Effects of supplementation with different amounts of malic acid to Tuj lambs diets on fattening performance, rumen parameters and digestibility

D. AKSU ELMALI1*, T. SAHIN2, I. KAYA2, Y. UNAL3

1Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary Medicine, University of Mustafa Kemal, 31040, Hatay, Turkey.
2Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary Medicine, University of Kafkas, 36040, Kars, Turkey.
3Department of Biology, Faculty of Letters and Science, University Agrı Ibrahim Cecen, 04000, Agrı, Turkey.

*Corresponding author: daksuelmali@hotmail.com

SUMMARY

In this study, the effects of concentrates including 0, 3 or 6 g/kg of malic acid on food intake, growth performance, food efficiency, nutrient digestibility and several rumen metabolite contents in Tuj lambs were analyzed. After a 15 days long adaptation period, a total of 15 male Tuj lambs, 3.5-4 months old, were randomly into 3 equal groups according to the malate contents (0, 3 or 6 g/kg) added to concentrates for 50 days. Neither the forage (hay meadow), concentrate, dry matter intakes, body weights and weight gains nor food efficiency were significantly modified in the supplemented lambs. In addition, no significant difference in the nutrient digestibility and in some rumen metabolite (rumen pH, ammonia and volatile fatty acids) concentrations were recorded between supplemented and controls lambs. It was concluded that malate can be included in rations for lambs up to 6 g/kg.

Keywords: Lamb, malic acid, mixed ration, concentrate, growth performance, food efficiency, digestibility, rumen metabolites.

RÉSUMÉ

Effets d’une supplémentation alimentaire par différents dosages d’acide malique sur l’engraissement, les paramètres ruminaux et la digestibilité des aliments chez les agneaux Tuj

Au cours de cette étude, les effets des concentrés contenant 0, 3 ou 6 g/kg d’acide malique sur l’ingéré alimentaire, la croissance pondérale, l’efficacité alimentaire, la digestibilité des nutriments et les concentrations intra-ruminales de différents métabolites ont été analysés chez des agneaux Tuj. Après une période d’adaptation de 15 jours, 15 agneaux Tuj, mâles, âgés de 3.5 à 4 mois, ont été répartis aléatoirement en 3 groupes égaux en fonction de la dose de malate (0, 3 ou 6 g/kg) ajoutée aux concentrés pendant 50 jours. Ni les quantités ingérées de fourrage (foin), de concentrés ou de matière sèche totale, ni les poids vifs et les gains de poids, ni l’efficacité alimentaire n’ont été significativement modifiés chez les agneaux supplémentés. De plus, il n’a été observé aucune différence significative de la digestibilité des nutriments, du pH ruminal, des concentrations intra-ruminales en ammoniac et en acides gras volatils entre les agneaux supplémentés et les contrôles non supplémentés. Il en a donc été conclu que le malate pouvait être incorporé dans la ration des agneaux jusqu’à 6 g/kg.

Mots clés : Agneau, acide malique, ration mélangée, concentrés, croissance pondérale, digestibilité, métabolites du rumen.

Introduction

Because of limitations in the use of antibiotics as growth promoters in animal nutrition, the development of alternative food supplements consisting of enzymes, probiotics and organic acids [2] is increasing in many countries. Some organic acids like malic, fumic and aspartic acids are commonly found in the nature as normal constituents of plant and animal tissues [7, 9]. Organic acids have antibacterial effects on the digestive system in animals. In addition to their growth stimulating effects, they can also be used as mineral and energy sources [6]. Malic acid (C4H6O5), is an important constituent of the energy metabolism and it is involved in the Krebs cycle [14]. In recent years, the use of malic acid and its salts as an antibacterial product or as a mineral and energy source in animal nutrition was investigated [4, 16, 17] and it is stated that organic acids and their salts can be an effective choice in animal nutrition as an alternative of antibiotic growth factors [7].

In some studies, the addition of malic acid into ruminant concentrates is reported to have a positive effect on daily live weight gain [13, 16] whereas in others [4, 17], no change in the daily weight gain was found. CARRO et al. [4] stated that the addition of malic acid into lamb rations didn’t affect forage and concentrate intake, but Mungói [17] stated that the forage intake increased and the concentrate intake decreased according to the gradual incorporations of malic acid into concentrates for lambs during the growth period. With the addition of malic acid into ruminant food, the changes in the rumen fermentation products and ruminal pHs are reported to be similar to the effects of ionophores [12]. Martin and Streeter [12] stated that the total volatile fatty acids and the ammonia nitrogen contents in the rumen from cows supplemented with DL malate yielded similar results with not supplemented controls. However, Mungói [17] reported that the total volatile fatty acids increased in ruminants supplemented with organic acids.
Today, it is necessary to largely explore the physiological and metabolic effects of organic acids in ruminants in order to propose them as alternative additives to antibiotics. In this study, the effects of the malic acid addition to the weaned lamb rations on growth performance, some rumen parameters and digestibility were investigated.

