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Proline and hydroxyproline metabolism: implications for animal and human nutrition

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Abstract

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Proline plays important roles in protein synthesis and structure, metabolism (particularly the synthesis of arginine, polyamines, and glutamate via pyrroline-5-carboxylate), and nutrition, as well as wound healing, antioxidative reactions, and immune responses. On a pergram basis, proline plus hydroxyproline are most abundant in collagen and milk proteins, and requirements of proline for whole-body protein synthesis are the greatest among all amino acids. Therefore, physiological needs for proline are particularly high during the life cycle. While most mammals (including humans and pigs) can synthesize proline from arginine and glutamine/glutamate, rates of endogenous synthesis are inadequate for neonates, birds, and fish. Thus, work with young pigs (a widely used animal model for studying infant nutrition) has shown that supplementing 0.0, 0.35, 0.7, 1.05, 1.4, and 2.1% proline to a proline-free chemically defined diet containing 0.48% arginine and 2% glutamate dose dependently improved daily growth rate and feed efficiency while reducing concentrations of urea in plasma. Additionally, maximal growth performance of chickens depended on at least 0.8% proline in the diet. Likewise, dietary supplementation with 0.07, 0.14, and 0.28% hydroxyproline (a metabolite of proline) to a plant protein-based diet enhanced weight gains of salmon. Based on its regulatory roles in cellular biochemistry, proline can be considered as a functional amino acid for mammalian, avian, and aquatic species. Further research is warranted to develop effective strategies of dietary supplementation with proline or hydroxyproline to benefit health, growth, and development of animals and humans.

Keywords

Proline; Nutrition; Biochemistry; Health; Growth

Introduction

Proline and its metabolite (hydroxyproline) are unique amino acids (AA) both chemically and biochemically (Hu et al. 2008; Kaul et al. 2008). They constitute one-third of AA in the collagen proteins which comprise approximately 30% of body proteins. On a per-gram basis, the requirement of proline for whole-body protein synthesis is the highest among all AA. However, the current edition of Nutrient Requirements of Swine (NRC 1998) concluded that L-proline (the physiological isomer) is not needed in the diets for gestating, neonatal, growing, finishing pigs or boars to achieve their maximal production performance. Likewise, the National Research Council (1998) does not provide data on proline contents in feed ingredients commonly used to formulate swine diets. Furthermore, proline is not considered a nutritionally essential or conditionally essential AA for humans without burns or injury (Elango et al. 2009). This is very unfortunate and reflects a lack of knowledge about proline biochemistry and nutrition in mammals.

Recent years have witnessed increasing interest in research on proline metabolism and nutrition (Phang et al. 2010; Srivastava et al. 2010; Wang et al. 2009a; Watford 2008), as well as metabolic diseases (Pérez-Arellano et al. 2010). Growing evidence shows that proline is a key regulator of multiple biochemical and physiological processes in cells. For example, proline is a signaling molecule, a sensor of cellular energy status, and a source of pyrroline-5-carboxylate (P5C) and superoxide anion (a free radical) participating in redox reactions in humans and animals (Phang et al. 2008, 2010). Additionally, proline plays an important role in differentiation of cells (including embryonic stem cells; Pistollato et al. 2010), as well as conceptus (fetus and associated extraembryonic membranes) growth and development (Wu et al. 2008). Furthermore, the seminal discovery that proline serves as a major AA for the synthesis of polyamines (key regulators of DNA and protein synthesis, as well as cell proliferation and differentiation) in the small intestine (Wu et al. 2000) and placenta (Kwon et al. 2003a; Wu et al. 2005) led to reconsideration of its crucial role in fetal and neonatal nutrition (Wu 2009). New developments in proline metabolism are shaping the

science and practice of animal and human nutrition. The major objective of this article is to review recent advances in this exciting area of AA research.

Chemical structures and functions of proline and hydroxyproline

Proline and hydroxyproline contains an α-imino group and, therefore, they are α-imino acids (Fig. 1). However, because proline is a substrate for protein synthesis like a-AA and hydroxyproline is its post-translational metabolite, they are loosely referred to as AA in biochemistry. Proline and hydroxyproline are major AA in the collagen proteins which contain three chains of polypeptides (two α1 chains and one α2 chain) and are major extracellular components in connective tissues (e.g., skin, tendon, cartilage, vessels of the vascular system, and bone). The helical region of collagen comprises the repeat of Gly–X–Y, where proline can be in the X or Y position and hydroxyproline occurs only in the Y position (Krane 2008). The unique ring structure of proline and hydroxyproline distinguishes them from other AA in terms of rigidity, chemical stability, and biochemical reactions.

Proline residues in the collagen proteins can be hydroxylated in the endoplasmic reticulum by collagen prolyl 4-hydroxylase or prolyl 3-hydroxylase in the presence of oxygen, ascorbic acid, α-ketoglutarate, and Fe^{2+} (Gorres and Raines 2010). The ratio of 4-hydroxyproline to 3-hydroxyproline in collagen proteins is approximately 100:1. Other prolyl 4-hydroxylases, including hypoxia-inducible transcription factor α, act on non-collagen proteins (Krane 2008). The hydroxylation of proline is a post-translational event because the hydroxylated residues appear after the collagen polypeptides are synthesized. This reaction also occurs in other proteins to regulate cellular oxygen sensing and physiological responses to hypoxia (Chandel 2010; Wenger and Hoogewijs 2010). Free hydroxyproline and 3-hydroxyproline are generated from the degradation of collagens or other proteins containing 4-hydroxylprolyl and 3-hydroxylprolyl residues, respectively (Phang et al. 2008, 2010).

