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Wastewater Grown Waterhyacinth as an Ingredient in Rabbit Food

ALVIN F. MORELAND, B. R. COLLINS, C. A. HANSEN AND R. O'BRIEN[1]

ABSTRACT

A rabbit ration consisting of 30% alfalfa was modified and two experimental diets formulated to contain 30% waterhyacinth and 0% alfalfa, or 20% waterhyacinth and 10% alfalfa. These pelleted diets were fed to rabbits for two generations, were palatable, and all animals thrived. First generation animals consuming the diet containing 30% waterhyacinth reached a final mean body weight significantly less (10.62%) than controls, however, there were no significant differences between control and 20% waterhyacinth diet. Second generation animals consuming the diets containing waterhyacinth were not significantly different from controls in final mean body weights and overall growth rates were unaffected by the diets in either generation, however, when the growth period was divided into segments, the rate of growth was variable in some time periods. Survivability, fertility, survival of offspring, and teratogenicity were not affected by the diets. Elemental analysis of muscle tissue from the rabbits showed levels below those reported as toxic.

[1]Professor, Department of Small Animal Clinical Sciences, University of Florida, P.O. Box J-6, HSC, Gainesville, FL 32610; Assistant Professor, Department of Small Animal Clinical Sciences, University of Florida; Assistant in Statistics, Department of Statistics, University of Florida; and Associate Research Scientist, Department of Statistics, University of Florida. Received for publication August 2, 1990 and in revised form November 15, 1990.

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INTRODUCTION

Waterhyacinth (*Eichhornia crassipes* [Mart] Solm) are looked upon by many as an environmental menace and provide, perhaps, the best example of explosive infestation of waterways by water weeds. However, the productivity of this plant has been the cause for examination of its potential usefulness. One such possible use is the removal of nutrient pollutants from municipal wastewater (1, 15, 25, 26) where this species may be the most promising for nutrient removal, due to its prodigious growth rate and free floating nature (3). Estimations have been made that 1 ha of waterhyacinth could potentially assimilate from domestic sewage the nitrogen and phosphorus generated by 1470 and 445 people, respectively, and produce 150 metric tons of dry matter ha^{-1} annually (23).

Since waterhyacinth extract nutrients from the water and subsequently store them in the plant tissues, they become a potentially useful food source. Alfalfa is recognized as, perhaps, the world's most valuable forage crop and calculations have been made that 777,000 km^2 of alfalfa could supply the minimum protein requirements of the human race with some left over for livestock (16). In contrast it is estimated that, under intensive cultivation, waterhyacinth could easily produce three times as much protein (3, 23).

Concentrations of the nutrient elements P, K, Mg, Cu, Zn, and Mn in waterhyacinth have been determined to be in the same range as in terrestrial forages; Na, and Fe were higher and Ca was lower (6).

Essential and toxic elements are passed through the food chain to man and animals. The toxicity of some elements depends upon the chemical form of the element, the dosage, route of administration, and frequency and duration of administration (9, 24). Some essential minerals may be toxic to plants *or* animals if given in excess and non-essential elements such as arsenic, cadmium, lead, and mercury are generally regarded as accidental contaminants although found in minute amounts in the newborn (9, 13). The concentration of essential and toxic elements in animal tissues depends mainly on the dietary concentration of the element, absorption of the element, concentration of other tissue elements, homeostatic control mechanism of the body for the element and the species of animal involved (24). Therefore, since wastewater is known to contain trace elements, often in elevated levels compared to soils, waterhyacinth grown in municipal wastewater if used for animal food could potentially be a source of toxic levels of trace elements in animal tissue, thus rendering the meat unsuitable for human consumption. Recent comprehensive reviews have indicated mineral element concentrations toxic to plants, animals, and man (9, 14) and a review of toxic and essential trace elements in meat of cattle, sheep, swine, and chickens has been published (5).

Comparisons of dried waterhyacinth grown in wastewater and alfalfa hay revealed 10.65% protein vs. 13%, total digestible nutrients (TDN) 46.2% vs. 48.0%, crude fiber 28.5% vs. 30.0%, and fat 0.91% vs. 1.8%. Thus, waterhyacinth grown in sewage effluent appears to have excellent potential as a nutritious food.

