Biochemical and pathological studies in rats following dietary supplementation with high levels of polyunsaturated fatty acids and vitamin E.

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SUMMARY

The effects of dietary supplementation with high levels of polyunsaturated fatty acids (PUFAs) and vitamin E and their interaction on biochemical and pathological parameters in rats were investigated. A total of 40 rats (*Rattus norvegicus*) were randomized in 4 groups, each containing 10 rats. Group 1 (control) was fed on basal diet. Group 2 was fed on basal diet with added PUFAs to attain a fat level of 24%. Group 3 received basal diet supplemented with 1500 ppm of vitamin E. Group 4 was fed basal diet supplemented with 24% PUFAs and 1500ppm vitamin E. Zoo-technical parameters on rats, including clinical picture and body weight changes were observed daily and weekly respectively. The rats were sacrificed after 20 weeks of feeding. Pathological examinations were done on the liver, kidney and heart. Thiobarbituric acid reactive substance concentration (TBArs) in the liver homogenates was determined for biochemical picture. At baseline Body weight and (TBArs) were homogenous in all the groups. Following treatments, average body weight in groups 4 and 2 was significantly higher than in group 1 and 3 and mean TBArs levels in the liver was significantly ($P<0.05$) higher in group 2 rats compared to groups. Furthermore, high dietary supplementation of vitamin E showed no deleterious effects on rats and no pathological changes in the liver, kidney and heart tissues were observed in the treated and control groups. The current study reveals that, peroxidative stress attributable to high levels of PUFAs supplementation in rats maybe counteracted by supplementing PUFA with high level of vitamin E.

Key words: Antioxidants, basal diet, PUFAs, vitamin E

INTRODUCTION

Human and animal foods containing polyunsaturated fatty acids (PUFAs) are increasingly being preferred to those containing saturated fatty acids (SFA). There are evidences that consumption of high levels of SFA is associated with diseases **(**Jakobsen *et, al.,* 2004; Xu *et al.,* 2006). It is observed that increasing the concentration and/or proportion or both of especially the n-3 series of PUFAs in diets modulates prostanoid biosynthesis with associated clinical advantages of decreased risk of coronary heart disease, microthrombosis and immune-related inflammatory diseases (Gian, 2009). Also, PUFAs contain high levels of other nutritive substances; for instance: vegetable oils are rich in vitamin E (Indrajit, *et al.,* 1988; Balthazary, 1991). However, excessive supply of PUFAs may result in PUFA overload to animals. PUFAs are easily attacked by molecular oxygen to form lipid peroxides and active free oxygen radicals. These products of fatty acids peroxidation produce harmful products, which can damage cells and subcellular membranes, (Bayani *et al.,* 2009; Yun-

Zhong *et al.,* 2002). If uncontrolled, these by-products of peroxidation produce pathological changes, which affect various animal tissues and organs (Niki, 2009).

Vitamin E is the principal agent that can counteract peroxidative damage to cells (Bastiaan *et al.,* 2003). Unesterified forms of vitamin E especially α-tocopherol are antioxidants which under normal conditions maintain the balance between oxidative and anti-oxidative processes in the body (Bastiaan *et al.,* 2003). It has been found that depletion of vitamin E is hastened by increased daily intake of PUFAs, and that PUFAs are the single most important factor in determining the daily requirements of vitamin E under normal conditions (Valk, and Hornstra, 2000).Though prophylactic against numerous diseases, evidence shows that, daily intake of 300 IU and above of vit E can be deleterious to humans and animals (Miller *et al.,* 2005). There is insufficient knowledge on health beneficial effects and appropriate combination of PUFAs and Vitaminin E for the best performance in animals. This experiment was undertaken to determine the biochemical and pathological effects of feeding high levels of PUFAs, vitamin E and combination of both in rats.

