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FOOD-BASE FLAVOR ADDITIVE IMPROVES BAIT ACCEPTANCE BY RICEFIELD RATS

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FOOD-BASE FLAVOR ADDITIVE IMPROVES BAIT ACCEPTANCE BY RICEFIELD RATS[1]

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Abstract: Philippine ricefield rats (*Rattus rattus mindanensis*) that were accustomed to eating rice grain showed greatly increased preference for granulated rice when it was enriched by the volatiles from ground unpolished rice. This was the best of 11 formulations involving 8 rice components that were tested with an automated brief-exposure preference tester. This formulation was also highly preferred ($P < 0.005$) over untreated granulated rice in an overnight paired preference test. In a similar test, rice volatiles significantly ($P = 0.04$) improved the acceptance of a marginally palatable bait. In a test with groups of rats in 3- × 3-m enclosures, the rice volatiles formulation was preferred ($P < 0.05$) over whole-grain rice, untreated granulated rice, and granulated rice with soybean oil. Finally, cage tests were conducted to determine if the rice volatiles would increase the acceptance of an acute toxicant bait. Rats given a choice between granulated rice with and without 0.2 percent zinc phosphide ate more of the untreated material and showed 50 percent mortality; rats given a choice between untreated granulated rice and the rice volatiles formulation with zinc phosphide ate more (> 2×) of the treated bait and showed 88 percent mortality. These results indicate that intensifying the flavor cues associated with a familiar or preferred food is a fruitful approach in the development of an effective bait enhancer.

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The familiar problem of bait acceptance in rat control programs readily applies to the Philippine ricefield rat. In their 1971 Annual Report (p. 61) scientists of the Philippine Rodent Research Center (College Laguna, Philippines) stated: "We suspect that bait acceptance is a major factor limiting the effectiveness of acute toxicants as now used for rodent population reduction in Philippine ricefields; methods to improve acceptance need further investigation." Because of this need, we began a program to develop highly palatable baits for rat control in ricefields.

The food preference behavior of wild rats is very complex. The drive to satisfy energy requirements affects preference (Schein and Orgain 1953, Cornwell and Bull 1967, Brooks and Bowerman 1973). Novelty also can be important (Welker and King 1962, Bronson 1966, Long and Tapp 1967, Valle 1970). A novel stimulus may direct an animal's exploratory behavior and lead it to the bait, but consumption of the bait may or may not follow, depending on how much wariness the same novel cue elicits. As the bait's novelty decreases, so does its ability to direct exploratory behavior (Long and Tapp 1967). Furthermore, novel factors such as taste, odor, color, and texture may provide cues for bait aversion (shyness).

However, response to a familiar food is more predictable. Previous experience is an important factor (Barnett and Spencer 1953, Jackson 1965, Cornwell and Bull 1967, Reif 1969, Bull 1972, Howard and Marsh 1974). In tests and observations of feeding behavior, we have found that rats consistently prefer familiar foods over unfamiliar ones. The durability and strength of this response seem to increase when the familiar food has been a major diet item and is satisfying the animal's nutritional needs. Tigner (1972) found that when there is enough rice grain in the paddies to sustain ricefield rats, it makes up 60–85 percent of the

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volume of food in their stomachs. Thus, the most familiar food of the rats that damage rice plants is the rice grain itself. Could a rice fraction that intensified the familiar flavor cues of rice provide the key to developing a highly palatable bait? The series of studies described below was designed to answer this question.

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GENERAL METHODS

All test animals were wild ricefield rats livetrapped in the Philippines, shipped by air to the laboratory at Denver, and held in individual cages for acclimation at least 30 days. For at least 2 weeks before testing (at least 1 week in phase 5), all were fed whole-grain, unpolished California Brown Pearl rice *ad libitum* (a few rat chow pellets were also offered in each cage so that the rats could balance their nutritional requirements, but they consistently ate mostly rice). During laboratory tests (phases 1, 2, 3, and 5), rats were held in individual 20- × 35-cm cages and offered only rat chow pellets between testing periods. For uniformity, all tests were conducted with California Brown Pearl rice in a granulated formulation, with or without test materials added.

We used a domestic rice variety because import restrictions make it difficult to obtain sufficient quantities of a Philippine variety for large-scale testing. Our earlier work and that of Hougen et al. (1971) indicated that the flavor of California Brown Pearl rice was close to that of the varieties the rats had probably encountered in the Philippines and that the 1 or 2 weeks during which the domestic variety was their principal diet was sufficient to assure that it was a familiar food.