**Material and Methods**

**EXPERIMENTAL ANIMALS AND DIET REGIMEN**

In the present study, 15 male Tuj lambs, 3.5 to 4 months old, weighing 33.8 kg in average, were used in the Education, Research and Practice Farm of Kafkas University. After an adaptation period of 15 days in which animals were treated for endo- and exoparasites (Ivomec® (Ivermectin), Bayer Ilac, Istanbul, Turkey), lambs were allotted in 3 equal groups homogeneous in weight according to the diet regimen: Malic acid was added to the concentrates at the doses of 3 g/kg and 6 g/kg in the groups M3 and M6, respectively for 50 days, whereas the third group served as negative control. Lambs were fed with a ration consisting of an average of 85% concentrate and 15% forage (hay meadow which the length was adjusted to 7-10 cm approximately) twice a day at 08:00 and 17:00, with an average of 10% more than the daily hay and concentrate intake of animals that is present in the mangers. Fresh drinking water was supplied *ad libitum*. The rations were prepared to meet the daily nutrient requirements for weaned lambs [18].

Dry matter (method 925.10), crude protein (method 984.13), crude ash (method 942.05) crude cellulose (method 962.09) and crude fat (method 920.39) in concentrate mix and hay were analysed according to the methods specified in AOAC Official Methods [1]. Metabolic energy of forage and concentrate were calculated according to the methods specified in AOAC Official Methods (Turkish Standards Institution) [23]. In the trial, concentrates containing 15.70% crude protein and 3169 kcal/kg OM (organic matter) metabolic energy were used. The composition of the concentrates and nutrient composition of the mixed (forage and concentrates) ration distributed to weaned lambs for 50 days.

**GROWTH PERFORMANCE ANALYSIS**

Total daily food intake was assessed by weighing the excess food from the previous day, in the morning. Daily forage intake, concentrate intake and total ration intake were assessed by weighing the excess concentrate and hay after passing through a sieve with 5 mm-pores.

The starting weight at adaptation period was assessed by weighing the animals for two consecutive days at the beginning (day 1), in the middle (day 25) and at the end (day 50) of the trial. Body weight gains were calculated by subtracting the previous weight from the next weight. The daily body weight gains were assessed by dividing the weighing results by the interval between two weighings. Food conversion rates were assessed by calculating the total daily dry matter intake for 1 kg weight gain.

**DETERMINATION OF SOME RUMEN PARAMETERS AND DIGESTIBILITY**

On days 1, 25 and 50, rumen contents were collected from lambs throughout a rumen catheter. Samples were collected 2-3 hours post-feeding in the morning and were immediately analyzed for pH using a pHmeter (Fisher Scientific Model 25). For determination of the rumen ammonia nitrogen contents, 3 to 5 drops of concentrate H2SO4 were added to 10 ml of rumen fluids, which was then filtered through four layers of cheesecloth. Samples were centrifuged immediately at 3000x g for 15 minutes at room temperature (23 ± 2°C). The concentrations of rumen ammonia nitrogen and total volatile fatty acids were assessed by Markham Steam Distillation method [10].

Classic digestibility experiment was conducted in assessing the *in vivo* digestibility intensity of rations [19]. In the last 5 days of the trial, 10% of daily collected faeces from each animal was taken homogenously and placed into deep freeze after the daily amount of faeces from each animal was assessed by weighing. The faecal samples that were stored in the deep freeze were ground after drying in the air circulation drying oven at 60°C for 48 hours. Dry matter, crude ash and crude protein analyses were performed in the ground faecal samples [1].
Dry matter, organic matter and crude protein digestibility rates were then calculated [19].

**STATISTICAL ANALYSIS**

Statistical calculations of the groups were made according to variance analysis method. Duncan test was performed for the significance control of the difference between groups [5]. Statistical analyses were performed using SPSS 11.5 program [22]. Data were represented as mean ± S.E.M. (standard error of mean). Differences were considered as significant when \( P \) values were less than 0.05.

**Results**

Average daily food consumption (g of dry matter/lamb/day) of forage, concentrate and total ration in weaned lambs according to the experimental periods are given in Table II. No significant difference in forage, concentrate and total food consumption was found between groups whatever the considered period. Nevertheless, it was observed that the food intake of forage, concentrate and the whole ration tended to decrease in lambs supplemented with 6g/kg of malic acid, whatever the experimental period. In the same way, as shown in Table III, whereas body weights were similar between the 3 groups at the end of the adaptation period, lambs supplemented with 6g/kg malic acid systematically exhibited lower body weights and daily weight gains than lambs from the 2 other groups (not supplemented or supplemented with 3g/kg malic acid) over the whole trial period but differences between groups were not significant (\( P > 0.05 \)). Consequently, the food conversion ratios calculated for each half of the trial period and for the whole experimental period have not significantly differed between the 3 groups.

