Utilization of NPN by Poultry: A REVIEW

非蛋白態 窒素化合物의 利用

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요 약

비단백태 질소 화합물의 가금에서의 이용 가능성은 오래 전부터 많은 사람들의 관심의 대상이 되어 있었다. Urea 나 Diammonium Citrate 같은 NPN 들이 부로일러에서 非必須 아미노산들의 공급을 목적으로 이용될 수 있다는 많은 증거들이 있다. 그 mechanism 은 이들에 의해 공급되는 ammonia 가 α-ketoglutaric acid 에 결합되어 glutamic acid 가 되어 체내에서 transamination 방법에 의해 비필수 아미노산들의 합성에 기여할 수 있다는 것이다.

산란계에서도 마찬가지 방법으로 이용될 수 있으나 육계에서 보다는 이용 효율이 낮다고 한다. 비단백태 질소 화합물의 급여가 사료섭취량에 영향을 주고, 산란율을 저하시키며 卵重을 적게하는 원인이 되나 albumin 의 質을 높인다는 장점도 보고되고 있다.

이들 화합물이 가금에서 효율적으로 이용되기 위해서는 사료내 단백질 수준이 정상보다 낮아야 하며 필수 아미노산을 충분히 함유하고 있어야 한다. 장차 단백질 자원의 부족 현상이 더욱 심각해질 것을 예상할 때 비단백태 질소 화합물의 가축에서의 이용성을 높이는 연구는 매우 중요하다.

1. Introduction

Many kinds of non-protein nitrogen (NPN) substances have been considered for a long time as the substitutes for dietary proteins for animals and, recently, for human. Among them, urea, especially, has been investigated in detail for

the utilization as a protein substitute in animal feeds. Now urea is a useful substitute for part of the protein supplements in a concentrate mixture for ruminants, particularly when there is a shortage of protein supplements or when they are very high in price. However, urea-containing concentrate mixtures are never superior to mixtures in which the same amount of nitrogen is supplied by ordinary protein supplement (Morrison, 1959).

The utilization by chickens of nitrogen from various non-protein sources has been the subject of much research, because we can expect from the supplementation of NPN to a diet that chicken may synthesize all of non-essential amino acids from nitrogen in NPN by usual biochemical routes as in most vertebrate.

Early work on the utilization of ammonium compounds suggested that urea, ammonium citrate and ammonium phosphate were not utilized by chickens when these compounds were added to diets containing from 11 to 20 per cent protein (Bice and Dean, 1942; Jones and Combs, 1953). However, Slinger *et al.* (1952) provided some evidence that a low level of urea (nitrogen equivalent 1.5% protein) could be utilized in a broiler diet containing 10.5% protein, but at higher dietary protein levels (15.5 or 17%) there was no evidence that urea was utilized.

Using a semipurified diet based on casein, Wixom *et al.* (1955) were unable to show a growth response in two-week-old chicks to diammonium citrate or alanine although additional glycine improved growth; yet Lee and McNab (1978), and Sullivan and Bird (1957) found that supplements of urea or diammonium citrate to low protein diets improved the growth rate of chicks. There is also ample evidence that, when added to diets which are based on crystalline amino acids, various non-protein nitrogen sources enhance growth of broiler chickens (Featherston *et al.* 1962; Farlin *et al.* 1968; Blair *et al.* 1972; Lee and Blain, 1972).

The extent to which utilization occurs is still a source of contention, but it appears that it depends on the type of diet to which the NPN source is added.

2. Effect of variation in NPN sources and levels on growth response

Dietary nitrogen serves to supply the needs of the animal in terms of essential amino acids and the materials necessary (amino groups) for the synthesis of those amino acids individually regarded as non-essential. Within the category of dietary non-protein nitrogen it is appropriate to include both free amino acids and non-amino nitrogen.

Non-Essential Amino Acids

L-glutamic acid appears to be utilized well by poultry (Scott *et al.*, 1963;

Dean and Scott, 1965; Young *et al.*, 1965; Renner, 1969), but the D-form is not utilized and has been reported to depress growth (Perterson *et al.*, 1971). Renner (1969) showed that either L-glutamate or L-aspartate could serve as the major source of non-essential nitrogen when non-protein energy was supplied by either glucose or soybean oil.

L-aspartate appears to be utilized as efficiently as L-glutamate (Sugahara and Ariyoshi, 1967a, Manoukas and Young, 1969; Renner, 1969; Kagan and Balloun, 1976), although one report suggests that L-aspartate is inferior to L-glutamate (Peterson *et al.*, 1971). Sugahara and Ariyoshi (1967 a) found that serum aspartic acid level was not increased markedly by increasing the level of this amino acid in the diet and they interpreted the result as indicating that the utilization of L-aspartic acid was efficient. D-aspartic acid has no nutritional value and when used to replace L-glutamic acid was found to cause a growth retardation.

