations [17–29].

The objective of this narrative review is to synthesize the available evidence and propose an operational framework for safety assessment of microbial enzyme and *Allium*-probiotic bioproducts intended for poultry. The framework complements, rather than replacing, existing enzyme, feed-additive, probiotic, and toxicology guidance by integrating those principles at the level of a complex final product. Throughout the review, statements derived from published studies or regulatory/scientific guidance are distinguished from implementation recommendations proposed by the authors.

2. Methodology

This article used a structured narrative-review approach. The literature was identified through PubMed and publisher databases, together with authoritative documents from the European Food Safety Authority (EFSA), the Organisation for Economic Co-operation and Development (OECD), the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), and the United States Food and Drug Administration Center for Veterinary Medicine (FDA/CVM), and Codex Alimentarius. Searches were updated through 13 August 2026 and emphasized publications from 2000 to 2026; older seminal sources were retained when they established widely used safety concepts. Search concepts combined poultry terms (poultry, broiler, and laying hen) with product terms (microbial enzyme, *Bacillus*, *Lactiplantibacillus*, probiotic, postbiotic, fermented *Allium*, onion, and chive) and safety terms (tolerance, toxicity, allergenicity, antimicrobial resistance, whole-genome sequencing, environmental safety, quality control, and stability).

Studies were selected for relevance to at least one of the following prespecified domains: product identity and manufacturing, production-strain biosafety, enzyme toxicology or allergenicity, target-animal tolerance, consumer or occupational safety, environmental exposure, AMR, product quality, or efficacy/mechanistic evidence needed to interpret but not substitute for safety. Because this was a narrative rather than systematic review, no meta-analysis or formal risk-of-bias score was applied. Evidence was mapped iteratively to the framework domains, with particular attention to where published poultry studies did not include dedicated safety endpoints. Author-group studies are identified as illustrative local case examples and are not treated as independent replication.

3. Scope, terminology, and product categories

This review uses "bioproduct" as an umbrella term for a defined final preparation that contains one or more biologically active microbial, enzymatic, or botanical fractions. The product categories considered are: (i) purified or partially purified microbial enzyme preparations; (ii) viable probiotic products, including spore-based products; (iii) fermented botanical substrates containing live microorganisms; (iv) enzyme-rich fermented products containing multiple active fractions; and (v) preparations containing inanimate microbial cells or components that may qualify as postbiotics when the accepted definition and demonstrated health benefit are met [27, 28, 30]. The term probiotic is reserved for live, strain-defined microorganisms administered in adequate amounts with a documented benefit. The term postbiotic is not used for an undefined fermented matrix merely because it contains metabolites.

Microbial enzymes such as cellulases, xylanases, beta-glucanases, proteases, amylases, phytases, and mannanases may improve nutrient release or alter substrates available to the gut microbiota [31–33]. Their safety dossier differs from that of a viable probiotic because the relevant hazards and exposures differ. Likewise, a fermented *Allium* product containing viable bacteria, enzymes, organic acids, and transformed phytochemicals requires a broader characterization than a purified enzyme or a single-strain probiotic. The framework therefore applies common risk principles while retaining product-category-specific evidence requirements.

The review draws on established principles for microbial production organisms, food/feed enzymes, target-species safety, probiotic qualification, and toxicology [17–29, 34, 35]. These sources are guidance or consensus references, not a single universal regulatory code. The proposed framework adds the following integration step: it asks whether the identity, hazard, exposure, quality, and safety evidence are coherent for the same final product and intended conditions of use.

4. Operational whole-bioproduct risk-assessment model

The operational model begins by separating hazard, exposure, and risk. A hazard is an intrinsic property with potential to cause harm; exposure describes the dose, route, duration, and population contacting that hazard; and risk reflects the likelihood and severity of harm under the intended conditions of use. Thus, detection of an AMR gene, allergenic sequence, phytochemical, or contaminant does not by itself establish meaningful risk without characterizing exposure and biological relevance. Conversely, low exposure does not justify omitting hazard identification. Product definition therefore precedes interpretation as follows: a purified enzyme dossier may focus on enzyme identity, sequence, activity, impurities, digestibility, and toxicology, whereas an enzyme-rich fermented product also requires characterization of the production organism, botanical substrate, fermentation process, viable/non-viable cells or spores, residual DNA, metabolites, contaminants, storage stability, and batch consistency [17–24].

