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REVIEW PAPER

SIGNIFICANCE OF ENDOGENOUS AMINO ACID LOSSES IN THE NUTRITION OF SOME POULTRY SPECIES: A REVIEW

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ABSTRACT

The accurate data on protein and amino acids digestibility of feed components is one of the ways to enhance nitrogen and protein utilization and to mitigate nitrogen pollution in a poultry farm. Digestibility and bioavailability of dietary nutrients depend on different factors like the type of feedstuffs, nutrients utilization, fiber contents, and diet adjustments in poultry. Diet formulation depends on dietary sources of protein, which is responsible to improve or maintain amino acid digestibility. The nutrients digestibility and absorption of the diet always decrease with increasing endogenous amino acid losses (EAAL). A lot of factors such as age, species and breeds play important role in EAAL contents. Furthermore, the high values of EAAL have been recorded in early ages in comparison with advanced ages; this result may be owed to the incomplete development of the gastrointestinal tract (GIT) and low digestion in early age as reported by several researchers. The estimation method and factors of the environment are strongly effective on the accurate measurement of EAAL. The aim of this review article is to compare the results of from different methods and highlight the issue EAAL of various poultry species including broiler chickens, laying hens and turkey poults. Besides, this paper illustrates some comparative studies among the aforementioned species according to different ages and methods to correctly understand the nutritional values of feed components, nutrient digestibility and utilization. Based on the review of existing literature this paper direct the attention of researchers and poultry producers to improve the accuracy of estimating EAAL among various poultry models, and to improve the productive and reproductive performance on commercial basis.

Key words: endogenous amino acid losses; pollution; ammonia; age; poultry.

INTRODUCTION

The optimization of nutrients utilization of poultry diets is very important for stabling production and enhancing performance as well as reducing environmental pollution. Actually, the nutrient requirements of birds mainly depend on protein and amino acids digestibility in different feedstuffs (Kim *et al.,* 2015; Reda *et al.,* 2015; Soomro *et al.,* 2017a,b; Abou-Kassem *et al.,* 2018). In poultry, the formulation of diets on the basis of digestible amino acids has improved amino acids utilization and nutrients digestibility (Alagawany *et al*., 2011; Alagawany *et al*., 2014; De Lange *et al*., 2003; Adeola *et al*., 2016). Several studies have been conducted on broiler chicken and turkey poults with various methods of endogenous losses of amino acids (EAAL) estimation at different weeks of age (Ravindran *et al*., 2004; Ravindran and Hendriks, 2004; Adedokun *et al*., 2007b). The absorption of endogenous amino acids is different in various poultry birds depending on the nutrient digestibility, requirements and physiological status of the gastrointestinal tract (GIT). The endogenous amino acid origin is influenced by digestive enzymes; salivary enzymes, gastric secretion, bile salt pancreatic enzymes, and mucins or mucoproteins (Ravindran and Hendricks, 2004). The EAAL are highly influenced by the digestibility of endogenous amino acids. Broiler and turkey birds recorded the largest amount of EAAL at 5 days of age (Adedokun *et al.* 2007a). On the same context, amino acids digestibility and age of birds played a vital role in EAAL (Batal and parsons, 2002; Huang *et al*., 2006). The EAAL fractions differ in different species of poultry such as broiler chickens, laying hens, turkey, caecetomized rooster and poults. The variation in EAAL values may be attributed to age, strains and experimental conditions (Adedokun *et al*., 2007a, 2007b, 2007c, 2008, 2009). In poultry, many methods have been employed to evaluate amino acids (AAs) and other nutrients digestibility to minimize EAAL and improve nutrient absorption.

The EAAL assessment in poultry is essential to evaluate the nutrients digestibility of ingredients for feed formulation and requirements of AAs (Zhai and Adeola, 2011; Favero *et al*., 2014). This assessment has many challenges to accurately estimate the losses in poultry birds. Furthermore, several factors affecting AAs losses in poultry such as age, analytical methods, nutritional factors and environmental factors.The present article throws light on some comparative studies on EAAL in few species of poultry (broilers, layers and turkey poults).

