COMPARATIVE CLINICAL STUDIES OF THREE PLANT LEAVES AS SOURCES OF PROTEIN USING ALBINO RATS

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Summary

The leaves of *Euphorbia hirta* (locally called "kaka wie adwe"), *Ipomea involucrata* (Iehowa dua) and *Launaea taxaracifolia* (wild lettuce) were used to assess their effects in animal feeding trials when utilized as source of protein, using albino rats. The animals fed on all the leaf protein diets (at inclusion levels of 5%, 8% and 10%) maintained similar haematological picture as those fed on the control (casein) diets. Their haemoglobin, red blood cell and white blood cell counts were not significantly different from those animals fed with the control diets ($P>0.05$). Methionine supplementation did not improve the red blood cell and haemaglobin content of animals fed with *Euphorbia hirta, Ipomoea involucrata* and *Launaea taxaracifolia* leaf protein diets. The activities of aspartate aminotransferase and alkaline phosphatase (ALP) were not influenced by any of the leaf proteins. However, alanine aminotransferase activity was significantly elevated in the animals fed with the test protein diets ($P<0.05$). The histopathological studies did not reveal any adverse pathological changes in any of the animals fed with the leaf protein diets.

Introduction

Protein deficiency has been cited as the most prevalent form of malnutrition in the world today (F.A.O., 1985) especially in Africa due to contributory factors like ignorance, poverty, poor food habits and illogical traditional beliefs. Ghana, like most tropical countries, has abundance of plants that grow all year round, the leaves of which could be utilized to supplement the much expensive and generally unavailable animal protein. The need to research into leaf protein becomes more compelling when one considers the fact that leaf proteins have been considered to be one of the most promising protein sources that have the potential to meet the growing demand for food proteins in the world. They are also reported to contain carotene and chlorophyll, which are resistant to lethal doses of radiation as well as xanthophyll, which enhances skin pigmentation of livestock and egg yolk (I.B.P., 1970).

Due to some of these seemingly beneficial qualities of vegetable proteins, the tendency of advocating the increased use of plants from both conventional and unconventional sources as food without in-depth and comprehensive knowledge of their chemical and nutritional value as well as possible adverse pathological effects on the consumer is very high. Some plants have been reported to contain certain toxicant and anti-nutritive principles that tend to decrease their nutritive value (Marfo *et al*, 1988) and cause growth depression, pancreatic enlargement and changes in pancreatic enzyme composition (Niam *et al*., 1982).

The leaves of *Euphorbia hirta, Ipomea involucrata and Launaea taxaracifolia* are used in some parts of Ghana as vegetables as well as anti-anaemic adjuncts. To promote their wider utilization, this study was undertaken to determined the effect their consumption would have on the health of the user by investigating the effect of the leaves (when consumed as sources of protein) on blood cells counts, haemoglobin and the activities of certain marker enzymes namely aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. Histopathological assay on some selected visceral organs were also conducted.

Materials and Methods

Sample preparation

Tender, fresh and green leaf samples of *E. hirta* (kaka wie adwe), *L. taxaracifolia* (wild lettuce) and *I. involucrata* (Iehowa dua) cultivated at the Department of Horticulture, Univer-

vated at the Department of Horticulture, University of Science and Technology (U.S.T.), Kumasi, Ghana were used in these studies. All leaf samples were harvested while young, between 5 and 6 o'clock in the morning. The samples were washed thoroughly in running water and air-dried at 60°C for 48 hours. These were then ground into fine powder, stored at -5°C and used for the diet formulation.

