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Understanding functional benefits of Isoleucine and Arginine on poultry performance and health.

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Introduction

Poultry industry is rapidly moving towards utilizing the best ingredients to precisely feed the birds for better performance, welfare, health, and profitability. One of the practices that evolved over time with research is better optimization of amino acid utilization in the diets thus changing the crude protein landscape. Historically, if we compare the modern diets to diets from 1980, significant changes are visible in the use of unbound or crystalline amino acids leading to reduced levels of CP and reduced unutilized nitrogen in the diets. Multiple feed-grade amino acids are available in a cost-effective manner in poultry diets. Multiple research has been conducted over 80 years to better understand the optimal requirement of lower limiting amino acids in poultry, formulation with digestible amino acid values, and ideal amino acid formulation to achieve precision feeding of nutrients. Those findings enabled the nutritionist to incorporate crystalline amino acids, remove CP minimum, and crystalline amino acid maximum in the diets to lower the feed cost, reduce CP, reduce excess nitrogen in hindgut and improve the health of the birds and improve litter quality (Kidd 2000; Maia et al., 2021). A 1960 diet would only contain the Methionine as synthetic amino acid, whereas the 2023 diet would contain up to 5/6 limiting amino acids in USA-based broiler diets. The 4th, 5th and 6th limiting amino acids in USA-based poultry diets are usually Valine (Val), Isoleucine (Ile) and Arginine (Arg) depending on the ingredients used. With almost 90% of the industry using the 4th limiting amino acid, there is a need to understand the optimal requirement of 5th and 6th limiting amino acids for performance and other biological functionality. With Ile and Arg being the 5th and 6th limiting

amino acid in most broiler, turkey, and layer diets, this article will discuss the importance of Ile and Arg in birds and the effect on performance and health.

Isoleucine

Isoleucine is a six-carbon essential aliphatic amino acid that is a part of the branch chain amino acid (BCAA) along with Valine and Leucine (Leu). Isoleucine along with Val and Leu account for about 22% of the total body protein content and about 35% of the dietary essential amino acid in skeletal muscle mass (Harper et al., 1984). Ile including BCAA enters the blood circulation from the intestine and mostly escapes the hepatic metabolism and appears in the systemic circulation (Brosnan and Brosnan, 2006). When the circulating volume of BCAA is measured at arteriovenous exchanges, muscles absorb about 50% and the splanchnic bed absorbs about 25% of the circulating BCAAs and the remainder is used by brain and other tissues (Fernstorm 2005; Bifari and Nisoli 2017). As BCAA are hydrophobic they are involved in the formation of alpha helices and beta sheets of proteins. Apart from muscle protein synthesis, Ile is also involved in reproduction, antioxidant capacity, hormonal regulation, innate and adaptive immunity regulation, and glucose and lipid metabolism.

Isoleucine being a part of the branch chain is highly affected by the amount of other Val and Leu in the diet. Branched-chain AA are structurally similar and share the first steps in catabolism. Excess of any one of the BCAA can result in increased degradation of the other two members of BCAA (Harper et al., 1984; Maynard et al., 2021a, Maynard et al., 202b; Kidd 2021a; Kriseldi et al., 2022). Normally in corn, SBM-based diets, Leu is over the requirement for the birds. This can result in increased catabolism of Val and Ile and cause marginal deficiency as we use 4th or 5th crystalline limiting amino acid. Thus understanding the effect of different levels of Leu in the diet on the requirement of Ile and Val is important not to lose the performance, health and welfare of birds. This understanding can change the antagonism to synergism. As we look at the ratio of Ile to Leu in the corn, SBM, DDGS, wheat, and other alternate ingredients, SBM has the lowest ratio of Ile to Leu (Fig 1). However, as our diet moves from Starter to grower to finisher, we reduce the SBM and include more corn, DDGS, and wheat to increase energy in the diet. This leads to a higher dLeu:Lys ratio in later diet and a wider Ile:Leu increasing any possible antagonistic effect. Every BCAA research concludes that higher Leu level in the diet hampers

the FCR, feed intake, and BWG however the magnitude of the effect can differ between breeds, sex, age, and amount of other 2 members of BCAA (Ile and Val) present in the diet (D'Mello and Lewis, 1970a; D'Mello and Lewis, 1970b; Wessels et al., 2016; Maynard et al., 2021b; Kidd et al., 2021b; Kriseldi et al., 2022). However, Leu should not always be considered a nuisance for performance. Leu can directly affect the mTOR pathway to affect metabolism, and improve protein synthesis and white muscle yield. Utilizing the Ile and Val to balance the higher Leu provides an opportunity to increase the performance of the birds.

