SUPPLEMENTATION OF APPLE POMACE WITH NONPROTEIN NITROGEN FOR GESTATING BEEF COWS. IV. PESTICIDE ACCUMULATION IN COWS

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SUMMARY

Samples of fresh apple pomace were collected from 14 loads of apple pomace as delivered from the processing plant in October 1968 and from stored apple pomace that had been obtained in October 1967 and fed during a feeding trial from mid-November 1967 through April 1968. All samples were analyzed for dicofol (kelthane), tetradifon (tedion), DDT, DDD and DDE. For the pesticide residues in fresh apple pomace, the difference between the low and high load means was greater than 10-fold and these differences between loads were significant (P<.01) for DDT, DDD and DDE. The pesticide residue content of stored apple pomace during a feeding trial ranged from .36 to 1.16 ppm for dicofol, .07 to .53 for tetradifon, 1.00 to 3.57 for DDT, .38 to .94 for DDD and .30 to .74 for DDE, which demonstrated potentially wide variation in pesticide intake during an apple pomace feeding period. Accumulation of pesticide residues in the fat depots of 28 pregnant beef cows was significant when apple pomace was fed for 160 days. Accumulation of residues was not affected by the type of nitrogen supplementation or by the inclusion of oil in the diet. Accumulation of residues appeared greater when pesticides were added to a simulated apple pomace diet than when apple pomace was fed. Tetradifon, DDT and DDD accumulated at a similar rate in fat tissue; 29% as fast as DDE. Dicofol accumulated 8% as fast as tetradifon. Total DDT residues (sum of DDT, DDD and DDE) were highest in heart fat, lowest in brisket and external rib fats and intermediate in internal rib, caul, kidney, ruffle and perianal fats of one trial, which indicated that depot location should be considered when obtaining samples for the determination of pesticide residue concentration in a carcass.

(Key Words: Apple Pomace, Urea, NPN, Residues, Pesticides, Cows.)

INTRODUCTION

Chemical residues are an important consideration when incorporating byproducts into animal diets. DDT residues in apple pomace (Bovard et al., 1961; Wilson et al., 1971) have prevented the recommendation of this byproduct as a feed for ruminants. Winter feeding trials conducted from the fall of 1965 to the spring of 1967 indicated that apple pomace containing as little as .6 to 1.2 ppm of DDT residues caused an accumulation of 2.2 to 13.7 ppm of DDT residues in perianal fat of pregnant beef cows and in their calves after parturition (Rumsey et al., 1969). These trials also indicated that dicofol (kelthane) and tetradifon (tedion) residues accumulated in perianal fat when apple pomace was fed. The purpose of the current study was to gain additional information on pesticide residues associated with the feeding of apple pomace diets to gestating beef cows during a time of diminished use of chlorinated hydrocarbon pesticides in orchards.

One trial was designed to investigate the variability of the dicofol, tetradifon and DDT residue content in fresh apple pomace obtained from a single processor in October 1968. Two trials were designed to compare the accumulation and distribution of these pesticide residues among various tissues in beef cows fed either apple pomace (November 1967 to April 1968) or a simulated apple pomace diet (November 1969 to April 1970) supplemented with non-protein nitrogen or natural protein.

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EXPERIMENTAL PROCEDURES

Residues in Apple Pomace. Apple pomace samples collected during previous apple pomace feeding trials conducted by the authors indicated a wide variation in the DDT residue content of apple pomace as fed. The present experiment was conducted to determine the residue variation in apple pomace before storage. Preliminary results were reported by Bovard et al. (1971). Duplicate samples (approximately .45 kg of wet apple pomace per sample) were obtained before storage (October, 1968) from each of 14 truckloads of apple pomace. All apple pomace was obtained locally, but did not necessarily originate from the same orchard. The fresh samples were frozen until analyzed at the Virginia Department of Agriculture and Commerce (VDAC) Laboratory in Richmond, VA, according to the procedure of Engel et al. (1965). Data were analyzed statistically to test differences in pesticide residue content among loads and to determine the standard error for samples within loads. The stored apple pomace was fed to pregnant beef cows during the winter of 1968 to 1969, cited as trial 2 by Fontenot et al. (1977). Unfortunately, the tissue samples collected for residue analysis during this study were lost during storage because of a power failure that resulted in thawing of samples in the freezer.

