FURTHER STUDIES ON URINARY 3-METHYLHISTIDINE EXCRETION AS AN INDIRECT INDEX OF DIETARY AMINO ACID ADEQUACY IN RATS

*OLUMAFEMI O. BALOGUN[1], AMOS A. ADEYEYE[1] AND ELIZABETH A. BALOGUN[2]
[1]Department of Biochemistry, [2]Department of Physiology and Biochemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.

SUMMARY

As a further step in the determination of the sensitivity of urinary 3-methylhistidine (3-MeH) to essential amino acid adequacy in diet, 36 male albino rats of the Wistar strain were divided into six groups of six rats per group. Each group with an initial mean liveweight of 106.52 ± 0.54g was fed in individual metabolic cages on a basal diet supplemented with graded levels of DL-methionine. Total methionine in diet ranged between 0.25 and 1.05%. The study lasted 10 days. The response of urinary 3-methylhistidine excretion to the graded levels of methionine in diet was compared to responses obtained from growth performance characteristics, plasma urea concentration, liver nitrogen and creatinine excretion. With the exception of feed intake, all other indices of dietary protein adequcy and efficient amino acid utilization viz growth rate, protein efficiency ratio, serum urea concentration, and creatinine excretion were significantly (P 0.05) to P 0.01) influenced by dietary methionine level. Maximum growth rate, liver N and urinary 3-methylhistidine were observed in rats given 0.45% total methionine in diet. Supplementing the basal diet to contain 0.45% total methonine significantly (P 0.01) decreased serum urea concentration. Urinary 3-methylhistidine excretion was found by regression analysis to be positively correlated to body weight gain (r = 0.73), feed intake (r = 0.61), urinary creatinine excretion (r = 0.74) and liver N (0.72) but negatively correlated to dietary methionine level (r = –0.41). We suggested that urinary 3-MeH excretion be added to the list of available indicts for dietary amino acid adequacy.

INTRODUCTION

The validity of urinary 3-methylhistidine (3-MeH) excretion as an index of muscle protein breakdown *in vivo* in laboratory animals and species of agricultural importance continues to increase (Nishizawa *et al.*, 1977; Haris & Milne, 1980). Omstedt *et al.*, (1978) reported that a good correlation exists between protein quality and total urinary 3-MeH, the higher the protein quality, the greater the excretion of total 3-MeH. Similarly our earlier report Balogun & Balogun (1982) indicates that total 3-MeH in urine is sensitive to dietary methionine and lysine deprivation in rats. This tends to suggest that this urinary metabolite could be a good indirect index of dietary amino acid adequacy. We also gave a theoretical basis for the possibility of using total urinary 3-methylhistidine as another indirect indication of dietary protein utilization. However, more information is imperative to validate the inclusion of this metabolite in the list of indices of dietary protein quality or dietary amino acid adequacy. The present paper deals with further investigation on the sensitivity of urinary 3-MeH in evaluating the quantitative amino acid particularly methonine requirements of laboratory animals and subsequently species of agricultural importance when compared with known indices of dietary amino acid adequacy.

*Present address:
Department of Animal Production, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria.

MATERIALS AND METHODS

Thirty-six male 8 weeks old albino rats of the Wistar strain, weighing between 90 and 118g were housed individually in metabolic cages for 15 days. After 3 days adaptation during which they were fed on

commercial rat cubes (Pfizer Livestock Products Limited, Ikeja, Nigeria) the animals were divided into six groups. The initial weight of each group was 106.52 ± 0.54g. Rats within each major group were fed on one of the basal diets supplemented with graded levels of DL-methionine. The percentage composition of the 10% crude protein and 3-MeH-free basal diet was as follows: Corn starch, 66.0; Casein, 9.0; Groundnut oil, 5.0; Mineral mixture (Rotruck and Boggs, 1977), 4.0; Vitamin mixture (Rotruch and Roggs, 1977), 1.0; Sucrose, 10.0; and cellulose, 5.0. The basal diet which contained 0.27 and 0.25% cystine and methionine respectively by calculation was supplemented with increasing amounts of DL-methionine in 0.2% increment to give total methionine levels ranging from 0.25 to 1.25% of diet. Dietary N was equalized by the use of L-glutamic acid. Animals were individually housed in metabolic cages, fed and watered *ad libitum*. After 2 days of dietary treatment, urine collection was commenced for a 10 day period. The urine was acidified to prevent N losses. Analyses were carried out on bulked urine samples. At the end of the 10 day urine collection, the rats

TABLE 1

Influence of Dietary Methionine Level on Urinary 3-Mehtylhistidine Excretion and Other Indices of Dietary Amino Acid Adequacy in Rats[1]

Parameter	*Methionine level (% of diet)*						
	0.25	0.45	0.66	0.85	1.05	1.25	SE_m
Feed intake (g/day)	11.23	12.77	11.70	11.38	11.67	12.52	$0.25^{n.s.}$
Body weight gain (d/day)	1.83^a	3.35^e	2.12^b	2.50^d	2.55^d	2.36^c	0.21*
Efficiency of Feed conversion[2]	6.14^d	3.81^a	5.52^c	4.55^b	4.58^b	4.72^b	0.33*
Protein Efficiency Ratio (PER)[3]	1.48^d	2.11^c	1.42^a	1.71^b	1.65^b	1.43^a	0.11**
Serum Urea-N (mg/100ml)	13.87^c	9.24^b	9.43^b	7.47^a	8.10^a	7.73^a	0.97**
Liver nitrogen:							
Absolute (mg/g)[9]	196^b	261^e	178^a	225^c	228^c	245^d	13*
Relative (mg/100GBW)[4]	156^a	194^c	150^a	145^b	181^b	173^b	7*
3-Methylhistidine excretion:							
Total (umole/10 days)	14.15^c	26.62^d	4.62^a	10.70^b	9.36^b	12.02^{bc}	3.03**
umole/100g BW^2/10 days	11.52	20.63	4.16	8.93	7.64	9.29	2.27**
Creatinine excretion in urine:							
Total (umole/10 days)	94.54^C	89.42^c	33.54^a	35.54^a	62.13^b	58.27^b	10.57*
umole/100g BW^2/10 days	90.00^d	55.66^c	28.13^a	30.50^a	54.50^{bc}	44.15^b	9.21*

