Amino Acids
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INVITED REVIEW

Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth

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Abstract Glycine, proline, and hydroxyproline (Hyp) contribute to 57% of total amino acids (AAs) in collagen, which accounts for one-third of proteins in animals. As the most abundant protein in the body, collagen is essential to maintain the normal structure and strength of connective tissue, such as bones, skin, cartilage, and blood vessels. Mammals, birds, and fish can synthesize: (1) glycine from threonine, serine, choline, and Hyp; (2) proline from arginine; and (3) Hyp from proline residues in collagen, in a cell- and tissue-specific manner. In addition, livestock (e.g., pigs, cattle, and sheep) produces proline from glutamine and glutamate in the small intestine, but this pathway is absent from birds and possibly most fish species. Results of the recent studies indicate that endogenous synthesis of glycine, proline, and Hyp is inadequate for maximal growth, collagen production, or feed efficiency in pigs, chickens, and fish. Although glycine, proline and Hyp, and gelatin can be used as feed additives in animal diets, these ingredients except for glycine are relatively expensive, which precludes their inclusion in practical rations. Alternatively, hydrolyzed feather meal (HFM), which contains 9% glycine, 5% Hyp, and 12% proline, holds great promise as a low cost but abundant dietary source of glycine, Hyp, and proline for ruminants and nonruminants. Because HFM is deficient in most AAs, future research efforts should be directed at improving the bioavailability of its AAs and the balance of AAs in HFM-supplemented diets. Finally, HFM may be used as a feed additive to prevent or ameliorate connective tissue disorders in domestic and aquatic animals.

Keywords Nutritionally nonessential amino acids · Feather meal · Livestock · Poultry · Fish

Abbreviations

AA	Amino acid
HFM	Hydrolyzed feather meal
Hyp	Hydroxyproline
α-KG	α-Ketoglutarate
P5C	Pyrroline-5-carboxylate
RER	Rough endoplasmic reticulum

Introduction

Glycine (aminoacetic acid) is the simplest amino acid (AA) in nature. It was first isolated from acid hydrolysates of protein (i.e., gelatin) in 1820 by the French chemist H. Braconnot (see Meister 1965 for review). This nutrient is as sweet as glucose; hence, its name was derived from the Greek word "*glykys*", meaning sweet. In 1838, G.J. Mulder reported that glycine could also be obtained from gelatin and meat using alkaline hydrolysis. It is now known that glycine is a major constituent of all the types of collagen (the primary extracellular matrix protein) in animals (Devlin 2006) and elastin (Chow et al. 1989), and plays an important role in nutrition and metabolism (Table 1). Because glycine had traditionally been classified as a "nutritionally nonessential AA" for mammals (including humans, pigs and rodents) and fish due to the presence of its endogenous synthesis in the body, this AA was not previously considered in the formulation of diets for these animals (Wu et al. 2013).

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Table 1 Physiological functions of glycine in animals. Adapted from Wang et al. (2013), Wang et al. (2014a), and Sun et al. (2016)

	Direct action of glycine or the functions of its metabolites
Direct action of glycine	Protein synthesis (particularly accounting for 1/3 of amino acids in collagen and elastin); cell signaling; inhibition of calcium influx through activation of the glycine-gated channel in the cell membrane; inhibitory neurotransmitter in the central nervous system; co-agonist with glutamate for *N*-methyl-D-aspartate receptor receptors; antioxidant; anti-inflammation; one-carbon metabolism; conjugation with bile acids
Function of glycine metabolites	
Serine	Protein synthesis, one-carbon metabolism, and gluconeogenesis; conversion into choline via a series of reactions requiring methionine; conversion into ethanolamine through formation of phosphatidylserine; synthesis of D-serine (a neurotransmitter) in the brain
Porphyrins and heme	Hemoproteins (e.g., hemoglobin, myoglobin, catalase, and cytochrome c); production of carbon monoxide (a signaling molecule); storage of iron in the body
Bilirubin	Natural ligand of aryl hydrocarbon receptor in the cytoplasm
Creatine[a]	Antioxidant; antiviral; antitumor; energy metabolism in heart, skeletal muscle and brain; neurological and muscular development and function
Glutathione[b]	Free radical scavenger; antioxidant; cell metabolism (e.g., formation of leukotrienes, mercapturate, glutathionylspermidine, glutathione–nitric oxide adduct and glutathionylproteins); signal transduction; regulation of gene expression; apoptosis; cellular redox; immune response
Nucleic acids[c]	Coding for genetic information; gene expression; cell cycle and function; protein and uric acid synthesis; lymphocyte proliferation; facilitation of wound healing
Uric acid	Antioxidant; the major end product of amino acid oxidation in avian species

The rates of some glycine-dependent metabolic pathways vary among species

[a] Requiring arginine, methionine, and glycine as substrates

[b] Requiring cysteine, glutamate, and glycine as substrates

[c] Requiring glutamine, aspartate, and glycine as substrates

However, growing evidence shows that the amount of glycine synthesized in vivo is insufficient to meet metabolic demands [i.e., the synthesis of proteins (including collagen), glutathione, and heme] in these species (Hou et al. 2016; Liu et al. 2017). Although mild insufficiency of glycine is not threatening for survival, a chronic shortage may result in suboptimal growth, impaired immune responses, and other adverse effects on health and nutrient metabolism (Wang et al. 2013).