Some rumen parameters (pH, ammonia nitrogen and volatile fatty acid contents) measured in lambs supplemented with malic acid and in negative controls at the 50th day, were summarized in Table IV. Although the rumen pHs have not significantly differed between the 3 groups on days 1, 25 and 50, it was observed that this parameter has remained stable.

<table>
<thead>
<tr>
<th>Period</th>
<th>Food intake</th>
<th>Malic acid addition (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>233 ± 4</td>
</tr>
<tr>
<td>Day 1 - Day 25</td>
<td>Concentrate</td>
<td>932 ± 50</td>
</tr>
<tr>
<td></td>
<td>Whole ration</td>
<td>1165 ± 51</td>
</tr>
<tr>
<td>Day 25 - Day 50</td>
<td>Forage</td>
<td>212 ± 2</td>
</tr>
<tr>
<td></td>
<td>Concentrate</td>
<td>1256 ± 99</td>
</tr>
<tr>
<td></td>
<td>Whole ration</td>
<td>1468 ± 100</td>
</tr>
<tr>
<td>Day 1 - Day 50</td>
<td>Forage</td>
<td>224 ± 3</td>
</tr>
<tr>
<td></td>
<td>Concentrate</td>
<td>1084 ± 73</td>
</tr>
<tr>
<td></td>
<td>Whole ration</td>
<td>1307 ± 74</td>
</tr>
</tbody>
</table>

Table II: Average food consumption (g of dry matter/lamb/day) of forage, concentrates and total ration in weaned lambs according to the dietary supplementation with malic acid (3 g/kg and 6 g/kg into concentrates). Results are expressed as mean ± standard error of the mean (SEM).

<table>
<thead>
<tr>
<th>Growth parameters</th>
<th>Periods</th>
<th>Malic acid addition (g/kg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>Adaptation</td>
<td>33.06 ± 2.25</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>32.84 ± 1.92</td>
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<tr>
<td></td>
<td>Day 25</td>
<td>38.46 ± 2.16</td>
</tr>
<tr>
<td></td>
<td>Day 50</td>
<td>43.24 ± 2.66</td>
</tr>
<tr>
<td>DWG (g/lamb/day)</td>
<td>Day 1-Day 25</td>
<td>224.8 ± 21.8</td>
</tr>
<tr>
<td></td>
<td>Day 25-Day 50</td>
<td>191.2 ± 29.4</td>
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<td></td>
<td>Day 1-Day 50</td>
<td>208.0 ± 25.1</td>
</tr>
<tr>
<td>FCR (kg/kg)</td>
<td>Day 1-Day 25</td>
<td>5.31 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>Day 25-Day 50</td>
<td>8.20 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>Day 1-Day 50</td>
<td>6.53 ± 0.54</td>
</tr>
</tbody>
</table>

Table III: Growth performance and food conversion in weaned lambs according to the dietary supplementation with malic acid (3 g/kg and 6 g/kg into concentrates). Results are expressed as mean ± standard error of the mean (SEM).
DIETARY MALIC ACID SUPPLEMENTATION IN LAMBS

(around 5.34-5.36) over the whole experimental trial in lambs supplemented with 3g/kg malic acid and has tended to increase at the beginning (day 1) and at the end (day 50) of the trial when lambs received 6 g/kg malic acid. The ammonia nitrogen content significantly declined in the rumen from malic acid supplemented lambs since the first day of the supplementation in a dose dependent manner \((P < 0.05)\). However, thereafter, the ruminal ammonia production did not differ between the negative controls and the supplemented lambs albeit it remained slightly lower in ruminants receiving 6 g/kg malic acid. On the other hand, although differences were not statistically significant, it was recorded that the volatile fatty acid concentrations tended to increase in the rumen from supplemented lambs proportionally to the malic acid dosage. The dry matter, organic matter and crude protein digestibility rates were reported in Table V and no significant differences were found between the 3 experimental groups.

