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IMPORTANCE OF ARGININE AS IMMUNE REGULATOR IN ANIMAL NUTRITION

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ABSTRACT

Arginine grouped in a gluconeogenic amino acid extensively found in the animal's cells. Fast growing animals require more arginine in their diet as broilers and pigs. It plays a critical role in ammonia detoxification and protein biosynthesis. Arginine is not only involved in the synthesis and catabolism of a variety of nutrients in the animal body but mainly act as an immunomodulatory mediator. Along with nutritional function has immune-related functions. In urea cycle act as an important intermediate and also catalyzes the production of ornithine by enzyme arginase and converted into polyamines that involved later in cell proliferation and wound healing. Nitric oxide synthases catalyze the production of nitric oxide from arginine and NO is a cellular signaling molecule for immune regulation. Many hormones secretion increased by arginine particularly the growth hormones which could enhance the immune function. Arginine metabolism pathway and its downstream metabolites like nitric oxide and polyamines might be vital for the activation of T-cell and ultimately take part in adaptive immunity. For immune regulation, arginine could stimulate the production of different immune cytokines. In this paper, in the perspective to provide significance reference about arginine in the immune study, the arginine immune mechanism and use of arginine in animals will review.

Contribution/Originality: Present study has contributed significant scientific information on a particular subject area "arginine as an immune regulator". Many researchers have discussed the arginine as feed ingredients in the animal diet, but less scientific data is available related to immune functions of arginine. Therefore, this study is one of the very few studies which have reported that arginine use as an immune regulator in animal nutrition.

1. INTRODUCTION

Arginine as a basic amino acid consumed in diet as naturally was discovered over 100 years ago. Also some other food products contains arginine as constituents but in nuts, watermelon juice, seeds, algae, rice protein concentrate, meats, and soy protein isolate and seafood are rich in arginine [1]. First time in 1886 by German scientist, arginine was isolated from lupin and pumpkin seedling [2]. It was reported that arginine is essential amino acid for optimal growth as well as for the maintenance and nitrogen balance in newborn suckling piglets [3]. In young rodents it was reported to be essential for their optimum growth [4]. Arginine has a wide range of

biological function, which is not only an important raw material for the synthesis of proteins, but also it is a precursors of substances such as polyamines, creatine and NO (nitric oxide) in the body [5]. In addition, arginine is anti-inflammatory, antioxidant and also reduces tissue radiation and oxidative damage and improve the body cellular functions in young animals. Also plays an important role in body immunity. Poultry chickens are entirely relay on dietary arginine as chicken cannot synthesize arginine to fulfil their body needs for protein synthesis and other immunological functions. It was reported that chicken can't synthesis arginine by urea cycle due to lack of enzymes carbamoyl phosphate synthetase I (catalytic glutamine and glutamate synthesis of citrulline) and synthesis of -5-carboxylic acid synthase (catalytic proline and aspartic acid turn into glutamate) and some other key enzymes. Therefore as a result, arginine dietary need is higher in birds than in growing animals [6]. Adult animals are able to synthesize essential amino acids on their own under normal circumstances. But in the state of hunger, trauma, stress and rapid growth, body synthesis of arginine is not enough to cope with the requirement of the animal body. Thus, arginine is also considered to be a conditionally essential amino acid [7]. Arginine has a wide range of biological functions and participates in tissue cell proteins formation within the body.

Moreover, also take part in the synthesis of urea, creatine, creatinine, NO, glutamine and pyrimidine [8]. In recent years, it has been found that arginine can pass NO pathway to participate in the body's nutritional immunomodulation, hence arginine as an immunomodulatory mediator has become a hot topic in animal nutrition studies. This review presents the role of arginine in the immunity of that we have found to drive the survival of mammals and birds in health as well as in diseased condition.

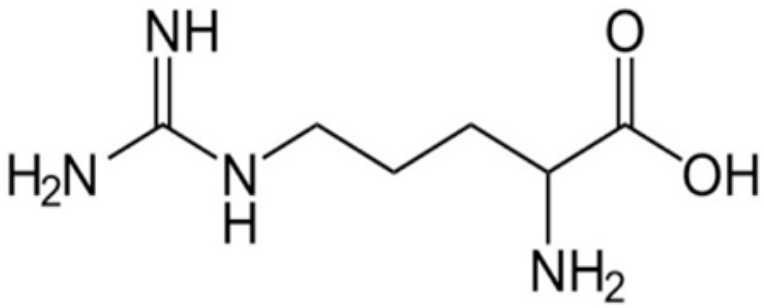

Figure-1. The structural formula of Arginine [9].
Source: McDonald, et al. [10].