Along with glycine, proline (pyrrolidine-2-carboxylic acid) is another abundant AA in collagen (Devlin 2006). Proline was discovered in 1901 as a component of casein's acid hydrolysates. One year later, 4-hydroxyproline (4-Hyp, 4-hydroxypyrrolidine-2-carboxylic acid) was identified as a product from the acid hydrolysates of gelatin. Subsequently, 4-Hyp was also found to be an abundant constituent of collagen. Studies in the 1960s revealed that Hyp is derived from the post-translational hydroxylation of proline in proteins (primarily collagen). In addition to their structural functions in collagens (Phang et al. 2010), both proline and Hyp have important metabolic and physiological roles (Table 2). For example, in most mammals (e.g., milk-fed, weanling

Table 2 Functions of proline and hydroxyproline in animal metabolism and physiology

Amino acid	Functions
Proline	Synthesis of arginine, polyamines, and proteins (including collagen and elastin)
	Regulation of gene expression and cell differentiation
	Activation of mTOR cell signaling for initiating protein synthesis (integrating nutrient and growth factor signaling in cells)
	Signaling via pyrroline-5-carboxylate, superoxide anion, and cellular redox reactions
	Synthesis of ornithine, arginine, polyamines, glutamate, protein and hydroxyproline
	Scavenging oxidants (including free radical species)
Hydroxyproline	Synthesis of glycine in multiple tissues of animals
	Maintenance of the structure of connective tissue
	Regulation of cellular redox reactions and serving as an anti-oxidative molecule
	Regulation of gene expression to promote removal of oxidants
	Scavenging oxidants (including free radical species)

animals, and gestating swine), proline is a major substrate for the synthesis of arginine, which is required for the production of nitric oxide to maintain normal hemodynamics and nutrient transport in the body (Wu and Meininger 2009). Much evidence shows that animals have particularly high requirements for proline (Wu et al. 2011). However, because of technical difficulties in the laboratory analysis of proline and Hyp (Wu 1993), research on their nutrition and metabolism in animals is limited.

In anticipation for the use of glycine, proline, and Hyp or their immediate precursorsas feed additives in the diets of livestock, poultry, and fish, the main objectives of this article are to highlight: (1) the cell- and species-specific synthesis of these AAs in animals; (2) energy needs of the biosynthetic pathways; (3) the production of mature collagen; (4) benefits of dietary glycine, proline and Hyp in collagen synthesis and growth performance of animals; (5) low-cost dietary sources of glycine, proline, and Hyp; and (6) future research direction.

Synthesis of glycine, proline and Hyp in animals

Glycine synthesis in animal tissues

In growing farm animals, including swine, poultry, and fish, the dietary intake of glycine meets at most 50% of glycine requirements for their maintenance plus growth (Hou et al. 2016). Thus, these animals must synthesize at least 50% of glycine needed daily to ensure their optimal health, growth, and feed efficiency. Available evidence shows that glycine is synthesized de novo from: (1) serine (which is produced from glucose and glutamate via serine hydroxymethyltransferase); (2) choline via the formation of sarcosine; (3) threonine via the threonine dehydrogenase pathway; and (4) Hyp (Fig. 1). Interconversion of glycine and serine may be quantitatively limited due to relatively low concentrations of folate in animal cells (Wang et al. 2013). Because of the low content of serine, choline, threonine, and Hyp in typical plant-based diets, glycine synthesis in animals is inadequate for their maximal growth (Wu 2014). For example, studies in the 1990s have shown that: (1) glycine synthesis from choline plus threonine contributes ≤6% of glycine needed by the young pig and (2) the production of glycine from dietary serine represents only ≤7% of total glycine synthesis (see Wang et al. 2013 for review). Hu et al. (2017) recently discovered that Hyp, which is an abundant AA in hydrolyzed feather meal (HFM) (Li et al. 2011), is actively converted into glycine in all the pig tissues that were studied, including the small intestine, liver, skeletal muscle, kidneys, and pancreas. In young pigs fed sow's milk that naturally contains a large amount of Hyp, this AA is a major substrate for endogenous synthesis of glycine. This recent finding challenges the traditional view that Hyp in its free or small-peptide form is merely a metabolic waste in animals, including pigs (Bushinsky et al. 2002; Khan et al. 2006; Mandel et al. 2004).

