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THE ENDOGENOUS AMINO ACID EXCRETION OBTAINED IN GROWING BROILER AND ADULT COCKERELS

Zuprizal[1], M. Larbier[2], and A.M. Chagneau[2]

ABSTRACT

Two trials were conducted to measure the uric acid excretion (UAE), the endogenous fecal nitrogen (EFN), in the endogenous amino acid (EAA) excretions in growing broilers and adult cockerels. Twenty-four broilers (50 % of each sex) were used both at 3 and 6 wk of age in the first trials and eighteen adult cockerels in the second one. In the second experiment, the influence of N-free diet intake on UAE, EFN, and EAA excretions was also investigated. The birds were fasted for 24-h and then forced-fed with moistened N-free diet (diet/water: 50/50). The N-free diet was composed of 48.25 % starch, 48.25 % glucose, 3.50 % mineral-vitamin premix. Excreta in both groups were collected during 24-h subsequent period after force-feeding. For growing broilers, the UAE, EFN, and EAA was significant (P<.05) lower in younger birds (at 3 wk of age) than in older ones (at 6 wk of age). In both ages of broilers, sex had no effect (P>.05), on UAE and EFN; however for EAA values there are some significant differences. In cockerel experiment, the UAE was significantly (P<.05) higher in fasted birds than in cockerels fed a N-free diet. On the other hand, the N-free diet increased the EFN and EAA excretions in adult cockerels.

Key words : Endogenous amino acids, Uric acid excretion, Growing broilers, Cockerels, N-free diet

INTRODUCTION

The dry forced-feeding methods of Sibbald (1976) and wet forced-feeding method of Lessire (1990) are currently used to measure the amino acid digestibility of feedstuffs for poultry (Likuski and Dorrell, 1978; Sibbald, 1979; Muztar and Slinger, 1980a; Parsons *et al.*, 1983; Green *et al.*, 1987a,b; Mohamed *et al.*, 1989; Zuprizal *et al.*, 1991a,b). In these methods, the indigenous amino acid values are particularly needed for calculation of true digestibility, and they are, generally, obtained by starved birds (Likuski and Dorrell, 1978; Sibbald, 1979; Muztar and Slinger, 1980a; Parsons *et al.*, 1981; Zuprizal *et al.*, 1991a,b) or by fed birds with a N-free diet (Parson *et al.*, 1983; Green *et al.*, 1987).

Several experiments have been carried-out to study the influence of dry matter and of dietary protein, cellulose (fiber), carbohydrate in intact or caeccectomised cockerels (Muztar and Slinger, 1980b; Sibbald, 1980; Parsons *et al.*, 1982; Parsons *et al.*, 1983; Green, 1988) and lying hens (Parsons, 1984) on endogenous amino acid excretion. However, little (or may be any) information has been reported concerning the values of endogenous amino acid excretion in young birds.

Since the apparent digestibility of protein and amino acids is affected by the age of the birds (Håkasson and Eriksson, 1974; Fonolla *et al.*, 1981; Hassan and Delpech, 1986; Zelenka and Liska, 1986;Carré *et al.*, 1991), it is necessary, therefore, to measure the endogenous amino acid excretion in different ages of the birds for calculation of

[1] Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia.
[2] Station de Recherches Avicoles, Institut National de la Recherche Agronomique, 37380 Nouzilly, France.

Table 1. Composition of N-free diet (%)

Ingredients	
Starch	48.25
Glucose	48.25
Trace mineral premix[1]	.10
Vitamin premix[2]	.50
Salt	.40
Calcium carbonate	1.00
Dicalcium phosphate	1.50
Total	100.00

[1] Provides per kilogram of diet: CO, .88 mg; Cu, 8.75 mg; I, 1.28 mg; Se, .15 mg; Zn, 100 mg; Fe, 35 mg; Mn, 110 mg.

