Research Note

AROMA AND FLAVOR ENHANCERS IN A LIQUID FEED SUPPLEMENT CONTAINING 90% OF LIQUID STREPTOMYCES SOLUBLES[1]

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Liquid steptomyces solubles (LSS) is a byproduct resulting from the fermentative production of the antibiotic erythromycin by Abbott Laboratories, Inc., in Barceloneta, PR. Although this material has limitations with respect to animal acceptance, due to its pungent odor and high acidity, it has long been sold in mixture with 30% of cane molasses as a liquid cattle feed on the island. As local production of molasses has declined and the cost of imported molasses has increased with time, the possibility of including a higher proportion of LSS and lower proportion of molasses in the mixture has become more attractive. However, such proportionate changes might be counter productive if animal acceptance were seriously impaired. One possible means to restore the loss of acceptability caused by a reduced molasses content is to use additives, such as aroma and flavor enhancers. The present experiment was undertaken with the objective of testing the acceptance by dairy heifers of liquid feeds (LF) containing only 10% of molasses, in combination with 90% of LSS, either without additives or with addition of only a commercial aroma enhancer at two levels of concentration, or of a commercial aroma and flavor enhancer, in comparison with the standard 70% LSS: 30% molasses product.

Three Brown Swiss and two Holstein heifers, 24 months of age or older, were used to compare the relative consumption of five different LF when offered in pairs. The distinguishing features of the LF tested are shown in Table 1.

There are 10 possible combinations of two of the five LF; thus 10 periods were needed to test all combinations in each animal. In successive pairs of periods (1+2, 3+4, 5+6, 7+8 and 9+10) each combination of two LF was included once. Also, during each of the first eight periods each individual LF was offered to two animals. However, in the last two periods deviation from this balanced pattern was unavoidable; thus B was offered to three animals in period 9 and to only one in period 10, while the reverse was true of C. This is shown in the following tabulation of assignment of animals to pairs of LF in each period:

	Period									
Animal	1	2	3	4	5	6	7	8	9	10
1	AB	BD	DE	AD	BE	CE	CD	AE	BC	AC
2	DE	AC	AB	BE	AD	BD	CE	BC	AE	CD
3	AD	CD	AC	BC	AE	AB	BE	BD	DE	CE
4	CE	AE	BD	CD	BC	AC	AD	DE	AB	BE
5	BC	BE	CE	AE	CD	DE	AB	AC	BD	AD

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TABLE 1.—*Formulas of the liquid feed compared in the experiment.*

Liquid feed	Percentage molasses	Percentage LSS	Additive
A	30	70	None (positive control)
B	10	90	0.025% aroma enhancer (lower level)
C	10	90	0.050% aroma enhancer (higher level)
D	10	90	0.090% aroma + flavor enhancer
E	10	90	None (negative control)

The periods were normally of 11-day duration, with the first five days serving for adjustment and the remaining six days for comparing intake of the LF. However, during periods 2 and 8, heavy rains with wind caused water to fall into some of the LF feeders, resulting in lost data and necessitating extension of the respective comparison phases for an extra two days and one day, to complete six days of usable data.

The management routine included housing the animals overnight (from approximately 15:00 to 06:00 h) in individual pens; these have a concrete floor and each was equipped with a water trough and two feeders for LF, located under a high sheet metal roof covering part of the area. The heifers were offered daily a weighed amount of each of two fresh LF greater than they had consumed the previous day. Leftover LF was weighed to determine intake. On alternate days, one and then the other of the two LF being compared, was placed in each previously washed feeder, thus avoiding possible confounding of animal preference for a given feeder with preference for the LF. At about 06:00 h daily the heifers were released from their pens and grazed together in an adjacent paddock of roughly 1-ha area on a sward of mixed grasses. Rainfall was abundant during the experiment and adequate pasture herbage was always available.

The LSS stored in 55-gallon drums tended to separate into a higher density free-flowing liquid fraction and a lower density semi-solid, viscous supernatant. Upon preparing 20-kg batches of LF, some of both of these LSS fractions were combined with the appropriate proportion of molasses and, where applicable, the additives. An electric drill fitted with a paint-stirring attachment was used to blend the mixture uniformly in a large pail. On two occasions during the course of the experiment, samples of LSS and molasses were taken for determination of dry matter content (AOAC, 1988).

