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# The non-volatile organic acids content in Italian green crops

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**Summary:** The objective of this study was to assess the concentration of main organic acids content in several green crops that are administered to cattle after different preservative processes. These acids, except *trans*-aconitic, are intermediate of citric acid cycle. Malic acid is involved in preventing ruminal acidosis in cattle and it represents, with soluble sugars, a rapidly fermentable source of energy. 72 samples of green crops of different species, at different vegetative stadium, were collected. All samples were analysed by HPLC with an ion exchange stationary phase. Simultaneous determination of citric, malic, *trans*-aconitic and fumaric acid were performed. Also chemical composition was determined. The samples were divided in: 20 samples of Italian Ryegrass (*Lolium italicum*), 8 samples of fescue grass (*Festuca pratensis*), 8 samples of triticale (*x Triticosecale*), 15 samples of corn (*Zea mays*, 13 samples of alfalfa (*Medicago sativa*) and 8 samples of white clover (*Trifolium repens*). Mean total organic acids content were respectively 29.7, 22.1, 23.3, 11.5, 43.0 and 31.6 g/kg DM. Malic acid was the main non-volatile organic acid in all crops, except corn, and it represent respectively 83.8, 87.1, 65.8, 22.9, 72.1 and 89.9% of the total amount. Citric acid, except corn and triticale, was the second one and its concentration was respectively 15.0, 12.2, 13.4, 7.4, 22.4 and 8.5 g/kg DM. *Trans*-aconitic is the most important non-volatile organic acid in corn (7.3 g/kg DM) and the second one in triticale (6.2 g/kg DM) while it is very low in all other forages. Malic acid was the most correlated with total organic acids content (r=+0.949; P<0,001). Excluding corn and triticale, citric, *trans*-aconitic and fumaric acids were all positively correlated (P<0.001) with ADL, DM, ADF and soluble protein and negatively correlate (P<0.001) with crude fat.

**Introduction**: The main organic acids in green crops, except *trans*-aconitic, are intermediate of citric acid cycle. Malic acid is the products of Hatch and Slack cycle of plant; an alternative way of classical photosynthetic Calvin cycle which produce glucose. When weather is hot and sunny organic acids content of plants increase (Timpa et al., 1986) but there is many differences among various species. These differences regard organic acids quantity and quality. For example alfalfa (Medicago sativa) and bermudagrass (*Cynodon dactylon*) are relatively rich in malic acid (Callaway et al., 1997). Corn and sorghum shows a relative high content of *trans*-aconitic acid (Poe et Barrentine, 1968; Rustamani et al., 1992).
These acids may be preserved in hay 50-70% or well in dehydrated forages (60-80%; Formigoni et al., 2003; Pezzi et al., 2006; Pezzi et al., 2008a). As well as sugars these acids are often converted in lactic or acetic acid in silages (Pezzi et al., 2008b). Malic acid is also involved in preventing ruminal acidosis in cattle (Evans et Martin, 1997; Martin et Streeter, 1995; Nisbet et Martin, 1990; Rossi et Piva 1999; Russell et Van Soest, 1984) and it represents, with soluble sugars and citric acid, a readily fermentable source of energy. Moreover organic acids increase solubility of all nutrients improving feeds digestibility (Salovaara et al., 2002; Martin et al, 1999; Martin et al, 2000).
This work want starting to evaluate the real intake of these non-volatile organic acid in cattle diets because they may be very important nutrients for rumen and animal metabolism.

**Material and methods**: 72 samples of seven different species of green crops were collected. All samples were analysed for chemical composition (dry matter, crude protein, soluble protein, non protein nitrogen, crude fat, crude ash, NDF, ADF and ADL) and for non-volatile organic acid content. This analysis was carried out by HPLC with an ion exchange stationary phase. Simultaneous determination of citric, malic, *trans*-aconitic and fumaric acid were performed. The samples were divided in: 20 samples of Italian Ryegrass (*Lolium italicum*), 8 samples of fescue grass (*Festuca pratensis*), 8 samples of triticale (*x Triticosecale*), 15 samples of corn (*Zea mays)*, 13 samples of alfalfa (*Medicago sativa*) and 8 samples of white clover (*Trifolium repens*). The

Statistical analysis was carried out with SPSS for Windows Version 13.0. Bivariate Pearson correlations were tested among all non-volatile organic acids and all the others parameters. Two-tailed significances were tested.