Arginine is essential to the body's production of nitric oxide, a key element in proper circulation. However, an amazing benefit of L-arginine is its ability to restore sinus rhythm, or the normal beating of the heart.

2. BIOSYNTHESIS AND METABOLISM OF ARGININE

Arginine in the body mainly comes from endogenous synthesis, food protein, and body protein turnover metabolism. About 40% of the arginine in the diet is decomposed and digested in the small intestine, and the rest enters the body circulation, which is the primary source of arginine in the body [9]; When animals fast, 80% of arginine in the body comes from the decomposition of the body's proteins; some other amino acids, such as glutamate, proline, and citrulline have the ability to convert or synthesize arginine. The kidneys are the leading site for the synthesis of arginine. Intestinal-renal axis is mainly involved in the endogenous biosynthesis of Arg in adult animal bodies. In adult animals, the small intestine is the leading site for the synthesis of citrulline. Citrulline is produced or absorbed in the mitochondria of intestinal epithelial cells from glutamine, proline or glutamate, absorbed from the intestine and enters the kidney for the synthesis of Arg. While in neonate animals, enzymes are present those are responsible for the production of Arg from this citrulline and carbon dioxide, ammonia and L-ornithine [11]. Entertainingly, the liver is not so much active organ for Arg extraction from blood because the liver uptake of citrulline is insignificant. For that reason, in pigs, approximately 100% citrulline and 90% Arg come from the intestine, bypass the liver [12]. Organ only plays a part in the urea cycle, under the action of transaminase, urea

and ammonia in birds were produced without entering the blood circulation. Two major enzymes arginine-succinate synthase and arginine-succinate lyase are involved in the production of Arg [13]. First, arginine-succinate synthase enzyme converts citrulline into arginino-succinate, and this will be converted into Arg by the enzyme arginine-succinate lyase [14].

2.1. Metabolism of Arginine

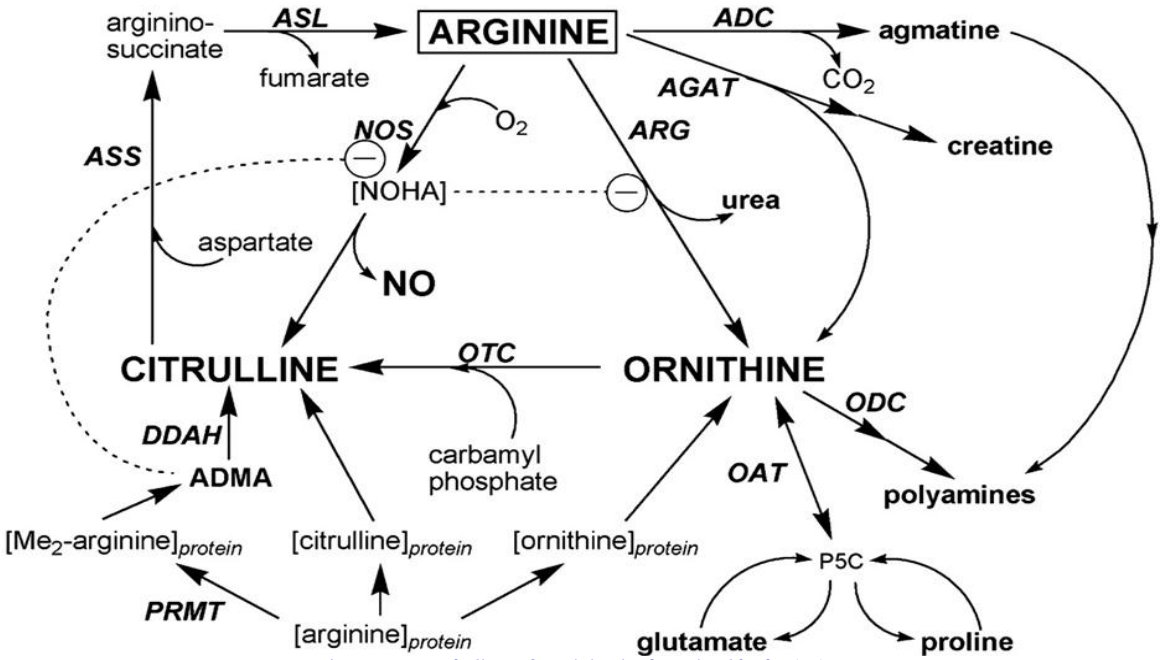

Figure-2. Metabolism of Arginine in the animal body [14].

