

## EVALUATION OF USE OF SISAL BY-PRODUCTS AS GROWTH PROMOTER FEED FOR RABBITS

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### SUMMARY

Forty eight (48) adult female rabbits were randomly assigned on the basis of diet into 4 groups, that is, Group A on control diet, B on untreated sisal leaf waste, C on ensilaged sisal leaf waste and Group D on hecogenin (sapogenin) extracted sisal leaf waste. In order to evaluate suitability of these diets as growth promoters rabbits were fed on the respective diet for 30 weeks. Blood was collected weekly and parameters namely Red Blood Cells, Haemoglobin, Packed Cell Volume, Differential Leucocyte Counts, Total protein, Glucose and Minerals were determined. At 31 weeks, rabbits were killed and organs (uterus, ovary, fallopian tube) were removed and stored at -20°C. The uterus/fallopian tubes were flushed with normal saline and flushings determined for mineral content. The results showed that untreated sisal leaf waste caused a marked decrease in Red Blood Cells, protein concentration and zinc content in blood. Mineral content in flushings declined with prolonged feeding. Body weights declined in Group B and C, but increased in A and D. Since Group A was on control diet and D on diet with reduced amounts of hecogenin, findings of this study indicated that reduced hecogenin content could provide some growth promoting effects. It is concluded that feeding untreated sisal leaf waste is detrimental in rabbits. However, these effects are ameliorated by extraction of sisal leaf waste hecogenin.

### INTRODUCTION

Steroids of many types, including cholesterol, oestrone, testosterone, cortisone and hexaesterol generally increase feed digestibility in vitro, possibly through action as a wetting agent (Hale et al., 1961; Valdex, 1985). It is through this same effect that some plants and grasses stimulate growth rates in domestic ruminants.

Certain natural plants containing steroids have been used in livestock feeding regimes, though to a limited extent, as growth promoters. For example, Sisal (*Agave sisalana*) containing large amounts of hecogenin, a steroidal sapogenin, with strong corticosteroidal properties, has been used as sisal leaf waste for feeding cattle in many

al., 1970; Ocampo, 1972; Rodriguez, 1984). Sisal leaf waste, as a by-product of sisal, contain large amounts of corticosteroids such that it was once used in East Africa in the commercial production of natural corticosteroids (United Kingdom Patent, 1942).

Sisal leaf waste feeding trials conducted in cattle both in East Africa and South America have revealed that it is through the enhanced anaerobic fermentation of organic matter and improved wetting that feeds mixed with sisal leaf wastes have increased digestibility and are utilized by livestock much more efficiently than those without (Frank, 1957; Sharaf and Zahran, 1965; Harrison, 1984). Although no feeding

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trials as yet have been conducted to examine the effects of sisal steroids on endocrine and reproductive organs, Ocampo, (1972), while working with mice in Mexico City reported that feeding of sisal leaf waste results into toxicity. He singled out sapogenins as the possible cause of the toxicity. Experience obtained elsewhere from feeding studies with plants containing large amounts of steroids have shown that sapogenins have toxic effects when used in large amounts (Chou et al., 1971; Göhl, 1975; Harrison, 1984). Furthermore, continuous accumulation of these chemicals in the body and increased residual concentrations in milk and meat might be an issue to human health. It is because of the reasons mentioned above that studies on the utilization of sisal leaf waste after a reduction of hecogenin are recommended. A simple way of reducing hecogenin content was suggested as that of mixing with large amounts of other feed ingredients (Göhl, 1975; Ferreiro et al., 1978; Obetsch and Owen, 1985).

Rabbit farming in the tropics has a great and increasing importance as source of cheap meat (Owen, 1978). Unfortunately, associated with requirements for improved rabbit breeds is an increased demand for cheap feeds. Sisal leaf waste produced in large quantities in Eastern Africa may be used as feed for rabbits to substitute and reduce use of grains highly demanded by human population i.e. maize. Sisal leaf waste feeding may not only reduce costs of raising rabbits, but may also add some advantages to such feeding regimes, because the waste might act as a growth promoter.