**Results and Discussion:** Chemical compositions of all seven forages classes are reported in Table 1. All data are comprised between a normality range. As expected legume forages, and particularly alfalfa, were higher in crude protein and ADL content while they showed the lowest values of NDF. Corn samples (harvested at ensiling time) showed the higher dry matter values.

**Table 1. Mean chemical composition of seven green crops species analysed.**

| | | *Lolium italicum* | *Festuca pratensis* | *x Tritico-secale* | *Zea mays* | *Medicago sativa* | *Trifolium repens* |
|---|---|---|---|---|---|---|---|
| Cases | N° | 20 | 8 | 8 | 15 | 13 | 8 |
| Dry Matter | % | 14.1 | 16.0 | 17.5 | 29.9 | 22.6 | 12.5 |
| Crude protein | % | 16.2 | 13.0 | 13.7 | 8.50 | 21.7 | 22.5 |
| Sol. Protein | % | 4.44 | 4.13 | 4.53 | 2.82 | 9.63 | 6.24 |
| NPN | % | 3.95 | 3.34 | 4.03 | 2.51 | 7.51 | 5.25 |
| Crude Fat | % | 2.61 | 2.56 | 2.39 | 2.29 | 1.78 | 2.55 |
| Crude Ash | % | 11.1 | 8.63 | 8.67 | 5.47 | 9.74 | 10.6 |
| NDF | % | 45.2 | 40.6 | 49.8 | 43.5 | 38.7 | 31.2 |
| ADF | % | 28.2 | 25.2 | 30.4 | 26.8 | 34.6 | 22.1 |
| ADL | % | 2.12 | 2.04 | 2.42 | 2.18 | 6.87 | 3.21 |
| ADF | %NDF | 62.5% | 62.2% | 61.1% | 61.7% | 89.4% | 70.7% |
| ADL | %NDF | 4.7% | 5.0% | 4.9% | 5.0% | 17.8% | 10.3% |

Non-volatile organic acids contents of green crops were showed in Figure 1 and in Table 2a (as absolute content) and Table 2b (as relative of total acids content).

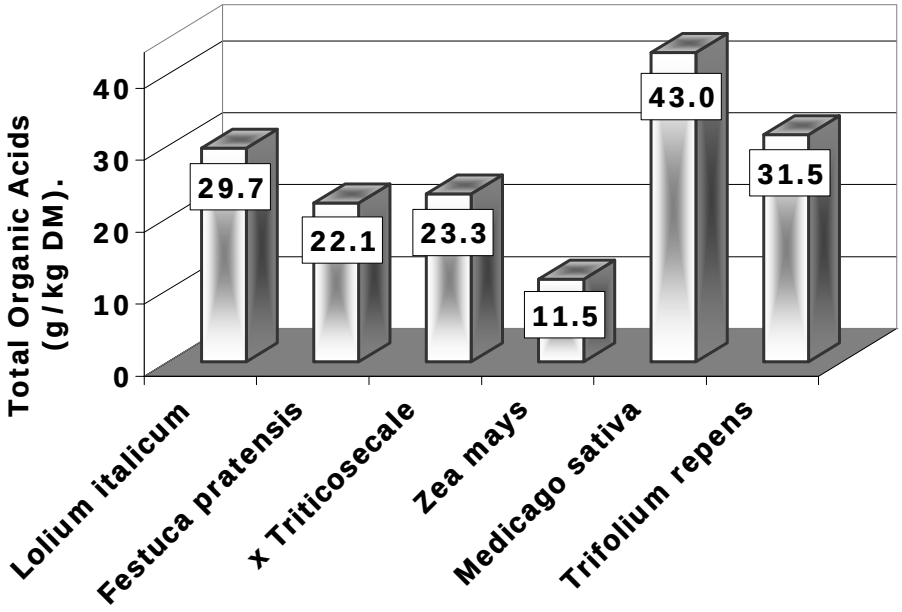
**Table 2a. Non-volatile organic acids absolute contents of green crops.**

| | | *Lolium italicum* | *Festuca pratensis* | *x Tritico-secale* | *Zea mays* | *Medicago sativa* | *Trifolium repens* |
|---|---|---|---|---|---|---|---|
| Cases | N° | 20 | 8 | 8 | 15 | 13 | 8 |
| Citric | g/kg | 4.46 | 2.69 | 1.73 | 1.54 | 9.64 | 2.78 |
| Malic | g/kg | 24.9 | 19.2 | 15.4 | 2.6 | 31.0 | 29.3 |
| T-Aconitic | g/kg | 0.125 | 0.014 | 6.196 | 7.261 | 0.762 | 0.288 |
| Fumaric | g/kg | 0.213 | 0.146 | 0.055 | 0.061 | 1.609 | 0.230 |
| Total | g/kg | 29.7 | 22.1 | 23.3 | 11.5 | 43.0 | 31.5 |

