Slight increases in the butterfat percentage were observed in three out of four experiments (3, 10, 11) when \(N,N'-\text{diphenyl-para-phenylenediamine} (\text{DPPD})\) was included in the ration of lactating cows for the purpose of inhibiting copper-induced oxidized milk flavor. These results suggested that DPPD, when fed to lactating cows, may affect lipid metabolism. However, no definite conclusions could be drawn, since feed intakes were not rigidly controlled or a system of equalized feeding was not employed in the above-mentioned experiments. The present study was, therefore, initiated to determine the effect of feeding DPPD on the butterfat percentage of the milk of lactating dairy cows which were fed equivalent energy for maintenance and production. In addition, DPPD was determined in the milk.

Twelve cows, two Ayrshires, six Holsteins, and four Jerseys, were paired according to breed and insofar as possible according to age and days in lactation. Each cow of a pair was assigned randomly to one of two sequences of including DPPD in the ration, namely: DPPD—No DPPD—DPPD and No DPPD—DPPD—No DPPD, designated as Sequence A and Sequence B, respectively. All animals were fed a ration consisting of 21 lb. dairy ration (16% crude protein), 29 lb. dried beet pulp, and 50 lb. chopped, field-cured, second-cutting alfalfa hay per 100 lb. of ration. The animals were fed estimated net energy (ENE) at 105% of the upper Morrison requirements (7), based on milk production and maintenance needs observed at the start of the experiment. The animals were subjected to a 5-wk. standardizing period, followed by three 2-wk. comparison periods according to the sequences described above, with each comparison period preceded by a 2-wk. period during which only the basal ration was fed. Feed intakes were adjusted to new maintenance and production needs at the end of the 2nd wk. of the standardizing period. To limit the occurrence of weighbacks, the ration allowance was reduced to 100% of Morrison's requirements, based on previously observed maintenance and production needs 2 wk. prior to the start of the first comparison period. ENE of the feeds was calculated from the formula of Moore et al. (6) derived from Forbes's data, based on total digestible nutrient (TDN) values calculated from proximate analyses according to Schneider et al. (8, 9). DPPD (95% feed grade) was fed at a level of 0.01% of total ration (90% dry matter basis). The antioxidant was premixed with linseed oil meal and added daily to each cow's r.m. grain allowance during the comparison periods. Cows not receiving the DPPD were fed an equivalent amount of linseed oil meal. DPPD intake averaged 1.40 g. per cow per day, with a standard error of 0.07.

All feeds, fed and refused, and milk yield were weighed to the nearest 0.1 lb. twice daily. Live weights were recorded to the nearest pound on the sixth and seventh day of each weekly period.

Milk samples were taken twice daily during the 2nd wk. of the three comparison periods for butterfat determination by the Babcock method (1), and 4% fat corrected milk (FCM) was calculated according to Gaines (4). Samples of feeds were taken at each feeding and composited each weekly period for proximate analysis (1), Table 1. Weighbacks were collected during the 2nd wk. of each of the three comparison periods for proximate analysis. TDN content of the weighbacks was computed from proximate analyses according to the method of Schneider et al. (8) and ENE as previously described. Once prior to the feeding of DPPD, and once during each comparison period, samples of milk from cows receiving the antioxidant were obtained for DPPD determination. The concentration of DPPD in whole milk was determined quantitatively, following its separation from the nonsaponifiable portion of milk, by paper
TABLE 1
Average proximate analysis of feeds and their estimated total digestible nutrient and estimated net energy content

<table>
<thead>
<tr>
<th>Feed</th>
<th>Dry matter (%)</th>
<th>Crude protein (%)</th>
<th>Nitrogen-free ether extract (%)</th>
<th>Ether extract (lb.)</th>
<th>Crude fiber (lb.)</th>
<th>TDN (lb.)</th>
<th>ENE (therms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy ration</td>
<td>91.4 ± 0.1</td>
<td>17.9 ± 0.3</td>
<td>56.0 ± 0.3</td>
<td>3.9 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>73.7</td>
<td>71.6</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>91.6 ± 0.1</td>
<td>8.8 ± 0.2</td>
<td>59.2 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>19.2 ± 0.1</td>
<td>67.8</td>
<td>63.1</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>92.3 ± 0.1</td>
<td>16.7 ± 0.4</td>
<td>37.7 ± 0.8</td>
<td>1.8 ± 0.1</td>
<td>29.8 ± 0.1</td>
<td>54.7</td>
<td>43.7</td>
</tr>
<tr>
<td>chopped</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean of 16 samples.