Waterhyacinth were well tolerated by cattle and sheep fed a pelleted ration with 33 percent organic matter from waterhyacinth, but was inadequate as the sole component of the ration (11). Ensiled waterhyacinth have been fed to cattle and sheep with excellent results when combined with additives which provide fermentable carbohydrates (2, 4) and consumption and weight gain was comparable to control diets in calves and sheep fed diets containing waterhyacinth as a portion of concentrate feeds (17, 19). Waterhyacinth and alfalfa were of value in channel catfish diets only when reduced to 10 percent of the diet by admixture with standard feed ingredients (12). However, the matrincha (*Brycon sp.*), an omnivorous indigeneous fish species from the Amazon grew well when fed diets containing waterhyacinth and were not adversely affected by adding 9.5% and 18.9% waterhyacinth while maintaining the protein level constant (20). Reports on feeding cooked waterhyacinth to swine have indicated variable results (8), however, addition of dried chopped waterhyacinth to the diets of pigs reduced the requirement of commercial dry feed and allowed acceptable growth (22). Fresh plants fed to poultry have not proven valuable, although ground and dried plants up to 7.5% of the diet did not affect weekly weight gain or feed conversion factors (21). Rabbits fed on diets containing waterhyacinth at 30 percent of the diet had reduced utilization of dry and organic matter, however, diets containing 10 percent waterhyacinth were similar to a control diet (10). Coefficients of digestibility of "fresh" waterhyacinth fed to rabbits were low but were improved by drying, addition of molasses, and pelletization, leading to the conclusion that incorporation in pellets at up to 25 percent of the diet seemed possible (7). The present study was undertaken to determine if the domestic rabbit (*Oryctolagus cuniculus*), prized by many as human food, could utilize waterhyacinth, grown in municipal wastewater as an ingredient in place of alfalfa in a rabbit ration, and if so, would the diets result in significant storage of excess trace elements in the muscle tissue of the rabbit.

MATERIALS AND METHODS

The study was carried out over two generations (F_0, F_1) beginning with thirty-two male and twenty-eight female rabbits (eight-week-old) of the New Zealand White breed obtained from a local breeder for generation F_0. All received an identifying tattoo number and were housed in a sheltered pole barn which was screened along three sides to approximate the housing regimen used by the rabbit industry. Suspended galvanized wire cages equipped with non-toxic black vinyl tubing and sipper valves for automatic watering were used. Galvanized food containers were mounted on the outside of the cage. Excreta fell to a concrete floor beneath the cages and was removed daily. Male breeders were shielded from view of female breeders by means of an opaque vinyl drape suspended between the respective cages. Initially, ventilation was by ambient air currents, however, as temperatures exceeded 32 C floor mounted fans were added to increase ventilation. During studies of the second generation, temperatures exceeding 35 C required installation of a water spray system which wet the screened sides of the building thus providing cooling. Plastic drapes were placed over the screens in winter.

Prior to beginning the study all animals were given a complete physical examination, blood was drawn for analysis of complete blood count, hemoglobin, hematocrit and a basic chemistry profile of 25 items. The blood analyses were examined by a veterinarian and animals judged not in reasonably good health were excluded from the study. Animals were treated prophylactically for coccidiosis prior to being randomly divided into three groups. One group of 10 females and 10 males received a diet containing 30% alfalfa which served as the control diet. One experimental group (WH) of 9 females and 11 males received basically the same diet as the controls except that the afalfa component was completely replaced by waterhyacinth. A second experimental group (WHA) of 9 females and 11 males received basically the same diet as the controls except two-thirds of the alfalfa component was replaced with waterhyacinth.

Three females in each group and 2 males of the control group died during the excessively hot and humid summer months of 1988. Because of concern that the remaining animals may have produced insufficient offspring to allow appropriate selection of the F_1 generation breeders, the decision was made to obtain replacement breeders for the deceased animals. These replacements, which were from the same breeder and of approximately the same chronological age as the original animals, were placed on the experimental diets 122 days after the original animals began consuming the diets and 30 days prior to initiation of breeding. Because they did not consume the diets for the full experimental period, data from these animals are not included as part of this study, however, their offspring were used in selection of the F_1 animals.

Dried waterhyacinth were analyzed to provide a basis for balancing the test and control diets. The dried waterhyacinth contained 9.92 to 10.65% protein and 0.11 to 0.91% fat. For comparative testing in this study, these two components in each food lot were adjusted by adding soybean meal and corn meal so that all three diets contained approximately the same 15% protein and 3.2% fat (Analysis of the initial lot of the experimental diets showed the fat was lower by 0.77% [WH] and 0.53% [WHA]). All diets were prepared, mixed and bagged by the Seminole Feed Division of Seminole Stores, Inc. of Ocala, Florida. The analysis of the initial lot of diets is reported in Table 1.

Because of the time required for completion of the study food was prepared in lots at approximately four month intervals. The initial (3/88) waterhyacinth were harvested from the Iron Bridge wastewater treatment facility in the city of Orlando, Florida and the subsequent (6/88 and 10/88) waterhyacinth from the treatment facility in Kissimmee, Florida. Proximate analysis of waterhyacinth was conducted as each food lot was prepared in order to balance the diets. Elemental analysis of waterhyacinth was done on only the original lot (Table 2).