Tissue Residues, Trial 1. Twenty-four pregnant beef cows and 16 nonpregnant cows were used in a factorially designed trial conducted from November 1967 through calving (April, 1968) as described by Fontenot et al. (1977). Half of the cows were fed stored apple pomace as the major component of the wintering diet and half were fed corn silage. The pesticide residue concentrations in the stored apple pomace samples collected monthly during the trial are shown in table 1. Within the corn silage and apple pomace groups, four lots of five cows each were supplemented with either cottonseed meal, urea, biuret or a mixture of urea and biuret.

Samples of perianal fat (6 to 10 g per sample) were surgically removed near the base of the tail from all the cows fed apple pomace and from the cows fed corn silage plus cottonseed meal. The perianal fat samples were obtained before the trial and 95 days after the trial was started. The cows fed corn silage plus cottonseed meal served as a control treatment. At the end of the trial (160 days), two previously pregnant cows from each of the apple pomace lots were killed, and samples of 16 tissues per cow were obtained to determine residue distribution among tissues. The 16 tissue samples included: eight fat tissues [heart (pericardial), ruffle (mesenteric), caul (omentum), internal rib, kidney (perirenal), perianal, brisket and external rib (Subcutaneous)]; five muscle tissues [diaphragm, heart, round (semitendinosus), rib eye (longissimus dorsi) and psoas major] and three organ tissues (liver, brain and kidney). Residues in the 0-, 95- and 160-day perianal fat from cows fed apple pomace and in the 0- and 95-day perianal fat from cows fed corn silage plus cottonseed meal were statistically evaluated to determine differences among treatments and sampling times (Steel and Torrie, 1960). Duncan’s (1955) multiple range test was used to test differences between individual treatment by time means. Residue content of all tissue samples collected at slaughter was statistically analyzed for differences among treatments and tissues by analysis of variance. A second analysis of variance was conducted on results from only fat tissue to test differences among treatments and tissues.

All silage and tissue samples were frozen until analyzed for dicofol, tetradifon, DDT, DDD and DDE with clean-up and gas-liquid chromatography methods used by Rumsey et al. (1969).

Tissue Residues, Trial 2. Forty-eight pregnant beef cows were used in a factorially designed trial that was conducted from November 1969 through calving (April, 1970), cited as trial 3 by Fontenot et al. (1977). All cows were fed a mixture of corn cobs and corn silage as the major component of the wintering diet plus additions of corn starch or corn oil, cottonseed meal or urea, and pesticides or no pesticides.

A mixture of pesticide chemicals, based on apple pomace analysis at the VDAC Laboratory, was added to the diets of the pesticide lots in an attempt to stimulate the pesticide residue content of an apple pomace diet. Chemical premixes were prepared by adding the chemicals in an acetone and ethanol solution to either cottonseed meal on corn meal, and these premixes were dried and added to the respective diets. The premixes were added to the corn cob-corn silage diets so that the total chemical concentration in the diets was 5 ppm (dry basis). The individual chemicals and concentrations (ppm) in the diets were: 1,1,1-trichloro-
2,2-bis(p-chlorophenyl)ethane (DDT), 2.12; 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD), .10; 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), .14; 1,1-dichloro-2,2-bis(p-chlorophenyl)benzhydrol (dicofol), 1.51; p-chlorophenyl 2,4,5-trichlorophenyl sulfone (tetrachloroethylene (DDE), .14; isopropyl 4,4'-dichlorobenzilate (chloropropylate), .53; 1,2,3,4,5,6-hexachlorocyclohexane (benzene hexachloride), .06; 2,4-dimethyl 4,4'-dichlorobenzilate (paraquat), .06; 0,0,0',0'-tetraethyl S,S'-methylene bis(phosphorodithioate) (ethion), .33, and S,S'-p-dioxane-2,3-diyl 0,0,0',0'-tetraethyl bis (phosphorodithioate) (dioxathion), .02. At the end of the trial (154 days), two cows from each pesticide lot were killed, and tissue samples were collected. Two cows from the starch, cottonseed meal and no pesticide group were killed and samples as controls.

