PROCESS NMR ASSOCIATES, LLC

Meat Authenticity by ^{1}H, ^{13}C, and ^{31}P NMR

Chemical basis, species evidence, method architecture, and validation strategy

A decision-grade white paper covering beef, pork, horse, poultry, duck, ostrich, finfish, game meats, and plant-based meat analogues

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Abstract

Nuclear magnetic resonance can be used to authenticate meat and meat-like products, but the scientifically defensible answer is **yes, within a defined product and reference domain** rather than yes as a universal species detector. The most mature implementation is ^{1}H NMR of two complementary extracts: a polar, aqueous extract that reports amino acids, dipeptides, osmolytes, creatine-related metabolites, carnitine, choline derivatives, and nucleotide breakdown products; and a nonpolar extract that reports fatty-acyl unsaturation, triacylglycerol structure, cholesterol, phospholipids, and oxidation products. Published studies have differentiated raw and processed beef, pork, lamb, and pooled poultry; screened beef against horse; quantified designed pork and duck adulteration in beef; separated chicken, chevon, beef, and donkey in small proof-of-concept cohorts; and discriminated selected fish species and production systems. The strongest broad meat studies report average classification near 97–99%, but these values apply only to the represented populations, matrices, processing states, and validation designs. They must not be translated into a universal accuracy or a trace-adulterant detection limit.

^{13}C NMR contributes lower-sensitivity but higher-dispersion information, particularly fatty-acyl placement at the sn-1,3 versus sn-2 positions of triacylglycerols and at the sn-1 versus sn-2 positions of glycerophospholipids, whose phosphate occupies sn-3. Direct ^{13}C evidence is strongest in fish oils and salmon/cod systems, where EPA/DHA content and regio-position encode species, feed, wild-versus-farmed status, and geographic production phenotype. Pork and beef have strong mechanistic differences, including the high sn-2 palmitate characteristic of porcine triacylglycerols, but a large externally validated ^{13}C-only terrestrial-meat classifier was not located. ^{31}P NMR is chemically selective for phospholipid classes, phospholipid degradation products, phosphocreatine, ATP, inorganic phosphate, and sugar phosphates. It is valuable for orthogonal compositional, freshness, and freeze-thaw evidence; added condensed phosphate is a plausible product-integrity application that still requires direct, product-specific validation. No validated broad ^{31}P species classifier was found for beef, pork, horse, poultry, duck, ostrich, or fish. It should therefore be treated as a supporting channel, not as the primary taxonomic decision.

Species readiness is uneven. Beef, pork, horse-versus-beef, pooled poultry, duck adulteration of beef, and selected fish groups have direct NMR evidence. Chicken and duck breed/age studies also demonstrate why age, diet, cut, and production system must be controlled. Ostrich is chemically plausible but remains an unvalidated NMR-authentication target. For plant-based meat analogues, published NMR studies establish compositional proof of concept in a few bounded product comparisons, not a validated multi-brand authenticity class. Bulk animal-versus-plant screening is a promising development hypothesis, but routine NMR cannot by itself prove the absence of trace animal

material or directly verify a low-level protein ingredient such as soy leghemoglobin. DNA or validated peptide methods remain the appropriate confirmatory tools for consequential species claims.

This white paper develops the chemical basis, evaluates the evidence by species and nucleus, proposes a 300 MHz-compatible workflow, and defines a validation program based on authentic reference materials, lot-grouped and nested validation, open-set rejection, blind external challenge samples, designed mixture series, model-transfer testing, and prespecified decision rules. Its central recommendation is a tiered platform: ^{1}H polar-plus-lipid screening, targeted ^{13}C lipid regiochemistry where it adds value, selective ^{31}P process/phospholipid interrogation, and orthogonal molecular confirmation when a regulatory, contractual, religious, or economic species decision depends on the result.

> **Principal conclusion.** NMR is well suited to rapid, reproducible, multicomponent screening of meat authenticity and composition. It is strongest when the question is narrowly defined, the authentic population is deliberately broad, and the method is allowed to return “indeterminate” or “outside model” rather than forcing every specimen into a known species class.

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1. Executive answer and decision boundaries

1.1 Can ^{1}H, ^{13}C, and ^{31}P NMR authenticate meat?

Yes—but each nucleus answers a different question, and none of them reads species identity directly. Species identity is encoded in DNA. NMR measures the chemical phenotype produced by genetics, physiology, diet, tissue type, postmortem metabolism, storage, processing, and formulation. That phenotype can be sufficiently characteristic for a well-designed classification or authenticity decision, and the published literature establishes that it often is. It can also drift when feed changes, when the sampled cut changes, when a product is cooked or cured, or when the unknown belongs to a class absent from the training set. The right framing is therefore "fit-for-purpose chemical authentication" rather than "universal taxonomic identification." Reviews of meat authentication and food-of-animal-origin testing reach the same general conclusion: robust control programs match the analytical platform to a defined fraud scenario and use orthogonal methods where needed [1,2].

The most defensible NMR roles are:

- **Rapid screening of representative lot samples or extracts.** NMR generates a wide chemical fingerprint with high analytical reproducibility and minimal compound-by-compound method development.
- **Compositional verification.** Lipid unsaturation, fatty-acyl regio-position, sterol class, phospholipid class, polar metabolite ratios, and postmortem chemistry can be measured or fingerprinted. Added-phosphate profiling is a proposed ^{31}P product-integrity application that requires its own validation.
- **Closed-domain classification.** When the allowed classes are known—such as beef, pork, lamb, and poultry in a defined raw-meat supply chain—multivariate models can assign samples with high within-domain performance [6,7].
- **One-class authenticity screening.** A model can learn the expected envelope of an authentic class and reject atypical or out-of-domain samples without claiming to identify the adulterant.
- **Mixture estimation after specific calibration.** Designed pork/duck/beef mixtures demonstrate feasibility, but the observed prediction errors are not compatible with blanket trace-level claims [9].
- **Investigation and triage.** NMR can determine whether a suspect result is driven mainly by fat composition, polar metabolites, plant ingredients, processing, oxidation, or phosphate treatment, thereby selecting the best confirmatory method.

NMR is not, by itself, the best final answer for an unknown species, a legal trace-level declaration, or a complex mixture containing many unrepresented components. In those cases, validated species-specific PCR, DNA barcoding/sequencing, or species-marker peptide LC–MS/MS should be included in the decision path [1,2,44–47,52]. Species DNA does not establish allergenic-protein abundance or clinical risk; an allergen claim requires an allergen-specific, validated protein method and a risk-appropriate sampling plan.

1.2 The analytical claim must be written before the method

A method described only as “meat authentication by NMR” is not yet a method. A validation-ready claim should state:

1. The authentic class or classes, including the biological species and whether subgroups such as chicken versus turkey must be distinguished.
2. The specimen type: raw muscle, adipose tissue, minced meat, cooked product, sausage, rendered fat, fish fillet, roe, or plant-based analogue.
3. The processing envelope: fresh, frozen-thawed, cooked, canned, smoked, brined, cured, fermented, or mixed with seasonings and binders.
4. The fraud or quality question: wrong species, undeclared admixture, wild-versus-farmed status, geographic or production origin, fresh-versus-frozen treatment, added phosphate, or animal-versus-plant composition.
5. The required decision threshold and consequence of false positive, false negative, and indeterminate results.
6. The confirmatory method and chain-of-custody pathway when the NMR screen is suspect.

This discipline prevents a common error: using an impressive closed-set classification result to imply performance on an undefined market population. A model trained on beef, pork, lamb, and pooled poultry will assign a horse, ostrich, venison, or plant analogue to one of those four classes unless the workflow contains an explicit outlier or open-set rejection rule. The assigned label may be mathematically inevitable but scientifically false.

1.3 Evidence grades used in this paper

The literature is graded according to what the experiment actually established, not according to the most optimistic interpretation of its title or headline accuracy.

Grade	Meaning	Typical evidence	Permitted conclusion
A — direct with independent challenge	Relevant matrix, locked or clearly specified workflow, and genuinely independent biological challenge lots	Beef-versus-horse challenge set; raw four-class polar model challenged with 40 external specimens	Feasible only within the represented domain; biological sample count and local validation remain decisive
B — direct proof of concept	Correct species/matrix with internal, grouped, or limited validation; designed mixtures may be included but without a broad external challenge	Processed multiclass models; beef/pork/duck mixtures; selected five-group fish model; pure chicken/chevon/beef/donkey cohort	Strong basis for method development, not universal deployment or a transferred detection limit
C — mechanistic support	Direct measurement of relevant chemistry without a blind species classifier	Pork-versus-beef TAG regiochemistry; pork fatty-acid qNMR; phospholipid class profiling	Marker plausibility and method design support only

Grade	Meaning	Typical evidence	Permitted conclusion
D — inference or adjacent evidence	Related matrix, production claim, freshness state, rendered fat, or other modality	Duck age models; broad plant-versus-animal oil database; single-pair ^{31}P salmon comparison; TD-NMR burger structure	Hypothesis-generating; a new product-specific validation is required
Gap	No fit-for-purpose NMR study located	Ostrich species authentication; routine ^{31}P multiclass meat classification	No claim of demonstrated NMR authentication

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

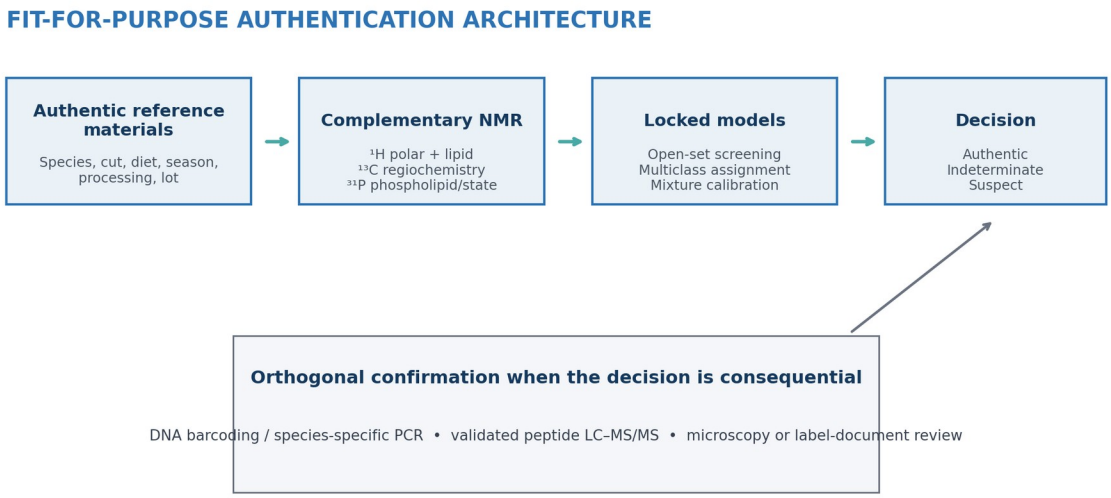

Figure 1. Fit-for-purpose NMR authentication architecture. NMR supports a bounded chemical decision; orthogonal molecular methods confirm consequential species findings.

