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# Arginine metabolism in uricotelic species

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**ABSTRACT.** Due to the lack of a complete urea cycle, uricotelic species, such as broilers, are not able to synthesize *de novo* arginine (Arg), thus depending exclusively on dietary Arg. High levels of dietary lysine (Lys) increase the demand for Arg because of the antagonistic relationship between these amino acids. The Arg-Lys antagonism promotes an expressive increase in the renal Arg activity and consequently induces the degradation of Arg and the decrease in the activity of glycine amidinotransferase, an enzyme that uses Arg in the synthesis of muscle creatin. Arg is considered an important modulator of immunological and physiological processes. The degradation of Arg produces ornithine, a precursor of polyamines that are key to cell division, DNA synthesis, and cell cycle regulation. Arg participates in the synthesis of nitric oxide (NO), a highly reactive free radical in cells and membranes and participates in several cell processes, including in neurotransmission and immune response. Arg is also considered a potent secretagogue of insulin, growth hormone, and IGF-I in the blood stream. Exclusively vegetarian diets may not provide an adequate supply of Arg, which is required for maximum production and for the immune system of current broiler lineages.

**Key words:** carbamoyl phosphatase synthetase, creatine, immune system, nitric oxide, polyamines, urea cycle.

**RESUMO. Metabolismo da arginina em espécies uricotélicas.** Devido à falta de um completo ciclo da uréia, espécies uricotélicas como os frangos de corte são incapazes de sintetizar arginina (Arg) *de novo*, por isso dependem exclusivamente da Arg dietética. Níveis elevados de lisina (Lys) dietética aumentam a exigência de Arg devido à relação antagonista entre estes aminoácidos. O antagonismo entre Arg e Lys promove expressiva elevação da atividade da arginase renal e conseqüentemente induz à degradação da Arg e leva à diminuição da atividade da glicina amidinotransferase, enzima que utiliza Arg na síntese de creatina muscular. Arg é considerada importante modulador da imunidade e de processos fisiológicos. A degradação de Arg gera ornitina, precursor das poliaminas que têm papel-chave na divisão celular, síntese de DNA e regulação do ciclo celular. A Arg é utilizada na síntese de óxido nítrico (NO), um radical livre altamente reativo, permeável às células e membranas que participa de vários processos celulares, incluindo a neurotransmissão e a imunidade. A Arg é também considerada um potente secretagogo da insulina, hormônio do crescimento e IGF-I. Dietas exclusivamente vegetais podem ser limitantes no fornecimento adequado de Arg para a maximização produtiva e do sistema imune das atuais linhagens de frangos de corte.

**Palavras-chave:** carbamil fosfato sintetase, creatina, sistema imune, oxido nítrico, poliaminas, ciclo da ureia.

## Introduction

Arginine (Arg) is considered an essential amino acid for birds, especially in the starter period. As the biochemical cycle of urea is not functional in birds, they cannot synthesize Arg and are therefore dependent on the supply of this amino acid from the diet. Among the animal species studied, birds have the greatest requirement for Arg (BALL et al., 2007) due to their lack of endogenous synthesis, the high rate of protein deposition by rapid growth of current broiler lineages, and to its antagonism with lysine (Lys).

The antagonism between Arg and Lys significantly increases the activity of renal arginase

and consequently induces Arg degradation (AUSTIC; SCOTT, 1975) and decreases the activity of glycine amidinotransferase, an enzyme that uses Arg and glycine as substrates with methionine in the synthesis of muscle creatine (JONES et al., 1967). Raising the level of Lys in feed can alter the ideal metabolic balance between the amino acids, especially the Arg:Lys ratio.

The negative effect of the antagonism between Lys and Arg in weight gain is not only due to reduced food consumption, as observed under amino acid imbalance, but also to other effects on metabolism and Arg utilization (HARPER et al., 1970). Therefore, there is a metabolic – but not

necessarily dietary – demand for Arg in this situation. This is particularly important with respect to variations in Arg requirement and to the negative effect of the Arg:Lys antagonism in chicks and adult birds (BALL et al., 2007).

Among the species studied, only birds, dogs, and rats have a distinct antagonistic relationship between Lys and Arg. However, the exact mechanism has not yet been fully elucidated in any of these species. Both amino acids share and compete for intestinal and renal transporters; however, there is no evidence that their antagonism is due to this competition (BALNAVE; BRAKE, 2002).