The aroma and aroma plus flavor enhancers used were pale yellow liquids, miscible but not truly soluble in water, of characteristic aroma, and specific gravity 0.84 and 0.85, respectively. They were supplied by the firm Feed Flavors, Inc. of Wheelling, IL[4], in one-pint plastic bottles, with instructions to store under refrigeration. The formulas of these commercial products are proprietary information, but they are likely mixtures of naturally occurring essential oils and/or synthetic aromatic compounds (Namur et al., 1988). The enhancers were added in weighed amounts to the LF.

Once during each period, body weights of the five animals were estimated from measurement of the heart girth with a calibrated tape. Body weight data were used only for expressing LF intakes on a relative basis.

Consumption of LF was the only response criterion under study in this experiment. Data on this variable were subjected to analysis of variance to test the effects of the suc-

[4]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

cessive pairs of periods (n = 5), animals (n = 5) and treatments (n = 5). The variance due to treatments was subdivided into specific contrasts to compare: (1) positive control (A) vs. three treatments including the use of additives (B, C and D); (2) negative control (E) vs. B, C and D; (3) treatments including aroma-only additive (B and C) vs. inclusion of aroma plus flavor additive (D); (4) treatment with lower (B) vs. with higher level (C) of aroma additive. The Bonferroni test was used to establish acceptance or rejection of the null hypothesis in these contrasts. In a second statistical approach, paired student T-tests were used to make head to head comparisons in each of the ten pairs of LF.

The samples of LSS and molasses were found to contain 29.0% and 79.5% dry matter, respectively. Thus the theoretical dry matter content of the 90% LSS: 10% molasses LF (B, C, D and E) was 34.0%, whereas that of the 70% LSS: 30% molasses mixture (A) was 43.5%.

Overall mean daily intake of LF was 1.43 kg per head on the as feed basis. Since two LF were offered simultaneously, the overall mean total LF intake was 2.86 kg. The five heifers showed marked differences in this respect. Mean total daily LF intake of individual animals ranged from 5.68 to 1.57 kg. The two Holsteins, with mean intakes of 5.68 and 3.20, markedly surpassed the 2.04, 1.81 and 1.57 kg values of the three Brown Swiss heifers. Animals constituted the single most importance source of variance ($P < 0.001$).

The variance due to periods did not quite reach significance at $P = 0.05$. In spite of the fact that LF intake per animal in periods 1 and 2 (2.07 kg) was markedly lower than during the remaining periods. Maximum total LF intake was observed in periods 5 and 6 (3.53 kg), whereas means of 3.15, 2.89 and 2.82 were recorded for periods 3+4, 9+10 and 7+8, respectively. The finding that under these conditions the animals required several weeks to become completely adjusted to eating the LF, as judged by a near leveling off of intake, could be a matter of practical concern in commercial herds.

The factor treatments also constituted a very important source of variance ($P < 0.001$). Mean (± std. error) daily intakes of the individual LF were: A, 2.31 ± 0.34; B, 1.24 ± 0.22; C, 1.18 ± 0.22; D, 0.93 ± 0.11; E, 1.48 ± 0.26 kg. Specific treatment contrasts showed that the positive control (A) was consumed in greater quantity ($P < 0.001$) than the combined three LF with additives (B, C and D), which gave a mean value of 1.12 kg. The latter value failed to equal even that of the negative control (E), though this was not a significant ($P > 0.05$) difference. The LF with aroma enhancer only (B and C) tended to be consumed in greater amounts (1.21 kg combined mean) than the LF with aroma plus flavor enhancers (D), but not significantly so. There was only a slight numerical advantage ($P > 0.05$) for the lower level (B) over the higher level (C) of aroma enhancer addition. When these mean daily as-fed intakes are expressed on a dry matter basis, the relative advantage in favor of treatment A is magnified (1.0 vs. 0.42, 0.40, 0.32 and 0.50 kg for B, C, D and E, respectively).