MATERIALS AND METHODS

Experimental Animals and Management

Experimental animals used were rats weaned at 14d old and weighing 18-20 g. The animals were assigned randomly into four groups, each of 10 rats. Group 1 formed the control and received the basal diet (Table 1). Group 2 received the basal diet and 24 % PUFAs (excessive PUFAs group). Group 3 was fed the basal diet and 1500-ppm vitamin E (excessive vitamin E group). Group 4 was fed the basal diet, 10% PUFAs and 1500 ppm vitamin E (excess in combination). The rats were provided with 15g of feed/d for the first two weeks of the experiment and thereafter they were fed 40 g feed/d.

The feed (basal diet)

Feeds were obtained from feed formulating firm (Interchick, Tanzania). The type and proximate analysis of the feed is presented in Table 1. Prior to the experiments, feed samples were sent to F. Hoffmann La Roche, Switzerland for analysis of vitamin E. Analysis of other components was done at the Department of Animal Science and Production SUA, Morogoro

Table 1. Composition of the basal diet fed to rats

Component	Quantity
Metabolizable Energy (Kcal/kg)	3000
Crude protein (CP%)	19.8
Ether extract (EE%)	13.7
Crude fibre (CF%)	13.7
Calcium (Ca%)	0.82
Phosphorus (P%)	0.66
Vitamin C (ppm)	40
Vitamin E (ppm)	13
PUFAs (%)	14

Supplementation of PUFAs and Vitamin E

The vitamin E was obtained by the courtesy of F. Hoffmann La Roche Company, Switzerland with trade mark Rovimix E-50 for vitamin E. Rovimix E-50 SD is a yellow powder containing dl-α-tocopherol acetate [dl-α-TA]. The vitamin was thoroughly mixed with the feed by using a feed mixer. Vegetable oil was added to a portion of the basal feed to attain a level of 24% fat, mixed thoroughly and fed to rats in group 2.Vegetable oil was added to another portion of the basal diet, which had added vitamin E to attain the same level of about 24% and fed to group 4 rats.

Zootechnical Observations

The experimental animals were observed daily for clinical health conditions where body weights were recorded weekly.

Tissue sample collection, Biochemical analysis and Pathological examinations

The animals were sacrificed at the end of the feeding experiment and the liver, kidney and heart tissues were observed for gross and histopathological changes.

Preparation of liver homogenate

About 0.8 g of liver sample from each rat was put in 10 ml of 1.15% KCl and homogenized using a hand mortar. The homogenate was stored at -22 °C until the day of analysis.

Biochemical analysis

Thiobarbituric acid test (TBA)

The peroxidative products of PUFAs are TBArs. In this test, the TBArs produce red pigmented end products, when they react with TBA, the fluorescence of which is determined by using spectrophotometer at 535 nm. 0.5 ml of the rat liver homogenate was mixed with 3.0 ml of 1-% phosphoric acid and 1.0 ml of 0.6% TBA. The mixture was heated for 60 min. at 95°C, and then cooled in ice. 4ml of n-butanol was added then mixed and centrifuged at 3000 r.p.m. for 10 minutes. TBArs formed from PUFAs in the samples react with TBA to form red-pigmented end products. The same procedure was applied to the standard. The fluorescence of the upper organic layer was determined by using spectrophotometer at 535 nm. The concentration of TBArs in the liver homogenate was calculated using the following equations:

The TBA reactive substance concentration = $\frac{\text{absorbency in sample x Conc. of standard}}{\text{Absorbency of standard}}$

Statistical Analysis

Statistical Package for Social Sciences (SPSS) was used for statistical analysis. The means were partitioned by Duncan's multiple range test.

RESULTS

All the rats in four groups were clinically healthy except for the death of four rats in the course of the experiment. The dead rats were; one from group 1 (control), two from group 2 (fed high level of PUFAs) and one from group 3 (supplemented with high level of vitamin E). Postmortem examination did not show pathological changes.

Body weight development results are shown in Table 2. The average body weights of rats in the first week were not significantly different in all groups. In the second week, the group which received high level of PUFAs had average body weights which differed from the control group and the group (3) which received high level of vitamin E. The average body weight for the group of rats fed high level of PUFAs was lower than that of the other groups. In the thirteenth and eighteenth week of the experiment, there was a significant difference between the average body weights of control and groups fed high levels of vitamin E on one side and PUFAs, PUFAs and vitamin E combined groups on the other side. The average body weights of the groups, which received high level PUFAs, PUFAs and vitamin E combined, were the highest.

