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Lysine in Cassava Based Diets: II. The Nutritional Evaluation of Acetylated Casein in Maize Meal, Corn Starch and Cassava Based Diets

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ABSTRACT

Casein was acetylated with sodium acetate/acetic anhydride and was found to be 98·5% acetylated by the FDNB available lysine method.

The PER of maize meal and corn starch diets decreased with increasing amounts of acetylated casein. The PER of a maize meal–casein diet was 2·21 but this decreased to 2·13 and 1·60 when acetylated casein was incorporated at 5 and 10% levels, respectively. The same trend was observed for corn starch diets with a PER of 1·60 for a casein–corn starch diet but PERs of 1·28 and 0·91 when acetylated casein was added at 8 and 16% levels, respectively.

In the presence of cassava, the nutritional value of acetylated casein was enhanced. Thus a meal made of 10% acetylated casein: 50% cassava: 25% maize meal had a PER of 2·06 compared to 1·60 for a diet consisting of 10% acetylated casein: 70% maize meal. At 16% acetylated casein: 70% cassava the PER was 1·75 compared to 0·91 in a 16% acetylated casein: 70% corn starch diet.

A similar trend was observed for NPU, NPR and serum urea levels for the respective diets considered.

Acetylated casein moderately elevated the activity of urine enzymes (LDH, GDH, alkaline and acid phosphatases) in corn starch based diets while cassava based diets appeared to depress the level of the enzymes.

INTRODUCTION

The use of amino-acid supplementation in feeds results in some modification. For example, fructose–glycine, fructose–methionine and

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fructose–leucine have been found as sugar–amino acid products in feeds where amino acids have been used as supplements (Erbersdobler, 1976). Lysinoalanine, besides its unavailability as a source of nitrogen, has nephrotoxic effects (Woodward *et al.*, 1975). Although most of these modified amino acids do not occur in large enough quantities in feeds to cause concern, Maillard reactions occur frequently under mild conditions of storage or processing. This is known to cause a fall in the nutritive value of proteins by decreasing digestibility and the available lysine content of the feed (Knipfel *et al.*, 1975)

The effect of heat on the Maillard reaction and the subsequent effect on the nutritive value of feedstuffs have been well documented for casein, egg, soy (Knipfel *et al.*, 1975), groundnut meal (Anantharaman & Carpenter, 1969) and buffalo casein–glucose mixture (Rao *et al.*, 1963). The mechanism of heat damage has been proposed by Carpenter and co-workers (Varnish and Carpenter, 1975; Hurrell *et al.*, 1976). As well as the Maillard reaction, the ε-amino group of lysine reacts with the carbonyl group of aspartic and glutamic acids and probably with the amide groups. This was demonstrated to occur by the isolation of ε-N-(γ-glutamyl)-lysine from wool keratin (Asquith *et al.*, 1970).

In the previous studies, feeding trials with leaf protein concentrate (LPC) led to the proposed use of LPC as a protein supplement (Oke, 1975) in human and animal diets with cassava as the energy base. However, recent findings of decreased digestibility and availability of lysine on heating LPC and carbohydrate–LPC mixtures (Adewusi & Oke, 1984) is cause for concern. It is therefore considered imperative to further investigate the effect of reactions of lysine on feed utilization. This study is designed to investigate the nutritional value and biochemical changes in maize meal, corn starch and cassava based diets where lysine is acetylated to make it unavailable.

MATERIALS AND METHODS

Preparation of acetylated casein

Casein (50 g) was mixed with 3 mol sodium acetate (crystals) and dissolved in 500 ml distilled water. The solution was ice-cooled and 86 ml of acetic anhydride was added over 1 h following the procedure of Bjanarson and Carpenter (1969). The resulting precipitate was washed with ethanol and then washed three times with 1 litre cold distilled water. The sample was air-dried and ground to a fine powder.

Nitrogen and available lysine determination

Nitrogen was determined by the micro-Kjeldahl digestion method while available lysine was determined by the method of Carpenter (1960) as modified by Booth (1971).

