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The Relationship Between Apparent and True Amino Acids Availability in Maize by Comparison of Precision-fed and Conventional Addition Methods

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Abstract: The experiment was performed to study the relationship between apparent and true amino acid digestibility at different levels of feed intake and to compare two procedures, precision feeding and CAM. Rhode Island Red (RIR) adult cockerels were used in the two bioassays, under standard conditions. In the first bioassay (precision feeding) the birds were not fed for 24 h and then various quantities of maize were fed. There were 6 replicates per treatment. Excreta voided during the 48 h of the experimental period were collected; dried and samples were assayed for gross energy, nitrogen and amino acids. In the second bioassay, after a 3-day adaptation period each bird was not fed for 24 h and during the third experimental period maize was fed CAM, in the same rations as those used in the first bioassay. During the 3 days of the experimental period, excreta were collected and frozen for amino acids and chemical analyses. Maize grain was fed at 10 levels (0 to 100 g) to starvation adult cockerels RIR and a group of unfed birds provided metabolic plus endogenous excreta. Birds were fed maize to determine apparent amino acids availability and corrected amino acids availability (true AAA) values. The latter were corrected for the amino acids in maize average 71.93 to 89.30%, respectively. The apparent available amino acids values of the maize, varied between amino acids and between levels of maize input but the true available amino acids values were relatively uniform. The apparent amino acids availability of the maize increase in a curvilinear with maize input. Apparent available amino acids values and dependent on maize intake at lower levels. Finally, the use of available amino acid in practice feed formulation is considered. Because, differences were observed for individual amino acid obtained of maize for birds. The results showed that mean apparent available amino acid values obtained by the precision-feeding procedure, were repeatedly higher than those obtained by CAM. The intercept obtained of regression analysis for precision feeding was higher than that given by CAM method. This may be due to the response of the birds to the methodology applied. It was concluded that procedures of feeding do not influence true available amino acids of maize at different ratio levels.

Key words: Precision feeding, Conventional Addition Method (CAM), maize, apparent, true available amino acid

INTRODUCTION

Knowledge of the availability of amino acid in feedstuffs is also important for consistent formulation of diets that meet the bird's amino acid requirements[1]. In order to improve the precision of diet formulation, many studies have been carried out to determine the availability of amino acids in various feedstuffs. Sibbald[2] uses the term bioavailability to define that portion of the ingested nutrient, which can be used for normal metabolic functions. Available amino acids are usually considered to be those actually supplied at the site of protein synthesis. A nutrient is bioavilable if, on entering a living tissue, it can be used for normal metabolic functions. Utilization of an absorbed molecule is proof of bioavailability, but excretion is not evidence of a lack of bioavailability[3].

The methods commonly used to determine availability of amino acids in poultry feedstuffs have been reviewed[4-7]. The original technique described by Sibbald[8] investigated the effect of raw material input on amino acid excretion and found that the latter increased in a curvilinear manner. Recently, increased emphasis has been placed on a precision-fed rooster bioassay[8] that measures true available amino acid. Not unlike other amino acid bioavailability techniques, this assay also has its limitations. Engster[9] reported a precision fed rooster assay as a means of measuring bioavailability of amino acid in a variety of feedstuffs for poultry. Likuski and Dorrell[10] and Sibbald[8] recommended the precision fed rooster bioassay as means of measuring the true available amino acid in feedstuffs. Studies comparing the two methodologies have been few and often limited to only one feed ingredient.

The majority of published values currently available on digestible amino acids for poultry are based on excreta analysis because of its simplicity and because the assay can be carried out on large numbers without sacrificing the birds[11-14]. Furthermore, excreta analysis does not measure digestibility as classically defined but rather amino acid metabolisabillty, because faeces and urine are voided together in birds. Green *et al.*[15] reported that the differences between cereals in apparent digestibility of amino acids were inconsistent and could more readily be attributed to differences between amino acid intakes than bioavailability and confined to higher values for nitrogen, aspartic acid, alanine, leucine in maize than in barley. The objective of the present study was to determine the availability of amino acid values of maize by used adult's birds and compare the two bioassay as precision-feeding method and Conventional Addition Method (CAM), for examine relationship between apparent and true amino acids available of maize. Therefore, determine the influence of feeding different amounts of maize on is its available amino acid and true available amino acid values decrease when amino acid consumption is reduced, all other conditions remaining constant.