TEST SERIES

Phase 1: Preference Tests with Different Rice Grain Fractions

In this first study, we tested 8 materials (11 concentrations) representing different fractions of rice grain to determine if any of them would increase acceptance if added to rice. All fractions were tested as ingredients in a basic granulated rice formulation prepared by adding 30 ml of a 25 percent ethanol solution of Plasdone (polyvinyl pyrrolidone) to a uniform mixture of 75 g ground rice material and 25 g Avicel (cellulose) filler (reference to trade names does not imply U.S. Government endorsement of commercial products). Unless otherwise specified, the 75 g of rice material was whole-grain California Brown Pearl rice ground to about 16 mesh size. This moist preparation was spread in a thin layer for drying; after the ethanol evaporated, the material was ground to 2 mm or smaller in a Wiley mill.

The eight rice fractions tested were as follows (none contained preservatives or other food additives):

(1) Cold-pressed rice bran oil (Hain brand). A diethyl ether solution of the oil was added to a round-bottom flask containing the granulated rice. The diethyl ether then was removed by rotary evaporation, leaving the oil as a uniform 0.2 or 2 percent coating on the granules.

(2) Acetonitrile extract of cold-pressed rice bran oil. Hain rice bran oil was extracted (in a separatory funnel) twice with equal volumes of acetonitrile. After removal of the acetonitrile by rotary evaporation, the oily extract was dissolved in diethyl ether and coated, as before, on granulated rice at 0.2 or 2 percent.

(3) Diethyl ether extract of rice. Ground (40 mesh) California Brown Pearl rice was extracted in a Soxhlet apparatus for 4 hours with diethyl ether. After removal of most of the ether by rotary evaporation, the extract was coated on granulated rice at 0.2 or 2 percent.

(4) Endosperm. Vemp brand stone-ground rice flour was mixed 1:1 (by weight) with ground California Brown Pearl rice and the mixture was granulated.

(5) Rice polish. El Molino brand rice polish was mixed 1:1 with ground California Brown Pearl rice and the mixture was granulated.

(6) Rice bran. El Molino brand rice bran was mixed 1:1 with ground California Brown Pearl rice and the mixture was granulated.

(7) Rice bran volatiles. Volatiles from 2 parts (by weight) of El Molino rice bran were trapped cryogenically onto 1 part of granulated California Brown Pearl rice by the process described below.

(8) Rice volatiles. Volatiles from 2 parts of ground California Brown Pearl rice were trapped cryogenically onto 1 part of granulated California Brown Pearl rice by the process described below.

A modified rotary evaporator was used for collecting the volatiles from rice bran and ground rice. A cold trap containing the granulated rice replaced the standard Friedrich condenser. The ground rice or rice bran was placed in a 2-l round-bottom flask that was modified by having 3 symmetrically spaced depressions (2 cm deep) on the side to serve as baffles. These tumbled the material during rotation, which in combination with a 50 C internal temperature supplied by an infrared lamp, provided appropriate conditions for the release of volatiles. A helium gas stream of 20 ml/minute (measured by soap bubble flow meter) swept the volatiles into a cold trap, which was immersed in an acetone-dry ice bath. At the end of a 3-hour collection period we thoroughly mixed the trap contents and then poured them into a capped bottle that was held under refrigeration until testing (within 48 hours).

Each of the 11 test formulations was compared separately against the control (granulated rice formulation without a rice fraction additive) in standard preference tests with the apparatus described by Thompson and Grant (1971). The only difference between the control and test formulations was the rice fraction being tested. This automatic taste preference tester is programmed so that the animal samples Food A alone for 3 seconds, Food B for 3 seconds, chooses between A and B presented simultaneously (accumulated 6 seconds), samples Food B, samples Food A, and finally chooses between B and A (i.e., positions reversed). Temporal and spatial position habits are minimized by alternately presenting the foods with sequence and positions reversed. The device is placed in the rat's home cage so that no animal handling is necessary. Tests are conducted during the beginning hours of a reversed 12-hour light cycle in a sound-attenuated room where the animal is isolated from visual and auditory interferences.

Six rats were used for each comparison; all had been conditioned to eat from the apparatus and had been tested for uniformity of response by the standard procedure described by Shumake et al. (1971). During the testing period, all were slightly food deprived by restricting their total daily ration (including food available during tests) to 12 g. Each rat was tested twice on successive days for a total of 32 choices. Preference was measured as the percentage that consumption of the test food contributed to the total eating time and to the amount of food eaten.

