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NUTRITIONAL VALUE AND CHOLESTEROL-LOWERING EFFECT OF WILD LETTUCE (*LAUNAEA TAXARACIFOLIA*) LEAF AS A SOURCE OF PROTEIN

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ABSTRACT

The nutritive value and cholesterol-lowering effect of wild lettuce (*Launaea taxaracifolia*) leaf when fed as a source of protein was assessed by using male albino rats (*Rattus norvegicus*) as an index of evaluation. The rats were fed on both methionine supplemented and unsupplemented wild lettuce leaf diets and elicited significant plasma hypocholesterolemic response ($p < 0.05$) at all levels of inclusions. There were no significant differences in the cholesterol levels as determined in some visceral organs namely liver, kidney and heart of animals fed on the wild lettuce diets ($p > 0.05$). Total lipid levels determined in the liver, kidney and heart of the animals fed on both wild lettuce leaf protein diets and casein-based diets (as control) were comparatively similar. However, lipid levels in the plasma of the animals maintained on the wild lettuce diets were significantly higher than those fed on the casein diets ($p < 0.05$). Total plasma protein was not affected by feeding animals with wild lettuce leaf. However, the rats fed with the lettuce leaf diets (both methionine supplemented and unsupplemented) gained comparatively lower growth response in terms of weight gain, protein efficiency ratio (PER) and feed efficiency ratio (FER) when compared with animals on the respective control diets ($p < 0.05$).

1. INTRODUCTION

The world is faced with the problems of atherosclerosis, cardiovascular diseases and associated complications. Epidemiological studies, animal experiments and clinical observations have now confirmed beyond reasonable doubts that raised serum cholesterol levels are strong risks factor for ischaemic heart diseases (IHD) and efforts to decrease serum cholesterol levels in an attempt to alleviate IHD have long been the preoccupation of medical research [1]. Other equally harmful effects of high plasma cholesterol is the deposition of nodules of cholesterol called xanthomas in the skin usually in the elbow, knuckles, knee, toes and more harmfully in the arterial plaques which leads to atherosclerosis [2].

The search for effective hypocholesterolemic agents with minimal side effects to address these disease problems is ever increasing. Major and minor nutrients and non-nutrients alike have been singled out for particular merit or disdain according to their lipaemic effect. Nutritional research has identified many dietary components, such as saponins, protein, etc., which tend to decrease plasma cholesterol levels [1, 2]. Majority of studies done with the view to unearthing hypocholesterolemic agents from plant sources have mainly been centred on only a few lineate sources particularly seeds and their products while little or nothing has been reported about the potential of leaves or the nutrients extracts to lower blood cholesterol when consumed as part of the regular meal [1]. Several studies in animals and man have indicated whole range of legumes, for instance, to be effective cholesterol-lowering principle when consumed on a habitual basis, especially when the background diet is high in fat and cholesterol [1, 2, 3].

Wild lettuce is eaten by a large number of Ghanaians, and is also generally acclaimed to possess great medicinal value [4]. The present study was undertaken to determine the potential of wild lettuce (*L. taxaracifolia*), a compositae, to lower cholesterol levels when consumed as source of protein on regular basis as well as to assess its nutritive value.

2. EXPERIMENTS

2.1 Sample preparation

Wild lettuce (*L. taxaracifolia*) leaves used in the study were obtained from the Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi.

The fresh green and tender leaves gathered early in the mornings were washed under tap water and pressure-cooked with minimum volume of water for 10 min, oven-dried at 60 °C for 48 h, milled into a fine powder and used for the diet formulation. The crude protein content of the leafy meal as well as that of casein were determined using the micro-kjeldahl method [5] and the levels factored into the formulation of the nine experimental diets.

2.2. Animal studies

The leaf powder was used in formulating test diets at 5 %, 8 % and 10 % protein levels of the total feed meal. Two test diets and a control were formulated. The first test diet was supplemented with 0.20 % methionine and the second without methionine addition, while the control diets contained casein as the only protein source at 5 %, 8 % and 10 % levels. To render the diets hypercholesterolemic, 0.1 % cholesterol was added as indicated in Table 1. To ascertain the cholesterol lowering potential of wild lettuce leaf protein, fifty-four male albino rats, each of an initial average weight of 50 g were randomly assigned to the nine treatment or formulated diets such that there were six animals to each of the nine experimental diets. The animals were housed individually and fed with 5 g of the formulated diets each day for a period of 28 days. The leftover of the feed was also weighed at the end of each day. Water was however, provided *ad libitum*.

