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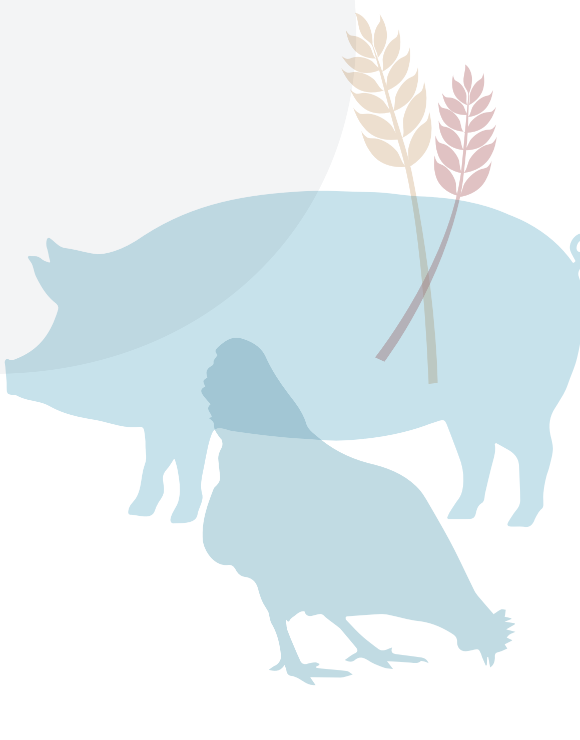

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Reevaluating the requirement ratios for broiler growth and yield

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INTRODUCTION

Providing a diet with the proper nutritional value including energy, minerals, amino acids, etc. is critical to recognizing the maximal growth potential and profitability of the modern commercial broiler. Over a 48 year period, the genetic selection of the modern broiler has resulted in over a 400% increase in growth rate, 50% reduction in feed efficiency and an 85% increase in breast filet yield (Zuidhof et al., 2014). The modern broiler has a lower feed intake per unit of body weight gain and the potential for more white meat accretion than the commercial broilers of decades prior. Broilers consuming less feed per unit of gain has led to formulating diets higher in amino acid density in commercial production for improved performance and meat yield (Dozier et al., 2008). This selection process has exerted marked changes in the broilers body composition which in turn influences their nutritional requirements especially regarding amino acids. Similarly, these body compositional changes combined with rearing management strategies such as raising without antibiotics, will in fact influence subsequent amino acid requirements in addition to lysine. A significant amount of research has been conducted on amino acid requirements for which a commercial synthetic product was economically available, such as threonine, however, only recently have significant efforts been made to re-examine changes in amino acid requirements of valine, isoleucine, and arginine.

Historically, valine, isoleucine, and arginine formulated values have typically exceeded minimum ratios recommended by primary breeders in traditional US based diets due to the use of crude protein minimums or the use of only 3 synthetic amino acids. Additionally, increasing any of these ratios could result in significant increase diet cost due to the lack of a commercially available synthetic forms. Therefore, a compromise between performance and diet cost at time must be made by formulating nutritionists. However, with the advancement in fermentation technology, commercially available forms of feed grade valine, arginine, and isoleucine allow for nutritionists to accurately formulate to the requirements of the modern commercial broiler to maximize performance, yield, and profitability.

Threonine

Threonine(Thr) is widely accepted as the 3rd limiting amino acid in broiler diets. Dozier et al., (2015) reported the digestible threonine to lysine ratio to optimize feed efficiency in broilers from 1 to 14 days of age was 69% in diets containing a coccidiostat. Additionally, in multiple studies, Dozier et al., (2016) reported that a digestible threonine to lysine ratio of 68% can optimize growth performance of broiler from 21 to 35 and 35 to 49 days of age although no impact on yield was observed.

The continued global growth of antibiotic free broiler production and increased use of coccidiosis vaccination can result in intestinal damage, increased pathogenic bacteria, intestinal cell turnover and mucin production. Threonine is the major component of intestinal mucin in animals, representing approximately 30% of its total amino acid content (Faure et al., 2002). Due to its importance of maintaining barrier function, mucin is not digested by the normal mechanism with the GIT and thus threonine which is secreted as mucin is eventually lost in the excreta or fermented by cecal microorganisms (Bortoluzzi et al., 2018). Therefore, any situation which induces mucin production would result in increasing the dietary threonine requirement. Induced mucin production associated with intestinal stress or increased bacterial load would shift endogenous amino acid flow and reduce the amount of threonine available for growth. For example, Corzo et al., (2007) demonstrated that the threonine requirement for broilers between 21 and 42 days was 4% higher for broilers reared on built-up vs new litter. Similarly, threonine requirement was reported to increase by 6% during a subclinical intestinal Clostridium infection (Star et al., 2012). Increasing the threonine level during time of intestinal challenge or stress is beneficial on the performance and health status of the animal. Threonine supplementation can reduce cecal pathogenic bacteria such as *Escherichia coli* and *Salmonella* and increase *Lactobacillus* colonies (Chen et al., 2017).

Additionally, threonine supplementation benefits intestinal maturity and integrity by increasing villus height, villus:crypt ratio, goblet cell density, jejunal Ig M and secretory Ig A (Chen et al., 2017). Therefore, nutritionist should consider health status and management conditions and the potential influence on intestinal integrity and endogenous amino acid flow when considering the threonine nutrient specification in the diet. Increasing threonine level should be considered if transitioning off of antibiotics or during periods of known intestinal stress as the increased flow of threonine to the gastrointestinal tract could limit growth potential of the dietary formulation.

Valine

Valine(Val) is recognized as the 4th limiting amino acid in early US poultry diets with the possible exception of diets containing significant amounts of animal protein meal (Maynard et al., 2020). Feed grade L-valine has been commercially available for many years and has recently seen substantial global growth. Having an economically viable source of valine provides nutritional flexibility for producers. Historically, dietary formulations have used valine ratios ranging between 74 and 76% of digestible lysine. However, most recent published data indicate that valine requirements of the modern broiler actually range between 78 and 84% of digestible lysine (Table 1). Kriseldi et al. (2020) recently confirmed the performance advantage of feeding a higher than typical digestible valine ratio by reporting a 50 g increase in d 33 body weight, a 2 point improvement in feed efficiency, and a $0.03 return over feed cost per bird advantage when increasing dietary valine from 74% to 78% of dig lysine in Ross 708 broilers.

Table 1. Reported optimal dig valine to dig lysine ratios

	Strain	Age	Parameter	Optimal Ratio
Duarte et al. (2013)	Cobb 500	22 to 42 days	Body weight gain	79
Duarte et al. (2013)	Cobb 500	22 to 42 days	Feed efficiency	84
Maynard et al. (2020)	Cobb 500	15 to 35 days	Feed efficiency	78
Agostini et al. (2018)	Cobb 500	0 to 35 days	Feed efficiency	78
Schedle et al. (2019)	Ross 308	0 to 36 days	Average daily gain	83
Schedle et al. (2019)	Ross 308	0 to 36 days	Feed efficiency	80

As previous discussed, the modern commercial broiler is a rapidly growing, efficient user of natural resources. In order to maintain adequate performance and welfare, proper bone mineralization and feather development is required to ensure skeletal development and skin protection. Valine plays an essential role in both of these vital activities. Valine concentration in the feather increases with age (Fisher et al., 1981), and inadequate dietary valine decreases feather protein and cysteine content. This results in the concave structure of the feathers as they bend away from the body (Farran and Thomas, 1992a). Poor feathering and feather quality results in skin scratches and injuries which can increase carcass condemnations. Additionally, inadequate dietary valine reduces the bone calcium content through decreased mineral deposition by depressing osteoblastic activity. Osteoblasts are responsible for producing the bone matrix of protein and minerals and their reduced activity results in elevated calcium excretion via the kidney (Farran and Thomas, 1992b) as opposed to bone deposition reducing bone strength (Ospina-Rojas et al., 2018). Improper bone development can result in rickets and tibia dyschondroplasia, reducing bird performance and compromising welfare. These observations can be exacerbated when the branched chain amino acid ratios become unbalanced, most likely associated with high levels of dietary leucine. Ingredients such as corn DDGs, peanut meal, and corn gluten meal have excess levels of digestible leucine which may cause imbalances in branched chain ratios within broiler and breeder diets; adjustment of these ratios by the addition of L-valine may benefit feathering, bone density, and performance.