The purple-onion/*B. subtilis* BSn5 system illustrates why this integration matters. Fermentation conditions that increase carboxymethyl cellulase activity also change microbial biomass, metabolite profiles, residual substrate, and potentially contaminant growth [8]. Accordingly, process optimization should not maximize enzyme activity in isolation; it should define an acceptable design space for potency, microbiological purity, metabolite profile, stability, palatability, and target-animal tolerance. This *Allium* example is used throughout the review as an illustration, not as evidence that all fermented *Allium* products share the same safety profile. The resulting five-step workflow and its cross-cutting characterization elements are summarized in **Figure 1**.

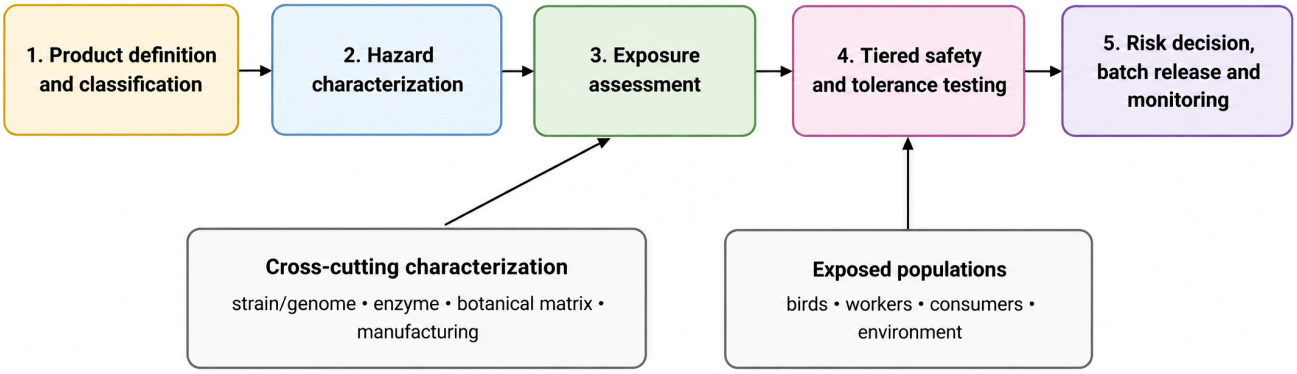

Figure 1. Operational whole-bioproduct risk-assessment framework. Product definition and classification precede hazard characterization, exposure assessment, tiered safety/tolerance testing, and a risk decision linked to batch release and monitoring. Cross-cutting characterization includes microbial strain/genome, enzyme identity and activity, botanical matrix, manufacturing controls, and the relevant exposed populations. Horizontal arrows indicate progression through the five assessment steps; upward arrows show how cross-cutting characterization and exposed-population information inform exposure assessment and tiered safety/tolerance testing.

In practical use, the framework follows these five steps: define and classify the final product and its intended active components; characterize hazards in the microorganism, enzyme, botanical matrix, contaminants, and process-derived constituents; estimate exposure for birds, workers, consumers, and the environment; perform Tier 1 testing and trigger higher-tier studies when hazard, exposure, uncertainty, or intended claims justify them; and integrate the evidence into conditions of use, batch-release criteria, risk-management measures, and post-market or field monitoring where appropriate. This sequence is the authors' proposed operationalization of existing safety principles rather than a jurisdiction-independent regulatory requirement.

5. Toxicological evaluation of microbial enzyme preparations

Enzyme proteins are frequently digested in the gastrointestinal tract. However, the risk from low oral exposure to whole enzyme proteins does not eliminate the need for a safety assessment. The safety assessment should include the enzyme sequence, the specificity and mechanism of action, the organism the enzyme was derived from, the likely level of exposure, the expected impurities, and the likely means of exposure. The oral safety of animals and consumers is not the same as the occupational safety of the workers who are exposed to the powder or aerosol. Enzyme dust is of particular concern because enzyme proteins can sensitize exposed workers and may cause respiratory allergy [17, 18, 25, 29].

For a novel enzyme bioproduct, a tiered toxicology package is more informative than a single endpoint. Literature-based hazard identification, enzyme-sequence comparison with known toxins/allergens, production-strain characterization, product specifications, and target-animal tolerance form a rational initial screen. Repeated-dose toxicity or genotoxicity studies may be appropriate when required by the applicable jurisdiction or when triggered by product novelty, exposure, residual production-organism material, unexpected metabolites, or other uncertainties; OECD Test Guidelines 408 and 471 are examples of established test methods [23, 24]. These examples should not be interpreted as mandatory tests for every poultry bioproduct.