1.4 Headline species readiness

Target	Current NMR readiness	Strongest direct basis	Major qualification
Beef	High for defined comparisons	Polar and lipid ^{1}H multiclass studies; beef-versus-horse screening; beef/pork/duck mixture study [6–9]	Cut, adiposity, feed, and processing must be represented
Pork	High as a class in defined models	Large ^{1}H multiclass studies and mixture work; distinctive TAG regiochemistry [6,7,9,19,20]	Trace pork claims require mixture-specific validation and molecular confirmation
Horse	Moderate for beef-versus-horse screening	External challenge using 60 MHz lipid ^{1}H NMR [8]	No mixture calibration in the pivotal study; lean horse may give weak lipid signal
Chicken and turkey	Moderate as pooled poultry; emerging separately	Large studies pooled poultry; chicken breed/production studies [6,7,13,14]	Pooled “poultry” is not a chicken-versus-turkey classifier

Target	Current NMR readiness	Strongest direct basis	Major qualification
Duck	Moderate in a specific adulteration design	Pork/duck in minced beef, plus duck metabolome studies [9,15–17]	Age and breed can dominate; no general trace-level declaration method
Lamb, goat, donkey	Moderate-to-low by target	Lamb in large multiclass studies; small chevon/donkey proof of concept [6,7,10]	Broader external cohorts and processed-product validation needed
Ostrich	Research-stage gap	General game-meat HR-MAS feasibility only [12]	No validated ostrich-meat NMR classifier located
Finfish	Moderate-to-high for selected bounded cohorts and claims	^{1}H polar profiles; ^{13}C lipid regiochemistry; low-field stingray work [23–30]	Several studies are small, internal, or supply-chain-specific; each model is bounded by species, origin, season, and processing
Plant-based meat analogue	Proof-of-concept only for bounded bulk comparisons	Narrow PBMA-versus-meat metabolomic/structural studies and broad oil chemistry [21,38,39]	No locked multi-brand external classifier or animal-mixture validation; NMR does not prove absence of trace animal material

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

2. Why species and product types differ chemically

2.1 Meat is a multicomponent, nonequilibrium biological matrix

Lean muscle is principally water and protein, with variable glycogen, free amino acids, dipeptides, nucleotides, organic acids, minerals, phospholipids, and intramuscular triacylglycerols. Adipose tissue contains much more neutral lipid and much less of the water-soluble metabolome. Fish white muscle, fish red muscle, mammalian red muscle, and avian breast muscle operate under different physiological demands and therefore maintain different metabolite pools. After slaughter or harvest, ATP production stops, phosphocreatine is consumed, glycogen becomes lactate, pH falls, nucleotides degrade, proteolysis proceeds, membranes hydrolyze, and lipids oxidize. The measured spectrum is consequently a snapshot of both biological origin and specimen history [3–5].

This is the fundamental opportunity and the fundamental risk. A high-dimensional spectrum contains many independent species-associated signals, so modest differences can combine into powerful classification. The same spectrum also records confounders. A classifier can learn freezer history, slaughterhouse handling, retailer, or sample-preparation batch if the experimental design accidentally aligns those variables with species. Good validation deliberately breaks those correlations.

2.2 Neutral lipids and fatty-acyl architecture

Triacylglycerols dominate adipose tissue and are a major authenticity channel in fatty meat. They contain three fatty-acyl chains esterified to glycerol at the sn-1, sn-2, and sn-3 positions. The spectrum reports several layers of information:

- **Total saturation and unsaturation.** Methyl, methylene, allylic, bis-allylic, and olefinic resonances describe the relative abundance of saturated, monounsaturated, and polyunsaturated chains.
- **Omega-family signatures.** The terminal methyl of omega-3 chains is displaced from the main terminal-methyl envelope. Fish, horse, and some feed-responsive tissues can therefore show distinctive omega-3 intensity.
- **Specific chain families.** Linoleate, α-linolenate, EPA, DHA, and conjugated or trans unsaturated structures alter recognizable ^{1}H and ^{13}C regions.
- **Glycerol and regio-position.** ^{13}C carbonyl and olefinic shifts can distinguish chains at sn-1,3 from those at sn-2. This is particularly useful in pork, in which palmitate is strongly enriched at sn-2, and in fish, where EPA/DHA position differs by species and lipid class [20,24,27].
- **Sterols and minor lipids.** Cholesterol supports an animal-fat signature. Plant sterols such as β-sitosterol, campesterol, and stigmasterol support a plant-oil signature, although refined formulations and mixed fats complicate simple marker logic.
- **Oxidation and hydrolysis.** Aldehydes, free fatty acids, lysophospholipids, and altered unsaturation ratios report age, heat, oxygen exposure, and processing as well as authenticity.

Ruminant tissues generally contain a more saturated and monounsaturated fat phenotype, shaped by rumen biohydrogenation and including ruminant trans and conjugated-linoleic-acid patterns. Pork, chicken, and duck commonly show more linoleate and total PUFA, strongly influenced by diet. Horse fat is comparatively unsaturated and often richer in α-linolenate. Marine fish commonly show EPA/DHA-rich profiles, but farmed fish can shift toward feed-derived plant-oil patterns. General ^{1}H oil/fat fingerprinting supports these marker families [18], while beef NMR/isotope work directly demonstrates that diet and geographic production can alter an animal-product profile [22]. These are useful population tendencies, not immutable species constants.

2.3 Structural phospholipids

Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidic acid, phosphatidylglycerol, cardiolipin, sphingomyelin, lysophospholipids, and their degradation products form membranes and organelles. Compared with storage triacylglycerols, membrane phospholipids are more closely linked to cell type and physiological function. They may therefore add discriminating information in lean meat and fish where neutral lipid is sparse. Their analysis is also more sensitive to extraction, aggregation, hydrolysis, oxidation, pH, ionic strength, and time after slaughter.

^{1}H detects headgroup and acyl resonances but with overlap. ^{13}C reveals carbonyl, olefinic, glycerol, and acyl-placement detail at glycerophospholipid sn-1 and sn-2; the phosphate-containing headgroup is attached at sn-3. ^{31}P directly detects phosphorus-containing headgroups and can resolve class-level profiles under a standardized solvent, detergent, chelator, pH, and temperature system [33,34]. The chemical promise is substantial, but the validation evidence for terrestrial species authentication remains limited.

2.4 Water-soluble metabolites

The aqueous metabolome adds an information layer largely independent of bulk fat. Important families include:

- **Imidazole dipeptides:** carnosine, anserine, and homocarnosine. Their abundance and ratios differ among mammalian, avian, and fish muscle and with muscle fiber type.
- **Energy metabolites:** creatine, phosphocreatine, ATP, ADP, AMP, inorganic phosphate, glycogen-derived glucose, and lactate.
- **Nucleotide degradation:** IMP, inosine, and hypoxanthine. These contribute both species/physiology information and a strong postmortem/freshness trajectory.
- **Carnitine system:** carnitine and O-acetylcarnitine, which are linked to fatty-acid transport and differ among meat categories in the published multiclass models [6].
- **Choline system:** choline, phosphocholine, glycerophosphocholine, betaine, and membrane-turnover products.
- **Amino acids and organic acids:** alanine, branched-chain amino acids, glutamate, glutamine, methionine, succinate, acetate, formate, fumarate, and others.
- **Fish osmolytes:** trimethylamine N-oxide, trimethylamine, dimethylamine, taurine, betaine, glycerophosphocholine, glycine, and arsenobetaine. These can encode habitat, depth, osmoregulation, trophic ecology, and spoilage [23,29].

Most of these compounds are not species-exclusive. Their value comes from relative concentration patterns across tens or hundreds of spectral variables. This is why multivariate fingerprints outperform single-peak threshold rules and why reference populations must include realistic biological variation.

2.5 Proteins, heme, carbohydrates, and formulation ingredients

Routine solution NMR of meat extracts is not a direct intact-protein sequencing method. Large proteins are broadened or removed during preparation, and their species-specific amino-acid sequence is better interrogated by DNA or peptide mass spectrometry. Nevertheless, proteins influence the low-molecular-weight pool through amino acids, dipeptides, creatine metabolism, and proteolysis. Heme proteins contribute color and redox chemistry but are not usually clean species markers in a one-dimensional extract spectrum.

Plant-based meat analogues add a different set of macromolecular and formulation variables: soy, pea, wheat, or other proteins; plant oils; starches and fibers; reducing sugars; flavor precursors; minerals; emulsifiers; organic acids; fermentation products; vitamins; and color systems. After protein removal, the remaining polar and lipid extracts can still be highly distinctive. A commercial analogue can also contain animal-like components by design or fortification, so authentication must distinguish "animal-like chemistry" from "animal biological material."

2.6 Processing is part of the phenotype

Cooking, curing, smoking, fermentation, freezing, thawing, high-pressure processing, canning, and storage alter the measured fingerprint. Heating denatures proteins, accelerates Maillard and Strecker chemistry, oxidizes lipids, and redistributes water. Freezing disrupts membranes and changes extractability. Curing adds salt, nitrite, sugar, phosphates, spices, and smoke components. In fish, ATP breakdown and TMAO reduction can change rapidly. Plant-based burgers can generate different Maillard products from animal burgers because their precursor pools and water/fat microstructure differ [31,32,37–39].

For this reason, raw and processed products should normally have separate models or at least separate validated subdomains. "Processing robustness" should never be assumed from a raw-meat study.

3. What each nucleus contributes

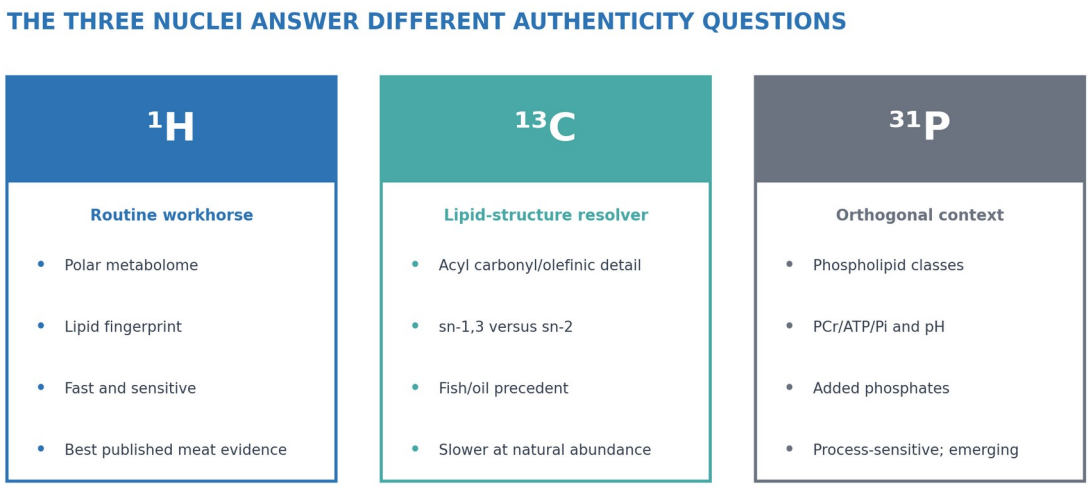

Figure 2. Complementary analytical roles of ^{1}H, ^{13}C, and ^{31}P NMR in a meat-authenticity platform.

3.1 ^{1}H NMR: the routine workhorse

^{1}H is the most sensitive of the three nuclei in ordinary food analysis and is present at essentially every organic molecular site of interest. A one-dimensional spectrum can be acquired in minutes on a high-field instrument and, as demonstrated by the 60 MHz beef-versus-horse work, can also support compact benchtop screening when the contrast is sufficiently strong [8]. Its main advantages are throughput, quantitative potential, mature pulse sequences, and simultaneous access to polar and lipid chemistry.

The principal limitations are a narrow chemical-shift range, overlap, sensitivity to pH and ionic conditions in aqueous extracts, water suppression, and the ease with which an unvalidated preprocessing choice can alter a chemometric model. Two-dimensional COSY, TOCSY, HSQC, and related experiments are extremely useful for assignment and method development but are usually too slow for routine lot screening. The practical strategy is to establish assignments with two-dimensional data, then lock a robust one-dimensional method.

3.2 ^{13}C NMR: dispersion and fatty-acyl regio-position

Natural-abundance ^{13}C is only about 1.1%, so direct-detected quantitative spectra require concentrated extracts, good probe sensitivity, and longer acquisitions. The reward is a chemical-shift range roughly twenty times wider than ^{1}H and excellent separation of carbonyl, olefinic, glycerol, and selected methylene environments. The small shift differences associated with sn-1,3 versus sn-2 esterification are particularly valuable.

Quantitative ^{13}C normally uses inverse-gated decoupling to suppress nuclear Overhauser enhancement bias and a repetition time long enough for the slowest relevant carbon. Faster INEPT-based methods transfer proton polarization and can produce useful fingerprints in minutes, but response depends on protonation, coupling constants, transfer delays, pulse calibration, and relaxation. An INEPT fingerprint is not automatically a mole-percent assay. HSQC provides much of the ^{13}C dispersion through proton detection and can be a practical compromise for assignment or selected fingerprinting on a 300 MHz platform.

