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CARCASS AMINO ACID COMPOSITION AND UTILIZATION OF DIETARY AMINO ACIDS BY CHICKENS*

A.J. Saunders[1], J.P.H. Wessels[2] and R.M. Gous
Department of Animal Science and Poultry Science, University of Natal, Pietermaritzburg, 3200

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OPSOMMING: KARKAS AMINOSUURSAMESTELLING EN BENUTTING VAN VOER AMINOSURE DEUR KUIKENS

In 'n proef waar verskillende eiwitbronne aan eenweek-oud kuikens behels het, was daar geen statistiese betekenisvolle verskille in die aminosuurinhoud van die kuikenkarkasse gevind nie, ten spyte van aansienlike verskille in liggaamsmassa as gevolg van die groot verskille in rantsoene wat gevoer is. Hoogsbetekenisvolle korrelasiekoëffisiënte is verkry tussen karkasvoginhoud en die inhoud van essensiële aminosure in die karkas. Karkasaminosuurontleding kon dus bereken word, onder sekere voorwaardes, vanaf die karkasvoginhoud deur gebruik te maak van voorafbepaalde regressievergelyking. Netto aminosuurbenuttingstudies het aangetoon dat lisien die eerste beperkende aminosuur by sonneblommeel is en dat isoleusien eerste-beperkend is by vismeel. 64% Van die isoleusien in vismeel en 75% van die lisien in sonneblommeel is deur die kuikens vir groei benut.

SUMMARY:

In an experiment involving the feeding of different protein sources to one-week old chickens, no statistically significant differences occurred in the amino acid content of the chicken carcasses, despite considerable differences in body mass changes as a result of the dissimilar diets fed. Highly significant correlation coefficients were obtained between body water content and the content of essential amino acids in the carcass. Carcass amino acid analysis could therefore, under set conditions, be calculated from the body water content by using predetermined regression equations. Net amino acid utilization studies indicated that lysine is the first-limiting amino acid in sunflower meal and that isoleucine is first-limiting in fishmeal. Sixty-four % of the isoleucine in fishmeal, and 75% of the lysine in sunflower meal was utilized by the chickens for tissue growth.

Amino acid composition of the carcass has received consideration as a means of determining the amino acid requirement of the animal. Studies in this field have been conducted by Williams, Curtin, Abraham, Loosli & Maynard (1954), Fisher & Scott (1954), Forbes & Rao (1959), Hartsook & Mitchell (1956). King (1963) showed that individual amino acids in the carcass could differ widely from the requirement by the animal for those particular amino acids used for purposes other than protein synthesis and subsequent retention. However, Pellet & Kaba (1972) reported no differences in the amino acid composition of rats fed a "nitrogen-free" diet and diets containing protein which originated from various sources.

The fact that body amino acid composition is relatively constant in fast or relatively-fast growing animals (Pellet & Kaba, 1972) and that a highly significant correlation exists between body water and body nitrogen content (Velu, Baker & Scott, 1972; Saunders, 1974) indicates that there may be a significant relationship between body water and body amino acid content. Regression equations expressing the relationship between body water and body amino acid content would simplify the calculation of net amino acid utilization (NAAU), and might lead to a wider application of this measure in amino acid availability studies. Simplification of the calculation of NAAU could be obtained if a correlation was found to exist between the carcass amino acid content and a more easily determined component such as body water.

The purpose of this investigation was two-fold. First, it was necessary to determine whether the amino acid content of chicken carcasses remained constant after these chickens had been subjected to varying dietary treatments. This is a necessary prerequisite if a relationship between body water and body amino acid content is to be at all meaningful. Secondly, NAAU values were calculated for fishmeal and sunflower meal in order to predict from these values their respective first-limiting amino acids as well as the availability of these amino acids in the two feed ingredients.

