S–carboxyethylcysteine (a constituent of *Acacia* seed) negatively affects Casein Protein Utilization by Rats

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ABSTRACT

Objective: Two rat bioassay experiments are reported. The first investigated the first limiting amino acid in *Acacia colei* and the second experiment investigated the effect of S-carboxyethylcysteine (CEC) (a compound present in *Acacia* seed) on protein utilization.

Methods: In the first experiment, Wistar strain rats were fed *Acacia colei* seed supplemented with three levels of methionine, cysteine and tryptophan (0.1, 0.2 and 0.4 %) while the second fed Wistar strain rats with CEC incorporated casein diets.

Results: Supplementation of *A. colei* with tryptophan had no significant effect on protein efficiency ratio (PER), cysteine recorded the highest PER value at 0.4 % level while PER increased significantly with increase in methionine content making methionine the first limiting amino acid. The methionine-induced growth rate was suppressed by the incorporation of CEC, which also had a negative effect on plasma amino acid levels.

Conclusion: The results indicated that methionine is the first limiting amino acid in *A. colei* and that CEC could affect the seed's protein utilization. *A. colei* seed could only be used effectively as famine food if it is complemented with other cereals known to be rich in sulphur amino acids.

Keywords: Acacia colei; Amino acid interactions; Limiting amino acid;

Protein utilization; S-carboxyethylcysteine

Introduction

Hunger, famine, malnutrition and their attendant diseases are major health concern in the semi arid Sahelian region of West Africa where rainfall is below 600 mm per annum [1] and crop failure is a common occurrence. In a normal year that is without drought, 150 million Africans are usually malnourished and go hungry [2].

To prevent famine, most especially in the arid regions where crop failure is common, it is imperative to introduce novel foods which can be obtained from drought resistant crops. Certain species of *Acacia* are potential drought resistant crops, which have been reported to thrive very well in dry zones of Africa in years where other crops such as millet, corn and sorghum have failed [3]. Harwood *et al.* [3], for example, reported rapid growth and heavy seed production of *Acacia* in several Sahelian countries, and drew attention to their potential as a human food in this region, given the documented consumption of their seeds in the traditional diets of Australian Aboriginal people.

Studies in our laboratory revealed *A. colei* and *A. tumida* to be rich in unsaturated fatty acids, vitamin and protein with tryptophan as the first and methionine as the second limiting amino acids using the digestibility corrected amino acid score procedure [4, 5]. In a preliminary feeding trial with rats using *A. colei* as the sole source of dietary protein, it was observed that the animals lost weight but this weight loss was alleviated by methionine supplementation. In addition, loss of hair was also observed in rats fed acacia diet without methionine supplementation. This observation seemed to suggest that the sulphur amino acids were in short supply or their metabolism *in vivo* was hampered thus making them the first limiting amino acids *in vivo* instead of tryptophan, which was found to be first limiting *in vitro*. Cysteine has been reported to be the major amino acid in hair keratin [6, 7] underscoring the importance of cysteine in hair formation. Cysteine is also used for the

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biosynthesis of glutathione - a major intracellular antioxidant [8]. Tryptophan is a precursor for serotonin, a brain neurotransmitter theorized to suppress pain [9].

Supplementation of plant foods with amino acids has been reported to improve protein utilization of plant proteins by man and animals [10, 11]. It has also been shown that the addition of dietary cysteine reduced the methionine requirement by approximately 40 % in both enterally and parenterally fed neonatal piglets [12] while methionine has been reported to spare cysteine in the infants supplied a generous amount of dietary methionine [13]. Polat [14] reported significant increase in the weight of tilapia fish fed soy bean meal (SBM) supplemented with 0.5 % methionine but supplementations above this level of methionine were not significantly different from the control diet. Muraia *et al.*, [10] reported that fingerling Carp fed SMB supplemented with essential amino acids (EAA) alone without methionine could not improve growth, protein deposition and feed efficiency, but when methionine was incorporated into the EAA – SMB diet, these parameters were significantly improved. Sarwar and Botting [15] observed from their study on rats fed infant formulas supplemented with graded levels of L – tryptophan (0.1, 0.5 and 1.0 %) that this amino acid had no effect on protein quality indices, based on rat growth but resulted in a dose – related increase in the concentrations of tryptophan in the plasma.

Harwood [16] reported the presence of S-carboxyethylcysteine, a non-protein (modified) sulphur amino acid at 4.45 mg / g level in *A. colei* seed. The metabolic function of S-carboxyethylcysteine (CEC) was not known but it (CEC) is structurally similar to the sulphur amino acids (Figures 1 a, b and c) hence the speculation that it may affect the metabolism of the latter. The present study is therefore an attempt to provide evidence to support or negate this speculation.

This study was therefore designed to investigate the discrepancy between the first limiting amino acid found *in vitro* (tryptophan) and *in vivo* (methionine). Since CEC,

present in *A. colei,* has been speculated to affect the metabolism of sulphur amino acids, the second experiment was also designed to investigate the effect of CEC on protein utilization using casein as a model to which CEC was incorporated at levels similar to what was obtained in *A. colei.* This would provide information on the likely effect of CEC in *A. colei* seed on the protein metabolism of people fed acacia-incorporated diets.

Materials and methods

Acacia colei seed, harvested from plants growing around Maradi, Niger Republic, was provided Mr. Tony Rinaudo, Director of Maradi Integrated Development Project (MIDP) of the Serving-In-Mission (SIM) International as a 100 kg batch. The seed was cleaned by removing stones, fragments of wood and resinous matter by hand and then milled in batches in a local mill. The milled *A. colei* seed was sieved (0.6 mm aperture size sieve) to remove the coarser fragments of the seed coat. The milled seed was then stored in plastic containers inside the refrigerator and used within three days of storage.

Casein, S-carboxyethylcysteine (CEC) and the amino acids - methionine, tryptophan and cysteine - were purchased from Sigma Chemical Co., St. Louis, Mo, USA.

Care of the animals and bioassay

The studies were conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving animals were approved by the Ethics Committee of the College of Health Sciences of the Obafemi Awolowo University, Ile – Ife, Osun State, Nigeria. The care of the animals was as described earlier [17] and followed the guideline of the good laboratory practice (GLP) of the Organization for Economic Cooperation and Development (OECD). The rats were housed individually in metabolic

cages (North Kent Plastic Cages Ltd, Home Gardens, Dartford, Kent, England) and acclimatized for four days.