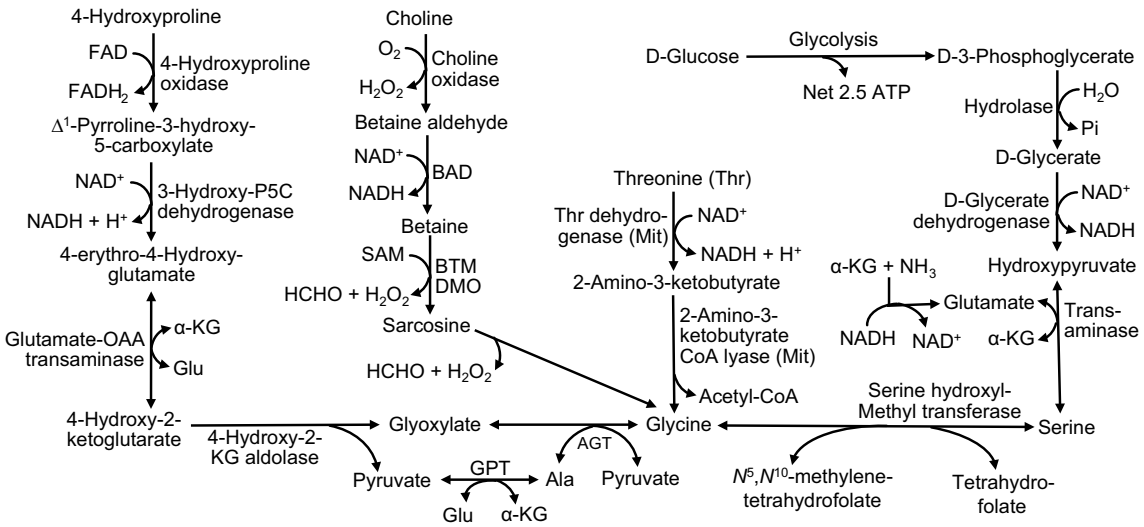

Fig. 1 Synthesis of glycine from serine, choline, threonine, and 4-hydroxyproline in animals. In addition to the D-glycerate pathway, serine can be formed from D-3-phosphoglycerate (an intermediate of glycolysis) via D-3-phosphoglycerate dehydrogenase, 3-phosphohydroxypyruvate-glutamate transaminase, and phosphoserine hydrolase (Wu 2013). *AGT* alanine–glyoxylate transaminase, *BAD* betaine aldehyde dehydrogenase, *BTM* betaine transmethylase, *DMO* dimethylglycine oxidase, *Glu* glutamate, *GPT* glutamate–pyruvate transaminase, *α-KG* α-ketoglutarate, *P5C* pyrroline-5-carboxylate

Proline synthesis in animal tissues

Advanced analytical techniques have greatly facilitated research on proline nutrition (Dai et al. 2014; Wu 1993). Arginine, glutamate, and glutamine are potential substrates for proline synthesis in a species- and cell-specific manner (Wu and Morris 1998). All animals can synthesize proline from arginine via arginase (both type I and type II), ornithine aminotransferase, and pyrroline-5-carboxylate (P5C) (Fig. 2), but the rates of synthesis vary greatly among species (e.g., mammals > birds = fish) (Li et al. 2009). In post-weaning mammals, the mammary tissue, small intestine, liver, and kidneys are quantitatively active tissues in converting arginine into proline, but the rates of proline synthesis from arginine are inadequate to meet optimal growth and connective tissue repair in the young and in stressed adults (Hu et al. 2015; Wu 2013; Wu et al. 2016).

The small intestine of livestock species (e.g., pigs and ruminants) can convert dietary glutamate, as well as dietary and arterial glutamine, into proline (Fig. 2), but this synthetic pathway is absent from other tissues of these animals due to the lack of P5C synthase (Wu 2013). In all tissues of birds, cats, ferrets, and likely many fish species, the absence of P5C synthase does not allow for proline production from glutamate or glutamine. Thus, adequate amounts of dietary proline are essential for maximizing growth performance and feed efficiency in farm animals (Hou et al. 2016).

