Contribution of Research with Farm Animals to Protein Metabolism Concepts: A Historical Perspective

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Abstract

The roles of proteins, carbohydrates, fats, and micronutrients in animal and human nutrition were broadly described during the late 18th and 19th centuries, and knowledge in protein nutrition evolved from work with all species. Although much of the fundamental and theoretical research in protein metabolism during the 20th century was conducted with laboratory animals, basic protein nutrition research in farm animals complemented those efforts and led to the development and use of new investigative methods (particularly in amino acid nutrition) as well as use of animal models in furthering the understanding of human protein metabolism. All these efforts have led to a contemporary hybrid model of protein nutrition and metabolism applicable to both humans and animal species. Now in the 21st century, farm animals are used in fetal and pediatric nutrition research, and data accruing for excess amino acid feeding in research with farm animals provide direction for assessment of pharmacological effects of amino acids when consumed in excessive quantities. Thus, as nutritional science is moving forward into nutrigenomics, nutriproteomics, and metabolomics, farm animal and human nutrition research interactions will likely continue with genetically modified farm animals produced for agricultural reasons (improved function and product quality) or those produced with human genes introduced to generate even better models of human protein metabolism. J. Nutr. 137: 706–710, 2007.

The concept that animals require dietary, indispensable organic nitrogenous substances for proper growth and maintenance was recognized ~2 centuries ago (1,2). Since then our understanding of protein/amino acid requirements and of the dynamics of protein metabolism including protein biosynthesis and degradation in humans and animals has grown immensely (1,2). Much of the early nutrition research was done with farm species because of a keen interest by early workers in agriculture. Farm species also played a unique role in early work in micronutrient deficiencies (3,4). The most useful data for application to protein nutrition concepts arose from pigs and chickens, but protein metabolism data from ruminants, because of differences in digestive physiology between ruminants and nonruminants/humans, have not been generally applied to human nutrition (4).

Nitrogen balance was used to study protein metabolism in animals and humans during the 19th and early to mid-20th century. Results indicated that N retention efficiency was much lower in growing humans (<10%) than animals (>50%) (3); the higher efficiency in N retention in animals is attributable to a greater growth rate (6) and earlier chronological age at sexual maturity than humans. Hence, during growth most of the dietary amino acids are used for protein accretion in animals, whereas humans partition most of their dietary amino acid intake toward maintenance (7). Although the N balance approach remained a viable tool in human studies, this experimental procedure did not allow the high animal growth rates typically attainable under production conditions. Thus, agricultural researchers favored empirical experimental designs (such as dietary protein intake vs. performance) (Fig. 1) to obtain operational protein requirements. However, such empirical growth studies are not plausible with growing humans or adults in maintenance. Goals of animal and human nutritionists have also differed as they apply to diet formulations. In agriculture, costs and overall production efficiency are key criteria; in human nutrition emphasis is on providing all required nutrients by consumption of a variety of animal- and plant-based foods that we enjoy, and costs, availability, and efficiency of nutrient usage are often not overriding concerns. This article does not review the benchmarks in protein metabolism research (for nutrition history, see other references

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A second-order polynomial relation (solid curved line) of dietary protein content and weight gain with 0.95 confidence limits (dashed lines) are plotted. A quadratic equation and statistical analysis can then be used to estimate a minimal (Xᵣ), likely adequate (Xᵢ), and maximum (Xₘ) dietary protein content to achieve most efficient and highest gains (Yᵣ).

Figure 1  Effect of dietary protein concentration on body-weight gain from a typical farm animal empirical feeding trial to establish an operational protein requirement. A second-order polynomial relation (solid curved line) of dietary protein content and weight gain with 0.95 confidence limits (dashed lines) are plotted. A quadratic equation and statistical analysis can then be used to estimate a minimal (Xᵣ), likely adequate (Xᵢ), and maximum (Xₘ) dietary protein content to achieve most efficient and highest gains (Yᵣ).

Common issues and time line in protein metabolism for animals

Prevailing conceptual understanding of protein metabolism at any given date or time greatly influenced experimental approaches and interpretations of protein nutrition results with farm animals, rodents, and human subjects. Earlier theories included the idea that dietary protein passed directly into blood proteins and tissue proteins and that the latter was the source of muscular energy (2,11), the concept of indispensable and dispensable/labile proteins within tissues (11), and as promulgated by Folin (12) the concept of an endogenous (obligatory) body N pool, thought to be constant and related to body size (maintenance), and an exogenous body N pool, which represents all processes related to dietary N. Folin’s theories (12) influenced Mitchell (13), who placed great emphasis on determining endogenous and total urinary and metabolic and total fecal N excretion with high precision in biological value assays (13). After the work of Schoenheimer (14) on the dynamic state of protein metabolism in the 1940s, our theoretical understanding of protein metabolism, synthesis, and turnover passed to the modern era (15–17), and influences of early theories of protein metabolism on protein nutrition studies waned. Table 1 presents a broad timeline overview of all N, protein, and amino acid metabolism research with contributions from farm animals noted from the 18th century to the present day.