The toxicological question is whether the defined final product produces adverse effects at the intended exposure and at appropriately justified higher exposures. A target-animal tolerance design should include the intended use level and one or more higher levels selected from product-specific exposure, palatability, and applicable guidance; multiples such as 3× or 5× may be useful research choices but are not universal regulatory thresholds. Endpoints should prioritize clinical signs, mortality, feed/water intake, hematology, serum chemistry, organ weights and pathology, and other claim- or hazard-specific measures. A no-observed-adverse-effect level (NOAEL) can be assigned only when the study design and highest tested dose support that conclusion. For example, a 42-day poultry safety study of an enzyme-expressing algal feed additive combined performance, hematology, clinical chemistry, and histopathology to support a NOAEL at the highest tested concentration [36].

6. Biosafety of microbial source organisms

Safety assessment starts with accurate microorganism identification. 16S ribosomal ribonucleic acid (rRNA) can support preliminary species identification for many bacteria but should not be presented as sufficient strain-level characterization for commercial development or a regulatory dossier. Whole-genome sequencing (WGS), comparative genomic analysis, strain-deposit information, and a documented bioinformatic pipeline provide a stronger basis for identity and safety assessment [19, 22, 26–28]. WGS findings should be reported with the databases, version/date, identity/coverage thresholds, and interpretation criteria used.

Bacillus spores offer processing and storage stability and some strains produce useful extracellular enzymes, but safety is strain-specific. AMR evaluation should distinguish intrinsic from acquired resistance, phenotype from genotype, chromosomal from mobile determinants, and gene detection from evidence of transferability. A defensible statement is that "no known acquired AMR or virulence determinants of concern were detected using the specified databases, thresholds, and pipeline," rather than claiming absolute absence. Phenotypic susceptibility testing remains important because in silico predictions are limited by database completeness and uncharacterized genes. Potential horizontal transfer should be considered when mobile elements or environmentally persistent viable organisms are present [2–5, 19, 22, 26, 37]. The recent poultry literature also frames *Bacillus*-based probiotics and fermented products as alternatives to antibiotic growth promoters; however, this efficacy-oriented rationale does not replace strain-specific safety assessment [38].

Lactic acid bacteria also require strain-level qualification. Probiotic status depends on a live, defined strain, an adequate viable dose, and a documented benefit [27, 28]. In local *Allium*-fermentation studies, *L. plantarum* 1582 and related isolates were selected for antibacterial activity, survival in the botanical matrix, fermentation performance, and gastrointestinal persistence [9, 13]. These selection traits are useful for product development but are not substitutes for WGS-based safety characterization, phenotypic AMR assessment, and targeted evaluation of mobile resistance or virulence determinants. Independent work with *Lacticaseibacillus rhamnosus* SN21-1 and *L. plantarum* SN21-2 illustrates a stronger combined approach by integrating genomic/phenotypic screening with 28-day broiler observations, blood biochemistry, and organ histology [39].

7. Immunogenicity and allergenicity

Immunogenicity must be used precisely. In vaccine science, it refers to antigen-specific immune induction. In enzyme and probiotic feed products, the more relevant concerns are unwanted sensitization, allergenicity, excessive inflammation, and immune dysregulation. Enzymes can be immunogenic as proteins, especially through inhalation exposure in workers; however, target-animal studies usually examine whether additives support balanced immunity rather than provoke harmful immune activation [25, 29].

For poultry, immune endpoints should be selected according to claim and plausible hazard. Serum immunoglobulins, cytokines, tight-junction expression, immune-organ indices, and pathogen load can be valuable mechanistic or efficacy endpoints [12–15], but they do not by themselves demonstrate immunological safety. Safety interpretation requires attention to adverse inflammation, immune dysregulation, clinical pathology, and dose–response. The absence of a statistically significant change in an immune biomarker in a small efficacy study is not equivalent to evidence of safety.

Allergenicity assessment for enzymes normally begins with sequence similarity to known allergens, molecular properties, protein stability, and exposure route. For botanical fermentation products, allergenicity is more complicated because fermentation can degrade, release, or transform proteins and small molecules. A prudent dossier should therefore separate occupational allergen risk, target-animal immunological tolerance, and consumer exposure through meat or eggs.