MATERIALS AND METHODS

The experiment was conducted with mature Rhode Island Red (RIR) cockerels, using both the CAM and precision feeding[16,8]. A locally produced maize (704) sample from Iran was used. Its chemical composition was dry matter 940 g kg^{-1}, crude protein 90.1 g kg^{-1}, crude fat 20.1 g kg^{-1} and crude fibre 20.6 g kg^{-1}. The cockerels were individually housed in metabolism cages (24 cm width, 59 cm length and 57 cm depth) in a temperature-controlled room (18°C) with 14 h of light per day in both bioassays. Each cage was fitted with an individual feeder and a nipple drinker. A total of 88 birds, drawn from the same population, were used for the two procedures. Between assays the birds were fed on maintenance diet and fresh water was available at all times. An aluminium tray was placed under each cage to allow droppings to be collected quantitatively. In both bioassays, modified plastic bags were fitted to the birds for the collection of droppings. For the precision feeding assay[8], the experimental period was 72 h and droppings were collected during the final 48 h. In the case of the CAM, the experimental period was 6-day: a 3-day pre-collection period and a 3-day collection period. The maize input was increased in 10 g increments in the both bioassay and the weights of maize consumed were recorded. An additional 6 birds were given no feed (72 h) and served as negative controls to provide a measure of the Endogenous Amino Acid Losses (EAAL). In the first bioassay (precision feeding) the birds were fed various quantities of maize, each sample being placed directly in the crops using a tube to ensure that known amounts of feed were ingested at specific times. In the second bioassay, which was performed according to the CAM, the duration of the feeding period was adjusted so that the adult cockerels voluntarily consumed all the maize they were offered. After precision feeding bags were immediately attached to each bird. The samples of dropping voided during the 48 h period were collected, weighed and frozen. Before analysis, the frozen samples were removed from the freezer, taken out of the bags and placed in an oven, to be dried at 90°C overnight. Samples of ground maize and excreta were assayed for gross energy by means of adiabatic oxygen bomb calorimeter and amino acid concentrations in the maize and excreta samples were determined by High Performance Liquid Chromatography (HPLC). Preparation for HPLC analysis involved hydrolysis of the sample according to the procedures described by Siriwan *et al.*[17]. Total intake of feed amino acid, excreta amino acids and excreta endogenous amino acid were measured for each bird. The experiment was conducted on the basis of a Completely Randomised Design, with 10 g increments of maize (4 replicates).

RESULTS

The data demonstrate the relationship between amino acid excretion and the maize intake. The correlation coefficient in both procedures was 0.63 and 0.65. The intercepts of the regression equations ranged from 1.37±0.81 for CAM to 3.44±0.53 for precision feeding. More importunity the intercept obtained for precision feeding (3.44±0.53) was higher than that for CAM (1.37±0.81) (Table 1). The intercepts of the linear regression provide estimates of the amino acid excretion of birds receiving no maize, that is, they are indicative of the combined metabolic and endogenous excretions. The regression coefficients are estimates of the intercept in the amount of amino acids excreted for each gram of maize input. Thus, regression equation of precision feeding was $Y = 3.44+0.042\chi^2$ and for CAM $Y = 1.37+0.072\chi^2$. The mean apparent available amino acid values obtained by precision feeding and CAM for maize were significantly difference ($p<0.05$) and the apparent available amino acid values from precision feeding tended to higher than CAM. But, true available amino acid observed for maize was not differences between two procedures. The true amino acid values obtained of maize (88.6 and 89.30%) were higher than apparent available amino acid values (79.03 and 71.93%). The main reasons for these

Table 1: Regression analysis and comparison of apparent available amino acid and true available amino acid values of maize by precision-fed and CAM (g)

Bioassay	R^2	Intercept (a)	Regression coefficient (b)	AAAA±SEM[1]	TAAA±SEM[2]
Precision fed	0.63	3.44±0.53	0.042±0.09	5.80±0.4^{b}	6.96±0.24^{a}
CAM	0.65	1.37±0.81	0.072±0.01	5.03±0.25^{c}	7.09±0.17^{a}

[1]Apparent available amino acid, [2]True available amino acid

Table 2: The mean apparent available amino acid and true available amino acid values with different inputs of maize (g)

Amino acid levels	10	20	30	40	50	60	70	80	90	100
AAAA[1]	2.18±1.4^{b}	5.73±0.34^{b}	6.05±0.34^{b}	6.43±0.56^{ab}	6.70±0.1^{bc}	6.21±0.24^{c}	6.33±0.3^{c}	6.67±0.24^{c}	6.70±0.23^{b}	7.06±0.4^{a}
AAAA[2]	2.36±0.3^{b}	3.73±0.3^{c}	5.40±0.07^{c}	5.60±0.52^{b}	6.48±0.6^{c}	6.17±0.2^{c}	6.46±0.1^{c}	6.51±0.1^{c}	6.00±0.3^{c}	6.30±0.29^{b}
TAAA[1]	4.11±1.6^{a}	7.70±0.52^{a}	7.36±0.37^{a}	7.41±1.01^{a}	7.49±0.1^{ab}	6.87±0.29^{b}	6.90±0.2^{b}	7.12±0.26^{b}	7.13±0.14^{a}	7.45±0.44^{a}
TAAA[2]	4.67±3.0^{a}	7.38±0.25^{a}	7.80±0.26^{a}	7.40±0.99^{a}	7.75±0.46^{a}	7.32±0.06^{a}	7.37±0.19^{a}	7.46±0.22^{a}	6.80±0.28^{b}	7.00±0.25^{a}
F-value	**	**	**	*	**	**	**	**	**	**