Endogenous secretions origination: Endogenous secretions originate from digestive secretions sources like bile, saliva, gastric, intestinal, and pancreatic secretions, epithelial intestinal cells and mucoprotein (De Lange *et al*. 2003; Ravindran 2016). In poultry, sources of endogenous origin are similar but the contribution of secretion may vary. In pigs, ileal endogenous protein and amino acid composition are constant and independent diet with technique of determination (Stein *et al*., 2007; Ravindran, 2016). Whilst, the endogenous AAs profile was similar in poultry but there were little differences between both species (Hess *et al*., 1998). Digestive secretions (saliva, bile and pancreatic secretion) are responsible for elevating and lowering flow of EAAs and endogenous secretions in poultry (Hodgkinson *et al*., 2003). The EAA flow and their losses were relatively affected by the amount of digestive secretions. The isolation methods of dietary and endogenous origin AAs from total endogenous losses are still facing many challenges. The accurate quantification of EAAL and true amino acid digestibility of feed ingredients are very important for the proper evaluation of digestive secretions ratio in GIT as reported by Makkar (2008). The intestinal epithelial cells and mucoprotein enhanced EAAL flow in animals (Moncada *et al*., 2003).

The relationship between amino acids excretion and nitrogen pollution: The main objective of feeding operations in most of the poultry production is to provide the actual amount and quality of dietary protein to improve productive performance with a minimum diet-cost. Reducing nitrogen and amino acid losses from the poultry farms should begin with proper management practices and feeding to decrease nitrogen excretion. Furthermore, improving the dietary AAs profile in the diet via theuse of crystalline amino acids may result in an additional reduction of the nutrient excretion, especially nitrogen (Han and Lee, 2000). A major part of excreted nitrogen can be speedily converted into ammonia gas, which may easily volatilize into surrounding environment. Volatile losses begin soon after nitrogen excretion, and these losses continue via handling processes of manure until the nutrients of manure are combined with soil (Rotz *et al*., 1999; Alagawany *et al*., 2014).

Crude protein (CP) in the diet can be decreased by supplementation of commercial amino acids or enzymes to decrease nitrogen excretion from poultry (Nahm, 2002; Alagawany *et al*., 2016a,b; Rehman *et al*., 2017; Alagawany *et al*., 2018). Reduction of 2 to 3% of CP-diet has been made without detrimental effect on productive performance and helps to reduce nutrient excretion, especially nitrogen (Han and Lee, 2000; Laudadio *et al*., 2012). On the same context, reduction of 4% of CP-diet in laying Japanese quail also has been made without negative impact on productive and reproductive performance and a low-CP diet supplemented with synthetic amino acids decreased nitrogen excretion and emissions (Alagawany *et al*., 2014).

Sutton *et al*. (1999) pointed out that low-CP diets supplemented with synthetic amino acids reduced ammoniaemission by 28 to 79%, the wide variation in excreted may be due to the reduction in CP-diet, the size of the animal, and the CP level in control diet.Moreover, ammonia is produced by bacteria from the nitrogenous components in the manure. For each % reduction in dietary CP level, a 9.5% reduction in ammonia emission was reported in previous studies (Kay and Lee 1997; Le *et al.* 2009).

Endogenous amino acid losses in poultry

Broilers: Several methods such as guanidinated casein (GuC) and enzyme hydrolysed casein (EHC) methods have been used to quantify the definite concentration of AAs in endogenous origin in broilers as reported by Ravindran *et al*. (2004). The high content of dietary crude fiber increased EAA flow (Montagne *et al*., 2003). Moreover, there are several factors like elevating the EAAL and decreasing nutrients digestibility affecting the production performanceof poultry birds. The digestible AAs play a critical role inminimizing these losses and enhancingdigestibility. Table1 presented many research data on the determination of EAAL for improving the digestibility of AAs in broilers, but the confusion in results is very clear to show the evaluation is unsatisfactory as claimed by Adedokun *et al*.(2009; 2007a; 2007c). Furthermore, there is still a question on the method, feeding and estimation trial for a precise measurement of EAAL in broiler chickens (Adedokun *et al*., 2007b; Ravindran *et al*., 2004; Kong and Adeola 2013). The EAAL ratios are little bit variation according to some trials (Table 1), and this variation may be due to different age of birds and methodology used. Additional studies thus are needed on birds'growing age for a better estimation of EAAL.