[2] Provides per kilogram of diet: vitamin A, 10,000 UI; vitamin D3, 1,500 UI; vitamin E, 15 mg; butylated hydroxytoluene, 125 mg; menadione, 5 mg; riboflavin, 4 mg; pantothenic acid, 8 mg; niacin, 25 mg; pyridoxine, 1 mg; vitamin B12, .008 mg; folacin, .2 mg; biotin, .1 mg; choline, 500 mg; thiamin, .5 mg.

their true digestibility.

The objective of the present study was to measure the endogenous amino acid excretion in growing broilers (at 3 and 6 wk of age) and adult cockerels. The influence of N-free diet intake on endogenous amino acid excretion in adult cockerels was also investigated.

MATERIAL AND METHODS

Birds and Management

The same twenty-four broilers (50 % each sex) were used at 3 wk and 6 wk of age in the first trial, and 18 cockerels (one year old) in the second one. The animals were kept in individual metabolism cages, where room temperature and relative humidity were controlled (22 ± 1 °C, 45-50 % RH). Water and feed were available *ad libitum*, except during forced-feeding experimental periods.

The birds were fasted for 24 h before forced-feeding, in order to ensure complete emptying of their digestive tracts. They were then forced-fed with 20 and 40 g of N-free diet (Table 1) at 3 and 6 wk of age, respectively. The 18 cockerels were devided into three groups of 6 birds each. They were then forced-fed with 100, 50, and 0 g (starved bird) of the same N-free diet above for groups 1,2,and 3, respectively. The diet, in both trials, was moistened with an approximately equal weight of water (diet/water:1/1), this being carefully mixed with the meal to give a homogenous paste. The procedure of wet forced-feeding technique and equipment were similar to those described by Lessire (1990).

During the forced-feeding experimental period, water was available *ad libitum*, and trays were positioned under cages for excreta collection. Excreta, in both trials, were collected daily during the subsequent 48-h. They were freeze-dried (after equilibration with atmospheric moisture) and ground to pass through a 0.5 mm screen.

Chemical analysis

The amino acid content of excreta was determined by ion-exchange chromatography using an autoanalyzer after hydrolysis in 6 M-aqueous HCl for 24 h at 115 °C. Values for aspartic acid, threonin, serin, glutamic acid, alanine, valine, isoleucine,

[1] BIOTRONIK, Amino Acid Analyzer L.C. 5000, Postfach 1330, D-6457 Maintal 1.

Table 3. Influence of N-free diet intake (g) on uric acid excretion (UEA), endogenous fecal nitrogen (EFN), and endogenous amino acids (EAA) excretions in adult cockerels (mg/48 h/bird)

N-free diet intake (g)	0 (starved)	50	100
UAE [1]	2772.2 ± 149.0^{b}	2263.2 ± 130.7^{a}	2289.2 ± 125.1^{a}
EFN	115.8 ± 6.0^{a}	140.6 ± 11.9^{a}	183.3 ± 9.9^{b}
EAA :			
Aspartic acid	34.9 ± 3.1^{a}	45.2 ± 5.0^{ab}	59.0 ± 2.1^{b}
Threonine	31.5 ± 2.3^{a}	46.7 ± 3.9^{a}	61.8 ± 1.2^{c}
Serine	28.8 ± 2.0^{a}	43.9 ± 3.6^{b}	60.0 ± 3.6^{c}
Glutamic acid	70.0 ± 4.8^{a}	88.5 ± 8.5^{b}	117.2 ± 1.6^{bc}
Alanine	24.9 ± 2.5^{a}	28.8 ± 3.7^{ab}	38.8 ± 1.3^{b}
Valine	21.2 ± 1.8^{a}	35.1 ± 3.2^{a}	42.3 ± 2.7^{b}
Isoleucine	19.5 ± 1.9^{a}	27.9 ± 5.3^{a}	33.3 ± 1.4^{a}
Leucine	30.0 ± 2.8^{a}	35.5 ± 3.6^{bc}	48.5 ± 1.3^{c}
Tyrosine	15.7 ± 1.5^{a}	22.4 ± 2.2^{b}	31.8 ± .8^{c}
Phenylalanine	20.9 ± 2.3^{a}	23.7 ± 2.6^{ab}	32.8 ± 1.0^{b}
Lysine	40.2 ± 3.0^{a}	43.5 ± 5.7^{a}	56.7 ± 4.0^{a}
Arginine	27.0 ± 2.1^{a}	34.0 ± 3.5^{ab}	44.4 ± 1.8^{b}
Cystine	24.8 ± 1.9^{a}	36.7 ± 2.4^{b}	42.3 ± 2.0^{c}
Methionine	9.7 ± .8^{a}	11.5 ± 2.4^{bc}	16.6 ± .8^{c}