[1]Mean of six rats; [2]Efficiency of feed conversion = g feed consumed/g livewight gain: [3]Protein Efficiency Ratio = gain in wt (g)/total protein consumed (g) during the duration of the experiment; [4]BW = Body weight; n.s. & not significant; *P 0.05 **P 0.01 $^{a,b,\cdots}$Means not sharing common superscripts in the same horizontal column are significantly (P 0.05) different from one another.

were mildly anaesthesized with diethylether and blood withdrawn from the dorsal aorta for measurement of serum total protein and urea. Records of daily feed consumption and liveweight of the rats were kept for the duration of the experiment. Nitcogen contents of basal diet and liver were estimated by standard procedures (AOAC, 1975). Serum urea-N concentration was estimated according to the procedures described by Fawcet and Scott (1960) while serum total protein was measured using the colorimetric method with Biuret reagent, according to the kit method of Boerhinger Manheim Gmlt Ltd., Germany. Creatinine was analysed colorimetrically with alkaline picrate (Jaffe reaction) similar to the standard manual procedures for blood and urine (Henry, 1964a,b) while 3-methylhistidine in urine was measured according to the procedures described by Ward (1978) after hydrolysing the urine samples with 12M HC1. The data were subjected to

analysis of variance (Snedecor, 1965) and treatment means compared by Duncan's (1955) multiple range test. Relations between urinary 3-MeH and other indices of dietary amino acid adequacy were measured by regression analysis (Snedecor, 1965).

RESULTS AND DISCUSSION

Data from growth performance, serum total protein and urea, liver nitrogen and urinary nitrogenous compounds are presented in Table 1. Increasing total methionine in diet from 0.25 to 0.45% significantly (P 0.05) improved the direct indices of dietary protein utilization viz body weight gain and PER. Serum urea concentration was significantly (P 0.01) lowered at the dietary total methionine level (0.045%) that gave maximum body weight gain. The liver N was significantly (P 0.05) higher in rats fed on 0.45% total methonine in diet. Urinary 3-MeH excretion was significantly (P 0.01) influenced by dietary methionine level. Urinary 3-MeH excretion in absolute and relative terms was maximal when rats were fed on diet containing 0.45% total methionine.

There was a good positive correlation (Table 2) between indices for efficient dietary amino acid utilization (i.e., growth rate, PER and liver N) and urinary 3-MeH excretion (r = + 0.72 to + 0.83). High positive correlation also existed between the index of muscle mass (creatinine) and urinary 3-MeH excretion (Table 2).

TABLE 2

Relations Between Urinary 3-Methylhistidine Excretion (Y in u mole) And Other Indices of Dietary Amino Acid Adequacy (X) in Rats Fed Varying Levels of DL-methionine In Diet.

X	*Regression equation*	*r*	r^2
1. Methionine level (% diet)	Y = 18.95 — 8.05 ×	— 0.41	0.17
2. Body weight gain (gm)	Y = —12.87 + 10.51 ×	+ 0.73	0.53
3. Feed intake (g)	Y = —73.36 + 7.26 ×	+ 0.61	0.37
4. Protein Efficiency Ratio (PER)	Y = —25.42 + 23.47 ×	+ 0.83	0.68
5. Serum Urea level (mg/100m)	Y = 7.28 + 0.62 ×	+ 0.17	0.03
6. Urinary creatinine (u mol)	Y = —P.37 +	0.17 ×	0.52
7. Liver nitrogen (mg)	Y = —25.74 + 0.17 ×	+0.72	0.52

Taking the total methionine in diet that gave maximum growth performance and maximum liver N as that representing dietary methionine adequacy for the normal growth and tissue protein synthesis in rats, it appears the methionine requirement of growing rats is 0.45%. This further confirms that the methionine rquirement of growing rats ranged between 0.4 and 0.5%. The fact that the total methionine level that resulted in maximum excretion of 3-Meh in urine was similar to that at which maximum growth performance was obtained is an indication of the sensitivity of 3-MeH to dietary amino acid adequacy in rats. Omstedt *et al.* (1978) have shown that the higher the protein quality, the higher the excretion of 3-MeH in urine. It is conceivable that, because of the high positive correlation between dietary protein quality and protein synthesis (muscle protein in particular), it would appear that 3-MeH excretion would be increased with the improvement of dietary amino acid balance, since, it is probably that, when muscle protein synthesis increases, the increased

excretion of total 3-MeH as an end product of muscle protein catabolism could be taken as an index of myofibrillar protein turnover (Balogun and Balogun, 1982).

Because of the similarity in the dietary methionine level adequacy for normal growth and tissue protein synthesis and the level that promoted maximum excretion of 3-MeH in urine (taking as an index of turnover rate of muscle proteins) and the good correlation between these indices, we suggest that urinary 3-MeH excretion be added to the list of available indices for dietary amino acid adequacy.

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