Experiment 1

Supplementation of A. colei with different levels of tryptophan, methionine and cysteine

Different levels (0.1, 0.2 and 0.4 %) of the first three limiting amino acids in our earlier *in vitro* studies [4] - tryptophan, methionine and cysteine - were incorporated into *A. colei* based rations. The *Acacia* basal diet was prepared by mixing 47.6 % *A. colei*, 10.0 % sugar, 2.8 % vegetable oil, 0.125 % vitamin / mineral mix (Daram Vita–Mix, Abeokuta, Nigeria) and corn starch was added to make 100 %. Each of the three amino acids at 0.1, 0.2 and 0.4 % levels was used separately to supplement the basal diet at the expense of corn starch, making a total of ten treatment diets. Each diet was iso-nitrogenous (10 % protein) with *A. colei* providing the whole protein apart from the added amino acids.

Six weanling rats of the Wistar strain with weights in the range 40 ± 2 g were used per treatment group (total of 10 groups). The animals were fed with each treatment diet *ad libitum* for 40 days, and were examined every day for signs of morbidity and / or mortality.

Feed intake was measured by the weigh-back technique in which the remnant feed for each rat was weighed daily and subtracted from the 10 g feed offered. All the weights were on dry matter basis. The animals were weighed twice each week and also at the beginning and the end of the experimental period. Weight gain was determined by difference and protein efficiency ratio (PER) was calculated as the weight gained by the individual rat divided by the protein intake.

Experiment 2

Effect of s-carboxyethylcysteine (CEC) on protein utilization by rats.

The basal casein diet used consisted of 13.7 % casein (crude protein content of 73 %), 20 % sugar, 5.0 % dietary fibre, 8.0 % vegetable oil and 0.125 % vitamin / mineral mix, with corn starch to make 100 %. Methionine at 0.1 and 0.2 % was added separately to two basal diets at the expense of corn starch to give casein + 0.1 % methionine and casein + 0.2 % methionine diets respectively. CEC (0.1 %) was added at the expense of corn starch to a diet containing the basal diet plus 0.1 % methionine for comparison with the treatment without CEC at that level. Three different levels of CEC (0.2, 0.4 and 0.8 %) were also added to the "basal + 0.2 % methionine" diet to provide three additional casein + 0.2 % methionine diets. Thus, there were seven diets as follows: (1) basal casein diet; (2) casein diet + 0.1 % methionine; (3) casein diet + 0.1 % methionine + 0.1 % CEC; (4) casein diet + 0.2 % methionine; (5) casein diet + 0.2 % methionine + 0.2 % CEC; (6) casein diet + 0.2 % methionine + 0.4 % CEC; and (7) casein diet + 0.2 % methionine + 0.8 % CEC. All diets were iso-nitrogenous (10 % crude protein) with casein providing the whole protein, apart from the methionine added.

Six weanling rats of the Wistar strain with weights in the range 40 ± 3 g were used per treatment group in this experiment. The animals were fed each treatment diet *ad libitum* for 70 days. The animals had access to water throughout the experimental period.

Free plasma amino acids

Blood samples were collected at the end of each experiment by cardiac puncture into test-tubes containing 1.0 ml of 3.8 % (w/v) sodium citrate as the anticoagulant. Plasma was collected by centrifuging at 12,000 rpm for 10 minutes and deproteinized with an equal volume of 10 % (w/v) trichloroacetic acid, heated at 100° C for 10 minutes and centrifuged

again at 12,000 rpm for 10 minutes. The supernatants containing the free amino acids were collected and tryptophan, methionine and cysteine were determined colorimetrically as described earlier [18, 19, and 20].

Urea determination

Urea level in the plasma was determined by the Urea-Berthelot colorimetric method using Randox Urea Kit (cat No. UR 1068, Randox Laboratories, San Diego, USA).

Statistical methods

The results were expressed as mean and standard deviation (X ± SD) of six determinations. Data were subjected to one way analysis of variance to determine the levels of significant difference by performing a multiple comparison post test (Tukey), with differences considered significant at $p \leq 0.05$. Correlation coefficients (Pearson r), which assumed a Gaussian distribution, were determined between weight gain and each of the plasma amino acids and also between PER and plasma urea level, using GraphPad InStat version 3.06 for Windows 2003 and were considered significant at $p \leq 0.05$.

Results

Morbidity and physical observations of rats fed A. colei diets supplemented with selected amino acids

All the animals survived until the end of the experimental period although some animals on the *Acacia* control, 0.1 % and 0.2 % (w / w) tryptophan supplemented *A. colei* diets exhibited signs of weakness. On the other hand, all the animals fed additional methionine; most especially those on 0.4 % (w / w) methionine supplementation appeared active and very healthy.

Supplementation of *A. colei* diet with 0.1 % (w / w) cysteine had a significant effect on both feed intake and weight gain (Table 1; Figure 2). There was a significant difference in feed consumption of rats fed 0.1 and 0.2 % cysteine supplemented diets but no difference in their weight gain. Increasing the level of supplementation to 0.4 % (w / w) cysteine did not significantly increase feed intake from that of 0.2 % (w / w) (Table 1) but significantly increased weight gain (Figure 2). The addition of methionine to the *A. colei* based diet increased both feed intake and weight gain in a dose dependent fashion. The increase in feed intake and weight gain produced by the addition of 0.1 % methionine to the *Acacia* diet (Table 1; Figure 3) was equivalent to that produced by *A. colei* supplemented with 0.4 % (w / w) cysteine (Table 1; Figure 2). Animals fed *A. colei* alone lost weight (Table 1) while supplementation of *A. colei* by 0.4 % (w / w) cysteine resulted in a weight gain (Figure 2) comparable to that of 0.1 % (w / w) methionine supplementation (Figure 3). The animals fed the three levels of tryptophan-supplemented *A. colei* based diets initially lost weight (Figure 4). Rats on 0.1 % (w / w) tryptophan - *A. colei* diet lost weight at the end of the experimental period just like in the control while supplementation by higher levels of tryptophan increased weight gain significantly ($p < 0.05$) (Table 1, Figure 4).

Supplementation of the *A. colei* diet by the different levels of cysteine led to significant ($p < 0.05$) improvement in PER but there was a non significant ($p < 0.05$) drop in the value obtained for this parameter at 0.2 % (w/w) cysteine supplementation. Supplementation by tryptophan had no significant effect on PER at all levels. Indeed, negative PER was recorded for 0.1 % (w / w) tryptophan - *A. colei* fed animals as well as those rats fed the *A. colei* control diet. PER obtained for animals fed 0.4 % cysteine supplementation and 0.2 % methionine diet were similar while 0.4 % (w / w) methionine - *A. colei* diet recorded the highest PER value of 2.0.