There were no pathological changes observed in the livers, kidneys and hearts obtained from all treatment and the control groups. However, biochemical changes by

TBArs test were observed and are as indicated in Table 3 below. The average concentration of TBArs for samples from group 2 which received high levels of PUFAs was higher than in the other treatment groups and the controls. The average concentration of TBArs for samples from group two which received high levels of PUFAs, was significantly higher than in the other groups, while there was no significant difference between the group supplemented high vitamin E, and high PUFAs and vitamin E respectively and the control.

Table 2. Body weights development in the rats

Time	Mean weight in grams			
	Group 1 Basal diet	Group 2 Basal diet + PUFAs	Group 3 Basal diet and vitamin E	Group 4 PUFAs and vitamin E
2 days post weaning	20.03±0.63	21.17 ±0.63	21.50±0.63	20.57±0.64
Week 2	49.38±1.4	45.81±1.4	47.84±1.4	45.91±1.4
Week 6	112.77 ±3.1	109.17 ±2.92	110.54±2.95	109.37±2.95
Week 13	155.82^{a} ±3.92	178.79^{b} ±4.16	154.51^{a}±3.92	181.30^{b}±3.37
Week 18	180.52^{a} ±6.07	214.99^{b} ±6.43	178.88^{a}±6.06	212.56^{b}±5.75

Note that: Group 1 (control), Group 2 (excessive PUFAs group), Group 3 (excessive vitamin E group); Group 4 (excess in combination). Means with different superscript letters differed significantly ($P \leq 0.05$).

Table 3.TBArs concentration in liver tissue homogenates of rats.

Sample	TBArs concentration in μg/g			
	Group 1 (control)	Group 2 (PUFAs)	Group 3 (α-tocopherol)	Group 4 (PUFAs+α-Tocopherol
1	15.42	21.20	14.20	16.20
2	19.94	18.20	16.20	17.41
3	18.72	24.26	14.60	18.21
4	15.56	23.50	15.50	16.24
5	17.67	17.50	16.30	17.24
6	14.68	22.40	17.01	15.20
7	15.89	21.20	14.32	17.34
8	17.22	23.30	15.50	14.20
9	16.12	-	15.80	17.40
10	-	-	-	18.00
Mean	16.47^{a}	21.45^{b}	15.49^{a}	16.74^{a}

Note that: Group 1 (control), Group 2(excessive PUFAs group), Group 3(excessive vitamin E group); Group 4 (excess in combination). Means with different superscript letters differed significantly ($P ,< 0.05$).

DISCUSSION

The health status of the experimental animals was good throughout the feeding experiment. Four deaths occurred at different times in the course of the feeding experiment. These mortalities cannot be associated with the feeding because they occurred in different groups including the control group and none in the high level PUFAs/vitamin E treated groups. On postmortem examination, there were no observable lesions ascribing the observed mortalities. The average body weights at day 2 post-weaning showed no significant difference between control group and the other groups (2, 3 and 4) which received high levels of PUFAs, vitamin E and their combination respectively. In the second week, the control group on basal diet had relatively higher average body weight than the treatments groups which was probably attributable to stresses caused by dietary transition in the treatment groups. In the sixth week there was no significant difference between the average body weights of the control and the treatment groups. In the thirteenth week average body weights in groups 1 and 3groups were relatively similar however differed significantly with as groups 2 and 4 which also had fairly similar average body weights. The same trend was observed up to the eighteenth week. The high PUFAs and high PUFAs and vitamin E fed groups were higher in terms of their average body weights. This can be explained by the fact that immature animals do not deposit fat significantly. While after maturity fattening occur significantly depending on the energy level of the diet provided. If the energy level in the diet is high, then fattening is faster.