Animal assay

The composition of the diets is shown in Tables 1 and 2. Weanling littermate rats of the Wistar strain from our animal colony were used in these feeding trials. The rats were collected at 23–24 days of age, numbered and housed individually in wire-screen bottom cages. They were weaned to the

TABLE 1
Composition of Maize Meal and Cassava Acetylated Casein Diets[a]

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Corn starch	76·5	—	—	—	—
Maize meal	—	70·0	70·0	70·0	25·0
Cassava flour	—	—	—	—	50·0
Rice bran	14·5	14·7	12·6	10·6	1·5
Casein	—	6·3	3·2	—	4·1
Acetylated casein	—	—	5·2	10·4	10·4
Palm-oil	5·0	5·0	5·0	5·0	5·0
Salt	0·5	0·5	0·5	0·5	0·5
Dicalcium phosphate (DCP)	1·65	1·65	1·65	1·65	1·65
Oyster shell	0·80	0·80	0·80	0·80	0·80
Vitamin/mineral premix[b]	1·05	1·05	1·05	1·05	1·05

[a] Diet 1 = protein-free diet; diet 2 = casein: 70 % maize meal diet; diet 3 = 5·2 % acetylated casein: 70 % maize meal diet; diet 4 = 10·4 % acetylated casein: 70 % maize meal diet; diet 5 = 10·4 % acetylated casein: 50 % cassava: 25 % maize meal diet.

[b] Vitamin/mineral premix is a commercial product which contains the following (per kg): vitamins A 220 000 I.U., B_1 39 mg, B_2 79 mg, B_6 10 mg, B_{12} 154 μg, D_3 22 000 I.U., E 120 mg; calcium pantothenate 110 mg; nicotinic acid 275 mg; folic acid 3·9 mg; choline chloride 5·75 mg; menaphthone 88 mg; calcium 94·5 g; phosphorus 63·6 g; NaCl 15 g; iodine 30 mg; cobalt 6 mg; copper 60 mg; iron 600 mg; magnesium 627 mg; manganese 90 mg; antibiotics (unspecified).

TABLE 2
The Composition of Corn Starch and Cassava Flour Acetylated Casein Diets[a]

Ingredients	*Diet 6*	*Diet 7*	*Diet 8*	*Diet 9*	*Diet 10*
Corn starch	85·7	72·2	71·3	70·1	—
Cassava flour	—	—	—	—	70·1
Rice bran	5·0	5·0	5·0	5·0	5·0
Casein	—	13·5	6·6	—	—
Acetylated casein	—	—	7·8	15·6	15·6
Palm-oil	5·0	5·0	5·0	5·0	5·0
Salt	0·5	0·5	0·5	0·5	0·5
Vitamin/mineral premix	1·2	1·2	1·2	1·2	1·2
DCP	1·6	1·6	1·6	1·6	1·6
Oyster shell	0·8	0·8	0·8	0·8	0·8
L-Methionine	0·2	0·2	0·2	0·2	0·2

[a] Diet 6 = protein-free diet; diet 7 = casein:corn starch diet (control); diet 8 = 7·8% acetylated casein71·3% corn starch diet; diet 9 = 15·6% acetylated casein:70·1% corn starch diet; diet 10 = 15·6% acetylated casein:70·1% cassava diet.

stock diet in the experimental cages for a week so that, at the commencement of the feeding trials, the rats were 30–31 days old and weighed 40–50 g. Water and food were given *ad libitum* and the weight of the animals was recorded every other day. The first 3 days were regarded as an acclimatization period. Collection of feces and treatment of carcass were as previously described (Adewusi & Oke, 1980).

PER was determined by the method of Osborne *et al.* (1919). NPU was determined by the method of Miller & Bender (1955) and true digestibility (TD) of the dietary nitrogen was obtained by the 'balance sheet' method of Mitchell (1923–24).

