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# Influence of chemical, enzymatic and phytogenic ensiling preparations on digestibility, degradability and PDI and NEL content of lucerne and red clover

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**Abstract**

Lucerne and red clover cut at the beginning of the flowering stage were conserved by chemical (formic acid; Na-formiate; Ca-propionate), enzymatic (based on cellulases, hemicellulases, amylases and glucose oxidases, including microbial inoculants) or phytogenic (plant oils) preparations. The effective degradability of crude protein (CP) was measured in two steers with rumen cannulas, intestinal digestibility of the undegraded protein (UDP) in two cows equipped with duodenal T-cannulas, and the apparent CP and organic matter (OM) digestibility in eight wethers. The PDI (protein digestible in the intestine) and NEL (net energy of lactation) were calculated using the above mentioned values. The differences among ensiling preparations were significant both in lucerne and clover, regarding CP effective degradability ($P < 0.01$), PDI ($P < 0.01$), and NEL ($P < 0.05$). CP effective degradability of red clover preserved with the enzymatic or phytogenic preparations was higher ($P < 0.01$) compared with the chemical agents. The mean of all PDI values of red clover ensiled with the phytogenic preparations did not differ ($P > 0.05$) from that of PDI values of chemically conserved clover. The UDP intestinal digestibility of the lucerne conserved by the chemical or enzymatic preparations was higher ($P < 0.01$) than that of clover. The difference in the UDP intestinal digestibility between samples conserved with formic acid or the enzymatic preparation was not proved ($P > 0.05$) either in the lucerne or in the red clover. Formic acid was recommended as the best preparation for ensiling lucerne and red clover regarding PDI and NEL content.

*Keywords:* Lucerne; Clover; Techniques, ensiling

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## 1. Introduction

The amount of the protein, which is available for digestion in the small intestine depends (regarding ensiled forages) not only on the protein content of the feed and its rumen degradability, but also on the extent of the changes of nitrogen compounds due to the action of the ensiling agents (Aufrère et al., 1994a). The microbial inoculants are safe and noncorrosive alternatives of the direct acidifiers, but significant improvement of either the preservation, or the animal production is not reported (Marshall et al., 1993). The influence of the enzymatic agents on the crude protein (CP) degradability is little explored (Hristov, 1993). The preparations based on the plant essential oils have been recently tested not only as the flavouring agents but also as conservation agents.

The objectives of the present experiment were to compare the direct (chemical) acidifiers with enzymatic conservants (in combination with microbial inoculants) and phytogenic preparations from the viewpoint of the influence on the nutritive value (apparent CP and OM digestibility, effective CP degradability, intestinal UDP digestibility, protein digestible in the intestine and net energy of lactation content) of lucerne and red clover.

## 2. Material and methods

### 2.1. Plant material

Lucerne (*Medicago sativa*, L.) and red clover (*Trifolium pratense*, L.) were harvested in the second cut in 1994. The harvest date was 11 August for clover and 30 August for lucerne, when both plants were at the same vegetative stage, beginning to flower. The nutrient content of lucerne and red clover is presented in Table 1. Glucose content was 15 and 3, fructose 0.1 and 0.1, sucrose 4.7 and 1.8 in lucerne and red clover, respectively.

### 2.2. Conservation

Both plants were cut to a length of 2–3 cm (cut by a harvester Claas Jaguar 690) and ensiled without wilting (DM content of the lucerne and clover was 23.3% and 17.1%, respectively). Characteristics of the used ensiling preparations, including amounts (recommended by the producers) are presented in Table 2. The same ensiling preparations were not available from the producers when either red clover (ensiled earlier) or lucerne samples were conserved (see Table 2). One tonne of each sample was conserved in three fibreglass vessels of diameter 1.5 m and height 2 m. The preservation time of the clover samples was 4 months; that of the lucerne samples 6 months.

### 2.3. Degradability measurements

The CP disappearances were measured in two steers (Czech Red and White breed) of average body weight 550 kg fitted with rumen cannulas of internal diameter 100 mm.

