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A PREFERENCE-TESTING SYSTEM FOR EVALUATING REPELLENTS FOR BLACK-TAILED DEER

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ABSTRACT: In a program to evaluate repellents for protecting Douglas-fir (Pseudotsuga menziesii) seedlings from browsing by black-tailed deer (Odocoileus hemionus columbianus), a preference-testing system was developed to supplement preliminary pen tests. The system uses an apparatus that presents individual test deer with a choice between two foods (usually feed pellets treated with a candidate repellent or a marginally palatable standard). The two foods are presented, in alternating positions, only long enough for the deer to make a choice; results are recorded in terms of percent choices made for the candidate repellent, or percent consumption. Tests thus far with a number of candidate repellents, including several chemical fractions derived from putrefied fish, have generally given clear-cut results, and the system appears very promising for this kind of evaluation. Although semi-tame deer and an experienced operator are required, the system uses very small amounts of candidate repellents, produces evaluations on a material in 1 to 2 days, can be used year-round, and permits observations of deer behavior during the choice process.

INTRODUCTION[1]

In the Pacific Northwest, browse damage to Douglas-fir (Pseudotsuga menziesii) by big game animals poses a major problem in forest regeneration (Black et al. 1969; Crouch 1969). West of the Cascade crest, black-tailed deer (Odocoileus hemionus columbianus) and Roosevelt elk (Cervis canadensis roosevelti) can cause enough injury to seriously retard growth of trees. Since 1960, the Denver Wildlife Research Center has been involved in research to alleviate this damage; repellents of both synthetic and biological origin have been used (Kverno et al. 1965). Some of the most promising biological repellents are fractions derived from extracts of putrefied salmon (Oncorhynchus spp.), or PF (putrefied fish). The chemical and biological problems we have encountered in identifying and testing PF fractions on deer have closely paralleled those encountered in developing the U. S. Navy's "Shark Chaser" shark repellent (Gilbert and Springer 1963), a product developed following observations of sharks being repelled by decomposing shark flesh (Tuve 1963).

Earlier work in isolating and testing PF fractions has been reported (Bullard and Campbell 1968). Recently, numerous compounds in the most active PF fractions were tentatively identified by mass spectrometry. It was not feasible to evaluate the repellency of these compounds by the procedures previously used at this laboratory (Dodge et al. 1967); these tests involved exposing treated seedlings to penned animals, which required large amounts of the experimental compounds, could not be used year-round, and frequently gave inconclusive results. We felt that a system based on a preference-testing device would overcome many of these difficulties. Crawford and Church (1971) recently reported testing procedures for evaluating taste stimuli in aqueous solutions, but these did not appear suitable for the comparisons we required. This paper describes apparatus and procedures we developed for preference tests with deer and elk and illustrates the kind of results we are getting with small amounts of such compounds as PF fractions.

PREFERENCE-TESTING SYSTEM

Apparatus

For tests with deer and elk, the general design of a solid-food preference-testing apparatus developed by Thompson and Grant (1971) seemed to fit our needs; their procedure was a modification of techniques used by Young and Kappauf (1962) and Young (1968) for exposing aqueous solutions to small mammals. A manually rotated plywood wheel, 78 inches in diameter, was constructed (Fig. 1). The apparatus is positioned so that it can be