The concentrations of dicofol, tetrachloroethylene, DDT, DDD and DDE were determined in heart fat, external rib fat, muscle tissue from the round and kidney tissue; methods were the same as those used in trial 1. Data were analyzed statistically as in trial 1.

RESULTS

Mean concentrations of pesticide residues and their standard errors for the fresh apple pomace obtained during the fall of 1968 and for the monthly samples collected from stored apple pomace during the preceding 1967 to 1968 winter trial are shown in table 1. Differences in residue content between loads of fresh apple pomace were significant (P<.01) for DDT, DDD and DDE. For all residues, the difference between the low and high load means was greater than 10-fold.

The mean residue concentrations in the apple pomace stored in 1967 to 1968 was similar to the fresh samples collected in 1968. Although the ranges of concentrations were less for the stored apple pomace than for the fresh apple pomace, considerable variation among samples was apparent. The residue content of the corn silage was similar for trials 1 and 2 and averaged (ppm): dicofol, .10; tetrachloroethylene, .10; DDT, .05; DDD, .03 and DDE, .03.

The accumulation of pesticide residues in the perianal fat of beef cows during trial 1 is shown in table 2. Residue concentrations (extracted fat basis) in the perianal fat of the cows fed apple pomace but samples before the study were similar to the concentrations in perianal fat obtained at 0 and 95 days on experiment

TABLE 1. RESIDUE CONCENTRATION IN FRESH AND STORED APPLE POMACE

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Dicofol</th>
<th>Tetrachloroethylene</th>
<th>DDT</th>
<th>DDD</th>
<th>DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean content of 14 loads, ppm</td>
<td>1.43</td>
<td>1.35-3.25</td>
<td>1.25-3.75</td>
<td>1.28-3.75</td>
<td>1.35-3.25</td>
</tr>
<tr>
<td>Range of load means, ppm</td>
<td>.07- .40</td>
<td>.07- .40</td>
<td>.07- .40</td>
<td>.07- .40</td>
<td>.07- .40</td>
</tr>
<tr>
<td>Standard error of samples within loads, ppm</td>
<td>.03- .13</td>
<td>.03- .13</td>
<td>.03- .13</td>
<td>.03- .13</td>
<td>.03- .13</td>
</tr>
</tbody>
</table>

Mean content of all samples, ppm

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Tetrachloroethylene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of sample means, ppm</td>
<td>1.43-3.66</td>
</tr>
<tr>
<td>Standard error of samples, ppm</td>
<td>.06- .15</td>
</tr>
</tbody>
</table>

Virginia Department of Agriculture and Commerce Laboratory, Richmond, Va.

Residue content among loads was different (P<.01).

Tetrachloroethylene was not detected in several samples; therefore, a meaningful standard error could not be computed.
from cows fed corn silage. The concentrations of dicofol, tetradifon and DDT (P<.01) and the concentrations of DDE and total DDT residues (P<.05) increased in the cows fed apple pomace during trial 1. The concentrations of dicofol, tetradifon and DDT were greater (P<.05) at 95 days than before the study and the concentrations of dicofol, tetradifon, DDT, DDE and total DDT residues (DDT plus DDD and DDE analogues) were greater at 160 days than at 95 days. The concentration of DDD was less (P<.05) at 95 and at 160 days than before the study.

The relative percentages of DDT, DDD and DDE in perianal fat are shown in the last three columns of table 2. For cows fed apple pomace, the percentage of DDT increased (P<.01) during the study and the percentage of DDE decreased (P<.05), particularly at 95 days. For cows fed corn silage, the trend for DDT and DDE from 0 to 95 days was similar but smaller. The percentages of DDD at 0 and 95 days for cows fed corn silage and at 0 days for cows fed apple pomace were similar. The percentage of DDD decreased at 95 and 160 days for the cows fed apple pomace.

The type of nitrogen supplementation did not affect the concentration of residues or the relative percentages of DDT, DDD and DDE in perianal fat.