The weights of the animals were taken at the end of each week. The total feed as well as that of protein consumed by each animal were computed at the end of the study period and data was used in calculating the protein efficiency ratio (PER) as ratio of gain or loss weight (g) to weight of protein (g) consumed, and feed efficiency ratio (FER) as ratio of gain or loss in weight (g) to weight of feed (g) consumed [6]. The protein contents of the diets were determined by Microkjeldahl Method.

2.3. Studies on some blood parameters

The levels of total cholesterol and total lipids in the plasma, liver, heart and kidney of animals fed with the treatment diets were determined by the methods described by Watson [7] and Folch et. al. [8], respectively. The Biuret and Dye techniques were used to determine total plasma proteins and albumin contents while globulin levels were quantified by the difference between plasma total proteins and albumin levels [9].

3. RESULTS

Animals fed with the control (or casein) diets gained significantly ($p < 0.05$) more weight, PER and FER than those maintained on equivalent leaf protein diets. For instance, the feed intake, protein intake and weight gain of animals fed with 5 % test protein diets were 114.8 g, 22.0 g and 1.90 g (unsupplemented diet respectively) and 114.1 g, 19.3 g and 1.7 g (supplemented diet respectively). The animals on equivalent 5 % casein diet recorded 156.0 g feed intake, 35.8 g protein intake and 51.1 g weight gain. For the 5 % protein diets, animals fed with the casein as well as the wild lettuce leaf protein diets elicited cholesterol response of 65.65 mg/dL (control diet), 11.61 mg/dL (unsupplemented diet) and 14.33 mg/dL supplemented diet). The results show differences of 51.37 mg/dL (between the control and supplemented diet) and 54.04 mg/dL (between

Table 1. Composition of Diets

Ingredients (g)	Casein Diet			Unsupplemented Lettuce Diet			Supplemented Lettuce Diet		
	5 %	8 %	10 %	5 %	8 %	10 %	5 %	8 %	10 %
Casein	133	218	267	-	-	-	-	-	-
Lettuce Leaf Protein Meal	-	-	-	403	645	806	403	645	806
Oil	200	200	200	200	200	200	200	200	200
Salt	10	10	10	10	10	10	10	10	10
Mineral/Vitamin	10	10	10	10	10	10	10	10	10
Starch	1645	1560	1511	1355	1114	952	1351	1109	948
Cholesterol	2	2	2	2	2	2	2	2	2
Methionine	-	-	-	-	-	-	4	4	4

the control and unsupplemented diets).

The contribution of methionine to plasma cholesterol levels became clearer though not significantly ($p > 0.05$). With respect to animals maintained on 8 % protein diets, those fed with the lettuce leaf protein diets elicited cholesterolemic response of 16.43 mg/dL (supplemented diet) and 14.03 mg/dL (unsupplemented diet) as against 68.57 mg/dL for the animals fed the casein (very rich in methionine) diet. When the plasma cholesterol levels of animals fed with the leaf protein diets were examined, the animals maintained on the diets supplemented with 0.20 % methionine exerted 2.40 mg/dL more cholesterol in the plasma than their counterparts fed with the unsupplemented diet.

The organ cholesterol levels in the animals fed with the casein diets were not significantly ($p > 0.05$) different from that of those fed with methionine supplemented or unsupplemented lettuce leaf diets. In effect, the animals fed the wild lettuce leaf protein diets had organ cholesterol levels comparatively similar to what was determined in animals fed with casein diets. The data in Table 2 show that animals fed with the lettuce leaf protein diets exerted significant ($p < 0.05$) hyperlipidaemic response than those maintained on the casein diets. Total lipids in the visceral organs, however, were not significantly different ($p > 0.05$) with respect to animals fed on both the casein and leaf protein diets.