The objectives of this study were therefore, firstly to evaluate the suitability of diets containing different forms of sisal leaf wastes as feed as well as growth promoters for

rabbits. Secondly, to evaluate the path model of toxicity due to sisal leaf waste on metabolic profiles and reproduction. Lastly to evaluate possibilities of reducing effects due to sisal leaf waste toxicity by extraction of hecogenin and mixing the same with large quantities of cheaper feedstuffs.

The determination of mineral content in organs and flushings was necessary in order to analyze effects of hecogenin on changes in metabolism in organs highly affected by steroid hormones; that is reproductive organs.

The endometrium is a tissue which is in a continuous flux of cellular morphology and physiology. Under the influence of sex hormones, the nature of the endometrial and fallopian tube epithelium changes. The endometrial and fallopian tube epithelium secretory fluids act to transport and nurture the fertilized ovum until implantation occurs. The composition of these fluids may be related to conception, hormonal changes and maintenance of pregnancy.

## MATERIALS AND METHODS

### Experimental Animals:

Adult female white rabbits (n=50) with an average weight of 2.2 kg were maintained in single cages under natural lighting and controlled temperature 25°C-30°C with tap water and rabbit feed (Table 1) offered ad-libitum. Feed analysis was performed using standard analytical methods outlined

Table 1: Feeding Regime and Experimental Design

FEED INGREDIENT (%)	TRIAL GROUPS			
	A <sup>1</sup>	B <sup>2</sup>	C <sup>3</sup>	D <sup>4</sup>
Sisal Waste (untreated)	0	10.0	0	0
Sisal Waste (treated)	0	0	10.0	10.0
Maize Bran	20.0	10.0	10.0	10.0
Sorghum (flour)	16.0	16.0	16.0	16.0
Fish meal	6.0	6.0	6.0	6.0
Mineral/Vitamin	2.0	2.0	2.0	2.0
Salt (NaCl)	0.3	0.3	0.3	0.3
Grass	1.0	1.0	1.0	1.0

NB:

- 1 = control Group A, on diet as according to King (1978).
- 2 = Trial Group (B) on sisal waste diet, prepared by freeze drying fresh sisal waste and grinding.
- 3 = Trial Group (C) on sisal waste silage prepared by ensilaging for 5 weeks, freeze drying and grinding.
- 4 = Trial Group D on diet prepared by extracting hecogenin by Sharaf and Zahran (1965) and modified by Chou and others (1971), freeze drying and grinding.
- 5 = Mineral/vitamin supplementation with Nutrafos<sub>3</sub><sup>(R)</sup> Hoechst (Frankfurt, West Germany).

Feed analysis was according to the official Analysis of Agriculture Chemists (A.O.A.C., 1970). All values are given as percent.

by Official Methods of Analysis of the Association of Analytical Chemists (A.O.A.C., 1970) Hecogenin extraction was carried out according to the method of Sharaf and Zahran (1965) and Chou et al. (1971). All rabbits were first acclimatized to the diets for a period of four weeks, and thereafter randomly allocated into four trial groups. Each group (n=12 rabbits) was fed on a respective trial diet for a period of 30 weeks.

#### Sampling and Analysis:

Rabbits were weighed and bled from the ear vein weekly. Blood was taken in EDTA containing bottles for the determination of haematological parameters, while another sample was collected and centrifuged at 1500 rpm for 15 minutes. Harvested serum was stored at -20°C until analysis. Determination for Red Blood Cells (RBC; No/10<sup>11</sup> litre), Haemoglobin (Hb; g/dl), Packed Cell Volume (PCV; litres/litre) and Differential leucocyte counts (%)

using a haemoglobinometer were performed in accordance with Schalm et al. (1975). Total protein and glucose concentrations were assayed according to standard laboratory procedures as described by Tietz (1976). Mineral (calcium, copper, magnesium, sodium, zinc, potassium) concentrations were analyzed weekly using the atomic absorption spectrophotometry (Kaneko, 1980). After 30 weeks of feeding trials, rabbits were killed by chloroform anaesthetization. Ovaries, uterus and fallopian tubes were removed under sterile surgical conditions and trimmed free of other tissues. In order to obtain flushings, each uterus and fallopian tube was flushed from the utero-tubal junction to the cervical of the uterine horn with a sterile buffer solution (0.9% NaCl). Flushings were received and kept frozen at  $-20^{\circ}\text{C}$ . The portions of the organs were blotted on acid washed and extremely low ash content paper, transferred to small polyethylene containers and frozen at  $-20^{\circ}\text{C}$ .