Arginine

Arginine(Arg) plays a crucial role in numerous metabolic and immunology pathways in poultry (Fig. 1). Arginine is considered an essential amino acid in broilers as they are unable to synthesize endogenous L-arginine because of the lack of a functional urea cycle and therefore, it is essential that the diet provides the optimal level to realize the genetic potential of the modern broiler.

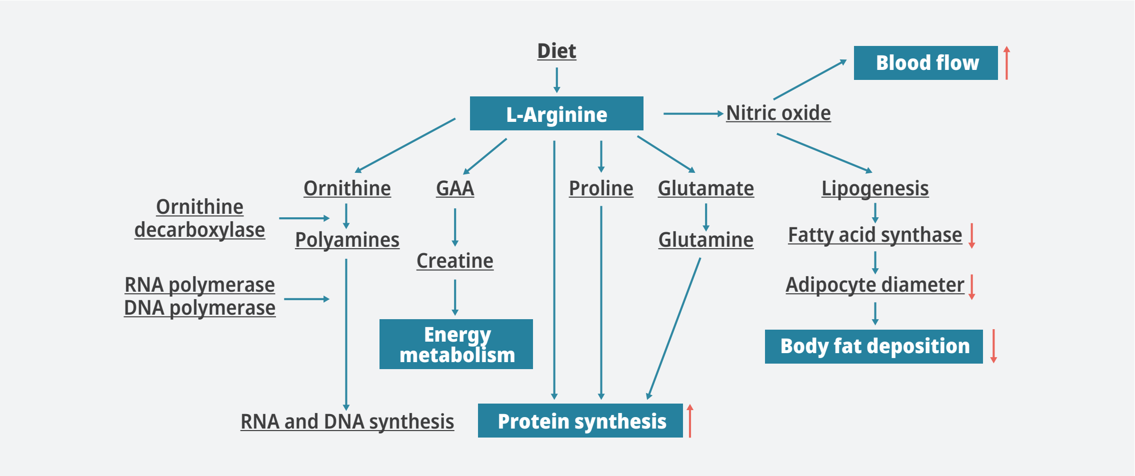

Figure 1. Destination pathways of dietary L-arginine in broilers.

L-arginine has garnered recent interest due to its role in vasodilation and blood flood and its potential benefit on meat quality, and with the recent introduction of feed grade L-arginine multiple published reports have linked elevated levels of arginine with reductions in breast muscle myopathies (Bodle et al., 2018; Zampiga et al., 2019). However, during these investigations, additional benefits on growth performance were observed. Bodle et al. (2018) reported an increase of 40 gram of body weight and 2 point 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 (Fig. 2). 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. This optimal ratio matches a recent meta-analysis reported by Dr. Opsina-Rojas of CJ Brazil identifying the optimal ratio for performance to be 114 to 115% of dig Lys (https://en.engormix.com/poultry-industry/articles/optimal-dietary-arginine-levels-t43741.htm).

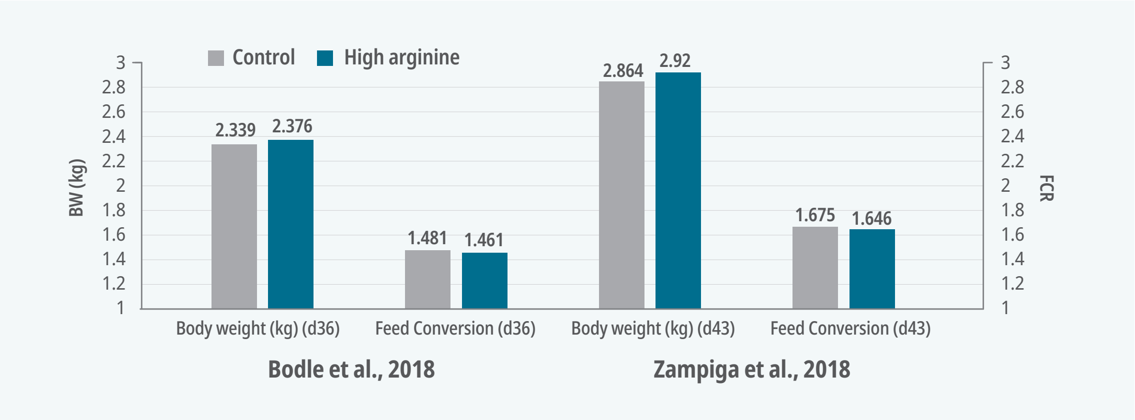

Figure 2. Body weight and feed efficiency of broiler fed increases in dietary arginine through 36 (20% increase) and 43 days of age (10% increase).

Isoleucine

Feed grade L-isoleucine(Ile) has been recently made available on the market place and now available for use in practical broiler diets. Depending on ingredient profile and nutrient specifications, Ile can fall anywhere between the 4th and 6th essential limiting amino acid. As such, in many cases Ile concentration in the diet exceeds the formulation specification. However, in instances of high animal protein and other low Ile containing ingredients, Ile may find itself behind threonine in order of limitation and thus formulation with the proper requirement is essential for optimal growth performance. Recently, Brown et al. (2020) reported the optimal ratio of Ile to Lys in starter Ross 708 broiler males from 1 to 18 days of age was 72% (Fig. 3). Dozier et al. (2012) reported a positive correlation between increasing dig Ile ratio and observed breast meat yield in two experiments. In both experiments, incremental increases in dig Ile ratio correlated with an observed increased in breast meat yield (Fig. 4).

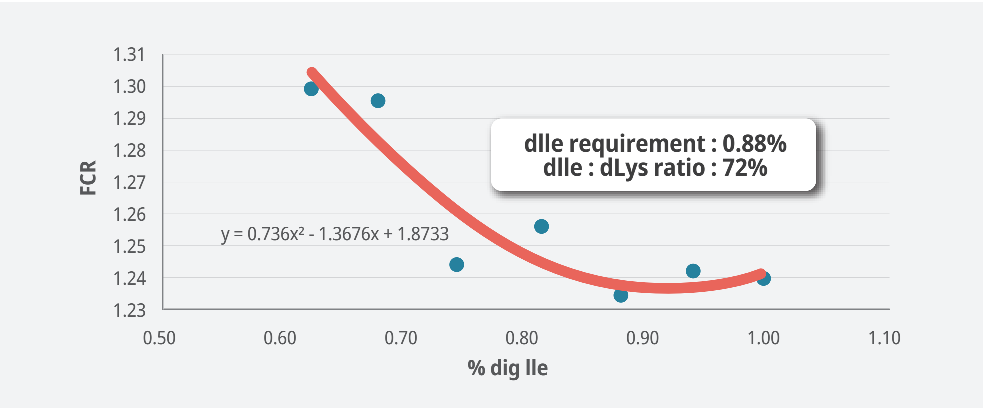

Figure 3. Feed conversion ratio of 1 to 18 day old Ross 708 broilers.
(Adopted from Brown et al., 2020)

