THE EFFECT OF PARTIAL REPLACEMENT OF BARLEY HAY BY BARLEY SILAGE ON THE PERFORMANCE OF LACTATING COWS IN EARLY LACTATION

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SUMMARY

Hay and silage made from barley, variety Morocco 628, were used in three feeding trials to investigate the effect of replacing part of barley hay and other roughages by barley silage on the milk yield, milk composition and body weight changes of lactating Friesian cows in early lactation. The digestibility coefficients of barley silage and barley hay (fed alone or with concentrates) were measured with Chios wethers sheep. Both hay and silage were of good quality. No effluent was produced from the ensiled material. Crude protein degradability in the rumen was higher with silage than hay, but the contribution of silage to the total crude protein requirements were very small because of the high DM intake from concentrates. Milk yield, milk composition and body weight changes were similar for the control and the silage fed cows within each trial, except that control cows lost weight in trial 3. Digestion coefficients and nitrogen balance in sheep were higher when silage was fed. However, digestion coefficients improved considerably when hay or silage were supplemented with a concentrate mixture, particularly hay diets. Calculated efficiency of utilization of energy or protein secreted in the milk was similar between the control and silage fed cows. It was concluded that the nutritive value of silage and hay made from barley cut at the same stage of maturity was similar and milk yield of lactating cows and milk composition were not affected from the partial replacement of the roughage component of the total diet by barley silage.

INTRODUCTION

In Cyprus, as in most Mediterranean countries and other countries with a Mediterranean type of climate, there is a surplus of forage in spring and a deficit during summer. Therefore, the conservation of forage either as hay or silage is necessary for better utilization of forage and balanced feeding of ruminants in periods of high nutrient de-
mand. Hay making is the only method of forage conservation in many Mediterranean countries, including Cyprus. Early cut hays are of excellent quality (Bertilsson et al. 1980) but usually hays are cut later than the optimum cutting stage and therefore their digestibility and protein content are reduced. The potential of hays for milk production is also reduced depending on mechanical losses, and rains during field drying or leaf shattering from the lack of humidity during raking and baling (Watson and Nash, 1960; McCulloch, 1989). Prolonged storage of hay at high environmental temperatures usually leads to leaf shattering. The quality of stored hay for cow feeding ranged from medium to poor and in many cases did not differ from the quality of baled straw (Antoniou, Personal Communication).

The use of winter cereals, mainly wheat, barley and oats, for silage making is a regular method of forage conservation in Israel (Kroll, personal communication) and Tunisia (Sansoucy, 1980). In California, the use of cereal forages either as hay or silage for dairy cow feeding is related to the emphasis given on reducing cost of production and maximizing output of cows and land (DePeters et al., 1989). Silage made from small grain cereals could replace alfalfa hay (DePeters et al., 1989; Marx, 1980, cited by DePeters et al., 1989) in the feeding of dairy cows in early lactation. In temperate dairy areas of Australia surplus pasture in the spring is conserved as silage or hay. Usually, silage it is of better quality and has a greater milk yielding potential than hay (Thomas and Mathews, 1991).

Information from feeding silage made from cereal forages (barley, wheat, oats) on the performance of high yielding cows in Cyprus is lacking. However, preliminary work on silage made from barley cut at the milk or dough stage showed that no effluent was produced, its nitrogen content and in vitro organic matter digestibility were high and no health problems were observed with dairy cows when silage replaced completely the hay portion of the diet. Furthermore, the metabolisable energy content of hay and silage were similar and the monetary value of silage based on its nutritional worth was 6 to 8% higher than that of hay considering the price of barley grain £84/t and the price of soybean meal ranging from £130 to £150/t.

The objective of the present work was to determine the nutritive value of hay or silage made from barley and to investigate the effect of feeding silage on the performance of high yielding dairy cows in early lactation.