Proline plays versatile roles in cell metabolism and physiology (Table 1). For example, proline is a major nitrogenous substrate for the synthesis of polyamines in the small intestine of neonatal pigs (Wu et al. 2000) as well as the placentae of gestating pigs (Wu et al. 2005) and sheep (Wu et al. 2008). This discovery is significant because both tissues are characterized by high rates of protein synthesis and cell proliferation. Pathways exist for the synthesis of polyamines from proline via proline oxidase and ornithine decarboxylase (Wu et al. 2005, 2008). Additionally, proline and its metabolite (P5C) are now known to regulate gene expression and cellular signaling pathways that are crucial to health and disease (Hu et al. 2008). Interestingly, proline can scavenge free radicals (Kaul et al. 2008), and this antioxidant property of proline may explain why its concentrations increase markedly in response to cellular oxidative stress (Verbruggen and Hermans 2008). Furthermore, results of recent findings suggest that proline may play a role in regulating the mammalian target of rapamycin (mTOR) activation pathway (van Meijl et al. 2010), which integrates signals from nutrients (glucose and AA), cellular energy status, growth factors, and various stress factors to affect cell growth and function (Li et al. 2009b; Liao et al. 2008). Therefore, proline acts in concert with arginine, glutamine, and leucine (activators of mTOR and regulators of polyamine production) to enhance protein synthesis in cells and tissues (e.g., the small intestine and skeletal muscle) (Wu et al. 2010a).

Although hydroxyproline has been traditionally considered to have little nutritional significance, it is now recognized as a substrate for the synthesis of glycine (an essential AA for chickens), pyruvate, and glucose (particularly important for neonates and ruminants) (Fig. 2). Hydroxyproline may also scavenge oxidants and regulate the redox state of cells

(Phang et al. 2008, 2010). Furthermore, hydroxyproline may greatly impact the nutrition of birds which cannot sufficiently synthesize glycine from other AA.

Developmental changes in proline and hydroxyproline in body proteins and physiological fluids

Concentrations of proline and hydroxyproline in tissue proteins increase markedly during fetal growth and development. For example, in the fetal pig, hydroxyproline increases from 0.84 to 3.64 g per 100 g protein between days 40 and 114 (term) of gestation (Wu et al. 1999). Proline and hydroxyproline account for 12% (wt/wt) of proteins in the animal body at birth and postnatally, with the ratio of proline to hydroxyproline being 2.25:1. Thus, 31% of protein-bound proline is hydroxylated after the polypeptide is synthesized. This indicates that requirements of proline for protein accretion substantially increase during both prenatal and postnatal periods (Wu et al. 2010b).

There are also marked changes in concentrations of free proline in the conceptus during pregnancy (Wu et al. 1995a, 2008). For example, between days 30 and 60 of gestation, concentrations of proline in porcine amniotic fluid increase by 141%, but those in porcine allantoic fluid decrease by 82%. Similarly, concentrations of proline in ovine amniotic fluid increased from 89 to 172 μM between days 30 and 60 of pregnancy (Kwon et al. 2003a). Interestingly, in contrast to gilts, concentrations of proline in ovine allantoic fluid increased by 172% during the same period. This reflects the differences in proline metabolism and requirements among animals or during the life cycle within the same species.

Proline metabolism

Proline synthesis

Proline synthesis from glutamine, glutamate, arginine, and ornithine in animals is cell-, tissue-, and species-specific (Wu et al. 2008). All mammals can synthesize proline from arginine via arginase (both type I and type II), ornithine aminotransferase, and P5C reductase, with the mammary tissue, small intestine (postweaning animals), liver, and kidneys being quantitatively the most active tissues (Wu et al. 2008). In mammary tissue, the major products of arginine catabolism are proline, ornithine, and urea (O'Quinn et al. 2002). Because proline oxidase activity is absent from mammary tissue, there is no degradation of arginine-derived proline in the lactating gland. This ensures maximal net production of proline from arginine by the lactating mammary gland. Because P5C synthase is also absent from mammary tissue, there is no formation of proline from glutamine or glutamate by this tissue (O'Quinn et al. 2002). Thus, arginase plays an important role in proline synthesis by lactating mammary tissue. Interestingly, the activity of P5C reductase is at least 50-fold greater than that of P5C dehydrogenase in lactating mammary tissue, thereby favoring the conversion of arginine-derived P5C into proline rather than into glutamate and glutamine (O'Quinn et al. 2002). The synthesis of proline from arginine helps to prevent an irreversible loss of arginine carbons in lactating porcine mammary tissue. These findings also provide a biochemical explanation for the observation that the output of proline in sow's milk greatly exceeds the uptake of plasma proline by the lactating mammary gland, whereas the output of arginine in sow's milk is much lower than the uptake of plasma arginine by the lactating mammary gland (Wu and Morris 1998). Because of extensive catabolism of arginine for proline synthesis via the arginase pathway and the lack of proline catabolism in lactating porcine mammary tissue, there is a relative enrichment of proline but a relative deficiency of arginine in milk proteins (Davis et al. 1994; Wu and Knabe 1994).

The small intestine of postweaning pigs degrades approximately 40% of arginine in the enteral diet, with proline being a major product (Wu et al. 2007a, b). Additionally, both

glutamate and glutamine in the enteral diet are almost completely degraded by the small intestine, with proline being a significant product (Reeds and Burrin 2001; Watford 2008). In the postabsorptive state, one-third of glutamine in arterial blood is extracted by the pig small intestine (Wu et al. 1994). Products of glutamate and glutamine degradation in enterocytes include not only proline but also ornithine, citrulline, arginine, and alanine (Wu et al. 1995b). Studies with jejunum-cannulated young pigs demonstrated net release of proline from the small intestine of food-deprived piglets (Wu et al. 1994). De novo synthesis and the hydrolysis of small peptides in enterocytes and the intestinal lumen may be sources of this gut-derived proline. Glucocorticoids are major hormones that regulate proline synthesis from arginine and glutamine in cells and tissues (Flynn et al. 2009).