Plasma amino acid concentration of rats fed amino acids supplemented diets

The free plasma amino acid content of rats fed *A. colei* diets supplemented with the various levels of amino acids is presented in Table 2. The free plasma methionine content increased by 28 % over the *A. colei* control with 0.1 % (w / w) cysteine supplementation. However, 0.2 % cysteine supplementation depressed the plasma level of methionine to the basal diet level while 0.4 % cysteine supplementation reduced it further by 54 %. The free plasma content of tryptophan also increased by 223 % over the control when 0.1 % (w / w) cysteine was added to the *A. colei* based diet but further increase in the level of cysteine supplementation elicited only a decrease in the free plasma tryptophan content by 37 % and 77 %, respectively. Free plasma cysteine content was high in *A. colei* (basal) diet but decreased by 35 % and 63 % when the basal diet was supplemented by 0.1 % and 0.2 % (w / w) cysteine, respectively.

Synergetic interaction was also observed among amino acids when *A. colei* seed was supplemented separately with methionine and tryptophan. For example, supplementation of *A. colei* diet with 0.1 % (w / w) methionine resulted in a significantly lower free plasma methionine content compared with the control *A. colei* diet. The highest concentration of free plasma methionine was obtained in the plasma of animals fed 0.2 % (w / w) methionine-supplemented *A. colei* with a decrease at 0.4 % (w / w) methionine supplementation. Supplementation of the basal *A. colei* diet with 0.1 % (w / w) methionine reduced the free plasma cysteine content by 62 % but the level was restored to close to that of the basal diet when the methionine supplementation was doubled to 0.2 %; however plasma cysteine concentration decreased significantly in animals fed 0.4 % (w / w) methionine supplemented *A. colei* diet (Table 2). Supplementation of *A. colei* based diet by 0.1 % (w / w) tryptophan decreased the free plasma methionine concentration by 26 % but

thereafter increased the content (of the free plasma methionine) by 33 % and 52 % in animals fed 0.2 % and 0.4 % (w / w) tryptophan-supplemented *A. colei* diets, respectively.

Plasma urea level of rats fed Acacia diets supplemented with some selected amino acids

The highest plasma urea level was recorded for animals fed 0.2 % (w / w) cysteine / *Acacia* diet and this was 200 % higher than the control – *Acacia* fed rats (Table 1). There was, however, a progressive decrease in the plasma urea level with increase in the level of methionine supplementation and a low negative but significant correlation (r = -0.4 at p < 0.05) between PER and plasma urea.

Results of Experiment 2: Morbidity and physical observations of Rats fed CEC supplemented casein diets

Morbidity such as eye infection was noticed 14 days into the experiment in one rat fed 0.2 % (w / w) methionine plus 0.4 % (w / w) CEC supplemented casein diet and also in two rats fed 0.2 % (w / w) methionine plus 0.8 % (w / w) CEC supplemented casein diet. Hair loss was noticed as from day 28 on one rat each fed the casein control diet and 0.2 % (w / w) methionine plus 0.4 % (w / w) CEC supplemented casein diet and on two rats fed 0.2 % (w / w) methionine plus 0.8 % (w / w) CEC supplemented casein diet. The extent of hair loss was estimated qualitatively by visual observation. All the animals, however, survived until the end of the experiment.

Supplementation of casein diet with methionine increased feed intake and weight gain progressively as observed in Experiment 1 for the *Acacia*-fed rats (Table 3 and Figure 5). The addition of CEC did not alter feed intake at 0.1 % but reduced it at higher CEC concentrations (0.2 – 0.8 %) though not in a dose-dependent manner (Table 3). As indicated in Figure 5, the incorporation of CEC inhibited the growth promoted by the

addition of methionine to below the casein control level. For instance, the inclusion of CEC at 0.1 % in 0.1 % methionine supplemented casein diet reduced weight gain and PER by up to 62 % with reference to the casein control while the inclusion of CEC at 0.2 % and 0.4 % to 0.2 % methionine supplemented casein diets reduced weight gain and PER by up to 78 % and 97 %, respectively (Table 3). Although the inclusion of 0.8 % CEC in 0.2 % methionine-supplemented casein diet significantly ($p < 0.05$) reduced PER to the value lower than that of 0.2 % methionine supplemented casein diet, the PER value of the rats fed this 0.8 % CEC incorporated diet was significantly higher than that of the 0.4 % CEC-incorporated, 0.2 % methionine-supplemented casein diet.

Plasma amino acid concentration of rats fed CEC supplemented casein diets

Free plasma methionine content was increased by 30 % over the casein control value in rats fed 0.1 % (w / w) methionine - casein diet while the content was reduced by 64 % when the level of methionine supplementation was increased from 0.1 % to 0.2 % (Table 4). Inclusion of 0.1 % (w / w) CEC in a casein diet supplemented by 0.1 % (w / w) methionine resulted in a 56 % reduction in the free plasma methionine content. Addition of CEC from 0.2 to 0.8 % did not significantly affect the free plasma methionine content of rats fed these casein based diets. Supplementation of casein diet with 0.1 % methionine led to a significant increase in the free plasma tryptophan compared with the casein control but the level was significantly lower with 0.2 % methionine supplementation. Inclusion of CEC also did not significantly affect the free tryptophan level in the plasma except for a reduction of 50 % at 0.4 % CEC supplementation level compared with casein control.

Supplementation of casein diets by methionine significantly increased the level of free cysteine in the plasma of the experimental rats compared with the animals on the casein control diet. CEC incorporation at 0.2 % reduced the free cysteine concentration in

the plasma by 23 % when compared with casein + 0.2 % methionine diet but CEC increased the free cysteine content at higher levels of inclusion. A strong correlation was recorded between weight gain and free plasma methionine and also between weight gain and free plasma tryptophan (r = 0.70 and 0.65 at p < 0.05, respectively) whereas there was no significant correlation between weight gain and plasma cysteine (r = -0.3; p > 0.05).

Plasma urea level of rats fed CEC supplemented casein diets

Plasma urea (Table 3) varied between 64 and 126 mg / dL for casein + 0.1 % methionine and casein + 0.2 % methionine + 0.8 % CEC diets respectively. Plasma urea was observed to increase in a dose dependent manner as CEC incorporation increased from 0.2 to 0.8 % with a strong negative correlation (r = - 0.78 at P < 0.05) between PER and the plasma urea level.