The levels of serum total protein, albumin and globulin as summarised in Table 3 indicate that the variation in the type and levels of protein fed the experimental animals did not effect any significant changes in the plasma protein levels ($p > 0.05$). In effect, there were no significant differences in the total protein levels in the plasma of animals fed with the casein-based diets as against those fed with the lettuce leaf diets.

4. DISCUSSION

4.1 Protein quality

The results of the experiments have shown the poor quality of wild lettuce leaf in animal feed when used as the main source of protein (Table 4). The feed intake of all the animals maintained on the leaf protein diets were quite similar, yet this did not reflect on the weight gain which in part depict the extent of growth when compared with animals fed with casein-based diets. The poor growth response of animals fed with the lettuce leaf diets could be attributed in part to the poor amino acid profile of wild lettuce leaves. In a previous study [10], the amino acid spectrum of wild lettuce leaves showed deficiency in more than one amino acids, particularly methionine, phenylalanine and threonine. The chemical score of the protein also ascertained that methionine was the most deficient amino acid in wild lettuce leaves.

The effect of essential amino acid imbalance on growth pattern may be attributed to the inability of the supplementation of methionine alone to arrest or improve the poor growth response of animals fed with the lettuce leaf diets. It would, therefore, be necessary to blend the leaves of wild lettuce with other vegetables rich in methionine, phenylalanine and threonine [11]. The low level of protein of wild lettuce leaf diets could also be due to the presence of some antinutritional bodies such as alkaloids, saponins, phytate, tannins, oxalate, etc., in plants, since tannins could moderate feed intake because of its astringent and bitter nature [12], while phytate in diets is reported to reduce the bioavailability and utilization of vitamins and several mineral ions [13, 14]. It has also affirmed that dietary phytate in rat feeding trials leads to significant reduction in growth rate [15]. Wallace *et al.* [10] have reported levels of 0.168 mg % tannins and 9.28 mg % phytate to abound in the wild lettuce leaf.

4.2. Hypocholesterolemic ability

The hypocholesterolemic ability of wild lettuce leaf when consumed as the main source of protein is shown in Table 5. All the animals fed with the leaf protein diets exerted significantly lower cholesterolemic response as against those maintained on the casein diets. This was amplified in the marked differences that was evident in the plasma cholesterol levels in animals fed with 5 % protein diets where a difference of 51.37 mg/dL was found to exist between those fed casein and supplemented diets and 54.04 mg/dL for those maintained on casein and supplemented diets. This observation could only be attributed to the type of protein used since all the diets contained the same constituents except the source of protein. Sub-

Table 2. Effect of Casein and Wild Lettuce Leaf (as sources of Protein) Diets on the Total Lipids content of Plasma and Visceral Organs of Male Albino Rats

Diet	Total Lipid Content			
	Plasma (mg/dl)	Liver (mg/g)	Kidney (mg/g)	Heart (mg/g)
Casein Protein Diets				
5 %	18.5 ± 0.44	5.29 ± 0.83	15.8 ± 0.24	1.60 ± 0.52
8 %	27.0 ± 0.23	6.09 ± 0.47	2.12 ± 0.43	1.67 ± 0.27
10 %	41.6 ± 0.45	6.93 ± 0.44	2.24 ± 0.62	1.79 ± 0.48
Wild lettuce Leaf Protein Diets				
(a). Unsupplemented				
5 %	28.5 ± 0.22	5.91 ± 0.45	1.99 ± 0.63	1.52 ± 0.71
8 %	46.7 ± 0.35	6.29 ± 0.26	2.21 ± 0.41	1.59 ± 0.27
10 %	58.0 ± 0.17	7.31 ± 0.42	2.25 ± 0.23	1.85 ± 0.42
(b). Supplemented with 0.2 % methionine				
5 %	28.0 ± 0.23	4.88 ± 0.95	18.9 ± 0.33	1.21 ± 0.24
8 %	43.7 ± 0.41	6.29 ± 0.63	2.10 ± 0.52	1.49 ± 0.37
10 %	55.3 ± 0.53	6.89 ± 0.46	2.23 ± 0.48	1.95 ± 0.28