[1] a,b; means ± (n=6 of each sex of the same birds at 3 and 6 wk of age) within a line with no common superscript differ significantly (P<.05)

leucine, tyrosine, phenylalanine, lysine and arginine were determined. For methionine and cystine, separate samples were subjected to a performic acid oxidation treatment by the method of Moore (1963), prior to hydrolysis. Tryptophan was not determined. The glycine and histidine values were not used in these results.

Endogenous fecal nitrogen (EFN) was precipitated by lead acetate according to the method of Terpstra and de Hart (1974). This was done to enable fecal nitrogen to be measured separately from urinary nitrogen. Uric acid in excreta was determined by method of Marquardt (1983).

Results were assessed by analysis of variance procedures and the comparison of means by Turkey's test. The calculations were performed using SYSTAT software program.[4]

RESULTS AND DISCUSSION

The excretion of uric acid (UAE), endogenous fecal nitrogen (EFN) and endogenous amino acids (EAA) in growing broilers and adult cockerels are shown in Tables 2 and 3, respectively.

Uric Acid Excretion (UAE)

Sex has no effect (P>.05) on uric acid excretion in both 3 and 6 wk of age. However, in both sexes, age has a significantly effect (P<.05), on uric acid excretion in growing broilers. The excretion of uric acid increases as the age of bird

[4] Wilkinson, Leland. Systats Inc. Evanston, IL 60201, USA.

increases from 3 to 6 wk. On 6-wk-old, the uric acid excretion was around two times more than in broilers at 3 wk of age. The UEA values at 3 wk of age for male and female broilers were 1140.9 and 1109.0, and those at 6 wk were 2026.4 and 1799.1 mg/48 h/bird, respectively. In chickens, uric acid is the excretory vehicle for four-fifths of the metabolized nitrogen, whereas ammonia accounts for 10-15% of total nitrogen (Griminger and Scanes, 1986). The results of lower in younger birds (3 wk-old) than those in older ones (6 wk-old) which may be indicated by a greater protein metabolic activity at 6 wk then at 3 wk of age.

However, sex had no effect on protein metabolic activity in young birds, but at 6 wk of age, male birds secreted more uric acid than female ones (difference not significant, P>.05).

For cockerels, the excretion of uric acid in starved birds was significantly (P<.05) higher than in ones fed a N-free diet (Table 3). However, the intake of N-free diet (100 vs. 50 g) had no effect on uric acid excretion in cockerels (2289 vs. 2263 mg/48 h/bird). Similar result were reported by Kussaibati and Leclercq (1985) who found the endogenous urinary nitrogen (EUN), mainly composed of uric acid, was far higher in starved cockerels than in those fed N-free diet or diet containing minimum protein requirement. Okumura *et al.* (1991) reported also a decrease in EUN when birds were fed a high energy N-free diet. These results confirm that protein catabolism is higher during starvation which could be related to a high utilization of body protein to satisfy the energy requirement of the animal. This process naturally leads to an increased excretion of nitrogen, in birds mostly as uric acid.

