WATER HYACINTHS (*Eichhornia crassipes*), GROWN IN MUNICIPAL WASTEWATER, AS A SOURCE OF ORGANIC MATTER IN RABBIT FOOD.

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Final report of a study conducted by Alvin F. Moreland, D.V.M. and Bobby R. Collins, D.V.M., Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida[1] for AMASEK, INC., 402 High Point Drive, Cocoa, Florida 32926.

Water hyacinths are looked upon by many as an environmental menace. They provide, perhaps, the best example of explosive infestation of waterways by water weeds. However, the successful and lush growth of this plant has been the cause for examination of its potential usefulness. One such use is the removal of nutrient pollutants from municipal wastewater. Water hyacinths are said to be the most promising species of water weed for nutrient removal, due to its prodigious growth rate and free floating nature. (Boyd, l970) Estimations have been made that one acre of water hyacinths could potentially assimilate from domestic sewage the nitrogen generated by 595 persons and the phosphorus generated by 180 people producing 67 tons of dry matter annually. (Steward, 1970) Thus, much effort has been focused on this potentially beneficial use.

Since the water hyacinths 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 300,000 square miles of alfalfa could supply the minimum protein requirements of the human race with some leftover for livestock. (Morrison & Pine, 1961) In contrast it is estimated that, under intensive cultivation, water hyacinths could easily produce three times as much per acre. (Boyd, 1970; Steward, 1970) Concentrations of the nutrient elements P, K, Mg, Cu, Zn, and Mn in water hyacinths have been determined to be in the same range as in land forages; Na, and Fe were higher and Ca was lower. (Easley and Shirley, 1974) Comparisons of dried water hyacinths arid alfalfa hay revealed 10.65% protein vs. 13%, TDN 46.2% vs. 48.0%, crude fiber 28.5% vs. 30.0%, and fat 0.91% vs. 1.8%. Thus, water hyacinths would seem to have excellent potential as a nutritious food. Efforts to determine the potential use of water hyacinths as a food have focused on culture of marine species which feed on aquatic weeds and on the harvest and processing of aquatic weeds for animal and human food. Water hyacinths were well tolerated by cattle and sheep fed a pelleted ration with 33 percent organic matter basis from water hyacinths, but was inadequate as the sole component of the ration. (Hentges, et al., 1972) Ensiled water hyacinths have been fed to cattle and sheep with excellent results when combined with additives which provide fermentable carbohydrates. (Baldwin, 1973; Byron, 1972) Food consumption and weight gain was comparable to control diets in calves and sheep fed diets containing water hyacinths as a portion of concentrate feeds. (Reddy & Reddy, 1979, and Osman, et al., 1975) Water hyacinths and alfalfa were of value in channel catfish diets only when reduced to ten percent of the diet by admixture with standard feed ingredients. (Liang & Lovell, 1971) However, the matrincha (*Brycon sp*.), an omnivorous indigeneous species from the Amazon grew well when fed diets containing water hyacinths and were not adversely affected by adding 9.5% and 18.9% water hyacinths while maintaining the protein level constant. (Saint-Paul et al., 1981) Reports on feeding cooked water hyacinths

to swine have indicated variable results, (Gopal, 1987), however, addition of processed water hyacinths to the diets of pigs reduced the requirements of commercial dry feed and allowed acceptable growth. (Solly et al., 1984) Green 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. (Soesiawaningrini et al., 1979) The present study was undertaken to determine if a non-ruminant herbivore, the domestic rabbit *Orvctolaaus cuniculus*, prized by many as human food, could utilize as a food ingredient water hyacinths grown in municipal wastewater.

MATERIALS AND METHODS

The study was carried out over two generations (F_0, F_1). Thirty-two, eight-week-old male and twenty-eight female rabbits (*Oryctolacrus cuniculus*) of the New Zealand White breed were obtained from a local breeder for generation F_0. All received an identifying tattoo number and were identified as to sex by the breeder. They were housed in a sheltered pole barn open, but 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 calculated to hold an approximate 2-3 day supply were mounted on the outside of the cage. Excreta was allowed to fall to a concrete floor beneath the cages and was removed by hose and water 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 90 degrees F. floor mounted fans were added to increase ventilation. During studies of the second generation temperatures exceeding 95 degrees F. required installation of a water spray system which wet the screened sides of the building thus providing cooling. During winter plastic drapes were placed over the screens.

During the summer months of 1988 very hot and humid conditions were encountered which, directly or indirectly, led to death of 3 females in each group and 2 males of the Control group. Because of concern that the remaining animals may produce 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 diets 122 days after the original animals began consuming the diets and 30 days prior to initiation of breeding. However, because they did not consume the diets for the full experimental period, data from these animals are not included as part of this study.