#### Statistical analyses:

The student t-test and the analysis of variance were used to determine the significance of observed differences as according to Snedecor and Cochran (1971).

## RESULTS

#### Haematological data:

Results on haematological values are recorded in Table 2. No differences were observed in the values of all haematological parameters at week 0 of the experiment. Similarly values for the control group showed close similarity in the haematological parameters during the 30 weeks of the feeding study. Generally, animals given sisal leaf waste in Group B, C and D had relatively lower Red blood

cell counts than those of Group A (control). Marked variations were observed in values of Group B and C starting at week 15. Also in these groups, significant changes in differential leukocyte counts occurred, with a marked increases in lymphocytes and eosinophils.

#### Total Protein and Glucose contents:

Results on total protein and glucose concentrations in blood are summarized and shown in Table 3. There was similarity in values for glucose and total protein between the four groups at week 0 of the experiment. This was mirrored in Group A throughout the experimental period. Decline in values were, however, observed in Group B, C and D with progress of the experiment.

#### Mineral values in organs and uterine flushings:

Mean values for concentrations of minerals in the organs and uterine flushings are represented in Table 4 to 6. Regarding mineral content in uterine flushings, values for calcium, magnesium and potassium were highest in Treatment A, while those for Group B, C and D were lowest - and similar. Zinc, copper and sodium demonstrated no changes between the four treatments though a tendency towards a decline in zinc and copper was observed in Group B and C. Mineral content in the organs for all the trace elements showed consistent similarity except for magnesium in which Treatment A had highest values, while Group B, C and D had lowest values.

#### Body weight changes:

Mean body weights and respective weights for reproductive organs of the rabbits are shown in Table 7. Results indicate that mean body

Table 2: Haematological values in rabbits on diets containing various amounts of sisal leaf waste.

Item determined	Duration (weeks)	Group			
		A	B	C	D
Red Blood Cell ( $N_0 \times 10^{12}$ /litre)	0	11.5±0.5	11.2±0.7	11.0±0.8	11.1±0.5
	15	11.2±0.8	9.5±0.8	9.7±0.7	10.5±0.9
	30	11.8±1.1	8.2±0.8	8.6±0.6	9.9±0.6
Haemoglobin (g/dl)	0	9.5±0.7	9.5±0.3	9.3±0.9	9.6±0.5
	15	9.6±0.8	9.4±0.5	9.3±0.8	9.5±0.4
	30	9.5±0.8	9.4±0.8	9.2±0.1	9.6±0.5
Packed Cell Volume (litres/litre)	0	41.5±2.9	41.0±2.8	40.0±2.8	40.9±2.6
	15	40.5±3.5	41.0±2.2	39.4±4.8	41.0±1.7
	30	40.0±2.8	41.0±1.8	40.0±2.5	41.0±2.6
Total leucocytes ( $N_0 \times 10^6$ /litre)	0	6.8±0.02	6.8±0.1	6.7±0.02	6.8±0.2
	15	6.8±0.01	6.1±0.04	6.4±0.03	6.6±0.3
	30	6.7±0.03	6.1±0.02	6.2±0.04	6.7±0.1
Neutrophils (%)	0	24±0.7	24±5.7	22±3.6	23±5.8
	15	23±2.5	20±2.5	19±1.5	24±3.5
	30	22±4.5	20±3.5	19±2.5	22±3.6
Lymphocyte (%)	0	65±0.5	64±0.8	64±0.2	64±0.5
	15	62±1.5	75±7.8	72±2.8	70±3.5
	30	64±1.5	72±3.5	74±7.8	68±3.8
Eosinophils (%)	0	6±1.5	6±1.8	7±1.5	6±1.0
	15	6±2.5	10±3.5	10±4.8	8±4.5
	30	6±1.6	10±4.5	10±4.1	10±4.1

**N.B.:**

A = Control group, B = fed on untreated sisal leaf waste, C = on sisal leaf waste silage and D = on hecogenin extracted sisal leaf waste. In all groups n = 12 rabbits. All values are given as Mean ± SEM.

weights of the rabbits at week 0 in both control and sisal leaf waste based treatment diets were the same. Similarly mean body and organ

weights were higher and similar for Groups A and D, than the values for rabbits in Group B and C.