8. *Allium*-probiotic systems: efficacy signals and safety-specific concerns

Allium species provide a useful running example because onion, shallot, chive, and garlic-related materials contain fructans, phenolics, flavonoids, and organosulfur compounds whose concentrations vary with species, plant part, harvest, processing, and fermentation [40–44]. These constituents can support microbial growth or biotransformation and may contribute antimicrobial or antioxidant activity, but those effects are efficacy or mechanistic signals. Fermentation can increase, decrease, or transform active constituents and can generate new organic acids and microbial metabolites; therefore, the pre-fermentation raw material and the final product both require characterization.

Published poultry studies report pathogen inhibition, changes in lactic acid bacteria or *Enterobacteriaceae*, intestinal morphology, tight-junction expression, cytokines, immunoglobulins, and performance [1, 6, 7, 12–16, 39]. These findings support biological plausibility and product development, but most are efficacy or mechanistic studies rather than dedicated safety studies. Several of the *Allium* studies cited in this review were conducted by the authors' research group [8–16]; they are presented as local case examples. Independent studies and assessments [1, 6, 36, 37, 39, 45] broaden the evidence base, but direct independent replication of the same fermented *Allium* formulations remains limited. This limitation is now explicit rather than being obscured by class-wide statements.

Allium-specific safety assessment should actively test potential disadvantages rather than infer safety from natural origin. Depending on composition and dose, relevant monitoring includes palatability and feed/water intake, gastrointestinal irritation, hematology, liver and kidney indicators, organ pathology, variability in organosulfur compounds, interactions between phytochemicals and microbial metabolism, pesticide and heavy-metal contamination, and sensory effects on meat or eggs. Long-duration exposure deserves particular attention in

laying hens and breeding birds. An eight-week chive-bulb study in laying hens included serum biochemistry and organ traits and reported no adverse organ effects at the tested inclusions [15], but it was not designed as a regulatory tolerance study or to establish a class-wide NOAEL. The principle that natural origin does not equal safety therefore applies equally to the *Allium* component, the microbial component, and the final fermented product. The separation between product definition and the four evidence types is illustrated in **Figure 2**.