Serum urea was determined by the method of De La Huerga *et al.* (1969) and thiocyanate was determined by the procedure outlined by Pettigrew & Fell (1972).

Enzyme assay

Urine excreted between 9 pm and 9 am on days 8 and 13 of the experimental period was collected, centrifuged and analysed for glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), and acid and alkaline phosphatases as outlined by Ngaha (1974).

TABLE 3
Results of Percentage Crude Protein, Available Lysine and Percentage Acetylation of Casein

	Casein	*Acetylated casein (1)*[a]	*Acetylated casein (2)*[a]
% Crude protein	80	49·6	69·4
Available lysine (g/16 gN)	7·80	0·13	0·11
% Lysine acetylated	—	98·3[b]	98·6[b]

[a] Two acetylations were carried out, one for each feeding trial. The difference in CP is probably due to moisture content.
[b] Values obtained from the calculation:

$$\frac{\text{Available lysine in casein} - \text{Available lysine in acetylated casein}}{\text{Available lysine in casein}} \times 100$$

RESULTS

The results for crude protein and available lysine content of casein and acetylated casein are shown in Table 3. Acetylation of casein by the method used (Bjanarson & Carpenter, 1969) was 98·5 %.

The protein content of each diet was determined to be 10 %.

Results of the feeding trials, available lysine and blood chemistry are shown in Tables 4 and 5.

The FDNB reactive lysine values were 5·84, 4·43 and 2·83 g/16 g N when 0, 5·2 and 10·4 % acetylated casein was incorporated into maize diets, respectively, while the value was 2·96 g/16 g N for a 10·4 % acetylated casein: 50 % cassava: 25 % maize meal diet (diet 5, Table 1).

The PER decreased with increased incorporation of acetylated casein in the diets. The highest PER of 2·21 was recorded for the casein–maize meal diet (diet 2); diet 3 (5·2 % acetylated casein: 70 % maize meal) had a PER of 2·13 while diet 4 (10·4 % acetylated casein: 70 % maize meal) had a PER of 1·60. The value (PER 2·06) obtained for 10·4 % acetylated casein: 50 % cassava: 25 % maize meal (diet 5) was considered high.

NPR values followed the pattern observed for the PER (Table 4).

The NPU values for the control (diet 2) and cassava based (diet 5) diets were both 67 and, for diets 3 and 4 were 61 and 42·6, respectively (Table 4). TD was high for all the diets (89·8–92·7 %) (Table 4).

Nitrogen content of the dry tissue was similar in all diets tested (120–126 mg N/g dry tissue).

TABLE 4
Results of Maize Meal and Cassava Acetylated Casein Diets Fed to Rats

Treatment	*PER*	*NPU*	*NPR*	*TD*	*Muscle nitrogen (mg N/g dry tissue)*	*Serum thiocyanate (μg/ml)*	*Serum urea (mg N/100 ml)*	*FDNB reactive lysine (g/16 g N)*
Diet 2	2·21 ± 0·34	67·6 ± 2·8	2·50 ± 0·33	92·1 ± 0·4	119·6 ± 3·4	1·5	27·4	5·84
Diet 3	2·13 ± 0·15	61 ± 1·0	2·48 ± 0·14	92·7 ± 1·0	125·7 ± 2·1	1·5	26·8	4·43
Diet 4	1·60 ± 0·10	42·6 ± 8·0	1·98 ± 0·11	92·5 ± 1·5	119·6 ± 5·1	1·4	33·3	2·83
Diet 5	2·06 ± 0·15	67 ± 5·0	2·45 ± 0·13	89·8 ± 0·7	125·8 ± 2·2	2·2	23·6	2·96

Serum thiocyanate was about 1·5 μg/ml for animals on diets 2–4 while those on the cassava diet (diet 5) had 2·2 μg/ml (Table 4).