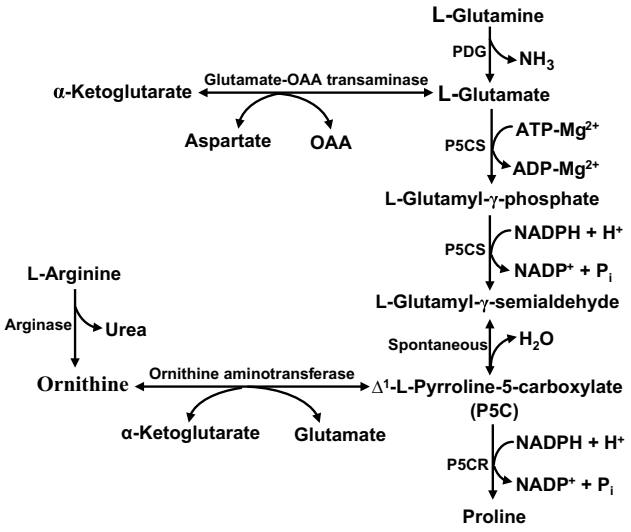

Fig. 2 Proline synthesis from arginine, glutamine, and glutamate in a cell- and species-specific manner. Arginine is converted into proline in all animals via the arginase pathway. In contrast, proline is synthesized from glutamine and glutamate in the small intestine of most mammals (including pigs, cattle, and sheep) via the pyrroline-5-carboxylate (P5C) synthase pathway, and this pathway is absent from birds and possibly most fish species due to P5CS deficiency or absence. *OAA* oxaloacetate, *PDG* phosphate-dependent glutaminase, *P5CS* pyrroline-5-carboxylate synthase, *P5CR* pyrroline-5-carboxylate reductase

Hyp synthesis in animal tissues

Unlike glycine and proline, Hyp is generated from proline-containing collagen but not from free AAs (Gorres and Raines 2010). Specifically, the Hyp residue is formed from the post-translational hydroxylation of proline in the newly synthesized collagen (Fig. 3). This reaction occurs in the rough endoplasmic reticulum by collagen prolyl 4-hydroxylase or prolyl 3-hydroxylase in the presence of oxygen, ascorbic acid, α-KG, and Fe^{2+} to generate 4-Hyp or 3-Hyp, respectively. The ratio of 4-Hyp to 3-Hyp in collagen proteins is approximately 100:1 (Wu et al. 2011). 4-Hyp and 3-Hyp are released from the degradation of extracellular and intracellular collagens. Matrix metalloproteinases (also called collagenases) are responsible for the hydrolysis of extracellular collagen in connective tissue (Malemud 2006).

Energy expenditure in the synthesis of glycine, proline, and Hyp in animals

Raw materials possess energy, and the conversion of large molecules into glycine (the smallest AA) is an exergonic process (Wu 2013). When 1 mol Hyp, 1 mol threonine, 1 mol glucose plus 1 mol glutamate, or 1 mol choline is degraded to form 1 mol glycine (Fig. 1), 4, 2.5, 2.5, or

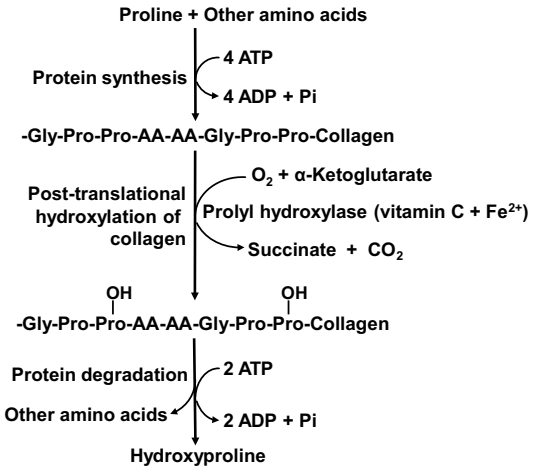

Fig. 3 Production of hydroxyproline from proline residues in collagen. Approximately 50% of proline residues in collagen are hydroxylated by collagen prolyl 4-hydroxylase and prolyl 3-hydroxylase, respectively, to generate 4-hydroxyproline and 3-hydroxyproline in the rough endoplasmic reticulum of fibroblasts. These two enzymes require oxygen, ascorbic acid, α-ketoglutarate (α-KG), and Fe^{2+} for catalytic activities. The degradation of extracellular mature collagen by matrix metalloproteinases (collagenases) releases 4-hydroxyproline and 3-hydroxyproline

2.5 mol ATP is produced, respectively (Table 3). The yield of ATP is 60% greater with Hyp than any other substrates.

As noted previously, most mammals can convert glutamine and glutamate into proline. The synthesis of 1 mol proline from 1 mol glutamine or glutamate requires 8 and 6 mol ATP, respectively (Table 3). In all animals, proline can be formed from arginine, but the rates differ markedly among species (Wu and Morris 1998). The production of 1 mol proline from 1 mol arginine needs 2.5 mol ATP. Thus, the arginine pathway is the least expensive for proline synthesis in terms of energy cost. This helps explain why arginase is widely spread in the animal kingdom for proline production.

All animals generate Hyp from proline, and this metabolic process requires a large amount of energy (6 mol ATP/mol Hyp), which includes 4 mol ATP for collagen synthesis and 2 mol ATP for collagen degradation (Fig. 3). For comparison, the removal of 1 mol ammonia as urea via the hepatic urea cycle requires 3.25 mol ATP, which is widely recognized as a pathway with a high energy cost (Wu 2013). Thus, direct provision of proline and Hyp or their immediate precursors in diets will minimize the expenditure of energy for the endogenous synthesis of these two AAs.