In contrast to mammals, birds have low arginase activity in tissues and, therefore, a limited ability to convert arginine into proline (Austic 1976). Therefore, proline is a nutritionally essential AA for avian species, including chickens (Graber and Baker 1971). Additionally, carnivores (e.g., cats and ferrets) lack P5C synthase in enterocytes and other cell types, and cannot convert glutamine and glutamate into proline in the body (Wu and Morris 1998). Thus, arginine is the only substrate for proline synthesis in these species. Owing to a high demand for dietary arginine for multiple synthetic processes and the lack of its endogenous synthesis, arginine is a nutritionally essential AA for carnivores. Dietary supplementation with proline may compensate for some arginine in these animals due to an inhibition of arginase by proline-derived ornithine.

Proline degradation

Except for mammary tissue, most tissues express proline oxidase activity (Wu et al. 2008). A byproduct of this mitochondrial enzyme is superoxide anion (O_2^-), which can be converted into H_2O_2 and other reactive oxygen species (Phang et al. 2008). In tissues and cells (e.g., porcine placenta and enterocytes of neonatal pigs) that do not contain arginase activity, proline is the only substrate for the synthesis of ornithine and, therefore, polyamines (putrescine, spermidine, and spermine) (Wu et al. 2000, 2005). This is of enormous importance in both nutrition and physiology because (1) polyamines are key molecules regulating DNA and protein synthesis, as well as cell proliferation, differentiation, and migration; (2) both placentae and neonatal small intestine grow very rapidly. In ruminants, placentae contain both arginase and proline oxidase, which helps to compensate for relatively low concentrations of proline in maternal blood (Kwon et al. 2003b).

Although all cells can recycle P5C into proline by P5C reductase and convert P5C into ornithine by ornithine aminotransferase, the utilization of P5C for the synthesis of citrulline is highly cell- and tissue-specific (Wu et al. 2008). Of particular note, only mammalian enterocytes are capable of synthesizing citrulline from P5C, indicating a unique role for the small intestine in proline metabolism (Wu et al. 2009). Although the mammalian liver can convert P5C into ornithine via the urea cycle, there is no net synthesis of arginine in this organ because exceedingly high arginase activity rapidly hydrolyzes arginine into ornithine and urea (Wu and Morris 1998). In liver and kidneys, P5C can be oxidized completely to CO_2 via the formation of □-ketoglutarate by P5C dehydrogenase. However, in placentae and enterocytes with limited P5C dehydrogenase activity, oxidation of proline to CO_2 is negligible (Chen et al. 2009; Wu et al. 2000, 2005). This prevents an irreversible loss of proline carbons and maximizes the availability of P5C for the synthesis of polyamines. Compelling evidence shows that polyamines play an important role in intestinal growth, function, and health during the neonatal period (Wu et al. 2000).

Because the entire molecule of P5C is incorporated into citrulline via ornithine aminotransferase and ornithine carbamoyltransferase in enterocytes, proline provides its

nitrogen and carbon skeleton for citrulline and arginine synthesis in the small intestine which expresses these two enzymes and P5C synthase (Wu and Morris 1998). A lack of knowledge or misunderstanding of these basic biochemical reactions can lead researchers to make erroneous conclusions regarding the contribution of proline carbons to endogenous synthesis of arginine. Such errors, which have recently occurred with glutamine studies, will surely not advance the field of mammalian arginine metabolism but rather will result in much misleading confusion in literature.

The arginine–proline cycle between mother and neonate

As noted previously, arginine is actively utilized to form proline in the lactating mammary gland, resulting in a deficiency of arginine and an abundance of proline in milk protein relative to needs by neonates (Wu et al. 2004). Concentrations of free arginine in milk are also relatively low (Wu and Knabe 1994). It is possible that arginine is a major factor limiting maximal milk production by mammals (Mateo et al. 2008), but much experimental data are required to test this hypothesis. Interestingly, milk-derived proline is a major precursor for the synthesis of citrulline (the precursor of arginine) in enterocytes of postnatal pigs (Dillon et al. 1999; Wu 1997). Thus, there is an arginine–proline “cycle” between mother and neonate. Although intestinal synthesis of citrulline and arginine partially compensates for an arginine deficiency in sow’s milk, one must wonder why there is extensive catabolism of arginine by the lactating mammary gland? There are several possible answers to this intriguing question. First, the uptake of proline from maternal blood by the lactating mammary gland may be inadequate for milk protein synthesis. Thus, arginine catabolism may be necessary to provide sufficient proline for maximizing protein synthesis by the lactating porcine mammary gland. Second, through the NADPH-dependent conversion of P5C into proline, arginine may regulate the cellular redox state and pentose cycle activity. The pentose cycle functions to provide NADPH and ribose-5-phosphate for a variety of metabolic processes. For example, NADPH is required for fatty acid synthesis, whereas ribose-5-phosphate is essential for purine synthesis and cell proliferation. This notion is consistent with the finding that dietary arginine supplementation to sows increases production of milk lipids (Kirchgessner et al. 1991) and piglet growth (Mateo et al. 2008). Third, arginine is the common substrate for both arginase and nitric oxide synthase, and thus, arginase may play an important role in regulating nitric oxide and polyamine synthesis by the lactating mammary gland. Although nitric oxide is quantitatively a minor product of arginine catabolism, it may play a crucial role in the regulation of mammary gland blood flow and thus the uptake of nutrients from blood by the lactating mammary gland (Kim and Wu 2009). Likewise, polyamines produced by mammary tissue regulate lactogenesis (Oka and Perry 1974) and greatly contribute to their abundance in sow’s milk (Motyl et al. 1995). There is little arginase activity in the small intestine of neonates, and yet, polyamines are essential for cell proliferation and differentiation (Wu 1998). Thus, milk-borne polyamines may be of nutritional importance for growth and development of the neonatal intestine. Finally, because the neonatal pig has a low capacity to synthesize proline (an essential AA for young pigs) (Ball et al. 1986), arginine catabolism via the arginase pathway in the lactating mammary gland will ensure an adequate supply of proline to suckling piglets to support tissue protein synthesis and extracellular matrix formation. Thus, through the arginine–proline cycle between mother and neonate, the mother sustains a capacity for milk synthesis and provides both proline and polyamines to her offspring, whereas the neonate can synthesize arginine and have both exogenous and endogenous polyamines required for protein synthesis and cell growth (Wu et al. 2009).