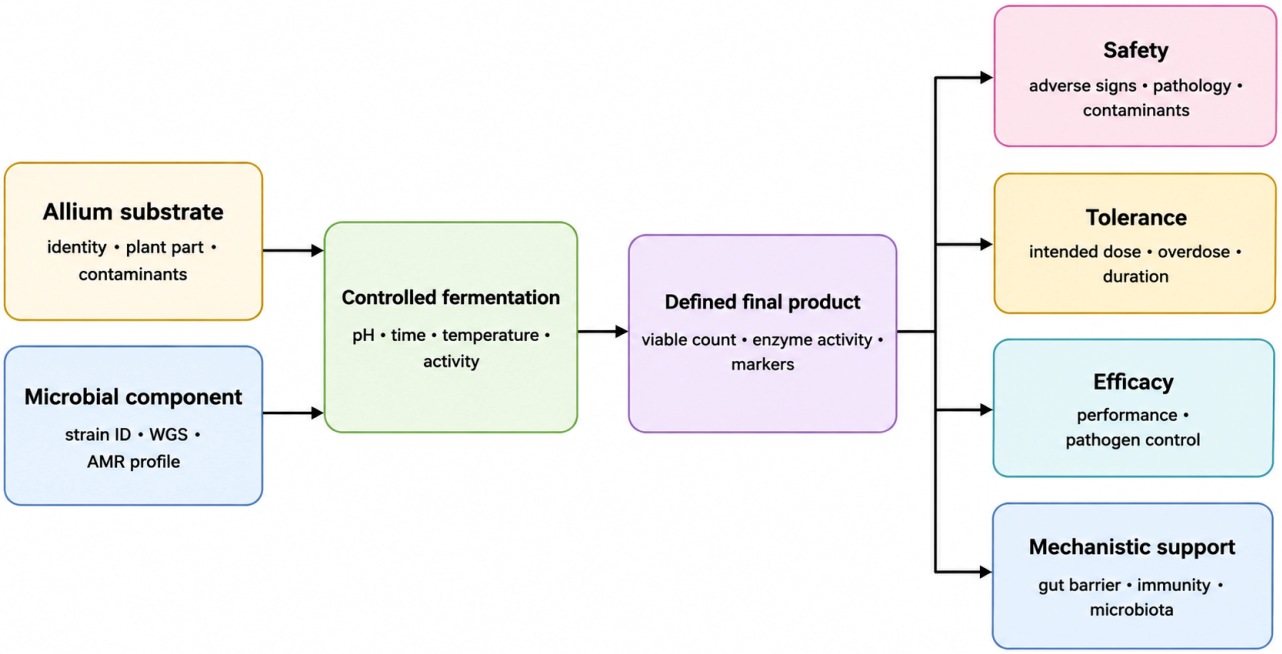

Figure 2. *Allium*-probiotic example showing the separation between product definition and evidence type. Botanical identity/contaminants and microbial strain/genomic characteristics feed into controlled fermentation and a defined final product. Evidence is then classified as safety, tolerance, efficacy, or mechanistic support; efficacy and mechanistic signals can inform biological plausibility but do not substitute for safety or tolerance evidence. Arrows indicate progression from botanical and microbial inputs through controlled fermentation to the defined final product and then from the final product to the four evidence classes. ID: identification; WGS: whole-genome sequencing; AMR: antimicrobial resistance; pH: measure of hydrogen ion activity (acidity/alkalinity).

9. Quality control and manufacturing specifications

Quality control bridges experimental effects and reproducible manufacturing. Enzyme activity must be defined by substrate, assay pH, temperature, incubation time, calibration/standard curve, calculation, matrix correction, and acceptance range. For cellulase-rich preparations, for example, carboxymethyl cellulase (CMCase) activity should be accompanied by the exact assay conditions and batch variation [8, 21, 32]. Viable microorganisms should be quantified using validated culture-based or equivalent methods appropriate to the strain/spore form, with identity checks after fermentation when relevant. Chemical markers may be measured by targeted chromatographic methods or validated spectrophotometric assays, while untargeted liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS) can be used when novel fermentation products or unexpected chemical changes require investigation.

For probiotic-enzyme-botanical products, batch release should combine identity, potency, purity, contaminants, and stability. Candidate parameters include viable/spore counts, enzyme activity, pH, moisture or water activity, organic-acid profile, selected phenolic/flavonoid/organosulfur markers, absence or limits for target pathogens and indicator organisms, mycotoxins when relevant, and heavy-metal/pesticide limits for agricultural raw materials [14, 40–44]. Stability should be evaluated in the marketed package and, for drinking-water products, at the intended dilution and contact time. The analytical approaches summarized below are implementation examples proposed by the authors; jurisdiction-specific validated methods should take precedence.

A common manufacturing mistake is to optimize only the endpoint which is most likely to carry the product claim. Maximizing enzyme activity, viable count, or antimicrobial inhibition may yield an unsafe or unstable product. The design space of a balanced optimization strategy must be defined for acceptable enzyme activity, viable cells, metabolite profile, contaminant control, palatability, and target-animal tolerance. Core safety domains in **Table 1** and quality-control specifications in **Table 2** for *Allium*-probiotic enzyme bioproducts are provided.

Table 1. Core safety domains and examples of acceptable evidence for microbial enzyme and *Allium*-probiotic bioproducts.

Domain	Minimum evidence	Example of acceptable evidence	Common weakness
Source organism	Strain identity; genomic safety screen; phenotypic AMR; relevant toxin/virulence assessment	WGS with documented database versions/thresholds plus susceptibility testing and strain deposit record	Species- or genus-level identification only
Enzyme preparation	Enzyme identity; activity method; impurities; sequence-based hazard review	Defined enzyme commission/enzyme name, validated activity assay metadata, batch potency and sequence comparison to known allergens/toxins	Activity value reported without method or purity context
Fermentation matrix	Botanical identity/plant part; process parameters; chemical markers; contaminants	Authenticated raw material, pH/time/temperature records, marker profile, heavy metals/pesticides where relevant	Raw-material and fermentation variability ignored
Toxicology/tolerance	Intended-use and justified higher exposures; adverse-effect endpoints	Clinical observations, intake, hematology/chemistry, organ weights/histopathology as hazard-appropriate; NOAEL only if design supports it	Efficacy trial treated as safety proof
Worker/consumer/environment	Exposure route; sensitization; residues/novel metabolites; shedding/persistence/AMR	Dust/aerosol assessment, product-specific residue or metabolite screen, manure/litter persistence and mobile-AMR evaluation when triggered	Exposure outside the bird omitted

AMR: antimicrobial resistance; WGS: whole-genome sequencing; NOAEL: no-observed-adverse-effect level.