Collagen synthesis in animals

Collagen synthesis and processing

Collagen is formed from AAs (mostly glycine and proline) by fibroblasts through the normal pathway of intracellular protein synthesis, which includes AA activation, initiation of peptide formation, peptide elongation, termination, and post-translational modifications (Wu 2013). The collagen precursor chains that are newly synthesized on ribosomes are called procollagens, which are then processed by the rough endoplasmic reticulum (RER) and Golgi (Myllyharju 2005). Specifically, procollagens are transported into the lumen of the RER to undergo a series of reactions, including (1) the hydroxylation of some proline and lysine residues by RER membrane-bound prolyl hydroxylase (procollagen-proline dioxygenase) and lysyl hydroxylase (procollagen-lysine 5-dioxygenase), respectively; (2) glycosylation (addition of galactose and glucose residues to certain hydroxylysine residues, as well as addition of long oligosaccharides to certain asparagine residues in the C-terminal); and (3) the generation of intrachain disulfide bonds between the N- and C-terminal polypeptides to align the three chains and form the triple helix. This procollagen complex moves into the Golgi for further processing (e.g., glycosylation), yielding large electron-dense aggregates. Within the Golgi apparatus, procollagens are packaged into membrane-bound vesicles for secretion into the extracellular space (Lavieu et al. 2014).

After their processing in the Golgi is completed, the procollagens are secreted by fibroblasts into the extracellular space through exocytosis (Myllyharju 2005). Extracellular enzymes (the procollagen peptidases) cleave the N-terminal and C-terminal AA sequences (called propeptides) to generate collagens. In addition, lysyl oxidase (an extracellular enzyme) acts on the ε-amino group of lysine residues to generate reactive aldehydes (α-aminoadipidic-δ-semialdehydes, also called allysines). The latter spontaneously form specific covalent cross-links between two triple-helical chains (Robins 2007) to stabilize collagen molecules (mature collagen) and contribute to fibril strength. Figure 4 summarizes the post-translational processing of procollagens to become mature collagens.

Collagen structure

Collagen, which accounts for one-third of the total protein in the postnatal animal, is the largest and most abundant protein in the body (Devlin 2006). The chemical structure of collagen is unique, because it is unusually rich in glycine, proline, and OH-Pro in the repeated form of tripeptides (glycine–proline-Y and glycine–X-Hyp, where *X* and *Y* can be any AA). On the molar basis, collagen contains 1/3 glycine, 0.7/3 proline plus Hyp, and 1.3/3 other AAs

Table 3 Energy expenditure in the synthesis of glycine, proline, and hydroxyproline by animal tissues

Amino acid synthesis	Metabolic pathway	ATP production (mol/mol product)	ATP requirement (mol/mol product)
Glycine	Hydroxyproline to glycine	4	–
	Threonine to glycine	2.5	–
	Glucose plus glutamate to glycine	2.5	–
	Choline to glycine	2.5	–
Proline	Glutamine to proline (only in mammals)	–	8
	Glutamate to proline (only in mammals)	–	6
	Arginine to proline (in all animals)	–	2.5
Hydroxyproline	Proline to hydroxyproline	–	6