[1]Precision method, [2]Conventional addition method, Mean values with different superscripts are not significantly different. *: Significant, **: Highly significant

corrections for metabolic fecal and endogenous urinary amino acid excretion.

The data indicated that the apparent available amino acid values of maize obtained by precision feeding and CAM were dependent on the amount of maize eaten at low feed intake. The apparent available amino acid values obtained by precision feeding were higher than those determined by CAM based on the value derived for the mean amount of maize eaten.

However, despite the substantial differences between values derived by the different methods, it is worthy to note that at low feed intake, the available amino acid values derived by precision feeding methods were not significantly lower than those obtained by CAM. Amount of apparent available amino acid values obtained by two procedure were indicated that at low levels (20 to 40 g) and higher levels as 90 and 100 g those had significantly differences ($p<0.05$) (Table 2).

The result indicated that there were not those same data obtained of experiment by using two procedures (Table 3). In this experiment a mount of apparent available amino acid arrived of two procedures had more differences contrast to true available amino acid.

There was a linear relationship between the amino acids (g) voided as excreta and the amount of amino acid intake of maize. From the intercepts, it can be seen that at zero amino acid intakes, the amino acid voided (i.e. the EAAL) was 0.45 and 0.56 (g) for precision and CAM feeding, respectively (Fig. 1 and 2).

The true available amino acid values for maize were 6.96 to 7.09 g/100 g, but apparent available amino acid values were 5.03 to 5.8 g/100 g (Fig. 3). The relationship between the latter two measurements was a hyperbolic curve with the apparent available amino acid value approaching the true available amino acid value at high maize intake. At low levels of maize input, apparent available amino acid increase with intake but after correction for metabolic and endogenous losses true available amino acid was independent of input. As the high levels of maize input, amount of apparent available amino acid and true available amino acid values were

Table 3: The mean apparent and true available of amino acid of maize by precision and CAM methods (%)

	Amino acid	Precision fed		CAM
	Apparent AAA	True AAA	Apparent AAA	True AAA
Aspartic acid	75.80	86.65	72.07	91.69
Threonine	76.60	86.64	66.11	85.98
Serine	82.97	91.10	70.99	89.35
Glutamic acid	86.76	93.09	81.40	90.98
Proline	85.61	92.37	78.86	92.97
Glycine	45.55	63.87	72.44	72.36
Alanine	81.21	88.30	72.19	87.21
Cystine	83.69	92.15	81.35	89.02
Valine	81.53	89.67	67.93	83.28
Methionine	72.96	91.28	49.49	85.30
Isoleucine	78.30	87.25	68.57	84.80
Leucine	89.32	93.63	84.09	93.64
Tirosine	84.19	89.93	77.02	93.87
Phenylalanine	87.37	92.80	79.05	90.19
Lysine	54.80	79.23	54.70	85.95
Histidine	81.67	87.77	79.05	89.13
Arginine	85.11	91.64	81.20	94.53
F-value	**	**	**	**

Apparent amino acid available = True AAA – EAAL/FI, True amino acid available = Apparent AAA + EAAL/FI

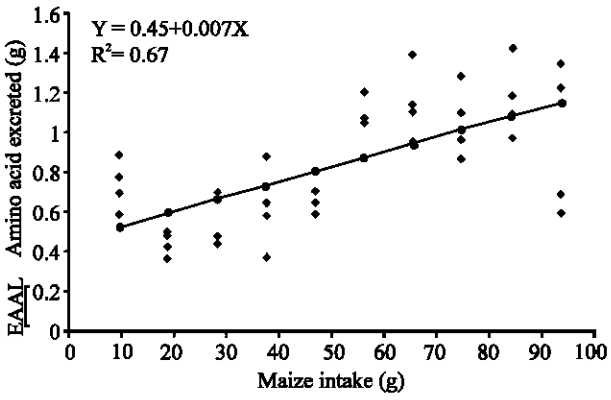

Fig. 1: The relationship between amino acid intake of maize and amino acid excretion (precision feeding)

independent of the amount of maize eaten. Therefore, the differences between apparent available amino acid and true available amino acid were caused by EAAL.