Discussion

Morbidity and physical observations of rats fed A. colei diets supplemented with some amino acids

The level of methionine in *Acacia* seed is 3.2 g / 100 g protein while that of cysteine is 2.9 g / 100 g protein, as shown in Table 5. Since cysteine is well known to be an important component of hair [6, 7, 21], it is expected that supplementation of *Acacia* diet with cysteine would prevent loss of hair but this was not the case, probably due to the presence of CEC, which could act as a competitive inhibitor to cysteine and / or methionine metabolism *in vivo*. None of the rats on 0.4 % (w / w) cysteine-*Acacia* diet exhibited loss of hair, probably because at this level excess cysteine in the diet was able to override the competitive inhibition by endogenous CEC in the *Acacia* seed.

The increase in feed intake with increase in methionine incorporation is in agreement with the study of Bateman *et al.,* [22] who reported an increase in feed consumption with increase in the levels of methionine in the diets of laying hen. When feed intake was calculated in gram per gram body weight (result not shown), there was no difference in feed consumption per treatment groups with the exception of rats on *A. colei* + 0.1 % (w / w) cysteine. This implied that the increase in feed intake was in consonance with increased weight gain.

Our earlier study [4] indicated that tryptophan was the first limiting amino acid but the present *in vivo* study again confirmed the sulphur amino acids as the first limiting. This discrepancy can be resolved if the S-carboxyethylcysteine component of the *Acacia* seed inhibited methionine absorption or incorporation during protein synthesis thus making the sulphur amino acids the first limiting. The result presented in this study suggested that this is indeed the case.

The loss of weight in the *Acacia* control and 0.1 % (w / w) tryptophan – *A. colei* diets could be due to amino acid imbalance and / or toxicity [23, 24]. The presence of CEC

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in *Acacia colei* seed [16] could inhibit methionine metabolism *in vivo* thus creating the amino acid imbalance. Sarwar and Botting [15] had earlier observed that the supplementation of infant formulas with L – tryptophan (0.1, 0.5 and 1.0 %) did not have any effect on rats' growth. From the nutritional viewpoint, the level of urinary urea is an indicator of protein quality. Deamination of amino acids leading to the formation of urea occurs when some amino acids are in excess of demand. Therefore, if *A. colei* is not first limiting in tryptophan, the diet's supplementation with tryptophan will not translate to growth, instead, it will result in amino acid imbalance and hence deamination. Plasma urea levels from the rats fed diets supplemented with tryptophan were higher than those animals fed the *A. colei* control ration, supporting this interpretation. The decrease in urea level with increase in methionine supplementation of *A. colei* diets seems to confirm that methionine is indeed the first limiting amino acid in *A. colei*. The loss in weight observed in *Acacia* control and 0.1 % (w / w) tryptophan – *A. colei* diets could be due to insufficiency in their methionine content especially when the metabolism of the methionine content of *A. colei* had been compromised by CEC. Methionine supplementation of *A. colei* diets resulted in the highest weight gain and PER values followed by cysteine-supplemented diets while the lowest values were for tryptophan-supplemented diets. If the first two are compared, rats fed *A. colei* diet supplemented with 0.2 % (w / w) methionine gained approximately twice the weight recorded for animals on 0.4 % (w / w) cysteine – *A. colei* diet while rats fed the 0.4 % (w / w) methionine-supplemented *A. colei* diet recorded three times the weight gain of rats fed the 0.4 (w / w) cysteine supplemented *A. colei* diet. Based on these findings, it appears safe to conclude that the first limiting amino acid *in vivo* is methionine, followed by cysteine while tryptophan may be the next.

Plasma amino acid concentration of rats fed amino acids supplemented A. colei diets

Interaction among amino acids as well as their metabolism is generally complex and difficult to explain; for instance, Ventrucci *et al*. [25] observed an increase in the rate of methionine absorption when pig diet was supplemented with leucine. An excess of dietary leucine on the other hand was observed to reduce the concentration of valine and isoleucine in the plasma of rat [26] and pig [27]. Wu [28] reported studies on the catabolism of amino acids by the small intestinal mucosa and observed that 30 to 50 % of most amino acids studied including methionine were not available to extra-intestinal tissues.

The complexity observed in the amino acid transport and regulation [29] may account for the irregular patterns of amino acid interaction observed for the plasma amino acids in this study (Tables 2 and 4). Cysteine utilization has been observed to be regulated by its conversion into glutathione if in excess [8]. It is also well known that in adults, adequate cysteine is synthesized from methionine via the trans-sulphuration pathway [30]. This could probably account for the increase in the plasma cysteine with increase in methionine supplementation in both experiments. Numerous studies have also shown that dietary cysteine can replace part of the methionine requirement in orally fed animals. Estimates of this cysteine sparing effect on methionine ranged from 40 – 70 % in growing pigs, 50 – 55 % in chicks, 17 – 90 % in humans and 50 – 65 % in rats [12]. Dietary cysteine has also been demonstrated to affect other metabolic pathways such as the control of the expression of 3-phosphoglycerate dehydrogenase in rat liver [31]. The plasma concentration of some amino acids in this study could be explained in part on the basis of their utilization. For instance, the plasma cysteine concentration decreased significantly with increase from 0 to 0.2 % (w/w) cysteine – *A. colei* supplementation indicating its increased utilization which was translated to improved growth rate. The 0.2 % (w/w) cysteine would therefore seem to

be the optimum supplementation level in *A. colei* diet since the plasma cysteine level increased dramatically in animals fed the 0.4 % (w/w) cysteine-supplemented *A. colei* diet.

The 62 % reduction in the free plasma cysteine content when the basal diet (*A. colei* alone) was supplemented with 0.1 % (w / w) methionine can be attributed to increased incorporation of cysteine into protein with corresponding increase in body weight. This is in agreement with the findings of Shoveller *et al.,* [12] who observed a decrease in the concentration of some amino acids including cysteine with increase in methionine intake.

Plasma urea level of rats fed amino acids supplemented A. colei diets

The progressive decrease in urea level with the increase in the amount of methionine supplementation in *A. colei* diets futher supported the interpretation that methionine is the first limiting amino acid and its limitation could cause the deamination of other amino acids that are in excess [23, 24].

Morbidity and physical observations of rats fed CEC supplemented casein diets

The CEC-supplemented casein experiment was designed to test the effect of S-carboxyethylcysteine on the nutritional status of rats. CEC was incorporated into the casein model diet at a concentration that would mimic the range found in *A. colei* seeds. Thus, the 0.4 % CEC incorporation corresponded to the 4.45 mg / g level of *Acacia* seed [16].