Waterhyacinths were processed by shredding through a chopper, squeezing in a press as chopped plants, drying in a rotating drum jacketed dryer, and after mixing with the other ingredients were pelleted and stored (15.5 C) similar to the control diet. As needed, a 7-day supply of feed was removed from refrigeration and stored at the rabbit facility at ambient temperature until used. Weighed quantities of diets were placed in the feeding devices three days per week and leftover food remaining in the device at each feeding time was weighed and reused as part of the food for the next feeding period. Waste food was collected in trays beneath the food device, weighed, and discarded. Daily food consumption was calculated by subtract-

TABLE 1. COMPOSITION OF THE INITIAL LOT OF CONTROL AND WATERHYACINTH DIETS USED IN THE FEEDING TRIAL WITH RABBITS.

	Experimental Diets		
Analysis	Control	WH	WHA
Crude Protein %	15.05	15.06	15.05
Crude Fat %	3.20	2.43	2.67
Fiber %	12.86	13.18	13.05
Calcium %	.78	.84	.98
Phosphorus %	.50	.49	.50
Salt %	.48	.48	.48
Vitamin A KIU/LB	3.01	3.01	3.00
Vitamin D3 KIU/LB	.25	.25	.25
Vitamin E IU/LB	5.01	5.01	5.00
Diet Composition (lbs)			
Wheat Middlings	605	605	605
Alfalfa Meal	600	0	200
Waterhyacinth Meal	0	600	400
Oats, Ground	370	370	370
Corn Meal	320	250	265
Soybean Meal	70	150	125
Calcium Carbonate	15	10	20
Trace Mineral Salt	10	10	10
Mono & Dical. Phos	4	0	2
Agri Gum	1	1	1
Vitamin Premix	1	1	1
Total	1996	1997	1999

TABLE 2. ELEMENTAL ANALYSIS OF WATERHYACINTH AND ALFALFA ON A DRY WEIGHT BASIS USED TO PREPARE DIETS FOR EXPERIMENTAL FEEDING OF RABBITS. ANALYSIS CONDUCTED ON PLANTS AT INITIATION OF STUDIES IN MARCH 1988. ND = NOT DETERMINED.

		Waterhyacinth	Alfalfa
Crude Protein %		10.65	13.00
Crude Fat %		0.91	1.80
Fiber %		28.50	30.00
Calcium %		1.88	0.75
Phosphorus %		0.31	.020
Sodium %		0.20	0.13
Chloride %		0.30	ND
Magnesium %		0.24	0.24
Potassium %		1.35	1.70
Sulfur %		0.27	0.24
Manganese	ppm	70.0	ND
Copper	ppm	9.1	ND
Cobalt	ppm	1.31	ND
Zinc	ppm	101.4	ND
Molybdenum	ppm	4.0	ND
Cadmium	ppm	0.2	ND
Nickel	ppm	3.3	ND
Lead	ppm	4.4	ND
Aluminum	ppm	500.0	ND
Mercury	ppm	0.43	ND
Selenium	ppm	0.12	ND

ing weight of leftover and waste from weight of diet supplied and dividing by the number of days. Animals were weighed prior to initiation of data collection and weekly thereafter.

All F_0 animals were fed their respective diets for two weeks prior to beginning data collection. After consuming the diets for five months, breeding was initiated. Females were placed in the cage of a male of the same dietary group and observed for copulation. Females failing to copulate with the first male were exposed to a second. If females failed to copulate with the second male they were rested for one week and a second attempt made. If they failed to breed on the second attempt they were recorded as failing to breed. All females were palpated for pregnancy 15 to 22 days after copulation. The breeding regimen described was repeated with females which were not pregnant. Females which failed to become pregnant on this second exposure were recorded as failing to conceive.

Pregnant females were provided a nest box ten days before expected kindling. Animals were observed daily and delivery of leverets was recorded as numbers liveborn or stillborn.

Breeders remained on the diets until leverets were weaned at eight weeks of age. Leverets dying prior to weaning were noted and recorded and post mortem examinations were conducted. Careful observations were made for the presence of congenital anomalies.

Animals for the F_1 generation study were randomly selected from the leverets weaned from the respective groups in the first generation (including offspring of the replacement breeders), and the same experimental design was repeated (minor differences noted below) except that each dietary group included 10 females and 10 males. The animals were not of uniform age upon initiation of data collection but all animals had been consuming the respective diets for their entire lifetime. Breeding of the F_1 animals was not initiated until the animals were at least five months of age, in contrast to the F_0 animals which were at least seven months of age, and the overall study period was 27 weeks instead of 40 weeks.