Proline and hydroxyproline nutrition

Composition of proline and hydroxyproline in postnatal animals and feedstuffs

The content of proline and hydroxyproline is 12 and 5.3 g/kg wet tissue in young pigs, respectively (Wu et al. 2010b). Proline represents 12% of proteins in the milk of mammals, including sows and cows (Davis et al. 1994; Wu et al. 1994). Indeed, proline is the most abundant AA in milk proteins (e.g., 30 g/kg dry matter in sow's milk), followed by glutamate, leucine, and glutamine (Haynes et al. 2009; Kim and Wu 2004). The abundance of proline in milk protein is consistent with a high requirement of proline for neonatal growth and development (Wu et al. 2010b). Notably, the content of proline is even higher in meat and bone meal, and poultry by-product meal than in milk (Table 2). Indeed, proline and hydroxyproline are most abundant in meat and bone meal, poultry by-product meal, and salmon proteins. Therefore, these animal products are excellent sources of proline and hydroxyproline for post-weaning animals and post-hatching birds. In general, animal proteins contain 3–6-fold greater amounts of proline than plant proteins per gram of feedstuff. Among plant proteins, proline is the second most abundant AA in barley, wheat, and wheat middlings, and the third most abundant AA in corn (grain) and sorghum (Table 2).

Digestion and absorption of proline

The circulating proline in plasma is derived from the diet, intracellular protein degradation, extracellular protein degradation, and endogenous synthesis (Wu et al. 2008). Peptide-bound proline is hydrolyzed by proteases in the luminal fluids of the stomach (pepsin) and small intestine (trypsin, chymotrypsin, elastase, carboxypeptidases A and B, and aminopeptidase) to yield dipeptides or tripeptides (Wu and Self 2005) (Fig. 3). The mucosa of the small intestine secretes proline peptidase (prolidase) that specifically hydrolyzes proline-containing dipeptides (Sjostrom et al. 1973). Prolidase also hydrolyzes hydroxy-proline-containing dipeptides. These small peptides (containing proline or hydroxyproline) in the lumen of the small intestine can be directly transported into enterocytes (absorptive epithelial cells) by H^+ gradient-driven peptide transporters, whereas free proline is taken up into cells primarily by the Na^+-dependent system IMINO transporter and system NBB transporter (present on the brush border for the transport of neutral AA), as well as the Na^+-independent system L transporter (Brandsch 2006). The entry of proline into enterocytes represents the first step for its utilization as an essential substrate for synthetic pathways (including synthesis of proteins and ornithine). Because proline is extensively degraded by enterocytes and luminal microorganisms (Bergen and Wu 2009), only ~60% of dietary proline enters the portal circulation (Wu et al. 2008).

Proline as a nutritionally essential AA for poultry, young mammals, and fish

There are remarkable differences in proline metabolism among species. Proline is a nutritionally essential AA for poultry (Baker 2009; Graber et al. 1970), young mammals (including piglets) (Ball et al. 1986), as well as wounded animals and humans (Barbul 2008) because of inadequate endogenous synthesis via the arginase and P5C synthase pathways relative to needs. Additionally, endogenous synthesis of proline from glutamate cannot meet the requirements for proline by many species of fish (Li et al. 2009a). Therefore, proline is now considered as a conditionally essential AA for fish in both early life and adult stages (Li et al. 2009a; Zhang et al. 2006). Interestingly, dietary supplementation with 0.07, 0.14, and 0.28% hydroxyproline (a metabolite of proline) to a plant protein-based diet enhanced weight gains of salmon (Aksnes et al. 2006). It is possible that hydroxyproline may spare proline by reducing proline catabolism or stimulate tissue protein synthesis through multiple signaling pathways.

The essential requirement for proline as a nutrient for poultry, young mammals, and wounded subjects is supported by several lines of experimental evidence. First, supplementing 0.0, 0.2, 0.4, and 0.8% proline to a chemically purified diet containing 1% arginine and 10% glutamate dose-dependently increased daily weight gains (from 11.88 to 13.38 g/day) of young chickens without affecting their feed intake (an average of 114 g/chick) (Fig. 4). Second, supplementing 0, 0.35, 0.7, 1.05, 1.4, and 2.1% proline to a proline-free chemically defined diet containing 0.48% arginine and 2% glutamate dose-dependently improved daily weight gains (from 342 to 411 g/day) (Fig. 5) and feed efficiency (gram feed/gram gain, from 1.66 to 1.35) of young pigs, while reducing concentrations of urea in plasma by one-half (Kirchgessner et al. 1995). Notably, increasing the dietary content of proline from 0.0 and 2.1% enhanced daily nitrogen retention from 1.27 to 1.53 g/kg bodyweight$^{0.75}$. Similarly, we found that supplementing 1% proline to a corn- and soybean meal-based diet enhanced villus height, small-intestinal weight, and growth performance in weanling pigs (Table 3). Third, dietary proline is necessary for promoting tissue repair and nitrogen balance in both animals and humans with wounds and burns (Barbul 2008). These findings have important implications for proline as a dietary essential nutrient in humans and animals under certain physiological and pathological conditions.

It should be borne in mind that effects of dietary supplementation with any AA depend on its supplemental dose and the content of other AA in the diet (Ferreira et al. 2010; Stipanuk et al. 2009; Tan et al. 2009a, b). For example, when the basal diet contained no glutamine, proline supplementation had no effect on piglet growth (Chung and Baker 1993). Thus, optimal nutritional conditions for proline supplementation must be identified to realize its potential for improving growth and reproductive performance of animals. In this regard, supplemental proline may play an important role in fetal survival, growth, and development (Wu et al. 2008). Because of its regulatory roles in metabolism and physiology, proline can be considered as a functional AA in nutrition (Li et al. 2007; Wu et al. 2007b). We must recognize that the traditional view that proline is a nonessential AA for the adult mammal is solely based on nitrogen balance studies. Whether proline is a nutritionally essential AA for animals should be reevaluated through careful design of experiments and use of meaningful criteria (including functional needs such as maximal growth performance, fertility, embryonic/fetal survival and growth, and immunity) (Wu 2009).