Lee and Blair (1972) compared various sources of nitrogen on an isonitrogenous level (2.52% N) by adding to the synthetic diet of 14.4% protein level. L-glutamic acid and triammonium citrate gave the better result in growth response and in feed conversion efficiency than monoammonium citrate, diammonium citrate, urea, or triammonium phosphate.

Alanine does not appear to be as good a source of nitrogen for the synthesis of non-essential amino acids as glutamic acid. While DL-alanine at a low level in mixtures with other non-essential amino acids has given good results (Klain *et al.*, 1959; Adkins *et al.*, 1962; Manoukas and Young, 1969), L-alanine per se is probably at best as good as diammonium citrate (Sugahara and Ariyoshi 1967 a; Renner, 1969).

Adkins *et al.* (1962) and Renner (1969) found that DL-but not L-alanine depressed growth. The growth depression was attributable to the D-form since Sugahara *et al.* (1967) found that the D-form retarded growth, while Adkins *et al.* (1962) found that the D-form was toxic for chicks although no impurities were found in it. Sugahara and Ariyoshi (1967a) tested serum responses to the addition of various non-protein nitrogen compounds to the diet and found that adding L-alanine caused a marked increase in alanine concentration in serum, whereas the addition of L-glutamic acid did not give a marked increase in the serum level of this amino acid. They interpreted this result as indicating that the utilization of L-alanine was inefficient.

Excess essential amino acids can also be utilized for the synthesis of non-essential amino acids. The results of Fell *et al.* (1959) and Sugahara *et al.*(1961) indicate that the D- and L- forms of methionine, phenylalanine and proline are utilized equivalently; D-leucine is slightly poorer than L-leucine; D-valine is one half as good as L-valine; D- forms of tryptophan, isoleucine and histidine are of limited use; and the D- forms of lysine, threonine and arginine have no nutritional value, the D-form of lysine perhaps depressing utilization of the L-form.

Proline has not been considered among the non-essential amino acids since there is now general agreement that it is essential at least for chicks fed purified

diets (e.g. Blair *et al.*, 1972). Glycine is utilized well by the bird for the synthesis of non-essential amino acids probably to the same extent as glutamic acid (Waterhouse and Scott, 1961a; Sugahara and Ariyoshi, 1967b).

There is considerable evidence for the interconversion of glycine and serine but the results of Sugahara and Ariyoshi (1967a) suggest that the conversion of serine to glycine is less rapid than the conversion of glycine to serine.

Non-Amino Nitrogen

At low levels in the diet (under 5 to 6 per cent), diammonium citrate appears to be utilized equivalently with L-glutamic acid (Young *et al.*, 1965), at least when the diets contain carbohydrates (Renner, 1969); equivalently with a mixture of non-essential amino acids (Featherston *et al.*, 1961 Featherston *et al.*, 1962a; Scott *et al.*, 1963; Blair and Young, 1970); and equivalently with intact protein (Young *et al.*, 1965; Chavez *et al.*, 1966).

Shannon *et al.* (1970) investigated the ability of diammonium hydrogen citrate and glutamic acid to meet the non-essential amino acid requirement by using diets containing, as the only sources of nitrogen, essential amino acids, or this diet supplemented with 11.1% diammonium hydrogen citrate or 12.0% glutamic acid. Diet containing glutamic acid, in addition, had a higher level of glycine and 1.0% proline.

Chicks given diammonium hydrogen citrate gained weight significantly faster than those given only essential amino acids but significantly more slowly than those given glutamic acid. Diets containing diammonium hydrogen citrate or L-glutamic acid were better in feed conversion efficiency than the essential amino acid diet. Carcass analysis revealed no significant treatment differences in carcass nitrogen or dry matter content, confirming that diammonium hydrogen citrate can be utilized to meet the non-essential amino acid requirement of the chick.

The results in Table 1 and Table 2 were shown by Lee and Blair (1972). Triammonium citrate and glutamic acid produced birds that were significantly heavier than those on other NPN sources such as monoammonium citrate, diammonium citrate and urea etc. and feed conversion efficiency was markedly improved (Table 1). In Table 2, chicks given diets in which one or two thirds of the glutamic acid were replaced by triammonium citrate (2C and 2D) also gave good results similar to those obtained from chicks given the glutamic acid as the sole NPN source. However, in contrast to the results in Table 1, chicks fed diet with triammonium citrate as the sole source of NPN showed lighter body weight and less feed intake than those fed diets containing glutamic acid.

But this difference appeared to be due to an improved growth rate with glutamic acid rather than a reduced growth rate with triammonium citrate.