Arginine from diet is absorbed in the small intestine and then taken up from the circulation by CAT into the liver, kidney, and endothelial cells. In addition dietary intake, arginine availability depends on endogenous release through protein degradation, on synthesis from citrulline by the enzymes.

3. IMMUNE MECHANISM OF ARGININE

Arginine is an essential nutrient for the growth and maturation of T cells. In normal healthy animal there is no need for additional Arg in diet, for T cells to play their immune functions, but in some pathological conditions, such as traumatic abdominal sepsis, infection and tumor, the myeloid suppressor cells (MSC) synthesized and theses cell could increase the synthesis of arginase enzyme to breakdown Arg in these pathological tissues. When Arg become deficient in the body, the T cells immune functions will disturb. In-vitro cell culture test showed that when the concentration of Arg was greater than 100Mmol/L, it could stimulate the T-cells proliferation and its function. Furthermore, there was an increases in cytotoxic T cells (CD8+) proliferation and memory T cells was also increased, when Arg is added to the culture medium. But secondary T cells (CD4+) do not depend on Arg [15]. Studies have shown that arginine promotes the proliferation of T cells by increasing the expression of CD8, CD3 receptors and increasing the synthesis of IL-2. When arginine in macrophages, metabolized in large quantities by arginase-1, this may be hinder the proliferation of T cells. Arg deficiency could affect the efficiency of T-cells as antigen presenting cells. Arg deficiency also downregulate the chain synthesis of CD3ζ and decrease in CD3 ζ mRNA or a reduction in its half-life [16]. The lack of arginine also inhibits the synthesis of RNA binding protein but does not inhibit the expression of mRNA. It can be combined with the 3'-end untranslated region (UTR) of D3 (cyclin D3) mRNA to enhance the stability of its mRNA, thus the balance of cyclin D3, suppress the T cells in the cell stage of G0 ~ G1, and the cellular phase of the GCN2 kinase signal pathway can be identified. The lack of

arginine leads to an increase in the expression of cyclin D3 and cyclin-dependent kinase 4 (CDK4), which in turn reduces the expression and phosphorylation of retinoblastoma proteins and binds to transcription factor (E2F1), and ultimately inhibiting T-cell proliferation [17]. The Arg deficiency mostly occurs due to an increase in the breakdown of Arg by MSC, which causes arginine to be metabolized in the macrophages. Interleukins IL-4 and IL-3 could be responsible for enhancing the expression of arginase-1 and cationic amino acid transporter 2B (CAT-2B), as CAT-2B can strengthen the metabolism of Arg in macrophages cells. In addition to the above effects, the increase in arginase-1 can also promote polyamine functions. The main function of polyamines is to promote cell differentiation and collagen synthesis, demonstrated in the body to promote wound healing [18].

4. IMMUNE REGULATION THROUGH NO-MEDIATED

An important metabolic pathway of arginine is the formation of NO under the action of iNOS enzyme in a normal healthy subject, while in some inflammatory and pathological conditions, such as coccidiosis, the expression of iNOS enzyme can be increased. NO is a biological messenger produced by specific tissues and cells in the body. On the other hand, Arg is the only a substrate for the synthesis of NO. Studies have shown that NO has a complex immunomodulatory effect in animal body [15]; [19]. The decline in the relative weight of lymphoid organs (spleen, thymus, and bursa) was observed when feeding poultry diet deficient in Arg to chicken Kwak, et al. [20]. Deng, et al. [21] detected that the improvement in primary antibody levels was significant against sheep red blood cells when increasing Arg level from 100% of NRC requirement (1994) to 130% of NRC requirements in chicken. Provision of about 0.3% extra Arg level above the NRC (1994) requirements for poultry significantly improve the titers of antibody against sheep RBCs [22]. It was studied that birds fed Arg 0.06% attained a significant proliferation in the number of a white blood cell. Consequently, Arg directly or indirectly by its metabolites can improve the immune response of chicken [23].