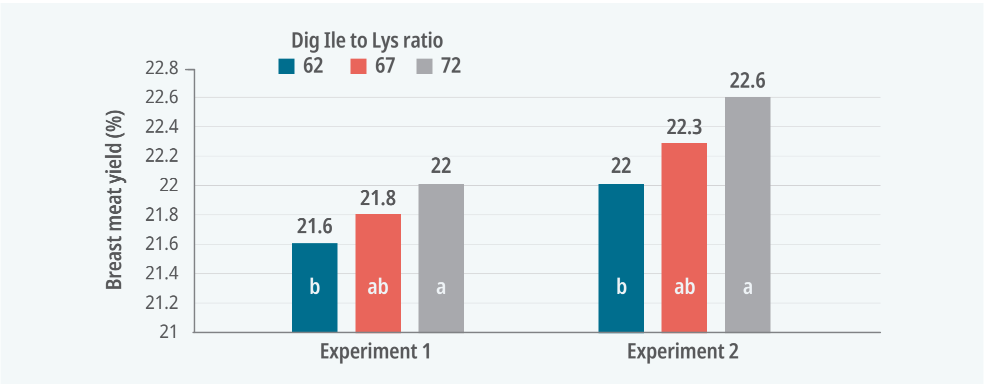

Figure 4. Effect of increasing dig Ile ration on Ross x Ross 708 male breast meat yield(%).
(Adopted from Dozier et al., 2012)

With the current commercial availability of the first seven limiting amino acids in broiler diets, understanding and identifying the proper requirement of each of these amino acid is necessary to maximize broiler performance, yield, and profitability. In a typical corn:soy diet, valine is the 4th limiting amino acid in the vast majority of formulations and thus can protect the other amino acid ratio specifications as they will have exceeded the formulation specification in the diet. However, the expanded use of alternative ingredients in an effort to reduce diet cost results in the switching of the 4th limiting amino acid. For example, high levels of animal protein in the diet will result in an Ile limitation, or high levels of corn DDGs can have a limitation on Arg especially in later phases of production with lower levels of soybean meal. Access to commercially available synthetic forms of each of these amino acids provides nutritionists the ability to capitalize on the benefits of reducing dietary crude protein and potentially reduce diet cost. However, when using the 4th limiting amino acid in a diet, nutritionists must accurately specify the 5th, 6th, and 7th limiting amino acids as each of these will closer approach the minimum specified in the formulation. Therefore, ratios used for these lower amino acids in the past were not as critical as they exceeded the formulation minimum but with the continued growth of producers using the 4th and in some cases the 5th limiting amino acid, incorrect ratios on these lower limiting amino acids may negatively impact performance, yield, and profitability. A significant amount of research in the re-evalaution of these optimal amino acid ratios has recently been conducted and continues in an effort to provide nutritionists to most current information possible when formulating diets. However, the optimal ratios reported seem to be slightly higher than historically used values which may be attributed to the continued selection and changes in body confirmation of the modern commercial broiler. Therefore, with the availability of these feed grade amino acids, nutritionist no longer have to comprise on the amino acid ratio specification in formulation software and can accurate formulate diets to properly meet the nutritional requirements of the broiler and to optimize performance and maximize profitability.

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Role of isoleucine as a member of branched chain amino acids in poultry

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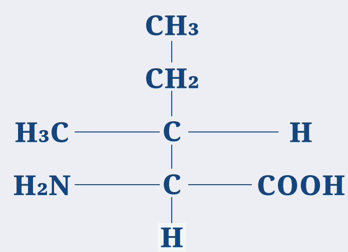
INTRODUCTION

In the present scenario of rising concern over the environmental issues, to reduce the nitrogen emission and to minimize the feed cost in the feed industry, lowering crude protein level with the addition of synthetic amino acids is the standard practice. In practical ration formulation a deficit of first-limiting amino acids can be prevented by supplying these amino acids in their free form particularly methionine, lysine, threonine, valine, arginine, and isoleucine (Ile). A progressive reduction of the dietary protein content can, however, lead to a situation where other amino acids, which are of no special concern in diets with normal protein levels, become limiting for performance. Isoleucine is considered as the fifth limiting amino acid in corn-soybean based diets and also can be a co-limiting amino acid together with valine in broilers diets when animal by products comprises 3% or more in the diet (Corzo et al., 2010).



Isoleucine

Isoleucine metabolism

Isoleucine is a branched chain amino acid (BCAA) and along with valine and leucine are essential amino acids. Although most of amino acids are catabolized in the liver, BCAAs are initially catabolized in skeletal muscle into BCKA (branched chain keto acid) with the involvement of branched-chain aminotransferase (BCAT) (leucine to α-keto isocaproate, valine to α-keto isovalerate, and isoleucine to α-keto-β-methyl-valerate). BCKA will be decarboxylated by branched-chain α-ketoacid dehydrogenase (BCKD) in the liver. Finally, these BCAA metabolites are catabolized by a series of enzymatic reactions to final-products (acetyl-CoA from leucine, succinyl-CoA from valine, and both acetyl-CoA and succinyl-CoA from isoleucine), which enter the TCA cycle (Fig. 1).

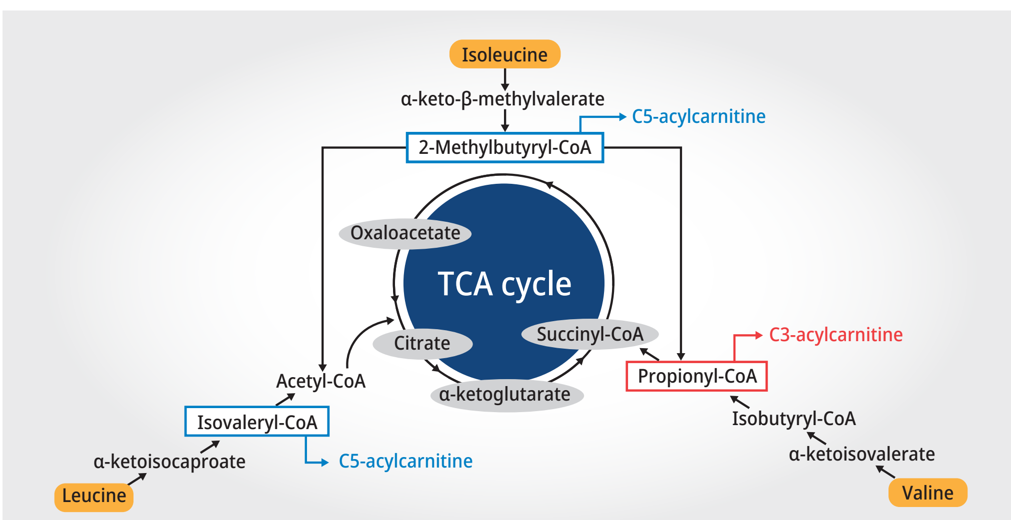


Figure 1. Pathway of branched chain amino acid catabolism.
(Adopted from Zhang et al., 2017)

Isoleucine requirement

The ideal ratio of Ile to Lys (dIle:dLys) for optimum growth performance and breast meat yield in broilers is considered as 67 (Kidd et al., 2004). According to a study by CJ Brazil (article in press) recommended SID Leu, Val, and Ile levels for optimal BW gain were estimated at 1.33, 0.96 and 0.84% for the starter phase; 1.23, 0.83 and 0.75% for the grower phase; and 1.16, 0.77 and 0.68% for the finisher phase, respectively. Similarly, SID Leu, Val, and Ile levels required for gain:feed optimization were estimated at 1.37, 0.94 and 0.87% during the starter phase; 1.23, 0.82 and 0.75% during the grower phase; and 1.15, 0.77 and 0.70% during the finisher phase, respectively. The NRC has increased its Ile recommendation for commercial layers to 650 mg/d per hen (NRC, 1994) from 550 mg/d per hen (NRC, 1984). For brown egg layers, NRC (1994) has recommended 715 mg isoleucine daily on 110 g of feed per hen. Harms and Russell (2000) suggested a daily requirement of Ile at 601 mg/d for a daily egg mass of 53g. The recommended dietary intake of dIle is 79% of lysine for single- comb white leghorn laying hens as per CVB (1996) (Table 2). The Ile requirements of turkeys and ducks are mentioned in Table 3.

