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Original Research Article

In-Vivo Evaluation and Comparison of Ileal Digestibility of Indispensable Amino Acids (IAA) of Formulated High Protein Cookies in Normal and Uremic Rats

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ABSTRACT

Objective: The aim of this study is to formulate the high protein cookies incorporated with egg white powder and to determine the ileal digestibility of Indispensible amino acid for the formulated high protein cookies in uremic female wistar rats.

Methods: Female wistar rats of age 8-12 weeks were selected for the study. The rats were grouped as control and uremic group. Nephrotoxicity was induced in wistar rats by administration of gentamicin 80 mg/g/day intraperitoneally. The rats were fed with cookies for 14 day period. Each day each rat received its diet for 10 minutes period as nine meals given at hourly intervals (0800-1600 hour). On the 14th day, from 5-7 hours, the rats were asphyxiated with carbon dioxide and decapitated. The 20 cm of ileum immediately anterior to ileocaecal junction was dissected out and ileal contents were flushed using distilled water, freeze dried and subjected to chemical analysis.

Results: The formulated cookie and ileal digesta were estimated for indispensible amino acid using high performance liquid chromatography. It was found that the rate of absorption of phenylalanine and methionine were found to be higher in uremic rats compared to the control group. The other indispensible amino acids were poorly absorbed at the terminal ileum in uremic rats showing significant decrease in absorption.

Conclusion: From the study it is concluded that the protein absorption is better in uremic rats and hence it can be recommended for subject with kidney disease. Hence kidney disease subjects should consume adequate high biological value protein to compensate the protein loss.

Key words: High Protein Cookies, Uremia, Indispensible amino acid, Protein absorption.

INTRODUCTION

Uremia is a clinical condition associated with electrolyte, fluid and hormonal imbalances with metabolic abnormalities which develop in parallel with deterioration of kidney function. Malnutrition is the serious threat for subjects with kidney disease. Uremia is the foremost reason behind the defects in protein and amino acid metabolism leading to malnutrition. [1] (Muscaritoi *et al*, 2009). Altered protein metabolism can be identified by decreased body weight, lean body mass and growth, altered plasma and muscle metabolism, altered plasma and amino acid levels, decreased serum and muscle protein concentration, reduced body pools of albumin, altered pool size, exchange rates and rates of degradation for certain amino acids like phenyl alanine and valine and altered activities of many enzymes that mediate synthesis or

degradation of amino acids. Indispensible Amino Acids plays a very important role in protein synthesis. When dietary protein intake is diminished, both humans and rat metabolic mechanism is activated to reduce the oxidative degeneration of essential amino acids. [2] (Tawa *et al*, 1992). Various alterations of blood amino acids levels have been described in uremic subjects. [3] (Goodship *et al*, 1990).

Amino acids are absorbed by different transport mechanisms from the intestinal luman depending upon whether they are present either in free form or as peptides. [4] (Mathew and Adibi, 1976). The availability of amino acids is determined primarily by their digestibility measured at the end of the small intestine, i.e. at the terminal ileum. This method of determination has been established that there will be no further amino acid absorption at the larger intestine. Moreover, the undigested amino acid are metabolised by microflora preventing them from appearance from excreta. To obtain the exact digestibility of dietary protein, the concept of "ileal digestibility" was obtained. It was suggested that during uremic condition, proteins of high biological value should be advised inorder to reduce the uremic load. Studying of metabolism of indispensible amino acids in the uremia condition is of vital need to prescribe the dietary protein to the kidney disease subjects quantitatively and qualitatively. The same concept would be applied to determine the ileal digestibility of dietary indispensible amino acid in the uremic condition in the rat model. Out of all high biological value protein, egg protein has the highest biological value with all indispensible amino acids required for protein synthesis. The objective of this study is to formulate the high protein cookie with egg white powder and also to determine the digestibility of indispensible amino acids at the terminal ileum in the uremic rat model and compare with the normal rats.