i. No Inhibits the Adhesion and Aggregation of Platelets

NO in the vasculature, regulates the flow of blood and vascular tone by the activating soluble guanylate cyclase in the smooth muscle of blood vessels. Moreover, it is essential for the migration or adhesion of platelets and leukocytes. By inhibiting enzyme cytochrome-C oxidase, it regulates the oxygen consumption of the mitochondria. Irregularities in NO production or transport in the blood may result in endothelial dysfunction with numerous cardiovascular abnormalities such as angiogenesis-associated disorders, hypertension and atherosclerosis, and so on Chen, et al. [24]. Studies have shown that NO can inhibit the adhesion of platelets to endothelial cells to a certain extent, mainly because, NO inhibits the expression of endothelial cell adhesion factors, such as cell selectin-E (CD62E), cell selector-P (CD62P) and endothelial cell adhesion molecule 1 (VCAM-1) and some others. NO can also inhibit the expression and function of neutrophils. In some inflammatory reactions, NO can regulate the nitrification of tyrosine by enzymes and some intracellular chemotactic signaling pathways to regulate the expression and activity of chemokines, and adhesion of neutrophils will also be controlled by iNOS activity [24]; [25].

ii. No is Resistant to Microbial Infections

NO and iNOS are essential in innate immune responses to several bacterial infections within the animal cells, including mycobacterial infections. It is thought that reactive nitrogen intermediates play an active role in host defense mechanisms against diseases (tuberculosis) [26]. The study found that NO can kill invasive microorganisms to some extent and relieve the symptoms of infection, but its exact mechanism is not entirely clear, and there may be so many ways. Studies have shown that NO can be used to regulate pathogenic microbes. NO regulates the microbial metabolism to inhibit its activity or to lose its activity directly, thus resisting microorganisms. In microorganisms, NO can be combined with ferritin and form a complex that inhibits the

activity of bacterial enzymes, which in turn inhibits the synthesis of ATP and DNA in cells and plays an antimicrobial role [27]. Another possible mechanism is that NO can interact with oxygen to produce stable free radicals (peroxynitrite), which may modify the NO functions and have the direct role of killing microorganisms. In some cells (monocytes and activated macrophages) can produce NO, but also provide some oxygen as free radical, the combination of these two produce active free radicals. For example, the combination of NO and H2O2 causes release of Fe2+ from bacterial DNA double-stranded, and the depletion of the antioxidant glutathione, leading to the death of bacteria. The interaction between NO and O2 can produce nitrogen peroxide; although it is not a free radical, it has a strong oxidation effect [27]. Also, nitric oxide can cause tyrosine nitration which is mediated by reactive species such as peroxynitrite anion and nitrogen dioxide, which acts as a killing of microorganisms.

iii. No-Mediated Toll-Like Receptor Signaling Pathway Relieves Inflammation

NO act as mediated in Toll-like receptor (TLRs) signaling pathway to relieves inflammatory response. TLRs are recently well studied and recognized as receptor family which mediates natural immunity in the animal body. The extracellular TLRs mainly includes TLR1, TLR2, TLR4, TLR5 and TLR6, which can form homologous dimer themselves or form heterologous dimer with other TLRs, which is used to identify the specific components of microorganisms, and then transmit the signal to NF-κB or MAPKs (mitogen-activated protein kinases), which in turn produces TNF-α, Cytokines such as IL-1, IL-2, IL-12 and IFN-γ are involved in immunomodulation and therefore play an essential role in the animal defense against pathogen infection [28]. For example, IL-6 is a cytokine that produces in response to the activation of the TLRs receptor outside the cell membrane, which is an inflammatory transmitter that provides a large number of inflammatory reactions in the body. It is found that adding a specific dose of Arg to the diet or culture system will produce more NO, which can significantly reduce the excessive expression of tissue or cell TLR4 and TLR5 and its downstream signaling molecules MyD88, p65, NF-κB, etc., and inhibit the excessive activation of the TLR4-MyD88 signaling pathway. In turn, the production of inflammatory factors such as IL-6 and TNF-α is reduced, and the inflammatory response is alleviated. NO is a key signaling molecule that mediates the TLR4 signal pathway of Arg regulation under immune stress, but its specific mechanism of action has yet to be further studied [29].

NO can resist tumor and regulate apoptosis. NO regulate the apoptosis by two ways; low doses of NO can induce the expression of heat shock protein and have anti-apoptotic effect, while high dose of NO treatment can cause the loss of mitochondrial cytochrome C by increasing the cell membranes permeability of mitochondrial cells, which leads to the occurrence of apoptosis [30]; [31]. In some pathological conditions, the inflammatory response will increase the expression of iNOS. The increase of iNOS expression leads to the addition of NO synthesis of the body, thus inducing the occurrence of intestinal apoptosis. However, apoptosis is also a self-immune defense response of the body; the body can be through apoptosis to clean up the damaged tissue, and can effectively remove the pathogenic bacteria from the cells. NO can increase tumor-inhibitory factor P53, change the expression of apoptosis-inducing factor Bax and anti-apoptosis factor Bcl-2 family, lead to the release of cytochrome C, protease activation, chromatin condensation, and DNA cracking to promote cell apoptosis. At the same time, NO also has the function of inhibiting tumor growth and promoting tumor cell death. Cellular toxin lymph nodes can secrete IFN-γ and TFN to stimulate the expression of iNOS in tumor cells and play an anti-tumor role [32].