3.3 ^{31}P NMR: selective but process-sensitive

^{31}P has 100% natural abundance and a useful sensitivity, with little interference from nonphosphorus compounds. In intact, in situ muscle under controlled calibration it reports phosphocreatine, ATP, inorganic phosphate, phosphomonoesters, phosphodiesters, sugar phosphates, and can estimate intracellular pH from the Pi chemical shift. A rapidly quenched or extracted preparation can preserve analyte abundance but not the original intracellular compartmental pH; it reports the extract conditions. In extracted lipids, ^{31}P reports phospholipid classes and degradation products. Condensed-phosphate

profiling in formulated meat is chemically plausible but should be described as a proposed product-integrity application until directly validated in the intended products.

The same selectivity makes ^{31}P easy to misinterpret. PCr, ATP, and Pi change quickly after slaughter; chemical shifts depend on pH, metal binding, ionic strength, temperature, and reference convention; and phospholipid peak resolution depends on the solvent/detergent system and aggregation. In the fish freeze-thaw study, ^{31}P used internal repeated validation, achieved about 80.9% mean correct classification, and underperformed the ^{1}H strategy [32]. The solid-state wild/farmed salmon paper compared only one wild and one farmed specimen [35]. These are development precedents, not mature classifier evidence. ^{31}P should therefore be deployed as a mechanistic and orthogonal channel with tightly standardized sampling.

3.4 Why data fusion is preferable to a single “magic marker”

The most important species effects are often distributed across many peaks, and different confounders affect different chemical blocks. A feed change can strongly alter lipids while leaving some polar ratios comparatively stable. Postmortem time can strongly alter nucleotides and phosphagens while leaving storage triacylglycerol structure more stable. Combining independently prepared polar and lipid blocks can therefore improve both separation and interpretability. Decker and coworkers showed this directly: polar and nonpolar models each performed well, and their combined interpretation was especially useful for mixed commercial products [6,7].

Data fusion should be designed, not improvised. Each block should be quality-controlled and scaled so that a large number of lipid bins does not automatically overwhelm a smaller but biologically informative polar block. Block scores, concatenated features, multiblock PLS, or late fusion of calibrated class probabilities can all be defensible if locked before validation.

4. The lipid evidence in detail

4.1 Interpreting a ^{1}H lipid spectrum

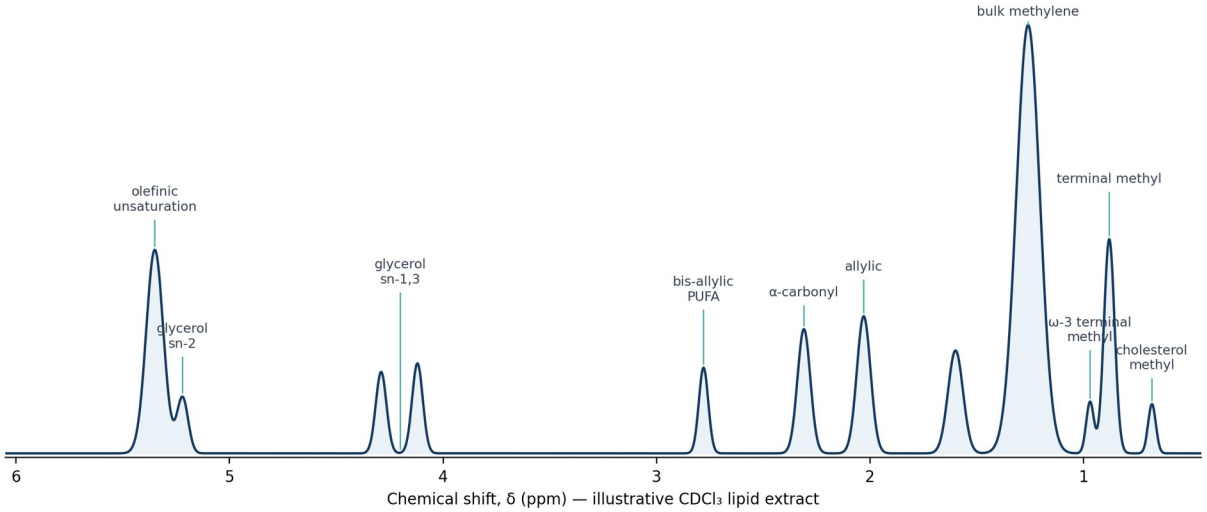

Figure 3. Illustrative—not experimental—^{1}H lipid spectrum showing regions that commonly contribute to species and composition models. Exact positions depend on solvent, temperature, concentration, and composition.

Approximate region, ppm	Dominant assignment	Authentication information	Important qualification
0.65–0.72	Cholesterol angular methyl and related sterol signals	Supports animal sterol content and cholesterol abundance	Overlap and concentration dependence; not species-exclusive
0.84–0.92	Terminal methyls of most fatty-acyl chains	Chain-family balance and normalization anchor	Large composite peak; limited selectivity alone
0.94–1.00	Terminal methyl of omega-3 chains, especially α-linolenate-rich patterns	Horse and marine/omega-3-rich lipid contribution	Requires adequate resolution and matrix-specific calibration
1.20–1.40	Bulk acyl methylene envelope	Chain abundance, saturation, and average chain environment	Dominant signal can mask minor components
1.55–1.65	β-methylene to carbonyl	Total esterified/free fatty-acyl contribution	Free fatty acids and esterified lipids may overlap
1.95–2.10	Allylic methylene	Total unsaturation	Affected by all unsaturated chain families
2.25–2.38	α-methylene to carbonyl	Fatty-acyl ester/free-acid structure	Extraction and hydrolysis influence profile
2.70–2.86	Bis-allylic methylene	PUFA abundance; linoleate/linolenate/EPA/DHA contribution	Oxidation selectively reduces PUFA signals
4.05–4.35	Glycerol sn-1,3 protons	Triacylglycerol amount and glycerol environment	Phospholipid/mono- and diacylglycerol overlap possible

Approximate region, ppm	Dominant assignment	Authentication information	Important qualification
5.15–5.28	Glycerol sn-2 proton	Triacylglycerol structure	Can overlap nearby olefinic envelope
5.28–5.45	Olefinic protons	Total double bonds and unsaturated-chain pattern	Conformation and mixture overlap limit specificity
9.2–10.1	Aldehydes	Lipid oxidation and processing history	Usually quality/freshness evidence, not species identity

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

Note: The regions are deliberately approximate. A validation protocol must specify solvent, temperature, concentration, referencing, phase/baseline procedure, integration or bin boundaries, and excluded regions.

4.2 Beef, pork, horse, and poultry lipid tendencies

Beef fat commonly presents a ruminant phenotype with higher saturated and monounsaturated contribution than pork or poultry, plus feed-responsive ruminant trans and conjugated structures. Pork fat usually contains more linoleate and a distinctive positional distribution: a large fraction of palmitate occupies sn-2. Kildahl-Andersen and coworkers quantified this contrast by ^{13}C NMR, reporting approximately 63–65 mol% sn-2 palmitate in their porcine material, whereas total sn-2 saturated fatty acids in the bovine material were much lower [20]. This is an excellent mechanistic discriminator, but the study was a compositional comparison rather than a blind classifier.

Horse fat contains more unsaturated and often more α-linolenic fatty acids than beef. In the pivotal benchtop study, the olefinic, bis-allylic, and approximately 0.97 ppm omega-3 terminal-methyl regions drove a naïve Bayes beef-versus-horse screen [8]. Poultry and duck are generally more PUFA- and linoleate-rich than beef, but avian lipids respond strongly to feed and age. These tendencies support multivariate models; they are not fixed specification limits.

4.3 Fish lipids and positional information

Fish provide the strongest direct case for ^{13}C lipid authentication. Marine oils contain long-chain omega-3 fatty acids such as EPA and DHA, and their distribution among triacylglycerol and phospholipid positions differs by species and production system. Standal and coworkers differentiated gadoid categories using phosphatidylcholine and phosphatidylethanolamine profiles, especially DHA sn-2 specificity [24]. Other studies differentiated salmon, mackerel, and herring oils using carbonyl-region positional information [27], and classified wild/farmed or production origin in Atlantic salmon [25,26].

These results are analytically powerful but biologically conditional. Aquaculture feed formulations can change substantially over time; season, size, tissue, and harvest location matter; and the fingerprint of a processed capsule is not the same as that of a raw fillet. A fish model should be updated when feed regimes or supply regions change.

4.4 Plant oils and phytosterols

Plant-based analogues often use coconut, canola/rapeseed, sunflower, soybean, or blended oils. Coconut oil is highly saturated and rich in medium-chain fatty acids; canola is usually oleate-rich with characteristic linoleate/linolenate balance; sunflower ranges from high-linoleic to high-oleic types. Plant sterols, tocopherols, and minor polar lipids are candidate formulation markers, but β-sitosterol/campesterol evidence in a commercial PBMA comparison comes from non-NMR mass spectrometry rather than a validated NMR marker assay [43]. Guyader and coworkers demonstrated broad untargeted ^{13}C separation across a large database of edible fats and oils and approximate detection of animal oil in plant oil under their specific design [21]. Sparse replication for individual terrestrial animal species means the study supports a development hypothesis for animal-versus-plant screening, not a ready-made beef/pork/chicken/duck or PBMA classifier.

Formulation surveillance is essential. A classifier that separates one product generation by coconut oil may fail when the manufacturer changes to canola or sunflower oil. The method should model product family and formulation era, not only brand name.

5. The polar-metabolite evidence in detail

5.1 Imidazole dipeptides and muscle physiology

Carnosine and anserine buffer protons and participate in muscle redox chemistry. Their relative abundance differs among muscle types and species, making them common drivers in meat and fish models. Poultry often contains substantial anserine, while mammalian red meats can show different carnosine/anserine balance. Duck breed and age studies show that these same metabolites vary within a species [15–17]. A robust model therefore treats the dipeptide profile as one block of evidence rather than a categorical single marker.

5.2 Creatine, phosphocreatine, lactate, and pH trajectory

Creatine and phosphocreatine support rapid energy buffering. After slaughter, phosphocreatine falls, ATP degrades, and lactate accumulates as glycogen is consumed. The final balance reflects muscle fiber type, stress, temperature, time, and freezing as well as species. In aqueous ^{1}H spectra, creatine and lactate are intense and reproducible, which makes them attractive classifier variables but also makes them potential batch or freshness proxies. ^{31}P adds PCr, ATP, and Pi evidence only when collection time and temperature are tightly controlled. Intracellular pH estimation requires intact/in situ tissue and a calibrated Pi chemical-shift relationship; an extract provides only extract-pH context.

5.3 Nucleotides and freshness

ATP proceeds through ADP and AMP to IMP, inosine, and hypoxanthine. Fish freshness metrics explicitly exploit this pathway, and NMR readily observes many of the intermediates [29,31,37]. Meat-species models also use inosine and hypoxanthine. Because the trajectory is time- and temperature-dependent, training samples should be balanced for postmortem interval and storage, and freshness variables should be monitored independently.

5.4 Carnitine, choline, betaine, and osmolytes

Carnitine and O-acetylcarnitine were important variables in the large raw/processed meat study [6]. Choline, phosphocholine, glycerophosphocholine, and betaine report membrane turnover and methyl-group metabolism. Fish add TMAO, TMA, DMA, taurine, and arsenobetaine. Saglam and coworkers reported strong separation among iridescent shark and selected flatfish groups using precisely this osmolyte-rich aqueous profile [23]. These molecules also respond to habitat, depth, microbial spoilage, and processing, so a species model should preserve the mechanistic interpretation instead of treating them as anonymous bins.

5.5 Plant-based analogue metabolites

A protein-depleted extract of a plant-based burger may contain sugars and sugar alcohols, oligosaccharides, organic acids, amino acids, nucleotides or yeast-derived flavor precursors, plant choline/betaine compounds, fermentation products, vitamins, salts, and Maillard precursors. Compared with animal meat, it may lack or strongly reduce creatine, carnosine, anserine, and the canonical postmortem nucleotide trajectory unless those compounds or close analogues have been added. Cooking changes both groups but not identically because their precursor pools and microstructure differ [38].