Table 3: Total Protein and Glucose content in rabbits on diets containing various amounts of sisal leaf waste.

Item Determined	Duration	Group			
		A	B	C	D
Glucose (nmol/litre)	0	12±1.5	11±1.8	12±0.8	12±1.2
	15	12±1.5	9±0.8	10±0.8	9±0.8
	30	11±2.8	9±1.8	9±0.6	
Total Prot. (g/dl)	0	4.8±0.3	4.7±0.3	4.8±0.3	4.8±0.6
	15	4.9±0.3	2.5±0.8	3.5±0.3	2.9±0.8
	30	4.8±0.3	2.5±0.8	2.4±0.6	2.7±0.5

N.B.

A = Control group, B = fed on untreated sisal leaf waste, C = on sisal leaf waste silage and D = on hecogenin extracted sisal leaf waste. In all the groups n = 12 rabbits. All values are give as Mean ± SEM.

Table 4: Mineral content in the Fallopian tube of rabbits on Sisal leaf waste

Organ and Group	Item determined						
	Zinc (mg/d)	Calcium (mg/d)	Copper (mg/d)	Magnesium (mg/d)	Sodium (mg/d)	Potassium (mg/d)	
Fallopian tube	A	78.4±26	440±0.5	4.9±0.5	445±72	0.31±0.2	0.47±0.1
	B	67.9±16	112±12.5	4.7±0.5	208±93	0.40±0.10	0.21±0.10
	C	80.0±32	165±20.5	4.5±0.8	219±60	0.46±0.2	0.24±0.1
	D	72.9±18	150±20.6	4.6±0.9	280±50	0.40±0.2	0.35±0.1

N.B.

A = Control group, B = fed on untred sisal leaf waste, C = sisal leaf waste silage and D = on hecogenin extracted sisal leaf waste. In all the groups n = 12 rabbits. All values are given as Mean ± SEM.

Table 5: Mineral content in the Uterus of rabbits on Sisal leaf waste

Organ/Group	Item determined					
Uterus	Zinc (mg/d)	Calcium (mg/d)	Copper (mg/d)	Magnesium (mg/d)	Sodium (mg/d)	Potassium (r g/d)
A	126.3±48.3	159±38	26.4±3.6	520±25	1.98±0.2	1.83±0.5
B	58.2±48.3	158±38	13.8±3.6	324±58	1.78±0.5	0.72±0.6
C	70.6±48.3	161±38	16.4±6.5	334±58	1.88±0.7	1.42±1.8
D	69.6±58.5	160±35	15.4±6.5	350±55	1.67±0.8	1.42±2.5

N.B.

A = Control group, B = fed on untreated sisal leaf waste, C = sisal leaf waste silage and D = on hecogenin extracted sisal leaf waste. In all the groups n = 12 rabbits. All values are given as Mean ± SEM.

Table 6: Mineral content in uterine/oviductal flushing in rabbits on sisal leaf waste

Group	Item determined					
	Zinc (mg/d)	Calcium (mg/d)	Copper (mg/d)	Magnesium (mg/d)	Sodium (mg/d)	Potassium (mg/d)
A	65.5±2.1	2.13±0.3	1.44±0.5	0.99±0.2	2.14±0.0	1.49±0.2
B	39.9±3.0	2.10±0.5	1.39±0.5	0.78±0.3	2.10±0.0	1.39±0.4
C	40.5±4.6	1.82±0.4	1.28±0.5	0.68±0.2	2.20±0.0	1.42±0.4
D	79.2±2.5	1.99±0.3	1.48±0.6	0.89±0.2	1.98±0.2	1.45±0.4

N.B.

A = Control group, B = fed on untreated sisal leaf waste, C = on sisal leaf waste silage and D = on hecogenin extracted sisal leaf waste. In all the groups n = 12 rabbits. All values are given as Mean ± SEM.