The Arg:Lys antagonism appears to be multifaceted. According to Balnave and Brake (2002), high concentrations of cations, such as potassium, in diets containing excess Lys alleviate the negative effect of their antagonism on the performance of animals. Potassium stimulates Lys-ketoglutarate reductase, an enzyme involved in the catabolism of Lys. On the other hand, high levels of chloride have an effect on Arg catabolism. Conventional diets with high levels of chlorides can induce a deficiency in Arg.

Besides being a protein constituent, Arg is also involved in the synthesis of creatine and polyamines, proline, as a substrate for the synthesis of collagen and nitric oxide (NO), and in the secretion of growth hormone (GH) and insulin-like growth factors (IGF).

The biosynthesis of creatine, which also involves the participation of methionine, produces phosphocreatine, an essential component of the energetic metabolism of muscles (KESHAVARZ; FULLER, 1971).

Polyamines have nutritional importance in the growth and development of the intestine in newborns (LÖSER et al., 1999) and are essential in the proliferation of endothelial cells. They are also associated with angiogenesis and wound healing (HUI et al., 2002). As proline is the main amino acid in the synthesis of collagen and the production of the extracellular matrix, it plays an important role in vascular remodeling. NO is a highly reactive free radical in cells and membranes. It participates in several cell processes, including in neurotransmission, endothelium-dependent vasorelaxation, and in immunity. High concentrations of NO can be induced by a variety of inflammatory stimuli, such as bacterium lipopolysaccharides and cytokine (DUSSE et al., 2003; HIBBS JR. et al., 1988). NO also has pronounced effects on bone tissue physiological mechanisms, controlling bone remodeling (COLLIN-OSDOBY et al., 1995).

The synthesis and secretion of GH are essential for promoting growth, the development, and the metabolism of mammals and birds. One of the best known effects of the GH occurs in the epiphyseal disk, where it stimulates the synthesis of IGF-I, which acts paracrinally inducing mitogenesis and results in the growth of long bones (ISAKSSON et al., 1987). IGF-I is also involved in numerous anabolic and catabolic events in skeletal muscles, such as the aggregation of myofibrillar protein by combination of the effects on protein synthesis and degradation (COLEMAN et al., 1995) and satellite cell differentiation and proliferation (FLORINI et al., 1996), which are fundamental for muscle hypertrophy (FERNANDES et al., 2009).

#### Arg metabolism

The classification of animals as amoniotelic, ureotelic, and uricotelic is based exclusively on the predominance of nitrogenated metabolic compounds in excreta. Mammals and birds have distinct excretory nitrogenated metabolic compounds. Birds excrete nitrogen preferentially as uric acid, while mammals excrete urea (LENINGHER, 2002).

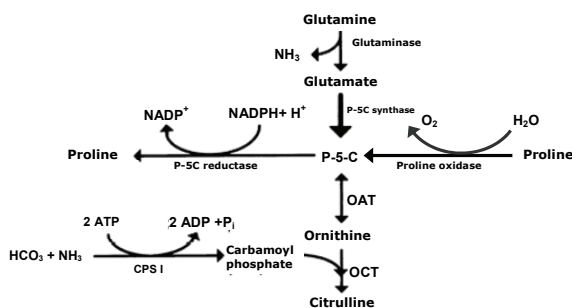
The acknowledged physiological non functionality of the urea cycle (Krebs-Hanseleit Cycle) in domestic birds (TAMIR; RATNER, 1963) is partially due to the absence of enzyme carbamoyl phosphatase synthetase, which catalyses the first step of the mechanism of detoxification of ammonia that leads to the synthesis of urea and Arg. In uricotelic species, the mitochondrial enzyme that exerts a parallel function is glutamine synthetase; however, in the end of the cycle, only uric acid is produced, which is Arg-independent. The C and N atoms of the uric acid molecule derive from aspartate, CO<sub>2</sub>, glycine, and glutamine (CHRISTMAN; MOSIER, 1929).

The first step of the synthesis of Arg in mammals is the formation of compound pyrroline-5-carboxylate (P-5-C) from glutamate or proline (Figure 1). However, the expression of all the enzymes necessary for the synthesis of Arg is restricted to the liver and the intestine mucosa, where citrulline is formed by the action of P-5-C synthetase and ornithine aminotransferase, which can be immediately converted into Arg (WU, 1997) or released into the blood stream. Citrulline is captured by the kidney and converted into Arg, which is then sent to other tissues through specific transporters.