The most robust PBMA classifier will combine the polar and lipid blocks and include multiple brands, lots, reformulations, cooking states, and manufacturing sites. A model based on one brand versus one beef lot will mostly learn product identity, not the general concept of animal versus plant.

6. Species-by-species evidence assessment

6.1 Beef

Beef is the best-supported terrestrial anchor class. In the Decker polar study, 379 raw samples included 105 beef specimens, and all ten beef specimens in an external 40-sample raw-meat challenge were correctly assigned when the four-class model was applied [6]. The companion lipid study included a

larger raw/processed cohort and achieved high average internal performance, although raw beef showed some confusion with lamb [7]. The external 40-sample result does not validate the processed models, which relied on internal Monte Carlo cross-validation. The published evidence therefore supports development of a dual-extract beef screen against pork, lamb, and pooled poultry, followed by local external validation for each processing domain.

Beef-versus-horse discrimination is also directly demonstrated. Jakes and coworkers trained on 76 beef and 62 horse lipid extracts at 60 MHz. Their second test set contained 31 new beef samples that generated 91 extracts/spectra and six new horse samples that generated 16 extracts/spectra; 90 of the 91 beef extracts were accepted, and all 16 horse extracts were rejected as non-beef [8]. These are extract-level outcomes from 37 independent biological samples, not results from 107 independent specimens. The result is nevertheless notable because it uses a compact benchtop instrument and a simple lipid extraction. The paper did not calibrate beef/horse mixtures, so it does not establish an adulterant limit of detection. Low-fat horse tissue can yield weak lipid signal, and beef fat could dominate a mixed extract. The defensible label is **bounded beef authenticity screen**, not trace horse quantification.

Beef also participates in the designed pork/duck adulteration study, where multiblock ^{1}H data supported both classification and composition regression [9]. Reported prediction RMSEP values of approximately 0.111 and 0.082 in binary and ternary models correspond to errors on the order of 11.1 and 8.2 composition percentage points when the response is expressed as a fraction. The small, designed study is useful process-screening proof of concept, not a 1% regulatory declaration or broad external validation.

6.2 Pork

Pork is represented extensively in the large ^{1}H multiclass studies [6,7]. Lipid markers include linoleate/PUFA abundance, total unsaturation, cholesterol and phospholipid-associated signals; the polar model uses the joint profile of dipeptides, carnitine, choline, nucleosides, amino acids, and organic acids. Pork fatty-acyl composition can also be quantified by ^{1}H NMR [19], and ^{13}C provides an unusually informative positional marker through sn-2 palmitate [20].

For halal, kosher, or contractual trace-pork decisions, NMR should remain a screen unless a product-specific mixture validation demonstrates the required threshold across realistic pork tissues and processing states. Species-specific DNA or peptide confirmation is preferred because a small amount of lean pork may contribute little lipid and because heat, curing, and protein/fat partitioning can change the relationship between gravimetric mixture percentage and NMR signal. A clinical allergen claim is a different question and requires an allergen-specific validated protein method; species DNA alone does not quantify allergenic protein or risk.

6.3 Horse

Horse is directly supported only in a narrower domain than beef and pork. The 60 MHz study provides strong raw-lipid screening evidence against beef [8], driven by higher horse-fat unsaturation and α-linolenate-related signals. No broadly validated cooked, seasoned, multispecies horse-adulteration calibration was located. A practical program would use the benchtop or 300 MHz lipid screen to flag non-beef character and then confirm equine material by species-specific PCR or peptide analysis.

6.4 Chicken and turkey

The large Decker studies pooled chicken and turkey as “poultry” [6,7]. That class is useful if the claim is mammalian meat versus poultry, but it does not establish chicken-versus-turkey resolution. Separate chicken studies show that NMR can distinguish breed or production system using dipeptides, β-alanine, glucose, lactate, amino acids, IMP, and other metabolites [13,14]. They also show how easily cut, age, feed, and farm become major sources of variance.

The recommended strategy is hierarchical: first discriminate mammalian, avian, fish, and plant-based matrices; then use a dedicated, balanced avian model if chicken, turkey, and duck must be separated. Breast and thigh should be treated as separate strata or explicitly balanced factors because fiber type and metabolite pools differ substantially.

6.5 Duck

Duck has direct evidence as an adulterant class in minced beef, alongside pork [9]. Pure duck metabolomics further demonstrates differences between Pekin and Linwu ducks and shows strong age effects on anserine, carnosine, creatine, myo-inositol, succinate, nicotinamide, and related metabolites [15–17]. In one study, age effects exceeded breed effects [17]. A duck classifier that lacks age and breed balance risks authenticating husbandry history rather than species.

Duck fat is relatively unsaturated and diet-responsive. Combining polar metabolites with lipid data is therefore preferable. As with horse and pork, a general trace-duck claim cannot be inferred from pure-class discrimination or from the RMSEP of a small designed-mixture study.

6.6 Lamb, goat, and donkey

Lamb appears as a distinct class in the large raw-meat studies but showed some confusion with beef in the lipid model [7]. This is chemically unsurprising because both are ruminants. The polar block and data fusion should be emphasized. Goat/chevon and donkey were included with chicken and beef in a small 600 MHz study of six animals per class [10]. OPLS-DA separation and identified metabolite differences

establish feasibility, but the absence of mixture samples and an independent external set prevents a quantitative adulteration claim.

These species should enter a production program only after the initial beef/pork/poultry system is stable. Each needs multiple breeds, cuts, regions, diets, ages, and processing states.

6.7 Ostrich and other game meats

No fit-for-purpose, validated NMR authenticity classifier for ostrich meat was located in the literature search. Ostrich should therefore be described as an **R&D target**, not a demonstrated application. Its lean, red, avian muscle is likely to present a distinctive joint lipid and polar phenotype, but that hypothesis must be tested against the most confusable classes: beef, horse, venison, turkey, and other ratites if commercially relevant.

An Animal Frontiers perspective summarizes exploratory HR-MAS work on game meats—including deer, hare, rabbit, pigeon, and partridge—and describes species-associated variation in dipeptides, amino acids, carnitine, nucleotides, sugars, organic acids, glycerol, and fatty-acyl variables [12]. It does not provide a primary classifier validation, complete sample accounting, an external challenge, or adulteration limits. It is adjacent feasibility evidence that helps frame an ostrich study, not performance evidence for ostrich or game-meat authentication.

6.8 Finfish

Finfish authenticity is both highly promising and highly conditional. Aqueous ^{1}H profiles can differentiate selected species through osmolytes, amino acids, dipeptides, creatine, lactate, choline derivatives, and arsenobetaine. Saglam and coworkers reported 100% recognition among five represented groups—iridescent shark, European plaice, rock sole, witch flounder, and common sole—even when some pangasius products contained citric acid and lysine additives [23]. The result is direct proof of concept in that five-group domain, not a universal fish identifier. The cited evidence set does not justify extending these conclusions to shellfish, crustaceans, mollusks, or caviar.

^{13}C lipid work is especially informative. Gadoid discrimination used DHA sn-2 specificity in phosphatidylcholine and phosphatidylethanolamine, with 78% LDA and 100% Bayesian belief-network results under the study's internal validation in a 27-fish, one-location/season cohort [24]. Those figures are not operational external performance estimates. Atlantic salmon studies directly addressed wild-versus-farmed and geographic/production origin—not species identity—using fatty-acid and regio-position fingerprints [25,26]. Fish-oil work separated salmon, mackerel, and herring [27]. Solid-state ^{13}C/MRI adds direct-tissue feasibility for wild/farmed salmon comparisons [36], while the ^{31}P study was

only a one-wild/one-farmed case comparison [35]. These models are sensitive to feed, season, size, tissue, catch stress, and origin.

Tuna metabolomics demonstrates the scale of those confounders: species, size, geography, muscle type, and on-board storage condition all influence the profile [29]. Low-field 60 MHz work on Amazonian stingrays analyzed 202 wild and 150 market fillets, but the market material came from only three batches and lacked species-resolved labels; it therefore broadens feasibility and traceability evidence without providing an independent species-accuracy validation cohort [30]. The authors also warn that cold-chain differences can masquerade as biological origin.

Finfish product forms should be modeled separately. Fillet, red muscle, white muscle, rendered oil, smoked fish, and canned products are not interchangeable. Roe/caviar would require its own evidence base. Freshness and freeze-thaw controls are mandatory because aspartate, fumarate, phenylalanine, nucleotide degradation, phospholipid breakdown, and ^{31}P energy metabolites all respond to treatment [31,32,37]. The ^{31}P freeze-thaw model in [32] was internally validated and less accurate than the ^{1}H strategy, so it is a supporting development result rather than a preferred operational method.

6.9 Plant-based meat analogues and “Impossible-type” products

Direct NMR evidence supports compositional proof of concept between selected PBMAs and conventional meat. Correia and coworkers used 600 MHz ^{1}H NMR on six prototype PBMAs, one commercial pea/rice-protein PBMA, and a 60:40 beef/pork burger before and after cooking [38]. In that dataset, anserine, carnitine, carnosine, creatine, creatinine, glutamine, glycine, hypoxanthine, and niacinamide were observed only in the meat product, whereas citrate, histamine, water-soluble lipids, sucrose, and trigonelline occurred only in the PBMA group. AMP and IMP occurred in both meat and the commercial analogue, which is an important warning against calling nucleotide signals inherently meat-specific. Cooking and prior extrusion materially changed both fingerprints. The experiment demonstrated separability and candidate markers within that narrow comparison; it was not a blinded authenticity validation and contained neither trace-meat mixtures nor an Impossible Burger.

Wiesman and coworkers compared 15 Angus beef and 15 commercial soy-PBMA burgers by 23.4 MHz time-domain NMR, diffusion, moisture analysis, and microscopy [39]. Beef showed a denser fibrous myofibrillar structure and a dominant relaxation component near 45 ms; the soy analogue showed a more porous gel-like matrix and broader relaxation behavior. The study supports fast structural screening but not species or trace-adulterant claims: the apparent thousands of datasets largely arose from repeated measurements of 30 burgers, and no locked external classifier or mixture detection limit was reported.

An “Impossible-type” product requires careful wording. FDA GRAS Notice 737 concerns a soy leghemoglobin preparation from a strain of *Pichia pastoris* and an intended use up to 0.8% soy leghemoglobin protein in ground-beef analogue products [40]. FDA’s 2018 no-questions letter did not itself authorize use as a color additive. The later color-additive listing, 21 CFR §73.520 and the associated 2019 final rule, limits soy leghemoglobin protein to 0.8% by weight of the uncooked ground-beef analogue [41]. These sources establish the regulatory ingredient context, not the current recipe of every branded product; formulations and labels should be checked at the time of sampling.

Routine small-molecule ^{1}H NMR should not be claimed to identify soy leghemoglobin specifically. Leghemoglobin is a roughly 16 kDa heme protein; intact protein resonances are broad, the heme center can introduce paramagnetic effects, cooking denatures the protein, and standard metabolomics preparation often removes macromolecules. A finished product can be recognized through its total formulation fingerprint without the spectrum proving the presence of that particular protein. The safety evaluation of the preparation used targeted chromatography rather than NMR and was not an authenticity study [42].

Animal-versus-plant contrast is a promising hypothesis when multiple blocks agree: creatine/carnosine/anserine and animal sterol/lipid patterns on one side; plant-oil TAGs, carbohydrates, organic acids, additives, and plant-processing chemistry on the other. An independent mass-spectrometric metabolomics study found extensive compositional differences between a soy-based analogue and grass-fed beef, including animal-associated anserine and hydroxyproline and plant-associated β-sitosterol and campesterol [43]. Because that study was not NMR and examined only one commercial formulation, phytosterols remain candidate NMR markers rather than validated PBMA NMR features.