METHODOLOGY

Formulation of the cookie and protein free feed

Among the bakery products, cookies were consumed extensively all over the world. These are important food product used as snacks by children and adults. [5] (Hussain *et al*, 2000). Cookies differ from other baked products like bread and cakes because of their low moisture content which ensures that they are free from microbial spoilage and confer a long shelf life on the product. [6] (Wade, 1988).

Egg white powder was purchased from SKM egg products ltd, Erode. The other baking ingredients were purchased from local market. The ratio of the ingredients used in the preparation of the cookies is presented in the Table 1. Both the egg white powder and high protein cookie with egg white powder is examined for the indispensible amino acid content using High Performance Thin Layer Chromatography (HPTLC).

Table 1: Composition of high protein cookie

Ingredients	Proportion (g/per 100 g)
Egg white powder*	19.7
Refined Wheat flour	32.39
Virgin Coconut oil	14.47
Vegetable fat	13.15
Sugar	19.7
Titanium oxide	0.5

*SKM egg products Ltd, Erode

Titanium oxide was included in each diet as indigestible marker. The flow chart of the cookies preparation is shown in the Figure 1. Protein free feed was prepared according to Moughan and Rutherford [7] and the composition of the feed is shown in the Table 2. Wheat starch was purchased from Borha Inc, Chennai, purified cellulose was obtained from Jugu-Orchem Pvt Ltd, Chennai and the vitamin mineral mix was purchased from Virbac Animal health care ltd, Mumbai.

Table 2: Composition of protein free feed

Ingredient	Composition (g/kg air dry weight)
	Protein free feed
Wheat starch#	755.7
Purified cellulose*	50
Sucrose	100
Vitamin and mineral mix**	39.3
Virgin coconut oil	50
Titanium Oxide	5

#Bohra Inc, Chennai*Jugu-orchemPvt Ltd. **Virbac Animal Healthcare Pvt Ltd,

Protein free feed was given for the estimation of endogenous flow of amino acids in the intestine and for the determination of exact digestibility of the test protein (Cookies incorporated with egg white powder).

Induction of Uremia

After obtaining the Institutional Animal Ethics Committee (IAEC) for animal trial, female wistar rats of approximately 200-240 g of body weight were housed in the individual cages and were maintained in the room temperature of 22±2˚C with 12h light and dark cycle. The grouping of the animals is shown in the Table 3.

Table 3: Grouping of study animals

GROUP A	GROUP B
N= 24	N=24

Group A was considered as control group and Group B as uremic group. Both the group animals were acclimatized with for the period of seven days. During the period of acclimatization, the animals were well fed and water was provided ad-libitum. After the period of acclimatization, the group B animals were injected with amino glycoside gentamicin with 80 mg/g/b wt (i.p) for the period of seven days. Group A animals were injected with distilled water simultaneously. On the seventh day, after body weight measurement, blood was collected at retro-orbital sinus and renal parameters like urea and creatinine were estimated for the evaluation of nephrotoxic effect gentamicin. Nephrotoxicity of gentamicin was further confirmed by histopathology of kidney and is shown in Figure 2.

Feeding regime

After induction of uremia, both the groups were further grouped into two groups to study the protein digestibility of the formulated high protein cookie. The grouping is shown in the Table 4

Table 4: Grouping of study animals according to test diet

NORMAL RATS (N=24)		UREMIC RATS (N=24)	
Protein free diet	Cookies	Protein free diet	Cookies
n-12	n-12	n-12	n-12

All the rats were acclimatized for five days and caged in individual cages. Water was available all the time. Twelve rats were fed with each diet in both the group. The rats were provided with the diets for 14-day period. Each day each rat received its diet as nine meals given at hourly intervals (0900 to 1700 hours). Each meal was freely available for a 10 minute period. The feed containers were weighed after each meal. Water was available at all times. On the fourteenth day of the study, from 5 to 7 hour after the start of feeding, each rat was asphyxiated with carbon dioxide gas and then decapitated. The 20 cm of the ileum immediately anterior to the ileo-cecal junction was dissected out. The dissected ileum was washed with distilled water to remove the blood and hair and was carefully dried on the tissue paper. The digesta were then gently flushed from the ileum section with distilled water from a syringe. The digesta from the rats fed the test diets were then freeze dried and subjected to chemical analysis. Amino acids present in the test diet and the digesta were estimated using HPLC system using the instrument Agilent 1100 series. Titanium oxide was estimated according to the procedure followed by Titgemeyer *et al*. [8]