Table 1. Ideal digestible amino acid profiles for broiler chickens expressed as percentage of lysine

Source	Cobb (2018)				Ross (2019)		
Age (Days)	0-8	9-18	19-28	>29	0-10	11-24	>25
Ile	*63*	*64*	*65*	*66*	*67*	*68*	*69*
Lys	100	100	100	100	100	100	100
Met	38	40	41	41	40	41	42
Met+Cys	75	76	78	78	74	76	78
Thr	68	65	65	65	67	67	67
Val	73	75	75	75	75	76	76
Arg	105	105	105	105	107	107	107
Leu	-	-	-	-	110	110	110
Trp	16	16	18	18	16	16	16

Table 2. Ideal amino acid profiles for single-comb white leghorn laying hens[1]

Amino acid	NRC (1994)[2]	CVB (1996)[3]	Coon and Zhang (1999)[4]	Lesson and Summer (2005)[5]	Rostagno (2005)[6]	Bregendahl et al. (2008)[7]
Ile	*94*	*79*	*86*	*79*	*83*	*79*
Lys	100	100	100	100	100	100
Arg	101	-	130	103	100	-[8]
Met	43	50	49	51	50	47
Met +Cys	84	93	81	88	91	94
Thr	68	66	73	80	66	77
Trp	23	19	20	21	23	22
Val	101	86	102	89	90	93

[1]Amino acid requirements expressed as a percentage of the requirement or recommendation for lysine.
[2]Calculated from total amino acid requirements.
[3]Calculated from digestible amino acid recommendations.
[4]Based on digestible amino acid requirements.
[5]Calculated from total amino acid recommendations for 32-to-45-week-old laying hens.
[6]Digestible amino acid basis.
[7]Based on true digestible amino acid requirements for maximal egg mass in 28-to-34-week-old laying hens.
[8]The arginine:lysine ratio was estimated to be 107 or less.

Table 3. Isoleucine requirement of Turkeys and White Pekin Ducks (%) (90% DM) NRC (1994)

<table>
<tr><th colspan="7">Growing Turkeys (Males)</th><th>Breeders</th><th>Laying hens</th></tr>
<tr><td>Weeks</td><td>0-4</td><td>4-8</td><td>8-12</td><td>12-16</td><td>16-20</td><td>20-24</td><td></td><td></td></tr>
<tr><td>Ile</td><td>1.1</td><td>1.0</td><td>0.8</td><td>0.6</td><td>0.5</td><td>0.45</td><td>0.4</td><td>0.5</td></tr>
<tr><td colspan="7">White Pekin Ducks</td><td colspan="2" rowspan="2">Breeders</td></tr>
<tr><td>Weeks</td><td colspan="3">0-2</td><td colspan="3">2-7</td></tr>
<tr><td>Ile</td><td colspan="3">0.63</td><td colspan="3">0.46</td><td colspan="2">0.38</td></tr>
</table>

Isoleucine content in raw materials

The approximate isoleucine (%) in the common feed ingredients is given in Table 4.

Table 4. Isoleucine content (%) of commonly used feed ingredients

Ingredients (As fed basis)	Isoleucine %	
	Total %	Digestible %
Barley	0.42	0.34
Canola meal (38%)	1.51	1.25
Corn	0.29	0.26
Corn gluten meal	2.30	2.19
Cotton seed meal, mech extracted	1.31	0.93
Cotton seed meal, direct solv.	1.33	0.95
Fish meal, white	3.00	2.55
Flax seed	0.95	0.81
Linseed meal flax, expeller	1.70	1.49
Linseed meal flax, solvent	1.80	1.58
Meat bone meal	1.70	1.41
Millet, pearl grain	0.52	0.46
Oats grain	0.53	0.47
Poultry by product meal	2.10	1.79
Rice bran, unextracted	0.39	0.30
Rice grain rough	0.33	0.27
Safflower seed meal, expeller	0.28	0.22
Sorghum, milo, grain	0.60	0.53
Soybean meal, expeller	2.18	1.94
Soybean meal, solvent	2.50	2.22
Sunflower meal, expeller	2.40	2.14
Sunflower meal, solvent	1.39	1.25
Wheat, hard grain	0.69	0.61
Wheat, soft grain	0.43	0.38
Wheat bran	0.60	0.47
Wheat middlings	0.70	0.58

Amino acid digestibility expressed as standardized ileal digestibility. Amino acid values are standardized for 88% dry matter (Source: Hy- Line. W-36 commercial layer management guide).

Beyond performance roles of isoleucine

Immunity

Immune cells oxidize BCAA as fuel sources and incorporate BCAA as the precursors for the synthesis of new immune cells, effector molecules, and protective molecules. Lack of BCAA in diet impairs many aspects of immune function and increases susceptibility to pathogens (Zhang et al., 2017).

Isoleucine and leucine contribute to immunity through the mammalian target of rapamycin (mTOR) signalling pathway. mTOR plays a vital role in the regulation of innate and adaptive immune responses and also various immune functions like promoting differentiation, activation and function in T-cells, B-cells and antigen presenting cells (Soliman, 2013). Isoleucine level also have a strong correlation with the excretion of β-defensin. Deficiencies of BCAA (leucine, isoleucine, valine) cause involution of the thymus (Konashi et al., 2000). Isoleucine could become marginal and its limitation could impair the immune function responses when hens are fed low protein diets (Konashi et al., 2000).

Feed consumption

The mTOR signalling pathway plays a vital role in the brain to detect nutrient availability and regulate energy balance (Cota et al., 2006). As isoleucine is also associated with mTOR signalling thus, low level of isoleucine can cause reduced feed intake.

BCAA deficient diet dramatically reduces feed intake by activating the GCN2 signalling pathway, which might participate in lipolysis (down-regulating lipogenesis genes or up-regulating lipolysis genes) in the liver and adipose tissue.

Glucose transportation

The function of isoleucine in enhancing glucose uptake and muscular glucose transporter expression (GLUT1 and GLUT4) was also demonstrated in C2C12 myotubes (Zhang et al., 2017). GLUT1 and GLUT4 are vital glucose transporters in muscle. Similarly, SGLT1 and GLUT2 are important glucose transporters in the small intestine. Isoleucine could potentially increase muscle growth and intestinal development and health by up-regulating the protein expression of GLUT1 and GLUT4 in muscle and enhancing the expression of SGLT1 and GLUT2 in the small intestine (Fig. 2).

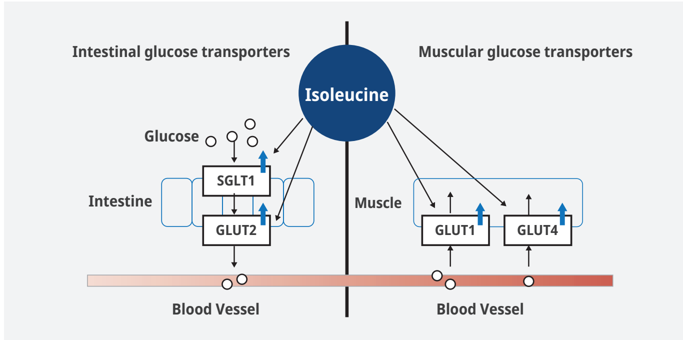


Figure 2. Isoleucine up-regulates intestinal and muscular transporters.
(Adopted from Zhang et al., 2017)

CONCLUSION

Lowering the crude protein level in poultry diet is a trend in poultry industry to address the current environmental pollution and for optimization of feed cost and isoleucine plays vital role in maintaining the amino acid balance in a low crude protein diet. Again, isoleucine along with valine and leucine also have positive influence on nutrient metabolism as well as immunity and gut health which can be focused further to have a clear impression.

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Coccidiosis in poultry: anticoccidial global market and trends

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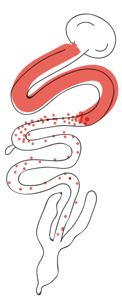
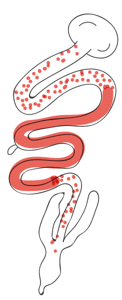
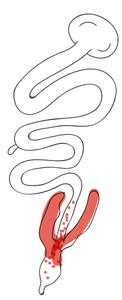
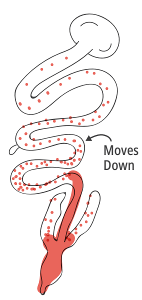
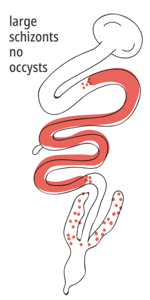
What is coccidiosis?