5. ROLE OF ARG IN HORMONES AND CYTOKINES SECRETION

Arg stimulates the secretion of growth hormone, prolactin, insulin, placental lactogen, and glucagon [33]. Arg can promote the secretion of some hormones and cytokines to regulate immunity by the production of immunoglobulins; these immunoglobulins play important role in humoral immunity of the body. L-Arg can promote the production of specific secretory immunoglobulin (sIgA) in the intestinal associated lymphatic tissue (GALT), which can enter the gut selectively coated with gram-negative bacteria, forming antigen-antibody

complexes that prevent bacteria from attaching to the epithelial cells, while stimulating intestinal mucus secretion and accelerating the flow of mucous layers, It can effectively prevent bacteria from sticking to intestinal mucosa [34]. Arg has the ability to improve nitrogen balance, and especially the biosynthesis of functional proteins. Arg can improve the number and percentage of monocytes, the percentage of lymphocytes and their proliferative activity, significantly increase the level of spleen IL-2 and IFN-γ expression, and reduce the level of TFN-α expression. Arg can significantly enhance the secretion of growth hormone, insulin, glucagon, corticosteroids, and other hormones. The flow of these hormones is involved in the maintenance of the body's internal nitrogen balance and internal environment stability, as well as play an essential role in the improvement of the body immune function [1].

6. APPLICATION OF ARGININE IN ANIMAL PRODUCTION

Arg is an essential amino acid for its physiological and pharmacological properties, because of these abilities Arg become essential and hotspot in animal nutrition research. Its application in animal production is mainly focused in pigs and poultry, and in recent years has been used in rabbit research, while the application in cattle is primarily focused on male reproduction. Chicken is unable to synthesize arginine due to the lack of methotrexate in the body, so arginine is an essential amino acid in poultry. Coccidiosis is a common disease in poultry production, mainly caused by the infection of the small intestine in poultry, which will cause the loss of poultry appetite, production can be reduced, and even cause hemorrhagic molecule like NO is produced as a result of Arg metabolism [35]. Synthesis of NO increased significantly in *Eimeria* (*E.tenella*, *E.maxima*, and *E.acervulina*) infection in poultry [36]; [37]. Significant declined in plasma Arg level was observed in birds infected with *Eimeria acervulina* [37]. Heat stress can cause a physiological and metabolic disorder in the animal body. As a result, the formation of a large number of free radicals and the body's antioxidant function becomes compromised, resulting in tissue damage [38]. The activity of antioxidant enzymes in the body can indirectly reflect the strength of antioxidant capacity. Adding 0.5% Arg to the diet can significantly improve the activity of antioxidant enzymes in the serum of broiler under heat stress condition, which shows that Arg can improve the antioxidant ability of heat stress broiler, relieve the degree of lipid peroxidation of muscle and also reduce the harmful effects caused by heat stress [3]. Also, studies found that the lack of arginine in the diet significantly reduced the weight of the thymus ($P < 0.01$) and spleen ($P < 0.05$) in the broiler, reducing the proportion of heterophils in peripheral blood and reduced in antibodies titers against Newcastle disease [39].enteritis, so that the intestinal wall thickening, fall off mucous membranes, and then cause poor absorption. Addition of Arg at the level of 0.3 or 0.6% with vitamin E in a poultry diet brings significant improvements in the production of serum antibodies (IgG and IgM) and also significant increase was observed in heterophils and monocytes oxidative burst activity in the birds that infected with *Eimeria* (*E. tenella*, *E.maxima* and *E. acervulina*) [40]. The results showed that the addition of Arg could improve the humoral immunity level of broiler and enhance the resistance to coccidiosis [34]. A free radical molecule like NO is produced as a result of Arg metabolism [35]. Synthesis of NO increased significantly in Eimeria (E.tenella, E.maxima, and E.acervulina) infection in poultry [36];[37]. Significant declined in plasma Arg level was observed in birds infected with Eimeria acervulina [37]. Heat stress can cause a physiological and metabolic disorder in the animal body. As a result, the formation of a large number of free radicals and the body's antioxidant function becomes compromised, resulting in tissue damage [38]. The activity of antioxidant enzymes in the body can indirectly reflect the strength of antioxidant capacity. Adding 0.5% Arg to the diet can significantly improve the activity of antioxidant enzymes in the serum of broiler under heat stress condition, which shows that Arg can improve the antioxidant ability of heat stress broiler, relieve the degree of lipid peroxidation of muscle and also reduce the harmful effects caused by heat stress [34]. Also, studies found that the lack of arginine in the diet significantly reduced the weight of the thymus ($P < 0.01$) and spleen ($P < 0.05$) in the broiler, reducing the proportion of heterophils in peripheral blood and reduced in antibodies titers against Newcastle disease [39].