A consequence of this metabolic dissimilarity between birds and mammals is that birds are dependent on exogenous sources of Arg, as this

amino acid is not produced by nitrogen depuration in birds. For this reason, Arg is considered an essential amino acid in birds.

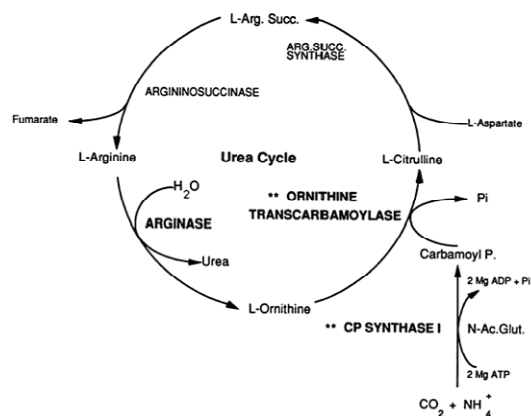
The essential character of Arg was acknowledged over 60 years ago. The first studies demonstrated that Arg can be synthesized from citrulline, but not from ornithine. This finding provided a satisfactory explanation for the classical observation that citrulline, but not ornithine, can replace Arg in birds' diet, whose mechanism was still unknown. Tamir and Ratner (1963) attributed birds' incapacity to synthesize ornithine, citrulline and Arg to the absence of another enzyme key to the urea cycle, ornithine carbamoyltransferase (Figure 2).



**Figure 1.** Synthesis of citrulline and ornithine in mammals.

Abbreviations: CPS I: carbamoyl-phosphate synthetase, OCT: ornithine carbamoyltransferase; OAT: ornithine aminotransferase; P-5-C: pyrroline-5-carboxylate. Adapted from Wu et al. (1995).

In a pioneer study of swines and rats, Windmueller and Spaeth (1981) and later Dhanakoti et al. (1990) demonstrated that the endogenous synthesis of Arg in the kidney depends on the synthesis of ornithine and citrulline in the intestine. Wu et al. (1995) studied the expression of the enzymes involved in the enterocytes of birds (P-5-C synthetase, ornithine aminotransferase, and ornithine carbamoyltransferase), besides carbamoyl phosphatase synthetase, which gives the compound carbamoyl phosphate. Surprisingly, these authors observed no activity for P-5-C synthetase, which converts glutamate into P-5-C, and only very low activity for ornithine aminotransferase, indicating a low conversion of P-5-C into ornithine. The lack of conversion of glutamate into ornithine and of the intermediate compound P-5-C also results in a very low conversion rate of ornithine into proline, of about 4% in swines. P-5-C is a common precursor in the synthesis of ornithine and proline (Figure 1), and because of this it is also considered an essential amino acid in birds.



**Figure 2.** Urea cycle in mammals.

In uricotelic species, the cycle is not complete because of the lack of enzymes\*\* ornithine carbamoyl transferase and \*\* carbamoyl-phosphate synthetase. Adapted from Wu et al. (1995).