The distribution of residues among various tissues on a whole tissue basis is shown in table 3. For all residues, the average concentrations in the muscle and organ tissues were not different. The average concentrations of dicofol, tetradifon, DDT, DDE and total DDT residues were greater (P<.01) in fat tissues than in muscle and organ tissues. DDD was also greater (P<.05) in fat than in muscle and organ tissues. On the average, the relative percentage of DDT was greater (P<.05) in the fat tissues than in the muscle and organ tissues, but the opposite trend was true for DDD and DDE. The relative percentage of DDD was greater (P<.05) in muscle and organ tissues than in fat, and the relative percentage of DDE was greater (P<.05) in muscle than in fat tissues.

The concentrations of tetradifon, DDT and DDD were not different (P>.05) among fat tissues, dicofol was greatest in internal rib fat and greater (P<.05) there than in kidney, ruffle, perianal, external rib and heart fat. Dicofol was lowest in kidney and ruffle fats and lower (P<.05) there than in internal rib and caul fat. DDE was greatest in heart fat and was
<table>
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<tr>
<th>Tissue(^a)</th>
<th>Dicofol(^b)</th>
<th>Tetradifon(^b)</th>
<th>DDT(^b)</th>
<th>DDD(^c)</th>
<th>DDE(^b)</th>
<th>Total of DDT and analogues(^b)</th>
<th>Relative percent of DDT and analogues</th>
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<tr>
<td>Fat, average</td>
<td>.14</td>
<td>.17</td>
<td>1.12</td>
<td>.40</td>
<td>.99</td>
<td>2.30</td>
<td>48.7</td>
</tr>
<tr>
<td>Heart</td>
<td>.13(^{fg})</td>
<td>.13</td>
<td>1.89</td>
<td>.40</td>
<td>1.87(^{f})</td>
<td>4.15(^{f})</td>
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<tr>
<td>Ruffle</td>
<td>.10(^{fg})</td>
<td>.17</td>
<td>1.63</td>
<td>.62</td>
<td>1.44(^{fg})</td>
<td>3.69(^{fg})</td>
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<td>Caul</td>
<td>.20(^{fg})</td>
<td>.20</td>
<td>1.18</td>
<td>.24</td>
<td>1.30(^{fg})</td>
<td>3.55(^{fg})</td>
<td>39.9</td>
</tr>
<tr>
<td>Internal rib</td>
<td>.25(^{h})</td>
<td>.21</td>
<td>1.02</td>
<td>.50</td>
<td>.78(^{ghi})</td>
<td>2.48(^{ghi})</td>
<td>40.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>.08(^{g})</td>
<td>.20</td>
<td>.86</td>
<td>.10</td>
<td>.97(^{ghi})</td>
<td>1.93(^{ghi})</td>
<td>47.5</td>
</tr>
<tr>
<td>Perianal</td>
<td>.11(^{ig})</td>
<td>.12</td>
<td>.97</td>
<td>.09</td>
<td>.78(^{hi})</td>
<td>1.84(^{hi})</td>
<td>52.8</td>
</tr>
<tr>
<td>Brisket</td>
<td>.17(^{gh})</td>
<td>.14</td>
<td>.60</td>
<td>.07</td>
<td>.45(^i)</td>
<td>1.12(^{hi})</td>
<td>53.6</td>
</tr>
<tr>
<td>External rib</td>
<td>.11(^{gh})</td>
<td>.19</td>
<td>.89</td>
<td>.07</td>
<td>.37(^{i})</td>
<td>1.33(^{hi})</td>
<td>66.8</td>
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<tr>
<td>Muscle, average</td>
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<td>.16</td>
<td>.14</td>
<td>.32</td>
<td>.61</td>
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<tr>
<td>Diaphragm</td>
<td>.03</td>
<td>.03</td>
<td>.27</td>
<td>.24</td>
<td>.47</td>
<td>.98</td>
<td>27.9</td>
</tr>
<tr>
<td>Heart</td>
<td>.05</td>
<td>.04</td>
<td>.15</td>
<td>.14</td>
<td>.31</td>
<td>.60</td>
<td>25.2</td>
</tr>
<tr>
<td>Round</td>
<td>.03</td>
<td>.05</td>
<td>.14</td>
<td>.10</td>
<td>.23</td>
<td>.48</td>
<td>30.1</td>
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<tr>
<td>Rib eye</td>
<td>.03</td>
<td>.04</td>
<td>.11</td>
<td>.10</td>
<td>.30</td>
<td>.51</td>
<td>21.9</td>
</tr>
<tr>
<td>Psoas, major</td>
<td>.06</td>
<td>.04</td>
<td>.10</td>
<td>.12</td>
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<td>.49</td>
<td>20.9</td>
</tr>
<tr>
<td>Organ, average</td>
<td>.04</td>
<td>.04</td>
<td>.14</td>
<td>.17</td>
<td>.26</td>
<td>.57</td>
<td>25.9</td>
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<td>.05</td>
<td>.04</td>
<td>.15</td>
<td>.15</td>
<td>.22</td>
<td>.52</td>
<td>28.7</td>
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<tr>
<td>Brain</td>
<td>.04</td>
<td>.03</td>
<td>.13</td>
<td>.18</td>
<td>.34</td>
<td>.65</td>
<td>20.3</td>
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<tr>
<td>Kidney</td>
<td>.04</td>
<td>.04</td>
<td>.13</td>
<td>.18</td>
<td>.21</td>
<td>.52</td>
<td>25.3</td>
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<tr>
<td>(\bar{X})</td>
<td>.03</td>
<td>.03</td>
<td>.32</td>
<td>.19</td>
<td>.27</td>
<td>.60</td>
<td>4.0</td>
</tr>
</tbody>
</table>