Animals which became ill during the study were examined by a veterinarian and treated as recommended and all animals dying during the course of the study were examined post mortem for cause of death. In addition, upon completion of the feeding and breeding protocols, 5 males and 5 females from each group in the F_0 generation and 2 males and 2 females from each group in the F_1 generation underwent necropsy examination for pathological changes and a fifty gram sample of semimembranosus, semitendinosus or gastrocnemius muscle was obtained and submitted to a commercial laboratory (Triple "S" Lab, Inc., Box 678, Loveland, CO 80537) for elemental analysis of the heavy metals: selenium, cadmium, mercury, arsenic, lead, nickel, chromium, vanadium, and aluminum. The samples from two males and two females from each group in each generation were similarly analyzed, in addition, for manganese, copper, cobalt, zinc, iron, and molybdenum. The samples were reduced to ash and the determination made by using an argon spectrometer.

Statistical analyses of the data were conducted by employing procedures in the SAS System (Cary, North Carolina). Rate of growth analysis of the F_0 generation included fitting a nonlinear growth curve for each rabbit that survived the study period and estimating a nonlinear growth curve for each rabbit that did not survive the study period. These growth rates, which are most sensitive to the earlier weeks of the study, were analyzed using analysis of variance (ANOVA) to determine the main effects of diet and sex, as well as their interaction. Because of the variable ages of the F_1 animals at initiation of data collection and the potential effect of variations in climate at the variable ages, rate of growth was not analyzed for that generation.

Food consumption and weight gain prior to initiation of breeding was analyzed for both generations. For the F_0 generation the period was divided into 4 time intervals of 40 days each, whereas the F_1 generation was divided into 2 similar time intervals. To study the relationship between weight gain and food consumption, two sets of analyses were performed. First, the Pearson product moment correlation between food consumed and weight gained was calculated for each time interval and diet, and transformed to a Fisher's Z in order to test for diet differences in these correlations. Bonferroni planned comparisons with an overall significance level of 0.05 were made for each time interval. Finally, analysis of covariance (ANCOVA) was performed for each time period to test the diet main effects, food consumption, and their interaction.

Two-way ANOVA's for the effects of diet, sex, and the interaction of diet and sex on food consumption and weight gain were conducted.

Overall weight gain was analyzed by ANOVA with *post hoc* pairwise comparisons of least squares means.

A Chi Square test was performed to compare diets within a generation with respect to survival of the animals. ANOVA was used to compare diets within generations and proportion of offspring weaned based on litter size, and proportion of offspring weaned based on the number born alive per female. ANOVA on the square root transformation of litter size was used to compare diets within a generation.

Suppression of fertility was determined by Fisher's exact tests comparing diets to number of exposures (1, 2, or >2) of females to males required for conception. Fisher's exact test was also calculated comparing diets to whether the females conceived or did not conceive.

To determine differences of heavy metal concentrations in muscle tissue a two-way ANOVA was performed to test for generation and diet main effects and interaction effect.

RESULTS AND DISCUSSION

Palatability. All rabbits consumed the diets from the outset although several F_0 animals, previously accustomed to a different rabbit food used by the breeder, were observed to waste up to 150 grams of all of the diets daily by scratching them from the feeder. This practice ceased after a few days as animals became accustomed to the new diets. Though fluctuations occurred, average daily consumption during the first 82 days of the diet experiments was: F_0, control 171.8 gm (se=7.3), WH 163.9 gm (se=3.6), and

WHA 166.9 gm (se = 4.2); F_1, control 164.8 gm (se = 5.7), WH 171.7 gm (se = 4.3), and WHA 175.4 gm (se = 4.9).

Less of the waterhyacinth diets were initially consumed compared to the control alfalfa diet by the F_0 animals. However, all diets were not totally accepted on first exposure. WH animals of the F_0 generation consumed slightly less than controls initially but ate more in later stages, however, with the F_1 animals who had eaten the diets since they began to take solid food prior to weaning, the animals consuming the WH and WHA diets consumed slightly more than the controls. Therefore it appears that naive rabbits find the WH diets palatable, whereas, animals previously accustomed to a different diet find WH diets less palatable initially but accept them as a satisfactory diet after becoming accustomed to them.

Survivability. Forty six F_0 animals survived the experimental period leaving the final group configuration: control = 6 female(F), 8 male(M); WH = 6F, 11M; WHA = 5F, 10M. Fifty three F_1 animals survived the experimental period leaving the final group configuration: control = 9F, 10M; WH = 9F, 9M; WHA = 9F, 7M. Cause of spontaneous death of all adult animals was related to infections with the bacterium *Pasteurella multocida* or to heat stress. The Chi Square test revealed no relationship between the number of rabbits surviving and their diet for either generation.