Preference scores for the 11 test formulations are summarized in Table 1. Four of the formulations were significantly better than the control ($P \leq 0.1$) for either eating time or food consumption. Because the rice volatiles formulation was the only one better than the control for both measurements, in addition to having the highest mean preference score in each case, it was ranked highest and, therefore, selected for advanced testing.

Phase 2: Paired Preference Test

The brief-exposure foods-together test used in phase 1 is a very rigid one and seldom reveals great differences. Therefore, the rice volatiles formulation was tested against the control by an alternative procedure widely used in rodent control research, a paired preference test measuring overnight consumption.

Methods.—Twelve individually caged Philippine ricefield rats (6 males and 6 females) were offered a choice between the rice volatiles and control formulations (same as used in phase 1) during 16 hours per night for 4 consecutive nights. The test materials were placed 5 cm apart in food cups at the front of each cage and their positions were alternated every night to decrease position habitat bias. Weighed amounts of the baits were placed in the cage each evening and the unconsumed remainder was weighed the following morning. The difference, corrected for any spillage caught on papers under the cage, was recorded as the quantity consumed overnight. Preference was measured as the percentage the rice volatiles formulation made up of the total food consumed.

Results.—The total food consumption of individual rats during nightly periods was variable (range, 2.7–23.0 g) but showed no particular patterns. Most of the 12 animals appeared to have some preference for feeding from either the right or left food cup; because the two test foods were alternated, preference scores for these animals tended to be either higher or lower on nights 1 and 3 than on nights 2 and 4. Nevertheless, for the 12 rats over 4 nights, there were only 10 occasions when animals ate more of the control than of the treated food. Overall, the rice volatiles formulation accounted for 64.1 ± 2.9 percent (standard error) of the total food eaten (range, 26.9–97.9 percent). Analysis by paired *t*-test showed that the difference in consumption due to treatment was highly significant ($P \leq 0.005$). Thus, the rats showed a consistent preference for the rice volatiles formulation in overnight consumption tests, in spite of the confounding influence of position habits and post-ingestional factors that have made some critical of this testing method (Young 1968, Thompson and Grant 1971).

Table 1. Preference of ricefield rats given a choice between the granulated control formulation (75 percent ground rice, 25 percent cellulose) and a treated formulation (same material plus additive), six rats tested twice for each treatment.

Treatment	% total eating time (mean ± SE)	% total food consumed (mean ± SE)
2% rice bran oil	39.7 ± 12.7	38.8 ± 11.4
0.2% rice bran oil	46.7 ± 6.6	48.8 ± 2.9
2% rice bran oil acetonitrile extract	54.0 ± 8.8	49.7 ± 5.4
0.2% rice bran oil acetonitrile extract	46.1 ± 2.4	48.2 ± 4.7
2% ground brown rice ether extract	52.9 ± 5.6	45.3 ± 3.7
0.2% ground brown rice ether extract	52.4 ± 5.7	51.1 ± 4.3
50% endosperm	51.7 ± 6.0	56.0 ± 5.1*
50% rice polish	56.7 ± 4.02*	52.4 ± 13.5
50% rice bran	61.8 ± 10.9*	55.5 ± 8.2
Trapped rice bran volatiles	51.1 ± 8.9	47.1 ± 3.6
Trapped whole rice volatiles	68.5 ± 19.0*	65.3 ± 20.0*

* Significantly greater than the control percentage ($P \leq 0.1$) by a 2-tailed paired test.

Phase 3: Improving a Marginally Palatable Food

One of the problems encountered in applying acute-toxicant baits is the decrease in palatability caused by the chemical. Phase 3 was a preliminary check to determine if rice volatiles would increase the acceptance of a marginally acceptable food.

Methods.—By testing a series of granulated rice formulations of increasing cellulose content, we found that a 75 percent concentration was the threshold of rejection (lowest concentration that elicits food rejection) for ricefield rats with minimal food deprivation. Shumake et al. (1971) obtained similar results with Norway rats (*Rattus norvegicus*) and wheat formulations. Consequently, for the control formulation in this test, we modified the basic granulated formulation of phase 1 to contain 25 percent ground rice and 75 percent cellulose. The treated formulation was prepared by cryogenically trapping volatiles from 2 parts of California Brown Pearl rice onto 1 part of this material. The 2 foods were compared in a 4-night paired preference test by the same methods and test rats as in phase 2.