In the corn starch based diets, the highest PER was recorded by control animals on diet 7 (PER 1·60) while animals on diets 8 and 9 (7·8% acetylated casein: 71% corn starch and 15·6% acetylated casein: 70% corn starch diets, respectively) had PER values of 1·28 and 0·91, respectively (Table 5). Animals on cassava based diet 10 (15·6% acetylated casein: 70% cassava) recorded a PER of 1·75 which was superior to the control PER of 1·60 (Table 5).

NPU and NPR followed the same pattern as the PER (Table 5). TD was very high for all diets, ranging from 98·6% for diet 9 to 99·7% for diet 7 (Table 5).

Table 6 shows the activity of the different urinary enzymes. Urine samples were collected on days 8 and 13 of the experiment.

GDH. The GDH activity ranged from 1·0 to 27·1 nmol/min/12 h excretion. The highest value, 27·1, was obtained in diet 8 (7·8% acetylated casein: 71% corn starch) on day 8 of the experiment.

LDH. The LDH activity was highest in the urine of diet 8 animals, with 33·3 nmol/min/12 h excretion followed by diets 9, 6 and 10 (5·2, 4·8 and 4·8 nmol/min/12 h excretion, respectively). The casein control diet (diet 7) had the lowest enzyme activity of 3·3 nmol/min/12 h excretion.

Alkaline phosphatase. The activity of this enzyme was highest in the urine of control animals on diet 7 (casein-corn starch diet, 57 nmol/min/12 h excretion) while the 7·8% acetylated casein: corn starch

TABLE 5

Results of Corn Starch–Cassava–Acetylated Casein Diets Fed to Rats

Treatment	*Feed intake (g)*	*Weight gain (g)*	*PER*	*NPU*	*NPR*	*TD*
Diet 7 (control)	102	16·3	1·60 ± 0·2	64 ± 5	2·0	99·7
Diet 8	98	12·5	1·28 ± 0·2	48 ± 6	1·7	99·3
Diet 9	91	8·3	0·91 ± 0·1	34 ± 7	1·4	98·6
Diet 10	102	17·8	1·75 ± 0·2	67 ± 5	2·2	99·4

TABLE 6
The Effect of Corn Starch and Cassava Acetylated Casein Diets Fed to Rats on Urinary Enzyme Activities (nmol/min/12 h excretion ± SD)

Treatment	*GDH*	*LDH*	*Alkaline phosphatase*	*Acid phosphatase*
Day 8 urine samples				
Diet 6	23·5 ± 5·0	4·8 ± 1·0	4·0 ± 2·2	4·5 ± 2·0
Diet 7	8·1 ± 2·0	3·3 ± 0·3	57 ± 12	4·0 ± 0·1
Diet 8	27·1 ± 4·0	33·3 ± 4·8	49 ± 24	3·6 ± 1·5
Diet 9	6·7 ± 1·5	5·2 ± 0·5	6·2 ± 0·8	4·4 ± 0·6
Diet 10	1·0 ± 0·2	4·8 ± 0·8	3·5 ± 1·0	0·6 ± 0·1
Day 13 urine samples				
Diet 1	1·9 ± 1·8	2·6 ± 1·2	7·8 ± 3·0	7·6 ± 0·4
Diet 2	1·2 ± 0·8	4·1 ± 2·6	31·8 ± 10·1	11·2 ± 4·8
Diet 3	16·7 ± 8·7	12·4 ± 4·0	19·7 ± 1·1	9·6 ± 4·2
Diet 4	19·5 ± 5·0	9·7 ± 1·0	25·3 ± 1·0	3·7 ± 0·4
Diet 5	6·7 ± 1·0	1·9 ± 0·9	12·6 ± 6·2	7·2 ± 2·0

diet (diet 8) had 49 nmol/min/12 h excretion. The other three diets (diets 6, 9 and 10) recorded low activities between 3·5 and 6·2 nmol/min/12 h excretion (Table 6).

Acid phosphatase. The activity was between 3·6 and 4·5 nmol/min/12 h excretion for all diets except diet 10 (15·6% acetylated casein:cassava) where the activity was just 0·6 nmol/min/12 h excretion.