In the past 5 years, a good amount of research has been published from several research labs in different parts of the world to shed more light on the importance and balance of Ile and Val based on the Leu level in the diet. A better understanding of the level of Ile needed for optimized performance depends on the Val and Leu level in the diet, genetics of birds, sex, and performance parameters measured. Further questions need to be asked if the BCAA antagonism will differ based on the diet type, calorie level in the feed, and high vs. low CP. In a series of experiments conducted by Burnham et al. (1992) and Kidd et al. (2004) weight gain of broiler decreased as the Ile content of the diet decreased, however, the feed intake on a marginally deficient diet increased to the maximum then decreased. Corzo et al. (2010) evaluated the limitations of Ile and Val in corn, SBM, and MBM diets for broilers. The study suggests that abdominal fat was reduced with the combined supplementation of Ile and Val, breast meat was sensitive to Ile whereas live performance was sensitive to Val level in the diet. Kriseldi et al., (2022) reported similar findings on the effect of Ile and Val supplementation on higher Leu levels in the diet. Looking at the Ile, Leu, and Val together is the absolute way to understand the effect of these BCAA. However, titration studies also need to be conducted to validate the result of the three-way interaction studies as well as to understand where the starting points are for those individual amino acids. A series of experiments were conducted on 0-18d, 14-28d, and 28-42d broilers to determine the optimal digestible Ile:Lys ratio on BWG, FCR, and yields (Brown et al., 2021a; Brown et al., 2021b; Brown et al., 2022). Most of the ratios of dIle:dLys ranged between 62 and 74. The ratio could vary depending on the parameter of measurement, the ratio of Val and Ile in the diet, age of the bird. A series of experiments have been conducted to evaluate the interaction, synergism, and antagonism of Ile, Val and Leu in broilers (Kidd et al. 2021b; Kidd et al. 2021c; Maynard et al. 2021a; Maynard et al. 2021b). The studies suggest that

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the optimal level of Ile is not a fixed number but more depends on how Val and Leu play an interactive role in the trio effect. It also depends on the desired outcome such as BWG, FCR, or processing yield.

Research in humans and mice has been conducted to evaluate the functional roles of Ile and BCAA on glucose metabolism, fatty acid metabolism, and immune function. Further research needs to be conducted to understand how the BCAA affects avian metabolism beyond protein synthesis. Further research utilizing the existing data on advanced modeling and validation studies can help to improve the accurate prediction of the Ile level according to the Val and Leu level to get the best out of the present BCAA in the diet. As dietary Ile in the diet changes, accounting for the level of Val and Leu may be necessary to achieve the optimized response.

Arginine

Arginine plays a crucial role in numerous metabolic and immunology pathways in poultry (Figure 2). Arginine is considered an essential amino acid in poultry as they are unable to synthesize endogenous L-arginine because of the lack of a functional urea cycle and therefore, the diet must provide the optimal level to realize the genetic potential of the modern broiler. Only recently, a feed-grade source of L-arginine has become available as an economically viable source of dietary arginine for nutritionists to utilize in current formulations.

In modern commercial poultry diets, arginine could potentially be the 4^{th}, 5^{th}, or 6^{th} limiting amino acid depending on dietary ingredient profile. If arginine is the 4^{th} limiting amino acid, a cost-saving opportunity exists with the use of L-arginine in addition to the benefits of decreasing dietary crude protein. If Arg is the 5^{th} or 6^{th} limiting amino acid, care should be taken to make sure dArg:Lys ratio is above the optimal requirement for birds while using the 4^{th} limiting amino acid. Diets that contain ingredients low in arginine as compared to valine and isoleucine as relative to requirement, such as corn DDGS, bakery meal, and corn gluten meal, are where nutritionists need to observe their arginine ratio in the diet to improve functionality, reduce diet cost and reduce crude protein.

L-arginine has garnered recent interest due to its role in vasodilation, blood flow, nitric oxide production, gut health, and a potential benefit on meat quality and reproductive performance.