Methionine has been reported by many authors to be the limiting amino acid in casein, and methionine supplementation in casein diet for rats increases its biological value substantially [32]. The result obtained in our study corroborated this earlier observation.

The sluggish movement and eye infection of the rats on 0.2 and 0.4 % CEC incorporated casein diets could indicate some chronic toxicity of S-carboxyethylcysteine, despite the fact that all the animals survived the experiment. CEC probably inhibited

methionine uptake and metabolism (Table 4). Shortage of methionine may then affect the cysteine level leading to hair loss observed in animals fed *A. colei* diets. This could also account for the decrease in the growth rate of rats fed CEC in methionine-supplemented casein diets.

The reversal of the initial increase in PER value achieved through methionine supplementation to the casein diets by CEC (Table 3) further underscored the negative effect of this compound on protein utilization. The reason for the significantly higher ($p < 0.05$) PER value observed for 0.8 % CEC incorporated 0.2 % methionine supplemented casein diet compared with 0.4 % CEC incorporation is not known.

Plasma amino acid concentration of rats fed CEC-supplemented casein diets

The 30 % increase in free plasma methionine content over the casein control value in rats fed the 0.1 % (w / w) methionine - casein diet cannot be easily explained even though the weight gain of rats on this diet was significantly higher than the control. The 64 % reduction in plasma methionine observed when the level of methionine supplementation of casein was increased from 0.1 to 0.2 % confirmed that casein is limited in methionine and its supplementation with methionine at 0.2 % level elicited further protein utilization. The observed 23 % reduction in the free cysteine concentration in the plasma of rats fed the 0.2 % CEC-incorporated casein + 0.2 % methionine diet compared with the casein + 0.2 % methionine diet could be explained on the basis of CEC's probable inhibition of methionine uptake resulting in reduced concentration of this amino acid. The reduction in methionine concentration would in turn reduce cysteine as a result of lower conversion of methionine to cysteine, since the reduction was not translated to weight gain but a 78 % reduction. The effect of CEC on the free plasma amino acid concentration observed may explain the decrease in the weight gain of rats as CEC incorporation increased. The plasma

concentration of methionine and tryptophan, but not cysteine, generally decreased with reference to the casein control diet as the concentration of CEC increased (Table 4). This decrease would no doubt negatively affect the *in vivo* protein synthesis, hence the decrease in the weight gain observed with the corresponding increase in CEC concentration. Apart from the negative effect of CEC on amino acid uptake, CEC could also inhibit protein synthesis due to its structural similarity to methionine and cysteine (Figures 1 a, b and c, respectively). The CEC content of *Acacia* could also explain the decrease in weight gain observed in an earlier nutritional study of three levels of millet / *Acacia* diets when *Acacia* level in millet / *Acacia* diet was increased from 20 to 40 % (Adewusi *et al.* unpublished data).

Plasma urea level of rats fed CEC supplemented casein diets

Plasma urea level is a good indicator of the protein quality of feedstuff as observed earlier [33, 34]. The result presented in Table 3 indicated a significant increase in the plasma urea level with CEC incorporation. From the nutritional point of view, the level of urea seemed to indicate a substantial deamination of amino acids when they are in excess in the plasma due to the presence of factor(s) such as CEC that could interfere with their utilization in protein synthesis.

Conclusion

The result of the first experiment indicated that methionine-incorporation in *A. colei* based diet improved the protein utilization of the seed in a dose dependent fashion, confirming it to be the first limiting amino acid in the seed. The result of the experiment also revealed cysteine to be the second limiting followed by tryptophan. The second experiment revealed that the presence of CEC in *A. colei* seeds could reduce feed intake of

rats and could also limit protein utilization, probably by inhibiting methionine and / or cysteine uptake and utilization in protein biosynthesis.

Acacia colei seed could therefore be optimally used in human diets if it is complemented with cereals and other plant protein sources that are known to be rich in sulphur amino acids such as acha (*Digitaria exilis* and *Digitaria fonio*) [35]. These cereals have previously been reported to give the best complementation to *A. colei* seed in rat feeding studies [36].

Acknowledgments

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Table 1: Feed intake[a], Weight gain[b], Protein Efficiency Ratio (PER) and plasma urea of rats fed amino acids (tryptophan, methionine and cysteine) supplemented *A. colei*[c] diets (dry weight basis).

Diet	Feed intake (g)	Weight gain / loss (g)	PER	plasma urea (mg / dL)
A. colei alone	91 ± 2.7^{d}	-2.1 ± 1.5^{f}	-0.3 ± 0.1^{c}	7.0 ± 2.3^{c}
A. colei + 0.1 % Cysteine	88 ± 26.9^{d}	5 ± 2.4^{d}	0.9 ± 0.5^{b}	4.8 ± 0.3^{f}
A. colei + 0.2 % Cysteine	134 ± 25.3^{b}	8 ± 2.1^{de}	0.4 ± 1.0^{bc}	14.0 ± 0.8^{a}
A. colei + 0.4 % Cysteine	146 ± 11.8^{b}	17 ± 5.4^{c}	1.2 ± 1.0^{ab}	5.6 ± 0.6^{e}
A. colei + 0.1 % methionine	164 ± 27.2^{b}	16.3 ± 0.3^{c}	1.0 ± 0.3^{b}	8.2 ± 0.7^{c}
A. colei + 0.2 % methionine	218 ± 19.5^{a}	32 ± 4.1^{b}	1.5 ± 0.3^{ab}	7.2 ± 0.3^{c}
A. colei + 0.4 % methionine	252 ± 23.2^{a}	49 ± 4.0^{a}	2.0 ± 0.2^{a}	6.5 ± 0.1^{d}
A. colei + 0.1 % tryptophan	107 ± 3.1^{c}	-2.5 ± 1.0^{f}	-0.2 ± 0.3^{c}	11 ± 1.9^{b}
A. colei + 0.2 % tryptophan	124 ± 25.4^{c}	0.9 ± 0.4^{e}	0.1 ± 0.0^{bc}	7.2 ± 0.8^{c}
A. colei + 0.4 % tryptophan	133 ± 11.1^{c}	2.0 ± 1.2^{e}	0.2 ± 0.2^{bc}	7.8 ± 0.7^{c}

a) The average weight of the total feed consumed by each treatment group from day one until the end of the experiment.

b) The difference between the final weight at the end of the experiment and the initial weight on day one.

c) *A. colei* (sieved)

Values are means ± standard deviation of mean of six determination.

Values in the same column with the same superscripts are not significantly different at the 5 % probability level.