Diet formulation

Twenty-one experimental diets were formulated using the leaves as sources of protein (as test proteins) and casein as the control protein (Table 1). All the diets were designed such that the protein levels were 5%, 8% and 10% of the total feed meal. Two sets of test diets were prepared. One set was fortified with 0.20% methionine while the other had no methionine

TABLE 1
Composition of Diets (Weight in Grams)

DIETS	CONSTITUENTS (G)					
			*Premix			
Casein	**Protein**	**$Met**	**Mineral**	**Vitamin**	**Oil**	**Corn**
A (5%)	133	-	20	20	200	1627
B (8%)	218	-	20	20	200	1542
C (10%)	267	-	20	20	200	1493
Unsupplemented *E. hirta* Diets						
D (5%)	688	-	20	20	200	1072
E (8%)	1011	-	20	20	200	749
F (10%)	1077	-	20	20	200	683
Supplemented *E. hirta* Diets						
G (5%)	688	4	20	20	200	1068
H (8%)	1011	4	20	20	200	745
I (10%)	1077	4	20	20	200	679
Unsupplemented *I. involucrata* Diets						
J (5%)	540	-	20	20	200	1220
K (8%)	8641	-	20	20	200	896
L (10%)	1079	-	20	20	200	681
Supplemented *I. involucrata* Diets						
M (5%)	540	4	20	20	200	1216
N (8%)	864	4	20	20	200	892
O (10%)	1079	4	20	20	200	677
Unsupplemented *L .taxaracifloia* Diets						
P (5%)	403	-	20	20	200	1357
Q (8%)	645	-	20	20	200	1115
R (10%)	806	-	20	20	200	954
Supplemented *L. taxaracifloia* Diets						
D (5%)	403	-	20	20	200	1353
E (8%)	645	-	20	20	200	1111
F (10%)	806	-	20	20	200	950

$Met ==> Methionine

*Premix : Vitamin mix (mg/kg): retinyl palmitate, 4000 IU; D-L-tocopheryl acetate, 50 IU; cholecalciferol, 1000 IU; menadione sodium bisulphite, 0.8; cyanocobalamin(vitamin B-12), 10; biotin, 0.2; folic acid, 2; calcium pantothenate, 16; niacin, 30; pyridoxine. HCl, 7; riboflavine, 6; thiamin. HCl, 6. Mineral mix (g/kg) : calcium, 5200; phosphorous, 4000; potassium, 3600; sodium, 1020; chloride, 1560; sulphur, 337; magnesium, 507; iron, 35; copper, 6.0; manganese, 35.0; zinc, 30.0; chromium, 2.0; iodine, 0.2; selenium, 0.1000g.

supplementation. The diets were thoroughly mixed, packed into tightly stoppered containers and stored at 4°C.

Animal studies

One hundred and twenty-six male albino rats (*Rattus norvegicus*) of average initial weightof 50 g were used in this assay. They were divided into twenty-one groups of six rats in each group, housed singly and fed the experimental diets for a period of twelve weeks. Each rat was given a daily ration of 5g of the diet while water was given *ad libitum*.

The animals were sacrificed on the last day of the feeding trial after the final weights were taken. The liver, heart and kidney were removed, blotted dry, weighed and preserved in portions of 10% formal-saline for the histopathological studies.

2 ml of blood from each animal was placed in sequestrin for haematological studies and another 5 ml collected in dry tubes and centrifuged at 3,000 x g using Hettich EB 8S model. The serum samples so formed were used for the enzymatic assay, namely, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase (ALP).

Alanine and aspartate aminotransferase activities were determined by the method of Reitman and Frankel (1957) while the method described by King and Armstrong (1934) was used for the alkaline phosphatase activity. The haematocrit and improved Neubauer methods (Coles, 1974) were used for the haemoglobin (Hb), red blood cell and white blood cell counts. The eosin-haematoxylin method as described by Lillie (1965) was employed for the histopathological studies.

Statistical Analysis

The student 't' test was used to test for significant differences between test diets(leaf) and that of the control (casein).

Results and Discussion

Effect of leaves as sources of protein on haemaglobin (Hb) and blood cells

The feeding of the leaves as sources of protein and their effect on the Hb levels as well as blood cell count as presented in Table 2 demonstrates that animals fed on the test diets had similar Hb levels and blood cell counts and these were not significantly different from the results obtained for animals on equivalent control diets ($P>0.05$). However, the inclusion of leaf protein in wheat flour diets has been reported to improve the haemoglobin level of blood significantly (Kawatra *et al.*, 1974).