Table 1

Dietary treatments, mean live-weight, food intake and food conversion efficiencies of chicks used in experiment 1

	Dietary treatments (g kg diet)						
	1A	1B	1C	1D	1E	1F	1G
Basal diet mixture	826.2	826.2	826.2	826.2	826.2	826.2	826.2
Glutamic acid	.....	120.0	.....	.....	.....	.....	.....
Monoammonium citrate	.....	.....	170.5	.....	.....	.....	.....
Diammonium citrate	.....	.....	.....	92.3	.....	.....	.....
Triammonium citrate	.....	.....	.....	.....	66.2	.....	.....
Urea	.....	.....	.....	.....	.....	24.5	.....
Triammonium phosphate	.....	.....	.....	.....	.....	.....	40.5
Calcium lactate	.....	.....	.....	.....	.....	.....	125.6
Sucrose	173.8	53.8	03.3	81.5	107.6	149.3	7.7
Total N content	13.8	25.2	23.9	26.7	23.4	25.6	23.3
Mean live-weight at 21 d (g)	89.4^{c}*	154.4^{a}	63.6^{d}	130.7^{b}	171.0^{a}	117.6^{b}	123.3^{b}
Mean food intake (g)	181^{d}	252^{bc}	129^{e}	279^{b}	307^{a}	235^{c}	237^{c}
Food conversion efficiency (g, gain g food intake)	0.269^{c}	0.456^{a}	0.192^{d}	0.319^{bc}	0.420^{a}	0.329^{b}	0.346^{b}

* Values in rows with the same superscript are not significantly different at $P = 0.05$.

Table 2

Dietary treatments, mean live-weights, food intake and food conversion efficiency of chicks used in experiment 2

	Dietary treatments (g kg diet)						
	2A	2B	2C	2D	2E	2F	2G
Basal diet mixture	826.2	826.2	826.2	826.2	826.2	826.2	799.1
Glutamic acid	.....	120.0	80.0	40.0	.....	.....	.....
Triammonium citrate	.....	.....	22.1	44.2	66.2	.....	.....
Uric acid	.....	.....	.....	.....	.....	34.3	.....
Dried autoclaved poultry manure	.....	.....	.....	.....	.....	.....	200.9
Sucrose	173.8	53.8	71.7	89.6	107.6	139.5	.....
Total N content	14.4	24.6	24.8	24.4	24.8	24.8	26.0
Mean live-weight at 21 d (g)	164.6^{c}*	245.2^{a}	249.6^{a}	243.7^{a}	205.5^{b}	149.8^{c}	240.1^{a}
Mean food intake (g)	224^{c}	316^{a}	346^{a}	340^{a}	274^{b}	213^{c}	319^{a}
Food conversion efficiency (g. gain/g food intake)	0.314^{c}	0.471^{a}	0.445^{a}	0.436^{ab}	0.399^{b}	0.243^{d}	0.451^{a}

* Values in rows with the same superscript are not significantly different at $P = 0.05$.

McNab *et al.* (1972) showed poorer growth rate in birds fed. diets containing triammonium citrate or diammonium hydrogen citrate compared to that in chicks fed commercial broiler mash, when dietary protein levels were lower than necessary for maximal growth. And at higher inclusion levels (4.8%). both salts caused a significantly deleterious effect on the chicks performance during the 1-to 3-week stage of growth. They have concluded, though chicks have the biochemical potential to convert the nitrogen from these salts into amino acids (McNab *et al.*, 1970), they do not seem to gain any benefit in vivo from the inclusion in protein-based diets.

The utilization of urea appears to be similar to that of diammonium citrate. When either replaced L-glutamic acid as the sole source of nitrogen for synthesis of non-essential amino acids their utilization for chick growth were equivalent each other but were still poorer than that of L-glutamic acid (Lee and Blair, 1972; Kagan and Balloun, 1976). Urea levels less than 2 per cent were found to give as good but not perhaps as efficient growth as a mixture of non-essential amino acids when growth was 4 to 5g per day (Featherston, Bird and Harper, 1961; Featherston, Bird and Harper, 1962a), but when the levels of amino acids in the diet were increased so that growth was 8 to 9g per day urea was not as effective as the mixture of non-essential amino acids.

Blair (1972) summarized details of the compounds that have been used as sources of nitrogen for the synthesis of non-essential amino acids (Table 3).