Procedure

An experiment with one-week old chickens was conducted in which combinations of fishmeal and sunflower meal were tested to determine the ratio of these two protein sources that would support maximum nitrogen retention (Saunders & Wessels, 1975). At the end of the seven-day test period, carcasses were dried in a moisture-extracting oven operated at 95°C for a period of 7 days (Saunders & Wessels, 1975). The carcasses used in this experiment were from treatments where chickens were fed fishmeal or sunflower meal as

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(1) Nulaid (Pty) Ltd., P.O. Box 3006, Johannesburg, 2000.

(2) Fishing Industry Research Institute, University of Cape Town, Private Bag, Rondebosch, 7700.

the only protein sources, and from a "N-free" treatment where a diet devoid of nitrogen was fed (Diets are shown in Table 1). Pooled samples consisting of the 2 heaviest and 2 lightest carcasses from each of 3 randomly-selected replications were taken from each of the three treatments.

Table 1

Composition (g/kg) and calculated analysis of diets used in the experiment

Ingredient	Fishmeal Diet	Sunflower meal Diet	N-free Diet
Sunflower oilcake meal		446,0	
Fishmeal	260,0		
Maize starch	236,3	216,5	193,0
Sucrose	200,0	200,0	200,0
Sunflower oil	90,0	90,0	90,0
Monocalcium phosphate		20,5	
Limestone		10,0	
Mineral premix*	15,0	15,0	15,0
Vitamin premix*	1,0	1,0	1,0
Choline Chloride	1,0	1,0	1,0
Cellulose	196,7		500,0
Calculated analysis:			
Nitrogen	28,1	28,1	1,0
Calcium	9,0	9,0	9,0
Phosphorus (avail.)	5,8	5,8	5,8
ME (MJ/kg)	7,3	7,3	7,3

* Mineral premix consisted of (g/kg): KH_2PO_4 479, NaCl 365, ferric citrate 23, $MgSO_4$ 114, $MnSo_4 \cdot H_2O$ 9, KI 0,46, $CuSO_4$ 0,58, $ZnCO_3$ 9, Na_2MoO_4 H_2O 0,46

** Vitamin premix consisted of (mg/kg): thiamin 35, riboflavin 23, pyridoxine 8, biotin 0,8, preroylmonoglutamic acid 5,7, menaphthone 7, cyanocobalamin 0,03, nicotinic acid 354, retinol (1982 µg/g) 212,7, cholecalciferol (2000 µg/g) 10,2, α-tocopherol (250 mg/g) 6, maize starch 95.

Each pooled sample was thoroughly mixed by passing this material through a mincing machine 3 times. Carcass fat was extracted from the samples using the method of du Preez, Wessels, Stokoe & van der Merwe (1971), after which the fat-free samples were ground to a powder using a ball mill. A total of 6 nitrogen and amino acid determinations was conducted on each pooled sample, using an amino acid analyser (Model 120B, Beckman Instruments Inc., Fullerton, Cal., U.S.A.)*

* Samples for the amino acid analyser were hydrolysed using the conventional procedure of hydrolysis in 6M–HCl (Dennison, 1976).

N content was determined using the Kjeldahl procedure (A.O.A.C., 1965). Methionine and cystine were estimated in samples oxidised with performic acid (Moore, 1963).

Carcass amino acid content was expressed in terms of total carcass N content (expressed as mg/g N).

Net amino acid utilization was calculated according to the formula of Harper & de Muelenaere (1963), namely,

$$NAAU = \frac{\text{Amino acid content of carcass on test diet} - \text{Amino acid content of carcass on "N-free" diet}}{\text{Amount of amino acid consumed}} \times 100$$

Results

Initial body mass, final body mass and percentage change in body mass of chickens fed the 3 experimental diets for 7 days are summarised in Table 2. The amino acid composition of the chicken carcasses is presented in Table 3, each value representing the mean of 6 determinations. No statistically significant F-value was found, which indicated that no difference existed between chickens on the 3 diets in respect of any of the amino acids analysed.