**Table 7: Terminal Mean Weights of Organs in Rabbits on Sisal Waste Feeding**

Organ weighed	Experimental Group			
	A	B	C	D
Body Weight (Kg)	2.23	1.74	2.15	2.10
Uterus (g)	7.53	2.51	4.84	7.45
Fallopian Tube(g)	0.63	0.28	0.54	0.67
Thyroid (g)	0.11	0.09	0.07	0.12
Ovary (g)	0.37	0.14	0.28	0.33
Pituitary Gland (g)	0.03	0.01	0.01	0.28
Adrenal Gland (g)	0.39	0.39	0.37	0.37

**N.B.:**

A = Control group, B = fed on untreated sisal leaf waste, C = on sisal leaf waste silage and D = on hecogenin extracted sisal leaf waste. In all groups n = 12 rabbits. All values give as Mean  $\pm$  SEM.

**DISCUSSION**

Values for the haematological parameters reported in this study for rabbits offered the control diet were within the range reported for other breeds of rabbits (King, 1971; Schalm et al., 1986). Rabbits in Group B fed on untreated sisal leaf waste and Group C on ensilaged sisal leaf waste had significant lower Red blood cell and leucocyte counts with increased lymphocyte and eosinophil percent than Group D. Group D had been offered sisal leaf waste (with partially extracted hecogenin content). The results of Group B and C were consistent with results found in literature on effects of saponin on haematological parameters (Arave et al., 1977;

Bernische et al., 1978). Rabbits in Group D, reconfirmed the

observations by Gohl (1975), Arave et al. (1977) and Ferreiro et al. (1978) who stated that the effects of sisal leaf waste on haematological values of domestic animals are due to saponin content.

A trend in the changes described above was also observed in total protein and glucose value (Table 3) within rabbit Group B, C and D when the feeding had been extended over 15 weeks. Changes in glucose and protein values can be explained to be due to the corticosteroid like effects inherent of hecogenin. High amounts of corticosteroids have been shown to decrease glucose and protein content in rabbits. These findings clearly showed that sisal leaf waste feeding has effects on the above mentioned parameters.

Minerals in tissue and uterine flushings (Table 4, 5 and 6) illustrated constant changes which were associated to the feeding of sisal leaf waste. These effects were however, more pronounced after feeding experimental diets for at least 15 weeks. Most interesting changes were those due to zinc. Since zinc concentration in tissues in the rabbit indicate and correlate to the levels of enzymes activity, especially carbonic anhydrate, an enzyme connected with both implantation and steroid hormonal activity (Iritani et al., 1971; Aitken, 1974); data obtained in this study on zinc therefore, reflect though indirectly derangement in bodily metabolic processes. Other minerals, such as copper, were not affected by sisal leaf waste feeding, indicating that there might have been a differential suppression of minerals by the sisal leaf waste feeding. Furthermore, the endometrium is a rich source of tissue calcium (Beir, 1970), yet the calcium concentration in this study was not affected by the feeding of sisal leaf waste. Although findings on minerals from uterine flushings similarly indicated a change in concentration of some of the minerals after feeding sisal leaf waste, the effects are however slow, being more marked after 20 weeks of feeding experimental diets. The search for the earliest responses of hecogenin by using uterine flushings in this study has led us to agree to a popular concept that response of a particular tissue to a hormone is expressed in altered metabolism which leads to altered tissue activity.

At slaughter, body and organ weights of rabbits in Group B and C were lower than those of rabbits in Group A and D (Table 7), even when expressed on metabolic body weight basis. In contrast, Group A and D showed an increase in body weights. For Group D, where increase in body

weights can be associated with hecogenin, the results have led us agree with previous statements that, it is impossible to perform a 100% hecogenin extraction by using the method described by Sharaf and Zahran, (1965). The remaining hecogenin content, though very low, could still be responsible for the observed booster effects on growth.

In conclusion, sisal leaf waste as a substitute roughage feed for rabbits causes detrimental effects. Extraction or reduction of the effects due to hecogenin (sapogenin) may reduce some of these effects, boost growth rates but might inhibit reproduction due to change in the uterine/fallopian tube environment. Although the practice of sisal leaf waste feeding might be beneficial to large animals (cattle) it is not recommended for small animals like rabbits.

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