Table 2: Free Plasma Amino acids (tryptophan, methionine and cysteine) of Rats fed Amino acid -supplemented *A. colei* [a] Diets.

Diet	Methionine	Tryptophan	Cysteine
		(µmol / L)	
A. colei alone	1649 ± 154 [e]	31.54 ± 0.04 [g]	129 ± 45 [c]
A. colei + 0.1 % cysteine	2105 ± 168 [c]	101.9 ± 1.96 [d]	83.5 ± 13.2 [e]
A. colei + 0.2 % cysteine	1649 ± 201 [e]	64.64 ± 3.43 [f]	30.6 ± 5.8 [h]
A. colei + 0.4 % cysteine	757 ± 235 [g]	15.18 ± 4.41 [h]	134 ± 43 [c]
A. colei + 0.1 % methionine	1320 ± 570 [f]	79.82 ± 18.12 [e]	49.6 ± 9.9 [g]
A. colei + 0.2 % methionine	3231 ± 362 [a]	62.68 ± 3.92 [f]	112 ± 45 [d]
A. colei + 0.4 % methionine	1823 ± 556 [d]	77.38 ± 17.14 [e]	82.7 ± 39.7 [f]
A. colei + 0.1 % tryptophan	1227 ± 543 [f]	656 ± 220 [a]	217 ± 69 [a]
A. colei + 0.2 % tryptophan	1629 ± 261 [e]	475 ± 117 [b]	181 ± 7 [b]
A. colei + 0.4 % tryptophan	2480 ± 67 [b]	142 ± 11 [c]	221 ± 43 [a]

a) *A. colei* (sieved)

Values are means ± standard deviation of mean of six determination.

Values in the same column with the same superscripts are not significantly different at 5 % probability level.

Table 3: Feed intake[a), weight gain[b), protein efficiency ratio (PER) and plasma urea of rats fed casein supplemented with S-carboxyethylcysteine [CEC] and methionine [met][c).

Diet	Feed intake (g)	Weight gain / loss (g)	PER	plasma urea (mg / dL)
Casein + 0.1 % met	$157 \pm 18.6^{\,b}$	$32.3 \pm 8.6^{\,b}$	$2.1 \pm 0.5^{\,a}$	$64 \pm 10.8^{\,c}$
Casein + 0.2 % met	$176 \pm 21.3^{\,a}$	$44.1 \pm 7.7^{\,a}$	$2.5 \pm 0.6^{\,a}$	$56 \pm 6.2^{\,c}$
Casein + 0.1 % met + 0.1 % CEC	$161 \pm 19.3^{\,a}$	$12.9 \pm 2.8^{\,c}$	$0.8 \pm 0.2^{\,c}$	$91 \pm 28.2^{\,b}$
Casein + 0.2 % met + 0.2 % CEC	$136 \pm 16.2^{\,c}$	$9.5 \pm 1.2^{\,d}$	$0.7 \pm 0.1^{\,c}$	$69 \pm 12.9^{\,b}$
Casein + 0.2 % met + 0.4 % CEC	$161 \pm 20.0^{\,a}$	$1.3 \pm 1.5^{\,f}$	$0.08 \pm 0.3^{\,e}$	$97 \pm 3.7^{\,b}$
Casein + 0.2 % met + 0.8 % CEC	$141 \pm 18.2^{\,c}$	$4.2 \pm 1.9^{\,e}$	$0.3 \pm 0.4^{\,d}$	$126 \pm 7.0^{\,a}$

a) The mean weight of the total feed consumed by each treatment group from day one until the end of the experiment.

b) The difference between the final weight at the end of the experiment and the initial weight on day one.

c) Mean ± standard deviation of six determinations.

Values in the same column with the same superscript are not significantly different at 5 % probability level.

Correlation between PER and urea is (r = -0.78 at P<0.05).

Table 4: Free amino acids (tryptophan, methionine [met] and cysteine) content of plasma of rats fed casein supplemented with S-carboxyethylcysteine and methionine.

Diet	Methionine	Tryptophan	Cysteine
		(μmol / L)	
Casein + 0.1 % met	$2627 \pm 181^{\,a}$	$147 \pm 34^{\,a}$	$289 \pm 49^{\,e}$
Casein + 0.2 % met	$938 \pm 201^{\,c}$	$98 \pm 44^{\,c}$	$496 \pm 41^{\,c}$
Casein + 0.1 % met + 0.1 % CEC	$1160 \pm 335^{\,c}$	$98 \pm 44^{\,c}$	$306 \pm 83^{\,e}$
Casein + 0.2 % met + 0.2 % CEC	$952 \pm 134^{\,c}$	$98 \pm 39^{\,c}$	$380 \pm 33^{\,d}$
Casein + 0.2 % met + 0.4 % CEC	$1347 \pm 335^{\,c}$	$49 \pm 29^{\,d}$	$579 \pm 116^{\,b}$
Casein + 0.2 % met + 0.8 % CEC	$1086 \pm 335^{\,c}$	$98 \pm 29^{\,c}$	$661 \pm 116^{\,a}$

Values are means ± standard deviation of mean of six determination.

Values in the same column with the same superscripts are not significantly different at the 5 % probability level.

CEC = S-carboxyethylcysteine

Table 5: Amino acid profile of *Acacia colei* (g / 100g protein)

Amino acids	
Asp	19.0
Thr	8.3
Ser	12.5
Glu	30.5
Pro	8.9
Gly	11.8
Ala	9.3
Val	11.6
Met	3.2
Ile	8.8
Leu	17.2
Tyr	8.4
Phe	9.9
Lys	14.3
His	7.6
Arg	15.1
Cys & Cy	2.9
Trp	2.2
Met + Cys	6.1
Phe + Tyr	18.3

Source: Adewusi, *et al.,* 2003
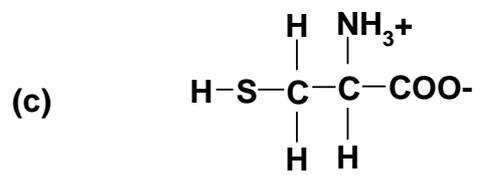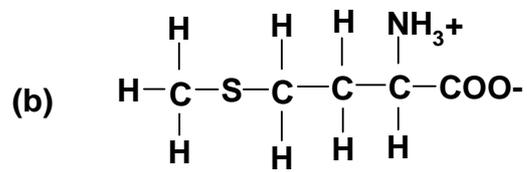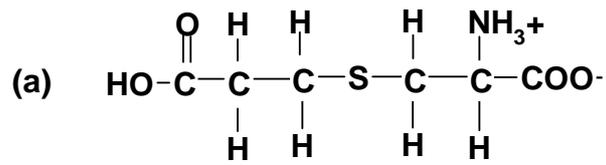
(a)