Table 2

Mean initial body mass, final body mass and percent change in body mass of chickens fed three diets during a seven-day experimental period

	"N-free" diet	Sunflower diet	Fishmeal diet
Initial body mass (g)	54,20	54,20	54,20
Final body mass (g)	43,58	81,07	92,44
Per cent change	–19,0	49,6	70,6

Correlation coefficients for body water content and, respectively, body lysine, histidine, arginine, threonine, valine, methionine, isoleucine and phynylalanine are presented in Table 4, together with F-values for significance due to linear regression, and regression equations of body water content on body amino acid content. All correlation coefficients were statistically significant ($P < 0,01$) indicating a high correlation between body water content and individual amino acid content in the body. A Z-transformation (Rayner, 1969) was used to test whether the lowest correlation coefficient (that of 0,80 between body water and body lysine) and the highest (0,92 for threonine) differed significantly from each other. An u-value of 0,69 proved that the two coefficients did not vary significantly from one another, and on this basis the regression equations were computed using combined data from all carcasses. In all cases the F-value due to the linear regression proved significant ($P < 0,01$) this indicating that the regression equations fitted the data well.

Table 3

Amino acid composition (mg/gN) of chicken carcasses after seven days of growth on three diets, each value representing the mean of six analyses

Amino Acid	Diet "N-free"	Fishmeal	Sunflower meal	S.E.M.[1]	C.V.[2]	F[3]
lysine	330,7	346,6	330,1	44,6	13,3	0,26
histidine	131,7	123,7	128,6	9,0	7,1	1,18
arginine	406,6	403,0	410,2	29,8	7,4	0,09
asparitic acid	487,7	491,6	485,5	26,4	5,4	0,08
threonine	206,4	219,5	210,4	15,2	7,2	1,16
serine	230,5	224,7	223,8	17,4	7,7	0,27
glutamic acid	821,3	809,5	813,3	59,7	7,3	0,06
proline	375,3	376,9	347,3	31,8	8,7	1,64
alanine	408,1	391,1	405,1	46,3	11,6	0,08
cystine	114,7	108,9	114,4	8,8	7,8	0,23
valine	309,6	303,0	307,6	19,0	6,2	0,82
methionine	107,0	106,0	108,5	11,4	10,6	0,19
isoleucine	250,5	252,4	245,4	24,2	9,7	0,14
leucine	432,1	429,4	438,1	22,9	5,3	0,23
tyrosine	186,1	176,9	183,7	14,2	7,8	0,21
phenylalanine	258,1	261,7	273,0	18,3	6,8	0,57

1 standard error of a single treatment mean

2 coefficient of variation (per cent)

3 F-value obtained by analysis of variance

Table 4

Correlation coefficient and regression equations, with levels of significance, of body water content on body amino acid content of fourteen-day old chickens fed diets based on fishmeal or sunflower meal

Carcass amino acid	Correlation coefficient	F-value in test of regression	Regression equation[1]
Body water content on body:			
lysine	0,80**	17,77**	Y = – 194,12 + 15,27 X
histidine	0,82**	20,53**	Y = – 36,29 + 5,14 X
arginine	0,86**	28,40**	Y = – 151,29 + 16,89 X
threonine	0,92**	55,10**	Y = – 19,68 + 7,89 X
valine	0,90**	42,60**	Y = – 46,14 + 11,66 X
methionine	0,88**	34,33**	Y = – 4,00 + 3,85 X
isoleucine	0,90**	42,63**	Y = – 141,71 + 11,10 X
leucine	0,91**	48,17**	Y = – 37,42 + 16,07 X
phenylalanine	0,86**	38,50**	Y = – 33,34 + 9,99 X

** significant at $P < 0,01$

1 where Y = Body amino acid content (mg)
X = Body water content (g)

Table 5

Net amino acid utilization (NAAU) of fishmeal and sunflower meal

Amino Acid	NAAU	
	Fishmeal	Sunflower meal
lysine	37,34	75,57
histidine	34,53	24,87
arginine	56,29	31,08
threonine	36,47	42,93
methionine	28,38	27,07
valine	52,65	45,35
isoleucine	63,80	43,13
leucine	44,42	45,62
phenylalanine	53,88	38,55

Discussion

The similarity in amino acid composition of chickens fed 3 entirely different diets in respect of amino acid content occurred in spite of the significantly improved body mass gain of chickens fed the fishmeal diet compared with the gain of those on the sunflower meal diet, both increases being significantly greater than the gain in body mass of chickens on the "N-free" diet.