Baker (18) listed a number of issues that remain unresolved in animal and human amino acid/protein nutrition. Originally, priority issues from a practical nutrition perspective included what amino acids are required, how well different food/protein sources supply these amino acids qualitatively and quantitatively, and how efficiently protein sources are utilized by animals and humans. Thus, efforts to determine which amino acids are dietary essentials, efforts to determine or refine amino acid and/or protein requirements during growing and maintenance stages, and assessment of efficiency of N utilization or protein quality of foods were the mainstays of protein nutrition research for many years (5,13,19,20). From an animal model perspective, since about 1900–1910, most basic work in protein metabolism was conducted with laboratory rodents; however, parallel work was often conducted with farm species. In fact, newer emerging concepts of amino acid nutrition and efficiency of N utilization were rapidly accepted by the animal industry. Hence, translational research was widely conducted with farm animals typically using growth trials with incremental nutrient concentrations (Fig. 1). When plasma amino acid concentrations emerged as a response criterion for amino acid adequacy, work with farm animals made a significant contribution to development of indirect assays for amino acid adequacy. Techniques such as the indicator amino acid (IAAO)2 and direct amino acid oxidation (DAAO) methodologies arose in principle from work with farm animals (21–27).

Contributions from research with farm animals to protein nutrition concepts

In the 19th century, Magendie developed experimental methods for animal feeding experiments. Utilizing diets of pure carbohydrates and fats, he showed that food nitrogen was essential (28). Thereafter, to study nutrition and physiology of lactation, Boussingault developed the concept of basic elements (C, N, P, O) balance studies with dairy cows in the mid-19th century (29). Voit (30) and others (1,2) and soon many others used this approach to study N metabolism and nutrition in farm animals, laboratory rodents, and humans, but work with farm animals was usually oriented more to the immediate objective of improving food production. From the end of the 19th century into the early 20th century, work on basic protein metabolism (2,11,12) was conducted with laboratory animals and was then extended to farm animals. During this time period the concept of a defined/single-feed (restricted ration) diet, attributed to Magendie, also reemerged at the Wisconsin Agricultural Experiment Station (3,4). The “restricted ration” approach was useful not only in delineating the value of a given feed to support protein accretion but also in identifying specific micronutrient deficiencies (3). It was further noted that when growth of pigs on certain feeds was slower than with combinations of other feeds in the absence of micronutrient deficiencies, this could be related to the amount and quality of the nitrogenous component (protein) and amino acid content present in the various feedstuffs (31). Utilizing rats, Osborne and Mendel (19) reported that nutritive failure occurred when certain protein sources were fed alone; such nutritive failure could be remedied by addition of missing amino acids to such diets either from intact proteins or specific amino acids. From such studies, the idea of protein supplementation emerged, which has been widely applied to this day in diet formulation in animal production systems (31).

It had already been established that urea and uric acid were the endproducts of N metabolism in mammals and avian species, respectively (2,4). In addition, the use of N-free diets had been introduced in nutrition studies, and methods of protein isolation, characterization, and amino acid analysis emerged. Thus, as the 20th century dawned, biochemists and nutritionists had a set of experimental procedures (N balance, N-free diet, purified and chemically defined diets) accompanied by emerging and established analytical methods (such as protein isolation and characterization, N analysis, urea-N analysis) (32) to determine amino acid content of proteins, to investigate protein/N requirements for growth and maintenance of farm animals, rodents,

2 Abbreviations used: DAAO, direct amino acid oxidation method; EAA, essential amino acids; IAAO, indicator amino acid oxidation method; PAA, plasma amino acid concentrations.
TABLE 1  A timeline of protein metabolism and nutrition research issues and accomplishments during the 18th, 19th, 20th, and 21st centuries\(^1,2\)

<table>
<thead>
<tr>
<th>Century</th>
<th>Issues and accomplishments</th>
<th>Contributions by farm animals</th>
</tr>
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<tbody>
<tr>
<td>18th</td>
<td>Gaseous nitrogen not involved in animal N metabolism, N necessary in diet.</td>
<td>X</td>
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<tr>
<td>19th</td>
<td>Development and refinement of organic chemistry, early protein isolation and characterization, nitrogen balance concept and wide application. Emergence of theories of protein metabolism.</td>
<td></td>
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<tr>
<td>20th</td>
<td>Single-ingredient diets, N or nutrient lack. Concept of supplementation by mixing feeds. Protein quality, identifying essential and nonessential amino acids</td>
<td>X</td>
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<tr>
<td></td>
<td>Protein and amino acid requirements; growth studies, factorial/N balance methods. Limiting amino acid concept.</td>
<td>X</td>
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<tr>
<td></td>
<td>Amino acid imbalance; amino acid antagonism.</td>
<td>X</td>
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<td></td>
<td>Dynamics of protein metabolism-turnover, use of isotopes</td>
<td>X</td>
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<tr>
<td></td>
<td>Protein [energy] malnutrition; protein synthesis mechanism, Uterine and fetal nutrition</td>
<td>X</td>
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<tr>
<td></td>
<td>Application of plasma amino acids in assessment of protein status; indicator amino acids. In vivo tracer kinetics to study protein synthesis and degradation</td>
<td>X</td>
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<tr>
<td></td>
<td>Further refinement in protein/amino acid requirement studies as applied to humans; direct and indicator amino acid methods</td>
<td>X</td>
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<tr>
<td></td>
<td>*Amino acids as regulatory and signal transducer molecules.</td>
<td>X</td>
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<td></td>
<td>Pediatric protein metabolism; role of gut, muscle, and liver development</td>
<td>X</td>
</tr>
<tr>
<td>21st</td>
<td>Pharmacology of excess amino acid intakes, Amino acid nutrigenomics; use of animal models</td>
<td>X</td>
</tr>
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1 Historical record of contribution specific to the issue by farm animals (left column, same line) is marked by X in the right column.
2 This table is an overview, and space limitations prevented direct citations for each specific contribution by farm animals; however, many specific contributions are discussed in the text with appropriate citations.