Table 3. Effect of Casein and Wild Lettuce Leaf (as sources of Protein) Diets on the Plasma Protein Levels of Male Albino Rats

Diet	Total Protein (%)	Albumin (%)	Globulin (%)
Casein Protein Diets			
5 %	6.75 ± 0.35	3.02 ± 0.16	3.37 ± 0.31
8 %	7.28 ± 0.49	3.41 ± 0.29	3.87 ± 0.12
10 %	7.54 ± 0.45	3.75 ± 0.89	3.79 ± 0.68
Wild lettuce Leaf Protein Diets			
(a). Unsupplemented			
5 %	6.65 ± 0.15	3.00 ± 0.14	3.15 ± 0.05
8 %	6.17 ± 0.97	3.27 ± 0.39	3.59 ± 0.68
10 %	6.53 ± 0.27	3.47 ± 0.18	4.07 ± 0.82
(b). Supplemented with 0.2 % methionine			
5 %	7.06 ± 0.08	2.97 ± 0.49	3.16 ± 0.56
8 %	7.13 ± 0.11	3.23 ± 0.20	3.45 ± 0.59
10 %	7.64 ± 0.53	3.36 ± 0.88	2.23 ± 0.61

Table 4. Male Albino Rats' Growth Response to Casein and Wild Lettuce Leaf as Sources of Protein Diets.

Diet	Protein Intake (g)	Feed intake (g)	Weight Gain (g)	PER	FER
Casein Protein Diets					
5 %	35.8 ± 0.14	156.0 ± 8.13	51.1 ± 1.51	1.43 ± 0.25	0.32 ± 0.10
8 %	43.3 ± 0.25	163.3 ± 0.28	63.2 ± 0.95	1.46 ± 0.25	0.38 ± 0.07
10 %	50.2 ± 0.23	169.6 ± 0.44	77.5 ± 0.69	1.55 ± 0.59	0.46 ± 0.11
Wild lettuce Leaf Protein Diets					
(a). Unsupplemented	22.0 ± 0.32	114.8 ± 1.52	1.9 ± 0.88	0.09 ± 0.02	0.02 ± 0.01
5 %	24.1 ± 0.71	120.6 ± 0.43	2.7 ± 0.21	0.11 ± 0.08	0.02 ± 0.01
8 %	35.7 ± 0.90	125.7 ± 0.17	5.0 ± 0.38	0.14 ± 0.09	0.04 ± 0.02
10 %					
(b). Supplemented with 0.2					
% methionine	19.3 ± 0.92	114.1 ± 0.94	0.11 ± 0.02	0.11 ± 0.02	0.02 ± 0.01
5 %	20.5 ± 0.44	116.4 ± 0.25	0.13 ± 0.02	0.13 ± 0.02	0.02 ± 0.01
8 %	29.1 ± 0.35	124.1 ± 1.46	0.14 ± 0.12	0.14 ± 0.12	0.03 ± 0.01
10 %					

Table 5. Effect of Casein and Wild Lettuce Leaf (as sources of Protein) Diets on the Cholesterol Levels of Plasma and Visceral Organs of Male Albino Rats

Diet	Total Lipid Content			
	Plasma (mg/dl)	Liver (mg/g)	Kidney (mg/g)	Heart (mg/g)
Casein Protein Diets				
5 %	65.65 ± 1.00	5.84 ± 0.74	2.11 ± 0.52	4.32 ± 0.51
8 %	68.57 ± 0.29	7.09 ± 0.81	2.26 ± 0.19	4.82 ± 0.96
10 %	72.62 ± 1.12	8.28 ± 0.97	2.39 ± 0.92	6.29 ± 2.55
Wild lettuce Leaf Protein Diets				
(a). Unsupplemented				
5 %	11.61 ± 0.79	5.53 ± 1.07	2.02 ± 1.33	0.65 ± 0.39
8 %	14.05 ± 0.57	7.28 ± 0.92	2.19 ± 0.77	1.08 ± 0.29
10 %	17.83 ± 0.83	7.91± 0.89	2.28 ± 0.27	1.30 ± 0.49
(b). Supplemented with 0.2 % methionine				
5 %	14.33 ± 1.15	5.56 ± 0.36	1.71 ± 0.30	0.59 ± 0.14
8 %	16.43 ± 0.65	6.98 ± 0.23	2.24 ± 0.49	0.82 ± 0.34
10 %	22.39 ± 1.45	8.00 ± 0.73	2.25 ± 0.75	0.96 ± 0.45