Table 1
Chemical composition of lucerne and red clover before and after ensiling including silage quality parameters [a] ($n = 3$)

| | Treatment | | CP [b] | | ADF [c] | | NDF [d] | | ADIN [e] | | NDIN [f] | | LA [g] | | VFA [h] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | g kg$^{-1}$ DM | | | | | | %CP | | | | g kg$^{-1}$ DM | | | |
| | | | mean ± s.e. | | | | | | | | | | | | | |
| RC [i] | Material to ensiling | | 181 | 2.1 | 333 | 7.2 | 374 | 7.7 | | | | | | | | |
| | C [j] | Formic acid | 203 | 3.5 | 291 | 4.9 | 378 | 5.9 | 8.0 | 0.4 | 11.6 | 0.3 | 129 | 3.6 | 45 | 2.7 |
| | | Silo Meister | 204 | 2.8 | 304 | 6.5 | 395 | 4.8 | 5.8 | 0.2 | 11.1 | 0.1 | 139 | 0.7 | 30 | 2.6 |
| | | Elmosil Grün | 199 | 3.0 | 293 | 6.6 | 391 | 7.0 | 6.5 | 0.1 | 10.2 | 0.3 | 130 | 3.0 | 43 | 3.1 |
| | E | Bactozyme | 211 | 1.9 | 330 | 4.1 | 377 | 5.2 | 5.6 | 0.3 | 9.1 | 0.4 | 135 | 2.5 | 33 | 2.2 |
| | | Bactensil2000 | 206 | 3.1 | 339 | 5.2 | 401 | 5.6 | 7.3 | 0.1 | 8.9 | 0.3 | 150 | 3.5 | 39 | 1.4 |
| | P | Aromasil S | 207 | 2.9 | 319 | 4.8 | 390 | 8.0 | 6.2 | 0.3 | 10.9 | 0.3 | 146 | 3.9 | 36 | 0.3 |
| | | Aromasil SN | 212 | 2.8 | 318 | 5.8 | 389 | 6.9 | 6.1 | 0.1 | 9.1 | 0.1 | 123 | 3.2 | 40 | 2.5 |
| L | Material to ensiling | | 180 | 0.9 | 352 | 2.9 | 403 | 5.6 | | | | | | | | |
| | C | Formic acid | 205 | 2.1 | 331 | 5.3 | 357 | 9.1 | 3.1 | 0.2 | 4.7 | 0.1 | 106 | 2.8 | 56 | 2.1 |
| | E | Bactosyme | 211 | 2.0 | 348 | 6.2 | 402 | 7.8 | 4.1 | 0.4 | 3.0 | 0.3 | 116 | 3.1 | 50 | 2.2 |
| | | Clampsyme + + Pioneer | 207 | 3.0 | 385 | 7.1 | 460 | 7.0 | 4.2 | 0.1 | 3.1 | 0.1 | 130 | 3.3 | 48 | 1.7 |
| | Co | Formic acid + + Aromasil S | 208 | 2.5 | 329 | 5.6 | 360 | 8.6 | 4.1 | 0.2 | 4.6 | 0.1 | 109 | 2.8 | 56 | 1.3 |

[a] Butyric acid not found in any sample; pH of all samples in the range 4.0–4.2
[b] Crude protein
[c] Acid detergent fibre
[d] Neutral detergent fibre
[e] Acid detergent insoluble nitrogen
[f] Neutral detergent insoluble nitrogen
[g] Lactic acid
[h] Sum of volatile fatty acids
[i] RC - red clover; L - lucerne
[j] C - chemical, E - enzymatic, P - phytogenic preparations; Co - combination of the preparations

The diets (fed twice daily) for the clover and lucerne samples, respectively were as follows (kg of fresh matter): meadow hay 2, maize silage 9, wheat 1, wheat straw 3; and meadow hay 3, lucerne silage 5, wheat 1, wheat straw 3, respectively.

The ensiled lucerne or clover samples, dried at 60°C were ground to pass through a 1 mm screen and weighed in 5 g portions into 14 × 8 cm synthetic bags (Uhelon, Hedva Mor. Třebová) with aperture size 40 μm. For the disappearance measurements within each of the six incubation intervals 2, 4, 8, 16, 24 and 48 h, six bags were used. The bags were fixed in the rumen between two rings of a segmented carrier equipped with a weight. The rings were screwed on the carrier central axis. Eighteen bags in six rows by three were attached on one carrier. When removed from the rumen, bags were rinsed under running water for 1 min, washed in a tap water bath for 15 min, and dried at 60°C.