If a future multi-brand PBMA model is externally validated, defensible report language could be **“consistent with the validated plant-based product class”** or **“animal-associated chemistry not detected above the validated decision limit in this matrix.”** This review does not establish such a validated class. The wording must never become “animal-free,” “vegan certified,” or “zero animal.” For trace cross-contact or label enforcement, universal vertebrate or species-specific qPCR and validated peptide LC–MS/MS methods provide the required molecular sensitivity [44–47]. A practical PBMA development program can evaluate NMR for bulk composition and formulation integrity, then route suspect or high-consequence samples to those molecular methods.

6.10 Evidence matrix: demonstrated, plausible, and unproven

Species/product	^{1}H NMR	^{13}C NMR	^{31}P NMR	Overall position
Beef	Direct multiclass, horse-screen, and mixture evidence	Strong compositional/regio-position basis	Quality/postmortem and additive evidence, not species	Ready for scoped screening and local validation
Pork	Direct multiclass and mixture evidence	Strong sn-2 palmitate basis	Process/additive evidence, not species	Ready for scoped screening; confirm trace claims molecularly
Horse	Direct 60 MHz lipid screen versus beef	No direct validated classifier located	No direct classifier located	Screening-ready only for defined beef-versus-horse domain
Chicken/turkey	Direct pooled-poultry evidence; chicken breed studies	Sparse species validation	No direct classifier located	Mammal-versus-poultry feasible; chicken-versus-turkey requires new model
Duck	Direct adulteration study plus pure-metabolome support	Sparse species validation	No direct classifier located	Feasible with dedicated age/breed-balanced model
Lamb/goat/donkey	Lamb direct; goat/donkey small proof of concept	Limited	No direct classifier located	Development needed for each claim
Ostrich	No direct classifier located	No direct classifier located	No direct classifier located	R&D gap; no current validation claim
Finfish	Direct proof of concept for selected species, origin, and treatment	Strong bounded selected-species/oil and salmon evidence	Limited internal or single-pair process evidence	Demonstrated only in selected cohorts; model per species universe and product form
Plant-based analogue	Narrow direct compositional comparisons; no locked multi-brand classifier	Broad plant-versus-animal oil support, not a PBMA classifier	Formulation/process context only	Promising bulk-screening hypothesis; multi-brand external validation and animal-mixture studies required

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

7. Recommended analytical architecture

7.1 Separate the analytical blocks before combining the data

The most practical platform uses one homogenized specimen to produce at least two analytical aliquots. One aliquot is used for a protein-depleted aqueous extract; the other is used for a lipid extract. A third aliquot is retained frozen for repeat analysis, molecular confirmation, or alternate extraction. A ^{31}P phospholipid extract can be prepared from the lipid fraction or from a dedicated aliquot if that channel is in scope.

The sequence matters. A classifier should not combine polar and lipid variables until each spectrum passes independent identity, signal-to-noise, line-shape, referencing, and blank checks. If the lipid fraction fails because the tissue is very lean, the result should not be silently replaced by a model trained on successful lipid extracts. It should be reported as a partial-data or indeterminate result according to a prespecified rule.

Analytical block	Primary purpose	Routine nucleus	Typical specimen contribution	Recommended role
Aqueous, protein-depleted extract	Dipeptides, amino acids, creatine, carnitine, choline derivatives, nucleotides, organic acids, fish osmolytes	^{1}H	Lean muscle and fish are information-rich	Primary screening block
Neutral-lipid extract	Fatty-acyl unsaturation, omega family, TAG glycerol, cholesterol, oxidation	^{1}H	Adipose and fatty products are information-rich	Primary screening block
Concentrated TAG/phospholipid fraction	Acyl chain identity and sn-position	^{13}C or ^{1}H–^{13}C HSQC	Requires sufficient lipid and clean extract	Targeted second-tier block
Standardized phospholipid or tissue preparation	Phospholipid classes and degradation; intact/in situ PCr/ATP/Pi; proposed added-phosphate profiling	^{31}P	Highly sensitive to matrix and sampling history	Orthogonal/process block
Intact tissue	Water mobility, lipids, low-MW metabolites without extraction	HR-MAS ^{1}H or TD-NMR	Requires specialized hardware or lower chemical specificity	Development or rapid structural screen

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

7.2 Sampling and homogenization

Sampling error can exceed spectrometer error. Fat, connective tissue, surface seasoning, and water are heterogeneous within a retail unit. The laboratory should define the test portion and homogenization plan in mass terms, not simply cut “a representative piece.” For minced products, multiple increments from the package should be composited. For intact cuts, the sampled anatomical location and visible external fat policy should be recorded. For fish, white versus red muscle and skin inclusion should be fixed. For plant-based analogues, crust and center should be considered separately after cooking because thermal gradients alter water and Maillard chemistry.

At minimum, record species claim, cut/product type, lot, supplier, geography, production system when known, fresh/frozen state, number of freeze-thaw cycles, postmortem/harvest interval when available, storage temperature, packaging, ingredient list, cooking protocol, and analyst preparation batch. These fields are not clerical extras; they are variables needed to detect confounding and model drift.

7.3 Polar extraction: a 300 MHz-compatible starting point

The large multiclass study began with 0.5 g of freeze-dried, cryomilled meat powder and approximately 6 mL water, followed by centrifugation, filtration, and 3 kDa ultrafiltration, with a pH 6 phosphate/D_2O/TSP NMR mixture [6]. This is not equivalent to extracting 0.5 g of wet homogenate. A 300 MHz development study should either reproduce the dry-powder basis or explicitly optimize and bridge a wet-homogenate method for recovery, dilution, reproducibility, and class performance. A validated precipitation route can replace ultrafiltration only if recovery and membrane/precipitation biases are demonstrated. Perchloric-acid extraction provides efficient quenching and was used in several smaller meat studies, but it adds neutralization, salt, and safety considerations [10].

For a 300 MHz Varian/Agilent-compatible system, a water-suppressed one-dimensional experiment can be developed around 298–300 K, 64–128 transients, a 4-second or longer relaxation delay, sufficient acquisition time to define narrow peaks, and a locked receiver-gain policy. The exact pulse sequence—presaturated 1D-NOESY, excitation-sculpting, or another validated method—must be held constant. A qNMR subset should establish which signals have adequate relaxation and recovery; a fingerprint model need not convert every bin to concentration, but it must still be analytically reproducible.

TSP is convenient but can interact with residual macromolecules, and its apparent shift can respond to matrix conditions. DSS or an external reference may be considered. Whichever system is chosen, chemical-shift reference, pH, phosphate concentration, D_2O fraction, ionic strength, and temperature should be part of the controlled method.

7.4 Lipid extraction and ^1H acquisition

The pivotal beef-versus-horse study used a simple chloroform extraction, while the broad Decker study used a chloroform/methanol/water system and subsequently analyzed a defined lipid mass in $CDCl_3$/MeOD [7,8]. The extraction selected for routine use should recover neutral and polar lipids consistently without causing selective oxidation or hydrolysis. Chloroform-based methods are effective but require appropriate engineering controls, waste handling, and solvent-quality checks. Alternative solvents may be developed, but the reference library must then be rebuilt or formally bridged.

A 300 MHz lipid spectrum can often be acquired in 16–64 transients with a relaxation delay appropriate to the fingerprint or quantitative claim. For a classifier, it is more important to control extract mass or tissue-to-solvent ratio, temperature, shimming, receiver gain, phasing, baseline, and spectral exclusions than to maximize nominal resolution. For composition work, relaxation and integral linearity must be demonstrated. Include a solvent blank, extraction blank, pooled QC lipid, and a system-suitability mixture containing representative saturated, oleate, linoleate, linolenate, cholesterol, and triacylglycerol signals.

7.5 ^{13}C implementation

Direct ^{13}C should be reserved for questions where it adds information unavailable from routine ^{1}H: pork-versus-ruminant TAG position, fish EPA/DHA regio-position, detailed carbonyl/olefinic structure, or a targeted lipid-composition confirmation. A concentrated extract—often tens of milligrams of lipid in approximately 0.5–0.7 mL deuterated solvent—is needed. Inverse-gated decoupling with long repetition is the reference approach for quantitative work. At 300 MHz, fully relaxed direct ^{13}C can require hours; throughput and sensitivity should be verified before it is placed in a routine decision path.

Three alternatives can improve practicality:

- Use direct ^{13}C only on NMR-suspect specimens or on a statistically selected subset of authentic references.
- Use a faster refocused INEPT fingerprint, accepting that response is sequence-dependent and validating it as a classifier rather than a mole-percent assay [21].
- Use ^{1}H–^{13}C HSQC to exploit ^{1}H sensitivity and ^{13}C dispersion for assignments or a targeted two-dimensional fingerprint. Quantitative HSQC requires its own calibration and should not be assumed from peak volume alone.

7.6 ^{31}P implementation

Three related ^{31}P questions should not be conflated. An intact or in situ tissue method can interrogate energy metabolites and estimate intracellular pH from calibrated Pi shifts. A rapidly quenched aqueous extract can preserve PCr/ATP/Pi abundance but reports extract pH, not the original intracellular compartmental pH. A phospholipid extract interrogates membrane classes and degradation products. The first two demand a tightly controlled interval from slaughter to quenching and are usually unsuitable for heterogeneous retail specimens. The phospholipid route is more compatible with an orthogonal authenticity investigation but requires a standardized solvent or detergent system, chelation of paramagnetic metals, pH control, and a validated reference/standard.

The fish freeze-thaw study used a cholate/EDTA/D_2O system with an internal phosphorus reference and collected 1024 scans [32]. That is a useful development precedent, not a universal recipe. Ahmmed and coworkers showed that analysis conditions materially affect quantitative phospholipid measurement [34]. A laboratory method should therefore validate response factors, decoupling/NOE effects, concentration linearity, stability in the NMR tube, and peak assignment under the exact matrix conditions.

For meat products containing added phosphate, ^{31}P is a plausible separate product-integrity test for orthophosphate, pyrophosphate, tripolyphosphate, and hydrolysis products. No direct meat-product validation study for that claim is included in this evidence set. It should therefore be labeled a proposed

application until matrix-specific recovery, speciation, stability, calibration, and decision performance are demonstrated.

7.7 HR-MAS and time-domain NMR

^{1}H HR-MAS can analyze milligram quantities of intact tissue with much of the chemical resolution of a solution spectrum. Ritota and coworkers studied 47 young bulls spanning cattle breeds, buffalo, and muscle type, supporting compositional and breed/muscle discrimination rather than a general cattle-versus-buffalo species classifier [11]. The game-meat perspective is adjacent exploratory evidence rather than a validated classifier [12]. HR-MAS reduces extraction but requires a dedicated MAS probe, careful rotor loading, temperature control, and attention to tissue degradation during spinning. It is attractive for R&D or a specialized laboratory, not a drop-in method for a conventional liquids probe.

Time-domain NMR is fast and robust for total water/fat and relaxation-based microstructure. The burger study demonstrates animal-versus-plant structural contrast [39]. TD-NMR does not identify molecular species with the specificity of high-resolution spectroscopy. Moisture, binder, salt, cooking, freeze-thaw, and particle size can all alter relaxation, so it is best used as a rapid front-end screen or formulation-control tool.

7.8 Proposed acquisition hierarchy for Process NMR Associates

Tier	Analysis	Approximate operational objective	Decision use
0	Sample records, homogenization, moisture/fat screening, retained aliquot	Prevent sampling and metadata failure	Eligibility and stratification
1A	Polar ^{1}H on 300 MHz	Broad metabolite fingerprint in roughly 15–30 minutes after preparation	Primary class and one-class screen
1B	Lipid ^{1}H on 300 MHz	Fatty-acyl/sterol fingerprint in roughly 5–15 minutes after preparation	Primary class and mixture screen
2	Data fusion and targeted qNMR ratios	Resolve discordant polar/lipid evidence; estimate calibrated mixtures	Screen decision with uncertainty
3A	Direct or INEPT ^{13}C; selected HSQC	Confirm TAG/phospholipid regiochemistry	Targeted technical confirmation
3B	^{31}P phospholipid method; proposed additive method	Determine membrane/process explanation and test additive hypotheses	Orthogonal evidence, not primary species label
4	qPCR/DNA sequencing or validated peptide LC–MS/MS	Establish biological species at claim-relevant sensitivity	Consequential confirmation

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

Note: Time estimates are development targets, not validated commitments. Preparation, batching, instrument configuration, concentration, and required signal-to-noise determine actual throughput.