**Discussion**

In the present study, the dietary supplementation with malic acid at 3 g/kg and 6 g/kg in weaned lambs for 50 days has not significantly altered neither the growth performance, nor the food consumption and the food efficiency. In agreement with that, CARRO et al. [4] have also observed no effect of dietary malic acid supplementation on the forage and concentrate intakes in growing lambs. By contrast, Mungói [17] has reported that the forage intake increased in supplemented lambs according to the malic acid dosages (from 0 to 9 g/kg). In the present study, a slight decrease in the dry matter and concentrate consumptions was also observed in lambs supplemented with 6 g/kg malic acid but the forage intake was remained similar to the other groups, even weakly depressed. However, the various proportions of concentrate mixes used in the different studies may lead to conflicted results in the effects of malate addition on food intake. The lack of effect of the dietary malate supplementation in growth performance in lambs (Table III) was also reported by CARRO et al. [4] and by MUNGOI [17]. However, in some studies, it is stated that daily live weight gain [13, 20] and food conversion [20] increased with the addition of malic acid. Even, Mungói [17] reported also a difference in food efficiency around 18% in lambs supplemented with 9g/kg malate and not supplemented controls and that the food conversion ratio tended to decrease in animals supplemented with increased amounts of malate. These conflicted results would be related to differences in the growth phases of lambs.

CARRO et al. [4] reported that ruminal pH values were 6.99 and 6.92 respectively with the addition of 0 and 4 g/kg malic acid into the concentrates of growing lambs and that differences with not supplemented controls were not significant [4, 13]. It was generally stated that ruminal pH increased prominently with the addition of malate [3, 11-13, 15, 17], leading

![Table IV: Variations of the rumen pH, ammonia nitrogen (NH3-N) and volatile fatty acid (VFA) concentrations in weaned lambs according to the dietary supplementation with malic acid (3 g/kg and 6 g/kg into concentrates). Results are expressed as mean ± standard error of the mean (SEM).](image)

![Table V: Dry matter, organic matter and crude protein digestibility rates (%) of the rations given to weaned lambs according to the dietary supplementation with malic acid (3 g/kg and 6 g/kg into concentrates). Results are expressed as mean ± standard error of the mean (SEM).](image)
to alleviate subclinical acidosis and to increase performance [13]. However, the ruminal pH values recorded in the present study were lower, ranging from 5.23 to 5.83 (Table IV); these discrepancies between studies may be attributed to various food contents and rumen content sampling methods. Although significant differences in ruminal pH values were not evidenced according to the amount of the malate supplementation, it was observed that ruminal pH remained stable with 3g/kg malate (around 5.34 and 5.36) and was slightly higher when 6 g/kg malate was added into concentrates.

Whereas the dietary addition of 6 g/kg malate has induced an abrupt decline in the ruminal ammonia nitrogen content compared to the controls ($P < 0.05$), no significant difference was found during and at the end of the experiment between groups (Table IV). The origin of significant decrease in the ammonia content detected at the beginning of this study may be related to individual differences in the nutrient digestibility of lambs. CARRO et al. [4] also reported no significant effect of dietary malate supplementation on ruminal ammonia content, but Mungói [17] found that the ruminal ammonia nitrogen content has weakly decreased in lambs fed with concentrates including 9 g/kg malate (4.97 mg/100 mL) compared to the not supplemented controls (5.33 mg/100 mL) and in another study [3], the ruminal ammonia nitrogen contents have significantly increased with the addition of malate.

Total volatile fatty acids weren’t significantly affected by the malic acid supplementation in the present study although they tended to increase in lambs supplemented with the highest dose (6 g/kg) (Table IV). Similarly, CALLAWAY and MARTIN [3], CARRO et al. [4], stated that the ruminal content of total volatile fatty acids has significantly differed according to the malate addition whereas significant changes were observed by MARTIN et al. [13], the volatile fatty acid formation increasing with the malate addition [17], particularly in females at the beginning of lactation [8].

At the end of the trial, dry matter, organic matter and crude protein digestibilities were similar between groups supplemented or not with malate (Table V). In agreement, CARRO et al. [4] reported that the addition of 4 g/kg and 8 g/kg malic acid into concentrates in growing lambs had no effect on organic matter and crude protein digestibility rates, the organic matter digestibility being within 81.7-82.2% range and the crude protein digestibility within 76.3-76.8% range. In the present study, the organic matter digestibility was slightly decreased and that of crude protein was slightly increased compared to the values reported before, and these differences would be due to the type of concentrates used in the different studies. Other studies also found no significant effects of malate supplementation on dry matter [21], organic matter [8, 15, 21] and crude protein [8, 21] digestibility. However, Mungói [16] stated that the use of 2 g/kg malate in concentrate of lambs decreased dry matter and organic matter digestibility rates.

As a conclusion, the addition of 3 g/kg and 6 g/kg malic acid into concentrates has induced no significant alteration in growth performance, food intake and food efficiency and has not significantly altered the rumen function and the dry matter, organic matter and crude protein digestibilities in Tuj lambs. Consequently, malic acid up to 6 g/kg can be successfully incorporated in lamb concentrates.

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References