Laying hens: As described by Moughan and Rutherfurd (1990); Adedokun *et al*. (2007a, b); Adedokun *et al*. (2009), the estimation of EAA flow in layers was employed using various methods for the evaluation of endogenous losses. Meanwhile, EAAL were higher in laying birds compared to broilers because layers are reared for a long time. Over the years, the management of the laying hen and composition of the diet fed have gone through many changes and alterations. This is because the diet fed to commercial laying hens can vary greatly, depending upon factors including the strain of bird, production goals, age, and weather conditions. So, EAAL ratio in laying hens is higher than that in turkey poults and broilers. Table 2 shows endogenous AAsevaluation.

Caecetomized rooster: Several methods have been used to estimate ileal EAAL. The classical methods, including the regression method, the use of nitrogen-free diet (NFD), and the fasted cecectomized rooster method, are the most widely used (Parsons *et al*. 1982; Adedokun *et al.* 2011).This method is practiced in monogastric animals since a longtime, however the dietary nutrients digestibility as well as ingredients selection are still a part of discussion, because of increase in the flow and EAAL. Meanwhile, Adedokun *et al*., (2009) reported that the EAA flow in cecectomized roosters at 104 wk of age was higher (3.5- to 12-fold) than in broiler chickens and layers. Moreover, no significant effect (NFD vs. HDP) was observed in the EAA flow in precision-fed and fasted cecectomized roosters, but the EAA in the fasted roosters was lower relative to cecectomized roosters fed NFD or HDP diets (Adedokun *et al.,* 2009). In another study Ravindran and Hendriks, (2004) estimated that the EAAL in (70 weeks old) roosters, using the peptide alimentation method. The results showed that the concentrations of serine and isoleucine were noted higher in roosters compare to layers and broilers. Song *et al.* (2007) evaluated the effect of nitrogen-free diet (NFD) and NFD+5% casein diet on EAAL in (27 weeks old) Lohmann Brown roosters and layers. They showed that casein supplementation up to 5% could improve the endogenous nitrogen and EAAL in roosters and layers, the endogenous nitrogen and AAs losses in roosters were noted higher compare to layers. Kessler *et al.* (1981) investigated the EAAL in Intact and cecectomized adult male, Single Comb White Leghorns rooster in unfed control roosters for a 24 to 48 hr fasting period. They found that histidine and methionine losses were unchanged in both intact and cecectomized roosters. However, all other reported AAs were changed in cecectomized roosters. Table 2 shows that body weight of roosters was heavy and feed intake was also improved than broilers and laying hens.

Turkey poults: The flow EAA was increased in early age (5 days old) turkey poults in comparison with advanced age at 21 daysof age (Adedokun *et al*., 2007a,b). In turkey poults, increased EAA flow was observed by age and diets of poults.The researchers suggested that feeding interval and ingredients significantly affect nutrients digestibility. There are only a few reports on IEAA or EAAL in turkey poults especially at early ages through the first 3 weeks of age. Adedokun *et al*. (2007a,b) and Adedokun *et al*. (2008) showed that the EAA flow in turkeys and broilers was similar (Table 3). On the other hand, the amount and profile of basal IAA_{end} determined with nitrogen free diet (NFD) may vary due to several factors such as choice of analytical method, types of feed ingredient used, age of poultry birds and environmental condition. Therefore it is necessary to include NFD in every AA digestibility experiment for accurate calculation of EAAL. The recommended NDF formulation is shown in Table 4.

Effect of animal age on EAAL

Pre-starter phase: Protein andAAs are vital nutrients that must be supplied in adequate amount and proportions to support optimal animal performance.The EAAL is higher during early age of poultry birds because the digestive tract of growing birds has adapted to tremendous changes in early life. Investigations are still in progress to explore these issues to improve animals' prestarter phase for better productions in the future. Adedokun *et al*. (2007a) stated that the EAAL were increased by 50% in the early phase in comparison with the growing phase. Not only the age of poultry birds influence on EAAL but the method of estimation also important to accurately measure the EAAL (Adedokun *et al*. 2007c). On the same context, Ravindran (2016) demonstrated that the estimation methods have more impact on EAAL evaluation. These procedures need to be optimized by conducting more researches in this direction, as theoutput and scientific data make more appropriate the estimation methodology of EAAL. There are differences in rearing and keeping, as well as diet formulation and ingredient selection.