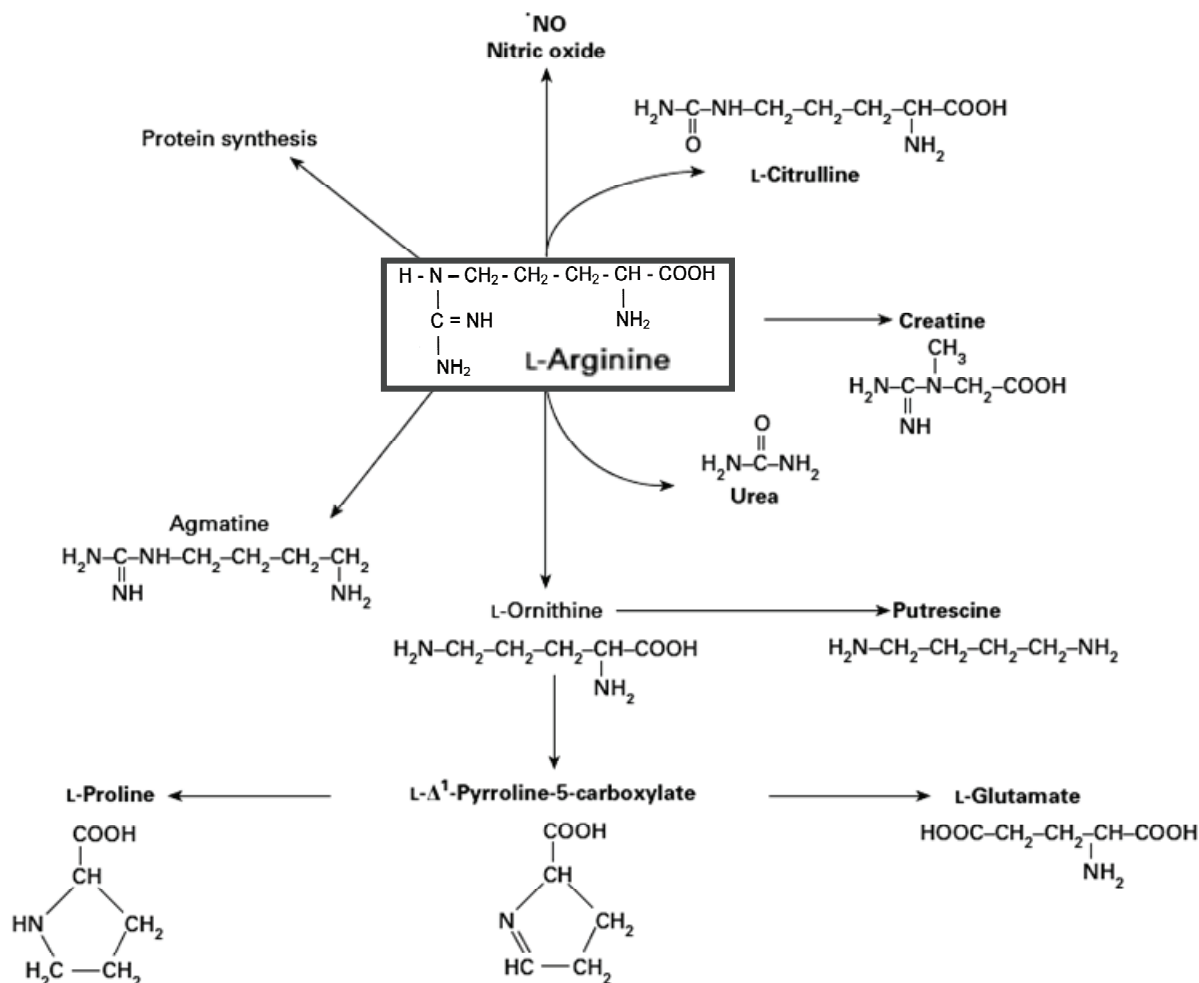
Despite the absence of the intestinal-renal axis and the supply of citrulline for the renal synthesis of Arg in birds, some tissues can convert citrulline into Arg, the renal tissue being the site of greatest conversion. Endothelial cells, macrophages, and neutrophils can also recycle Arg from citrulline produced by nitric oxide synthetase.

Wu and Morris (1998) describe Arg as "one of the most versatile amino acids in animal cells". In fact Arg is required for the synthesis of several compounds, such as ornithine, polyamines, proline, creatine, proteins, nitric oxide (NO), and citrulline, besides glutamate and agmatine in mammals. Arg is also considered a powerful secretagogue, increasing the release of insulin, the growth hormone, and IFG-I in the blood stream (NEWSHOLME et al., 2005) due to the fast depolarization of the plasma membrane associated with the transport of amino acids with positive lateral chain (Figure 3).

All these reactions depend on the intracellular concentration of Arg, which is determined by two paths: 1) the synthesis of Arg from other substrates, in the case of uricotelic species, citrulline, a mechanism that is little efficient in organic homeostasis conditions, and/or 2) the transport of Arg from the extracellular medium to intracellular compartments, a mechanism that depends on the availability of dietary Arg and the regulation of the synthesis of proteins.