Conclusion and perspectives

Proline and hydroxyproline are unique AA for maintaining cell structure and function. There are remarkable species differences in proline metabolism and requirements among vertebrate animals during the life cycle. However, emerging evidence consistently points to proline as an important regulator of cell metabolism and physiology. Therefore, proline can be considered as a functional AA for humans, livestock species, poultry, and fish. This promising role of proline is expected to be translated into enhanced efficiency of nutrient utilization and improved health in organisms. Although much is known about proline needs for wound healing in humans (Barbul 2008), there is a paucity of information about roles for proline in growth and development of the fetus and neonate, as well as lactation performance of mothers. Indeed, there are few experimental data for the essentiality of requirements of dietary proline by mammals. Given the significant problem of intrauterine growth retardation (IUGR) in both humans and livestock species (Wu et al. 2004, 2006), it is surprising that there are no data in literature regarding (1) impacts of IUGR on postnatal proline metabolism and dietary requirements in offspring; (2) a potential role for alterations of IUGR-associated proline metabolism in the pathogenesis of chronic disease (e.g., hypertension, cancer, obesity, and diabetes) in adults; or (3) effects of supplementing proline to IUGR offspring on their growth and health. These questions can be addressed using animal models, such as pigs (Li et al. 2010; Rhoads and Wu 2009; Suryawan et al. 2009),

sheep (Reynolds et al. 2006; Satterfield et al. 2010; Wang et al. 2010), and rodents (Blachier et al. 2010; Zeng et al. 2008). Additionally, multidisciplinary research is necessary to identify optimal conditions for dietary supplementation with proline to mammals (particularly neonates with IUGR), birds, and fish to improve their health, reproductive performance, growth, and development. Such endeavors can be greatly facilitated by using recent advanced tools, such as genomics (Jobgen et al. 2009; Palii et al. 2009), transcripteomics (Mutch et al. 2005), proteomics (Wang et al. 2009b), metabolomics (He et al. 2009), and bioinformatics (Fu et al. 2010). Collectively, elucidation of the complex mechanisms responsible for the actions of proline on cells is expected to expand its applications to solve major practical problems in livestock production and human medicine.

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Abbreviations

AA	Amino acid
IUGR	Intrauterine growth retardation
mTOR	Mammalian target of rapamycin
NRC	National Research Council
P5C	Pyrroline-5-carboxylate

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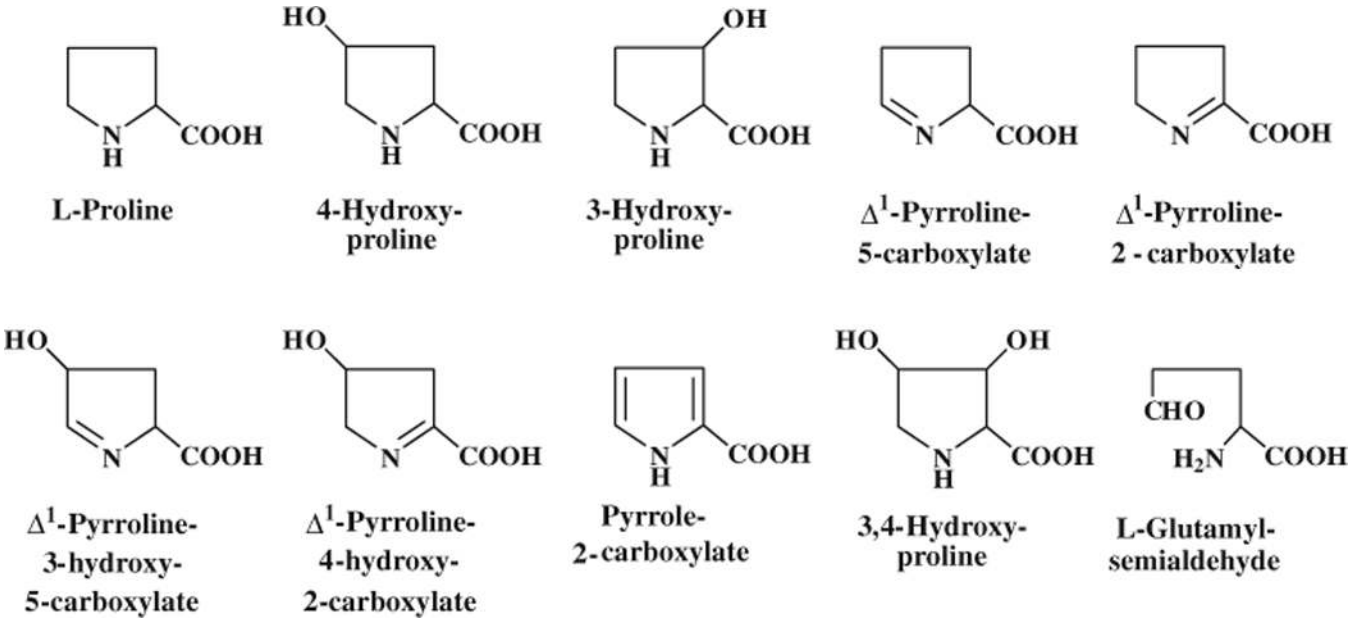

Fig. 1.