Grower stage: The impact of age on LEAA estimates has been elaborated in poultry (Ravindran and Hendriks 2004). Jansman *et al*., (2002) showed that EAAL was highly influenced by age of birds. Moreover, improvement in animal health and function of digestive tract reduced EAAL. The growing stage of broilers needs to optimize the utilization of amino acids through formulating diets on DAA basis (De Lange *et al*., 2003). Many studies were conducted on feed formulation and diet utilization, but these studies did not focus on nutrient utilization and excretion. The digestibility and absorption of nutrients play animportant role on EAAL and nitrogen emission. Furthermore, Adedokun *et al*. (2007a) pointed out that EAAL flow in broiler chickens at 5 days of age was approximately twice that noted on 15 or 21 days of age,while the change in flow through the same period in turkey poults was about 3-fold higher than that of broilers. These results point to 2 things. First, under a given set of conditions, the concentration of amino acids of endogenous origin in the ileal digesta on d 5 could affect the values of apparent digestibility of different feed ingredients. This brings into question the extent to which digestibility of amino acid of feedstuffs increases after the first 7 days of age. Furthermore, the reduction in LEAA flow on d 15 relative to d 5 (53% reduction for chicks and 68% reduction for poults, total AAs) and the lack of significant variation in LEAA flow between d 15 and 21 is very interesting. The impact of age on EAA flow might become less significant as a bird matures is confirmed by the findings of Ravindran and Hendriks (2004) when EHC (180 g of EHC/kg of diet) was fed to 6 wk of age broiler chicks, 70 wk of age laying hens, and 70wk of age roosters.

Dietary factors affecting EAAL: Determination of EAAL was affected by several directly and indirectly factors like dietary protein, marker index, mucin, phytate, dietary fiber, gut performance, age, species and methodology. As shown in Fig. 1a the basal IAA_{end} is defined as the inevitable loss of AA in the digestive tract of animals, which is related to the amount of DM intake but otherwise unrelated to dietary composition (McDonald *et al*., 2011). Furthermore, EAAL, protein excretion, and nitrogen pollution were influenced by feed formulation, diet composition, bird's age, productive status, health status and dietary amino acids and fiber contents (Adedokun *et al*., 2011; Alagawany *et al*., 2014). However, in poultry, ELAA and protein were affected by non-digested dietary amino acid (NDDAA); basal endogenous amino acid losses (BEAAL) and specific endogenous amino acid losses (SEAAL) (Fig. 1b). Many research studied have been conducted regarding the accurate estimation of endogenous losses and to minimize the EAAL, however it still in progress (Fig. 2) (Stein *et al*., 2007; Adedokun *et al*., 2011).

Anti-nutritive factors: Table 5 documents several results by many trials regarding anti-nutritional factors. The anti-nutritive factors in the diets directly and indirectly influenced the flow of EAAL in poultry (Ravindran, 2016). These factors including cellulose and pectin (De Lange *et al*. 1989); soybean trypsin inhibitor (Barth *et al*., 1993); neutral detergent fiber NDF (Schulze *et al*. 1994); sources of NDF (Schulze *et al*., 1995); non starch polysaccharide (NSP) (Morel *et al*. 2003); tannins (Steendam *et al*., 2004); phytic acid (Woyengo *et al*. 2009) were commonly found in poultry. Therefore, it is necessary to modify the basic reasons causing these factors.

There are similar factors in poultry, but these factors are few in poultry that influence the endogenous flow of AAs and their losses such as NSP (Angkanaporn *et al*. 1994), phytic acid and cellulose (Kluth and Rodehutscord, 2009). Reducing digestibility of ingredients and utilization of dietary AAs supplementation increased endogenous losses. However, increasing diet utilization and nutrients absorption directly reduced the EAAL. It's documented that diet and feed formulation needs to be revised for reducing endogenous losses and increasing dietary amino acid digestibility (Onyango *et al*., 2009).

Table 1. Endogenous amino acid losses in broiler chickens fed Nitrogen free diets (mg/kg of DMI)