Table 2. Influence of age and sex on uric acid excretion (UEA), endogenous fecal nitrogen (EFN), and endogenous amino acids (EAA) excretions in growing broilers (mg/48 h/bird)

Age	3 wk		6 wk	
Sex	Male	Female	Male	Female
UAE [1]	1140.9 ± 53.3[a]	1109.0 ± 32.0[a]	2026.4 ± 79.1[b]	1799.1 ± 97.0[b]
EFN	106.5 ± 12.4[a]	109.9 ± 7.4[a]	199.6 ± 9.9[b]	192.6 ± 4.0[b]
EAA :				
Aspartic acid	41.5 ± 3.2[a]	51.8 ± 2.4[b]	68.5 ± .3[c]	75.3 ± 1.1[c]
Threonine	26.7 ± .7[a]	30.9 ± 1.1[b]	38.3 ± 1.2[c]	43.3 ± .3[d]
Serine	29.6 ± 1.7[a]	29.3 ± .6[a]	39.8 ± .8[b]	49.3 ± .1[c]
Glutamic acid	52.2 ± 3.6[a]	60.7 ± 2.2[a]	81.8 ± .3[b]	96.6 ± .7[c]
Alanine	26.0 ± 1.6[a]	26.6 ± 1.2[a]	38.3 ± .2[b]	42.0 ± .6[b]
Valine	25.3 ± 1.7[a]	28.3 ± .9[a]	40.2 ± .2[b]	43.2 ± .7[b]
Isoleucine	17.7 ± 1.6[a]	20.0 ± .9[a]	27.6 ± .3[b]	29.9 ± .4[b]
Leucine	25.0 ± 1.1[a]	31.8 ± 1.3[b]	47.1 ± .2[c]	50.4 ± .5[c]
Tyrosine	17.5 ± 1.6[a]	21.3 ± .6[b]	26.7 ± .4[c]	32.4 ± .3[d]
Phenylalanine	18.9 ± 1.4[a]	21.2 ± .6[a]	30.2 ± .1[b]	33.2 ± .5[b]
Lysine	33.0 ± 1.3[a]	35.6 ± .8[a]	45.8 ± 1.2[b]	48.7 ± 1.8[b]
Arginine	33.3 ± 2.0[a]	38.7 ± 1.0[b]	44.2 ± .4[c]	56.8 ± .4[d]
Cystine	29.8 ± 4.0[a]	32.1 ± 1.9[ab]	43.6 ± 2.0[bc]	51.2 ± 3.8[c]
Methionine	6.8 ± .7[a]	11.7 ± 1.7[ab]	16.4 ± 1.4[bc]	17.8 ± 1.7[c]

[1] a,b; means ± (n=6 of each sex of the same birds at 3 and 6 wk of age) within a line with no common superscript differ significantly (P<.05)

Endogenous Fecal Nitrogen (EFN) and Endogenous Amino Acids (EAA) Excretion (UAE)

In an experiment with growing broilers, EFN values, obtained after separation of nitrogen fecal from urinary according to Terpstra and de Hart (1974), were significantly (P<.05) lower in broilers 3-wk than in ones 6-wk-old. The EFN values for male and female broilers at 3 wk of age were 106.6 and 109.9 and those at 6 wk of age were 199.6 and 192.5 mg/48 h/bird, respectively. Similar results have been observed also for endogenous amino acids (EAA) excretion. The EAA values increased significantly (P<.05), in both sexes, with the increasing of age of broilers from 3 to 6 wk (Table 2). However, sex had no effect (P>.05), in both ages, on EFN values. As for EFN values, for the most part of amino acid the EAA excretion was not affected by sex of the birds. However, at 3 wk of age, higher value for aspartic acid, threonine, leucine, tyrosine, and arginine has been found in female broilers than in males. Similar results have been observed also, at 6 wk of age, for threonine, serine, glutamic acid, tyrosine and arginine values which were higher in female than in male birds.