and people, to determine dietary essentiality of amino acids, and to assess the role of the amino acid profile in nutritive quality of proteins. Such efforts were the main focus of N nutrition research until emphasis slowly changed from nutritional explorations to increased studies of mechanisms of protein synthesis and breakdown as illustrated by the work on the dynamics of body constituents by Schoenheimer (14).

Starting in the 19th century, proteins [as named by Mulder (2)] were more frequently isolated and purified, and their composition was analyzed. What eventually emerged was that proteins are comprised of some 20 amino acids (4). Thus, it became important to understand which of these 20 amino acids are dietary essentials or nonessentials. By the late 1930s, principally based on work by Rose and coworkers (4,31) at the University of Illinois, Thr, Met, Val, Ile, Leu, Phe, Trp, Lys, His, and Arg had been established as the essential amino acids (EAA, now often referred to as indispensable); this list may be modified by special needs of certain species and physiological states. Somewhat later the EAA for humans were established by Rose with N balance studies using defined diets and purified amino acids (20). EAA were established concomitantly by growth studies and N balance studies in pigs and chickens (33,34).

Agricultural researchers pioneered rapid application of EAA knowledge to diet formulations of food animals. Through empirical feeding studies, N balance trials, and later using indirect response criteria such as plasma amino acids, combined with amino acid content analyses of feeds and protein supplements (21–24), the first, second, and sometimes third limiting amino acids were established. From this then developed wide application of specific supplementation schemes and use of pure amino acids to balance diets for pigs, chickens, turkeys, and farm-raised fish during production. As recognized for many years (35), whenever significant quantities of dairy foods and other animal products are in the diet, protein requirement data (36) may not be directly relevant to dietary practices; however, EAA requirement data are critical in medical research with current priorities on EAA needs during pregnancy, during illnesses such as infectious diseases, during recovery from malnutrition, and during excessive consumption of amino acids (37). From a broader perspective, newly revised AA requirements based on AA oxidation techniques developed in farm animals (see below) will play a vital role in food security planning and public health policy at national and international levels.

Empirical growth trials to establish amino acid requirements are costly with food animals because growth or product yields by animals involved in N balance studies are typically lower than expected under usual production conditions. Experimental protocols that allowed high productivity and nutritional assessment criteria that would not modify production rates were therefore needed. This necessitated development of indirect approaches, and plasma amino acid concentrations (PAA) emerged as useful indicators of protein/amino acid status under well-controlled experimental conditions (22,38,39). The use of PAA was embraced by workers studying farm animals, and extensive work followed in the late 1960s through the 1970s to test and refine the application of PAA as response criteria for amino acid nutrition in pigs, chickens, and ruminants (21–24,27). Growth, N balance, and PAA breakpoint curves were studied in combination, and it was established that under tightly controlled experimental conditions, PAA responses may be used directly in protein status assessment of production animals as shown in Figure 2 (21–24,27). Concurrently PAA-based response indices were used by nutrition workers addressing endemic childhood malnutrition (40).

The concept of PAA as a response criterion of EAA adequacy was then extended to metabolic approaches (25–27,41). When
an EAA is limiting performance, a major fraction of this amino acid is used for protein synthesis with only slight oxidation to carbon dioxide. Raising the dietary supply of this amino acid above its requirement resulted in increased deamination and oxidation to carbon dioxide and decreased oxidation of other EAA (25). These observations led to the development by Guelph workers (led by Henry Bailey) of the indicator amino oxidation method (27,41). Such work with animal models helped lay the foundation to what ultimately evolved as the DAAO (Fig. 2) and IAAO (Fig. 2) methods to determine amino acid needs in humans as most notably employed by Young and Pencharz and Ball and their respective coworkers (42–44). The DAAO and IAAO have been extended to study amino acid needs in infants (42–44), sheep (42), young pigs receiving diets with varying levels of lysine or threonine, and for uterine and fetal nutrition research (47–49), for studies in pediatric protein nutrition and amino acid dynamics (44,45,49), and for assessments of the effect of excessive amino acid intakes in young, growing, and mature animals (7,37).

**Literature Cited**