The red blood cell (rbc) count of animals on the test diets were not significantly different from the rbc count of those on equivalent control diets ($P>0.05$). The results therefore, seems to suggest that the leaves under study when fed as protein source would not be inferior to casein (the control protein) in so far as the formation of Hb and rbc is concerned. The implication is that the consumption of these leaves as sources of portein and as part of the diet on regular basis would improve the Hb and rbc count to the same extent as when animal protein is consumed. Earlier studies on one of the plants, *I. involucrata* indicated that it has anti-anaemic potential (Burkill, 1985 and Akodam, 1986). Infusions prepared from *I. involucrata* reportedly contained haematinic principles like thiamine, ascorbic acid and nicotinic acid (Akodam, 1986).

The consumption of the whole plant means therefore, the ingestion of those haematinic and antianaemic principles as well. This strongly suggest that the whole leaf meal of *E. hirta, I. involucrata* and *L. taxaracifolia* may be adjuncts to the treatment of anaemic condition.

The wbc counts of the animals on the test diets were not significantly different ($P>0.05$) from those maintained on equivalent control diets. A case in point is when the wbc counts of animals fed diets B (8% casein), E (8% *E. hirta* without methioinine supplementation) and H (8% *E. hirta* with methionine supplementation). The wbc count for B was 6.00×10^9 while those of E and H were 6.19×10^9 and 6.24×10^9 respectively. Methionine supplementation of the leaves did not have significant effect on the wbc counts ($P>0.05$). This observation indicate that the consumption of these leaves would not result or induce in any infectious disease state.

TABLE 2

Haematological results of animals fed casein and leaves as sources of protein

Diets	Hb(mg/dl)	RBC Count(x 10^{12})	WBC Count(x 10^{9})
Control Diets			
A(5%)	12.62 ± 1.01^{a}	6.30 ± 0.02^{a}	6.40 ± 0.50^{a}
B(8%)	14.92 ± 1.01^{a}	7.20 ± 1.11^{a}	6.00 ± 1.20^{a}
C(10%)	16.10 ± 0.67^{a}	7.52 ± 0.45^{a}	5.90 ± 0.41^{a}
Unsupplemented *E. hirta* diets			
D(5%)	13.14 ± 0.99^{a}	6.97 ± 0.80^{a}	6.24 ± 0.02^{a}
E(8%)	15.50 ± 0.31^{a}	7.39 ± 1.03^{a}	6.19 ± 0.11^{a}
F(10%)	17.30 ± 0.05^{a}	7.99 ± 0.47^{a}	6.36 ± 1.20^{a}
Supplemented *E. hirta* diets			
G(5%)	13.35 ± 0.26^{a}	6.87 ± 0.34^{a}	5.96 ± 0.45^{a}
H(8%)	15.66 ± 0.53^{a}	7.23 ± 0.17^{a}	6.24 ± 1.52^{a}
I(8%)	17.42 ± 1.52^{a}	8.47 ± 0.20^{a}	5.02 ± 0.22^{a}
Unsupplemented *I. involucrata* diets			
J(5%)	13.97 ± 0.46^{a}	6.40 ± 0.93^{a}	6.43 ± 0.10^{a}
K(8%)	15.50 ± 1.77^{a}	7.31 ± 0.71^{a}	6.24 ± 0.52^{a}
L(10%)	16.93 ± 2.78^{a}	8.43 ± 0.34^{a}	5.53 ± 0.22^{a}
Supplemented *I. involucrata* diets			
M(5%)	12.08 ± 0.97^{a}	7.11 ± 0.83^{a}	6.43 ± 0.72^{a}
N(8%)	15.52 ± 0.77^{a}	7.28 ± 0.08^{a}	6.54 ± 0.86^{a}
O(10%)	16.77 ± 0.78^{a}	8.43 ± 0.34^{a}	6.13 ± 0.12^{a}
Unsupplemented *L. taxaracifolia* diets			
P(5%)	12.31 ± 0.65^{a}	6.54 ± 0.12^{a}	5.82 ± 0.22^{a}
Q(8%)	14.40 ± 1.72^{a}	6.73 ± 0.52^{a}	5.47 ± 0.34^{a}
R(10%)	14.90 ± 1.31^{a}	7.50 ± 1.11^{a}	5.04 ± 0.97^{a}
Supplemented *L. taxaracifolia*			
S(5%)	13.50 ± 0.04^{a}	7.10 ± 0.43^{a}	6.20 ± 0.21^{a}
T(8%)	15.28 ± 0.55^{a}	7.29 ± 0.21^{a}	6.32 ± 1.12^{a}
U(10%)	16.52 ± 0.67^{a}	7.53 ± 0.15^{a}	5.22 ± 0.11^{a}