The head to head treatment comparisons summarized in Table 2 show that A emerged victorious when matched against each of the other LF, by margins ranging from 1.85 to 0.88 kg of daily intake. In two of the four cases (A vs. C and A vs. E) the positive control was consumed in greater quantity by all five heifers and the mean difference was significant ($P < 0.05$), whereas in the other two cases (A vs. B and A vs. D) the opposing LF was consumed in greater quantity by one or two of the five animals, respectively, and the mean difference was not significant.

Although the negative control (E) lost decisively when matched against A, it tended to be superior in competition with each of the three LF including additives, by non significant differences ranging from 0.86 to 0.41 kg. Treatment B tended to be inferior to both of the controls, but when matched against C it showed the opposite tendency in all five heifers; in competition with D it registered a higher mean value, but was consumed in lesser amounts by three of the five animals. Finally C was consumed in greater amounts than D by four of the five heifers, but the mean difference (0.72 kg) was not significant.

TABLE 2.—*Head to head treatment comparisons of daily liquid feed intake.*

Treatment compared and mean intakes (kg)	Mean difference (kg)	Frequency of winning[1]	Level of significance[2]
A 1.77 vs. B 0.88	0.89	4:1	NS
A 2.68 vs. C 0.90	1.78	5:0	<0.05
A 1.87 vs. D 0.99	0.88	3:2	NS
A 2.92 vs. E 1.07	1.85	5:0	<0.05
B 1.82 vs. C 1.07	0.75	5:0	NS
B 1.12 vs. D 0.91	0.21	2:3	NS
B 1.15 vs. E 1.56	-0.41	2:3	NS
C 1.82 vs. D 1.10	0.72	4:1	NS
C 0.94 vs. E. 1.80	-0.86	2:3	NS
D 0.74 vs. E 1.47	-0.73	0:5	NS

[1]Contest won by first mentioned treatment: won by second mentioned treatment.
[2]NS = not significant at P = 0.05.

Overall mean estimated body weights of four of the heifers showed a narrow range of only 13 kg (448 to 435 kg), whereas the remaining Brown Swiss animal was of somewhat smaller size (375 kg). The mean of all five heifers was 428 kg. Daily total LF intake (2.86 kg) expressed on a dry matter basis was equal to 1.06 kg, which represents only 0.25% of the mean body-weight of the heifers. Therefore, the LF in general represented a minor part of the total ration. Dry matter intake from grazed herbage is not known, but a level on the order of 1.25 to 1.5% of body-weight daily might be a reasonable expectation for these heifers that gained weight at a rate of about 0.5 kg daily over the course of the experiment. On the basis of this assumption, the LF provided roughly 1/6 of the dry matter ingested. An exception to this general situation was the highest-consuming animal, that reached an impressive maximum daily total LF intake of 7.2 kg, when offered A and C, in the 10th and final period, equivalent to 3.0 kg of dry matter.

The principal conclusion to be drawn from these results is that the objective of finding an additive able to improve animal acceptance of a LF containing 90% LSS and 10% molasses was not achieved. The LF containing 30% molasses was clearly better liked. The marked positive effect of molasses addition to LSS on animal acceptance was established in the earliest studies on this type of LF (Randel, 1981; Randel and Vallejo, 1982; Korber and Randel, 1982). Indeed, the natural flavor and aroma of cane molasses has long been prized as an inducement to animal consumption of diverse diets. A commercial liquid additive, known as Aromolass™ and claimed to be a synergist designed especially to intensify the flavor and aroma of cane molasses, was placed on the market in the late 1950s (Tribble, 1962). The additives used in the present study were of a different nature, having a vaguely citrus-like odor, and they showed no promise for the intended purpose. These enhancers are known to be effective in promoting palatability when added to many diets composed of conventional feedstuffs, but the odor and flavor of LSS may be so strong as to overwhelm their effect. Ironically, in this experiment as the level of addition of these enhancers increased, LF intake decreased. Adding flavor enhancers to LF containing high proportions of LSS, to improve animal acceptance, does not appear to be a promising approach.

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