For the incubation interval $t = 0$ h, weight losses were determined by washing 6 bags in a tap water for 15 min. Soluble nitrogen fraction was determined after the nitrogen content in the residue had been measured (see Table 3).

In all samples the CP content was determined by the Kjeldahl method. CP degradability was calculated according to Orskov and McDonald (1980) using a lag time (Denham

Table 2
Ensiling preparations used in the experiment

| | Name | Composition | Plant | Amount |
|---|---|---|---|---|
| C [a] | Formic acid | 84% solution | Lucerne<br>Clover | 4 l $t^{-1}$ |
| | Silo Meister [b] | Na-formiate<br>Ca-propionate | Clover | 3.5 kg $t^{-1}$ |
| | Elmosil Grün [b] | Ca-propionate<br>Lactose<br>Microelements<br>Macroelements | Clover | 5 kg $t^{-1}$ |
| E | Bactozyme [c] | Cellulases<br>Glucose oxidases<br>Streptococcus faecium<br>Lactobacillus plantarum<br>Lactobacillus casei<br>Pediococcus spp. | Lucerne<br>Clover | 150 ml $l^{-1}$<br>(enzymes)<br>15 g $t^{-1}$<br>(bact.) |
| | Bactensil [d] 2000 | Cellulase<br>Hemicellulase<br>Amylase<br>L. plantarum<br>L. acidophylus<br>P. pentesaceus | Clover | 3 l $t^{-1}$<br>(enzymes)<br>20 g $t^{-1}$<br>(bact.) |
| | Clampzyme [e] + Pioneer [f] | Cellulases<br>Hemicellulases<br>Amylases<br>Proteases<br>L.plantarum<br>S. faecium<br>$CaCO_3$ | Lucerne | 150 ml $t^{-1}$<br>(enzymes)<br>20 g $t^{-1}$<br>(bact.) |
| P | Aromasil S [g] | Plant oils | Clover | 300 g $t^{-1}$ |
| | Aromasil SN [g] | Plant oils | Clover | 500 g $t^{-1}$ |
| Co | Formic acid + Aromasil SN | | Lucerne | 4 l $t^{-1}$<br>300 g $t^{-1}$ |

[a] C - chemical; E - enzymatic; P - phytogenic; Co - combination
[b] Salvana, Germany
[c] Medipharm, Czech Republic (cellulase activity 25000 ncat $ml^{-1}$; glucoseoxidases activity 4000 ncat $ml^{-1}$; lactacidogennic germ concentration $15 \times 10^9$ $g^{-1}$)
[d] Salvana, Germany (germ concentration $10 \times 10^9$ $g^{-1}$)
[e] Kantvik, Finland
[f] Jonston, USA
[g] Delacon, Austria (preparations based on essential oils of garlic and spearmint bound to the carrier)

et al., 1989). Rumen fractional outflow rate for the CP particles was considered to be 3.5% $h^{-1}$

### 2.4. Intestinal digestibility measurements

Two dry cows fitted with the T-piece cannula in the proximal duodenum were used. The diet (fed twice daily) consisted of 6 kg of lucerne hay, 14 kg of maize silage, 1 kg

Table 3
Influence of the ensiling preparations on crude protein degradability characteristics of lucerne and red clover ($n = 6$)

| | Preparation | | a [a] %CP | | b [b] %CP | | c [c] $h^{-1}$ | | EP [d] %CP | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | mean ± s.e. | | | | | | | |
| RC [e] | C | Formic acid | 40 | 1.7 | 68 | 3.5 | 0.03 | 0.011 | 32 | 1.5 |
| | | Silo Meister | 42 | 1.0 | 49 | 1.3 | 0.06 | 0.012 | 29 | 1.6 |
| | | Elmosil grün | 45 | 2.4 | 53 | 2.0 | 0.03 | 0.007 | 32 | 2.4 |
| | E | Bactozyme | 44 | 1.5 | 54 | 3.1 | 0.04 | 0.010 | 28 | 1.4 |
| | | Bactensil2000 | 41 | 1.8 | 46 | 2.9 | 0.12 | 0.025 | 28 | 2.3 |
| | P | Aromasil S | 45 | 2.8 | 49 | 2.6 | 0.05 | 0.008 | 27 | 1.5 |
| | | Aromasil SN | 44 | 2.8 | 51 | 3.0 | 0.05 | 0.008 | 28 | 2.2 |
| L | C | Formic acid | 62 | 1.2 | 33 | 2.3 | 0.07 | 0.005 | 16 | 0.8 |
| | E | Bactozyme | 71 | 1.1 | 21 | 2.4 | 0.08 | 0.003 | 15 | 1.1 |
| | | Clampzyme + + Pioneer | 65 | 1.5 | 26 | 3.1 | 0.08 | 0.005 | 17 | 1.1 |
| | Co | Formic acid + + Aromasil S | 65 | 1.4 | 25 | 2.3 | 0.11 | 0.038 | 17 | 0.8 |