The recent introduction of feed-grade L-arginine provides nutritionists the ability to increase the arginine ratio in an economically viable way without increasing dietary crude protein compared to increasing Arg form-bound protein sources. Recently multiple published reports have linked elevated levels of arginine with reductions in breast muscle myopathies (Bodle et al., 2018; Zampiga et al., 2019; Anderson et al., 2023; Brugaletta et al., 2023). During these investigations, additional benefits on growth performance were also observed. Bodle et al., (2018) reported an increase of 40 grams of body weight and 2 points reduction in feed efficiency at 36 days of age with increasing the ratio from 105 to 125. Similarly, Zampiga et al., (2018) observed an increase of 65 g of body weight and a 3-point improvement in feed efficiency with an arginine ratio increase of 10% at 43 days of age (Figure 3). These results are similar to data reported by Corzo et al. (2012) in which a 114% Arg ratio was found to optimize feed efficiency during the starter phase of production.

Arginine plays an important metabolic role in broiler and Turkey growth. The mechanism of action of Arg in a lot of pathways has not been fully elucidated in chickens and warrants more research. Supplementation of Arg increased growth hormone (GH) and insulin-like growth factor (IGF-1) gene expression and mTOR pathway stimulating the growth of poultry (Yao et al., 2008; Wu et al., 2011; Castro et al., 2019; Castro et al., 2020; Brugaletta et al., 2023). Arg also plays an important role together with Glycine in the synthesis of creatine to support muscle growth (Oliveira et al., 2022). Arginine through the ornithine pathway contributed to collagen synthesis improving the structural and elasticity of animal skin, and reducing skin scratch infections and parts defects from processing stress (Corzo et al., 2003; Oviveira et al., 2022). This may lower the condemnation in processing plants and improve grade A carcass. Arginine supplementation increased lean deposition of muscles and bone mineral density in broilers (Castro et al., 2019).

The benefits of a higher level of arginine has been reported in the improvement of gut health and disease challenge (Castro et al., 2020; Brugaletta et al., 2023). Barekatain et al., (2019) reported that feeding L-arginine to broilers increased body weight, reduced feed conversion, and increased villi surface area. Similarly, during periods of pathogen challenge, higher levels of dietary arginine can attenuate the overexpression of pro-inflammatory cytokines (Tan et al., 2013; Anderson et al., 2023) and increase jejunal villus height (Tan et al., 2014), thus improving digestibility and conserving nutrients for growth during an active immune response. Arginine

improved the intestinal permeability of birds challenged with different *Eimeria* species and upregulated the expression of Zonula occludens1 and Zonula occludens 2(Castro et al., 2020; Teng 2021). Additionally, Tan et al. (2015), determined the dietary arginine level required for maximal performance increased by 15% in infectious bursal disease vaccine-challenged broilers as compared to non-challenged control broilers.

Higher dietary arginine concentration can also be beneficial to reproductive performance. Increasing digestible arginine levels by 0.15% increased egg production in broiler breeder hens by 4% over a 30-week experimental period (Silva et al., 2012). Similarly, Duan et al., (2015) reported a 4% increase in egg production in 60-week-old broiler breeder hens during a 9-week experimental period with an increase of 0.20% in dietary digestible arginine level (Figure 4). Duan et al., (2015) attributed the increase in egg production with increasing digestible arginine to effects on the ovaries and ovarian follicles to stimulate the exudation of luteinizing hormone in the Pituitary gland. Additionally, increasing dietary arginine levels could be beneficial for breeder males. Administration of L-arginine has been shown to increase sperm count and motility in mammals. Sperm are known to be particularly susceptible to lipid peroxidation which impairs functionality and mobility and thus stimulating the production of nitric oxide with L-arginine could inactivate superoxide anions and improve sperm quality (Srivastava et al., 2006).

Conclusion

As the poultry industry adjusts management and nutritional practices based on consumer demand, market development, and scientific/research guidance it may be necessary to re-evaluate and refine our nutritional programs and recommendations. The development of feed-grade lower limiting amino acid and a better understanding of amino acid optimal levels provides nutritionists an opportunity to reduce diet cost, reduce crude protein, reduce disease challenges, and enhance performance and reproductive performance of poultry.

Understanding the 4^{th}, 5^{th} and 6^{th} limiting amino acids in the diet, their optimal requirement can support the best performance. The optimal value of Ile depends on the Leu and the Val level in the diet, ingredients available for diet formulation, performance parameter that is targeted to

achieve in the program, and the strain of birds used. An increase in the Arg ratio can improve feed intake and body weight gain in poultry. The scientific community has just recently begun to unlock and understand the benefits of dietary arginine in modulating an active immune response and enhancing intestinal maturation, health, and permeability. Performance improvement through Arg supplementation can vary depending on nutritional program and management strategies as Arg has numerous biological functions and participates in multiple biological pathways. A better understanding of the lower limiting amino acid functionality and requirement can support the best performance, health, and profitability in poultry as we move towards precision nutrition.