Results.—The rats' overnight food consumption was variable and similar to that in phase 2 (range, 1.9–24.7 g). Again, some animals appeared to show a preference for the right or left food cup, although this pattern was not as pronounced as before. In this test, rats ate more of the control than of the treated food during 16 of the 48 observations. Overall, the rice volatiles formulation accounted for 62.0 ± 3.36 percent (SE) of the total food eaten (range, 21.6–100 percent); analysis by paired t-test showed that the difference due to treatment was significant ($P = 0.04$). Thus, the presence of rice volatiles increased the consumption of a marginally palatable food. Although the mode of palatability change caused by a toxicant may differ from that caused by high cellulose content, these results confirmed that rice volatiles were worth further investigation as a bait enhancer.

Phase 4: Enclosure Test

All previous tests had been conducted with individual animals in laboratory cages. To determine if group behavior and nonconfinement would change the preference response, an 8-day preference test was conducted with rats in small enclosures.

Methods.—Four rice formulations were tested: whole-grain California Brown Pearl rice, the granulated control and rice volatiles formulations of phase 1, and a 1 percent soybean oil formulation prepared by adding a diethyl ether solution of the oil to the control formulation and removing the ether by rotary evaporation to leave a uniform coating. Whole-grain rice was included to evaluate the importance of texture, and the soybean oil formulation was included because it had been the leading candidate before the development of the rice volatiles formulation.

The tests were conducted in four 3- × 3-m enclosures within an 11-m diameter converted grain storage bin. Each enclosure contained five animals of the same sex; two held males and two held females. Temperatures were controlled at 22.5 ± 5 C. A reversed 14:10 light:dark cycle was maintained with lights on at 1700 and off at 0700. Water and shelter were available in the center of each enclosure. Four feeders, each containing a different formulation, were placed in the four corners; their positions were rotated each day to counteract position habits. The feeders, which were designed to allow simultaneous feeding by all rats and prevent contamination and spillage, were left in the enclosures for 7 hours a day (0800–1500); no other food was of-

Table 2. Daily consumption of four rice formulations by ricefield rats (five each) in four enclosures.

Day	Granulated rice with rice volatiles	Grams consumed (mean ± SE) Granulated rice with 1% soybean oil	Granulated rice control	Whole-grain rice
1	35.1 ± 3.2	22.8 ± 2.9	12.9 ± 2.7	8.1 ± 1.7
2	35.1 ± 3.2	21.9 ± 2.3	8.9 ± 1.6	6.6 ± 2.1
3	35.9 ± 3.3	25.9 ± 2.5	15.5 ± 2.7	5.3 ± 2.0
4	34.4 ± 2.8	16.4 ± 3.0	14.6 ± 2.9	6.4 ± 2.4
5	34.6 ± 2.6	19.8 ± 3.0	12.0 ± 2.3	5.8 ± 2.0
6	36.8 ± 3.1	21.6 ± 2.6	15.6 ± 2.6	4.5 ± 1.7
7	35.8 ± 2.7	15.0 ± 2.2	18.9 ± 2.4	5.5 ± 2.6
8	32.4 ± 1.7	21.9 ± 3.0	19.1 ± 3.3	7.0 ± 2.8
Mean	35.0[a]	20.6[b]	14.7[bc]	6.2[c]

[a-c] Mean followed by letter *a* is significantly different from those means not having *a*; those followed by *b* are significantly different from those not having *b*, etc. ($P < 0.05$) by Duncan's new multiple-range test.

fered. The weight of food missing from each feeder at the end of the 7-hour period was recorded as the weight of food consumed.

Results.—The rats' daily consumption of the four formulations is summarized in Table 2. Analysis of variance showed no significant differences due to sex, enclosure, or day, but a significant difference among treatments ($P < 0.05$). Mean separation by Duncan's multiple-range test showed significantly greater consumption of the soybean oil formulation than of the whole-grain rice and granulated rice control (which were not different), and significantly greater consumption of the rice volatiles formulation than of the other three. Consumption of the rice volatiles formulation averaged over twice that of the granulated rice control, a greater margin than shown in any previous tests.

Phase 5: Paired Test with Toxic Bait

The final step was to determine if rice volatiles would increase the acceptance of a bait treated with an acute toxicant and result in greater mortality.

Methods.—The general testing procedure was the same as used in phase 2. Test rats were divided into 2 groups of 16 each (8 males and 8 females). During a 3-day prebaiting period, all rats were offered granulated rice in 2 food cups for 7 hours a day; 1 cup contained 20 g of granulated untreated rice and 1 contained 20 g of the untreated rice volatiles formulation. All animals were given rat chow pellets ad libitum during the 17-hour periods between all choice periods. During the following 3 days (baiting period), 2 cups were again offered for 7 hours a day; 1 contained 20 g of untreated rice and 1 contained 20 g of the bait treated with 0.2 percent zinc phosphide (by weight). The bait carrier for group A was untreated rice; for group B, the rice volatiles formulation. Both baits were prepared by uniformly mixing zinc phosphide with the granulated rice material. As before, cup positions were alternated daily. Consumption from each cup was measured at the end of each 7-hour period, and mortality was recorded daily.