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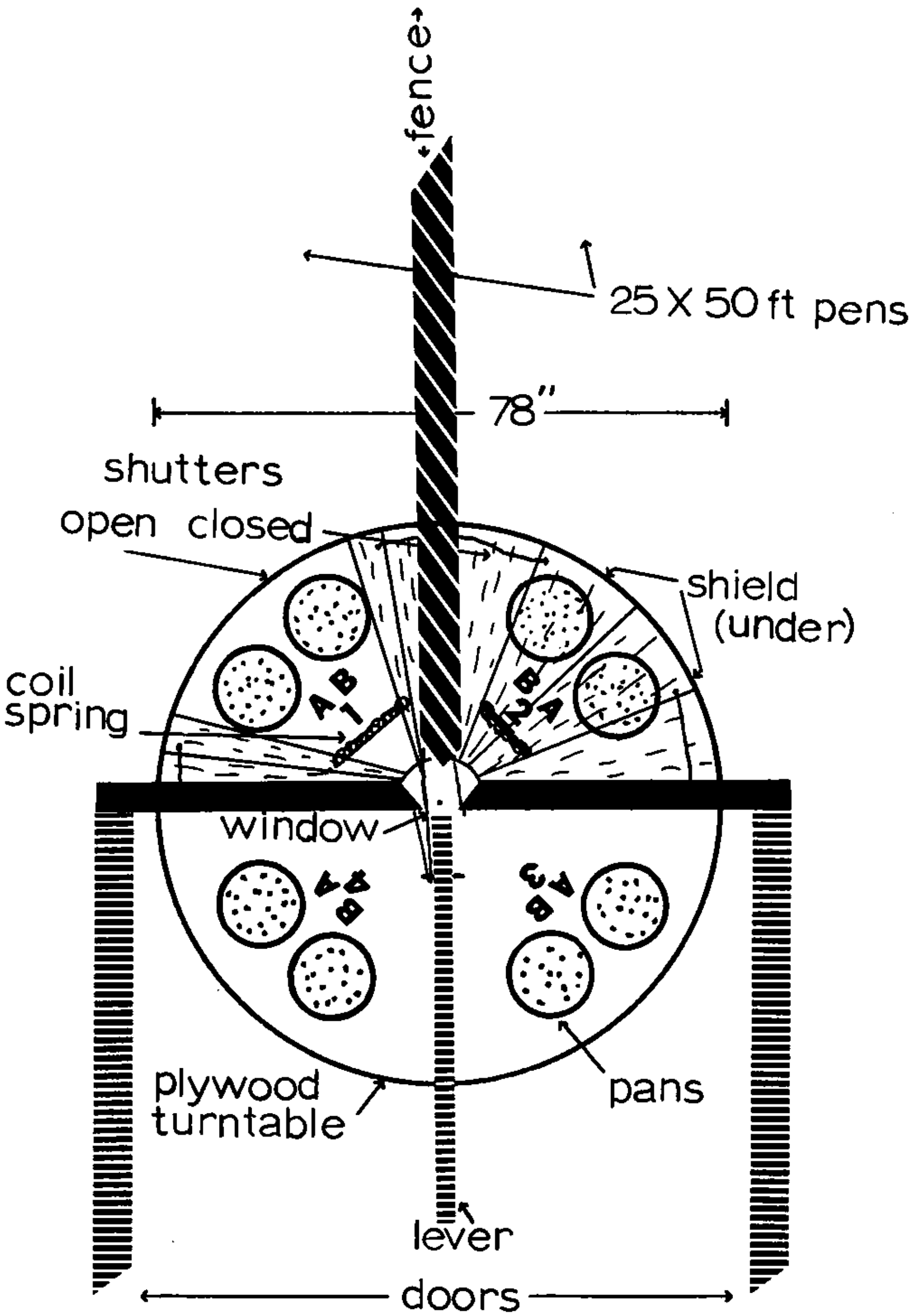

Fig. 1. Top view diagram of preference-testing apparatus.

opened to either of two pens. The wheel holds four pairs of stainless steel pans (11 inches wide by 8 inches deep) spaced 4 inches apart. The wheel is rotated until a pair of pans is in place for exposure; hand-operated shutters hide the pans until they are positioned. The shutters are opened, the wheel is left in place until the deer makes a choice (i.e., eats from one pan for 2 seconds), and the shutters are again closed as the wheel is moved to the next position (Fig. 2, 3). The operator is concealed in a shed, and observations are made through a one-way green plastic window. We have found that talking into a portable tape recorder is the most convenient way to record observations.