	Broiler chickens							
Items	Ravindran *et al.* (2004)	Adedokun *et al.* (2007a)	Adedokun *et al.* (2007b)	Adedokun *et al.* (2007c)	Goline *et al.* (2008)	Adedokun *et al.* (2009)	Soleimani *et al.* (2010)	Kong and Adeola (2013)
Essential Amino acids								
Arginine	280	159	272	168	230	122	469	575
Histidine	158	70	158	73	91	57	203	248
Isoleucine	287	159	242	162	200	119	327	541
Leucine	439	242	408	251	298	184	535	875
Lysine	209	182	273	181	173	140	383	630
Methionine	101	44	90	50	65	50	81	173
Phenylalanine	287	153	263	154	420	119	394	500
Threonine	512	263	454	274	434	236	538	804
Tryptophan	95	NA	71	NA	71	NA	50	68
Valine	417	205	389	214	270	180	398	660
Nonessential amino acids								
Alanine	293	167	308	177	217	140	349	572
Aspartic acid	607	329	568	340	430	248	574	1015
Cystine	262	138	212	136	143	87	103	1085
Glutamic acid	721	431	687	420	492	334	851	1337
Glycine	508	195	324	205	245	155	400	653
Proline	NA	245	381	240	289	163	341	675
Serine	424	268	401	260	343	169	547	711
Tyrosine	253	130	193	124	NA	98	265	390
Total Amino acids	5,817	3,935	6,843	3,952	4,368	3,076	6,810	12,779

[1]NA: data not available

Table 2. Endogenous amino acid losses in laying hens and roosters fed Nitrogen free diets (mg/kg of DMI).

Items	Laying hens	Rooster
	Adedokun *et al*. (2009)	Adedokun *et al*. (2009)
Essential Amino acids		
Arginine	263	1759
Histidine	128	846
Isoleucine	280	1354
Leucine	424	2338
Lysine	272	1693
Methionine	117	406
Phenylalanine	248	1405
Threonine	529	1853
Tryptophan	75	194
Valine	424	1992
Nonessential amino acids		
Alanine	287	1793
Aspartic acid	573	2640
Cystine	198	1087
Glutamic acid	670	4245
Glycine	308	10815
Proline	392	2030
Serine	390	1978
Tyrosine	211	1315
Total Amino acids	7214	41145

Table 3. Endogenous amino acid losses in turkey fed Nitrogen free diets (mg/kg of DMI).

Items	Turkey		
	Adedokun *et al*. (2007a)	Adedokun *et al*. (2007b)	Adedokun *et al*. (2008)
Essential amino acids			
Arginine	81.6	96.9	66.7
Histidine	68.7	94.4	71.6
Isoleucine	63.8	93.4	63.1
Leucine	69.1	94.4	67.7
Lysine	76.4	95.4	52.7
Methionine	73.6	97	76.6
Phenylalanine	67.3	94.9	71.7
Threonine	55.9	92.4	49.7
Tryptophan	NA	91.6	NA
Valine	62.3	92.8	63.1
Nonessential amino acids			
Alanine	63.7	92.6	47.8
Aspartic acid	81.4	94.4	48.2
Cystine	67.3	82.6	NA
Glutamic acid	79.1	95.3	78.6
Glycine	57.5	91.7	32.7
Proline	57.8	93.4	40.9
Serine	62.4	94.1	60.2
Tyrosine	56.3	91.5	48.8
Total Amino acids	1211.7	1770.6	998.6

[1]NA: data not available

Table 4. Mean of determined basal endogenous losses of AA (g/kg DM intake basis) with different methods in some previous studies in past decade.

Species	Broilers			Layers	Roosters	Turkeys			Ducks
Method	Nitrogen-free diet[a]	Casein diet[b]	Regression[c]	Casein diet[d]	Casein diet[d]	Nitrogen-free diet[e]	Casein diet[f]	Regression[g]	Nitrogen-free diet[h]
Essential Amino Acids									
Arginine	0.39	0.22	0.18	3	2.5	0.27	0.31	0.27	1.49
Histidine	0.18	0.13	0.13	2.4	2.2	0.15	0.17	0.14	0.51
Isoleucine	0.37	0.38	0.2	3.3	4.7	0.25	0.39	0.26	1.26
Leucine	0.56	0.37	0.27	4.9	4.7	0.41	0.49	0.4	2.15
Lysine	0.39	0.3	0.15	3.2	3.6	0.27	0.35	0.24	1.18
Methionine	0.11	0.1	0.06	1.2	1.1	0.09	0.12	0.1	2.23
Phenylalanine	0.37	0.33	0.28	3.4	2.9	0.26	0.28	0.24	1.21
Threonine	0.6	0.45	0.34	7.5	7	0.44	0.47	0.38	1.43
Tryptophan	0.09	0.09	0.08	NA	NA	0.07	0.07	NA	0.24
Valine	0.51	0.39	0.24	4.9	5.5	0.4	0.49	0.37	1.76
Non-Essential Amino Acids									
Alanine	0.39	0.28	0.19	5.2	3.1	0.31	0.37	0.29	1.33
Aspartic Acid	0.73	0.56	0.36	7.4	7.9	0.56	0.68	0.53	2.34
Cysteine	0.41	0.16	0.14	2.3	2.1	0.21	0.23	0.21	1.04
Glutamic Acid	0.98	1.11	0.41	10.5	15.1	0.7	1.14	0.52	3.03
Glycine	0.47	0.28	0.22	5.8	6.2	0.33	0.36	0.51	1.64
Proline	0.5	0.38	0.24	6.4	6.6	0.4	0.46	0.35	1.87
Serine	0.56	0.61	0.33	6.2	8.3	0.39	0.57	0.38	1.64
Tyrosine	0.3	0.17	0.11	2.7	2.3	0.19	0.22	0.18	0.7