[a] Soluble fraction
[b] Insoluble degradable fraction
[c] Degradation rate of fraction b
[d] Escape protein (100 - CP effective degradability, %)
[e] RC - red clover; L - lucerne; C - chemical agents; E - enzymatic preparations; P - phytogenic ppreparations: Co - combination of the preparations

of barley meal, and supplemented minerals. Intestinal digestibility was measured in the lucerne and clover samples conserved by formic acid and Bactozyme by the mobile bags method according to Frydrych, 1992 (briefly: 0.5 g samples of the rumen undegraded residues after 16 h incubation were weighed into polyester bags 3.8 cm × 4.5 cm with pore size 45 μm; 20 bags per sample were inserted into the small intestine after incubation in HCl/pepsin; bags recovered from faeces within 20 h /16–18 bags per sample/ were analysed).

### 2.5. PDI and NEL calculation

The content of protein digestible in the intestine was calculated using the PDI system (Verité and Peyraud, 1988). Briefly: PDI content is a sum of two fractions, PDIA, feed protein undegraded in the rumen, digestible in the intestine, and PDIM (PDIMN or PDIME), true microbial protein digestible in the intestine. PDIMN is based on microbial protein synthetized in the rumen from degraded dietary nitrogen, when energy and other nutrients were not limiting. PDIME is based on the microbial protein synthetized in the rumen from available energy, when degradable nitrogen and other nutrients were not limiting. True PDI is the lower value from two sums, PDIA + PDIMN or PDIA + PDIME where PDIA = 1.11CP(1 − P)D; PDIMN = 0.64CP(P − 0.1); PDIME = 0.093FOM ; P is CP effective degradability; D is UDP intestinal digestibility and FOM is fermentable organic matter. The PDIME values were calculated using FOM as a

difference: digestible organic matter – (UDP + crude fat + volatile fatty acids + lactic acid). The net energy of lactation content (NEL) was estimated using VanEs (1978) system modified by Vencl (1990): $NEL = ME(0.463 + 0.24\frac{ME}{GE})$; $GE = 0.00588CP + 0.01918OM$; $ME = 0.00137DCP + 0.01504DOM$ where NEL is net energy of lactation, GE is gross energy and ME is metabolizable energy in MJ $kg^{-1}$ DM; CP is crude protein, OM is organic matter, DCP is digestible CP and DOM is digestible OM in g $kg^{-1}$ DM.

The apparent CP and OM digestibility was determined in eight wethers (Merino breed) in the balance trial with a 10-day preparation period and a 10-day balance period (Vencl, 1985).

### *2.6. Chemical analyses*

Fibre fractions were determined according to Goering and VanSoest (1970). Volatile fatty acids and lactic acid were measured by isotachophoresis, soluble sugars (glucose, fructose, sucrose) by high performance liquid chromatography (Czech standard ČSN 467092, 1985).

Statistical evaluation was based on the variance ratio test and the *t*-test (Snedecor and Cochran, 1971).

## 3. Results

### *3.1. Comparison of lucerne and red clover*

Silages of both plants did not differ in CP content. However, lucerne silages tended to have higher ADF (acid detergent fibre) content (Table 1). ADIN (acid detergent insoluble nitrogen) content was lower ($P < 0.01$) in lucerne silages compared with corresponding red clover samples.

Silage quality parameters are also presented in Table 1. Lactic acid content in red clover conserved by formic acid or Bactozyme was higher ($P < 0.05$) compared with corresponding lucerne samples. On the other hand, volatile fatty acids (VFA) content was higher in lucerne samples.