Table 3

Compounds added to poultry diets for the synthesis of non-essential amino acids

Compound	Formula	Nitrogen content per cent	Utilization relative to L-glutamic acid
L-glutamic acid	$C_5H_9O_4N$	9.5	–
D-glutamic acid	$C_5H_9O_4N$	9.5	O : toxic?
L-alanine	$C_3H_7O_2N$	15.7	$<$
D-alanine	$C_3H_7O_2N$	15.7	O : toxic?
L-aspartic acid	$C_4H_7O_4N$	10.5	$\equiv$
D-aspartic acid	$C_4H_7O_4N$	10.5	O : toxic?
Ammonium acetate	$C_2H_7O_2N$	18.2	$\equiv$
Monoammonium citrate	$C_6H_{11}O_7N$	6.7	$<$
Diammonium citrate	$C_6H_{14}O_7N_2$	12.4	$\leqslant$
Triammonium citrate	$C_6H_{17}O_7N_3$	17.3	$\leqslant$
Ammonium lactate*	$C_3H_9O_3N$	13.1	$\leqslant$
Diammonium phosphate	$(NH_4)_2HPO_4$	21.2	$\leqslant$
Ammonium sulphate	$(NH_4)_2SO_4$	21.2	O

Biuret	$C_2H_5O_2N_3$	40.7	O
Urea	CH_4ON_2	46.6	⩽
Uric acid	$C_5H_4O_3N_4$	33.3	O : some toxicity?
Dried autoclaved poultry manure	–	about 5.7	⩽

* Actual mixture used was $(NH_4)_2HPO_4$ + $[CH_3.\ CHOH.\ COO]_2Ca.\ 5H_2O$

3. Utilization of Ammonium Nitrogen By Laying Birds

Though many evidences show that laying hens are able to utilize NPN in productive ways under certain circumstances, Blair (1972) suggests that the hen may be less efficient in utilizing urea than the chick. Chavez *et al.* (1966) found that increasing the protein level of a layer diet from 12.75 to 15.75% by supplementation with diammonium citrate or intact protein gave a similar response in egg production, but supplementation with an equivalent amount of urea did not significantly increase egg production. According to Michie (1971), 20% of the nitrogen of a 15% protein diet for layers could be replaced by urea without affecting egg production adversely, egg grading was, however, somewhat poorer.

Blair and Lee (1973) showed that egg production was not increased by urea supplementation (1.15% of diet) to the basal diet (11.5% protein). The lack of response to urea alone might have been due to a failure to increase food intake. Furthermore the addition of urea depress the intake of the diet supplemented with EAA. Eggs from the hens fed on the basal diet showed reduced albumen quality, as measured by Haugh units, but no effect were found on gross composition. Although urea supplementation resulted in the smallest eggs, which confirms the finding of Michie (1971), it also resulted in eggs of the highest albumen quality. Ammonium chloride at a 2% level in the diet has been found to increase the viscosity of egg albumen and to increase the chloride and total ionic concentration of albumen (Helbacka and Hall, 1958; Hunt, 1964).

Kazemi and Balloun (1973) showed egg weight, egg production, feed conversion, and feed consumption per kilogram of eggs were significantly better when the basal diet was supplemented by additional soybean meal than when urea or diammonium citrate were added. Serum total nitrogen and protein nitrogen concentrations paralleled the pattern of production data and were greater in serum of hens fed soybean meal diets. Serum uric acid was not significantly affected by diets and Haugh unit and shell thickness were not affected by urea and DAC supplementation.

Davis and Martindale (1973) compared the performance of laying hens on the control rearing diets, containing 11.5% crude protein, with two diets containing 13.2% crude protein, one in which the protein was from conventional sources and the other containing 11.5% conventional protein and urea

equivalent to 1.7% crude protein. At 20 weeks of age, half the birds from each rearing treatment received a low protein diet (14.7%) and the remaining birds received a 16.4% protein diet which included urea equivalent to 1.7% crude protein. Laying performance was recorded for the next 30 weeks.

Body weight of birds fed on the urea-containing rearing diet were similar to those of birds fed on the control diet (1.22 Kg) and less than those on the 13.2% protein diet (1.29 Kg). Urea in the laying diet caused a 2% increase in egg production and tended to improve feed conversion efficiency. In contrast, a 3.5% less efficient feed conversion was found during laying in birds which had received urea during rearing, compared to birds which had received the low-protein rearing diet. Birds fed the 13.2% protein diet, containing no urea, during rearing laid smaller eggs than birds fed on the 11.2% protein rearing diet. Recently in 1980, Keshavarz et al. also demonstrated a partial effectiveness of urea in the diet of laying hens. Addition of urea at a level of 1.07% to the basal diet containing 12% protein resulted in better egg production than the basal diet alone, though egg weight was a little lighter in the former group. All these data suggest a possible use of urea in the laying hen diet to spare natural protein resources.

4. Biochemical Aspects of Incorporation of Ammonium Nitrogen into Body Protein.

According to Lewis (1972), only those materials that can give rise to ammonia, normally within the alimentary tract, are of value (Fig. 1). Dietary non-amino nigrogen sources can be readily separated into ammonium salts and compounds like urea that can give rise to ammonia. In case of urea, however, a further penalty against its utilization is incurred. Within the alimentary tract it must be converted to ammonia and the ammonia to be utilized for protein synthesis, these two changes occurring in that order.