This is the little information about the effect of age and sex of birds on the values of endogenous fecal nitrogen and endogenous amino acid excretions. However, using adult cockerels, Muztar and Slinger (1981) and Crissey and Thomas (1983) showed that the endogenous amino acid excretions did not vary significantly with time. In their experiments, two trials in an interval of about 3 months (Muztar and Slinger, 1981) and 8 months (Crissey and Thomas, 1983) had not effect on the values of endogenous amino acid excretion in cockerels. However, in this current experiment, using growing broilers, the endogenous fecal nitrogen (EFN) and endogenous amino acid (EAA) excretion increased with the increasing of age from 3 to 6 wk. These results may be explained first by the increasing of the size of digestive tract in growing broilers with age which can secrete more endogenous materials proteins as sloughed cells, endogenous secretions from salivary glands, stomach, liver, pancreas and mucosal cells. These secretions are primarily enzymes, which are proteins or mucoproteins (Crissey and Thomas, 1985). In the second hypothesis, the higher values of EFN and EAA in broilers at 6 wk of age compared to those at 3 wk of age may be explained by the higher microbial protein amounts in the excreta of 6 wk-old than those of 3 wk-old. It is interesting, therefor, to study the effect of age on the excretion of the diaminopimelic acid (DAP), as the specific identification of microbial cell wall (Louisot, 1980), in the excreta of 3 and 6 wk-old broilers. However, using adult cockerels, Crissey and Thomas (1988) found that the means of DAP content of excreta from intact and caecectomised cockerels were not significantly different.

In the experiment with cockerels, the endogenous fecal nitrogen (EFN) and endogenous amino acids (EAA) excretions increased with the increasing of N-free diet intake (Table 3). The EFN values were 115.8, 140.6, and 183.3 mg/48 h/bird for starved birds, forced-fed with 50 and 100 g of N-free diet, respectively. Similar results have been shown for EAA values. For all amino acids tested, the EAA values of starved birds were significantly (P<.05) lower than those obtained in fed birds with n-free diet. However, the quantity of N-free diet intake (50 vs. 100 g) had only a significant effect (P<.05) on threonine, serine, valine, tyrosine, and cystine excretions. The values were higher in cockerels fed 50 g than in birds fed 100 g N-free diet.

In total fecal collection method, there are two principal ways to determine the endogenous amino acid excretion: by measuring the excretion of fasted birds, or by measuring the excreta from animal fed N-free diets or diets containing a small amount of highly digestible protein (Austic, 1983). However, results of the two methods above are not often similar. Sibbald (1979) found that the administration of graded levels of glucose (from 0 to 30 g) had no effect on the excretion of 13 amino acids by adult roosters. Crissey and Thomas (1983) also showed that lysine and methionine excretion by fasted males and males receiving corn starch or corn oil plus corn starch did not differ significantly. However, comparing two methods (fasted birds vs. N-free diet), the results of this current experiment indicates that the endogenous amino acids excretions in fasted birds are significantly (P<.05) lower than those obtained in birds fed a N-free diet. Similar results have been reported by several authors (Muztar and Slinger, 1980b) who found that the excretion of amino acids was significantly (P<.05) increased by the use of a N-free diet rather than fasting, Likuiski and Dorrell (1978) showed also that cockerels fed corn starch and corn oil excreted slightly more amino acids than fasted cockerels, and Parsons *et al.* (1983) showed that the amino acid excretion by roosters fed a low fiber. N-free diet was much greater than that of fasted rooster. This increasing of endogenous nitrogen and amino acid excretion when the birds were fed a N-free diet may be explained by the bacterial activity in the hind gut (Parsons *et al.*, 1983), as Mason and Palmer (1973) have shown that dietary carbohydrate has a substantial effect on excretion of endogenous nitrogen in rats. In their study, an increase in nitrogen excretion from potato starch was associated to a substantial raise in diaminopimilic acid in the feces, indicating an increasing in the bacterial synthesis of amino acids in the gut.

In conclusion, the results obtained in the current experiment indicated that, in growing broilers, the uric acid excretion (UAE), endogenous fecal nitrogen (EFN), and endogenous amino acids (EAA) values are lower in younger birds (at 3 wk of age) than in older ones (6 wk of age). However, sex has no effect on UAE, EFN and on most part of EAA values in both ages of broilers. In cockerels, the UAE was higher in fasted birds than in ones fed a N-free diet. However, the N-free diet intake increased the EFN and EAA excretions in adult cockerels.

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