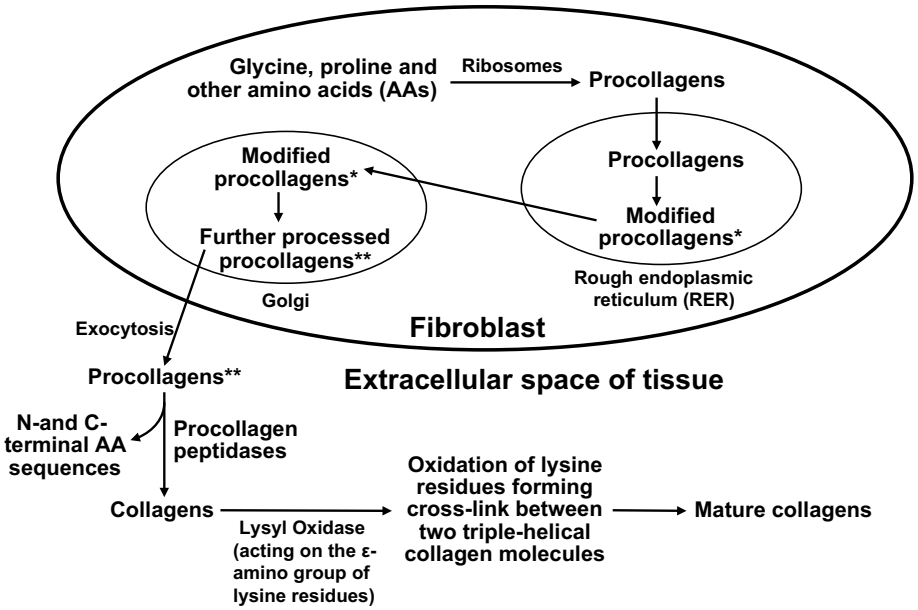

Fig. 4 Processing of procollagens by the rough endoplasmic reticulum (RER) and Golgi apparatus of fibroblasts, as well as the extracellular processing of procollagens to become mature collagens in connective tissue. Asterisk modifications in RER include: (1) the hydroxylation of some proline and lysine residues by proline and lysine hydroxylases, respectively; (2) glycosylation (e.g., addition of galactose and glucose residues to certain hydroxylysine residues); and (3) alignment of the three α-chains to form the triple helix. Procollagens are secreted into the extracellular space, where they undergo limited N- and C-terminal cleavage, and form cross-links between two triple-helical chains via allysines (intermolecular linkages) to become mature collagens. Double asterisk further modifications in the Golgi apparatus include glycosylation and the packaging of processed procollagens into membrane-bound vesicles for secretion

(Table 4), and glycine plus proline plus Hyp contribute to 57% of total AAs in collagen.

There are approximately 20 different types of collagen in the animal kingdom. Each mature collagen contains three polypeptide chains (called α chains) (Table 5), which may be the same or different and are organized in a triple-helical structure (Bella 2016). Individual α chains are identified by the following nomenclature: $\alpha n(N)_p$, where n is the identification number of the α chain, *N* is the collagen type, and *p* is the number of the polypeptide. For example, $\alpha1(I)_2\alpha2(I)$ denotes a heterotrimer of type I collagen consisting of two identical α1 chains and one distinct α2 chain, whereas $[\alpha1(II)]_3$ indicates three identical α1 chains of type II collagen. Different types of collagens may have different rates of turnover in connective tissue.

The distribution of collagens differs among organs, and the length of collagens varies with type (Table 6). In type I, II and III collagens, each polypeptide is 300 nm long (corresponding to about 1000 AA residues) (Chu et al. 1987; Burgeson and Morris 1987). For example, in type I collagen, each of the two identical α1(I) chains consists of 1056 AA residues. In fibrous collagens, collagen molecules pack together side by side through an interchain cross-link between allysine residues formed by the extracellular lysyl oxidase (Bella 2016). Thus, collagen confers the strength, rigidity, and flexibility of connective tissue, and is necessary for the growth, development, and health of all animals.

Benefits of dietary glycine, proline, and Hyp in collagen synthesis and animal growth

When animals (including swine, poultry, and fish) grow, connective tissue and other tissues accumulate in the body (Wu et al. 2014). Glycine, proline, and Hyp are highly abundant in collagen (Table 4) and elastin (Table 6). Elastin is another structurally important protein in connective tissue and contributes to the elastic properties of vertebrate organs (Debelle and Tamburro 1999). Therefore, the adequate provision of both glycine and proline is essential for maximal collagen synthesis, maximal growth performance, and optimal health of all animals, including mice (Shimizu et al. 2015), rats (Barbul 2008; Chyun and Griminger 1984), humans (Barbul 2008; Shaw et al. 2017), pigs (Hou et al. 2016; Wang et al. 2014b), poultry (Baker 2009), and many fish species (Li et al. 2009).

Li et al. (2011) reported that plant-source feedstuffs contain a low content of glycine, little to no Hyp, and a relatively low content of proline. For example, typical corn- and soybean meal-based diets provide at most only 48% of glycine and 60% of proline that are needed for protein accretion

Table 4 Composition of amino acids and sugars in types I–VII collagens (residues/1000 amino acid residues)

Amino acid	Type I		Type II	Type III	Type IV		Type V			Type VI			Type VII
	α1	α2	α1	α1	α1	α2	α1	α2	α3	α1	α2	α1	α1
Alanine	119	105	108	95	30	47	46	52	49	50	44	35	64
Arginine	49	51	52	48	22	42	45	50	42	59	68	83	48
Asp + Asn	46	45	36	42	45	50	50	51	42	87	81	85	51
Cysteine	0	0	0	2	2	3	0	0	1.3	10	6	14	6.7
Glu + Gln	77	70	95	71	88	64	91	84	98	119	108	110	105
Glycine	330	331	334	351	324	328	344	341	332	323	298	328	307
Histidine	4	10	2	6	6	7	8	11	14	7	9	11	31
Isoleucine	8	16	11	13	32	39	19	16	20	15	20	27	10.5
Leucine	20	33	27	23	50	56	39	35	56	21	30	23	49
Lysine	27	20	20	29	7	5	20	18	15	8	17	19	18
5-OH-Lysine	10	12	18	6	49	39	35	24	43	57	67	47	41
3-OH-Proline	1.1	1.2	1.1	0	1	1	2.9	2.5	2.2	NR	NR	NR	0.1
4-OH-Proline	114	105	96	126	118	107	109	109	92	78	71	52	84
Methionine	7	8	11	8	14	14	8	11	8.1	10	5	4	7.6
Phenylalanine	13	11	13	8	28	36	12	14	9.2	16	14	14	9.8
Proline	118	114	106	107	88	73	118	97	99	89	101	89	81
Serine	35	30	27	38	36	30	36	31	34	24	27	27	49
Threonine	18	18	22	13	20	28	19	26	19	14	16	19	26
Tyrosine	2	3	1	3	8	6	2	2	2.4	15	10	11	28
Valine	19	35	19	15	34	25	25	24	29	18	19	20	35
Glucose	1	1	5	1	43	NR	18	10	17	NR	NR	NR	NR
Galactose	1	2	10	2	45	NR	30	14	24	NR	NR	NR	NR