In uricotelic species such as chicks, it must first be recognized that there is a substantial loss of ammonia as such in the urine. For its retention within the body kidney function is important. A recent work has been shown that there was a greater loss of ammonia in urine after intravenous infusion if an acid salt (e.g., ammonium chloride) was administered rather than a neutral or basic salt (ammonium carbonate or bicarbonate). Over short periods it was possible to increase ammonia excretion if a small pH gradient was established between peritubular blood and the relatively more acid tubular urine. The loss of ammonia in chick urine is substantial and subject to metabolic regulation. Though there is an excretion of urea in the urine of the fowl it must originate from preformed arginine since the full complement of enzyme systems for the ornithine-urea cycle is not present (Tamir, 1963).

Insofar as partition of ammonia is concerned between retention as α-amino nitrogen or loss as uric acid there is little information available on the relative effectiveness of the enzyme systems involved. However, there seem to be inbuilt

mechanisms within both ureotelic (pig and man) and unicotelic species that contribute to substantial inefficiency in the utilization of ammonia as a source of α-amino nitrogen.

Lee *et al.* (1972) have shown that chicks can incorporate NH_4+ into α-ketoglutaric acid to form glutamic acid by means of glutamic dehydrogenase. In vitro studies with chick liver homogenates showed that DAC stimulated this incorporation even in the absence of α-ketoglutaric acid (McNab *et al.*, 1970). This is believed to be due the synthesis of α-ketoglutaric acid from citrate using the enzymes of the Krebs citric acid cycle. According to this scheme the relationship between citrate, NH_4+ and glutamic acid on molar basis would be 1:1:1. It was, therefore, perceived that MAC would be likely to give better growth than TAC as there would be less likelihood of ammonium toxicity. This did not occur: MAC caused a growth depression while TAC gave the best performance. As these diets were isonitrogenous they differed in citrate level. The level of citrate may therefore be a factor in determining growth rate; possibly also affecting the acidity and/or the palatability of the diets. There is also evidence that in rat heart muscle citrate can inhibit glycolysis by inhibiting the enzyme phosphofructokinase (Garland, Randle and Newsholme, 1963). Some preliminary studies have shown a depressed lactate level in the gut mucosa of chicks fed MAC compared with that in the chicks given TAC suggest-

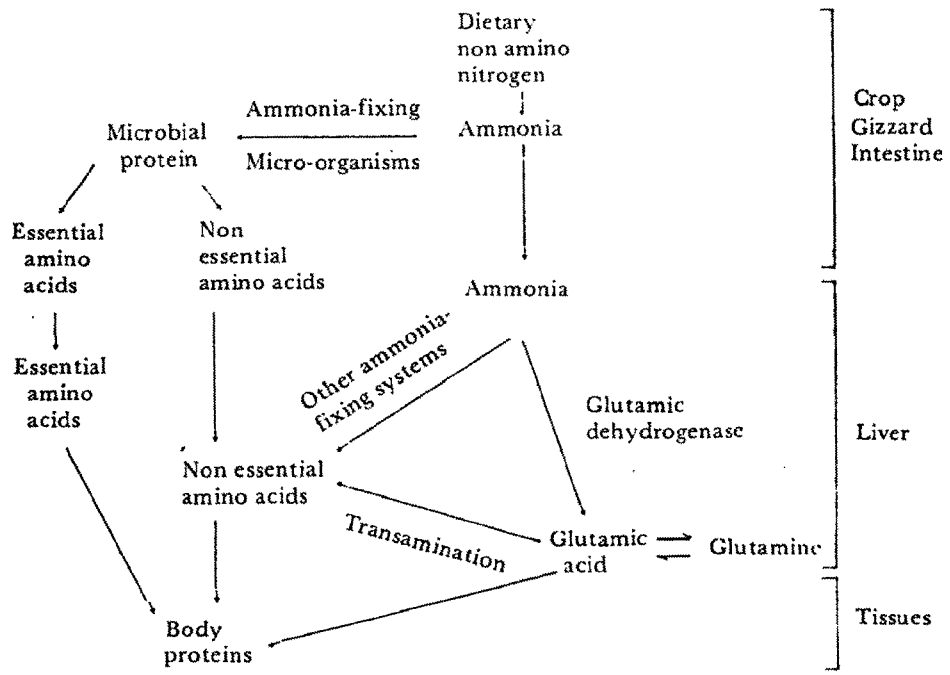

Fig. 1. Utilization of nonamino nitrogen in the chick

ing that glycolysis may be inhibited (P. J. Pritchard and D. J. W. Lee, unpublished). If this is the correct interpretation, utilization of carbohydrate from the diet would be less efficient.