Results.—Consumption and mortality for the two test groups are summarized in Table 3. Males and females did not differ significantly in any comparison made. During prebaiting, both test groups ate significantly more of the rice volatiles formulation than of the untreated rice; preference did not differ significantly between groups. During baiting, bait consumption was significantly greater for group B, given the rice volatiles bait, than for group A, given the con-

Table 3. Bait consumption and mortality in a paired test when ricefield rats (16 per group) were offered the granulated control or rice volatiles formulation treated with 0.2 percent zinc phosphide for 7 hours per day.

3-day period	Test group	Bait carrier	Grams/rat/day consumed (mean ± SE)			Total Zn_3P_2 dose, mg/kg/rat/day (mean ± SE)	% mortality
			Untreated volatiles	Untreated control	Zn_3P_2 bait		
Prebaiting	A		3.0[a] ± 0.4	1.7 ± 0.3			
	B		4.3[a] ± .6	2.1 ± .4			
Baiting	A	Granulated rice		2.0 ± 0.3	1.4 ± 0.3	19.8 ± 3.7	50
	B	Granulated rice with volatiles		1.2 ± .4	2.6[b] ± .5	33.9[b] ± 5.3	88[c]

[a] Within groups, consumption significantly greater for untreated rice volatiles than for control formulation ($P < 0.05$) by paired t-test.
[b] Bait consumption and Zn_3P_2 dose significantly greater for group B than for group A ($P < 0.05$) by unpaired t-test.
[c] Mortality significantly greater in group B than in group A ($P < 0.005$) by chi-square test.

trol formulation bait. In fact, the group B rats ate more of the zinc phosphide bait than of the untreated rice 66 percent of the time, almost twice as frequently as the group A rats, which did this only 36 percent of the time. The consistently greater bait consumption in group B was reflected in the significantly greater zinc phosphide doses (mean, 19.8 mg/kg in group A, 33.9 mg/kg in group B) and the significantly greater mortality (8 dead in group A, 14 dead in group B).

The 0.2 percent concentration of zinc phosphide was chosen as moderately toxic under laboratory conditions. At this level, we expected about one-half of the rats to begin experiencing sublethal intoxication and stop eating the bait before they received a lethal dose. This occurred in group A, but in group B most of the rats apparently ate the bait fast enough to ingest a lethal dose before detecting sublethal effects. Thus, adding rice volatiles to a well accepted, moderately toxic bait made it sufficiently attractive to largely overcome effects of bait shyness and result in high mortality.

DISCUSSION

In the laboratory, the rice volatiles formulation worked well as a bait carrier for an acute toxicant, even though untreated food was available. It also improved acceptance of a marginally palatable food. We suspect that it would work even better in the field as a carrier for a cumulative toxicant such as an anticoagulant, where bait shyness does not have to be overcome and the problem is merely to make the bait more attractive than the rats' everyday food.

During volatiles collection the weight increase of granulated rice in the cold trap ranged from 0.04 to 0.16 percent depending on the moisture content of the batch of rice. To test the influence of moisture on preference response additional replications of Phase 2 and Phase 5 were conducted. Water (0.09 percent) was added to the bait formulations not containing trapped rice volatiles. In both cases the results were essentially the same as they were in the first replication. Therefore, the preference response was due to the volatiles and not to moisture.

In any baiting program, more rats can be reached if olfactory attraction draws them from distances of several feet. Part of the excellent performance of the rice volatiles formulation in the enclosure test may have been due to such an attraction. Field tests are needed for more accurate assessment of this behavior.

Naturally, before rice volatiles can be made into a practical bait additive for the Philippine farmer, more work is needed. The volatiles used must correspond in flavor (compounds and concentrations) to those in the rice variety familiar to the target animals. Since the extremely low yield would make commercial stripping of the volatiles from rice too costly, our alternative is to develop a synthetic rice essence from the important compounds. Therefore, the flavor components need to be identified chemically. To remain effective for a reasonable time, the volatile components of the synthetic essence will have to be formulated in a keeper that will slow their release. Finally, field tests will be required to show that the final synthetic product is actually effective in the ricefields. We are already working on some of these.

From our success with rice volatiles, we believe that intensifying the flavor cues associated with a familiar or preferred food will prove to be a good starting point for the development of additives that increase bait acceptance by rats, and probably other species, in many situations.

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