Structures of proline and related metabolites. All of these proline metabolites occur in animals, with 4-hydroxyproline being the most abundant

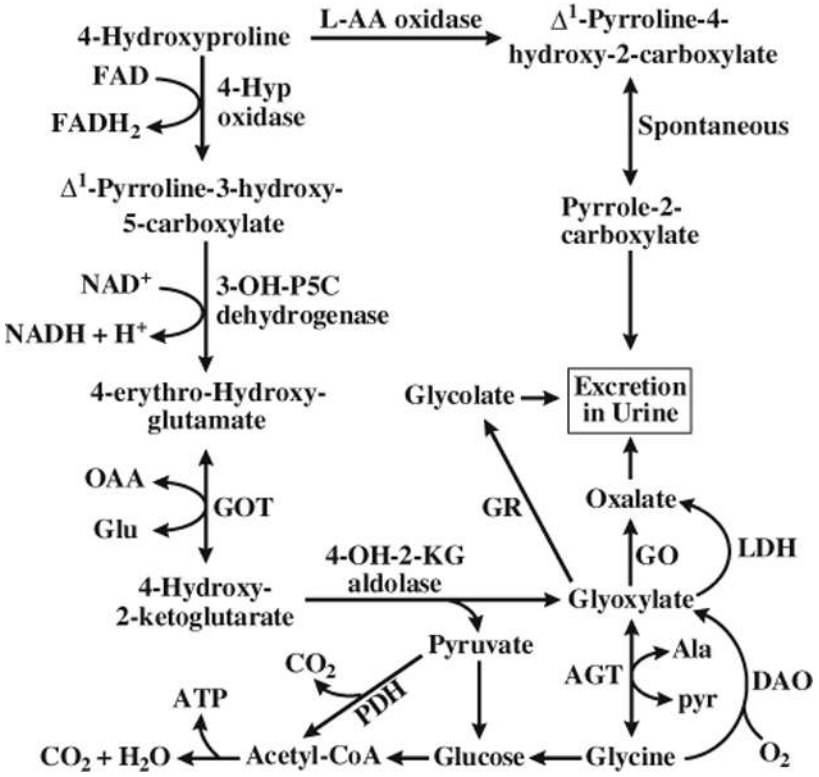

Fig. 2.

Metabolism of 4-hydroxyproline in animals. AA amino acid, AGT alanine-glyoxylate aminotransferase, *Ala* alanine, *DAO* D-amino acid oxidase, *Glu* glutamate, *GO* glycolate oxidase, *GOT* glutamate oxaloacetate transaminase, *GR* glyoxylate reductase, *4-Hyp* 4-hydroxy-proline, *4-OH-2-KG* 4-hydroxy-2-ketoglutarate, *3-OH-P5C* Δ1-pyrro-line-3-hydroxy-5-carboxylate, *LDH* lactate dehydrogenase, *OAA* oxaloacetate, *PDH* pyruvate dehydrogenase, *Pyr* pyruvate

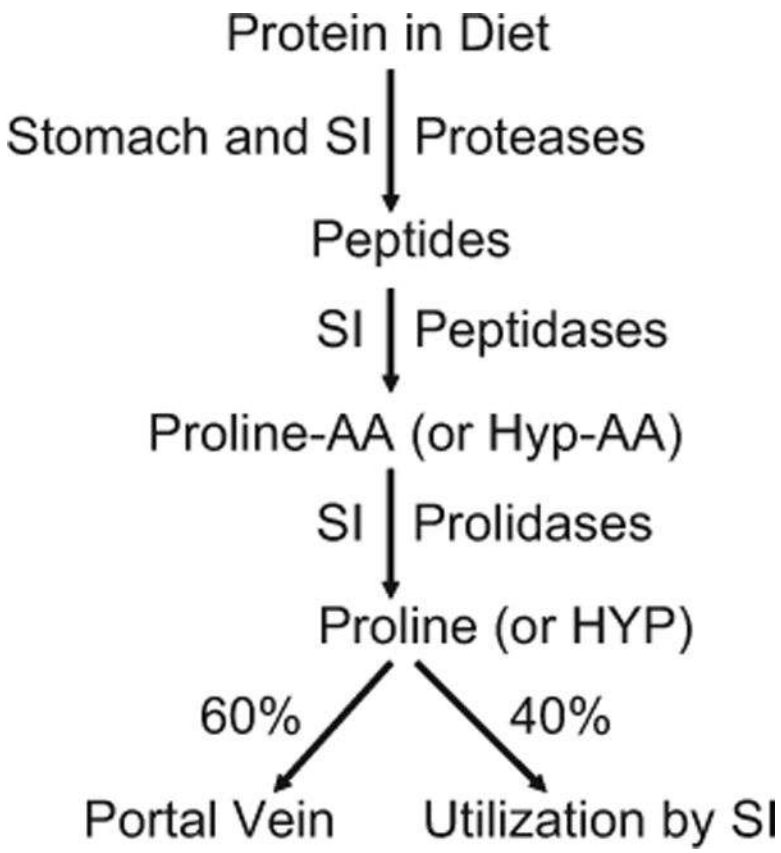

Fig. 3.

Digestion of dietary protein-bound proline or hydroxyproline (*Hyp*) in the gastrointestinal tract of the small intestine. Proteases and peptidases in the lumen of the small intestine hydrolyze proteins and large peptides, respectively, to eventually form proline- or hydroxyproline-containing dipeptides. These dipeptides are hydrolyzed by specific proline peptidases (prolidases) to yield free proline or hydroxyproline. Approximately 40% of luminal proline is catabolized by the mammalian small intestine, and the responsible cell types include enterocytes (Wu 1997) and bacteria (Dai et al. 2010). *SI* small intestine