**Table 2b. Non-volatile organic acids relative contents of green crops.**

| | | *Lolium italicum* | *Festuca pratensis* | *x Tritico-secale* | *Zea mays* | *Medicago sativa* | *Trifolium repens* |
|---|---|---|---|---|---|---|---|
| Cases | N° | 20 | 8 | 8 | 15 | 13 | 8 |
| Citric | % Total | 15.0% | 12.2% | 7.4% | 13.4% | 22.4% | 8.8% |
| Malic | % Total | 83.8% | 87.1% | 65.8% | 22.9% | 72.1% | 92.7% |
| T-Aconitic | % Total | 0.4% | 0.1% | 26.5% | 63.2% | 1.8% | 0.9% |
| Fumaric | % Total | 0.7% | 0.7% | 0.2% | 0.5% | 3.7% | 0.7% |

**Figure 1. Total non-volatile organic acids contents of green crops (g/kg of dry matter).**




Malic acid is the most representative of all non-volatile acid and its correlation with total organic acid is 0.949 (P<0.001; Table 3). Positive are also its correlation with citric and fumaric acid (respectively 0.660 and 0.595 both for P<0.001). In contrast, negative is resulted correlation of malic acid content with T-aconitic acid content (-0.715; P<-0.715; Table 3) and with DM (-0,65; P<0,001; Table 4). Interesting is the correlation between crude protein and total organic acids (0.52; P<0.001). Citric acid in legume forages and in grasses is the second acid. Corn, as well as sorghum, is rich in *trans*-aconitic acid and in triticale samples this acid is the second after malic acid.

**Table 3. Relationship among the non-volatile organic acids in green crops.**

| | | Malic | T-Aconitic | Fumaric | Total |
|---|---|---|---|---|---|
| Citric | r | 0.660 | -0.454 | 0.855 | 0.806 |
| | Sign. | 0.000 | 0.000 | 0.000 | 0.000 |
| Malic | r | 1 | -0.715 | 0.595 | 0.949 |
| | Sign. | | 0.000 | 0.000 | 0.000 |
| T-Aconitic | r | | 1 | -0.340 | -0.530 |
| | Sign. | | | 0.004 | 0.000 |
| Fumaric | r | | | 1 | 0.746 |
| | Sign. | | | | 0.000 |

**Table 4. Relationship between the green crops non-volatile organic acids and the chemical parameters.**

| | | DM | CP | Sol. Prot. | NPN | EE | CH | NDF | ADF | ADL |
|---|---|---|---|---|---|---|---|---|---|---|
| Citric | r | -0.204 | 0.632 | 0.725 | 0.691 | -0.578 | -0.013 | -0.306 | 0.602 | 0.827 |
| | Sign. | 0.063 | 0.000 | 0.000 | 0.000 | 0.000 | 0.468 | 0.024 | 0.000 | 0.000 |
| Malic | r | **-0.650** | 0.372 | 0.235 | 0.236 | -0.291 | 0.174 | -0.228 | 0.260 | 0.455 |
| | Sign. | 0.000 | 0.008 | 0.067 | 0.067 | 0.031 | 0.135 | 0.074 | 0.048 | 0.001 |
| T-Aconitic | r | 0.609 | -0.135 | -0.036 | -0.044 | 0.088 | -0.391 | 0.282 | 0.036 | -0.034 |
| | Sign. | 0.000 | 0.197 | 0.411 | 0.392 | 0.290 | 0.005 | 0.035 | 0.411 | 0.416 |
| Fumaric | r | -0.012 | 0.596 | 0.656 | 0.636 | -0.621 | -0.152 | -0.350 | 0.655 | 0.910 |
| | Sign. | 0.464 | 0.000 | 0.000 | 0.000 | 0.000 | 0.168 | 0.011 | 0.000 | 0.000 |
| Total | r | -0.496 | **0.519** | 0.468 | 0.454 | -0.449 | 0.035 | -0.245 | 0.462 | 0.695 |
| | Sign. | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.413 | 0.059 | 0.001 | 0.000 |

**Conclusions:** All green crops fed to cattle may have consistent amount of non-volatile organic acid which are very important for the nutritional value of feeds. It is important to underline that:

- Alfalfa shows the higher values of total amount of these acids and particularly of malic acid,
- Citric acid in legume forages and in grasses is the second acid after malic,
- Corn is very low in malic acid but is rich in *trans*-aconitic acid.
- In Triticale *trans*-aconitic acid is second acid after malic.