8. Chemometrics and model architecture

8.1 Preprocessing must be part of the locked method

Phase correction, baseline correction, referencing, alignment, water/solvent exclusion, binning or targeted feature extraction, normalization, transformation, scaling, and outlier handling can each change the answer. These steps must be version-controlled and applied identically to development and unknown samples. Recommended ^{1}H processing practices emphasize inspection of raw data, appropriate alignment, transparent normalization, and avoidance of preprocessing that manufactures separation [48].

Common options include:

- Full-resolution or intelligently binned spectra after locked alignment.
- Targeted integrals for interpretable compounds plus untargeted residual regions.
- Probabilistic-quotient, total-area, internal-standard, tissue-mass, or extract-mass normalization, selected according to the claim.
- Log, Pareto, or autoscaling, selected within the inner development loop rather than after viewing test performance.
- Separate block scaling for polar and lipid data before fusion.

Any preprocessing parameter estimated from the data—such as a mean spectrum, scaling factor, selected variable list, or alignment template—must be derived from the training partition only during validation. Estimating it once on the full dataset leaks information into the test set.

8.2 Exploratory visualization is not validation

PCA is useful for detecting gross outliers, batch structure, and confounding. A clean PCA plot is not a measure of predictive accuracy. Likewise, PLS-DA or OPLS-DA scores can look impressive even when sample numbers are small and variables are numerous. The validation must operate on predictions for specimens that were not used to tune preprocessing, feature selection, latent variables, or thresholds.

Permutation tests and random-label checks can expose obvious overfitting, but they do not replace external validation. Report R^2 and Q^2 only with prediction metrics, confusion matrices, and the exact split unit.

8.3 Split by biological unit and provenance group

Replicate extractions, replicate spectra, subsamples from one animal, and multiple packages from one production lot must remain in the same fold. Otherwise the model recognizes the individual or lot rather than the species. More stringent splits should hold out entire suppliers, farms, slaughter days, vessels, seasons, or manufacturing batches. A useful hierarchy is:

1. Inner grouped cross-validation: preprocessing and hyperparameter selection.

2. Outer grouped cross-validation: an unbiased development performance estimate.

3. Locked blind external validation: new biological lots from sources and dates not used in development.

4. Transfer validation: another instrument, probe, laboratory, or analyst if those uses are intended.

8.4 Closed-set, one-class, and open-set models

A multiclass model answers "which represented class is most likely?" A one-class model answers "is this sufficiently consistent with the authentic class?" An open-set architecture combines known-class assignment with rejection of unknowns. Meat-authenticity programs generally need the latter two capabilities because market specimens can contain unrepresented species, binders, additives, spoilage, or unusual processing.

The decision layer should therefore permit at least three outcomes:

- **Consistent with authentic class.** Class probability or one-class distance meets the locked acceptance rule and all QC gates pass.
- **Indeterminate/outside domain.** Spectrum or metadata falls outside the validated applicability domain, blocks disagree, or uncertainty is too high.
- **Suspect.** Evidence crosses a locked nonconformity threshold or a calibrated adulterant estimate exceeds the action level.

Forcing indeterminate samples into a species class creates false certainty and is not defensible.

8.5 Mixture calibration

Mixture models require gravimetrically prepared, matrix-matched specimens spanning both composition and biological diversity. A useful development design could include 0, 0.5, 1, 2, 5, 10, 20, 40, and 60% w/w adulterant, but these levels are challenge points—not promised detection limits. Each level should be made from multiple independent donor pairs and processed exactly as real products. Lean and fatty adulterant sources should be included because equal mass percentages can produce different lipid signals.

Report bias, RMSEP, residual pattern, prediction interval, false-positive rate at zero adulterant, decision capability near the action threshold, and robustness to cooking/storage. A nominally low average RMSEP can still be unacceptable if the model is biased at the regulatory threshold. Classification sensitivity at a concentration is distinct from quantitative LOQ.

8.6 Interpretability and causal checks

Variables selected by a classifier should be mapped back to plausible chemistry. A result driven by solvent residual, filter extractable, tube batch, pH shift, or baseline curvature is a method failure even if cross-validation is high. Useful causal checks include spike/recovery of candidate compounds, alternate extraction, targeted qNMR, GC-FID/GC–MS fatty-acid comparison, enzymatic or chromatographic phospholipid comparison, freshness K-value or TMA-N in fish, and controlled feed/age/cut challenge sets.

9. Validation strategy

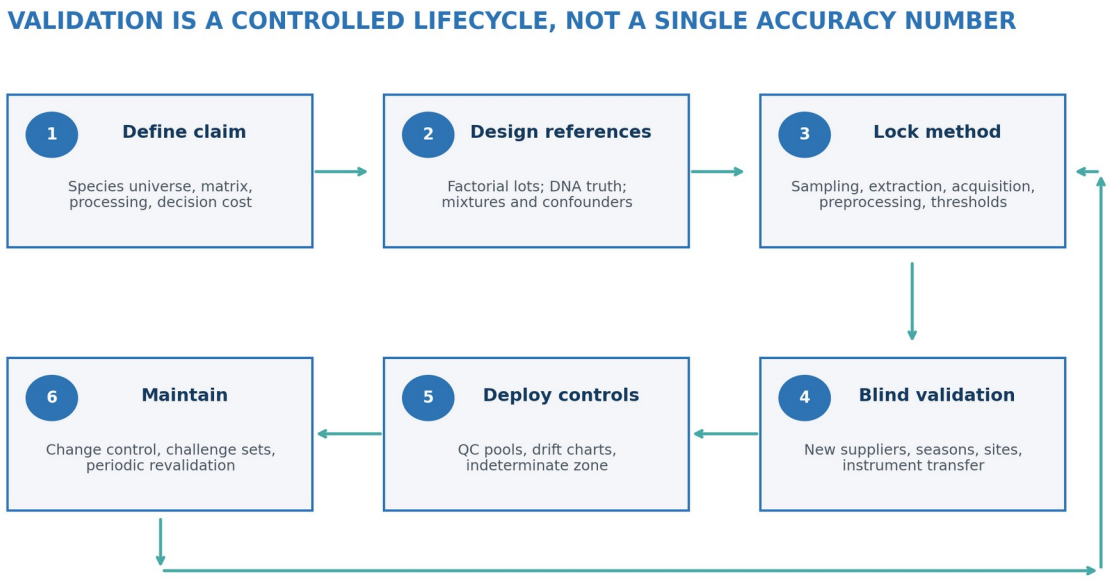

Figure 4. The validation and maintenance lifecycle for a non-targeted NMR authenticity method.

9.1 General validation principles

Non-targeted methods require the same fitness-for-purpose discipline as targeted assays, but their validation object includes the reference population, preprocessing pipeline, model, applicability domain, and decision rule. Reviews and consensus papers emphasize authentic reference materials, transparent

class definitions, independent validation, false-positive and false-negative control, and lifecycle management [49–51].

The validation protocol should be approved before the blind set is opened. It should identify which decisions are confirmatory and which are screening, the intended matrix, the species universe, processing boundaries, minimum signal quality, handling of missing blocks, model version, thresholds, acceptance criteria, and confirmatory workflow.

9.2 Reference population design

The reference collection must be broad enough that the target species signal exceeds legitimate within-species variation. A factorial plan should cover, where relevant:

- Multiple animals, farms, suppliers, slaughter/harvest days, regions, and seasons.
- Breed or strain, sex, age/size, diet/feed, production system, and wild/farmed status.
- Cut/muscle, visible fat policy, adiposity, and tissue depot.
- Fresh/frozen-thawed status, storage time/temperature, packaging atmosphere, and processing.
- Product formulation, ingredient suppliers, manufacturing site, and formulation generation for PBMAs.

Ground truth should be independent of the NMR features. Species identity should be verified by documented chain of custody plus DNA barcoding or validated species-specific PCR on a statistically appropriate subset, and preferably on every rare or high-value class. The reference collection should retain aliquots so that future model changes can be bridged.

Sample size cannot be reduced to one universal number. It should be justified from prespecified confidence-interval width, sensitivity/specificity or false-positive targets, expected class heterogeneity, intended subgroup claims, and the biological split unit. Low false-positive claims and heterogeneous market deployment can require far more independent lots than an exploratory feasibility study. Replicate spectra improve precision estimates but do not increase the number of independent animals or lots.

9.3 Analytical performance

Validate, separately for each analytical block:

- Within-run and between-day precision of selected integrals, spectral distances, and final decision score.
- Intermediate precision across analyst, extraction batch, tube lot, instrument state, and relevant probe or autosampler configuration.
- Concentration/extract-mass range, receiver-gain behavior, and spectral signal-to-noise gate.
- Temperature, pH, water suppression, shimming/line width, referencing, and storage stability in the NMR tube.

- Carryover, solvent and filter blanks, oxidation during preparation, and freeze-thaw stability of retained material.
- Robustness to small deliberate method changes within the operating range.
- System suitability using representative pooled QCs and chemically defined controls.

For direct quantitative ^{13}C or ^{31}P, also validate relaxation, decoupling/NOE bias, response factors, linearity, and traceability of the reference standard. For fingerprint classification, validate decision-score precision and classification stability rather than treating every bin as a quantitative analyte.

9.4 Predictive performance

For categorical decisions, report class-specific sensitivity, specificity, false-positive and false-negative rates, balanced accuracy, precision, predictive values at relevant prevalence, confusion matrix, and indeterminate rate. For one-class methods, report authentic acceptance and nonauthentic rejection against clearly defined challenge classes. For continuous mixture estimates, report bias, RMSEP, prediction intervals, and performance at the action level.

Confidence intervals are essential. “100% accuracy” on ten specimens is not equivalent to 100% on one hundred independent lots. The report should show the numerator and denominator and avoid excessive significant figures.

9.5 External and adversarial challenges

The blind external set should be acquired after model lock and should include new suppliers/lots/seasons, authentic edge cases, close biological neighbors, intended adulterants, unexpected species, processing variations, and formulations that stress the model. For a beef system, challenges might include buffalo, horse, lamb, venison, pork, poultry, plant analogue, high-fat beef, very lean beef, grass-fed and grain-fed beef, fresh and frozen-thawed material, and seasoned/cooked products if those are within the claim.

Adversarial design is not an invitation to manipulate the test; it is a way to discover the method's true boundary. A robust system should reject a new species as outside domain rather than confidently mislabeling it as the nearest known class.

9.6 Instrument and laboratory transfer

A model built at 600 or 400 MHz does not automatically transfer to 300 MHz. Peak overlap, line shape, digital resolution, probe response, water suppression, temperature calibration, and shimming differ. A 300 MHz library should be acquired directly on the intended platform or transferred through a documented standardization and bridging study. Transfer sets must span classes and confounders, not only pooled QC.

If deployment spans multiple instruments, include standard mixtures, common pooled samples, instrument-specific quality limits, calibration transfer or domain-adaptation rules fixed before validation, and a requalification trigger after probe service, console changes, pulse-sequence updates, or major software changes.

9.7 Ongoing performance monitoring

Each batch should include blanks, pooled authentic QC, and an appropriate challenge or control material. Monitor chemical-shift residuals, line width, water suppression, key integral ratios, PCA/Q-residual or other spectral-distance statistics, and final model score. Establish alert and action limits from stable historical data. A control chart detects instrumental drift; a population-drift chart detects changes in supply chemistry.

Revalidation triggers include new species or product claims, new suppliers/regions, feed or formulation changes, new processing states, new extraction chemistry, instrument or probe changes, sustained QC drift, increased indeterminate rate, or confirmed false decisions.