**MATERIALS AND METHODS**

Three trials were carried out with lactating Friesian cows, in three consecutive years, to investigate the effect of replacing part of the roughage diet with barley silage. Barley, variety Morocco 628, was sown at Athalassa ARI farm and was cut at the milk or milk to dough stage (April each year). In the third year the barley crop suffered before cutting from moisture stress, grains were not filled completely with carbohydrates and the basic leaves of the plants were yellowish and dry. Barley forage was cut and chopped with a forage harvester, ensiled in a concrete bank silo and sealed with a polyethylene sheet. During ensiling the material was well compacted with a tractor to expel oxygen and after sealing, the polyethylene sheet was kept in place with soil and used car tyres.

**Animals**

Eighteen lactating cows were used every year. They were 100±15, 74±14 and 60±12 days post partum in trials 1, 2 and 3, respectively. Cows were divided each year into two treatment groups on the basis of milk yield (approx. 27kg/cow/day) fat content of milk, liveweight and parity.

**Feeding**

The cows were offered the same concentrate mixture containing 18% crude protein (as fed) and one of the following roughage diets daily.

**Trial 1**

**Control.** Barley hay 3.5kg, barley straw 1.50kg and alfalfa hay 1.0kg.

**Barley silage.** Barley hay 2.5kg, barley straw 1.25kg and barley silage 7kg. (One kg of barley hay, 0.25kg straw and 1.0 kg alfalfa hay were replaced by 7kg silage). Barley was cut for silage at the milk stage.

**Trial 2**

**Control.** Barley hay 6.0 kg.

**Barley silage.** Barley hay 3.5kg and 7kg si-
lage. Barley was cut for silage at the milk to dough stage.

**Trial 3**

**Control.** Barley hay 6.0 kg and alfalfa hay 1.0 kg.

**Barley silage.** Barley hay 3.5 kg, alfalfa hay 1.0 kg and barley silage 7 kg. Barley was cut for silage at the milk to dough stage.

The composition of the concentrate mixture (kg/t) used in the three trials was barley grain 613, corn 100, soybean meal 236, wheat bran 25, dicalcium phosphate 2.5, limestone 18, sodium chloride 3.5 and vitamin and trace element mixture 2.0. The vitamin and trace element mixture provided per kg concentrate feed 8,000 IU vitamin A, 600 IU vitamin D, 15 I.U. vitamin E, 25 mg Mn, 30 mg Fe, 1.5 mg I, 1.5 mg Co, 45 mg Zn and 60 mg Mg.

The chemical composition of the roughages and of the concentrate mixtures are presented in Table 1.

**Management**

The daily allowance of the concentrate mixture for each cow was calculated on the basis of liveweight, parity and the energy secreted in milk (NRC, 1989) at the commencement of the trial and was kept constant during the experimental period. Metabolisable energy intake was calculated from published values of ME content of feeds (Hadjipanayiotou et al., 1983; NRC, 1989). Milk yield was recorded twice daily and fat (Gerber method) CP, TS, and ash contents of milk once weekly (MAFF, 1973). Liveweight was recorded on two consecutive days at the commencement and at the end of each trial. Cereal hay and alfalfa hay were fed at 07.00h, silage at 09.00h and cereal straw at 13.00h. The daily allowance of the concentrate mixture was offered individually to each cow in 5 meals, using an electronic management system for individual feeding.

Trial 1 lasted 6 weeks (after 2 weeks of gradual adjustment to silage) and trials 2 and 3, 12 and 10 weeks, respectively.

**Digestibility studies**

Digestibility coefficients of barley hay, and barley silage either alone or supplemented with concentrates were measured in Trial 3.

Ten Chios wether sheep of approximately 65 kg liveweight were used in two consecutive periods to determine digestion coefficients of four diets (Diet 1, 1.1 kg barley hay/head/day; Diet 2, 3 kg barley silage/head/day; Diet 3, 0.27 kg barley hay + 0.6 kg concentrates/head/day (14% CP); Diet 4, 0.7 kg barley silage + 0.6 kg concentrates/head/day (14% CP). Diets 1 and 2 were tested in period I (14 days) and diets 3 and 4 in period II (14 days).