Following paragraph and figure 2 are illustrated from Blair's (1972) review on utilization of ammonium compounds. "Unlike the ruminant the bird has no intestinal sac in which large quantities of bulky foods can be fermented so that food protein is largely degraded to ammonia by micro-organisms and becomes subsequently available to the animal as microbial or protozoal protein. It would therefore seem logical to propose that the gut flora would play an insignificant role in the utilization of ammonium nitrogen by the fowl and instead that a direct incorporation mechanism will be involved".

The possible influence of gut flora in this connection, however, should not be entirely dismissed. Intestinal bacteria may play some part in the incorporation of ammonia and might be expected to play a more significant role in the utilization of urea by providing the urease for its hydrolysis. (Okumua *et al.*, 1976). On the other hand, the evidence from Slinger *et al.* (1952), Jones and Combs (1953) and Bare *et al.* (1964) that antibiotics overcome the deleterious effects of ammonium compounds on growth rate and feed conversion efficiency suggests that the relationship between gut flora and the utilization of ammonium compounds may not always be beneficial to the animal.

There is little information on the biochemical transformations by which ammonium nitrogen is metabolised. Shannon, Blair and D'Mello (1969) showed that the nitrogen of diammonium citrate and diammonium phosphate was almost completely absorbed, and subsequent in vitro studies at Edinburgh indicated that the liver was an active site for the conversion of ammonium nitrogen to glutamic acid (McNab *et al.*, 1970; Lee *et al.*, 1972).

The most readily metabolised ammonium compound was diammonium citrate, suggesting that the anion was converted to α-ketoglutaric acid by enzymes involved in the tricarboxylic acid cycle and that the α-ketoglutaric acid was then converted to glutamic acid by glutamic acid dehydrogenase. This latter enzyme was found to be present in chick liver regardless of whether or not the birds received ammonium nitrogen in the diet, and there appeared to be no adaptation in enzyme level by the chick. Enzymes involved in the transamination of glutamic acid to alanine and aspartic acid were also found to be present in liver. Fig. 2 shows the probable scheme for the conversion of diammonium citrate to glutamic acid (Blair, 1972).

In vitro studies by Lee *et al.* (1972) indicated that radioactivity could be recovered in liver glutamate following incubation with ^{14}C-α-ketoglutaric acid + diammonium citrate, and that the levels of glutamic acid and total free amino acids in liver increased significantly following incubation with α-ketoglutarate + diammonium citrate.

Glutamine formation from glutamate plus ammonium ions is also known to take place in birds (Brown, 1970), and it is possible that glutamine is involved in ammonia transport (Olsen *et al.*, 1963; Brown, 1970).