All animals consuming the diets thrived and gained weight. Several animals in control and experimental groups died during the course of the study, however, deaths could not be attributed to effects of the diets. Severely hot and humid conditions occurred during both generations of the study. Each time the temperature exceeded 32 C the rabbits exhibited signs of heat stress, i.e., they lay stretched out in their cages, respiration was rapid, and they seemed reluctant to move. Following such episodes some animals became anorexic within one or two days, sneezed excessively, and developed diarrhea. Some responded to treatment while others did not. Post mortem examination usually revealed only intestinal inflammation and pulmonary inflammation which yielded the bacterium *Pasteurella multocida*. Control animals were affected as well as experimental animals.

Growth rate. Table 3 includes mean weights at beginning, 12, 24, 27, and 40 weeks (F_1 terminated at 27 weeks). Although differences in mean weights may be evident for F_0, ANOVA failed to reject the null hypothesis that the mean growth rate was equivalent for the three diets. The two-way ANOVA detected a significant sex effect (p = 0.0003) with males having a higher estimated growth rate parameter (12.6) than females (9.0).

Growth *rate* appeared to have been affected little, if any, by the waterhyacinth diets.

Weight Gain. Analysis of overall weight gain after 24 weeks on the diets and at termination of the study, which was 40 weeks F_0 and 27 weeks F_1, showed that after 24 weeks females had gained an average of 2218 gm (se = 7.0) and males 2010 gm (se = 6.0). The greater gain by the females shows a significant sex effect on weight gain (p = 0.0263). After 40 weeks (Table 3) F_0 control animals gained significantly (p = 0.0484) more (3.294 kg) than WH (2.811 kg) animals. Weights of F_1 animals after 27 weeks did not differ significantly between the diets (p = 0.05).

TABLE 3. LEAST SQUARES MEAN WEIGHT (KG) OF TWO GENERATIONS OF RABBITS CONSUMING CONTROL AND WATERHYACINTH DIETS.

	Control		WH[1]		WHA[1]	
			F_0 Generation			
Initial	1.501	(.356)[2]	1.475	(0.421)	1.541	(0.381)
12 Weeks	3.817	(0.580)	3.402	(0.425)	3.492	(0.428)
24 Weeks	4.348	(0.623)	3.915	(0.431)	4.052	(0.496)
27 Weeks	4.580	(0.684)	3.936	(0.449)	4.259	(0.515)
40 Weeks	4.795	(0.910)	4.286	(0.830)	4.630	(0.742)
			F_1 Generation			
Initial	2.412	(0.379)	2.437	(0.364)	2.094	(0.448)
12 Weeks	3.815	(0.361)	3.669	(0.355)	3.718	(0.586)
24 Weeks	3.937	(0.399)	3.886	(0.372)	3.872	(0.420)
27 Weeks	4.053	(0.414)	4.006	(.0432)	3.965	(0.489)
40 Weeks	ND		ND		ND	

[1]WH = 30% waterhyacinth 0% alfalfa; WHA = 20% waterhyacinth.
[2]Standard errors in parentheses.
[3]Not determined.

Overall weight gain was greater in the F_0 control animals than either the WH or WHA, however, no difference was observed in the F_1 animals. Because the original lot of the F_0 control diet was approximately 1/2% higher in fat content than the WH or WHA diets, this could possibly explain the greater weight gain. Unfortunately, through an oversight, analyses of the subsequent lots of the diets were not done after ingredients were mixed and cannot be compared. Since the F_1 animals were selected from litters of the F_0 animals and were therefore of different ages (all were born over a period of 5 weeks and the age variation was present in all dietary groups) at the time F_1 weight data collection began, this variation in age could have influenced the overall growth data. Also, environmental and seasonal influences could have had an effect. F_0 animals were reared mainly in the spring and summer whereas the F_1 animals were reared in the late fall and winter.

Food consumption and weight gain. Table 4 shows least squares means for food consumption prior to initiation of breeding divided into 40-day time intervals. Comparisons showed that in the first time interval for F_0 animals, the correlation between food consumed and weight gained was higher for the control diet than either WH (p = 0.00004) or WHA (p = 0.0000). ANCOVA showed that during the first 40-day period, average weight gain was significantly higher (p = 0.0126) for the control diet than WH. Average weight gain was Control 1.074 kg (se = 0.05) and WH 0.902 kg (se = 0.05). ANCOVA showed no significant differences for the other time periods.