Animals (5/diet) were in metabolic crates designed for the separate collection of urine and faeces. The diets were offered to meet maintenance requirements of energy. Diets 3 and 4 were offered at 30:70% roughage to concentrate ratio (DM basis). Concentrates and roughage were given in separate feeders twice daily. Feed intake was recorded daily and faeces and urine were collected daily for 7 days, following a 7-day adjustment period.

**Chemical analysis of feeds, faeces and urine**

Representative samples of feeds, faeces and urine were analysed for proximate constituents (AOAC, 1975) and neutral detergent fiber (NDF) (Harris, 1970). The gross energy of feeds and faeces was determined using an adiabatic bomb calorimeter.

**Ammonia nitrogen, pH and lactic acid measurements of silage**

Water extract of the fermented material was prepared by homogenizing 25 g of silage
with 100 ml water in a blender at full speed for 5 min. The contents were filtered through two layers of cheese-cloth and the pH of the filtrate was determined immediately. The samples were then processed and analyzed for ammonia nitrogen (NH$_3$-N) by steam distillation and titration of the distillate as outlined by Hadjipanayiotou (1988). Lactic acid in silage was determined as outlined by MAFF (1979).

Degradation studies

Rumen degradation studies and analysis of samples were carried out as described by Hadjipanayiotou (1992). Loss of crude protein at various incubation intervals was estimated by the exponential equation $P = a + b (1-e^{-ct})$ (Orskov and McDonald, 1979), where $P$ is the amount degraded at time $t$, $a$ is the rapidly degradable fraction, $b$ is the potential degradability at infinite time and $c$ is the fractional rate constant at which the fraction $b$ will degrade per hour.

Data collected were analysed within trials by one-way analysis of variance (Steel and Torrie, 1960).

RESULTS

The proportion of roughage to concentrate DM intake was 30:70% in trials 1 and 2 and 34:66% in trial 3, while the proportion of silage DM to the total DM intake was 10.4, 13.3 and 12.0% in trials 1, 2 and 3, respectively. Protein intake from the concentrate mixture ranged from 80 to 85% of the total protein intake and silage contribution to the total protein intake was 8.60, 6.55 and 5.65% in trials 1, 2 and 3, respectively. Protein degradability of barley silage in trial 1 was higher than that of barley hay (Table 2). There was no effluent loss from the silage in any of the three trials. Ammonia nitrogen concentration (NH$_3$-N) of silage in trial 3 was 3.85±0.82, pH 4.64±0.2 and lactic acid 1.07%.

Table 2. Estimates of CP disappearance of hay and silage incubated in the rumen of fistulated sheep

<table>
<thead>
<tr>
<th></th>
<th>Hay</th>
<th>Silage</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>25.90</td>
<td>68.20</td>
</tr>
<tr>
<td>b</td>
<td>64.30</td>
<td>17.06</td>
</tr>
<tr>
<td>c</td>
<td>0.032</td>
<td>0.045</td>
</tr>
<tr>
<td>$P_{0.02}$</td>
<td>64.7</td>
<td>78.5</td>
</tr>
<tr>
<td>$P_{0.05}$</td>
<td>52.55</td>
<td>77.2</td>
</tr>
<tr>
<td>$P_{0.08}$</td>
<td>47.65</td>
<td>76.9</td>
</tr>
</tbody>
</table>

$a$ = The rapidly degradable fraction; $b$ = the potential degradability at infinite time; $c$ = fractional rate constant at which the fraction $b$ will degrade per h.

Data in trials 2 and 3. Also the energy and protein excreted in milk as a percentage of the energy intake (gross or metabolisable) and protein intake were similar between the two treatments in trials 2 and 3 (Table 4).

Digestion coefficients of silage or silage supplemented with concentrates (DM, OM, GE, CP and NDF) were significantly higher than hay or hay supplemented with concentrates (Table 5). Supplementation of hay with concentrates improved digestion coefficients of the total diet from 16% (NDF) to 49% (protein), whereas the improvement of digestion coefficients of the total diets when silage was supplemented with concentrates was less. Nitrogen balance of sheep fed silage was positive and significantly higher ($P<0.05$) than hay, when hay and silage were fed alone to sheep, but nitrogen balance was positive and similar when hay or silage were supplemented with concentrates (Table 5).