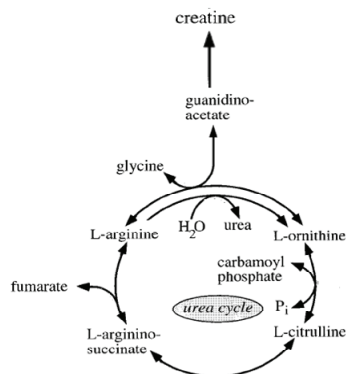
#### Arg and the synthesis of creatine

Arg is also catabolized for the biosynthesis path of creatine (Figure 4). Arg transfers a guanidine group to glycine through the action of amidinotransferase, forming ornithine and guanidinoacetate. In a second reaction, catalyzed by guanidinoacetate N-methyltransferase, guanidinoacetate is methylated by S-adenosylmethionine to form S-adenosylhomocysteine and creatine (BLOCH; SCHOENHEIMER, 1941; BORSOOK; DUBNOFF, 1945).



**Figure 3.** Main paths of participation of Arg in the cellular metabolism. Adapted from Wu and Morris (1998).

An advantage of the synthesis of creatine in the liver of mammals is that the ornithine produced in this reaction can be recycled to Arg; therefore, this process does not consume Arg (BROSAN; BROSAN, 2004).



**Figure 4.** Two-cycle path proposed for the synthesis of urea and creatine in the liver of mammals. Abbreviations: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine. Adapted from: Brosnan and Brosnan (2004).

In exclusively vegetarian diets, the Arg requirement for the synthesis of creatine is increased, as vegetables are not sources of creatine. On the other hand, animal byproducts are great sources of creatine, and diets supplemented with these ingredients can be considered Arg-“savers” in the metabolism of uricotelic species.

#### Arg and the synthesis of ornithine, polyamines, and proline

For Arg to be used in the synthesis of polyamines (putrescine, spermine, and spermidine) or proline, it needs to be hydrolyzed into urea and ornithine by arginase (MEIJER et al., 1990; WU; MORRIS, 1998). As birds cannot synthesize ornithine, practically all ornithine in the plasma derives from the metabolism of Arg (AUSTIC; NESHEIM, 1971; CHU; NESHEIM, 1979; NESHEIM et al., 1968; STUTZ et al., 1971). The urea in the plasma is associated with the supplementation of Arg, 40-60% of urea excreted by birds results from the synthesis of ornithine (RUIZ-FERIA et al., 2001).

In mammals, two distinct forms of arginase are well known (arginase I and II). They are codified by different genes and differ in molecular and immunological properties, tissue distribution, intracellular location, and regulation of expression (WIESINGER, 2001; ASH, 2004).

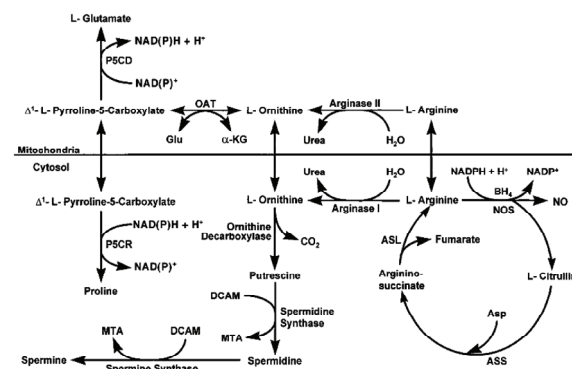
Arginase I, a cytosolic enzyme found in liver cells, is an important component of the urea cycle, but it is practically inexistent in other tissues, while the expression of arginase II, a mitochondrial enzyme, is found in a number of nonhepatic tissues, such as the kidneys, small intestine, and brain (BROSANAN; BROSANAN, 2004). This intracellular compartmentation of arginases has important implications for the metabolism of arginases in mammal cells. Due to their co-location with ornithine decarboxylase in the cytosol, arginase I can preferentially direct ornithine to the synthesis of polyamines, while arginase II can direct ornithine to the production of proline and glutamate, as both arginase II and ornithine aminotransferase are located in the mitochondria (Figure 5).

On the other hand, this enzyme is not well characterized in uricotelic species. Grazi et al. (1975) described the presence of two arginases, hepatic and renal; however, both are located in the mitochondria. According to these authors, the pool of cytoplasm Arg can be hydrolyzed by mitochondrial arginase. The expression of this enzyme is detected outside the membrane and for this reason it can act on Arg. Bedford et al. (1987) observed a relative lack of arginase activity in the liver of birds, indicating a minimum potential for the regulation of the synthesis of polyamines in its tissue. Therefore, it is possible to attribute the control of the synthesis of polyamines through the degradation of Arg to renal arginase.