ANOVA for diet and sex effect on food consumption showed significant sex effects in time intervals 3 (p = 0.0169) and 4 (p = 0.0012). In interval 3 the males consumed 5947.53 gm (se = 157.31), females 6586.18 gm (se = 202.72). In interval 4 males consumed 2823.42 gm (se = 87.30), females 3321.40 gm (se = 112.50).

ANOVA for effect of diet and sex on weight gain showed significant sex effect in intervals 2 (p = 0.0012) and 3 (0.0001). In interval 2 the males gained an average of 0.385 kg (se = 0.03), females 0.548 kg (se = 0.03). In interval 3 the males gained an average of 0.259 kg (se = 0.02), females 0.441 kg (se = 0.03).

TABLE 4. CONSUMPTION OF CONTROL AND WATERHYACINTH FOOD BY TWO GENERATIONS OF RABBITS PRIOR TO BREEDING.[1]

	Interval 1	*Interval 2*	*Interval 3*	*Interval 4*
		F_0		
Control	7239.95	6984.50	5947.29	2892.59
	(253.76)[2]	(227.45)	(220.47)	(122.35)
WH[3]	7249.06	6517.08	6268.09	3159.59
	(256.63)	(217.50)	(216.20)	(119.98)
WHA[3]	7313.77	6739.27	6585.18	3165.03
	(256.63)	(226.32)	(229.76)	(127.51)
		F_1		
Control	7315.80	6525.30	ND[4]	ND
	(234.04)	(226.56)		
WH[3]	7511.60	6912.70	ND	ND
	(234.04)	(226.56)		
WHA[3]	7603.08	6169.81	ND	ND
	(240.44)	(232.77)		

[1]Values are least squares means in grams. Intervals = 40 days.
[2]Standard errors in parentheses.
[3]WH = 30% waterhyacinth 0% alfalfa; WHA = 20% waterhyacinth.
[4]Not Done.

Significant differences in correlations between food consumption and weight gain for the F_1 generation were not detected. ANCOVA revealed that during the first 40 day time period average weight gain was significantly less for WH animals than either WHA ($p = 0.0002$) or controls ($p = 0.0037$). The average weight gain was WH 0.549 kg (se = 0.05), WHA 0.805 kg (se = 0.05), and control 0.740 kg (se = 0.05). ANCOVA for the remaining time period showed no significant differences. Two-way ANOVAs for food consumption showed no significant effects for either time period.

Fertility. Table 5 displays breeding performance data. All F_0 control, WH, and WHA females bred on exposure to one of two males except one WHA female who failed to breed. Three F_1 control, one WH, and two WHA females failed to breed. All others bred on exposure to one of two males. Fisher's exact tests failed to detect differences in fertility of the breeders between diets in either generation.

TABLE 5. BREEDING PERFORMANCE OF TWO GENERATIONS OF FEMALE RABBITS CONSUMING CONTROL AND WATERHYACINTH DIETS.

	No of Matings on Exp to first male				No of Matings on Exp to 2nd male				Failed to Breed
	1	2	3	4	1	2	3	4	
					F_0				
Control	1	3[1]	2	0	0	1[1]	0	0	0
WH[2]	1	5	0	1	0	0	0	0	0
WHA[2]	1	0	0	1	1	2	0	0	1
					F_1				
Control	1	4	0	0	0	2	0	0	3
WH[2]	1	0	4[3]	1	0	3	0	0	1
WHA[2]	1	4	3[3]	0	0	0	0	0	2

[1]One animal bred but failed to conceive.
[2]WH = 30% waterhyacinth 0% alfalfa; WHA = 20% waterhyacinth.
[3]One animal bred but died prior to parturition.

Survival of offspring. Table 6 gives litter size and survival data for the offspring. ANOVA did not detect differences between the number of leverets born, born and survived until weaning, or born alive which survived until weaning and the diet of the doe for either generation. However, the adverse temperature and humidity conditions previously referred to likely had an effect on offspring survival. Nursing mothers subject to the stresses of environmental extremes may not produce adequate quantity or quality of milk. Also, leverets are normally placed in a bed of fur which prevents heat dissipation and, under high temperature conditions, could be severely stressful. Therefore, since the losses of leverets prior to weaning occurred as frequently among the control animals as among the experimental diets, the losses are likely a result of these external factors.

Teratogenicity. One congenital malformation was observed, an F^1 leveret in the control group had a skeletal deformity involving the right forearm and carpus.

Since the only malformed leveret observed was a control animal, it probably occurred as a result of chance. Teratogenic effects occur in normal rabbit populations with a very low incidence. Sample sizes in this study were small, but no indication was found of any appreciable teratogenic effects of the experimental diets.