RESULTS AND DISCUSSION

Proximate analysis of the test feed

The HPTLC report of indispensible amino acid content of both egg white powder and high protein cookie incorporated with egg white powder is shown in the Figure 1. The proximate analysis of the formulated cookie is presented in the Table 5.

Table 5: Proximate analysis of cookies

Nutrients (per 100 g)	Contents
Energy (Kcal)	125.3
Carbohydrate (g)	30.20
Protein (g)	10.03
Fat (g)	7

The protein free feed has the nutrients 10% sucrose, 5% virgin coconut oil, 1% vitamin mineral mixture and 5% of fiber. It is devoid of protein and the remainder was wheat starch up to 100%. The HPTLC analysis of indispensible amino acids in egg white powder and egg white powder cookies is shown in the Figure 1

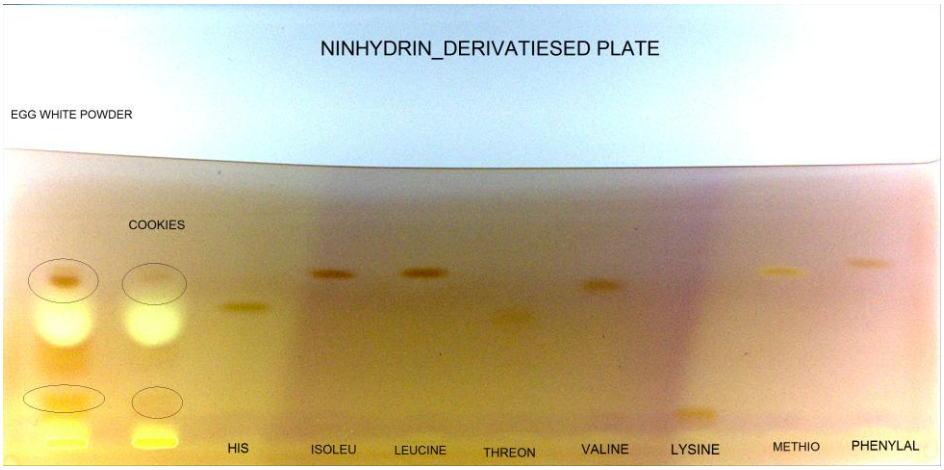


Figure 1: HPTLC analysis of egg white powder and high protein cookies with egg white powder

The amino acid composition of the cookies using HPLC is shown in the Table 6

Table 6: Amino acid content of cookies with egg white powder

Indispensible amino acid (IAA)	Egg white powder (µg/ 1 mg sample)	Cookies (µg/ 1 mg sample)
Histidine	0.033	0.015
Valine	0.008	0.172
Methionine	0.075	0.665
Isoleucine	0.185	0.145
Phenylalanine	0.009	0.007
Threonine	0.002	0.005
Leucine	0.723	0.989
Tryptophan	0.004	0.011
Lysine	0.374	0.007

Nephrotoxic effect of gentamicin on body weight and serum urea and creatinine

Injection of gentamicin intra peritoneally for seven days in the dose of 80 mg/kg/days daily to the rats caused nephrotoxicity compared to the normal rats and is manifested by significant ($p<0.05$) increases in serum and creatinine values. It was also found that there was significant ($p<0.05$) decrease in the feed intake and body weight of gentamicin induced rats compared to normal rats (as shown in Figure 2). The result of urea and creatinine is shown in the Table 7.