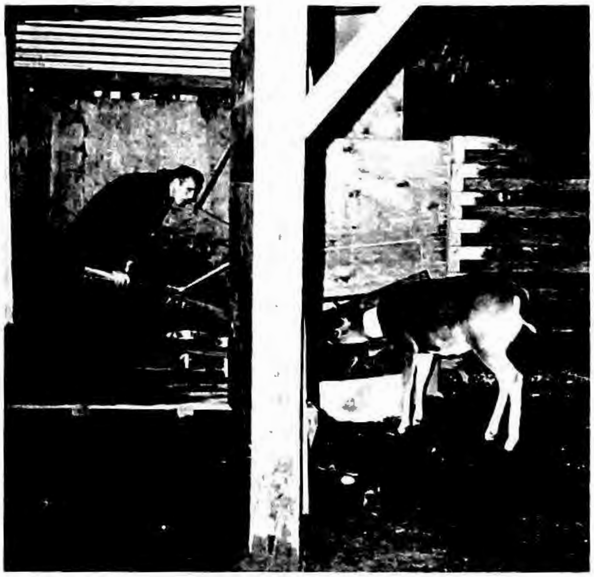

Fig. 2. Preference-testing apparatus with one door open to show observer and shutter-opening lever.

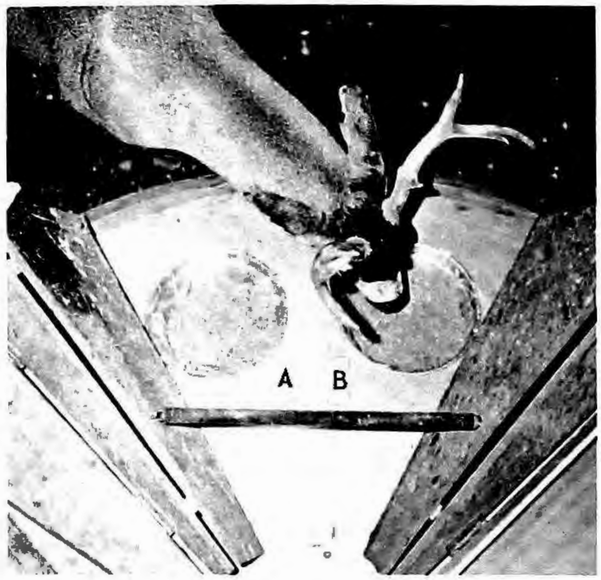

Fig. 3. Arrangement of A and B pan positions with shutters open.

Food Base

Our standard food base is the custom-prepared pelleted deer feed (Appendix A) we use as supplemental food for captive deer and elk. For testing, these pellets (which have been cooled several days after manufacturing) are uniformly coated in a food mixer with 0.4 percent fresh corn oil ("control" pellets) or with treatment formulations in 0.4 percent corn oil. For each test, feed for the various pans (all from the same batch of pellets) is prepackaged in polyethylene bags. These are placed in the pans and serve as a liner that can be changed with each deer (in earlier tests with unlined pans, it appeared that some deer accidentally or intentionally contaminated the pans with odors--possibly pheromones as described by Müller-Schwarze 1971). Normally, 250 grams of pelleted feed is presented in each bag; consumption is determined by reweighing the bags after the test.

During procedure development, several materials--apple juice concentrate, granular dextrose, and freshly ground leaves of salal (Gaultheria shallon)--were tested to establish consistent feeding behavior. The addition of salal did increase acceptance somewhat, but we discontinued it because of the possibility of seasonal changes in palatability combined with increasingly individualized responses by test deer. Recently, Douglas-fir--chopped branches (fresh and dried), pelleted branches, and pelleted seedlings--has shown promise as a food base or additive. Initially, we found that chopped dried foliage was preferred over chopped fresh foliage. Pelleted branches mixed with control pellets were poorly accepted as 30 percent of the mix but were well accepted as 10 percent of the mix. In further testing, pelleted seedlings (less roots; forced-air-dried at 150°F for 12 hours and pelleted at 12 percent moisture content) were well accepted as 25 percent of the food base. Including Douglas-fir as part of the test feed should give a food base that is more closely related than standard grain-based pellets to the Douglas-fir seedlings that deer will encounter in the field, both in general palatability (Longhurst et al. 1968) and in possible effects of volatile terpenes (Maarse and Kepner 1970).

Test Design

Initially, test design followed that used with rats (Young and Kappauf 1962; Thompson and Grant 1971): single pans containing the test foods were offered separately before the two foods were presented together for a choice. This appeared to confuse the deer and resulted in unnecessary consumption (deer will eat only a certain quantity before leaving the test apparatus). Since deer generally made clear choices without this extra step, we decided to present only pairs of treatments.