NA= Not available

[a] Means of previous studies (Cozannet *et al*., 2011; Kong and Adeola, 2013; Toghyani *et al*., 2015).
[b] Means of previous studies (Adedokun *et al*., 2007c; Golian *et al*., 2008).
[c] Means of previous studies (Adedokun *et al*., 2007,b; Golian *et al*., 2008).
[d] Ravindran and Hendriks (2004)
[e] Means of previous studies (Adedokun *et al*., 2007a).
[f] Means of previous studies (Adedokun *et al*., 2007a).
[g] Means of previous studies (Adedokun *et al*., 2007a).
[h] Means of previous studies (Kong and Adeola, 2013).

Table 5.Studies evaluating the effects of purified forms of fibre or anti-nutrients on ileal endogenous amino acid losses reported by Ravindran (2016).

Reference	Background	Factors examined
de Lange *et al*. (1989)	Protein-free diet	Cellulose, pectin
Barth *et al*. (1993)	Guanidinated casein	Soybean trypsin inhibitor
Schulze *et al*. (1994)	Highly digestible protein	Level of neutral detergent fibre
Schulze *et al*. (1995)	Highly digestible protein	Source and level of neutral detergent fibre level
Morel *et al*. (2003)	Enzyme-hydrolysed casein	Non-starch polysaccharides
Steendam *et al*. (2004)	Isotope dilution and enzyme-hydrolysed casein	Condensed tannins
Woyengo *et al*. (2009	Guanidinated casein	Phytic acid
Broiler chickens		
Angkanaporn *et al*. (1994)	Guanidinated casein	Non-starch polysaccharide
Cowieson and Ravindran (2007)	Enzyme-hydrolysed casein	Phytic acid
Cowieson *et al*. (2008)	Enzyme-hydrolysed casein	Phytic acid
Kluth and Rodehutscord (2009)	Regression method	Cellulose
Onyango *et al*. (2009)	Glucose	Phytic acid

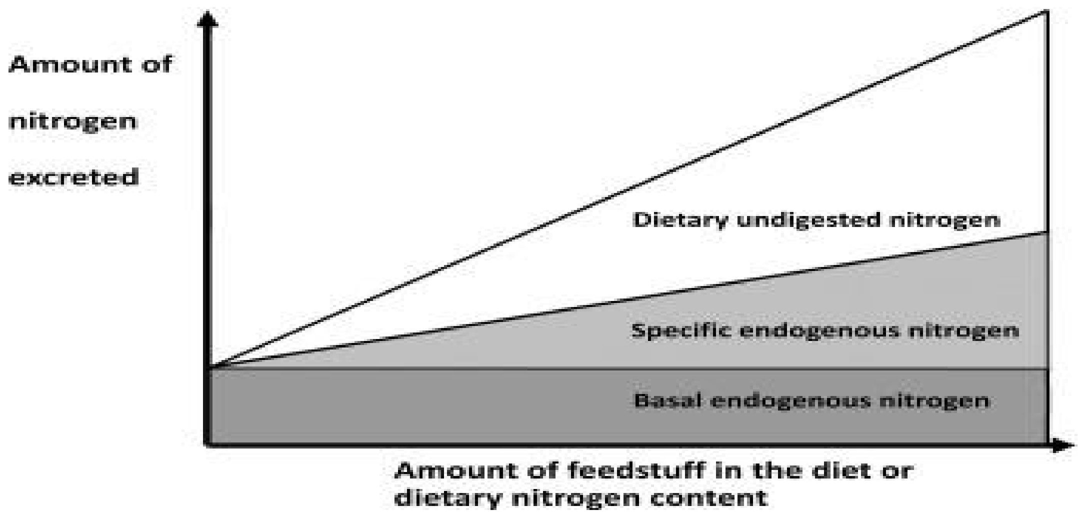

Fig. 1a. Partition of ileal nitrogen flow (adapted from *McDonald et al.* 2011)