Further trials are needed to better understand the variability of these acids content in various cultivation condition and during different preservative processes as well as hay making and ensiling.

**References:**


1. Callaway TR, Martin SA, Wampler JL, Hill NS, Hill GM. (1997). *Malate content of forage varieties commonly fed to cattle*. J Dairy Sci. 80(8):1651-5.
2. Evans JD, Martin SA. (1997). *Factors affecting lactate and malate utilization by Selenomonas ruminantium.* Appl Environ Microbiol. 63(12):4853-8.
3. Formigoni A., Biagi G., Piva A., Pezzi P. (2003). *The drying method affects the organic acid content of alfalfa forages.* Atti XV Congr. Naz. A.S.P.A. Parma, Italia. 243-245.
4. Martin SA, Bertrand JA, Sauls B, Hill GM. (2000). *In situ disappearance of malate from alfalfa and bermudagrass hay*. J Dairy Sci. 83(2):308-12.
5. Martin SA, Streeter MN, Nisbet DJ, Hill GM, Williams SE. (1999). *Effects of DL-malate on ruminal metabolism and performance of cattle fed a high-concentrate diet.* J Anim Sci. 77(4):1008-15.
6. Martin SA, Streeter MN. (1995). *Effect of malate on in vitro mixed ruminal microorganism fermentation.* J Anim Sci. 73(7):2141-5.
7. Nisbet DJ, Martin SA. (1990). *Effect of dicarboxylic acids and Aspergillus oryzae fermentation extract on lactate uptake by the ruminal bacterium Selenomonas ruminantium.* Appl. Environ. Microbiol., 56: 3515-3522.
8. Pezzi P., Fusaro I., Giammarco M., Angelozzi G. (2008a). *Malic acid content as hay quality marker. Atti LXII Conv. Naz. SISVet., San Benedetto del Tronto (*AP), Italy, 217-218.
9. Pezzi P., Fusaro I., Giammarco M., Manetta A.C., Vignola G., Formigoni A. (2008b). *Le concentrazioni degli acidi organici quali indicatori per la valutazione qualitativa degli insilati di mais.* XL Congresso Nazionale della Società Italiana di Buiatria. Castel San Pietro Terme – BO, 22-24/05/08. Buiatria Vol. II-2008. 53-60.
10. Pezzi P., Giammarco M., Fusaro I., Manetta A.C., Formigoni A (2006). *Variazioni del contenuto di acidi organici durante l'essiccazione dell'erba medica*. IV Convegno AISSA. 5-6/12/06 - Mosciano Sant'Angelo (TE).
11. Poe, WE; Barrentine, BF (1968). *Colorimetric Determination of. Aconitic Acid in Sorgo*. J. Agric. Food Chem. 16(6): 983-984.
12. Rossi F, Piva G*. (1999) Effect of malic acid on growth and metabolism of Megasphaera elsdenii - a rumen bacterium [cattle].* Proceedings ASPA Congress –Recent Progress in Animal Production-Science (Italy). V.1 p. 342-344.
13. Russell JB, Van Soest PJ. (1984). *In vitro ruminal fermentation of organic acids common in forage.* Appl Environ Microbiol. 47(1):155-9.
14. Rustamani M.A., Kanehisa K., Tsumuki H., Shiraga T. (1992). *Further observations on the relationship between aconitic acid content and aphid densities on some cereal plants*. Bull. Res. Inst. Bioresour. Okayama Univ. 1:9-20.
15. Salovaara S, Sandberg AS, Andlid T. (2002). *Organic acids influence iron uptake in the human epithelial cell line Caco-2.* J Agric Food Chem. 50(21):6233-8.
16. Timpa JD, Burke JJ, Quisenberry JE, Wendt CW. (1986). *Effects of Water Stress on the Organic Acid and Carbohydrate Compositions of Cotton Plants.* Plant Physiol. 82(3):724-728.