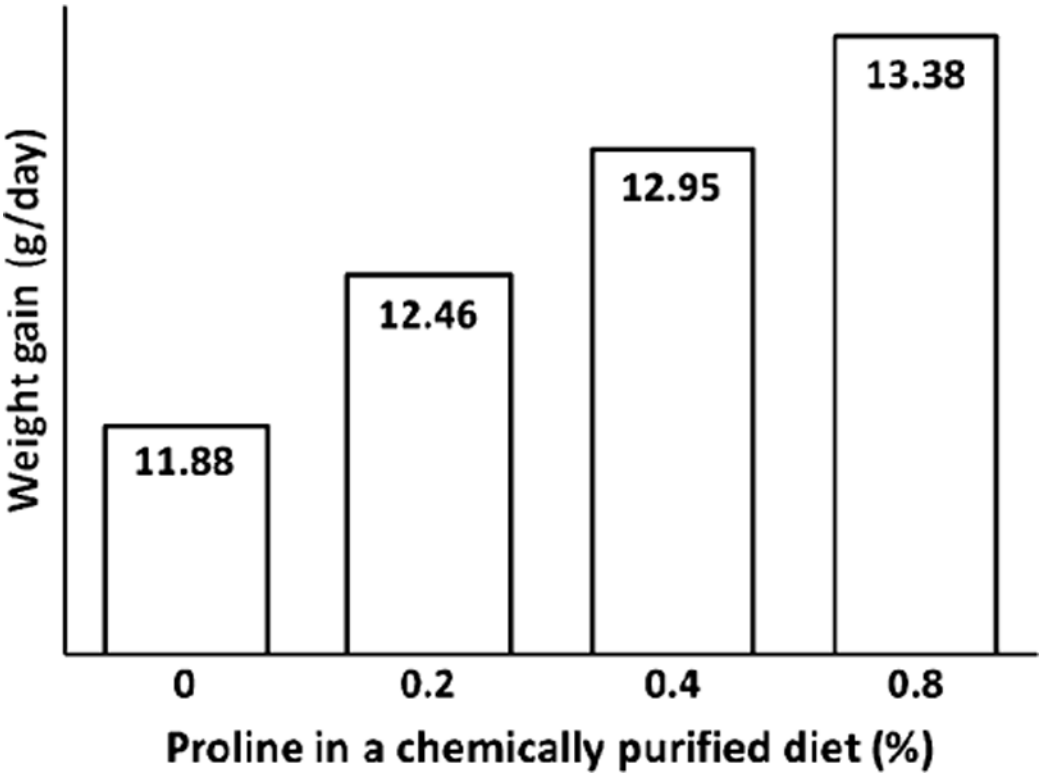

Fig. 4.

Effect of supplementing proline to a chemically purified diet on growth performance of young chickens. Data are taken from Graber et al. (1970). Chicks (the cross of New Hampshire males to Columbian females) were fed a purified diet supplemented with 0, 0.2, 0.4, and 0.8% L-proline for 6 days. The basal diet contained the following amino acids (% of diet): L-arginine–HCl, 1.21; L-histidine–HCl·H_2O, 0.41; L-lysine–HCl, 1.19; L-tyrosine, 0.45; L-tryptophan, 0.15; L-phenylalanine, 0.50; DL-methionine, 0.35; L-cystine, 0.35; L-threonine, 0.65; L-leucine, 1.20; L-isoleucine, 0.60; L-valine, 0.82; glycine, 1.20; and l-glutamate, 10.00. Supplementing L-proline to the basal diet resulted in a linear increase ($P < 0.01$) in weight gain

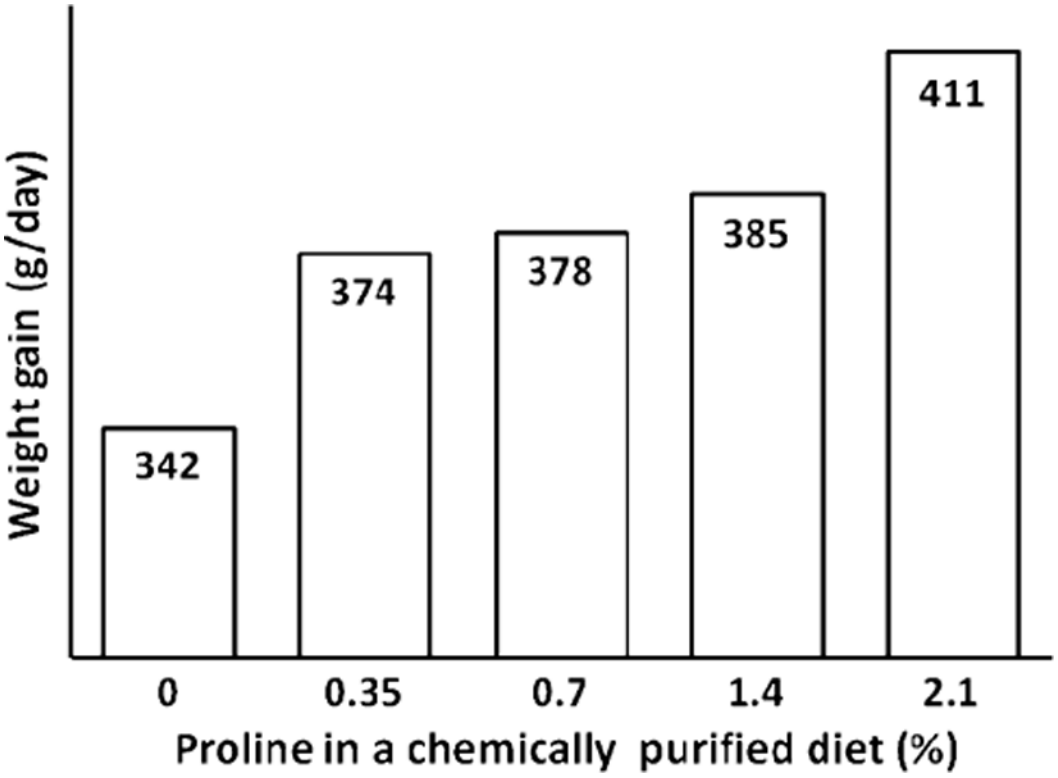

Fig. 5.