The pattern of the activity of these enzymes did not vary much on day 13 of the experimental period. It was only the GDH activity which rose steeply in animals placed on diet 9 (15·6% acetylated casein:corn starch).

DISCUSSION

Acetylated casein was used as a model to study the effect of 'tied-up' lysine in the diet on the growth of rats. Many models have been used to study the resistant nature of the linkages of the ε-amino group of lysine to digestive enzymes and nutritive values. Thus Neuberger & Sanger (1943) reported that ε-N-acetyl-1-lysine appeared to promote growth in lysine-deficient rats both as an oral supplement and when given by intramuscular injection. Bjanarson & Carpenter (1969) fed ε-N-acetyl-lysine, ε-N-propionyl-lysine, acetyl-bovine plasma albumin (acetyl-BPA), formyl-lactalbumin and propionyl-lactalbumin to rats as models of reducing sugar–lysine reaction complexes. They found that acetyl-lysine supported growth to about 50 % of growth recorded for the control while propionyl-lysine did not support growth. Acetyl-BPA, formyl- and propionyl-lactalbumin supported growth to 67, 77 and 43 % of the control, respectively. Heated proteins and heated acetylated proteins supported growth to a lesser extent.

An attempt has been made to assess the nutritive value of diets containing acetylated casein and the possible effect of cassava on its utilization. With maize meal diets, the FDNB reactive lysine decreased with increasing acetylated casein content. The corresponding PER and NPU values also decreased (Table 4). This decrease in biological performance indicators is likely due to the reduction in the availability of the essential amino acid lysine. In diet 5 (10·4 % acetylated casein: 50 % cassava: 25 % maize) the FDNB reactive lysine value (2·96 g/16 g N) was very close to the 2·83 g/16 g N obtained for diet 4 (10·4 % acetylated casein: 70 % maize). The PER of diet 5 (2·06) was, however, higher than the PER of 1·60 obtained for diet 4. The NPU data followed the same trend.

The low serum urea level, which is a measure of protein quality (Eggum, 1971) corroborates results obtained using other biological performance indicators (PER, NPU, NPR) which shows that acetylated casein is well utilized when fed with 50 % cassava in the diet.

In the corn starch based diets the PER, NPU and NPR decreased with increasing levels of acetylated casein. Using NPU and NPR, that take into consideration the nitrogen used for maintenance, it is possible to calculate approximately the decrease in the nutritive value of casein caused by acetylation. In this experiment, 47 % of the nutritive value of casein was lost through acetylation using NPU data and 30 % using NPR (Table 5). It was again shown that cassava enhanced the utilization of acetylated casein.

The mechanism by which cassava increases the nutritive value of acetylated casein is unknown but may be connected with the fact that the mammalian kidney contains an enzyme, ε-lysine acylase, which is capable of hydrolysing the ε-N-formyl and ε-N-acetyl linkages of lysine (Paik and Benoiton, 1963; Leclerc and Benoiton, 1968). Corn starch based diets containing acetylated casein increased the excretion of GDH, LDH, and acid and alkaline phosphatases while cassava based diets containing acetylated casein decreased these to control levels and often below control levels (Table 6). These enzymes are used as indicators of kidney damage (Ngaha, 1982) and, in particular, increased membrane permeability. We therefore speculated that acetylated casein in corn starch based diets may be disrupting the kidney structure, increasing the permeability of the kidney membranes, thereby causing leakage of the enzymes. Enzymes leaked out may include ε-lysine acylase which hydrolyses acetyl-lysine. Cassava may be helping to decrease leakage.

In conclusion, it has been clearly demonstrated that 'tied-up' lysine not only causes a decrease in the nutritive value of the protein source but could also be harmful to the animal. In the presence of cassava this deleterious effect is decreased. The practical implication of these findings is that cassava may be a better carbohydrate source because the ε-amino group of lysine of the protein may be 'tied-up' due to storage or processing conditions.

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