Trace elements in muscle tissue. The two-way ANOVA (Table 7) revealed no significant differences for the interaction or main effects for copper, arsenic, manganese, molybdenum, selenium and aluminum. However, significant interactions were found for nickel, chromium, and vanadium. The F_1 WHA animals had significantly higher mean levels of nickel ($p \leq 0.0007$) than any other group, whereas, F_1 control animals were comparable to F_1 WHA but had significantly higher mean levels ($p \leq 0.0118$) than any F_0 group. The F_1 WHA group had significantly higher mean levels of chromium than any other group ($p \leq 0.01$).

TABLE 6. LITTER SIZE AND SURVIVAL OF OFFSPRING FROM TWO GENERATIONS OF RABBITS CONSUMING CONTROL AND WATERHYACINTH DIETS.

	Control		WH[1]		WHA[1]	
	F_0	F_1	F_0	F_1	F_0	F_1
Number of Females	7	7	6	8[2]	6	8[3]
Ave. Litter Size	5.14	6.29	7.50	8.11	5.50	6.89
	(5.55)[4]	(1.70)	(1.38)	(2.93)	(4.55)	(1.76)
Ave No. Liveborn Per Female	4.71	5.57	5.00	3.56	4.83	4.33
	(4.92)	(1.99)	(3.16)	(3.24)	(4.26)	(2.87)
Ave No. Liveborn That Died Prior Weaning/Female	3.29	2.43	3.17	2.00	2.17	1.11
	(4.19)	(2.70)	(2.32)	(2.00)	(2.79)	(2.32)
Weaned/Born[5]	0.214	0.502	0.233	0.195	0.321	0.482
	(0.30)	(0.48)	(0.31)	(0.37)	(0.35)	(0.48)
Weaned/Born Alive[6]	0.220	0.519	0.252	0.207	0.378	0.537
	(0.30)	(0.49)	(0.31)	(0.37)	(0.42)	(0.51)

[1]WH = 30% waterhyacinth 0% alfalfa; WHA = 20% waterhyacinth.
[2]One other pregnant with eleven fetuses died from heatstress.
[3]One other pregnant with nine fetuses died from heatstress.
[4]Standard errors in parentheses.
[5]Average ratio of the total weaned of total born per female.
[6]Average ratio of number weaned of those born alive per female.

TABLE 7. TRACE ELEMENT ANALYSIS OF MUSCLE TISSUE FROM TWO GENERATIONS OF RABBITS FED CONTROL AND WATERHYACINTH DIETS (LEAST SQUARES MEAN PARTS PER MILLION, DRY MATTER).[1]

Element	WH[2]		WHA[2]		Control	
	F_0	F_1	F_0	F_1	F_0	F_1
Manganese	2.25	2.63	3.05	3.55	3.52	2.78
	(0.47)	(0.47)	(0.47)	(0.47)	(0.47)	(0.47)
Copper	1.53	11.05	1.33	5.68	1.18	3.88
	(3.09)	(3.09)	(3.09)	(3.09)	(3.09)	(3.09)
Cobalt[3]	0.18	0.27	0.17	0.30	0.13	0.44
	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)
Zinc[3]	33.43	44.68	31.50	53.03	30.73	38.00
	(4.21)	(4.21)	(4.21)	(4.21)	(4.21)	(4.21)
Iron[3]	13.73	15.05	15.75	6.08	28.05	4.83
	(4.62)	(4.62)	(4.62)	(4.62)	(4.62)	(4.62)
Molybde-	0.25	0.28	0.23	0.28	0.20	0.50
num	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)
Selenium	0.04	0.07	0.05	0.07	0.047	0.04
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Cadmium[3]	0.02	0.57	0.07	0.37	0.09	0.28
	(0.06)	(0.10)	(0.06)	(0.10)	(0.06)	(0.10)
Mercury[3]	0.92	1.13	0.99	1.48	1.02	1.37
	(0.07)	(0.11)	(0.07)	(0.11)	(0.07)	(0.11)
Arsenic	0.28	0.11	0.46	0.30	0.33	0.11
	(0.09)	(0.15)	(0.09)	(0.15)	(0.09)	(0.15)
Lead[3]	5.34	0.62	4.92	1.30	6.14	0.94
	(0.63)	(1.00)	(0.63)	(1.00)	(0.63)	(1.00)
Nickel	0.21	0.27	0.21	0.69[4,5]	0.24	0.43[5]
	(0.04)	(0.06)	(0.04)	(0.06)	(0.04)	(0.06)
Chromium[3]	0.13	0.35[5]	0.13	0.58[4]	0.14	0.44[5]
	(0.03)	(0.05)	(0.03)	(0.05)	(0.03)	(0.05)
Vanadium	0.37	0.31	0.36	0.70[6]	0.38	0.55[6]
	(0.04)	(0.06)	(0.04)	(0.06)	(0.04)	(0.06)
Aluminum	21.87	46.98	18.51	42.63	20.19	12.83
	(6.99)	(10.88)	(6.88)	(10.88)	(6.88)	(10.88)