Effect of supplementing proline to a chemically purified diet on growth performance of young pigs. Data are taken from Kirchgessner et al. (1995). Young pigs were fed a purified diet supplemented with 0, 0.35, 0.7, 1.4, and 2.1% L-proline. The basal diet contained the following amino acids (% of diet): L-lysine–HCl, 1.82; DL-methionine, 0.48; L-cystine, 0.44; L-threonine, 1.06; L-tryptophan, 0.26; L-isoleucine, 0.86; L-leucine, 1.58; L-phenylalanine, 0.86; L-tyrosine, 0.86%; L-histidine, 0.56; L-valine, 1.08; L-alanine, 1.67; L-arginine, 0.48; L-aspartate, 3.61; L-glutamate, 2.02; glycine, 1.54; and L-serine, 2.24. Supplementing L-proline to the basal diet resulted in a linear increase ($P < 0.01$) in weight gain

Table 1

Functions of proline in cellular metabolism and physiology

Regulation of gene expression and cell differentiation
mTOR activation (integrating nutrient and growth factor signaling in cells)
Proline signaling via pyrroline-5-carboxylate, superoxide anion (a free radical), and cellular redox reactions
Polyamines glutamate and protein syntheses
Hydroxyproline generation
Arginine synthesis in mammals (particularly important for milk-fed neonates)
Scavenging oxidants

Table 2

Composition of dry matter (DM), crude protein (CP), and amino acids in feedstuffs (%, as-fed basis)

Nutrient	Barley[a]	Corn, grain[a]	Cotton seed meal[b]	Fish meal[c]	Rice protein concentrate[d]	Salmon protein hydrolysate[d]	Soy bean meal[e]	Spray dried plasma protein[d]	Sorghum[a]	Wheat, grain[a]	Wheat middlings[a]
DM	91.4	90.8	93.9	91.9	92.7	91.4	87.7	90.9	90.8	89.9	91.6
CP	13.9	9.60	43	60.1	67.5	92.7	44.0	78.0	10.3	12.4	16.9
EAA											
Arg	0.70	0.54	4.35	3.78	5.26	5.47	3.23	4.57	0.42	0.61	1.15
His	0.31	0.29	1.12	1.23	1.65	1.59	1.14	2.61	0.24	0.29	0.46
Ile	0.48	0.35	1.11	2.51	2.91	2.16	2.07	2.90	0.40	0.40	0.52
Leu	0.95	1.14	2.14	4.41	5.31	3.97	3.41	7.51	1.36	0.81	1.04
Lys	0.44	0.32	1.62	4.46	2.21	5.05	2.80	6.90	0.24	0.35	0.64
Met	0.16	0.21	0.66	1.69	1.77	1.89	0.59	0.69	0.20	0.20	0.23
Phe	0.78	0.47	2.07	2.38	3.52	2.10	2.29	4.38	0.54	0.58	0.68
Thr	0.45	0.36	1.17	2.54	2.12	2.62	1.78	4.33	0.29	0.35	0.53
Trp	0.11	0.05	0.43	0.50	0.81	0.48	0.50	1.38	0.07	0.11	0.16
Val	0.67	0.48	1.62	2.96	4.13	2.78	2.18	5.20	0.54	0.54	0.78
NEAA											
Ala	0.51	0.72	1.34	4.62	3.47	5.93	1.97	4.18	0.98	0.44	0.77
Asp[f]	0.77	0.67	3.37	6.70	5.39	6.18	5.15	7.35	0.70	0.60	1.09
Cys	0.22	0.22	0.70	0.62	1.45	0.42	0.69	2.73	0.20	0.28	0.34
Glu[g]	3.87	1.85	7.76	9.15	10.9	10.0	8.26	11.5	2.20	3.78	3.66
Gly	0.55	0.41	1.39	6.04	2.77	12.0	1.95	2.76	0.36	0.54	0.90
Pro	1.79	0.82	1.33	3.91	2.94	6.17	2.88	4.44	0.87	1.28	1.22
Ser	0.58	0.46	1.56	2.57	2.36	2.60	2.26	3.98	0.46	0.57	0.73
Tyr	0.42	0.39	1.14	2.19	3.32	1.32	1.70	4.04	0.44	0.38	0.49

EAA nutritionally essential amino acids, *NEAA* nutritionally nonessential amino acids

[a] Lin et al. (1987)

[b] Glandless cottonseed meal (LaRue et al. 1985)

[c] Knabe et al. (1989) for essential amino acids; values for nonessential amino acids were determined by authors of the present article using HPLC (Li et al. 2009c)

[d] Gottlob et al. (2006)

[e] Hansen et al. (1993). Proline was determined using HPLC (Wu 1993)

[f] Aspartate plus asparagine

[g] Glutamate plus glutamine

Table 3

Effects of proline supplementation on growth performance of weanling piglets

Treatment	Dry matter intake Days 21–35 [g/(kg bodyweight × day)]	Plasma proline (mM)	Bodyweight		Bodyweight gain Days 21–35 (g/d)	Jejunal villus height[a] (μm)	Small intestinal weight (g)
			Day 21 (kg)	Day 35 (kg)			
Alanine	32.7 ± 1.9	0.32 ± 0.02	5.82 ± 0.05	8.04 ± 0.08	159 ± 3.8	274 ± 6.1	291 ± 4.2
Proline	33.1 ± 2.1	0.41 ± 0.03†	5.85 ± 0.06	8.39 ± 0.09†	181 ± 4.5†	319 ± 7.7†	323 ± 5.0†

Values are means ± SEM, n = 18 for feed intake, jejunal villus height, and small-intestinal weight; *n* = 36 for other parameters. Pigs were weaned at 21 days of age to a corn- and soybean meal-based diet (Wu et al. 1996) supplemented with 0.775% L-alanine (isonitrogenous control) or 1% L-proline. There were two pigs per pen. Amino acids were added to the basal diets at the expense of cornstarch. On day 35 of age, blood samples were obtained from jugular vein 1 h after feeding, and plasma was obtained for proline analysis (Wu 1993)

† $P < 0.05$ versus the Alanine group

[a] A different study was conducted to determine jejunal villus height on day 7 postweaning (Wu et al. 1996) as described above, except that pigs were euthanized on this day to obtain the jejunum. The depth of lamina propria was 240 ± 5 and 253 ± 6 μm, respectively, for the alanine and proline groups

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