Two treatments (A and B) are compared in each test. These are arranged in four alternated positions (AB, BA, AB, BA) to eliminate position bias. Tests to determine uniform acceptance of control pellets in both the A and B positions are made before and during each testing series.

During most series, 4 to 10 deer are individually tested once or twice daily for 1 to 2 weeks, depending on availability of deer, feeding behavior, and test requirements. A test consists of at least 10 but usually not more than 30, 2-second choices. Normally a deer completes 20 to 25 choices in 6 to 10 minutes.

Choice preference (acceptance) and consumption for each test are rated as follows:

$$\text{Percent choice (or consumption)} = \frac{\text{Number of choices (or consumption) of test food} \times 100}{\text{Total choices presented (or total consumption)}}$$

Further comparisons are made using chi-square analysis and analysis of variance.

Training Deer

Deer to be trained are first routinely fed from exposed test pans. Once they learn that the pans contain feed, all other sources of food are eliminated. Each deer is then gradually introduced to the noise and motion of the operating test apparatus while it is feeding. Some deer are easily trained; some learn only after feeding with trained deer; and some never overcome their fear of the apparatus and cannot be used. The adaptability of any one deer to feeding and making choices cannot be predicted from its relative tameness. Deer that are aggressive toward other deer during group training do not necessarily feed well when tested alone. We have found that most deer suitable for testing accept the mechanism after several days of training. The one calf elk tested was readily trained.

Feeding Schedule

Numerous sequences of feeding, testing, and food deprivation were tried at various times of the day. Ultimately, the following schedule was selected: deer are allowed to feed overnight on control pellets; feed is removed at 8 a.m.; testing is conducted from 10 a.m. until noon; control pellets are provided from noon until 1 p.m.; and a second test is run from 3 to 5 p.m. This regime appears to provide the most consistent feeding behavior and to keep the deer in better condition than longer periods of food deprivation.

Repellent Standard

TMTD (tetramethylthiuram disulfide), which has been extensively used as a hare and deer repellent (Besser and Welch 1959) was chosen as the "standard" repellent for comparison with experimental materials, and tests were conducted to find a formulation that would give about 30 percent acceptance. (Marginal acceptance is needed for comparing repellents; a standard with too much or too little repellency would tend to be 100 percent rejected or 100 percent accepted.) Three formulations--45 percent TMTD suspension, 95 percent TMTD powder, and Eastman Organic Chemical's[1] practical grade TMTD (bisdimethylthiocarbamyl disulfide)--were tested on pelleted feed at concentrations from 1.0 to 0.01 percent active TMTD. Striking differences were observed when a 0.5 percent concentration of powdered 95 percent TMTD was compared with the same concentration in a corn oil suspension; 65 percent of the suspension was accepted, while the powder coating was completely rejected. The best marginal acceptance was obtained with a formulation of 0.01 percent practical grade TMTD in 0.4 percent corn oil, and this served as a standard for most subsequent tests comparing repellent activity. In tests comparing this formulation with control pellets, deer chose the TMTD about 30 percent of the time, but all deer preferred the control pellets.

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[1] Use of trade names does not imply endorsement of commercial products by the Federal Government.

Some deer are quite sensitive to TMTD, rejecting it in favor of control pellets even at 0.01 percent active, but this rejection is readily lost when the same deer are given a choice between TMTD and a more active repellent. Chronic feeding on TMTD could possibly cause digestive disorders in deer, but this was never observed in our studies. In normal testing, maximum daily consumption of TMTD at 0.01 percent active is less than 400 mg per deer.

Measuring Taste

Odorless quinines were tested to determine how well the system could discriminate repellency based on taste. Deer did not distinguish between control pellets and pellets treated with 0.001 percent quinine hydrochloride in corn oil. Quinine sulfate at 0.1 percent was definitely detected by two adult does and an adult and yearling buck, but not until several seconds after tasting. Altering the treatment positions effectively cancelled any learned position cues, so the deer became confused and choices and consumption for quinine sulfate and untreated feed pellets were nearly equal. This test indicates that our system is not suitable, at least without modification, for evaluating repellency based on a delayed taste response.