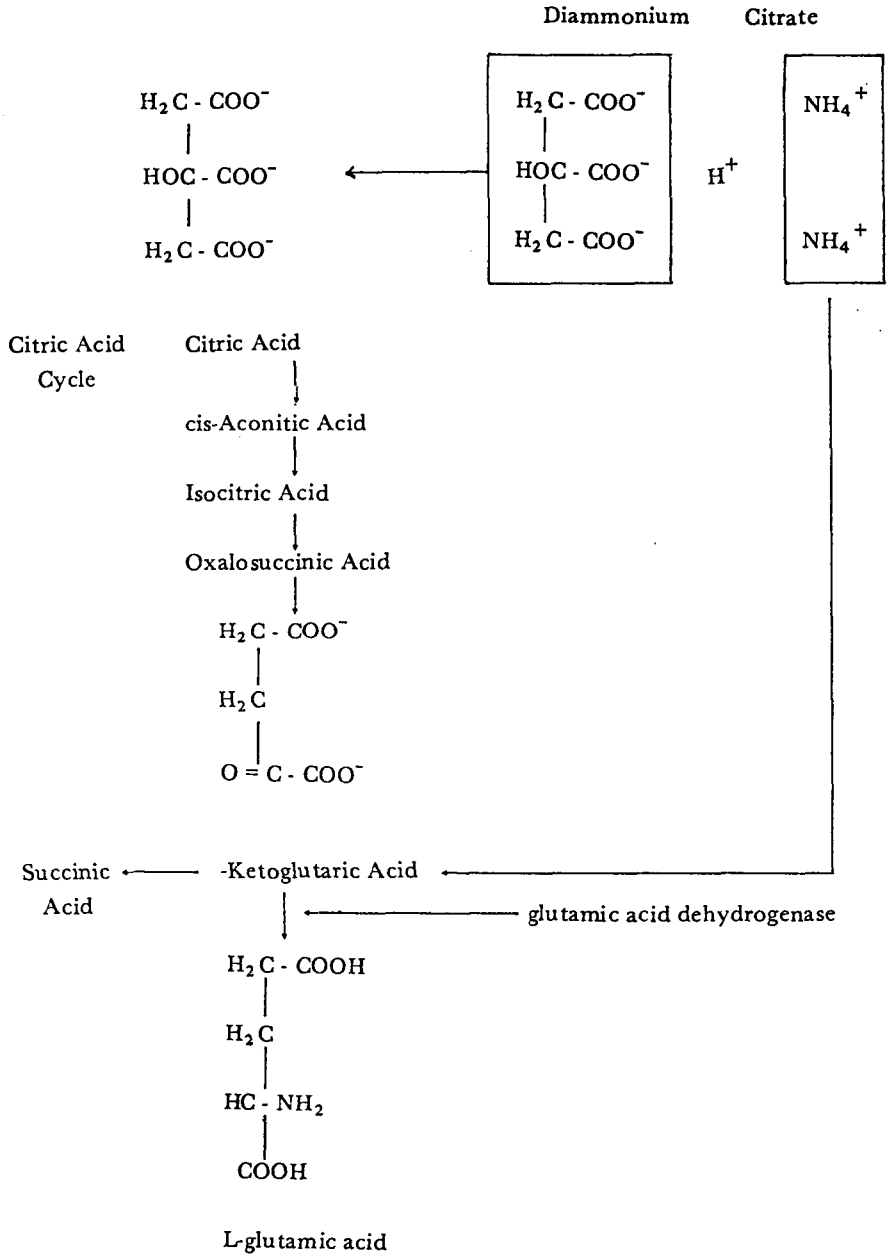

Fig. 2. The probable scheme for the incorporation of diammonium citrate by chick liver

The suggested scheme for the conversion of diammonium citrate to glutamic acid indicates that a molecule of α-ketoglutaric acid accepts one NH_2 group to form one molecule of glutamic acid. Thus only one ammonia group from the diammonium salt is utilised per reaction.

Diammonium citrate at relatively high levels has been found to result in a depression of growth or egg production which is possibly due to toxicity from excess ammonia (Blair and Young, 1970). The use of diammonium citrate may result in an excess of ammonia in the liver, and perhaps the monoammonium and triammonium salts would be expected to be better and poorer sources, respectively, since the former salt should yield no ammonia excess and the latter salt a high excess. However, Lee and Blair (1972) found that these salts were less well utilized for growth than diammonium citrate.

Since both pyridoxal phosphate and pyridoxamine phosphate are active as coenzymes in transamination (White *et al*, 1968), one can speculate that perhaps the use of glutamic acid or ammonium compounds as sources of nitrogen for the synthesis of non-essential amino acids increases the dietary pyridoxine requirement.

In the ruminant, non-protein nitrogen compounds that give a slow release of ammonia seem to be preferable to compounds that give a rapid release of ammonia, since the rumen may be unable to utilise the ammonia as fast as it is released. In such a situation ammonia toxicity may result. Since the bird absorbs ammonium nitrogen and uses it directly, perhaps soluble rather than insoluble compounds would be more suitable. Solubility is probably also important for the efficient intestinal hydrolysis of some compounds. Such a consideration might help to explain the utilization of urea but not uric acid by the bird (Lee and Blair, 1972).

5. Enzymatic Aspects of Nitrogen Utilization

Enzymes that would be involved in α-amino acid synthesis from non amino nitrogen are L-glutamate dehydrogenase, aspartate transaminase, carbamyl phosphate synthetase, and alanine transaminase which are mostly concerned in the urea-ornithine cycle except alanine transaminase. Mammals can synthesize arginine from carbamyl phosphate and ornithine via the urea cycle, avian species, on the other hand, have no carbamyl phosphate synthetase.

Lee *et al* (1970) showed that the activity of glutamate dehydrogenase in the liver from birds fed essential amino acids (A) as the only sources of nitrogen was significantly higher than the activity of this enzyme in the liver from birds fed this diet supplemented with 11.1% diammonium hydrogen citrate (B) or 12.0% glutamic acid (C). There was no significant difference between the activities of groups B and C. In addition, the results of incubation of [α-C^{14}] ketoglutaric acid with liver homogenates from birds of each treatment appears that the amount

of glutamate recovered from the livers of group B was lower than that recovered from group C which in turn was lower than that recovered from group A.

But Davis and Martindale (1972) showed that the glutamic dehydrogenase activity per unit liver weight and total glutamate dehydrogenase activity increased with dietary protein level but there were no consistent responses to diammonium citrate supplements. They analyzed this difference was due to the fact that the intact protein sources were used in their studies so that non-essential amino acids were present as alternatives to diammonium citrate as sources of non-essential nitrogen, as they would be in practical diets. They concluded also that liver glutamate dehydrogenase activity did not provide a useful index of the utilization of NPN.