Table 7: Serum Urea, Creatinine and Body weight of the study animals before and after injection with gentamicin

Renal parameter	Group A (N=24)	Group B (N=24)
Urea (mg/dl)	41.42±2.6	73.23±2.1
Creatinine (mg/dl)	1.3±0.5	3.2±0.7

Mean±SD are significant at $p<0.05$ using one way ANOVA test.

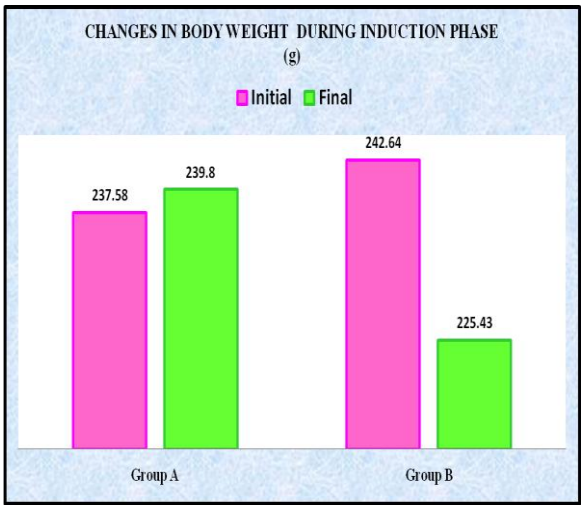
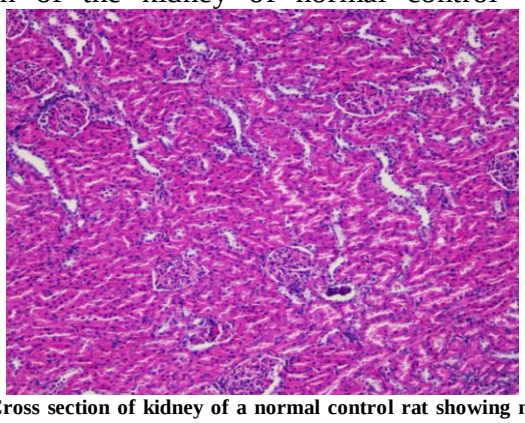


Figure 2: Body weight changes during induction period

Histological procedure

Using 10% neutral formalin solution, kidney specimens were cleaned. The specimens were then trimmed and dehydrated in ascending grades of alcohol and cleared with xylene. They were embedded in paraffin boxes, sectioned at 4-

6 microns thickness and stained with Hematoxylin and Eosin (H and E). Diagnostic terminology was done according to Peter *et al.* [9] Figure 3 shows the cross section of the kidney of normal control animals showing normal renal parenchyma (glomeruli and renal tubules) where as Figure 4 shows the cross section of kidney of gentamicin treated animals showing the marked necrosis of renal tubules.



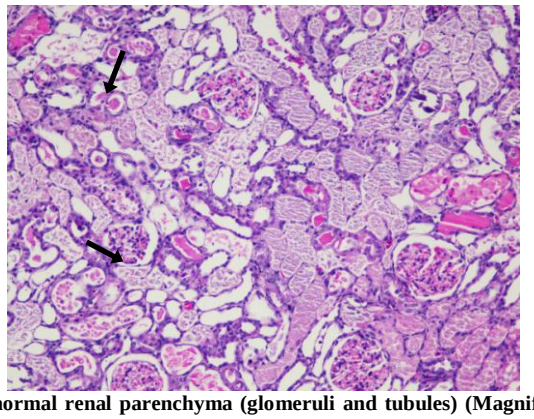


Fig 3- Cross section of kidney of a normal control rat showing normal renal parenchyma (glomeruli and tubules) (Magnification 10×)

Fig 4- Cross section of kidney of a gentamicin induced rat showing marked necrosis of renal tubules (Magnification 10×)

Amino acid content in the ileal digesta in normal and uremic rats

There was some difference observed in the amino acid composition of ileal digesta when normal and uremic rats were compared. The total feed intake and the composition of indispensible amino acids in the ileal digesta is shown in the Table 8