DISCUSSION

In the present trials silage and hay were made from the same barley crop (Morocco 628), cut at the milk (trial 1) or the milk to dough stage of grain (trials 2 and 3). Ensiling was completed in 2 to 3 days, whereas hay making in 9 to 14 days. Hay was green and leafy with high protein content because there were no losses during harvest and drying in the field and was stored only for a short period until the experiments with cows were completed.
<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th></th>
<th></th>
<th></th>
<th>Trial 2</th>
<th></th>
<th></th>
<th></th>
<th>Trial 3</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Barley</td>
<td>SE</td>
<td>Control</td>
<td>Barley</td>
<td>SE</td>
<td>Control</td>
<td>Barley</td>
<td>SE</td>
<td>Control</td>
<td>Barley</td>
</tr>
<tr>
<td>Milk Yield (kg/cow)</td>
<td>24.08</td>
<td>24.20</td>
<td>1.65</td>
<td>23.50</td>
<td>24.00</td>
<td>1.38</td>
<td>25.10</td>
<td>24.60</td>
<td>1.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>3.55</td>
<td>3.20</td>
<td>0.16</td>
<td>3.36</td>
<td>3.53</td>
<td>0.09</td>
<td>3.83</td>
<td>3.80</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.24</td>
<td>3.24</td>
<td>0.09</td>
<td>3.10</td>
<td>3.11</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Solids (%)</td>
<td>8.33</td>
<td>8.26</td>
<td>0.16</td>
<td>8.33</td>
<td>8.26</td>
<td>0.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solids Non Fat (%)</td>
<td>0.76</td>
<td>0.74</td>
<td>0.01</td>
<td>0.76</td>
<td>0.74</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liveweight (kg/cow)</td>
<td>590</td>
<td>567</td>
<td>21.8</td>
<td>613</td>
<td>588</td>
<td>22.13</td>
<td>574</td>
<td>570</td>
<td>19.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+2</td>
<td></td>
<td></td>
<td>+13</td>
<td></td>
<td></td>
<td>-20</td>
<td>+1</td>
<td>4.73**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate intake (kg/cow/day)</td>
<td>13.62</td>
<td>13.50</td>
<td>0.63</td>
<td>14.4</td>
<td>14.2</td>
<td>0.83</td>
<td>13.56</td>
<td>13.60</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake (g/cow/day)</td>
<td>2760</td>
<td>2630</td>
<td>135</td>
<td>3135</td>
<td>3105</td>
<td>160</td>
<td>3162</td>
<td>3184</td>
<td>95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Energy and protein excretion in milk and conversion efficiency of gross or metabolisable energy and protein intakes in energy and protein in milk

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Barley silage</th>
<th>SE</th>
<th>Control</th>
<th>Barley silage</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excretion in milk (Cow/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross energy (MJ)</td>
<td>66.40</td>
<td>69.44</td>
<td>4.18</td>
<td>75.23</td>
<td>73.40</td>
<td>3.45</td>
</tr>
<tr>
<td>Protein (kg)</td>
<td>0.78</td>
<td>0.78</td>
<td>0.04</td>
<td>0.77</td>
<td>0.76</td>
<td>0.03</td>
</tr>
<tr>
<td>Conversion efficiency (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross energy</td>
<td>19.80</td>
<td>20.60</td>
<td>0.73</td>
<td>22.64</td>
<td>21.62</td>
<td>0.63</td>
</tr>
<tr>
<td>Metabolisable energy</td>
<td>32.40</td>
<td>34.20</td>
<td>0.71</td>
<td>38.37</td>
<td>37.31</td>
<td>0.99</td>
</tr>
<tr>
<td>Protein</td>
<td>24.74</td>
<td>24.94</td>
<td>0.56</td>
<td>24.35</td>
<td>23.90</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Silage had a pleasant aroma indicative of good fermentation. However, there were substantial losses from secondary deterioration of silage by air entering the silage after it has been disturbed, because of the small quantity of silage fed daily to the experimental animals and the high environmental temperatures. In Australia silage wastage was about 26% under experimental conditions with dairy cows and only 7% under commercial conditions. (Thomas and Mathews, 1991).