TESTING FOR REPELLENT ACTIVITY

Materials so far tested for repellent activity by our system have included a number of naturally occurring and chemically synthesized compounds and mixtures. Some have been merely screened for repellency; others have been tested at progressively reduced concentrations to identify and compare marginal differences in acceptability. The following summary of four test series is given as an illustration of the kind of results we have been getting. Results are expressed in terms of percent choice (acceptance). Consumption data gave essentially the same results but are more difficult to compare because amounts consumed varied considerably with individual deer.

Wild Ginger

Wild ginger (*Asarum caudatum*), a plant native to Washington, demonstrated potential repellent value in three tests. When fresh leaf particles were substituted as 5 percent of the feed pellet mix, acceptance was only 2 percent. Dried leaves in the pellets were much less repellent; acceptance was 20 percent for 3.3 percent leaves, 36 percent for 0.4 percent leaves. Individual deer reacted strongly, however, even at this low concentration. An adult doe completely rejected food, even though apparently hungry. An adult buck chose the mixture only two times (12 percent acceptance) before rejecting it. The specific chemical activity responsible for this repellency has not yet been determined.

PF Fractions

PF fractions G and GM were compared with S in initial tests at 0.02 percent concentrations. Methods of obtaining G, a main subfraction, and S, derived from G, have been described by Bullard and Campbell (1968). Fraction GM is obtained by a process developed by Weyerhaeuser Company (Dr. Katashi Oita, pers. comm.).

Bucks and does consistently rejected the S treatment in favor of the G; rejection by bucks was generally the stronger. One adult buck completely rejected both G and S treatments. He vigorously kicked the feed pans in apparent attempts to turn the wheel for better feed. After much delay and apparent increasing hunger, he chose the G treatment, feeding on it for 16 consecutive choices while rejecting the S treatment. When GM and S were compared, all deer but one completely rejected the S treatment; the exception, an adult doe, chose more S than GM.

PF Fractions in Douglas-fir

The potential value of using xylem sap transport (Hinckley 1971) for a pseudo-systemic treatment of seedlings with PF fractions was investigated. Tests were conducted with chopped foliage made from Douglas-fir seedlings that had been root-soaked in a 2-percent water solution of fraction G or GM to check potential xylem sap transport. Fraction G dispersed in the solution, and its odor could be readily detected in the clipped terminal foliage. However, these seedlings used less than 1 ml water per gram of seedling weight, and the foliage became dry and brittle. Fraction GM appeared to act like an oil in the solution and could not be detected in clipped terminals; these seedlings used about 3 ml water per gram and retained normal appearance.

The G- and GM-treated foliage, mixed with control pellets, were compared with each other in initial tests with three bucks and one doe. After initial sampling, one buck definitely rejected the G-treated mixture (overall acceptance, 21 percent for G, 79 percent for GM). The other deer, however, did not appear to notice the foliage treatment (acceptance, about 50:50). Additional testing of translocatable repellents is strongly indicated.

Components of Selected PF Fractions

Tests were conducted on 12 chemical groups, artificial mixtures approximating the composition of 12 solubility classes tentatively identified in the mass spectral analysis of PF fractions. Again, these dissolved or liquid mixtures were suspended in corn oil and coated on feed pellets at low concentrations. Table 1 summarizes test results with four of the chemical groups.

Table 1. Representative preference tests of selected repellent materials on four black-tailed deer.

		Percent choices/deer			
Test no.	Treatment[a]	Adult male 1	Adult male 2	Yearling female	Yearling male
1	A = untreated	100	100	45	50
	B = 0.01% Group 1[b]	0	0	55	50
2	A = untreated	100	90	50	55
	B = 0.01% Group 4[c]	0	10	50	45
3	A = untreated	99	100	77	42
	B = 0.01% Group 2[d]	1	0	23	58
4	A = untreated	100	100	65	50
	B = 0.01% Group 10[e]	0	0	35	50
5	A = 0.01% TMTD	100	100	100	50
	B = 0.01% Group 2	0	0	0	50
6	A = 0.01% TMTD	100	100	92	46
	B = 0.005% Group 2	0	0	8	54
7	A = 0.01% TMTD	61	50	86	53
	B = 0.001% Group 2	39	50	14	47
8	A = 0.01% TMTD	91	82	83	63
	B = 0.001% Group 2 + 0.001% Group 1	9	18	17	37
9	A = 0.01% TMTD	57	33	88	52
	B = 0.001% Group 2 + 0.001% Group 4	43	67	12	48