Birds can enzymatically degrade Arg through arginase activity. However, in contrast to mammals, the activity of arginase in the kidney is 30 times greater than in the liver (TAMIR; RATNER, 1963). Despite the fact that there is no functional urea cycle in birds, they excrete urea, which can be taken as a “measure of degradation of Arg by arginase”. The activity of renal arginase and the excretion of urea increase with both the ingestion of Arg and protein.

Ruiz-Feria et al. (2001) mention only renal arginase as being important in the degradation of Arg in birds and that there may be differences between broiler lineages or even within lineages in relation to the activity of this enzyme. Thus, birds with high enzymatic activity could degrade Arg at higher rates, favoring the synthesis of polyamines

and proline. Nevertheless, it is unknown if there is a preferential path in relation to the directing of ornithine to the synthesis of proline or polyamines as in mammals.



**Figure 5.** Metabolism of Arg, ornithine, and citrulline in mammal cells.

Abbreviations: ASL, argininosuccinate lyase; Asp, aspartate; ASS, argininosuccinate synthetase; BH4, tetrahydrobiopterin; DCAM, decarboxylated S-adenosylmethionine; Glu, glutamate; MTA, methylthioadenosine; NO, nitric oxide; NOS, nitric oxide synthetase; OAT, ornithine aminotransferase; P5CD, pyrroline-5-carboxylate dehydrogenase; P5CR, pyrroline-5-carboxylate reductase;  $\alpha$ -KG,  $\alpha$ -ketoglutarate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine. Adapted from Wu and Morris (1998).

Putrescine is formed by decarboxylation of ornithine and spermine and spermidine are obtained from putrescine in the presence of decarboxylated S-adenosylmethionine, derived from methionine, thus involving the participation of both amino acids, Arg and methionine. These biogenic amines are important for cell division, protein synthesis, and tissue growth (GONZALEZ-ESQUERRA; LEESON, 2006).

Polyamines are also considered nutritionally important for growth and development of the intestine in newborns (LÖSER et al., 1999). Furthermore, as polyamines are essential for the proliferation of endothelial cells, they are associated with angiogenesis and wound healing (HUI et al., 2002). Likewise, proline, a major amino acid in the synthesis of collagen and production of the extracellular matrix, plays an important role in vase remodeling.

Studies from the 1970's showed that the rate of conversion of Arg into proline is less than 10% of the required for normal growth in birds (AUSTIC, 1973). Later, these studies were confirmed by Wu et al. (1995), who demonstrated the absence or low activity of key enzymes in the production of proline in birds, reinforcing the essentiality of proline, mainly in the initial phase of development.

The tissue contents of polyamines in birds seem to be particularly responsive to dietary manipulation of amino acids, possibly due to the lack of a functional urea cycle (SMITH, 1981). Bedford et al. (1987)

observed an increase in the activity of renal arginase, followed by an increase in the availability of renal ornithine and an increase in the putrescine concentration after the Arg:Lys ratio was altered through Lys level increase. In contrast, high concentrations of ornithine can inhibit the activity of ornithine decarboxylase due to the fast formation and accumulation of putrescine (DIRCKS et al., 1986).

Bedford et al. (1987) observaram o aumento da atividade da arginase renal, seguido de aumento da disponibilidade de ornitina renal e elevação da putrescina, após alteração da relação Arg:Lys pelo aumento dos níveis de Lys. Por outro lado, elevadas concentrações de ornitina podem inibir a atividade da ornitina descarboxilase pela rápida formação e acúmulo da putrescina (DIRCKS et al., 1986).

This rise in the activity of renal arginase is also observed when great levels of Arg are supplied (STUTZ et al., 1971). The excessively fast degradation of Arg into ornithine and urea can compromise the synthesis of NO, which is the only physiological path of production of this compound. Additionally, according to Prabhakar et al. (1997), the rise in the plasma urea level can inhibit the action of nitric oxide synthetase (NOS), enzyme that catalyzes the reaction that converts Arg into NO.

#### Arg and the synthesis of nitric acid

Arg is converted into citrulline and NO stoichiometrically (1:1) by catalytic action of a group of isoenzymes called nitric oxide synthetases (NOS) (FÖRSTERMANN et al., 1991; GRIFFITH; STUEHR, 1995), this being the only physiological path of production of NO.