(b)

(c)

Figure 1: Structure of (a) S-carboxyethylcysteine (b) Methionine and (c) Cysteine
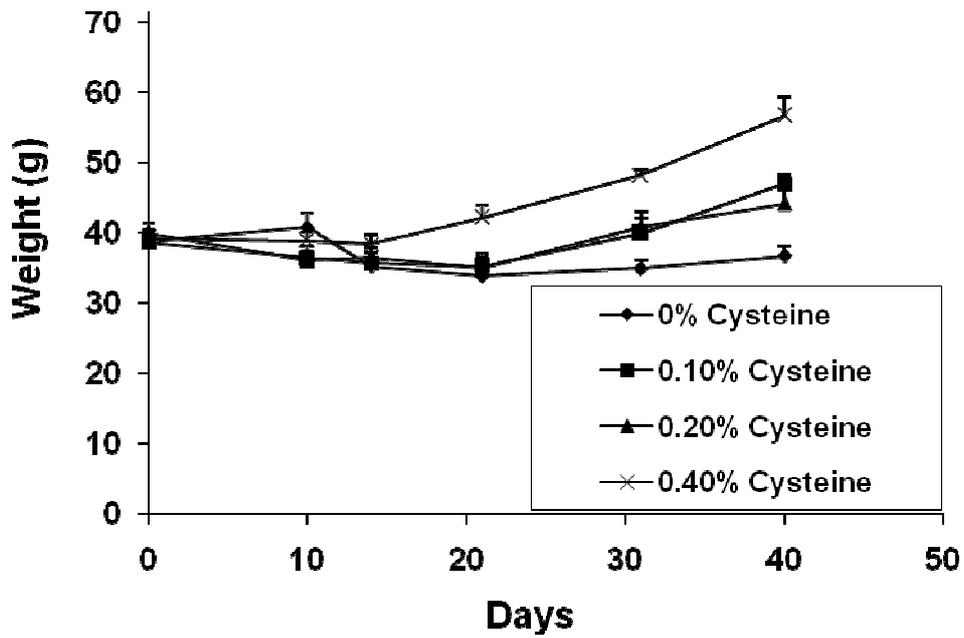
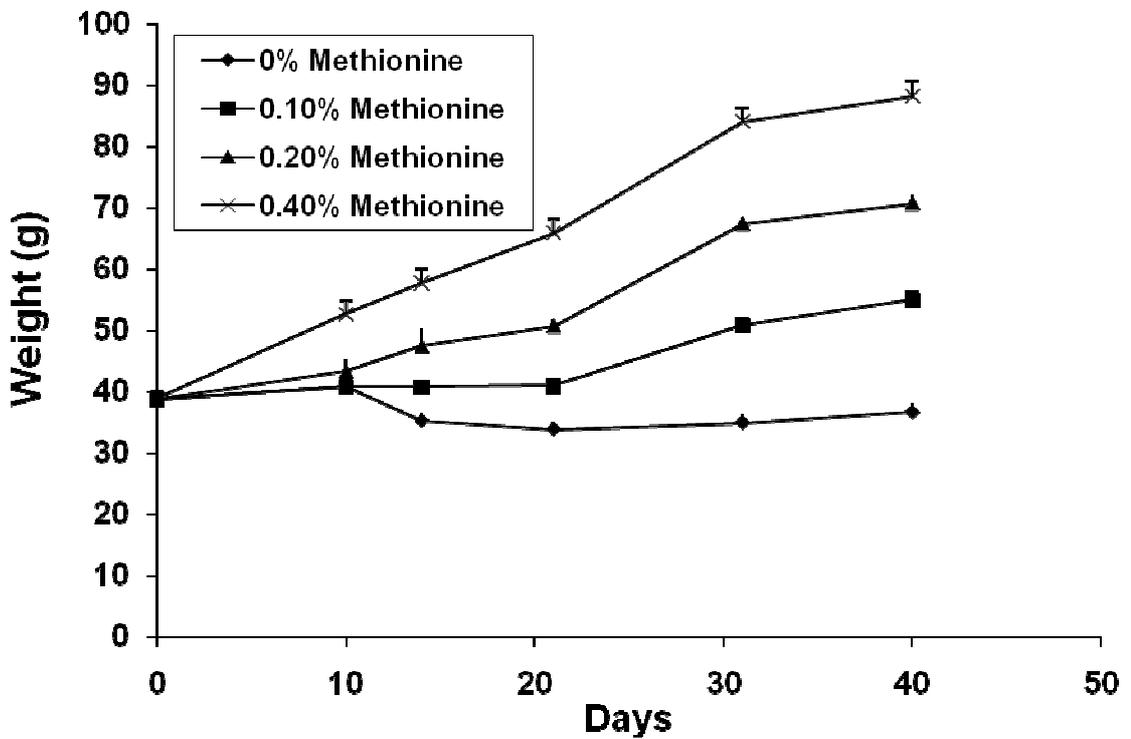**Figure 2: Weight gain of rat fed Cysteine supplemented *A. colei* diets**