Coccidiosis is a severe intestinal tract disease in animals caused by coccidian protozoan parasite (*Eimeria)*. This disease impacts commercial meat production and leads to serious economic loss to farms. Annual loss caused by coccidiosis has been estimated over 2.4~3 billion dollars in worldwide. Poultry industry is considered as the most effected. (Williams et al., 1999).

In poultry, at least seven species of *Eimeria* have been widely recognized and identified as parasitizing different regions of intestine. The following five major species named *E. tenella, E. maxima, E. acervulina, E. necatrix* and *E. brunetti* have been considered as the most economical affecting species (Shirley et al., 1986, Thenmozhi et al., 2014).

The life cycle of *Eimeria* is complex and comprised of three stages: (1) first stage is sporogony which occurs in litter under the condition of humidity, oxygen supply and temperature; (2) second stage is called merogony or schizogony (asexual reproduction) continues in the cells of intestinal epithelium and (3) last stage is called gametogony (sexual reproduction), which also occurs in the epithelial cells. After these steps, the formation of immature oocyst are completed and will be excreted by feces (Lal et al., 2009). The disease transmission starts when the host ingests sporulated (matured) oocysts. According to Reid et al. (1990), the sporulated oocyst can survive up to 602 days in the exogenous environment. During last few years, lots of research has focused on the development of anticoccidial drugs targeting each or both stage of sexual and asexual or blocking the formation of oocysts.

Differential characteristics of 5 major *Eimeria* species in chicken					
Species	*E. acervulina*	*E. maxima*	*E. tenella*	*E. brunetti*	*E. necatrix*
Target location	 Duodenum, jejujum	 Duodenum, jejujum, ileum	 Caeca	 Ileum, Rectum	 Upper jejujum, middle ileum
size (average)	18.3 x 14.6µ	30.5 x 20.7µ	22.0 x 19.0µ	24.6 x 18.8µ	22.0 x 19.0µ
Pathogenicity*	++	++	+++	+++	+++
Symptoms	Anemia, light enteritis, loss of appetite	Diarrhea, dropping	Bloody droppings, reduce in feed intake	Enteritis, Occasionally bloody	Bloody enteritis, drops in feed intake

*: + low pathogenic, ++ moderately pathogenic, +++ highly pathogenic

Figure 1. Differential characteristics of 5 major *Eimeria* species in chicken.
(Adopted from engormix.com)

Anticoccidial global market size

As mentioned, global economic losses caused by coccidiosis is enormous and the increased outbreaks of coccidiosis drives the growth of anticoccidial drugs market. Global anticoccidial market estimated about 1,354 million dollars in 2020, at CAGR of 3.61%. Among livestock fields, the major field of anticoccidial market is poultry and this is composed of 53% of total, estimated 720 million dollars. By classifying the area, Asia-Pacific region was estimated to account for the largest share of the feed anticoccidial market in 2020 estimated 415 million dollars, due to lenient regulations on the usage of anticoccidial in feed and increasing livestock population in the region. More specific, the major products market size of feed anticoccidials such as ionophore and synthetic compounds is projected to reach USD 429 million by 2025 from USD 345 million in 2020, at a CAGR of 4.4%.

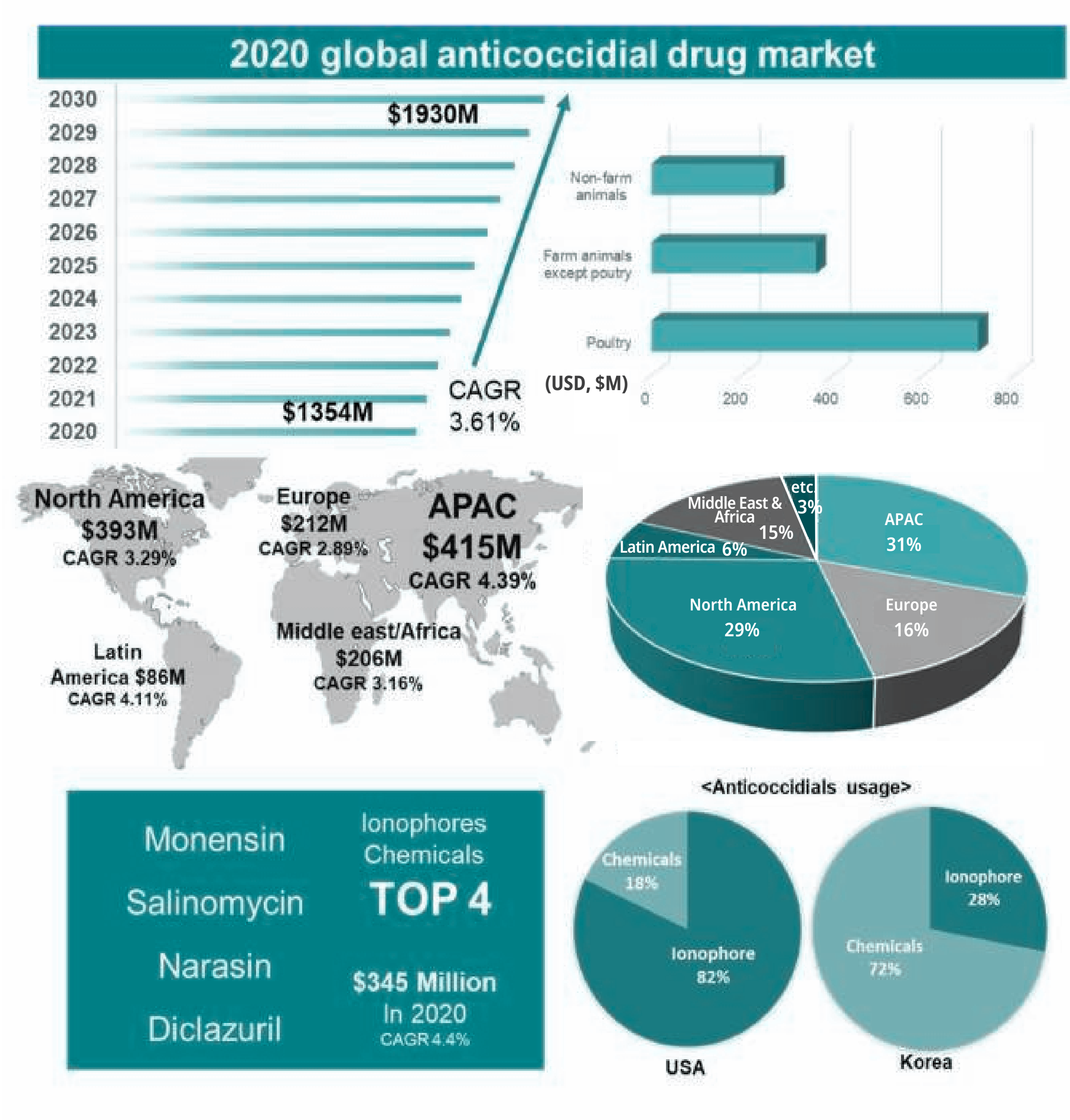


Figure 2. Global anticoccidial market size (per livestocks, nations, products).
(Adopted from Market Data Forecast and Mordor Intelligence)

Anticoccidial trends: now and future

There are two main categories of anticoccidial drugs, ionophore and chemically synthetic compounds. At first, ionophores such as monensin, narasin, salinomycin have the function of disrupting ion gradients across the cell membrane of the parasite. On average, over 70% of global farms have been treating ionophore products against coccidiosis. Second, chemical compounds such as diclazuril affect later phases of coccidia differentiation and inhibit the synthesis of oocysts wall (Verheyen et al., 1989). In Korea, the use of chemical compounds is over 70% and this is higher than ionophore.