It has long been recognised that the level of protein intake affects the amount of protein in the body and that different tissues respond diferrently to protein deprivation. Calet (1967) illustrated that the body N content of the chicken depends not only on the nature of the protein ingested, but with a given protein, it also depends on the growth rate of the animal. A faster growth rate is associated with a proportionately greater body N content. This confirms work by Wilson (1954), Widdowson & McCane (1960) and Dickenson & Widdowson (1960) who illustrated that various tissues do not develop at the same rate when the plane of nutrition or growth rate is varied. Nevertheless this differential growth pattern is apparently not accompanied by any demonstrable change in the amino acid composition of the young animal carcass as a whole, even with a 20% loss in body mass as the results of this experiment indicate. This result is in agreement with work reported by Pellet & Kaba (1972) who used rats as experimental animals.

Because body amino acid composition appears to be relatively constant in fact-growing animals, and considering the highly significant correlation between body water and body nitrogen content (Saunders & Wessels, 1975), the relationship between carcass water content and carcass amino acid content was investigated. The correlation coefficient between body water content and each of the 9 amino acids analysed proved highly significant ($P < 0,01$). The equations expressing individual amino acids as a function of body water content be used to estimate body nitrogen content. However the use of such equations is subject to certain limitations, and a set of equations should be established for every set of test conditions, taking into account the age, sex and strain of the test animal as well as the dietary protein level fed to the chickens.

Little consideration has been given in the literature to those proportions of various amino acids consumed during NPU experimentation that are actually used for protein synthesis. The majority of amino acids that penetrate the gut wall are used for protein synthesis, but a varying proportion are metabolised. All available amino acids must be of potential use for protein synthesis, even if, at the moment of this test, they are then used as a source of energy. Only small quantities of absorbed amino acids leave the organism without any utilization and consequently all the amino acids which are absorbed and utilized can be defined as being "available" (Erbersdobler, 1976). In the case of the first-limiting amino acid in a diet this would be utilized completely for protein synthesis. It could be argued, therefore, that as NAAU refers to those fractions of the absorbed amino acids that are utilized for tissue growth, this value would also give an indication of the availability of the first-limiting amino acid in that diet.

The amino acid for which the highest NAAU value is obtained would necessarily be the limiting essential amino acid, so it is possible from this method to ascertain which amino acid is first-limiting in each ingredient, and also the availability of that amino acid to the chicken.

From the calculations of the NAAU of fishmeal and of sunflower meal it is evident that isoleucine is first-limiting in fishmeal (Table 5) 63,8% of this amino acid being utilized for tissue synthesis by chickens in this experiment. Similarly, lysine is apparently the first-limiting amino acid in sunflower meal, with a NAAU value of 75,57% . This confirms the results published by Saunders & Wessels (1975) with regard to the first-limiting amino acids of these 2 feed ingredients.

On the basis of these results, the availability of lysine in sunflower meal would be 75,57% of the lysine content (3,22%) namely, 2,43% . The sunflower meal used contained 41 % crude protein. Similarly, the fishmeal used in this experiment (59% crude protein; 4,09% lysine) was shown to have 63,80% of the lysine available to the chicken, i.e. 2,61% available lysine.

The calculation of NAAU could be simplified by the use of regression equations of body water content on body amino acid content, thereby eliminating the need to analyse chicken carcasses for amino acid content. Such results are subject to the limitations mentioned above, and would obviously not be as accurate as direct measurements would be. NAAU values can then be calculated for different food ingredients, and used to determine the first-limiting amino acid of each ingredient, and the availability of this amino acid in the corresponding ingredient. Unfortunately it is not possible to use this method to determine the availability of any other than the first-limiting amino acid in each ingredient.

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