Figure 3: Weight gain of rats fed Methionine supplemented *A. colei* diets
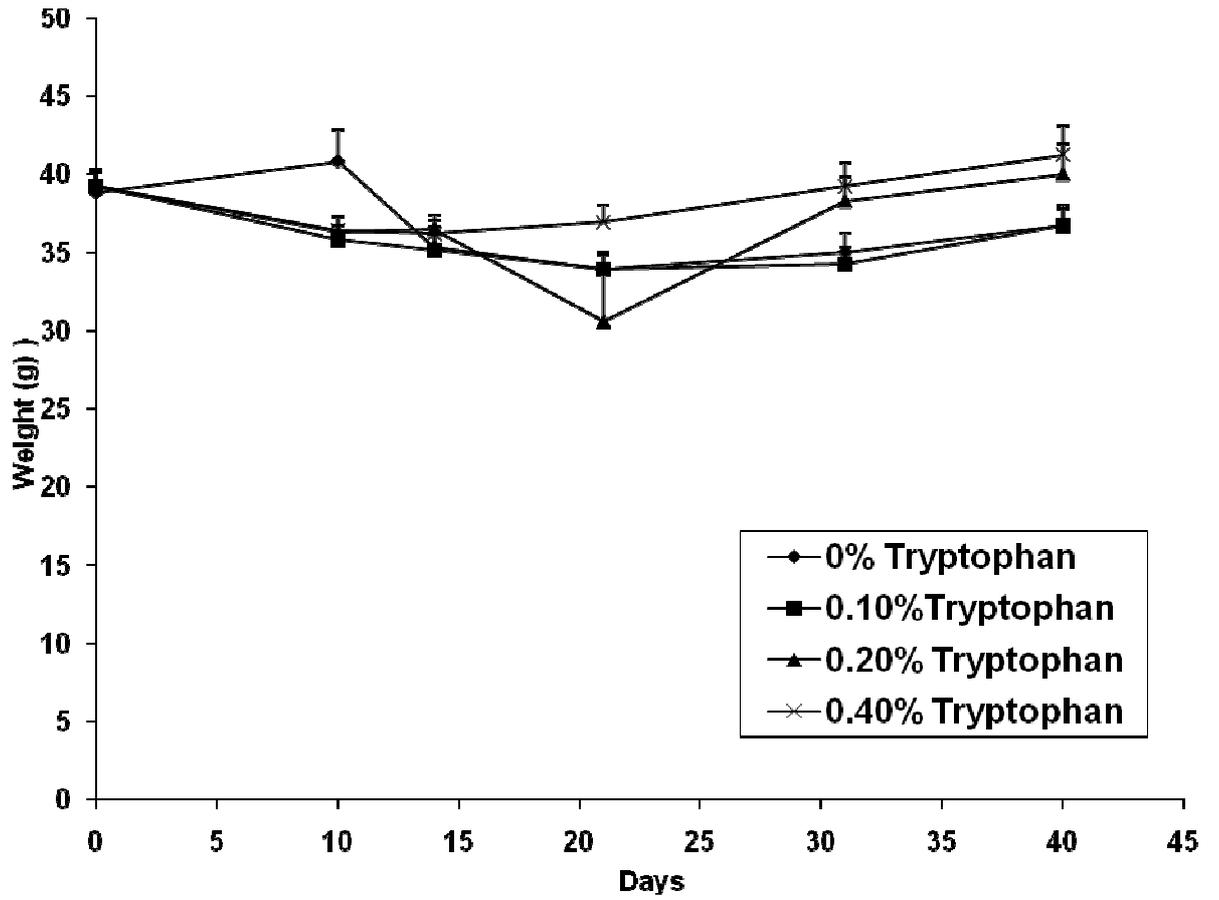
Figure 4: Weight gain of rat fed with Tryptophan supplemented *A. colei* diets
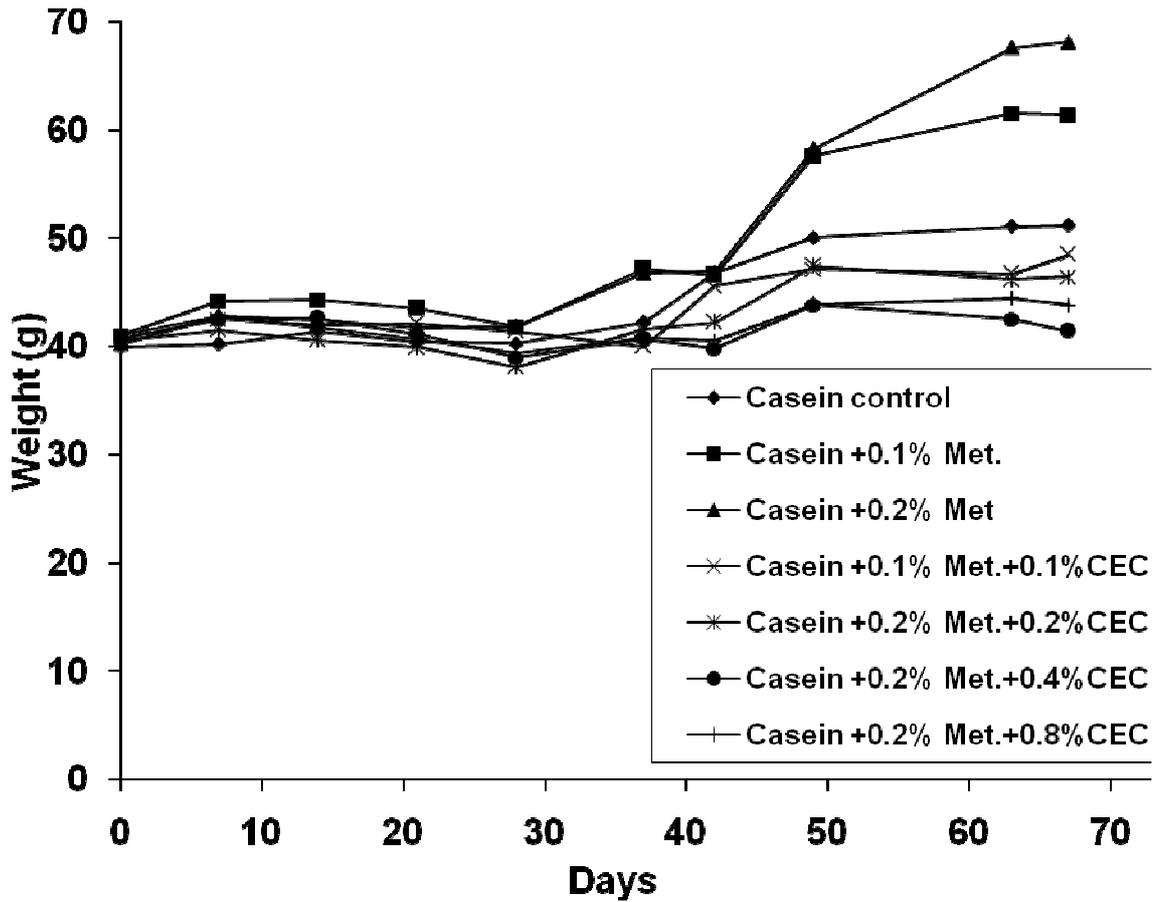
Figure 5: Weight Gain of Rats fed Casein Supplemented with CEC and Methionine Diets

Figure 1: Structure of (a) S–carboxyethylcysteine (b) methionine and (c) cysteine

Figure 2: Weight gain of rat fed cysteine-supplemented *A. colei* **diets**

Figure 3: Weight gain of rats fed methionine-supplemented *A. colei* **diets**

Figure 4: Weight gain of rat fed with tryptophan-supplemented *A. colei* **diets**

Figure 5: Weight gain of rats fed casein supplemented with CEC and methionine Diets