10. Reporting and confirmatory decision rules

10.1 Suggested report vocabulary

Avoid language that exceeds the evidence. Preferred expressions include:

- "The specimen is **consistent with** the validated beef reference population."
- "The specimen is **outside the applicability domain** of the current model."
- "The lipid and polar blocks are discordant; the result is **indeterminate** pending repeat extraction and molecular confirmation."
- "The estimated pork fraction is X% w/w with a validated prediction interval of Y–Z% for this product class."
- "Animal-associated chemistry was not detected above the validated NMR decision limit in this PBMA matrix." *(Use only after a multi-brand, matrix-specific PBMA method has actually been validated.)*
- "^{31}P findings are consistent with the validated phospholipid/process interpretation; they do not independently establish species." Added-phosphate language should be used only after direct product-specific validation.

Avoid "proved authentic," "100% species identification," "zero animal," or "LOD 2%" unless that exact decision and matrix were independently validated. Detection limits from simple oils, rendered fats, or a different species/product must not be transferred to meat tissue.

10.2 Confirmation triggers

Molecular confirmation should be triggered when:

- The species claim has legal, religious, ethical, contractual, or high economic consequences.
- The NMR result is suspect, indeterminate, or outside domain.
- A low-level adulterant decision is below the validated NMR quantitation range.
- The specimen is highly processed, protein/fat partitioning is unusual, or an unknown species is possible.
- The animal-versus-plant question concerns trace cross-contact or vegan certification.
- A clinical allergen question is present; route it to an allergen-specific validated protein method rather than treating species DNA as an allergen assay.
- The sample may contain multiple animal species.

NMR and molecular methods answer different questions and can legitimately disagree. NMR may detect a bulk plant-oil formulation while PCR detects trace animal DNA; conversely, intense beef fat can dominate a mixture while lean adulterant DNA remains detectable. The final interpretation should integrate mass fraction, tissue type, processing, and method sensitivity rather than declaring one platform "right" in all circumstances.

10.3 A practical decision pathway

1. Verify chain of custody, product class, and model eligibility.

2. Acquire polar and lipid ^{1}H data with batch QC.

3. Apply locked preprocessing, model, open-set rejection, and uncertainty rules.

4. If both blocks agree and all gates pass, issue a screening conclusion within the validated claim.

5. If blocks disagree or the sample is outside domain, repeat preparation once according to the deviation procedure; do not tune the model to the sample.

6. Use targeted ^{13}C or ^{31}P when it can explain the discordance through lipid regiochemistry, phospholipid degradation, or phosphate treatment.

7. Route suspect or consequential samples to DNA/proteomics confirmation and integrate the findings in a final technical opinion.

11. Recommended phased development program

11.1 Phase 0 — define claims and assemble truth materials

Begin with four separate intended-use domains rather than one global model:

- **Domain A:** raw mammalian and poultry muscle—beef, pork, chicken, horse, duck, and later lamb/goat.
- **Domain B:** cooked and processed meat products, stratified by product family.
- **Domain C:** finfish, grouped by realistic substitution problems and product form; shellfish, crustaceans, mollusks, and roe/caviar require separate evidence.
- **Domain D:** plant-based analogues versus animal products, stratified by protein/oil/formulation family and cooking state.

Ostrich should be a defined expansion study, not folded into Domain A after the fact. Acquire DNA-verified reference material, record the confounder metadata, and reserve a blind external set before any model is tuned.

11.2 Phase 1 — establish the 300 MHz dual-^{1}H platform

Develop and lock the polar and lipid methods on the existing 300 MHz system. Use two-dimensional experiments on pooled and representative samples for assignment, then convert routine acquisition to one-dimensional fingerprints. Establish system suitability, pooled QC, batch acceptance, spectral processing, data storage, audit trail, and version control.

Initial feasibility should answer:

- Can the 300 MHz spectra reproduce the marker regions reported at 400–700 MHz?
- Does dual-block data separate beef, pork, chicken, horse, and duck across independent sources?
- Which variables encode species and which encode cut, fat content, storage, or supplier?
- How frequently does one block fail or disagree?
- Can known unknowns—lamb, venison, fish, and PBMA—be rejected as outside domain?

11.3 Phase 2 — mixture and processing validation

Prepare multi-donor binary and ternary mixture designs around economically and contractually meaningful thresholds. Test raw, cooked, frozen-thawed, seasoned, and high/low-fat strata as required by the claim. Lock the qualitative and quantitative decision rules and conduct the blind external validation.

The endpoint is not the highest possible cross-validation accuracy. It is a documented false-positive/false-negative balance, uncertainty at the action level, and a manageable indeterminate rate on new lots.

11.4 Phase 3 — targeted ^{13}C and ^{31}P extensions

Add ^{13}C first where the business or scientific question benefits from regio-position: pork-versus-beef fat, finfish species/oil, or unexplained lipid discordance. Add ^{31}P for finfish freeze-thaw/phospholipid studies or controlled postmortem research. Treat added-phosphate analysis as a proposed product-specific

development target until directly validated. Do not make either nucleus a mandatory routine step unless it improves external decision performance enough to justify time and cost.

11.5 Phase 4 — transfer, accreditation, and maintenance

Conduct instrument/laboratory transfer, analyst qualification, proficiency challenge, and controlled-document release. Define model ownership, software versioning, retraining rules, retained-data requirements, and annual performance review. If the method will support accredited or regulatory work, engage the quality unit and relevant authority early to agree on non-targeted-method expectations.

11.6 Recommended initial priority

The highest-value first **development and pilot target** is a dual-extract ^{1}H model for raw/minced beef, pork, chicken, horse, and duck on the 300 MHz platform, with one-class rejection and PCR/proteomic confirmation. No integrated externally validated five-class 300 MHz method presently supports deployment. The pilot should proceed to operational use only after local blind external validation, mixture challenges, and acceptance criteria are passed. It aligns with the strongest published evidence, leverages existing hardware, and creates a reference infrastructure that can later support lamb/goat, processed products, PBMAs, and ostrich.

12. Limitations and scientific cautions

No NMR authenticity model is timeless. Biological supply chains and formulations evolve. Feed changes alter fat; breeding and slaughter weights change tissue composition; aquaculture substitutes plant oils for marine oils; PBMA recipes change; cold chains differ; and new fraud strategies target known methods. Model maintenance is part of the analytical method.

Recurring limitations include small or local cohorts, uneven class sizes, closed-set models without unknown rejection, cross-validation by spectrum rather than animal or lot, internal accuracy without blind external validation, and “adulteration” studies that compare only pure species. Other recurring problems are prediction errors without action-level performance, confounding among species and supply-chain variables, transfer of performance from oils or rendered fats to lean meat, and use of a single formulation to represent all PBMAs.

No validated NMR ostrich classifier or broad ^{31}P meat-species classifier was found. PBMA studies support compositional proof of concept—not a validated multi-brand class—and trace-free vegan certification is not an NMR-only claim. These gaps define the experiments required before success can be claimed.

Appendix A. Detailed species and claim matrix

Claim	Best NMR block	Direct evidence	Development need	Confirmation
Beef versus pork, lamb, pooled poultry	Polar + lipid ^{1}H	Large raw and processed cohorts [6,7]	Local suppliers, cuts, processing; open-set rejection	PCR/peptides for disputes
Beef versus horse	Lipid ^{1}H, potentially benchtop	Independent challenge [8]	Multi-donor mixtures; cooked/seasoned matrices; low-fat specimens	Equine PCR/peptides
Pork fraction in beef	Polar + lipid ^{1}H; ^{13}C as structural support	Designed mixtures with duck [9]	Action-threshold calibration; lean/fatty pork; processing	Porcine qPCR/peptides
Duck fraction in beef	Polar + lipid ^{1}H	Designed binary/ternary mixtures [9]	Age/breed/feed-balanced donors; external lots	Avian/duck molecular assay
Chicken versus turkey	Polar + lipid ^{1}H	No direct broad classifier located	Dedicated hierarchical avian cohort	Species-specific PCR
Lamb versus beef	Polar + lipid ^{1}H	Direct multiclass evidence; some lipid confusion [6,7]	Ruminant-focused model; feed/geography balance	DNA/peptides
Goat or donkey adulteration	Polar ^{1}H	Small pure-species proof of concept [10]	Independent cohorts and mixtures	DNA/peptides
Ostrich versus beef/horse/poultry	Polar + lipid ^{1}H; exploratory HR-MAS	No direct ostrich classifier	DNA-verified ratite cohort; closest-neighbor challenge; mixtures	Ratite/ostrich PCR or sequencing
Selected finfish species	Polar ^{1}H; lipid ^{13}C	Multiple direct but bounded selected-species studies [23,24,27,30]	Species universe, season, site, size, muscle, storage; external lots	DNA barcoding
Wild versus farmed salmon	Lipid ^{13}C/^{1}H	Direct bounded production-origin cohorts [25,26,36]	Current-feed and region updates; external transfer	Records, isotopes, genetic/traceability evidence
Fresh versus frozen-thawed finfish	^{1}H; exploratory ^{31}P	Direct ^{1}H studies; internally validated, lower-performing ^{31}P result [31,32]	Product-specific storage challenge	Document/quality methods
Meat versus PBMA	Polar + lipid ^{1}H; TD-NMR optional	Narrow direct comparison studies [38,39], not a locked classifier	Multi-brand/formulation/lot/cook state; blind external set; animal mixture series	Universal vertebrate qPCR or peptides
Soy leghemoglobin identity	Not routine small-molecule NMR	Regulatory context only [40–42]	Dedicated protein-specific method if needed	Targeted protein assay
Added condensed phosphate	^{31}P	Proposed mechanism; no direct meat-product validation cited	Matrix-specific recovery, calibration, speciation, and hydrolysis/stability validation	Ion chromatography or validated reference method

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

Appendix B. Marker and confounder dictionary

Marker family	NMR channel	Species/product information	Major confounders
Total SFA/MUFA/PUFA	Lipid ^{1}H and ^{13}C	Ruminant versus monogastric versus marine phenotype	Feed, tissue depot, season, oxidation
α-Linolenate and omega-3 terminal methyl	Lipid ^{1}H	Horse-rich and omega-3-rich lipid patterns	Feed, plant oils, marine oils, resolution
EPA/DHA amount and sn-position	^{13}C, supported by ^{1}H	Fish species, oil, wild/farmed/feed phenotype	Feed formulation, season, size, tissue
sn-2 palmitate	Quantitative ^{13}C	Strong porcine TAG mechanistic marker	Tissue depot, breed/diet; measurement time
Cholesterol	Lipid $^{1}H/^{13}C$	Animal-lipid support	Fortification, mixed formulations, concentration
Plant sterols	Lipid $^{1}H/^{13}C$	Candidate plant-oil support; current PBMA evidence is non-NMR [43]	Oil type/refining, fortification, low abundance
Carnosine/anserine	Polar ^{1}H	Mammal/avian/fish muscle phenotype	Muscle type, age, breed, processing
Creatine/phosphocreatine	Polar ^{1}H and ^{31}P	Muscle phenotype and energy state	Postmortem time, pH, temperature, freezing
Lactate	Polar ^{1}H	Muscle metabolism and processing	Stress, glycogen, postmortem interval, microbial change
Carnitine/O-acetylcarnitine	Polar ^{1}H	Meat-class information in multiclass models	Muscle and metabolic state
Choline/GPC/betaine	Polar ^{1}H; selected ^{31}P	Membrane turnover and fish/plant osmolytes	Storage, hydrolysis, formulation
IMP/inosine/hypoxanthine	Polar ^{1}H	Meat/fish profile and freshness	Time, temperature, freeze-thaw, microbial activity
TMAO/TMA/DMA, taurine, arsenobetaine	Polar ^{1}H	Fish/osmoregulatory phenotype	Habitat, spoilage, processing, additives
Phospholipid classes	^{31}P and ^{13}C	Membrane phenotype and degradation	Solvent, pH, aggregation, oxidation, time
PCr/ATP/Pi; calibrated intact-tissue pH	^{31}P	Postmortem/freshness state; intracellular pH only in intact/in situ tissue	Sampling delay, stress, temperature, freezing, extraction
Sugars, citrate, trigonelline, plant organic acids	Polar ^{1}H	PBMA/formulation support	Recipe, fermentation, cooking, fortification
Aldehydes and free fatty acids	$^{1}H/^{13}C$	Oxidation/hydrolysis quality state	Oxygen, heat, storage, extraction artifacts

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

Appendix C. Validation checklist

C.1 Intended use and governance

- [ ] Authentication claim, species universe, matrix, processing state, action level, and consequence are written.
- [ ] Screening versus confirmatory status is explicit.
- [ ] Model owner, software version, data retention, change control, and revalidation triggers are assigned.
- [ ] Chain of custody and retained-aliquot procedure are defined.