Data in Table 3 presented as mean values + SD. Casein diets (A-C) are compared with unsupplemented and supplemented leaf protein diets. Superscripts in common letters for diets being compared indicate no significant difference (P>0.05).

Effect of leaves (as protein sources) on enzyme activities

Examination of the enzyme activities (Table 3) shows that the levels and types of protein generally did not influence the activities of aspartate aminotransferase as well as that of alanine phosphatase ie. the activities of these marker enzyme were not significantly difference (P>0.05) from their counterparts on equivalent control diets. For example, animals fed with 5% protein registered enzyme (aspartate aminotransferase) activities of 26.96 IU/L (control), 27.04 IU/L (unsupplemented *E. hirta*), 26.89 IU/L (supplemented *E. hirta*), 26.37 IU/L (unsupplemented *I. involucrata*), 27.05 IU/L (supplemented *I. involucrata*), 25.88 IU/L (unsupplemented *L. taxaracifolia*) and 26.67 IU/L (supplemented *L. taxaracifolia*).

In the alanine aminotransferase activity all the animals fed with the test proteins recorded significantly higher activity than the animals maintained on the control diets (P<0.05). The reasons for the apparent higher alanine aminotransferase activity in response to the consumption of the test diets as compared to the control diets cannot be clearly advanced. However, it has been suggested that higher alanine

TABLE 3

Enzyme activities of animals fed with the Control (Casein) and experimental (leaves fed as source of protein) Diets

Diets	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
Control (casein)			
A(5%)	15.33 ± 0.15^a	26.96 ± 0.37^a	36.23 ± 0.56^a
B(8%)	16.50 ± 1.50^a	27.94 ± 0.52^a	38.03 ± 1.64^a
C(10%)	18.34 ± 1.07^a	29.00 ± 1.73^a	39.17 ± 0.23^a
Unsupplemented *E. hirta*			
D(5%)	20.84 ± 0.79^b	27.04 ± 1.45^a	35.97 ± 0.54^a
E(8%)	21.89 ± 1.02^b	28.06 ± 0.14^a	38.50 ± 0.99^a
F(10%)	24.08 ± 0.35^b	29.66 ± 0.51^a	40.16 ± 0.92^a
Supplemented *E. hirta*			
G(5%)	20.95 ± 0.61^b	26.89 ± 1.40^a	37.15 ± 0.03^a
H(8%)	21.89 ± 1.02^b	28.06 ± 1.25^a	37.20 ± 0.99^a
I(10%)	24.08 ± 0.35^b	29.66 ± 0.51^a	40.04 ± 1.14^a
Unsupplemented *I. involucrata*			
J(5%)	19.79 ± 0.21^b	26.37 ± 0.51^a	36.75 ± 0.03^a
K(8%)	21.04 ± 0.62^b	27.99 ± 0.64^a	38.27 ± 0.69^a
L(5%)	24.22 ± 0.56^b	30.51 ± 0.21^a	39.58 ± 1.90^a
Supplemented *I. involucrata*			
M(5%)	19.05 ± 0.64^b	27.05 ± 0.38^a	35.75 ± 0.14^a
N(8%)	20.23 ± 0.39^b	28.21 ± 1.31^a	38.25 ± 0.64^b
O(10%)	23.97 ± 1.29^b	30.51 ± 0.82^a	39.58 ± 1.09^b
Unsupplemented *L. taxaracifolia*			
P(5%)	18.67 ± 1.03^b	25.88 ± 0.04^a	32.43 ± 0.63^b
Q(8%)	19.85 ± 0.37^b	26.79 ± 0.23^a	33.56 ± 0.66^b
R(10%)	23.77 ± 0.78^b	29.83 ± 0.82^a	35.06 ± 0.29^b
Supplemented *L. taxaracifolia*			
S(5%)	19.14 ± 0.96^b	26.67 ± 0.73^a	33.67 ± 0.29^a
T(8%)	20.05 ± 0.33^b	26.33 ± 1.08^a	34.07 ± 0.87^b
U(10%)	23.98 ± 0.27^b	29.81 ± 0.79^a	35.94 ± 0.54^a