As far as the degradation characteristics are concerned (Table 3), measured soluble nitrogen fraction (a) was on average 60% lower and escape protein twice as high in the red clover silages compared with lucerne. Lucerne had significantly ($P < 0.01$) higher CP degradability in the samples conserved by the same preparations (formic acid, Bactozyme), on average by 14%. As a consequence, the PDI content of the clover samples was higher ($P < 0.01$) on average by 20 g/kg DM. Also NEL content was higher ($P < 0.05$, by 0.3 MJ $kg^{-1}$ DM) in clover (Table 4).

### *3.2. Comparison of silage preparations*

ADIN content of red clover conserved by formic acid (8.0% of CP) tended to be higher compared with the other red clover silages (Table 1). On the other hand, lactic

Table 4
Comparison of the effect of the individual ensiling preparations on the nutritive characterictics of lucerne and red clover. Duncan test (OM digestibility, CP digestibility $n = 8$; CP degradability, PDI, NEL $n = 6$).

| | Preparation [a] | | OM dig (%) | CP dig (%) | CP deg (%) | PDI (g kg$^{-1}$ DM) | NEL (MJ kg$^{-1}$ DM) |
|---|---|---|---|---|---|---|---|
| RC [b] | C | Formic acid | 65.1 $^{c,d}$ | 66.8 $^{e,f}$ | 67.9 $^{c}$ | 79 $^{c}$ | 5.4 $^{c}$ |
| | | Silo Meister | 65.6 $^{c}$ | 68.8 $^{c}$ | 71.3 $^{d}$ | 73 $^{d}$ | 5.4 $^{c}$ |
| | | Elmosil Grün | 64.1 $^{d}$ | 66.2 $^{f}$ | 67.9 $^{c}$ | 72 $^{d}$ | 5.2 $^{d}$ |
| | E | Bactozyme | 64.3 $^{d}$ | 68.2 $^{c,d,e}$ | 72.5 $^{d}$ | 73 $^{d}$ | 5.2 $^{d}$ |
| | | Bactensil 2000 | 65.0 $^{c,d}$ | 69.6 $^{c}$ | 72.5 $^{d}$ | 67 $^{e}$ | 5.3 $^{c}$ |
| | P | Aromasil S | 64.8 $^{c,d}$ | 67.4 $^{c,d,e}$ | 72.6 $^{d}$ | 70 $^{d}$ | 5.2 $^{d}$ |
| | | Aromasil SN | 64.9 $^{c,d}$ | 68.7 $^{c,d}$ | 72.5 $^{d}$ | 74 $^{d}$ | 5.2 $^{d}$ |
| L | C | Formic acid | 65.1 $^{c}$ | 78.6 $^{c,d}$ | 82.9 $^{d}$ | 60 $^{c}$ | 5.1 $^{c}$ |
| | E | Bactozyme | 63.2 $^{d}$ | 80.1 $^{c}$ | 85.2 $^{c}$ | 51 $^{f}$ | 4.9 $^{d,e}$ |
| | | Clampzyme + + Pioneer | 64.3 $^{d}$ | 78.3 $^{d}$ | 83.0 $^{d}$ | 55 $^{e}$ | 5.0 $^{c,d}$ |
| | Co | Formic acid + + Aromasil S | 62.0 $^{d}$ | 77.9 $^{d}$ | 82.9 $^{d}$ | 57 $^{d}$ | 4.8 $^{e}$ |

[a] Preparation producer: see Table 2
[b] RC - red clover; L - lucerne; C - chemical agents; E - enzymatic preparations; P - phytogenic preparations; Co - combination of the preparations; OM dig - organic matter apparent digestibility; CP dig - crude protein apparent digestibility; CP deg - crude protein effective degradability; PDI - protein digestible in the intestine; NEL - net energy of lactation; DM - dry matter
[c,d,e,f] The means with unlike superscripts in columns are significantly different ($P < 0.01$) within RC and L,resp.

acid content of red clover conserved by Bactensil 2000 (150 g kg$^{-1}$ DM) or Aromasil S (146 g kg$^{-1}$ DM) was higher ($P < 0.05$) in comparison with the sample conserved by formic acid (129 g kg$^{-1}$ DM). From this viewpoint (lactic acid content) we found differences between Bactozyme and Bactensil 2000, or between Aromasil S and Aromasil SN (Table 1).