[1]Standard error in parentheses below the means.
[2]WH = 30% waterhyacinth 0% alfalfa; WHA = 20% waterhyacinth.
[3]F_0 significantly different from F_1 ($p \leq 0.0009$ except Fe $p = 0.012$).
[4]Significantly different from other group means ($p \leq 0.01$).
[5]Comparable; significantly different from F_0 ($p \leq 0.01$).
[6]Comparable; significantly different from other group means. ($p \leq 0.01$).

No difference was detected between mean chromium levels for the F_1 control or WH diets; but, both groups had significantly higher mean levels ($p \leq 0.0006$) than any F_0 group. F_1 control and WHA animals had significantly higher mean vanadium levels ($p \leq 0.0145$) than any other groups, but no difference was detected between the means of the F_1 control and WHA animals.

Of the remaining trace elements no significant differences were found between the diet groups. However, significant differences were found between the generations for zinc ($p \leq 0.0009$), cobalt ($p \leq 0.0003$), cadmium ($p \leq 0.0001$), mercury ($p \leq 0.0001$), iron ($p \leq 0.012$), and lead ($p \leq 0.0001$). The least squares mean levels for the F_0 *vs.* the F_1 generations were: zinc 31.88 ppm (se = 2.43) *vs.* 45.57 ppm (se = 2.43), cobalt 0.16 ppm (se = 0.03) *vs.* 0.34 ppm (se = 0.03), cadmium 0.056 ppm (se = 0.04) *vs.* 0.408 ppm (se = 0.06), mercury 0.974 ppm (se = 0.04) *vs.* 1.327 ppm (se = 0.06), iron 19.18 ppm (se = 2.67) *vs.* 8.65 ppm (se = 2.67), and lead 5.465 ppm (se = 0.36) *vs.* 0.952 ppm (se = 0.58).

Differences observed in levels of trace elements in muscle tissue between the generations could be due to source of the non-waterhyacinth dietary ingredients because of the soil composition in which they were grown and to influences of climate and season on the growth of the plants. Likewise, total body weight differences between the animals of the two generations may have had an effect, for example, a 50 gm sample of muscle from a 4 kg animal represents a lower percentage of total muscle mass than a 50 gm sample from a 3 kg animal. Thus, if the whole animal stores the element proportional to its muscle mass it could have an important effect on the concentration per gram of tissue.

Significant differences in mineral content of waterhyacinth taken from lakes in different geographic regions of Florida at different times of the year have been reported (18). Since the waterhyacinth used in these diets were harvested at different times of the year and from different wastewater treatment facilities, this could have affected the trace element content of the plants.

Differences in trace element content of the alfalfa and grain components of the diets could have had an effect on levels in muscle tissue of animals consuming the diets. Unfortunately elemental analysis of the alfalfa and other dietary components was not done, nor was it done on the waterhyacinth from the Kissimmee facility. Nevertheless, the results show that cobalt, zinc, cadmium, mercury and chromium concentration in muscle tissue was significantly higher in F_1 animals in all dietary groups which indicates a probability that they were present in higher levels in dietary ingredients common to all of the F_1 diets. This strongly suggests that the waterhyacinths were not the source. Iron and lead were significantly higher in muscle tissue of F_0 animals in all dietary groups indicating a strong probability that they were present in higher levels in dietary ingredients common to *all* of the F_0 diets and, again, strongly suggesting that the waterhyacinths were not the source. Nickel and vanadium were higher in muscle tissue of F_1 control and WHA animals which suggests alfalfa, which was common to these diets, but not found in the WH diet, as the source. Thus, although higher levels of several trace elements in muscle tissue were noted between the generations, these differences could not be solely attributed to the waterhyacinth component of the diets.

Guideline levels for toxic concentration of trace elements in mammalian muscle tissue intended for human consumption apparently have not been established. However, levels present in the muscle tissue of rabbits consuming the control diet and diets containing WH and WHA in this study were below the levels reported as toxic in reviews (9, 14).

These extensive studies conducted over 2 generations of rabbits conclusively show that growth of rabbits, in general, is not adversely affected by waterhyacinth in the diet. It is known that waterhyacinth grown in sewage effluent potentially can contain high concentration of potentially toxic elements. This did not seem to be a problem in this study, but the elemental contents of effluents can vary widely and it must be recognized that in some effluents accumulation of toxic elements may occur.

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