The level of crude protein was higher and that of NDF lower in hay and silage used in trial 1 (Table 1) compared to those of hay and silage in trial 3 (Table 1), because barley crop in trial 3 suffered from moisture stress (prolonged period without rains) resulting in yellow and dry leaves and thus the production of forage of lower quality.

It was suggested that there is no relation between preservation of wilted silage and acidity as in un wilted silage (Murdoch, 1980) and that preservation is the result of increased osmotic pressure or to reduced buffering capacity (Wieringa 1960; Playne and McDonald, 1966). The only similarity between wilted silage and silage made from barley is the high dry matter of the ensiled material and it may be suggested that preservation of small grain cereals at high dry matter is similar to that of wilted silage. The overall effect of conservation of forage on the feeding value of silage or hay is that wilted silage (with high dry matter content, similar to that of winter cereal cut at the milk to dough stage) has equal or better feeding value than hay (Wilkins, 1988).

The proportion of roughage to concentrate DM intake in the present trials was 30-34:70-66%. In Mediterranean climates insufficient quantities of roughage lead to high concentrate supplementation particularly in early lactation (Economides, 1985). As a result both energy and protein intakes are largely met from the concentrates and the contribution from the roughage source, including silage, is limited.

Protein degradability in the rumen of fresh forage, hay and silage ranges from 0.71 to 0.90 (MAFF, 1986). The degradability of protein in the rumen was higher with silage (Table 2). This is in agreement with the work of Rohr and Daenicke (1985), and Makoni et al. (1991). Although protein degradability of silage in the rumen is high, nitrogen use is improved when animals are fed silage together with a carbohydrate source or high energy intake (Edwards, 1989; Wilkins, 1988; Rohr and Daenicke, 1986). Therefore, when forage is scarce and must be fed in minimal quantities, silage is more important for fiber and energy and not for protein (McCulloch, 1989).

There was no effluent loss during ensiling which usually leads to loss of nutrients and pollution problems. This is because effluent loss from ensiled cereal crops cut at the milk to soft dough stage of grain ceases when the dry matter of the ensiled material is about 30% (McCulloch, 1989; Murdoch, 1980).

Milk yield and milk composition of silage fed cows in the three trials was similar
Table 5. Digestibility coefficients of barley hay and barley silage either alone or supplemented with concentrates

<table>
<thead>
<tr>
<th></th>
<th>Hay</th>
<th>Silage</th>
<th>SE</th>
<th>Hay plus concentrates</th>
<th>Silage plus concentrates</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>57.60</td>
<td>65.10</td>
<td>1.09**</td>
<td>73.30</td>
<td>76.50</td>
<td>0.56**</td>
</tr>
<tr>
<td>Organic matter</td>
<td>59.50</td>
<td>66.50</td>
<td>1.07*</td>
<td>75.90</td>
<td>79.90</td>
<td>0.49**</td>
</tr>
<tr>
<td>Gross energy</td>
<td>55.30</td>
<td>65.10</td>
<td>1.04***</td>
<td>73.50</td>
<td>77.80</td>
<td>0.52***</td>
</tr>
<tr>
<td>Protein</td>
<td>47.50</td>
<td>60.50</td>
<td>2.34**</td>
<td>70.90</td>
<td>75.70</td>
<td>1.23*</td>
</tr>
<tr>
<td>NDF</td>
<td>53.70</td>
<td>61.10</td>
<td>1.75*</td>
<td>62.50</td>
<td>66.00</td>
<td>1.61NS</td>
</tr>
<tr>
<td>Nitrogen Use (g/day)</td>
<td>12.19</td>
<td>14.16</td>
<td>0.82NS</td>
<td>17.12</td>
<td>16.32</td>
<td>0.00</td>
</tr>
<tr>
<td>Nitrogen intake (g/day)</td>
<td>6.40</td>
<td>5.61</td>
<td>0.46NS</td>
<td>4.96</td>
<td>3.97</td>
<td>0.20**</td>
</tr>
<tr>
<td>Nitrogen excretion (g/day)</td>
<td>6.91</td>
<td>6.40</td>
<td>0.57NS</td>
<td>9.50</td>
<td>9.98</td>
<td>0.47NS</td>
</tr>
<tr>
<td>Nitrogen balance (g/day)</td>
<td>-1.12</td>
<td>+2.15</td>
<td>0.67*</td>
<td>+2.66</td>
<td>+2.37</td>
<td>0.54 NS</td>
</tr>
</tbody>
</table>