Table 2. Suggested quality-control specifications and illustrative analytical approaches for enzyme-rich fermented poultry bioproducts.

Specification group	Examples of parameters	Illustrative analytical approach	Purpose
Identity	Microbial strain; botanical species/plant part; enzyme nomenclature	WGS/strain record; botanical authentication; enzyme sequence/nomenclature	Prevents ambiguity and mislabeling
Potency	Enzyme activity; viable/spore count; organic acids; claimed antimicrobial activity	Validated activity assay; plate count/spore count; HPLC/GC as appropriate	Defines active components and release criteria
Purity/contaminants	Pathogens; coliforms; molds; mycotoxins; heavy metals; pesticides	Culture/PCR confirmation; validated chemical residue methods	Controls biological and chemical hazards
Stability	Potency and viable count during storage and in drinking water	Real-time/accelerated stability with prespecified acceptance limits	Supports shelf-life and practical-use claims
Batch consistency	pH, moisture, activity, microbial counts, marker compounds	Trend analysis across independent lots with predefined ranges	Demonstrates reproducible manufacturing

WGS: whole-genome sequencing; HPLC: high-performance liquid chromatography; GC: gas chromatography; PCR: polymerase chain reaction; pH: measure of hydrogen ion activity (acidity/alkalinity).

10. Target-animal safety, tolerance, efficacy, and mechanistic evidence

For poultry products, four evidence classes should be distinguished. Safety endpoints detect adverse effects under intended use (e.g., clinical pathology or organ lesions). Tolerance endpoints test whether birds withstand the product across a justified dose/duration range. Efficacy endpoints test the intended benefit (e.g., performance or pathogen control). Mechanistic/supportive endpoints explain how an effect may occur (e.g., cytokines, tight-junction genes, microbiota, and antioxidant markers). Improved growth, villus morphology, or immunoglobulins cannot exclude subclinical toxicity, and the absence of statistically significant adverse effects in a small efficacy study is not equivalent to a dedicated safety evaluation [20, 34, 35]. Consistent with this distinction, probiotic, prebiotic, and postbiotic interventions are widely discussed in terms of gut-microbiota and immune modulation [46].

Target-animal studies should prioritize clinically interpretable adverse-effect endpoints and add mechanistic measurements only when they address a defined hazard or claim. Broiler tolerance studies can include body weight, feed and water intake, feed conversion, mortality, clinical observations, hematology/serum chemistry, necropsy and organ pathology, and product-specific endpoints. Layer studies require longer exposure and should consider egg production/quality, liver and kidney indicators, minerals, organ traits, and potential transfer of undesirable residues or sensory changes into eggs. Drinking-water products additionally require palatability, water consumption, dilution stability, and drinker-line microbiological assessment.

Challenge studies can improve biological relevance but generally answer efficacy or mechanistic questions unless they include a prespecified safety/tolerance component. Pathogen strain, challenge dose, route, timing,

clinical scoring, humane endpoints, and containment must be justified. Seasonal stress can also modify exposure and interpretation; for example, heat stress alters intake, gut function, immunity, and microbiota [47–50]. Such effect modifiers should be recorded and, when relevant, included in the study design rather than assumed to be equivalent across production environments.

11. Environmental, occupational, and consumer-facing biosafety

Safety assessment extends beyond the treated bird. For viable probiotics or spore-formers, an environmental plan can quantify the product organism in litter/manure before, during, and after use; assess persistence after storage or composting; examine water-line biofilms or runoff when relevant; and monitor AMR determinants when mobile elements or resistant phenotypes are identified. Culture, strain-specific quantitative polymerase chain reaction (qPCR), and WGS-based confirmation can be combined depending on the question. Worker exposure should consider dust/aerosol generation, dermal contact, enzyme sensitization, and practical risk controls such as formulation, containment, and personal protective measures [2–5, 25, 29]. These are risk-based assessment options proposed by the authors rather than mandatory tests for every product.

Consumer-facing safety should include product-specific contaminants and, when biologically plausible, residues or novel biotransformation products in meat or eggs. Fermentation can generate metabolites not present in the raw botanical material; therefore, targeted analysis of known hazards may be supplemented by comparative LC-MS/GC-MS profiling when the process is novel or produces an unexpected chemical fingerprint. Changes in meat oxidative stability, egg quality, color, odor, or taste should be reported as product-quality outcomes and should not substitute for residue or toxicological evidence [15, 40–44].