Values for type VI collagen are obtained from (Chu et al. 1987), and values for all collagens from Burgeson and Morris (1987)

NR not reported

Table 5 Major types of collagen in animals

Type	Composition	Arrangement	Representative tissues
I	2 α1(I) chains + 1 α2(I) chain	Fibrils	Bone, dentin, interstitial tissues, ligaments, skin, and tendon
II	3 α1(II) chains	Fibrils	Cartilage and vitreous humor (eyeball)
III	3 α1(III) chains	Fibrils	Blood vessels, skeletal muscle, heart, and skin
IV	2 α1(IV) chains + 1 α2(IV) chain	Two-dimensional network	All basal laminaes in tissues
V	3 α1(V) chains	Fibrils with GD	Bone, dentin, interstitial tissues, ligaments, skin, and tendon
VI	α1(VI) + α2(VI) + α3(VI) chains	Associated with type I collagen	Mainly extracellular matrix of skeletal muscle
IX	α1(IX) + α2(IX) + α3(IX) chains	Associated with type II collagen	Cartilage and vitreous humor (eyeball)

GD globular *N*-terminal domain

in 30-day-old growing pigs (Hou et al. 2016). Insufficient endogenous synthesis of these two AAs limits maximal collagen synthesis and growth performance of young pigs (Hou et al. 2016) and poultry (Baker 2009), as well as many fish species studied (Dabrowski et al. 2010; Liu et al. 2014). Thus, dietary supplementation with collagen precursor AAs, such as 0.5–2% glycine (Wu 2015) and 1% proline (Wu et al. 2011), enhances intestinal villus height and nutrient absorption, as well as whole-body weight gains and collagen production in post-weaning pigs. Similar results have been reported for broiler chickens. For example, supplementing 0.3% glycine to a 17% crude-protein diet increased fat absorption, mucin production, whole-body weight gain and collagen deposition, and feed efficiency in 21–35-day-old broilers (Ospina-Rojas et al. 2013). Likewise, supplementing 0.2% glycine to an 18% crude-protein diet for 5–21-day-old broilers enhanced skeletal muscle growth, whole-body collagen production, and the efficiency of nitrogen retention (Corzo et al. 2005). Furthermore, dietary supplementation with 0.3% glycine enhanced growth rate and resistance

Table 6 Composition of amino acids in elastin (residues/1000 amino acid residues)

Amino acid	Porcine elastin[a]		Chicken mature elastin[b]	Bovine mature elastin[c]	Salmon mature elastin[c]
	Soluble	Insoluble			
4-OH-proline	7	8	19	9	10
Asp + Asn	3	5	4	10	35
Threonine	10	11	7	11	37
Serine	8	11	5	11	36
Glu + Gln	12	16	13	18	49
Proline	92	90	127	116	82
Glycine	245	256	361	320	387
Alanine	187	181	177	228	126
Cysteine	<1	<1	–	–	3
Valine	103	92	166	127	48
Methionine	0	0	0	Trace	4
Isoleucine	14	14	19	25	14
Leucine	41	41	50	62	47
Tyrosine	14	12	11	9	46
Phenylalanine	14	12	20	32	16
Lysine	37	5	3	9	18
Histidine	0	0	1	1	8
Arginine	4	5	5	9	32
Tryptophan	0	0	–	–	–

[a] Adapted from Davidson (1987)

[b] Adapted from Keeley and Labella (1972)

[c] Adapted from Chow et al. (1989)

Table 7 Composition of amino acids in hydrolyzed feather meal. Adapted from Li et al. (2011)