Level of significance

*** P<0.001
** P<0.01
* P<0.05
NS Non Significant
to that of the control cows. Liveweight changes were also similar in two out of the three trials. Similar performance response was observed when cereal silage replaced 34 or 66% of alfalfa hay with high producing cows in early lactation (DePeters et al., 1989). However, when cereal silage replaced 66% of alfalfa hay or decreasing DM intake of alfalfa from 8kg to 2.50kg daily and increasing cereal silage to 5.50kg, overall DM intake was reduced. This reduction was possibly a result of gut fill, since the high silage diet had the highest NDF concentration and average consumption of NDF was similar for the three groups of cows (DePeters et al., 1989). Dry matter intake was not reduced when silage replaced 34% of alfalfa hay (2.75kg D.M. daily). Silage and hay made from the same herbage have been compared in feeding trials with dairy cows (Merrill and Slack, 1965). These trials indicated that although the silage was consumed in smaller quantities than hay, differences in milk yield were generally small and rather conflicting. Dry matter digestibility of silage which is cut earlier than hay, because ensiling is less weather dependent, was greater than that of hay and milk production of cows fed silage was superior (Thomas and Mathews, 1991). Other workers have suggested also that the nutritive value of silage, when ensiled at the correct time is superior to hay (Yiakoumettis, Personal Communication; Sansoucy, 1980).

The results of the digestibility trials are in agreement with digestibility studies carried out with three qualities of cereal hays either alone or supplemented with different levels of concentrates (Blaxter and Wilson, 1963). In these studies digestibility coefficients increased with increasing concentrate allowance, the increase being greatest for the ration containing the poorest quality hay, possibly because the presence of concentrates promotes a more efficient digestion of the fibrous materials. On the other hand milk production was not affected when silage made from cereals cut at different stages of maturity (bloom, milk or dough) was fed to dairy cows in association with concentrates (Polan et al., 1968; Snyder, Polan and Miller, 1979).

The calculated efficiency of utilization of energy or protein in milk (energy or protein secreted as a percentage of gross or metabolisable energy and protein intakes) were similar in both trials 2 and 3 (Table 4). The average value of the two groups of cows in trial 2 was 21.22% and in trial 3 21.11% for gross energy, 35.38 and 35.75% for metabolisable energy and 24.54 and 24.42% for protein in trials 2 and 3, respectively. These results are in agreement with those of Van Es, (1969). From a study of 280 balance trials Van Es (1969) concluded that the efficiency of utilization of ME for lactation did not differ for hays and silages of similar ME concentration. He also concluded that “our ability to predict the ME content of silage and the efficiency with which it is used for various functions by the animal is poor”.

It may be concluded from the present studies that hay or silage made from the same variety of barley, cut from the same field at milk or milk to dough stage of grain maturity, had similar effect on the milk yield and milk composition of lactating cows when fed in association with a high proportion of concentrates in early lactation.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs M. Theodoridou, Mrs M. Karavia and the staff of the Central Chemistry Laboratory for technical assistance and Drs M. Hadjipanayiotou and T. Antoniou for reviewing the paper.

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