AMR is a cross-cutting One Health concern. Candidate probiotic/production strains should be assessed for acquired resistance, mobile determinants, phenotype-genotype concordance, and transferability when indicated. Environmental monitoring becomes more important when viable organisms are shed at high levels or when mobile AMR elements are identified. The aim of reducing antimicrobial use does not justify introducing a new microbial or genetic hazard [2–5, 22, 26].

12. Operational dossier and regulatory positioning

A practical dossier can be organized into the following five evidence levels: Level 1, product identity and classification; Level 2, manufacturing and exposure description; Level 3, quality/potency and batch consistency; Level 4, hazard-based safety and target-animal tolerance together with worker, consumer, and environmental assessment; and Level 5, efficacy under the intended conditions of use. Advancement to higher-tier testing should be triggered by unresolved hazards, high or prolonged exposure, novel metabolites, mobile AMR determinants, sensitization potential, unexpected adverse signals, or regulatory requirements. Batch release should link directly to the identity, potency, purity, and stability attributes shown to be relevant in the safety package.

The framework is not a substitute for jurisdiction-specific law. EFSA and OECD documents cited here provide European risk-assessment guidance and internationally recognized test methods [19–24, 29, 34, 35, 37]. In the United States, the FDA/CVM evaluates animal-food ingredients through pathways that include animal-food additive petitions and generally recognized as safe (GRAS) determinations under U.S. law [51]. Codex Alimentarius provides international risk-management principles relevant to foodborne AMR and the food/feed

continuum [52]. Requirements can therefore differ by jurisdiction, product category, claims, and intended use; the authors' framework is a scientific organizing tool that must be adapted to the competent authority.

Terminology should remain evidence-based. "Natural" is not a safety category. "Probiotic" applies to a live, strain-defined microorganism with a documented benefit, whereas "probiotic product" refers to the marketed formulation that contains the microorganism. "Postbiotic" should be reserved for preparations of inanimate microorganisms and/or their components that confer a health benefit, not used as a synonym for cell-free supernatant or undefined fermented matrix [27, 28, 30]. "Enzyme-rich fermented product" is preferable to "enzyme supplement" when multiple active fractions remain in the final preparation. The distinctions among these evidence layers and their safety interpretation are summarized in **Table 3**.

Table 3. Classification of safety, tolerance, efficacy, mechanistic, and consumer-facing evidence in poultry bioproduct studies.

Evidence layer	Typical endpoints	Primary classification	Safety interpretation
Adverse-effect monitoring	Clinical signs, mortality, intake, hematology, serum chemistry, organ pathology	Safety	Directly supports safety when adequately powered and exposure-relevant
Dose/duration margin	Intended dose plus justified higher exposures; prolonged exposure	Tolerance	Defines tolerated exposure range; may support NOAEL if design is adequate
Performance/pathogen control	Growth, FCR, survival, pathogen/oocyst load, production efficiency	Efficacy	Benefit signal; cannot substitute for safety
Gut/immune/oxidative response	Villus/crypt, tight junctions, cytokines, immunoglobulins, microbiota, antioxidant markers	Mechanistic/supportive	Explains plausibility; abnormal changes may trigger safety investigation
Product quality/consumer outcomes	Meat/egg quality, residues, novel metabolites, sensory traits	Quality + consumer safety	Quality benefit is distinct from residue/toxicology assessment

FCR: feed conversion ratio; NOAEL: no-observed-adverse-effect level.

13. Discussion

13.1. Research gaps and limitations

Several recurring evidence gaps are now clear. Many poultry efficacy studies characterize growth, gut morphology, cytokines, or microbiota without a prespecified safety hypothesis, overdose group, organ pathology, or sufficient duration to assess tolerance. Enzyme studies often omit assay metadata needed for reproducibility, while probiotic development may stop at species-level identity or in vitro AMR screening rather than genome-resolved strain assessment. *Allium* studies frequently emphasize antioxidant or antimicrobial benefits without equally detailed characterization of dose, organosulfur variability, contaminants, sensory effects, or long-duration layer/breeder exposure. Occupational sensitization and environmental persistence are also commonly omitted. **Table 4** shows why an efficacy study, a dedicated tolerance study, and a regulatory dossier provide different types of evidence rather than interchangeable proof.

Table 4. Representative poultry studies and their safety relevance.