Unfortunately, the emergence of resistant strains, especially after a prolonged use of drug shuttle program, is a real problem. Thus, vaccines are the only preventative methods against this disease but the cost and time of production of live vaccines is inefficient.

Because of these reasons, new alternative products have emerged, most of which are natural compounds extracted from plants or synthesized by microorganisms. Some of these compounds are antioxidants that damage the parasite, thus preventing the infection. In this context, plant extracts such as essential oils have emerged as an alternative and complementary approach to control coccidiosis. Also, modern approaches towards the discovery of novel resistance-breaking drug candidates may be anticipated. Genomic analysis of all major *Eimeria* species related to the poultry coccidiosis has been accomplished (Reid et al., 2014) and this may allow the identification and validation of recombinant vaccines as alternative control measures against chicken coccidiosis.

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Types and characteristics of killed bacteria as a feed additives

Yang-Su Kim
CJ BIO, HQ

SUMMARY

✓Currently, *Escherichia coli (E. coli)* and *Corynebacterium (Coryne.)* are mainly used as amino acid industrial fermentation strains for food or feed additives. These are classified into Gram-negative and Gram-positive bacteria, respectively.
✓The biggest difference between Gram-negative bacteria and Gram-positive bacteria is the presence or absence of Lipopolysaccharide (LPS), a component of the cell wall (Gram-negative bacteria have LPS, but Gram-positive bacteria do not have LPS).
✓LPS is an intracellular toxin (endotoxin), which is not affected when consumed under healthy situations, but maybe harmful to health if it enters the blood due to internal organ wounds.

Difference between Gram-negative bacteria and Gram-positive bacteria

Strains are largely classified by morphology, nutrition, flagella number, and cell wall [1]. Among various classification methods, a bacterial identification method by gram's stain, which can find a suitable strain for cultivation or sterilization of bacterial species, is commonly used [2]. The Gram staining method developed by Danish scientist Hans Christian Gram in 1884 is a very important staining method that defines the characteristics of bacteria because the staining result varies depending on the cell wall component (structural difference of the cell wall) that determines the molecular pattern derived from microorganisms. Even now, it is very important and useful tool in the research of bacteria. Introducing the classification criteria by Gram staining method and the differences are detailed in Table 1 below.

For both Gram-negative and Gram-positive bacteria, the cell wall consists of peptidoglycan (PG). However, in the case of Gram-negative bacteria, in addition to PG, there is an additional layer of lipopolysaccharide(LPS) composed of lipoprotein and protein, and this LPS layer has somatic antigen (O antigen) Lipid A, which has the characteristic of exhibiting toxicity (i.e., endotoxin) (Fig. 1). Due to the risk of these Gram-negative bacteria, companies in the pharmaceutical industry worry about the harmful effects such as pyrogenicity, lethality, Schwartzman reactivity [3], adjuvant activity and macrophage activation. It is essential to remove endotoxins from parenteral agents due to their biological activity [4].

Table 1. Difference between Gram-positive bacteria and Gram-negative bacteria classified by Gram staining method

	Gram-positive bacteria	Gram-negative bacteria
Define	Positive results for Gram stain test (crystal violet stain, dark purple or purple)	Negative results in Gram stain test (no crystal violet color, red or pink)
Species	*Staphylococci, Streptococci, Pneumococci, Enterococci, Bacilli, Clostridia, Corynebacterium, Listeria, Actinomyces*	*Enterobacteriaceae (E. coli, klebsiella, salmonella, shigella), Bacteroides, Pseudomonas, Vibrio (cholera), Campylobacter, Legionella, Neisseria, Hemophilus, Bartonella*
Cell wall	Monolayer	Double layer
	High elasticity (peptidoglycan content about 80%)	Low elasticity (peptidoglycan content about 2-12%)
	Resistance to alkali	Sensitive to alkali
	No lipopolysaccharide	Lipopolysaccharide presence
	Contain of teichoic acid	No teichoic acid
	Easy to decompose by lysozyme	Less sensitive to degradation by lysozyme action
	Cell wall thickness up to 15 to 30nm thick (sometimes 80nm)	Cell wall is as thin (8-12nm)

Other characteristics	Very narrow if there is no or no periplasmic space	Periplasmic space available
	Lipid content is as low (1-4%)	The lipid content is as high (11-22%)
	No outer membrane	Outer membrane
	No porin (protein membrane)	Porin presence
	Endospore production under adverse conditions	Endospore not produced

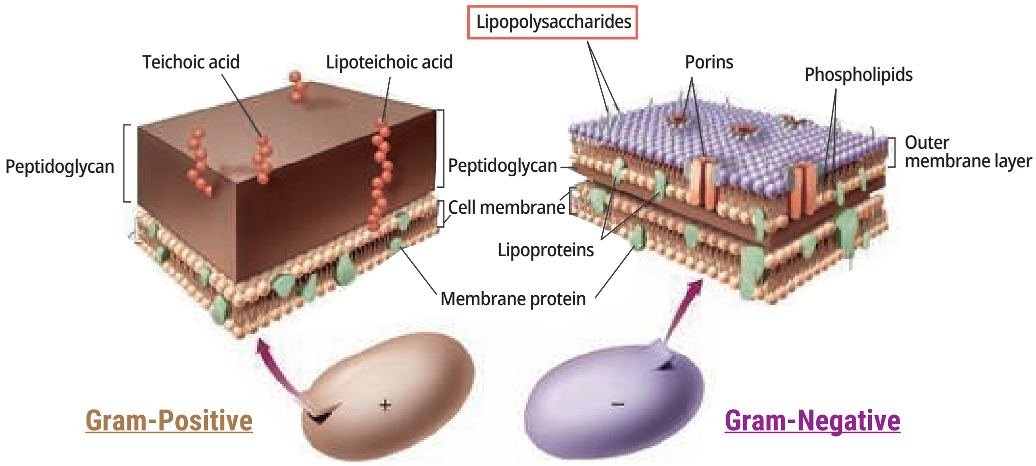

Figure 1. Structural differences between Gram-positive and Gram-negative bacteria [5].
(Adopted from microbiologyinfo.com)

Industrial use of microorganisms (amino acid production, etc.)

Mankind has been using microorganisms since 700 BC, and through antibiotics and biopolymers, it has recently been used in a variety of fields, from biofuels (fuel cells) to disease treatment (toxic element detection biosensors, cancer/ulcer disease elucidation). Both the Gram-negative bacteria and Gram-positive bacteria described above have important industrial usage. Among the traditional bulk products are ethanol, amino acids, organic acids, inorganic feeds, health foods, nutritional supplements, artificial sweeteners, and cosmetics (Table 2).
The amino acid market for feed additives has been developed along with the growth of the global livestock industry (because of an increased demand for vegetable protein sources and competition in feed costs). The volume and type of essential amino acids produced each year are increasing. (Demand for global L-lysine has been growing at CAGR of 14.7% during 2001-2015 [6]. China is the world's largest L-lysine producer, accounting for about 65% of the world's lysine production capacity [7,8]). The quantitative increase of amino acid production and the expansion of the product portfolio have been greatly improved by the fermentation and purification technology development and the strain improvement programs. The production of amino acids (L-glutamic acid) by bacteria was first produced in 1907 by *Coryne*. Thereafter, the development of efficient mutant strains for commercial production over the 1940-1950s increased economic viability with the mass production of various essential amino acids [9].