On the other hand, in uricotelic species, this reaction is the only path of endogenous synthesis of Arg, through the catalytic action of argininosuccinate lyase and argininosuccinate synthetase on citrulline. According to Su and Austic (1999), activated macrophages can recycle up to 20% of Arg derived from citrulline.

NO is a gaseous free radical capable of freely diffusing through the plasma membrane. It is highly reactive and is synthesized and used by endothelial cells, neurons, macrophages, and tissues, including the hypophysis (FEELISCH et al., 1994; HOBBS; IGNARRO, 1996; MARLETTA, 1993). Many roles have been attributed to NO, many of which are controversial.

The appearance of NO as a cell signaling agent is another important discovery in human and animal physiology of the last years. NO participates in several phenomena, such as vase relaxation dependent on the endothelium, cytotoxicity

mediated by macrophages, and inhibition of platelet activation, adhesion, aggregation. NO is the main cytotoxic mediator of immune-activated effector cells and is one of the most important regulating molecules of the immune system (HIBBS JR. et al., 1988; DUSSE et al., 2003).

The literature describes three isoforms of NOS, one induced NOS (iNOS or NOS 2) and two constitutive NOS, endothelial and neuronal (cNOS or eNOS 3 and nNOS 1). NO is produced by eNOS 3 in endothelial cells and works as a vase dilator, an angiogenesis factor, and a survival factor of endothelial cells (HOBBS; IGNARRO, 1996; DIMMELER; ZEIHNER, 1999). In the central nervous system, the NO is produced by neuronal form, the nNOS 1, which participating in the neurotransmission and the control of the blood flow in the brain (BRENMAN; BREDT, 1996). Both forms are  $\text{Ca}^{2+}$ /calmodulin-dependent (ADAMS, 1996) and are continuously expressed in the absence of inducing agents, with basal synthesis in picomolar concentrations. The half-life of gaseous NO and of NO dissolved in biological fluids is 3-4 s on average and it is rapidly converted into stable oxidation products, nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) (GRISHAM et al., 1999).

In contrast, iNOS does not depend on calcium for activation, but the synthesis of mRNA of iNOS is necessary for its activity. iNOS is not detectable in basal conditions. LPS or bacterial endotoxins, together with cytokines, such as  $\text{TNF}\alpha$ , IL-1 $\beta$  or IF- $\gamma$ , induces the synthesis of iNOS in 2-4h after exposure to the agent. As iNOS requires protein synthesis for its expression and as its activity lasts over 24h and it synthesizes NO in nanomolar concentrations, a concentration 1,000 times greater than that of cNOS, NO is the key cytotoxic element in the elimination of bacteria (HIBBS JR. et al., 1988; MACMICKING et al., 1997; MARLETTA, 1993).

The expression of iNOS results from either localized or diffuse inflammatory response resulting from infection or tissue damage. According to Salvemini et al. (1996), NO is a powerful vasodilator and its involvement in the inflammatory response can be related to its ability to increase vascular permeability and edema by changing the local blood flow and increasing the production of pro-inflammatory prostaglandins.

The expression of iNOS in endotoxemia is cytoprotecting, inhibiting microthrombosis by the prevention of the adhesion of platelets and of damages caused by free radicals. On the other hand, the NO cytotoxicity results from its direct action or



its reaction with other compounds released during the inflammatory process. The biochemical basis for the direct action of NO consists in its reaction with metals, especially iron, in the enzymes of its targets (MONCADA et al., 1991; JAMES, 1995). In this way, key enzymes are deactivated for the Krebs cycle, for the electron transport chain, DNA synthesis, and the cell proliferation mechanism.

In infectious process, activated cells, such as macrophages, neutrophils, and endothelial cells, simultaneously secrete NO and the reactive intermediates of oxygen; the indirect cytotoxic action of NO consist mainly of its reaction with these oxygen intermediates. As NO is a free radical, one of its electrons is unpaired and therefore reacts with molecules with this same characteristic. One of the most reactive derivatives is peroxynitrite ( $\text{ONOO}^-$ ), which is formed by the reaction of NO and the superoxide radical anion ( $\text{O}_2^-$ ), which oxidizes proteins, amino acids, lipids, and DNA, causing damage and cell death (LI et al., 2007).