Table 8: Indispensible Amino Acid content in the ileal digesta

Indispensible Amino Acid (IAA) (µg/ 1 mg sample)	Normal control rats (N=24)		Uremic rats (N=24)	
	Protein free feed (n-12)	Cookies (n-12)	Protein free feed (n-12)	Cookies (n-12)
Threonine	0.029	0.706	0.121	0.650
Histidine	0.019	1.107	0.049	0.693
Valine	0.322	0.579	0.020	0.393
Methionine	0.010	0.473	0.012	0.720
Isoleucine	0.552	0.837	0.399	0.444
Tryptophan	0.041	0.744	0.035	2.371
Leucine	0.029	0.748	0.024	0.884
Phenylalanine	0.023	0.532	0.021	0.650

Indispensible amino acid (IAA) should be provided adequately for the normal protein synthesis. For past three decades many researchers suggest that subjects with kidney disease engender protein energy malnutrition. [10-12] Moreover, uremia is a catabolic state. [13,14] Catabolic state of the uremia can be evident by greater prevalence of malnutrition in the subjects with chronic kidney disease. It has been postulated that in renal failure, indispensible amino acids may be utilized more efficiently than protein and may decrease urea production. [15] It may further enhance the re-utilization of endogeneously formed urea and ammonia for synthesis of amino acids and protein. [16] Table 8 shows that the digestibility of indispensible amino acids after feeding the rats with cookies with egg white powder. It was found that the digestibility of methionine, tryptophan, leucine and phenylalanine was higher in uremic rats compared to normal control rats. But the absorption of other indispensible amino acids like threonine, histidine, valine and isoleucine was lower in uremic rats compared to normal rats.

The digestibility of the leucine was higher in the uremic rat model. This has the positive effect on protein synthesis after feeding the uremic rat with ample amount of good protein which prevents the protein degradation. Holliday *et al* [17]

found that after the infusion of ^{14}C leucine, in-vivo, after fasting, the decrement was higher in uremic rats. The digestibility of valine and isoleucine were lower in uremic rats. Alfred *et al*, [18] reported after feeding the rat with essential amino acid found that there were decreased plasma levels of isoleucine and valine. The lower digestibility of this particular amino acid may be the reason for decreased plasma levels.

The digestibility of phenylalanine was higher in uremic rats, suggesting that protein synthesis can be aided if the better amount of protein is provided to the uremic subjects as suggested by Baliga *et al*. [19]

Serum urea and creatinine values of study animals fed with cookies

From the study it was evident that there was significant decrease in the serum urea and creatinine levels of animals fed on cookies with egg white powder. The values of the urea and creatinine are shown in the Table 9

Table 9: Serum Urea, Creatinine and Body weight of the study animals before and after injection with gentamicin

Renal parameter	Normal control rats (n-24)	Uremic rats (n-24)
Urea (mg/dl)	39.2±1.2	40.21±2.3
Creatinine (mg/dl)	0.9±0.2	1.5±0.6

Mean±SD are significant at $p<0.05$ using one way ANOVA test.

CONCLUSION

This study evaluated the high protein cookies with egg white powder on ileal digestibility of indispensible amino acids in both normal and uremic rat model. This study is the new attempt to study the dietary protein quality evaluation in terms of ileal digestibility of indispensible amino acid digestibility rather than the previous method of evaluation of protein quality on the whole. From the results obtained, there are variations in the indispensible amino acid digestibility between normal control rats and uremic rats which is not clearly understood. It may be due to metabolic acidosis, the etiology of the uremia or may be due to poor intake by the uremic animals etc. On comparison, the protein absorption was better in uremic rats on basis of decreasing the uremic load. Hence the protein with high biological value can be promoted to the subjects with kidney disease.

The idea behind to carry out this study was to find the dietary protein metabolism in uremic condition because during uremia is the condition where dietary protein plays a vital role. This study may help in the dietary management of uremic subjects on the type of protein to be prescribed to the subjects with kidney disease qualitatively and qualitatively.

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