Table 2. Major microorganisms and their products applied in the food/feed industry

Bacteria	Raw ingredients	Products
Micrococcus sp.	sugar	**monosodium glutamate**
Leuconostoc mesenteroides	sucrose	**dextran**
E. coli, C. glutamicum, B. flacum, B. lactofermentum, Bacillus methanolicus	starch, glucose, molasses, diamino-pomelic acid (*E. coli* specific), aspartate (*C. glutamicum* specific), methanol, lignocellulose (*B. methanolicus* specific)	**Lysine, Threonine, Methionine, Isoleucine**
E. coli, C. glutamicum	starch, glucose, molasses	**Tryptophan**

Utilization of killed bacteria as a feed additives

Harmful by-products are not found in almost all amino acid products. Rather, useful organic ingredients remain in the recovered by-products and are utilized as resources, such as feeder protein and fertilizer. However, the development of amino acid production technology for feeds such as granular form by simplifying (or omitting) the refinery process. This was an economical inevitability using bacteria from the fermentation incorporated into the final product in the form of killed/deactivated bacteria. Several studies have shown that killed lactic acid bacteria are stable to the environment due to strong resistance to acid and heat, are highly concentrated, easy to handle, and used as food for beneficial bacteria that have settled in the intestine, thereby enhancing the immune-enhancing activity unique to lactic acid bacteria [10].

However, it is unclear to date, whether the efficacy of these killed bacteria is the same for all strains. For example, comparative data is relatively small as to whether the observed efficacy in Gram-positive bacteria such as lactic acid bacteria is the same in Gram-negative bacteria having LPS known as endotoxin in the cell wall. Moreover, heat deactivation of Gram-negative bacteria promotes the release of LPS, and it is known that relatively heat-stable LPS still exists after heat treatment [4]. For example, the U.S. Pharmacopoeia (USP) recommends dry-heat treatment at temperatures above 220°C for as long as possible to reduce endotoxins to ≥3-log [11]. However, these temperatures have not been applied in general feed manufacturing processes. Therefore, the effect of feed containing killed bacteria as a protein or amino acid source in feed on the performance of animals may differ between Gram-negative and Gram-positive bacteria.

CONCLUSION

Currently, *Corynebacterium glutamicum* (Gram-positive) and *Escherichia coli* strains (Gram-negative) are most commonly used at an industrial level for the production of essential amino acids used in food, feed and pharmaceuticals. Therefore, as mentioned above, the recent market launch and expansion trend of feed additives as granular type amino acids and/or alternative protein sources containing killed/deactivated bacteria suggested that information on the type as strain (whether it is Gram-positive bacteria or Gram-negative bacteria) used as the raw additive is needed. Now, a variety of killed bacteria are on the market for alternative protein (amino acids) sources for feed, and it is time to make informed decisions based on accurate information on raw additive.

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Salmonellosis in animals

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INTRODUCTION

Salmonella enterica subspecies *enterica* (Fig. 1) can be separated into more than 2,400 antigenically different serotypes. The greater number of incidents of salmonellosis in humans and livestocks originated from relatively few serotypes. *Salmonella* has long been recognized as an important zoonotic pathogen causing food-borne disease throughout the world. *Salmonella* infections occur in both domestic and wild animals. The common reservoir of *Salmonella* is the intestinal tract (Fig. 2). Therefore, *Salmonella* is usually present in feces excreted by animals and often contaminates animal origin products through fecal contact during production and slaughter. For that reason, they are called as carrier animals.

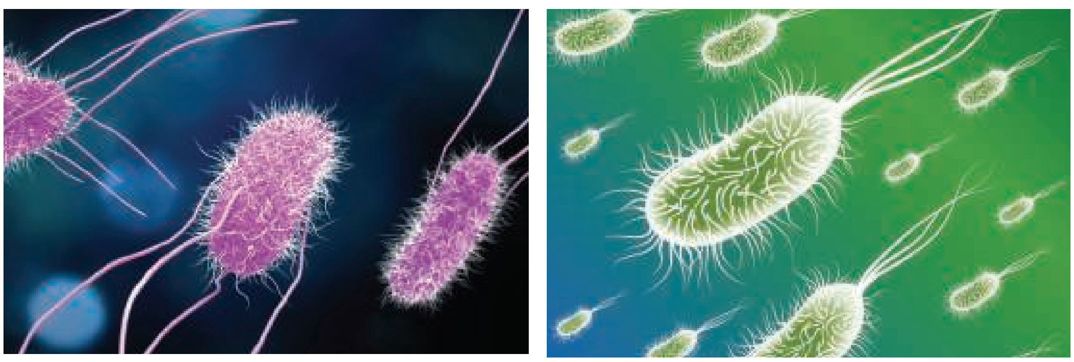

Figure 1. *Salmonella enterica* subspecies *enterica.*

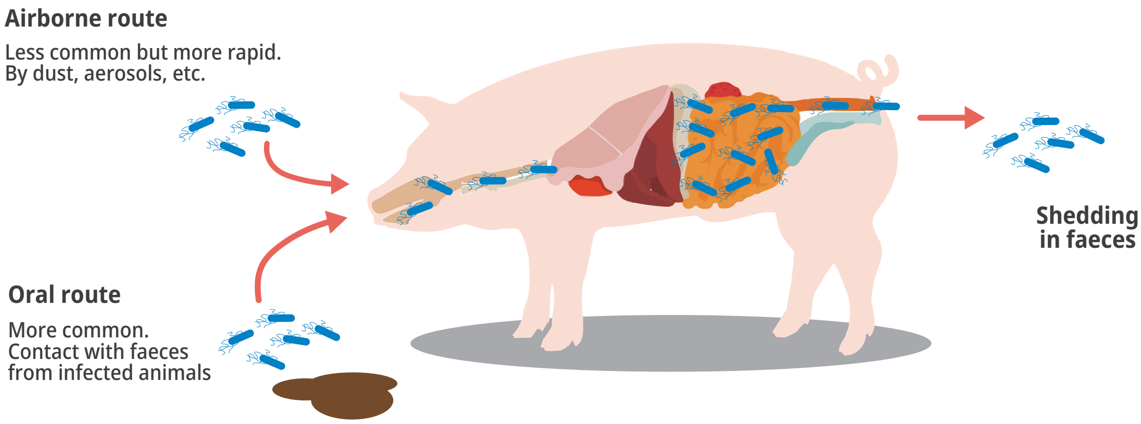

Figure 2. Infection route of Salmonellosis in swine.

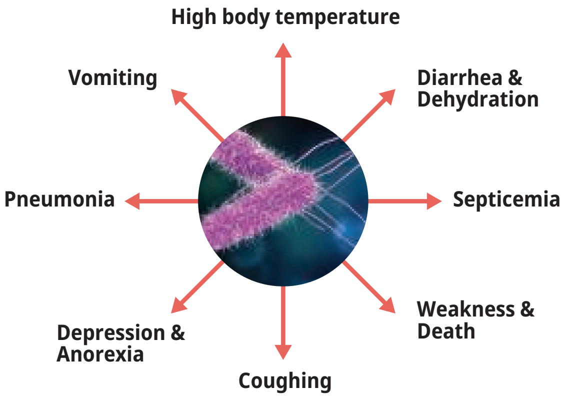

Figure 3. Symptoms of Salmonellosis.

Salmonella infections usually cause enteritis and diarrhea (Fig. 3). The bacteria can also aggress the body to cause septicemia and this aggression result in fever that commonly accompanies the enteritis caused by *Salmonella* infection. The affected animals are lethargic and do not eat. Very young, old, or immune-suppressed animals can be seriously affected by dehydration associated with diarrhea, develop septicemia or even die. In the case of survival, diarrhea may be seen for a while, but any recovering animal may be a carrier for various times. The bacteria can live in the intestinal lining, lymph nodes, and lymphoid areas such as the bird's ceca. This continuation of survival can also lead to the recurrence of *Salmonella* infections when the animal suffers from other diseases.

Salmonella infections in poultry

(A) Horizontal transfer of *Salmonella* between chickens occurs when virulent bacteria shed in the feces are ingested by feeding birds. (B) Only a small percentage of chickens entering processing plants are *Salmonella*-positive. (C) Up to 50% of carcasses become contaminated (indicated by cross-hatching) during processing. (D) Human infection occurs upon consumption of contaminated meat. (E) Transmission of *Salmonella* from hens to eggs. (F) The incidence of *Salmonella*-contaminated eggs (indicated by cross-hatching) increases greatly in the hatching cabinet and (G) within the first day after hatching. (H) *Salmonella*-infected chicks mature into infected adult birds. (I) Transfer of *Salmonella* to humans occurs upon consumption of contaminated eggs.