\(^a\) Each tissue mean is an average from eight cows.
\(^b\) Average for fat greater (\(P < .01\)) than for muscle and organ.
\(^c\) Average for fat greater (\(P < .05\)) than for muscle and organ.
\(^d\) Average for fat less (\(P < .05\)) than for muscle and organ.
\(^e\) Average for fat less (\(P < .05\)) than for muscle.
\(^{fg,i}\) Means for fat tissue in the same column which do not contain a common letter in the superscript are different (\(P < .05\)).
greater (P<.05) there than in internal rib, kidney, perianal, brisket and external rib fat. DDE was lowest in external rib fat and brisket fat and lower (P<.05) there than in caul, ruffle and heart fat. DDE content of ruffle fat was greater (P<.05) than that of perianal, brisket and external rib fat. The distribution of total DDT residues among fat tissues resembled the distribution of DDT and DDE. The relative percentage of DDT and DDE were not different (P>.05) among fat tissues, but the relative percentages of DDD tended to be greater in ruffle, caul and internal rib fat.

There was no difference in tissue residue concentration attributable to the use of urea vs cottonseed meal or corn starch vs corn oil in the diet (trial 2). The tissue residue data for the cows fed pesticides were, therefore, averaged, across treatments on a whole tissue basis and are shown in table 4. The average residue concentrations (ppm) of fat tissues in the control cows were: dicofol, .16; tetradifon, .20; DDT, 1.27; DDD, .68 and DDE, 2.11. The residue concentrations in tissues from the cows fed pesticides were higher than those in the controls, but the differences were not significant except for tetradifon and DDD (P<.05). Except for dicofol, the residue concentrations were greater (P<.05) in the fat than in the round or kidney tissues. The concentration of dicofol was greater (P<.05) in heart fat than in kidney tissue. There were no concentration differences between round and kidney tissues or between heart fat and external rib fat.

Differences in the relative percentages of DDT and DDE in trial 2 were similar to those noted in trial 1. In trial 2, unlike trial 1, the relative percentage of DDD was greater in the fat tissues than in round and kidney tissues.

**Discussion**

With an emphasis over the past several years on replacing persistent pesticides with more biologically labile chemicals, residue problems have decreased. However, Fries (1970) points out that because of the persistence of materials such as DDT in the environment, the food chain will be subject to low levels of residue contamination for many years. For example, DDT residues in the depot fat of grazing cattle were found to result from a carry-over of residues in the soil after the use of DDT in previous years (Harrison et al., 1969). Selected use of persistent pesticides for which there are
no substitutes is also a source of low level contamination in the environment. Therefore, it is important to know the nature and relative levels of residues in various parts of our environment, particularly if potential sources of animal feed are involved.