Prior to beginning the study all animals were given a complete physical examination, blood was drawn for laboratory analysis, and animals were treated prophylactically for coccidiosis prior to being divided into three groups by way of a table of random numbers. A control group of 10 females and 10 males received a standard commercial rabbit diet manufactured by the Seminole Feed Division of Seminole Stores, Inc. of Ocala, Florida. This standard diet was of the following guaranteed analysis.

GUARANTEED ANALYSIS

Crude Protein, not less than…….....15.00%
Crude Fat, not less than…….3.50%
Crude Fiber, not more than…….13.00%
Vitamin A, U.S.P. Units per Lb……3,000
Vitamin D3, U.S.P. Units per Lb…….250
Vitamin E, U.S.P., Units per Lb……...5

Ingredients

Alfalfa Meal, Soybean Meal, Ground Corn, Ground Oats, Wheat Bran, Defluorinated Phosphate, Salt, Zinc Oxide, Manganous Oxide, Ferrous Carbonate, Iron Oxide, Copper Oxide, Calcium Periodate, Cobalt Carbonate, Calcium Carbonate, Vitamin A Acetate, D-Activated Animal Sterol (Source of Vitamin D3), DL-Alpha Tocopheryl Acetate (Source of Vitamin E), Vitamin Bl2 Supplement, Riboflavin Supplement, Niacin Supplement, Calcium Pantothenate, Choline Chloride, Folic Acid, Pyridoxine Hydrochloride, Thiamin Mononitrate.

One experimental group (WH) of 9 females and 11 males received the same basic diet as the controls except that the alfalfa component was completely replaced by water hyacinths. A second experimental group (WHA) of 9 females and 11 males received the same basic diet as the controls except two-thirds of the alfalfa component was replaced with water hyacinths. Dried water hyacinths were analyzed to provide a basis for balancing the test diets and control diet. The dried water hyacinth feed contained 10-13% protein and 0.5% fat. For comparative testing in this study these two parameters were balanced so that all three diets contained approximately the same 15% protein and 3.5% fat. The water hyacinths were harvested from the Wastewater Treatment Facility for the city of Kissimmee, Florida, and 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 similar to the control diet. The diets were stored at 60 degrees F. As needed, a weeks supply 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 subtracting 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 15-22 days after copulation. If not pregnant the breeding process as described was repeated. If they failed to become pregnant on this second exposure they were recorded as failing to conceive.

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

Animals remained on the diets until laverets were weaned at eight weeks of age. Laverets 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 laverets weaned from the respective groups in the first generation (including offspring of the replacement breeders), and the same experimental design was repeated except that each dietary group included 10 females and 10 males, the animals were not of uniform age upon initiation of data collection, and 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. The source of the water hyacinths used for the F_1 animals was the Iron Bridge Wastewater Treatment Facility for the city of Orlando, Florida.

Animals which became ill during the study were examined by a veterinarian and treated as recommended. All animals dying during the course of the study were examined by a veterinarian post mortem for cause of death. In addition, upon completion of the feeding and breeding protocols, five 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[2] for analysis of the heavy metals: selenium, cadmium, mercury, arsenic, lead, nickel, chromium, vanadium, and aluminum.

Statistical analysis of the data was obtained by employing procedures in the SAS system[3]. 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 were analyzed using one-way analysis of variance (ANOVA) to determine if the growth rate differed between diets and two-way ANOVA to determine main effects of diet and sex, as well as the 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 were 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. Second, analysis of covariance (ANCOVA) was performed for each time period to test the diet main effects, food consumption, and the interaction of both.

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

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 171.76 gm (SE=7.34), WH 163.91 gm (SE=3.60), and WHA 166.86 gm (SE=4.23); F_1, Control 164.77 gm (SE=5.71), WH 171.71 gm (SE=4.27), and WHA 175.36 gm (SE=4.92).

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 *Pasteurella multocida* or to heat stress. The Chi Square test revealed no difference between the number of rabbits surviving and their diet for either generation.

Growth rate. Table 1 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).

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 gin (se=7.0) and males 2010 gin (se=6.0). The greater gain by the females shows a significant sex effect on weight gain ($p=0.0263$). After 40 weeks F_0 Control animals gained significantly ($p=0.0484$) more (3.373 kg) than WH (2.721 kg) animals. After 27 weeks weights of F_1 animals did not differ significantly between the diets.