[a]All treatments were based on feed pellets coated with 0.4% corn oil.
[b]Equal parts of benzothiazole, 2-methyl benzothiazole, and 2,5 diethyl benzothiazole.
[c]Equal parts of O-hydroxyacetophenone, m-hydroxyacetophenone, and p-hydroxyacetophenone.
[d]Equal parts of indole, skatole, and n-hexyl sulfide.
[e]Equal parts of 1-hexadecylamine, n-octylamine, and quinaldine.

The first four tests, initial assays to establish repellency, compared control pellets and pellets treated with a relatively high concentration (0.01 percent) of each chemical group. These data showed clear individual repellency for all four mixtures and suggested somewhat greater activity for groups 2 and 10 than for 1 and 4. There were relatively clear-cut similarities and differences among deer; in general, the two yearlings seemed less discriminating than the two adult bucks.

Tests 5 through 9 show differences that began occurring as concentrations were changed and groups of chemical components were combined to investigate synergism. In these tests, the candidate repellent mixtures were compared with the 0.01 percent TMTD standard. Chi-square tests showed that the lower concentration of Group 2 in Test 7 was significantly better accepted than the higher concentration in Tests 5 and 6. Synergistic activity was demonstrated in Test 8; low concentrations of Groups 1 and 2 were statistically as repellent as a higher concentration of Group 2 alone (Test 5). The yearling male, however, did not discriminate between any of these treatments and TMTD.

DISCUSSION AND CONCLUSIONS

We have been generally pleased with the preference-testing system described here. It has several obvious advantages over pen tests in evaluating candidate repellents; (1) It requires very small amounts of active ingredient; for example, a typical individual deer test with feed pellets treated at 0.01 percent requires less than 100 mg. (2) It is fast (1 to 2 days vs. 2 to 3 weeks for pen tests). (3) It can be used nearly year-round, whereas pen and field tests are restricted to the dormant season of Douglas-fir (approximately November 15 through April 15) and the summer growing season from May 15 to July 15. (4) It permits observations of deer behavior related to the taste or odor of candidate repellents, differences associated with sex and age, and individual differences; such indications provide a basis for additional testing. From the small amount of work so far done on elk, it appears that these advantages will hold for this species also.

As with any system, there are of course certain disadvantages as well. The most obvious is the necessity of using semi-tame deer that can be trained and handled. The number of such animals available is a limiting factor in test programs. In addition, an experienced operator must be present continuously during testing, and deer must be handled individually. Group tests were investigated but did not appear practical. Although deer which were normally unwilling to feed alone would join others feeding, the social dominance of some deer caused inconsistencies. In addition, reactions to odors left on the pans by other deer were particularly noticeable in group testing.

The great individuality among deer is a major problem and may necessitate more tests or more animals than expected when fine discrimination is required. Some of the preference-test differences in response to repellents may be related to sex, breeding condition, or age (for example, the two yearlings in Table 1 appeared less discriminating than the adults). These relationships require further study.

Finally, it should be emphasized that although the preference-testing system will help identify and compare active repellents, it is an "artificial" system, and further testing in enclosures and field installations is still required.

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APPENDIX A

DEER FOOD PELLET FORMULA (3/16 x 1/2-inch pellets):

	Percent	Pounds/ton
Ground corn	30.00	600
Millrun bran	13.75	275
Ground wheat	12.50	250
Beet pulp	10.00	200
Dehyd. alfalfa meal (18% protein)	10.00	200
Soybean meal (48-1/2% protein)	8.75	175
Molasses	7.50	150
Herring meal (72% protein)	5.50	110
Trace mineral salt	1.00	20
Dicalcium phosphate	1.00	20