Role of Arg in Pigs Production: Although in adult pigs, Arg is a conditional amino acid, experiments proved that it has a positive effect in all stages of pig growth. Piglets have an essentially higher requirements of Arg for their growth and metabolic functions. Because the ability of piglets to synthesize arginine is limited, and the milk of sows cannot provide enough arginine to meet the growth needs of piglets, which will lead to the damage of microvascular endothelial function of the body, the decline of intestinal digestion and absorption function, affect its growth performance, so arginine is the essential amino acid of newborn mammals [33]. I was found that adding appropriate amount of Arg to pig diet can significantly improve the antioxidant function of the body, in the event of stress, improve the level of arginine addition can ensure the normal intake of piglets, enhance the antioxidant capacity of piglets, and alleviate the adverse effects of oxidative stress on animal production [41]. Furthermore, found that Arg can promote the proliferation of small intestinal epithelial cells, increase the height of intestinal villi, improve weaning weight of piglets, reduce mucosal barrier damage, and control the metabolism of intestinal bacteria. Study revealed that the weight of duodenum, the weight of duodenal and ileum mucosa, the content of ileum mucosal DNA and protein, the height of ileum villi, and the cell proliferation index of ileum and jejunum cells were significantly improved compared to those in the control group that did not add Arg Sukhotnik, et al. [42]. Kim, et al. [43] added the Arg 0.2% and 0.4% through the milk feeding of piglets at the age of 7 to 21 days feeding. The results showed that the concentration of Arg in plasma increased by 30% and 61% respectively, and the body weight gain increased by 28% and 66%, indicating that 7 to 21 day. The addition of 0.4% Arg to the diet of day-old piglets has a significant effect on its body. Adding 1% Arg can increase the daily weight gain of growing fattening pigs, promote body tissue protein deposition, increase the content of protein and fat in the muscle, reduce the concentration of muscle lactic acid, and thus increase the carcass weight [44]. Furthermore, adding the right amount of arginine to fattening pig diet can improve meat quality, increase the content of intramuscular fat, reduce drip loss, and improve muscle tenderness, but the best effect when the added amount of Arg was 1%. The application of arginine in sows is mainly reflected in improving the reproductive performance of sows, and every nest of piglets is an important index to measure reproductive performance, mainly because Arg can promote the release of prolactin and growth hormone, while growth hormone has a positive effect on animal ovulation and reducing the death and loss of fertilized eggs. The addition of 1% Arg to the diet of sows during pregnancy and lactation has a potentially positive effect on the lactation performance of first-stage sows. At the same time, it was found that adding 1% arginine to the diet could increase the number of sows ' births by 22%, which could increase the number of live births by an average of two, and increase the weight of live birth by 24% [45]. Adding 1% Arg to the diet of sows in the later stages of pregnancy could improve the production performance of sows, possibly because Arg could regulate the expression of their respective genes by affecting the expression of umbilical vein and placenta in pigs. In order to regulate the umbilical vein and placenta angiogenesis, late development and functional improvement, through the umbilical vein and placenta from the mother to provide more nutrients to the fetus, improve the survival rate of the fetus and physical health status.

7. SUMMARY

At present, the research and application of Arg in various aspects are mainly focused on pigs and chickens, and also relatively few applications are in other animals. Application of Arg in animal nutrition are still need detail and further studies, not only from the synthesis process of Arg, to reduce the cost improvement process; it is also necessary to deeply study the functions of Arg in different livestock and poultry and different growth stages, quantify the optimum amount of different growth and development stages. For achieving better growth performance of animals Arg is important amino acid. It also provides a theoretical basis for feed production. In short, the application of Arg in animal nutrition has great prospects for development and research in animal nutrition.

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