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Inorganic Chemical Composition of Trout Food Pellets and Alfalfa Used to Sustain *Daphnia magna* Straus

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The original rationale for proposing the use of a synthetic diet to maintain laboratory cultures of Daphnia magna Straus, 1820 was due to the impression held by some government agencies and other investigators that such diets were nutritionally adequate (Winner et al 1977; Biesinger and Christensen 1972; Weber and Peltier 1980; Peltier and Webber 1984) or chemically defined (Winner et al 1977; Taub and Dollar 1968). The purpose of this study is to examine the distribution of 15 elements, among a variety of lots of trout food pellets and alfalfa, which are believed to be essential to aquatic life. This synthetic diet is fed to daphnids in liquid form. Only this liquid form has been subjected to chemical analyses since it is of major concern to discover the composition of the diet actually fed these freshwater organisms. It is unfortunate indeed that comparative chemical data for this particular diet in liquid form are unavailable, and furthermore it is believed that the present study is the first to rigorously address the chemical composition of synthetic diets as they are fed to aquatic organisms.

Another aspect of the synthetic diet is that organisms sustained in this fashion provide a different 48 hour static acute LC_{50} than those maintained on algal food (Winner et al 1977). For example, the author's laboratory has found that the LC_{50} for NaCl utilizing D. magna as the test organism maintained on the synthetic diet here described was 1661 (1453-1888) mg L^{-1}. The LC_{50} for NaCl for organisms maintained on algal diets was 4571 (4149-5090) mg L^{-1}. In addition, longevity and reproduction are severely reduced when D. magna is reared on this synthetic diet in autoclaved Lake Huron water (Cowgill et al In Press). Winner and his co-workers (1977) were able to sustain D. magna on this synthetic diet reared in pond water. It was hoped that chemical analyses of the liquid diet as fed might elucidate the chemical constituents available to D. magna and thus shed light on some of the observations that have been made with organisms thus maintained.

MATERIALS AND METHODS

The synthetic diet utilized in this study is a 4:1 mixture of a

liquid (dechlorinated Lake Huron water or processed Lake Huron water, cf Table 1) preparation of dry trout food (No. 4 pellets: Lot A - Central Soya, Portland, Michigan; Lot B-F - American Scientific Products, McGaw Park, Illinois) and dried alfalfa meal (ground dry alfalfa grass purchased from a local feed store and grown in Michigan. Three separate growing seasons are represented among the three lots tested). The trout food utilized here conforms to Fish and Wildlife Service Specification PR(11)-78. Formulation involves initial dry blending for 2 min followed by high speed blending (glass jar, Waring Commercial Blender) with 0.4 L of liquid for 2 min. The resulting suspension is transferred to a one liter graduated glass cylinder, made up to volume and allowed to settle for 30 min. The supernatant liquid is decanted and fed to D. magna at the rate of 3.2 ml per L of daphnid medium. The dry equivalent of 3.2 ml is 60 mg. Since it was of interest to learn the chemical composition of the synthetic food being fed to D. magna, the supernatant liquid is the sample chemically analyzed and presented in this paper.

In order to compare the chemical consistency among different lots of trout food and alfalfa, six lots of pellets were purchased and analyzed over a two year period. During the same length of time, three different lots of alfalfa were purchased. Only the formulated synthetic diets were analyzed. Three separate dry aliquots of each trout food were formulated with alfalfa and the appropriate water and analyzed. The alfalfa that comprised the remainder of the synthetic diet was allocated to each of two different lots of trout food pellets. Thus, a total of 18 samples of synthetic diet were studied which represent six lots of pellets and three lots of alfalfa. Sample A was formulated with dechlorinated Lake Huron water while Samples B through F were made with industrial water. Both types of water were supplied by the Midland, MI treatment plant.

The liquid synthetic diets analyzed for the presence of Na, K, Cu, Ca, Mg, Zn, P, Mo, Fe, Mn, Co and Ni, were acid digested with H_2SO_4 and HNO_3. The residue was taken up in 6N HCl. Liquid diets analyzed for Se were prepared by digesting the diet with H_3PO_4 and HNO_3 according to the method of Reamer and Veillon (1981). Samples of dechlorinated Lake Huron and industrial water were analyzed without a digestion step and are to be considered blanks.

Selenium was determined by hydride generation-atomic absorption spectrometry in all samples. Flame atomic absorption was utilized to detect the amounts of Na, K, Ca and Mg. The analysis for P, Zn, Mo, Fe, Mn, Co, Ni and Cu were carried out with inductively coupled plasma optical emission spectroscopy.