stantial number of studies have demonstrated that plasma cholesterol could be lowered greatly or significantly when animal protein are replaced by that of plant, especially, when the background diets are high in fat and cholesterol [16, 17, 18, 19].

Moreover, the contribution of methionine to plasma cholesterol response became more clearer from the study as all the control diets which had casein (very rich in methionine) as the main source of protein, had significant levels of plasma cholesterol. Even when lettuce protein diets are considered, the animals fed with 0.20 % methionine supplemented diets had 2.40 mg/dL more plasma cholesterol than those fed the unsupplemented diets though this was not significant. The difference, therefore, is attributed to the added methionine; which is a pre-requisite one-carbon donor during choline synthesis which in turn is required for the synthesis of low-density lipoprotein (LDL), the principal cholesterol carrier in the plasma [2].

It has been suggested that the hypercholesterolemic effect of casein is partially the result of the relatively higher methionine content of casein [1]. The results obtained in this study confirm previous observations [1, 3]. It has also been reported that the high ratio of lysine to arginine (Lys:Arg) in casein contributes significantly to hypercholesterolemic potential of casein [20, 1]. The relative abundance of lysine is postulated to inhibit liver arginase activity [21] and hence increase the availability of arginine for incorporation into the arginine rich apoprotein of low-density lipoprotein [1]. Hence, the Lys:Arg ratio of 0.75 quantified in wild lettuce leaf [10] is far lower than the ratio of 2.0 reported for casein [20]; the 0.90 and 1.08 reported in the well documented hypocholesterolemic soy and jack beans, respectively [22]. It is therefore, not surprising that the animals fed with the lettuce leaf protein diets elicited significant hypocholesterolemic response relative to those fed with the casein diets. The 0.424 % saponin reported to be present in wild lettuce leaves [10] may have contributed to the observed hypocholesterolemic ability of the lettuce leaf diets. The presence of saponin has been reported to combine with cholesterol and bile acids in the intestine to form large mixed micelles, the content of which are not available for absorption and the complexation of bile acids, represents an interruption of the enterohepatic cycle of bile acid which invariably leads to increased turnover of cholesterol to bile acids [13, 23].

The results obtained in this study in relation to the effect of the lettuce leaf protein on organ cholesterol showed that the organ cholesterol levels were not affected by the type of protein fed the animals, i.e., animals fed with the lettuce protein diets had comparable organ cholesterol as those fed with the casein diets. However, this observation is in contrast with earlier results reported [3, 24, 25, 26]. From these studies, it has became clear that reduction in serum cholesterol levels is accompanied by concomitant decrease in the cholesterol content of the liver, aorta, kidney and other tissues. The source of protein used in the reported studies may in part account for the varied responses and observations. For example, Marfo et. al., [3] fed jackbean seed protein as the main source of protein while wild lettuce leaf protein was used in the present study.

There were no significant variations in the total plasma protein of animals fed with casein-based diets as against those fed with the lettuce leaf diets. This observation gives the indication that the protein synthesizing function of the liver of animals fed the various types and levels of protein was not impaired. Similar observations were made on male albino rats that were fed jackbean seed protein as the only source of protein [3].

5. CONCLUSIONS

The present study has unearthed the hypocholesterolemic potential of wild lettuce leaf when fed as the sole source of protein. The results have shown that methionine fortification of the leaf protein elevated plasma cholesterol were not significant. However, hypolipidaemic effect characteristic of other plant sources was not observed in this study. It is therefore concluded that wild lettuce leaf in animal diets does not alter plasma protein levels. It would be advisable not to consume wild lettuce singly but to mix it with other foodstuffs or feedstuffs of known amino acid profile in order to raise its nutritive value.

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