Lee *et al.* (1972) studied the effect of a supplement of 12% glutamic acid or 11.1% diammonium citrate on the various enzymes in the chicken livers involving in the nitrogen utilization. Glutamic acid caused a depression of glutamate dehydrogenase levels but had no effect on aspartate transaminase and alanine transaminase compared with the controls from birds fed diet based on cereal protein, whereas diammonium citrate caused a decrease in alanine transaminase but had no effect on aspartate transaminase and glutamate dehydrogenase. Diet containing crystalline essential amino acids as the sole source of nitrogen depressed alanine transaminase and aspartate transaminase levels.

Since pyridoxal phosphate is the co-enzyme of the transaminase, it seemed possible that the dietary requirement for pyridoxine would be higher when non-essential nitrogen is supplied in the non-amino rather than the amino form. Lee *et al.* (1976) compared the relative value of growth and of aspartate transaminase activity in the liver as criteria of the pyridoxine status of the chicken. Chicks fed glutamic acid added at a level of 136.2 g/Kg diet containing only essential amino acids resulted in better growth than those fed diets with diammonium hyaroxycitrate. Aspartate tramsaminase activity was not affected by the nitrogen source, but varied significantly with dietary content of pyridoxine.

6. General Aspects of Ammonia Toxicity

Even though ammonia is normally produced by living cells, its toxic effects have been observed in such many cases as that feeding of non-protein nitrogen or diets with excesses or unbalanced supplies of amino acids increases the production of ammonia. Diammonium citrate at relatively high levels has been found to result in a depression of growth or egg production which is possibly due to toxicity from excess ammonia (Blair and Young, 1970).

Toxic manifestations are believed to result from the action of ammonia at intracellular sites and appear when : a) normal detoxification processes are impared, b) ammonia formation is too rapid, c) the quantities of ammonia

are excessive, and d) when the hydrogen ion and electrolyte relationships in body fluids raise the concentration of nonionic ammonia (NH_3) relative to ammonium (NH_4^+). Ammonia being lipid soluble is the favored form for penetration of cell membranes and factors depermining its concentration are of prime importance in producing toxicity (Manning, 1966).

The toxicity of ammonium salts varies and is believed to be due to changes in pH caused largely by the accompanying anion (Manning, 1966). Amino acid required of the urea cycle, such as arginine and mixtures of ornithine and aspartate, satisfactorily counteract toxicity of ammonia provided the liver has the required capacity to synthesize cycle enzymes (Greenstein *et al.*, 1955; Greenstein *et al.*, 1956; Harper, Benevenga and Wohlhueter, 1970). Arginine has been shown to have a protective function in rats against toxicity due to dosing with ammonium acetate (Meister, 1965) and glycine might be expected to play a similar role in the fowl (Blair, 1972).

A widely prevalent hypothesis proposed by Bessman and Bessman (1955) is that ammonia depletes intracellular α-ketoglutarate by reductive deamination to glutamate, a NADH-dependent reaction catalyzed by glutamic dehydrogenase. The equilibrium is far toward glutamate which would suggest that ammonia leads to the removal of α-ketoglutarate from the tricarboxylic acid cycle, reducing oxygen consumption and decreasing metabolic energy in direct proportion to α-ketoglutarate depletion. This is compatible with accumulation of pyruvate and lactate secondary to a relative lack of oxaloacetate for condensation with acetyl CoA to form citrate.

A readily demonstrable response to acute ammonia intoxication is a rise in blood glucose (Chalupa *et al.*, 1970; Chao and Tarver, 1953). Hyperglycemia, however, would not be expected since ammonia has been reported to stimulate glycolysis (Kloppick, 1967). Visek (1972) reports that the rise in blood glucose, pyruvate, and α-ketoglutarate are dose and time dependent.

7. Conclusion

Three major reactions operate to initiate entry of ammonia into anabolic pathways. These include glutamic acid, glutamine, or carbamyl phosphate, whose responsible enzymes are glutamic dehyrogenase, glutamine synthetase, and carbamyl phosphate synthetase which is not found in chicken, respectively. Chicken can incorporate ammonium from non protein nitrogen substances such as diammonium citrate, diammonium phosphate and urea into α-ketoglutaric acid to form non-essential amino acids via the formation of glutamic acid by means of glutamic dehydrogenase, even though there are possible hazards from excess amount of ammonia. The main criteria for the use of NPN by poultry to supply a portion of the total protein requirement is that the diet be relatively low in protein, but adequate in essential amino acids. Since there would appear

to be no case for including nonamino nitrogen as a supplement in the diet until a very substantial part of the essential amino acid needs were met by adding free synthetic amino acids, including nonamino nitrogen in the diet of animals other than ruminants would thus seem not to be a profitable proposition. However, a number of studies have still been conducted in recent years on the feasibility of supplying a portion of protein needs of monogastric animals with non-protein nitrogen compounds, whose importance are increasing day after day since we can never expect again the high protein plant and animal products be as plentiful or as low cost as in the past (Allen, 1973).

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