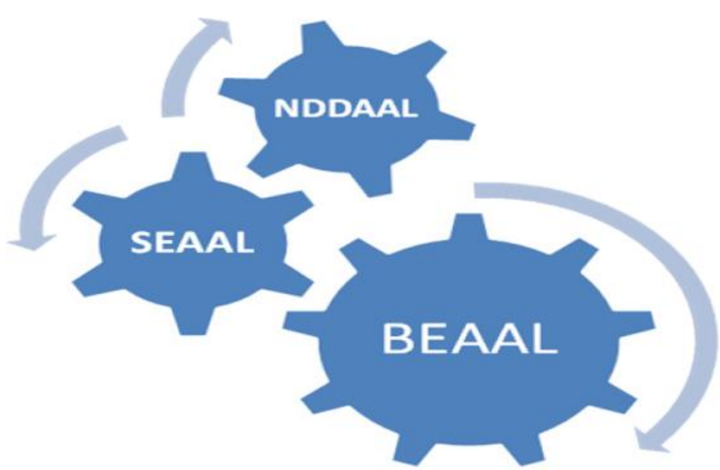

Figure.1b. Poultry ileal digesta the EAAL partitioning by dietary AAs fed.

*BEAAL, Basal ileal endogenous amino acid losses; SEAAL, Specific ileal endogenous amino acid losses; NDDAAL, Non-digestible dietary amino acid losses.

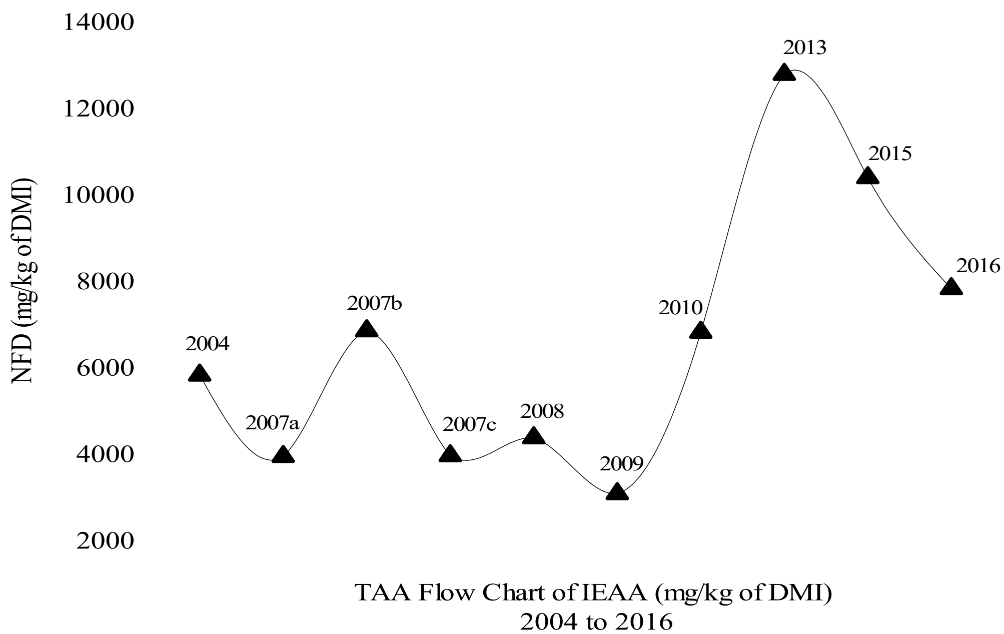

Fig. 2.Total flow chart of IEAA in poultry and pigs.

Conclusions: Adjusting EAAL in poultry birds is an important factor to enhance nutrient utilization, reduce nutrient losses and nitrogen excretion as well as to mitigate nitrogen emission and environmental pollution. The reduction in endogenous losses also improves the productive and reproductive performance as well as reduces feed cost. Based on the review of existing literature this review paper highlights theimportance of EAAL in poultry birds.

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