Food consumption and weight gain. Table 2 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=0.00l2) and 3 (0.0001). In interval 2 the males gained an average of 0.385 kg (se=O.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 1.
MEAN WEIGHT (KG)

	Control	WH	WHA
		F_0 Generation	
Initial	1.501 (0.356)	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

Standard Errors in Parentheses
WH=Water hyacinths replace 100% of the alfalfa in Control diet.
WHA=Water hyacinths replace 66-2/3% of alfalfa in Control diet.

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 ANOVA for food consumption showed no significant effects for either time period.

TABLE 2.
FOOD CONSUMPTION PRIOR TO INITIATION OF BREEDING (LEAST SQUARES MEANS, GRAMS) IN 40 DAY TIME INTERVALS

	Interval 1	Interval 2	Interval 3	Interval 4
			F_0 Generation	
Control	7293.95 (253.76)	6985.50 (227.45)	5947.29 (220.47)	2892.59 (122.35)
WH	7249.06 (256.63)	6517.08 (217.50)	6268.09 (216.20)	3159.59 (119.98)
WHA	7313.77 (256.63)	6739.27 (226.32)	6585.18 (229.76)	3165.03 (127.51)
			F_1 Generation	
Control	7315.80 (234.04)	6225.30 (226.56)	ND	ND
WH	7511.60 (234.04)	6912.70 (226.56)	ND	ND
WHA	7603.08 (240.44)	7169.81 (232.77)	ND	ND

ND=Not Done
Standard errors in parentheses

Fertility. Table 3 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.

Survival of Offspring. Table 4 gives litter size and survival data for the offspring.

The ANOVA did not detect differences between the number of laverets born, born and survived until weaning, or born alive which survived until weaning and the diet of the doe for either generation.

Teratogenicity. Only one congenital malformation was observed, an F_1 laveret in the control group with a skeletal deformity involving the right forearm and carpus. Therefore, no difference in incidence of malformations could be attributed to the experimental diets.

Tissue content of heavy metals. Table 5 displays the results of the analysis of muscle tissue for heavy metals. ANOVA revealed no significant differences in selenium and aluminum levels between the dietary groups and/or generations. However, the ANOVA yielded significant generation effects for cadmium, mercury, chromium and lead. Post hoc least squares mean levels of cadmium [0.056 ppm (se=0.04) F_0 vs. 0.408 ppm (se=0.06) F_1], mercury [0.974 ppm (se=0.04) F_0 vs. 1.327 ppm (se=0.06) F_1], and chromium [0.133 ppm (se=0.02) F_0 vs. 0.456 ppm (se=0.03) F_1] were significantly higher

(p=.0001) in the F_1 animals. The mean level of lead (5.465 ppm (se=0.36) F_0 vs. 0.952 ppm (se=0.58)] F_1, was significantly (p=0.0001) higher in F_0 animals; whereas, the mean level of arsenic was higher in F_0 animals but was of borderline significance (p=.0444)

TABLE 3.
FEMALE BREEDING PERFORMANCE

	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 Generation				
Control	1	3*	2	0	0	1*	0	0	0
WH	1	5	0	1	0	0	0	0	0
WHA	1	0	0	1	1	2	0	0	1
					F_1 Generation				
Control	1	4	0	0	0	2	0	0	3
WH	1	0	4#	1	0	3	0	0	1
WHA	1	4	3#	0	0	0	0	0	2

* One animal bred but failed to conceive
# One animal bred but died prior to parturition

TABLE 4.
LITTER SIZE AND SURVIVAL OF OFFSPRING

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

Standard error in parentheses

* In addition one doe pregnant with eleven laverets died due to heatstress.
# In addition one doe pregnant with nine laverets died due to heatstress.

TABLE 5.
HEAVY METALS ANALYSIS OF MUSCLE TISSUE
(Mean Parts per Million Dry Matter#)

	WH		WHA		Control	
	F_0	F_1	F_0	F_1	F_0	F_1
Selenium	0.044 (0.007)	0.068 (0.012)	0.045 (0.007)	0.068 (0.012)	0.042 (0.007)	0.035 (0.012)
Cadmium*	0.015 (0.060)	0.570 (0.095)	0.066 (0.060)	0.373 (0.095)	0.087 (0.060)	0.283 (0.095)
Mercury*	0.919 (0.067)	1.128 (0.106)	0.985 (0.067)	1.483 (0.106)	1.019 (0.067)	1.370 (0.106)
Arsenic	0.282 (0.094)	0.113 (0.148)	0.455 (0.094)	0.303 (0.148)	0.329 (0.094)	0.108 (0.148)
Lead*	5.337 (0.631)	0.618 (0.997)	4.921 (0.631)	1.295 (0.997)	6.138 (0.631)	0.943 (0.997)
Nickel	0.207 (0.038)	0.268 (0.060)	0.212 (0.038)	0.688[ab] (0.060)	0.236 (0.038)	0.425[b] (0.060)
Chromium*	0.130 (0.030)	0.348[b] (0.047)	0.129 (0.030)	0.583a (0.047)	0.141 (0.030)	0.438[b] (0.047)
Vanadium	0.369 (0.036)	0.308 (0.057)	0.361 (0.036	O.7OO[c] (0.057)	0.379 (0.036)	O.55O[c] (0.057)
Aluminum	21.87 (6.99)	46.98 (10.88)	18.51 (6.88)	42.63 (10.88)	20.19 (6.88)	12.83 (10.88)

Standard errors in parentheses below the means.