C.2 Reference materials

- [ ] Species truth is independently documented and molecularly verified as appropriate.
- [ ] Animals/lots, suppliers, regions, seasons, breeds, diets, ages, sexes, cuts, and fat levels are represented.
- [ ] Fish models balance species, site, season, size, muscle, catch/farm, and storage.
- [ ] PBMA models include brands, protein/oil systems, lots, sites, reformulations, and cooking states.
- [ ] Rare classes such as horse and ostrich have adequate independent biological replication.
- [ ] A blind external set is reserved before model optimization.

C.3 Sample preparation and acquisition

- [ ] Sampling increments, homogenization, test portion, tissue/fat policy, and storage are controlled.
- [ ] Polar and lipid extraction recovery, precision, stability, blanks, and safety controls are validated.
- [ ] NMR temperature, reference, pH/ionic conditions, pulse sequence, scans, delay, acquisition, gain, processing, and exclusions are locked.
- [ ] System suitability and pooled QC materials are defined.
- [ ] Missing-block and failed-QC rules are prespecified.

C.4 Model development

- [ ] Biological units and provenance groups—not replicate spectra—define folds.
- [ ] Preprocessing and feature selection occur inside the training loop.
- [ ] Hyperparameters are selected by nested grouped validation.
- [ ] Open-set or one-class rejection and applicability-domain rules are implemented.
- [ ] Variables are inspected for chemical plausibility and batch artifacts.
- [ ] Polar/lipid block scaling and data-fusion rules are locked.

C.5 Performance validation

- [ ] Blind external samples include new lots, suppliers, seasons, and edge cases.
- [ ] Sensitivity, specificity, false-positive/false-negative rates, confusion matrix, indeterminate rate, and confidence intervals are reported.

- [ ] Mixture bias, RMSEP, prediction interval, and action-level performance are reported.
- [ ] Processing, storage, oxidation, pH, fat content, and formulation challenges are included.
- [ ] Instrument, analyst, day, and laboratory transfer are tested when intended.

C.6 Deployment and maintenance

- [ ] Batch QC and spectral/model drift charts are active.
- [ ] Confirmation triggers and PCR/proteomics workflow are documented.
- [ ] Results use “consistent with,” “outside domain,” “indeterminate,” and “suspect” language.
- [ ] New supply sources and formulations undergo bridge testing.
- [ ] False decisions and increased indeterminate rates trigger investigation and revalidation.

Appendix D. Reference validation audit

D.1 Validation policy

Every bibliographic item cited in this white paper was checked as of **28 August 2026** against at least one primary or authoritative metadata source: the publisher record, PubMed/PMC, a Crossref-indexed institutional record, or an official FDA/eCFR/Federal Register page. DOI strings were matched to title, complete author list, journal, year, volume, and page range or article number. Official web sources were used when no DOI applies. This audit validates citation identity and traceability; it does not imply independent replication of each scientific result. Direct NMR comparisons, adjacent non-NMR evidence, regulatory sources, and molecular confirmation studies are labeled according to their actual evidentiary role.

Ref.	Permanent identifier	Metadata verification record
1	10.1016/j.meatsci.2010.06.001	Elsevier/publisher record and journal pagination checked
2	10.1016/j.foodchem.2017.11.007	Elsevier and Crossref-indexed institutional record; all nine authors checked
3	10.1111/1541-4337.12408	Wiley/publisher record; volume, issue, and pages checked
4	10.1016/S0924-2244(10)80001-9	Elsevier/Crossref-indexed record; two authors and pages checked
5	10.4081/ijas.2002.151	Journal publisher record; complete seven-author list checked
6	10.3389/fnut.2022.985797	Frontiers and PMC full text; authors, article number, methods, and outcomes checked
7	10.1021/acs.jafc.2c01871	ACS and PubMed record; authors, issue, and pages checked
8	10.1016/j.foodchem.2014.11.110	Elsevier, PubMed, PMC, and university repository; all nine authors checked
9	10.1016/j.foodcont.2023.110018	Elsevier/Crossref record; six authors and article number checked
10	10.3390/molecules26154643	Publisher, PubMed, and PMC; all nine authors and study design checked
11	10.1016/j.meatsci.2012.06.034	Elsevier and PubMed record; four authors and pagination checked
12	10.1093/af/vfad066	Oxford University Press record; complete seven-author list checked
13	10.1016/j.foodchem.2022.134004	Elsevier/Crossref record; complete six-author list checked
14	10.5851/kosfa.2020.e102	Journal publisher and PMC; five authors, issue, and pages checked
15	10.3382/ps/pew279	Poultry Science publisher record; five authors and pages checked

Ref.	Permanent identifier	Metadata verification record
16	10.1016/j.foodchem.2013.03.102	Elsevier/Crossref record; four authors and pages checked
17	10.1016/j.foodres.2020.109126	Elsevier/Crossref record; complete eleven-author list checked
18	10.1016/j.foodchem.2012.09.136	Elsevier/Crossref record; five authors, issue, and pages checked
19	10.1016/j.foodchem.2012.08.058	Elsevier/Crossref record; six authors and pages checked
20	10.1002/lipd.12277	Wiley and PubMed record; five authors, title, volume, and pages checked
21	10.1016/j.foodcont.2018.03.046	Elsevier and HAL/Crossref record; seven authors and pages checked
22	10.1016/j.foodchem.2003.08.021	Elsevier/Crossref-indexed record; six authors and pages checked
23	10.1016/j.focha.2023.100602	Elsevier/OpenAlex/Crossref and open manuscript; five authors checked
24	10.1016/j.foodchem.2009.12.074	Elsevier/Crossref record; three authors, volume, and pages checked
25	10.1021/jf8039268	ACS and PubMed record; six authors and pages checked
26	10.1016/j.foodchem.2009.03.026	Elsevier/Crossref record; five authors and pages checked
27	10.1007/s11746-009-1370-y	Springer/Crossref record; three authors, issue, and pages checked
28	10.1021/jf070736g	ACS/Crossref record; seven authors and pages checked
29	10.1016/j.foodchem.2021.131094	Elsevier and PubMed record; nine authors and article number checked
30	10.1016/j.microc.2026.117163	Elsevier/Crossref record; complete author list and article number checked
31	10.1016/j.foodchem.2020.126227	Elsevier and PubMed record; three authors and article number checked
32	10.1007/s00217-023-04383-x	Springer publisher record; six authors, volume, and pages checked
33	10.1021/jf4021812	ACS/Crossref record; five authors, issue, and pages checked
34	10.1016/j.lwt.2021.110991	Elsevier/Crossref record; five authors and article number checked
35	10.1016/j.rechem.2022.100423	Elsevier open article; five authors, volume, and article number checked
36	10.1039/C6AY03000A	Royal Society of Chemistry record; four authors and pages checked
37	10.1016/j.foodchem.2015.03.037	Elsevier/Crossref record; five authors and pages checked
38	10.1016/j.foodchem.2024.138705	Elsevier and PubMed record; six authors and article number checked

Ref.	Permanent identifier	Metadata verification record
39	10.1111/1750-3841.70376	Wiley publisher record; three authors, issue, and article number checked
40	FDA GRN 737; FDA response letter dated 23 July 2018	Official FDA GRAS inventory and agency response letter checked
41	84 FR 37573; 21 CFR §73.520	Official Federal Register final rule and current eCFR section checked
42	10.1177/1091581818766318	SAGE/publisher and PubMed record; five authors and pages checked
43	10.1038/s41598-021-93100-3	Nature/Scientific Reports and PubMed/PMC; seven authors and article number checked
44	10.1016/j.foodcont.2021.108001	Elsevier/Crossref record; two authors and article number checked
45	10.1007/s00217-020-03608-7	Springer/Crossref record; four authors and pages checked
46	10.1021/acs.jafc.3c01100	ACS/Crossref record; five authors, issue, and pages checked
47	10.1021/acs.jafc.4c12769	ACS/Crossref record; six authors, issue, and pages checked
48	10.1007/s11306-018-1321-4	Springer, PubMed, and Crossref; complete seven-author list checked
49	10.1021/acs.jafc.9b03085	ACS and PubMed record; complete six-author list and pages checked
50	10.1016/j.fochx.2022.100538	Elsevier, PubMed, PMC, and JRC record; complete nine-author list checked
51	10.1016/j.aca.2015.06.003	Elsevier/Crossref record; three authors and pages checked
52	10.1016/j.foodcont.2016.04.013	Elsevier/Crossref record; four authors and pages checked

Source: Process NMR Associates synthesis of the validated literature cited in this paper.

D.2 Claim-validation safeguards

- Reported sample counts and accuracies were taken from primary articles or authoritative abstracts; unresolved details were not invented.
- "100%" results from small, closed, or internally cross-validated studies are described as proof of concept rather than universal performance.
- Oil, rendered-fat, production-origin, breed, and freshness studies are labeled as supporting or adjacent evidence when they do not directly validate meat-species authentication.
- The beef-versus-horse study is not cited as a mixture-quantitation study, because it did not test mixtures [8].
- The chicken/chevon/beef/donkey study is not cited as a validated adulteration-LOD study, because it examined pure species and had no external set [10].
- The broad ^{13}C fat/oil database is not used to claim validated individual beef, chicken, duck, or lamb classification because those terrestrial classes were sparsely represented [21].

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Summary

^{1}H, ^{13}C, and ^{31}P NMR can support meat authenticity when the result is a validated chemical decision within a defined species, matrix, and processing domain. ^{1}H is the routine workhorse: complementary aqueous and lipid fingerprints have separated bounded raw and processed meat classes, screened beef against horse, modeled pork/duck mixtures in beef, and differentiated selected finfish cohorts. Published accuracy applies only to the represented biological lots and validation design; it is not a universal species accuracy or trace-adulterant limit.

^{13}C adds fatty-acyl identity and position: sn-1,3 versus sn-2 in triacylglycerols and sn-1 versus sn-2 in glycerophospholipids. It is strongest in selected finfish/oil and salmon-production studies; porcine sn-2 palmitate is a valuable mechanistic pork-versus-beef marker. ^{31}P is complementary for phospholipid classes, degradation, and controlled postmortem/freeze-thaw research. Intracellular pH requires intact/in situ tissue; extracts report extract conditions. Added-phosphate profiling is a proposed product-specific application here, and no broad validated ^{31}P species classifier was located.

Readiness is unequal. Beef and pork have the broadest evidence. Horse is supported only for bounded beef screening, with no mixture LOD. Poultry is often pooled; chicken-versus-turkey needs a dedicated model. Duck has direct mixture evidence, but age and breed are major confounders. Finfish results are promising but commonly small, internal, or supply-chain-specific. Ostrich remains an R&D gap. Plant-based analogues have narrow compositional proof-of-concept evidence, not a validated multi-brand class; NMR cannot certify trace-free vegan status or directly verify soy leghemoglobin.

The recommended first target for Process NMR Associates is a 300 MHz dual-extract ^{1}H development pilot for raw/minced beef, pork, chicken, horse, and duck, with one-class/open-set rejection and molecular confirmation. Deployment should follow only after DNA-verified, biologically broad references; grouped/nested development; blind external lots; designed mixtures; processing and confounder challenges; transfer testing; locked preprocessing and thresholds; and explicit authentic/indeterminate/suspect rules. Targeted ^{13}C or ^{31}P should enter only when it improves a defined decision. Under that architecture, NMR is a credible screening and compositional-authentication technology that complements—not replaces—DNA and targeted proteomics.