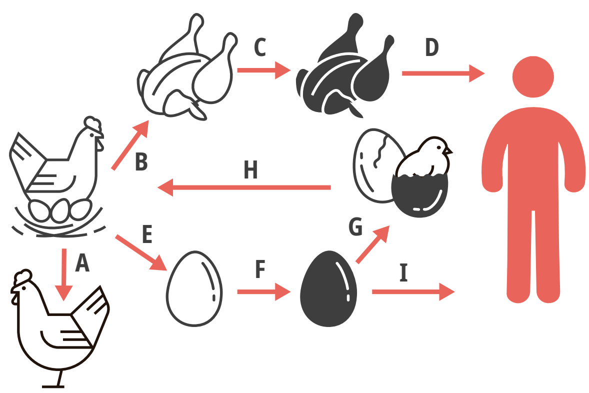

Figure 4. Salmonellosis in poultry and humans.

Poultry is one of the largest reservoir of *Salmonella* and significant risk to public health through consumption of contaminated eggs and meat (Fig. 4). Poultry products are frequently identified as important sources of *Salmonella* that cause human diseases. Eating contaminated eggs or chicken have been identified as a significant risk factor for *S*. Enteritidis infection [1]. Many of the serotypes that are most prevalent in humans such as *S*. Typhimurium and *S*. Enteritidis are also commonly found in poultry [2].
Poultry has many sources of infection including vertical transmission, contaminated feed and the environment. Asymptomatic excretion of *Salmonella* in the intestine causes the contamination of eggs in vertical transmission. As soon as hatching, oral ingestion by the chick or poult can result in large numbers of *Salmonella* entering the intestine and feces. This causes a rapid horizontal spread through the hatchery [3]. Poultry can be infected with a variety of *Salmonella* serotypes, mainly localized in the gastrointestinal tract with fecal excretion [4].

There are four diseases induced by *Salmonella* in poultry such as pullorum disease caused by *Salmonella enterica* serotype Pullorum, fowl typhoid (FT) caused by *S*. Gallinarum, arizonosis caused by *S. enterica* subsp. arizonae and paratyphoid caused by several serotypes and subspecies of *Salmonella* most particularly *S*. Typhimurium, *S*. Enteritidis, *S*. Infantis to name a few [4]. The pullorum disease (PD) caused by *S. enterica* serotype Pullorum, is egg transmitted and occurs primarily in the first few days of life, many dead-in-shell chicks are seen (white bacillary diarrhea). Fowl typhoid (FT) is a disease caused by *S. enterica* serotype Gallinarum, which is usually transmitted by the oro-fecal route and mainly affects adult birds [3]. The first incidence of FT was characterized by high mortality, especially during the first two months of the outbreak [4]. Arizonosis caused by *S. enterica* subsp. arizonae is an egg-transmitted infection mainly of young poultry, still sporadic in commercial flocks, can infect and induce disease in chickens or other species of birds. The bacteria is present in the ovary and oviduct of adult breeders and the chicks and poults hatched from infected breeders develop the disease. The disease is described by diarrhea with pasting feces in the cloaca, anorexia and elevated levels of mortality can be observed up to 50% [5].

Fowl paratyphoid caused by several non-host-specific *Salmonella* is an acute or chronic disease in poultry or mammalian species. The highest morbidity and mortality are usually observed during the first two weeks after hatch. Paratyphoid infections are important for public health through contamination and mishandling of poultry products. *Salmonella enterica* serotype Typhimurium, *S. enterica* Enteritidis, *S. enterica* Kentucky, and *S. enterica* Heidelberg are known as the most common reasons of *Salmonella* infections in poultry. *Salmonella enterica* serotype Typhimurium is mainly known to induce clinical salmonellosis in very young birds. Mortality rate range from less than 10% to over 80% in severe outbreaks. Resistance to infection develops rapidly during the first 72 hours of life, and has been attributed to maturation of macrophages and the development of a commensal flora in the gut leading to competitive exclusion of *Salmonella* [4]. The strains of *S. enterica* serotype Enteritidis are also highly virulent for young chicks [6]. *Salmonella enterica* serotype Enteritidis, and in particular strains of phage type 4 (PT4) can also cause asymptomatic and chronic infections in older birds including commercial layers and broiler breeders [7-9]. Epidemiological data show a clear association between food borne illness caused by serotype Enteritidis PT4 and the consumption of undercooked eggs [10]. *Salmonella enterica* serotype Enteritidis infections are mostly seen in fresh shelled eggs and egg products, in which the bacteria contaminate the interior components of the egg through transovarial infection. *Salmonella* enterica serotype Enteritidis infects the ova or oviduct of the hen's reproductive tract, which causes contamination of the albumen, vitelline membrane and possibly the yolk.

Salmonella infections in pigs

The organism, now known as *Salmonella enterica* serotype Choleraesuis, was first isolated from pigs when it was considered the cause of swine fever (hog cholera) [11]. The ability of *Salmonella* to cause disease in pigs depends on number of factors including the infected serotype and the age of the pig. Regional variation in occurrence of salmonellosis is loosely correlated to pig density, husbandry practices and co-mingling of pigs [4].

The *Salmonella* serotypes associated with clinical disease in pigs can be divided into two groups: the host-restricted serotypes represented by *S*. Choleraesuis and the ubiquitous serotypes represented by *S*. Typhimurium. The presence of *S*. Choleraesuis has sharply declined and is now only isolated sporadically. It was later understood that a diversity of antigenically distinct *S. enterica* serotypes can be isolated from pigs, some of which are of zoonotic as they transmitted to humans through the food chain and farm environment, where they typically cause acute but self-limiting gastroenteritis [12]. *Salmonella enterica* serotype Typhimurium is the most common serotype isolated from pigs. Likewise, *S*. Derby is strongly linked with pigs on both sides of the Atlantic Ocean, and is recognized as the second most common serotype in pigs. It is thought that oral intake is an important route of infection as a large amount of *Salmonella* are shed through feces of clinically infected pigs.

Aspiration of infected material into the upper respiratory tract is another possible route of infection. Pneumonia is a common feature of swine *S*. Choleraesuis infections [13]. Several studies have shown that pigs can be experimentally infected by intranasal inoculation. Pigs infected with *S*. Choleraesuis through the intranasal route experience more severe clinical signals than those infected through the oral route [14]. These observations indicate that the tonsils and lungs are important sites of invasion. Clinical salmonellosis in pigs is standardly of two forms; septicemia caused by host-restricted *S. enterica* serotypes such as Choleraesuis, and enterocolitis originated by wide host-restricted serotypes such as Enteritidis. Unsurprisingly, weaned pigs that are intensively reared are more at risk to be affected by *Salmonella* infections.

Like other host-specific serotypes, *S*. Choleraesuis can induce disease in both young and older animals, whereas *S*. Typhimurium typically lead to disease in pigs aged between 6 and 12 weeks of age, but seldom in adult animals. In older animal, subclinical infections with *S*. Typhimurium are frequent, leading to high transmission rates if active carrier animals are not detected. *Salmonella* enterica serotypes Choleraesuis typically cause septicemic forms of infection. *Salmonella enterica* serotypes Typhimurium typically causes enterocolitis [3].

CONCLUSION

Regulation is strengthening on meat production in many countries. Rearing of animals in unsanitary environments combined with insufficient control measures result in the transmission of many microorganisms including *Salmonella*. *Salmonella* has been recognized as a major zoonotic pathogen causing food-borne disease across the world. Unfortunately, many aspects of *Salmonella* are still unresolved despite significant amount of research aimed to control this microorganism. Therefore, it is imperative that *Salmonella* should continue to remain an important concern worldwide regarding research activity.

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