Rats (*Rattus norvegicus*) mature at about 50-70 days (Buckland *et al.,* 1981). The vegetable oil added provided extra energy. At thirteenth week the rats were mature enough to begin fattening and so gain more weight compared to those fed lower levels of fat (control and high vitamin E groups).

The result showed that group two (high PUFAs) differed significantly ($P \leq 0.05$) from control (1) and the other groups (3 and 4) fed high vitamin E and high vitamin E and PUFAs respectively. The concentration of TBArs was higher in high PUFAs fed group. This suggests that there were more peroxidative processes in the high PUFAs group as compared to control, high vitamin E and high PUFAs and vitamin E groups. These results are in agreement with those of Iritani *et al,* (1980) who found that the TBArs concentrations were higher in 5 and 10% corn oil fed rats compared to 0.5% corn oil fed rats and to Valk and Hornstra, 2000 who showed that increasing the degree of dietary fatty acid unsaturation in experimental animals increases the peroxidizability of the lipids and reduces the time required to develop symptoms of vitamin E deficiency. The higher the level of PUFAs in diet, the higher the rate of peroxidation and hence, the higher the concentration of TBArs (Cortinas *et al.*, 2005).

On supplementing high levels of vitamin E alone, there were no observable clinical, pathological or biochemical changes in the rats at 1500 ppm of the vitamin in the feed. The body weights development was not different from that of control group, but differed from the groups supplemented with high levels of PUFAs. These results conflict those reported by Christensen (1983) that massive oral doses of vitamin E produced toxic effects which include depressed coagulation of blood, possibly because of interference of vitamin E with vitamin K activity. Related observations reported by Miller, *et al.* (2005) showed increased mortality in high vitamin treated participants compared to control. March *et al.* (1973) found that reduced growth rate, reduced respiration rate of skeletal muscles

mitochondria, depressed bone calcification and prolonged prothrombin time occurred when 2200 IU vitamin E/kg feed was supplied to chicks. The contradiction could probably be due to the species differences between rats humans and chicken and the level of vitamin E. Probably 1500 ppm could be deleterious to chicks but not to rats and human. Yang and Desai (1977) reported that massive oral doses of vitamin E fed to rats for 8 months or more produced deleterious effects.

The fact that there was no difference between the control group and the high PUFAs/vitamin E fed group, while there was a difference between high PUFAs and PUFAs/vitamin E fed groups, suggest that vitamin E prevented peroxidative processes so that the TBArs concentration was similar to that of control group. This is in agreement with the findings by Kornbrust and Mavis (1979) and Ching *et al.* (2013), who found that vitamin E plays the role of major cellular antioxidant, especially in the highly oxygenated tissues of heart and lungs. Brandt *et al.*1990 found that α-tocopheryl acetate could reduce the effects of *Clupea spattus* oil and its oxidative degeneration products in mink. Results of this experiment seem to corroborate those of Brandt *et al.* (1990) on the role of vitamin E.

Moreover, there were no histopathological lesions associated with high levels of PUFAs and Vitamin E in the liver, heart and kidney tissue sections. These results differ from those of Corner *et al*, (1984), who found degenerative myocardial changes, ascites, and cachectic muscular atrophy and peri-acinar hepatic necrosis in cockerels. The reason for this disagreement could be due to species difference. Rat tissues could be less susceptible to higher oxidative stress than the chick tissues. Another reason could be that the feed had a reasonably high level of selenium, which has some similarities to vitamin E in terms of its activity as an antioxidant (Smith, 1977).

It is concluded that PUFAs are a good source of energy to animals and that the sources of PUFAs also contain other nutrients like protein and fat-soluble vitamins. Feeding of animals with diets containing high levels of PUFAs may result in undesirable side effects such as disease susceptibility due to increase in fat peroxidative processes. Vitamin E (α-tocopherol) has been shown to be a good anti-oxidative agent in the body. Therefore, faster fattening of animals by supplementing the sources of PUFAs can be achieved and yet, the side effects of high level of PUFAs in diet be avoided by simultaneous supplementation with vitamin E. However, the economics of production will have to determine the efficiency and level of supplementation for maximum productivity depending on the market forces.

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