Chlorides and S in liquids were analyzed by ion chromatography-conductivity detection (Small et al 1975; Stevens and Turkelson 1977; Rawa 1979).

The error of determination was $\pm 2\%$ in all cases. To obtain this

Table 1. Typical inorganic composition (mg L^{-1}) of waters used in this study.

Element	Dechlorinated Lake Huron[1]	SD[3] n=7	Processed Lake Huron[4]	SD[3] n=13
Na	4.7	1.3	4.6	1.0
K	1.1	0.06	1.1	0.04
Cu	ND(0.005)[2]		ND(0.007)	
Mg	7.6	0.31	8.1	1.3
Ca	30.3	0.94	17.3	1.2
Zn	0.03-ND(0.010)		0.03-ND(0.008)	
P	0.12	0.03	0.02	0.005
S	6.5	1.5	5.9	0.45
Se	ND($0.25X10^{-3}$)		ND($0.25X10^{-3}$)	
Mo	ND(0.007)		ND(0.007)	
Cl	12.2	1.3	13.0	0
Fe	0.03	0.017	0.025-ND(0.01)	
Mn	ND(0.001)		ND(0.002)	
Co	ND(0.005)		ND(0.005)	
Ni	ND(0.005)		ND(0.005)	

[1]synthetic diet sample A liquid
[2]not detected at levels in parenthesis
[3]standard deviation
[4]synthetic diet sample B-F liquid

precision it was often necessary to concentrate samples by heating under controlled temperature conditions.

RESULTS AND DISCUSSION

Typical analyses of dechlorinated Lake Huron water and processed Lake Huron water are shown in Table 1. It may be noted that major difference among the calculated means is that the processed Lake Huron water has been treated to bring about a decline in the Ca and P content. The remaining detectable elements vary only within their standard deviation about their mean for the two year period these data represent. In any case, the chemical contribution of Lake Huron water, be it processed or dechlorinated, is minimal as may be noted by examining the chemical composition of the synthetic diet with the chemical contribution of the formulation water (the blank) subtracted (Table 2 and 3).

The major constituents of the synthetic diet are shown in Table 2. Three aliquots of each lot of trout pellets and alfalfa were formulated and analyzed. Data presented for each lot, therefore, are the average of three individual aliquots taken from that lot and their resulting standard deviation. The last three columns show the range among the six means for a given element, the calculated Chi Square for that range and its level of

Table 2. Major chemical constituents (mg L^{-1}) of Lots A through F of formulated trout pellets + alfalfa. (SD=std. deviation; ND=not detected at levels noted in parenthesis). Appropriate blank chemistry has already been subtracted.

Element	A	SD	B	SD	C	SD	D	SD
Na	183.0	29.1	172.7	4.03	131.3	3.3	92.0	13.4
K	445.3	67.5	452.7	12.5	483.0	24.1	489.3	23.2
Mg	51.3	10.2	50.3	1.7	60.3	3.3	59.7	2.5
Ca	107.8	29.3	122.3	3.86	181.0	14.4	191.7	0.47
P	151.0	87.7	83.3	5.0	102.7	2.49	94.0	1.63
S	331.0	13.5	57.0	3.74	88.3	12.9	103.0	44.9
Cl	450.0	4.0	286.0	7.26	282.0	4.08	199.0	1.41

	E	SD	F	SD	Range	χ^2	P <
Na	91.0	12.8	210.0	5.72	91.0-210.0	47.0	0.001
K	458.0	27.2	464.0	9.93	445.3-489.3	2.1	NS
Mg	55.3	1.7	61.3	1.9	50.3-61.3	1.1	NS
Ca	170.3	3.1	162.7	4.0	107.8-191.7	23.5	0.001
P	84.0	4.32	94.7	6.6	83.3-151.0	19.6	0.001
S	112.7	5.56	113.7	9.74	57.0-331.0	193.5	0.001
Cl	233.3	4.19	399.0	2.16	199.0-450.0	97.1	0.001

significance. Table 3 presents the minor constituents found among the six lots and follows the same format as Table 2.