b Standard error.

c Per 100 lb. of feed.

chrotochromatography. Fifty-milliliter samples of whole milk were lyophilized, extracted with 50-, 25-, and 20-ml portions of peroxide-free ethyl ether. The supernatant was decanted after each extraction, centrifuged, and the combined ether extracts were evaporated under nitrogen to a volume of approximately 5 ml and then saponified with 2 ml. of saturated alcoholic KOH for 15 min. on a steam bath under nitrogen. Fifteen milliliters of distilled water were added to the saponification mixture and, after cooling, the mixture was extracted twice with 20 ml. of peroxide-free ethyl ether. The combined ether extracts were then washed four times with 25 ml. distilled H₂O, evaporated to dryness under nitrogen, and the resulting nonsaponifiable fraction was made up to a volume of 0.2 ml. with benzene. Separation of DPPD from other constituents in the nonsaponifiable fraction by paper chromatography, and subsequent estimation of its concentration, have been described previously (2).

The data of this double-reversal trial were analyzed as outlined by Lucas (5). Data for only the last seven days of each comparison period were used in the statistical analysis. One Jersey cow developed viral pneumonia and had to be removed from the experiment.

Table 2 lists the treatment means, based on average daily responses observed during the last week of each comparison period, for the various milk production criteria. No discernible responses were observed for the supplementation with DPPD in pounds of milk produced, the percentage butterfat, or the daily yield of pounds of fat and FCM. These results suggested, under conditions of short-time feeding, that a level of 0.01% DPPD does not alter the lipid metabolism of lactating dairy cows as measured by the butterfat percentage of the milk. The level of feeding DPPD, 0.01% of 90% dry matter ration, was chosen on the basis of consistent inhibition of copper-induced oxidized flavor (3, 10, 11) and, therefore, higher levels of feeding were not considered in this study. No ready explanation can be given why under ad libitum feeding conditions, as employed in the previous studies, small increases in the butterfat percentage were observed in three out of four trials. One possible reason may have been increased feed intake, but this was not determined in the previous studies (3, 10, 11).

Average daily intake of estimated TDN and ENE were calculated, using each cow’s actual feed intake and feed refusal and the proximate analyses for the particular week. As was expected, these values were similar between rations. To explore further the effect of feeding of DPPD, the efficiency of nutrient utilization for milk production was calculated. From individual energy intakes the maintenance requirement was deducted, employing 6.0 therms ENE and 7.0 lb. TDN per 1,000 lb.
live weight to the 0.67 power. The therms of ENE and pounds TDN required per pound of FCM were derived (Table 2) and found to be the same, whether DPPD was included in the ration or not. The relatively high values be the same, whether DPPD was included in ENE and pounds TDN required per pound of pound of FCI~[ (7) can, in part, be explained by the fact that the animals gained weight during each feeding period. It was realized that calculations which considered live weight change probably would have given a more exact estimate of nutrient utilization. However, the short periods used in this experiment, and the small differences in live weight increases between DPPD and no DPPD, did not seem to warrant the added calculations.

Concentration of DPPD averaged 12.7 μg/100 ml. of milk with a standard error of 1.5, based on duplicate analyses of milks obtained from cows fed DPPD, and on a per-gram of fat basis 3.30 μg ± 0.37. This resulted in an apparent mammary transfer of 0.14% ± 0.02 of the total DPPD fed. This value for mammary transfer was approximately four times as great as previously reported (11). It may possibly be a reflection of the quantitative method used in this study, as contrasted to the semiquantitative method used previously (11), and/or of greater efficiency of mammary transfer at the 0.01% level in this study as contrasted to the 0.10% level in the previous study.

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graphy, 2: 81. 1959.


DEVICE FOR MEASURING RATE OF MILK FLOW 1

The rate at which cow's milk flows has been investigated by a number of workers, using a variety of equipment to obtain and record observations (1, 2, 3, 4, 5, 6, 7, 8). An apparatus has been developed in this laboratory and found to perform exceptionally well as a means of recording the milking profile. It is essentially a continuous-feed kymograph (Figure 1), a self-contained, enclosed unit with an opening on one side through which notations can be made on the record. The weighing mechanism consists of a spring with a known stretch coefficient. In our studies we have used springs with stretch coefficients of 7 or 10 lb. per inch of expansion. The stylus is attached to a unit which operates on ball bearings to reduce friction. A steel clip extends from the unit and rests on a tubular collar fitted over and extending the full length of the weighing spring. The tube and collar unit may be adjusted to place the stylus at zero point, regardless of the tare weight involved. The driving

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