In this way, a high NO concentration produced by iNOS can have deleterious effects on mammal cells and mediate the pathogenesis of many diseases. However, Hartman et al. (2006) reported that bird cells are less susceptible to the harmful effects of free radical attack because of their high levels of circulating uric acid.

Recently, uric acid has been considered an important antioxidant (HARE; JOHNSON, 2003), as it eliminates free radicals, chelates metals in transition, and blocks the action of peroxynitrites. As uricotelic species do not have the enzyme urate oxidase, which degrades uric acid into allantoin, uric acid can directly act as an antioxidant. The concentration of uric acid in the plasma of birds is exceptionally higher than those of other vertebrates, and because of that, the activation of iNOS, which raises the levels of NO, probably does not have the same consequences reported in mammals (HARTMAN et al., 2006).

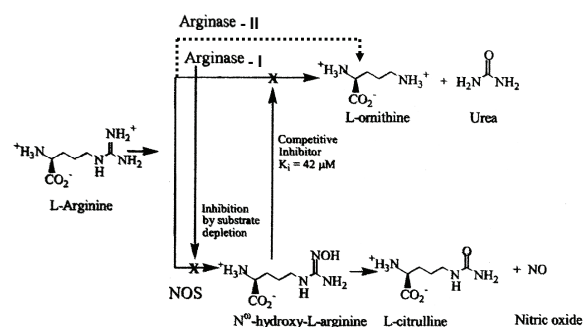
Another aspect to be considered in the metabolism of NO is the competition of arginase and iNOS for Arg as a common substrate that can lead to the reduction of the synthesis of NO in leucocytes. According to Wu and Morris (1998), although the affinity of iNOS for Arg is about 1,000 times greater than that of arginase, the velocity of reaction of arginase on Arg is about 1,000 greater than that of iNOS.

The competitive mechanism of enzymes in birds is little elucidated. Ash's (2004) revision discusses a reciprocal inhibitory mechanism between arginase I and iNOS in mammals that prevents the production

of overly high levels of NO. On the other hand the inhibition of the activity of arginase I would result in a high production of NO. In this way, arginase II would act by producing ornithine for the synthesis of polyamines and proline, which are necessary for tissue repair (Figure 6).

This consensual mechanism is extremely relevant for the functioning of the immune system. The activation of iNOS or arginase reflects the type of inflammatory response in diseases. For instance, infectious processes are associated with the predominance of iNOS, the release of NO, a microbicide substance and signaler of cell immunity, while trauma preferably induces arginase, which degrades Arg into polyamines and proline for tissue repair and fibrosis (BRONTE; ZANOVELLO, 2005).

In this sense, the supplementation of Arg through diet in immunocompromised birds could further increase the activity of arginase and result in less available Arg for the synthesis of NO (KEPKALLENHART et al., 2000). Alternately, Bansal et al. (2004) suggest the supplementation of citrulline, mainly in immunodeficiency conditions associated with high arginase plasma activity as a dietary strategy to restore plasma concentrations of Arg. In uricotelic species, the Arg-citrulline cycle is functional. Citrulline is not found in any body protein and birds cannot synthesize endogenous citrulline. Dietary supplementation is the only source of Arg for macrophages, through the NO-citrulline cycle (WIESINGER, 2001).



**Figure 6.** Relationship between arginase (I and II) and NOS activities.

Adapted from Ash (2004).

## Conclusion

The excessively fast degradation of Arg into ornithine and urea by arginase due to the high plasma concentration of Lys can compromise the synthesis of NO, this being the only physiological path for the production of this compound in birds.



Therefore, future studies of the metabolism of Arg in uricotelic species must seek a better understanding of the relationships between key enzymes, arginase and nitric oxide synthetase, which compete for the common substrate, which in turn can lead to the reduction of the synthesis of NO by leucocytes. This is particularly important with regard to situations in which the immune system is activated and high concentrations of NO are required.

In mammals, the plasma Arg homeostasis is influenced by the urea cycle, and consequently, not only by dietary Arg, but also by the nitrogen metabolism "status." On the other hand, birds basically depend on dietary Arg, and due to this direct relationship between dietary supplementation of Arg and the amount of Arg available for the metabolism, birds can be an invaluable model in the study of the effects of Arg in the immune response.

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