Variable	Content (%)	Variable	Content (%)
Dry matter	96.1	Hydroxyproline	4.95
Crude protein	82.1	Isoleucine	3.79
True protein	81.0	Leucine	6.75
Alanine	4.18	Lysine	2.16
Arginine	5.74	Methionine	0.75
Asparagine	1.67	Phenylalanine	3.95
Aspartate	2.92	Proline	11.8
Cysteine	4.16	Serine	8.80
Glutamine	2.86	Tryptophan	0.80
Glutamate	4.81	Threonine	3.97
Glycine	8.95	Tyrosine	2.04
Histidine	0.88	Valine	5.76

Values are expressed as % (g/g) on an as-fed basis. Molecular weights of intact amino acids were used to calculate the content of peptide-bound amino acids in hydrolyzed feather meal

to environmental salinity stress in juvenile Pacific white shrimp, *Litopenaeus vannamei* (Xie et al. 2014), whereas dietary supplementation with 0.5% glycine improved weight gain, anti-oxidative capacity, and immunity in Nile tilapia, *Oreochromis niloticus* (Xie et al. 2016). Finally, there are reports that dietary supplementation with 0.07, 0.14, and 0.28% 4-Hyp to a plant-based diet dose-dependently augmented whole-body weight gains and collagen deposition in salmon (Aksnes et al. 2008) and turbot (Liu et al. 2014).

Dietary sources of glycine, proline, and Hyp for animals

Adequate provision of glycine and proline in diets is essential for optimal health and growth of animals (Hou and Wu 2017). As noted previously, Hyp serves as a major precursor of glycine (Fig. 1) and an anti-oxidative molecule in the body (Table 2). Crystalline glycine, proline, and Hyp can be used as feed additives for the diets of swine, poultry, and fish. In addition, gelatin is an abundant source of these AAs. However, the synthetic AAs (e.g., proline and Hyp) and gelatin are expensive, which precludes their inclusion in practical animal rations. Alternatively, HFM, which contains 9% glycine, 5% Hyp, and 12% proline (Table 7), is potentially a low cost but abundant source of these three AAs for ruminants and nonruminants (Li et al. 2011). There is evidence that finisher pigs fed 85.9% corn- and 9.8% HFM-based diets did not grow as well as pigs fed 79.2% corn- and 18.5% soybean meal-based diets (Divakala et al. 2009). However, finisher pigs fed the corn- and HFM-based diets supplemented with a mixture of crystalline AAs (lysine, tryptophan, threonine, histidine, and isoleucine) in small amounts could utilize feed for lean-tissue gains as efficiently as those fed the corn- and soybean meal-based diets (Divakala et al. 2009). When pasture is inadequate, dietary supplementation with HFM can improve the growth and feed efficiency of grazing steers (Brown and Pate 1997; Brown and Adjei 2001). Likewise, inclusion of HFM along with supplemental lysine and methionine could support egg production by laying hens (Koelkebeck et al. 1999) and reduce abdominal fat accumulation in broilers during the finishing period (Cabel et al. 1988). Furthermore, HFM can replace 10 and 15% fishmeal in the diets of gilthead seabream (Nogueira et al. 2012; Laporte et al. 2007) and rainbow trout (Hertrampf and Piedad-Pascual 2000), respectively, without affecting their feed intake or growth rate. Taken together, these results show the potential of HFM as a cost-effective source of glycine, proline, and Hyp in the diets of livestock, poultry, and fish.

Future research direction

AAs are not balanced in HFM (Li et al. 2011). It is deficient in most AAs based on the patterns of its AAs (Hou et al. 2015; Wu 2014). Thus, identifying appropriate additions of crystalline AAs (e.g., aspartate, glutamate, glutamine, lysine, methionine, threonine, and histidine) to HFM-supplemented diets will be crucial to maximize the nutritive value of this protein ingredient for feeding farm animals (including cattle, goats, sheep, swine,poultry, fish, and shrimp). In addition, the standardized ileal digestibilities of AAs in HFM are about 55–60% for growing pigs and broilers, in comparison with 85–90% for heat-processed soybean meal (Bandegan et al. 2010). This indicates incomplete hydrolysis of the HFM ingredient by the gastrointestinal tract of nonruminants. Future research should focus on: (1) more complete hydrolysis of poultry feather meal; (2) use of bacterial source keratinases as feed enzymes in HFM-supplemented diets; (3) balance of AA composition in HFM-supplemented diets through addition of crystalline AAs or animal protein hydrolysates (Hou et al. 2017); and (4) inclusion of sufficient vitamin B_6 in diets to promote the conversion of Hyp into glycine. Finally, it will be imperative to determine whether dietary supplementation with HFM can be effective to prevent or ameliorate connective tissue disorders that commonly occur in domestic meat animals [e.g., cartilage abnormality or lameness in growing, gestating. and lactating swine (Halper 2014; Olstad et al. 2015)], horses [e.g., osteochondrosis (Olstad et al. 2015)], and fish [e.g., skin lesions (Tørud and Håstein 2008)].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article reviews published studies and does not require either the approval of animal use or human consent.

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