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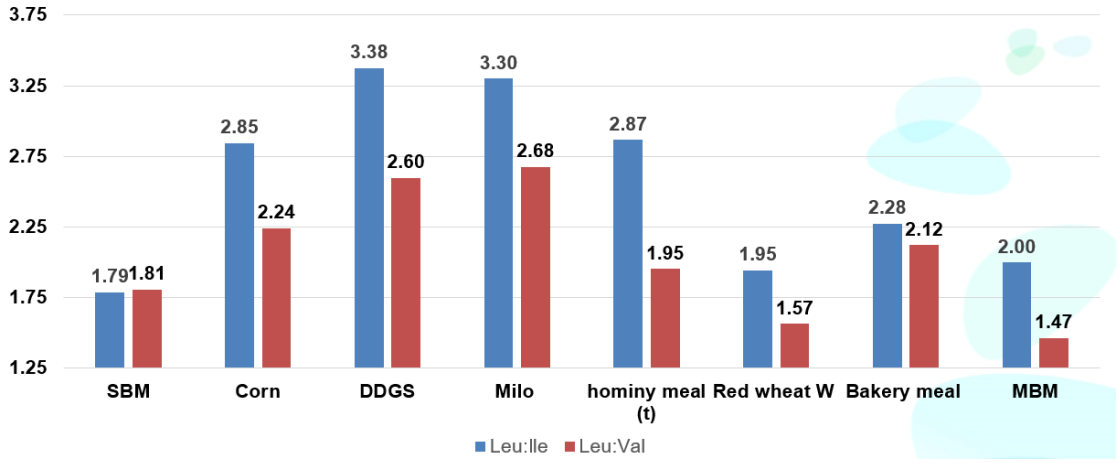

Fig 1: Leucine:Isoleucine (blue) and Leucine:Valine (red) ratio in different commonly used ingredients in the poultry diets.

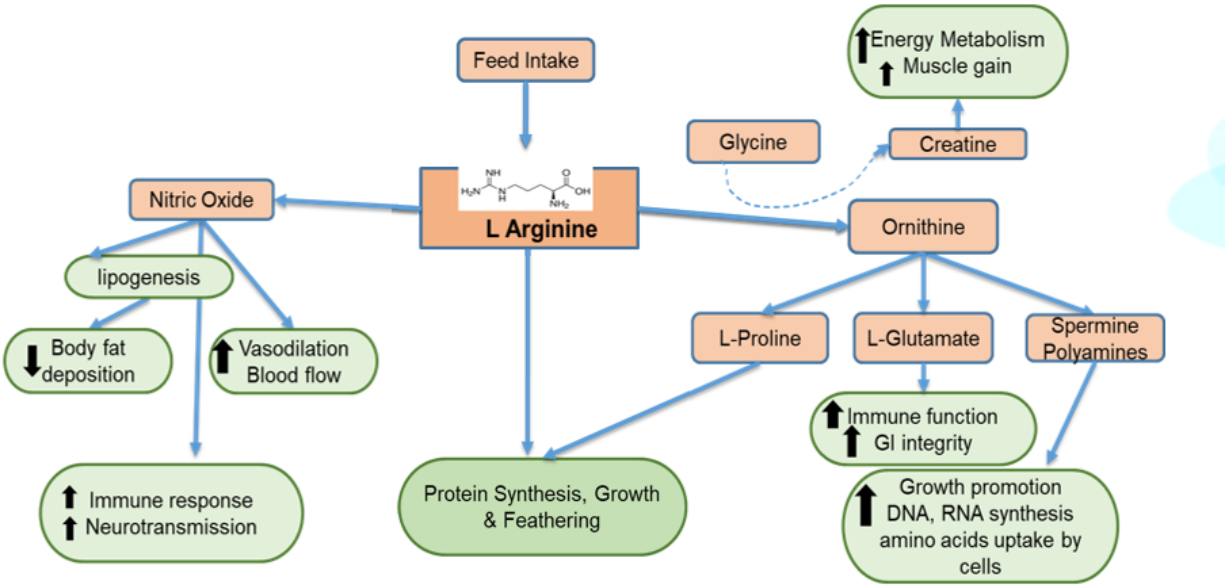

Fig 2: Destination pathways of dietary L-Arginine in broilers. (Adopted from Quimidroga.com)

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