Study/product	Design and exposure	Safety/tolerance endpoints	Safety-relevant finding	Major limitation/ independence
Hai et al. [12]—*L. plantarum* 1582-fermented chive	250 broilers; 1% or 3% product; *E. coli* challenge; to day 35	Primarily performance, immune and gut endpoints	No dedicated safety conclusion should be drawn	Efficacy/challenge study; author-group evidence
Hai et al. [13]—*L. plantarum 1582*-fermented chive	250 broilers; 1% or 3%; *Eimeria* challenge at day 14; to day 42	Performance, immune, barrier and lesion endpoints	Mechanistic/efficacy evidence, not formal tolerance	No overdose or organ-pathology safety package; author-group evidence
Hai et al. [14]—fermented purple onion/chive extracts	In vitro antibacterial testing plus chick pathogenicity assessment	Not a repeated-exposure additive safety study	Supports antimicrobial activity/pathogen characterization	Does not establish target-animal safety; author-group evidence
Hai et al. [15]—chive bulb extract	72 laying hens; 0.5%, 1.0%, 1.5%; 8-week feeding after adaptation	Feed intake, serum biochemistry, immunoglobulins, organ traits	No adverse organ effects reported at tested inclusions	Not designed as regulatory tolerance/NOAEL study; author-group evidence
Lou et al. [39]—*L. rhamnosus* SN21-1/*L. plantarum* SN21-2	Broilers; 0.2 mL of 10^9 CFU/mL suspension; 28-day safety observation; challenge component	Growth, blood biochemistry, liver/spleen/heart/ kidney histology	No significant adverse effects reported in safety component	Independent evidence; product/route differs from *Allium*-fermented matrix
Lee et al. [36]—enzyme-expressing algal biomass	42-day oral toxicity study; highest dose 5000 ppm	Performance, mortality, hematology, clinical chemistry, histopathology	NOAEL reported at 5000 ppm, highest tested dose	Independent example of dedicated tolerance; different product class
EFSA GalliPro Fit [37]—multi-strain Bacillus additive	Regulatory assessment for poultry under defined conditions of use	Strain identity, AMR, manufacturing, target-animal/user/ environment considerations	Illustrates integrated regulatory evidence package	Independent regulatory assessment; not an *Allium* product

CFU: colony-forming units; NOAEL: no-observed-adverse-effect level; AMR: antimicrobial resistance; EFSA: European Food Safety Authority; ppm: parts per million.

13.2. Future directions

Future studies should integrate genome-resolved strain selection, controlled fermentation, chemical fingerprinting/metabolomics, validated enzyme-activity methods, target-animal tolerance, practical shelf-life, and environmental persistence when relevant. Multi-omics can help attribute effects to live microbes, enzymes, organic acids, sulfur compounds, peptides, or host–microbiome changes, but omics should explain rather than replace classical safety endpoints [6, 7, 19, 22, 39, 49]. Independent replication of specific fermented *Allium*

formulations is particularly important because much of the direct evidence currently comes from a limited number of research groups.

Climate should be treated as a potential effect modifier in safety and efficacy studies. The cited evidence directly supports effects of heat stress and high ambient temperature on poultry growth, intestinal inflammation, immunity, and microbiota [47–50]. These references do not directly establish effects of cold-humid stress. Cold-humid production conditions may be locally relevant, but dedicated studies are required before they are incorporated as an evidence-based safety modifier. For heat-stress studies, temperature-humidity exposure, water/feed intake, product stability in drinking water, gut-barrier endpoints, and adverse-effect signals should be interpreted together.

14. Conclusions

Microbial enzyme and *Allium*-probiotic bioproducts can combine enzymes, live microorganisms or spores, botanical constituents, and fermentation-derived metabolites. Their potential benefits for gut function, pathogen control, immunity, and production do not establish safety. The safety question concerns the defined final product, its hazards, exposure, manufacturing consistency, and tolerated use conditions.

The central contribution of this narrative review is an operational whole-bioproduct framework that separates product classification, hazard characterization, exposure, safety, tolerance, efficacy, mechanistic evidence, quality control, and environmental/consumer considerations. It also makes it explicit where current *Allium*-poultry evidence is mechanistic or efficacy-oriented and where independent safety validation remains limited. Applying this framework can improve study design and dossier transparency, but regulatory requirements must be determined for the relevant jurisdiction and product category.

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Conflict of interest

The authors declare that they have no competing interests.

Data availability statement

No new data was created or analyzed in this study. Data sharing is not applicable to this article.

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