Selenium has not been detected in any sample of synthetic diet examined in this investigation. Lot numbers B through F contained substances that were undigestable even though many days were passed attempting to encourage this material to go into solution. Due to the presence of this undigested organic matter, Se could not be detected below 0.8 µg/L. Though each sample was spiked to determine recovery quantities, Se was not detected in any of the 15 samples of this series. Lot A samples presented no such problem and therefore the Se detection limit was 0.2 µg/L. Daphnia magna fed this synthetic diet contained no detectable Se at a detection level of 2 µg/L (Cowgill et al In Press). It is normal for D. magna to contain Se within the range of 1-2 mg/L (Cowgill et al In Press). Since Se is well known to be required for successful reproduction, the lack of Se in the synthetic diet used to maintain D. magna may explain the low mean brood size often observed (Cowgill et al In Press). In addition, Keating (1984) reported that inadequate quantities of Se in foods used to sustain daphnids caused loss of appendages and deterioration of the cutical finally resulting in mortality. It often occurs that fertility declines and sometimes other adverse effects are not noted. Thus the lack of detectable quantities of Se alone make the use of this synthetic diet inadvisable for aquatic species maintenance.

Table 3. Minor chemical constituents ($\mu g\ L^{-1}$) of Lots A through F of formulated trout pellets + alfalfa. (SD=std. deviation; ND=not detected at levels noted in parenthesis). Appropriate blank chemistry has already been subtracted.

Element	A	SD	B	SD	C.	SD	D	SD
Cu	1090	310	360	100	520	350	280	16
Zn	1840	390	850	320	960	130	930	90
Se	ND(0.2)		ND(0.8)		ND(0.8)		ND(0.8)	
Mo	110	19	20	0	20	0	17	5
Fe	8500	2000	3670	960	8470	6180	5670	2020
Mn	930	200	420	30	650	20	670	110
Co	430	120	90	0	3520	4870	80	9
Ni	160	30	110	30	1360	1730	130	20

	E	SD	F	SD	Range	X^2	P <
Cu	260	0	620	60	260-1090	51.0	0.001
Zn	1180	71	1470	54	850-1840	36.4	0.001
Se	ND(0.8)		ND(0.8)		ND	-	-
Mo	13.3	5	20	0	13.3-110	75.8	0.001
Fe	4270	820	4930	340	3670-8500	1916.9	0.001
Mn	610	68	730	57	420-930	192.7	0.001
Co	70	14	110	5	70-3520	3315.5	0.001
Ni	120	16	80	0	80-1360	1137.8	0.001

This synthetic diet consists of a 4:1 mixture of trout food to alfalfa. It is of interest to realize that though the Se content of alfalfa may vary, it is usually detectable (Cowgill et al 1980) and hence the absence of detectable Se suggests that the chemical contribution to the formulated diet is minimal. Thus, it may be proposed that the variations noted in Table 2 and 3 are chiefly due to the differences among the purchased lots of trout pellets.

Molybdenum presents an enigmatic situation. The quantity encountered in the formulated synthetic diet is doubtless sufficient. However, chemical examination (Cowgill et al In Press) of the daphnids consuming this diet failed to reveal detectable quantities of Mo in their tissues. Hence, though Mo is present it is in unavailable form to daphnids.

Of the 15 elements studied in the synthetic diet only K and Mg failed to show a statistically significant variation among the six lots of trout food pellets examined. Table 4 shows the extremes in the "percent coefficient of variability" among the three samples obtained from each lot. Clearly, even aliquots taken from a given lot lack chemical consistency. Thus, there is no quality assurance within lots among the concentrations of the 15 elements examined.

Table 4. Highest and lowest percent coefficient of variability (% CV) for each element among the three samples obtained from each lot.

Element	% CV		Element	% CV	
	Highest	Lowest		Highest	Lowest
Na	15.9	2.3	Zn	37.6	0.002
K	15.2	2.1	Se	ND	
Mg	19.9	3.1	Mo	37.6	0.0
Ca	27.2	0.3	Fe	73.0	6.9
P	58.1	1.7	Mn	21.5	3.1
S	43.6	4.1	Co	138.4	0.0
Cl	2.5	0.5	Ni	127.2	0.0
Cu	67.3	0.0			

Two different manufacturers contributed trout food pellets to this study. Only fat (5%), protein (36%) and fiber (7%) content of trout pellets are regulated. Various governmental agencies and other investigators have stated that synthetic diets are nutritionally adequate (Winner et al 1977; Biesinger and Christensen 1972; Weber and Peltier 1980; Peltier and Weber 1984). However, it is clear from the present study that this synthetic diet in the form that it is fed to D. magna is chemically not defined and provides insufficient selenium to sustain the organism.

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