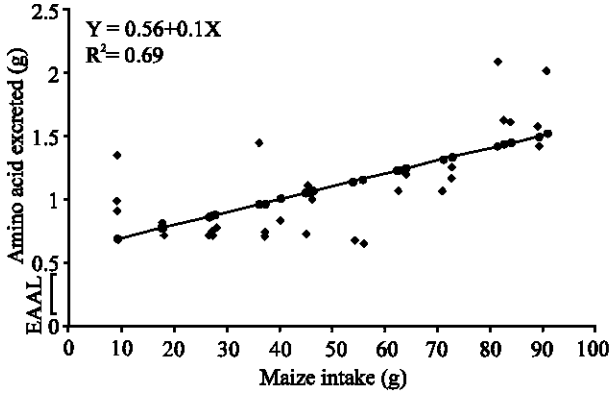

Fig. 2: The relationship between amino acid intake of maize and amino acid excretion (CAM methods)

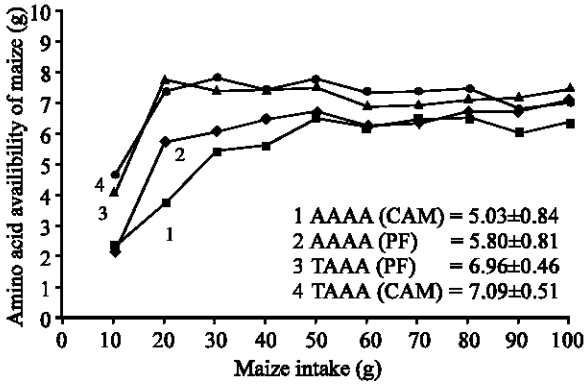

Fig. 3: Mean values of apparent and true amino acid available for maize input plotted by CAM and precision feeding (g)

DISCUSSION

The regression analysis carried out to estimate the availability of the amino acids in maize and the equations generated from the data obtained after applying the two methods have shown that there was a significant difference between values found for the apparent and the true availability values for the amino acid in maize, irrespective of the method used. In the precision feeding method this may have result from the fact that there was an increased amount of endogenous amino acid losses. Because the effect of microbial action in the lower intestine, particularly in the caeca and colon (resulting in the increased bacterial synthesis of amino acid)[4,14,18-22]. The sizes of the differences observed between the apparent and true availability of the amino acids in the present study suggest that the extent of amino acid metabolism by the micro flora of the hind-gut may be substantial. In an evaluation of the precision-feeding cockerel assay Engster[9] compared results from several laboratories and showed there to be good agreement for true available amino acids. Furthermore, Likuski and Dorrell[10] and Sibbald[8] also drew attention to the reliability of the precision-fed rooster assay in generating true available amino acid values.

In the experiment carried out here the intercepts of the regression equations determined from the data generated as a result of precision feeding were markedly different from those determined after applying the CAM. This means that the method chosen would influence the values derived for the apparent available amino acids, because they depends on the EAAL per unit of maize amino acid intake[8]. The value of the intercept from the precision-fed assay was 3.44±0.53 g (per 48 h), substantially than the values of 1.37±0.81 g (per 72 h) obtained from the CAM. The main explanation for this higher intercept (EAAL) is likely to be the result of greater microbial activity in intestine[23].

The relationship between the apparent availability of the amino acids and the maize intake is described by a hyperbolic curve, with the apparent availability value for the amino acids value for the amino acids approaching the true availability value at high maize intakes. The results of this experiment show clearly that the value apparent availability of the amino acids in maize depends on the intake[3]. This effect was attributed to the EAAL. At high maize intakes, EAAL have less effect on the values for the apparent available amino acids. It is generally accepted that EAAL in birds will vary with the quantity and nature of the feedstuffs ingested. Nitrogen retention by the cockerels also proved to be negative under both procedures at all intakes[14]. Consequently, with respect to the intakes of maize fed in both the precision-feeding method and CAM, the values determined for the apparent availability of the amino acids in maize were always lower than those for the true availability. The values for the apparent available amino acid obtained from precision feeding were higher than the corresponding values obtained from the CAM. Apparent available amino acid values should vary with the amount of feed eaten because, under standard conditions, the EAAL is relatively constant. When the maize intake was high the EAAL was relatively small but as the amino acid (maize) declined the endogenous amino acids excreted as a proportion of the total create relatively greater impact, with the result that apparent available amino acid values decline.

Furthermore, with both procedures the apparent available amino acid values were similar as the intake of maize increased. Also, the true availability values of the amino acids did not depend on the intake. Under the condition of the present experiment the true availability of

the amino acids in maize were independent of the bioassay applied and the intake of maize, although the values attributed to the EAAL varied.

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