Apple pomace, a potential feed for beef cattle, was shown by Rumsey et al. (1969) to contain DDT, dicofol and tetradifon residues at levels that caused residue accumulation in fat depots of beef cows. The present study indicated that apple pomace may present an additional residue problem because of the variation in the concentration of pesticides in the apple pomace. Differences in residue concentration obtained between truckloads of fresh pomace from the same processing plant were probably a result of differences among orchards in pesticide application rates as dictated by pest infestation levels. Analyses of stored apple pomace throughout the 1967 to 1968 feeding trial showed that storing did not remove the variability in the concentration of residues. Therefore, cattle consuming byproducts such as apple pomace can be expected to be exposed to varying levels of residue contamination throughout a feeding period. This variable exposure to residues in apple pomace did not impose an apparent health problem to the cattle in this trial because residues were not present at toxic levels; however, this type of exposure makes it more difficult to predict accumulation of tissue residues on the basis of the analysis of the material that is fed unless that material is monitored routinely.

The accumulation of DDT residues in the depot fat of beef cows fed apple pomace was below the current tolerance of 5 ppm⁵ and similar to that observed in the second experiment of an earlier study by Rumsey et al. (1969). After correction for differences in pesticide intake, residue accumulation appeared to be greater in the heart and external rib fats when the pesticides were added to the simulated apple pomace diet (trial 2) than when they were present as a contaminant in apple pomace (trial 1). Although confounded by years and type of diet, a comparison of results of trials 1 and 2 indicated that the source of dietary residues may affect the accumulation of residues in depot fats. Differences in the source of residue contamination have been noted previously by Ely et al. (1952).

Differences in the accumulation of individual residues were noted. After correction for differences in pesticide intake, tetradifon was found to accumulate in depot fat at a relative level similar to that of DDT and DDD but only 29% as high as that of DDE. Dicofol accumulated 8% as high as tetradifon and 2.3% as high as DDE. DDE accumulated approximately four times more than DDT and DDD. Fries and Kanc (1967) have reported that DDE, is more resistant than DDT to elimination from the body because of differences in the metabolism of the two compounds.

The distribution of low levels of DDT residues in the present study was found to be similar to the distribution of higher levels (Rumsey et al., 1967) except that the relative difference between fat vs muscle tissue was smaller. This change in distribution between fat and muscle was expected because the fat depots act as a reservoir for these compounds. In trial 1, the ranking of tissue on the basis of total DDT residue content was similar to that reported by Rumsey et al. (1967) at residue levels 100 times those found in the present study. On a whole tissue basis, heart fat was generally highest, internal rib, caul, kidney, ruffle, and perianal fats were intermediate and brisket and external rib fats were below average. In the current study, calculating the residue concentration on the basis of extracted fat did not change the relative distribution among fat tissues.

The difference between heart and external rib fat was not as great in trial 2 as in trial 1. The distribution was not the same for dicofol and tetradifon; however, a distribution trend for dieldrin similar to that for DDT (trial 1) was found in a different study (Rumsey and Bond, 1974). Although it is not apparent why differences in residue concentrations among depot fats should occur, these differences should be considered when carcasses are checked at slaughter.

Pesticide residues in apple pomace as a causative factor of the calf problem described by Fontenot et al. (1977) and Bovard et al. (1977) was not apparent in these trials. Pesticide residues in the apple pomace and subsequent tissue concentrations were low and the use of nonprotein nitrogen (NPN) in place of cottonseed meal did not change the tissue levels

⁵Code of Federal Regulations, Title 40, Part 180:147.
or distribution of these residues. These results suggest that protein supplement did not affect the fate of these residues in the animal. There is no evidence in the literature that pesticide residues at these low levels would interact with NPN to adversely affect the animal, and adding a combination of pesticides and NPN to a simulated diet did not cause apparent problems. Recent research has indicated that a trace mineral deficiency and ammonia toxicity are not causative factors (Rumsey, 1975), but utilization of NPN may be depressed because of rapid rumen washout (Rumsey et al., 1976). Addition of straw to an apple pomace-urea diet reduced rumen washout rate and had a mitigating effect on the calf problem (T. S. Rumsey, unpublished data) as did the addition of hay (Fontenot et al., 1977). Based on the information gathered in the current series of trials and in subsequent research, delineating the cause of the calf problem associated with feeding apple pomace and NPN requires further study.

LITERATURE CITED