Data in Table 3 presented as mean values + SD. Casein diets (A-C) are compared with unsupplemented and supplemented leaf protein diets. Superscripts in common letters for diets being compared indicate no significant difference ($P>0.05$).

transaminase activity is indicative of the inci dence of organ damage (Malhotra, 1980). Corroborative evidence from the histopathological studies on the other hand did not indicate any adverse organ damage.

Histopathological studies

The type and level of protein fed the experimental animals used in the study seemed not to have affected the state of the visceral organs appreciably. Except for the occurrence of few instances of granulomas, congestion and steatosis in the liver and heart of some of the animals eg. those fed with diets A,E,G, L and A,B,J,X respectively, there were no obvious pathological alterations in both the animals fed with the test as well as the control diets.

The near similar histopathological picture presented by both the animals fed with the control and test diets coupled with almost similar activities of the marker enzymes (except that of alanine aminotransferase activity) studied tends to rule out the likely incidence of apparent organ damage on the consumption of these leaves

TABLE 4
Histopathological results of selected visceral organs

Diet	Liver	Heart	Kidney
A	Occasional steatosis	Focal necrosis	Normal
B	Normal	Focal necrosis	Normal
C	Normal	Normal	Normal, mild congestion
D	Acute congestion but Normal	Focal haemorrhage	Normal, mild congestion
E	Widespread granulomas	Few granulomas	Normal, mild congestion
F	Normal	Few granulomas	Normal, mild congestion
G	Acute congestion	Few granulomas	Normal, mild hyperaemia
H	Normal	Few granilomas, focal necrosis	Normal, mild congestion
I	Few granulomas	Few granulomas	Normal
J	Few haemorrhage, occasional steatosis	Haemorrhage, necrosis & widespread granulomas	Normal
K	Normal	Normal	Normal, mild congestion
L	Acute congestion, mild steatosis	Normal	Normal, mild congestion
M	Normal	Normal	Normal, mild congestion
N	Normal	Normal	Normal, mild congestion
O	Normal	Focal haemorrhage	Normal, mild congestion
P	Normal	Focal haemorrhage	Normal, mild congestion
Q	Occasional granulomas	Focal necrosis	Normal, mild congestion
R	Occasional steatosis	Few granulomas	Normal, mild congestion
S	Focal necrosis and	Focal granulomas	Normal, mild congestion
T	Acute congestion	Focal necrosis by inflammatory cells	Normal, mild congestion
U	Normal	Necrosis of muscle	Normal, mild congestion

when included in the diet as protein source. proteins. This, therefore, implies that these leaves at various concentrations (up to 10%) could be used in the preparation of animal feed and possibly for human consumption without exposing these visceral organs (namely liver, kidney and heart) to injury.

Conclusion

The results of the studies revealed that all the leaf samples studied have great erythropoietic potential considering the comparative haemoglobin and red blood cell counts observed in rats fed with these leaves as well as casein as protein sources. The type and quantity of protein did not influence the activities of aspartate transaminase or alkaline phosphatase to any significant extent.

However, the activities of alanine transaminase in all the animals fed with the test diets tended to be significantly higher ($P<0.05$).

The histopathological studies on some selected visceral organs, albeit, did not indicate any adverse pathological alterations when these leaves were consumed as part protein sources as well as being part of the normal diet of the animals.

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