***** F_0 significantly different from F_1 ($p=0.0001$).

a Significantly different from other group means ($p \leq O.O5$).

b Comparable; significantly different from F_0 ($p \leq 0.05$).

c Comparable; significantly different from other group means ($p \leq 0.05$).

The two-way ANOVA revealed a significant interaction effect for nickel, chromium, and vanadium. 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. F_1 WHA animals had significantly higher mean levels ($p \leq 0.0345$) of chromium than any other group. 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.

DISCUSSION

The water hyacinth diets were initially consumed less well than the control alfalfa diet by the F_0 animals. However, all diets were not totally accepted on first exposure. In the F_0 generation the WH animals consumed slightly less throughout the study, however, in 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 quite 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.

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 90° F 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 inflammations which yielded *Pasteurella inultocida*. Control animals were affected as well as were the experimental animals.

Growth rate appeared to have been affected little, if any, by the water hyacinth diets. 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. 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.

Fertility and fecundity of breeders, and survival of offspring were not adversely affected by the experimental diets. However the adverse temperature and humidity conditions previously referred to also likely had an effect on offspring survival. Nursing mothers subject to the stresses of environmental extremes may not produce adequate amounts or quality of milk. Laverets 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 laverets prior to weaning occurred as frequently among the control animals as among the experimentals, the losses are likely a result of these external factors.

Since only one congenitally malformed laveret was observed, and since it occurred in a Control animal, it occurred as a result of chance and no indication exists of teratogenic effects of the experimental diets.

Differences observed in levels of heavy metals in muscle tissue between the generations could be due to source of the dietary ingredients 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.

Parra and Hortenstine (1974) reported significant differences in mineral content of water hyacinths taken from lakes in different geographic regions of Florida at different times of the year. Since the water hyacinths used for the F_0 diets in the present study came

from the Kissimmee Wastewater Treatment Facility and those for the F_1 diets from the Orlando Iron Bridge WTF and since harvest was at different times of the year, differences in mineral content of the plants should probably be expected. Orlando has a much higher concentration of industrial activity than Kissimmee which is primarily a residential community, therefore, differences in heavy metal content of sewage wastewater in which the water hyacinths were grown would be expected.

Differences in mineral content of the alfalfa and grain components of the diets could also have had an effect. Cadmium, mercury and chromium concentration in muscle tissue was significantly higher in F_1 animals in all dietary groups which indicates a strong probability that they were present in dietary ingredients common to all of the F_1 diets. Lead was significantly higher in F_0 animals in all dietary groups indicating a strong probability that it was present in dietary ingredients common to all of the F_0 diets. Nickel and vanadium, however, were higher in the F_1 Control and WHA diets which suggests alfalfa, which was common to these diets but not found in the WH diet, as the source.

These data support the observation that dietary concentration of mineral elements are reflected in tissue concentrations of animals consuming the diets.

SUMMARY

Diets containing dried water hyacinths were fed to rabbits for two generations. The diets were palatable and all animals thrived on the diets. First generation animals consuming the diets containing water hyacinths reached a final mean body weight slightly less than controls, however, second generation animals consuming the diets containing water hyacinths were not different from controls in final mean body weights. Overall growth rate was unaffected by the diets, however, when the growth period was divided into segments the rate of growth was variable in some segments. Survivability, fertility, survival of offspring, and teratogenicity were not affected by the diets.

Levels of heavy metals in muscle tissue of animals consuming the diets were analyzed. Muscle tissue concentrations of heavy metals were not significantly affected by the experimental diets but data suggested significant effects resulting from other ingredients common to experimental and control diets.

In conclusion, based on data obtained from this study water hyacinths grown in municipal wastewater which completely or partially replaced alfalfa as a source of organic matter in a commercially formulated rabbit food were satisfactory as an ingredient of the food.

Footnote 1
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Footnote 2
Triple "5" Lab, Inc., At Johnson's Corner On Interstate 25, P. 0. Box 678, Loveland, Colorado 80537

Footnote 3
Statistical Analysis System
SAS Institute, Inc.
Cary, North Carolina

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