The mean (calculated from all measurements within the given group of ensiling preparations) of CP degradability of the clover samples conserved by either enzymatic or phytogenic preparations was higher ($P < 0.01$) than that of chemically conserved clover samples. However, the enzymatic preparations did not differ ($P > 0.05$) from formic acid as far as the CP degradability of lucerne was concerned. The mean of the PDI values of the clover samples conserved by the enzymatic preparations was lower than that of the PDI values of the chemically conserved clover samples ($P < 0.05$). However, the influence of the phytogenic preparations on the PDI value of red clover was comparable with the influence of direct acidifiers ($P > 0.05$). Similarly, PDI of lucerne conserved by means of enzymes with microbial inoculants was on average by 7 g kg$^{-1}$ DM lower ($P < 0.01$) than in lucerne samples conserved by formic acid. Enzymatic and phytogenic preparations (tested as the whole sets) did not differ mutually ($P > 0.05$) in the effect on the nutritive value of red clover (CP effective degradability, PDI, NEL).

The comparison of the individual ensiling preparations from the viewpoint of the nutritive traits is presented in Table 4. Within the set of the enzymatic preparations,

clover samples preserved with Bactozyme had higher PDI value than those conserved with Bactensil 2000 ($P < 0.01$). However, opposite results were found in this study as far as the lucerne was concerned: samples with the mixture of Pioneer and Clampzyme (similar composition to Bactensil) had higher ($P < 0.01$) PDI than those with Bactozyme. Both tested phytogenic preparations had a similar ($P > 0.05$) effect on the nutritive characteristics in red clover.

We found highly significant ($P < 0.01$) differences in the UDP intestinal digestibility between red clover and lucerne in the samples conserved either by formic acid (68.9 and 70.3, respectively) or by Bactozyme (68.9 and 70.0, respectively). On the other hand, the differences between both preparations in the UDP intestinal digestibility either in lucerne or in red clover were insignificant ($P > 0.05$). Therefore the PDI of all lucerne and red clover samples, resp., was calculated using the mean value of the UDP intestinal digestibility: 70.2% and 68.9%, respectively.

## 4. Discussion

Higher ADF content of lucerne silages in comparison with red clover (with practically the same NDF content, Table 1) did not correspond with higher CP digestibility or CP degradability of the lucerne silages (Table 4). On the other hand, results of this study confirmed the data concerning the significance of ADIN for the prediction of CP digestibility (VanSoest and Sniffen, 1984). The coefficient of correlation between ADIN and CP digestibility in experiments in the current study was $r = -0.88$ ($P < 0.01$), and between ADIN and escape protein $r = 0.91$ ($P < 0.01$). A strong relationship was also found between ADIN (Table 1) and soluble nitrogen fraction (Table 3) with $r = -0.88$, $P < 0.01$. However, even the highest value of ADIN (red clover conserved with formic acid, 8% of CP) was below the limit value indicating the heat damage of the silage (Yu and Thomas, 1975).

As far as the silage quality parameters are concerned (lactic acid, VFA, Table 1), biological preparation Bactozyme did not differ from the mean of all silage samples within a given plant species. The main reason was probably low content of soluble sugars of both lucerne and red clover (see Section 2); the quality of the biological innoculants could not fully manifest. The favourable values of the silage quality parameters in the clover sample preserved with Aromasil S were possibly in relationship with the supposed ability of phytogenic preparations to restrain the growth of enterobacteria and clostridia in silages.

Aufrère et al. (1994b) found a CP degradability (determined by the in situ method on sheep) of lucerne (cut at pre-flowering stage of the first cut) conserved by formic acid 89.3%, which is nearly 6% higher than results in this study (82.9%, Table 4). The differences are most likely due to the different vegetative stage (beginning of the flower stage in the present experiment), and (or) the different cut, or due to the different species of the experimental animals. On the other hand, Komprda et al. (1993) reported nearly the same value as in the present experiment (83.1%) of CP degradability of lucerne from the bud stage of the first cut conserved by formic acid.

According to Selmerolsen (1994), the total digestibility in sacco and in vitro of the herbage from grasses and red clover was not influenced by the addition of cellulases and hemicellulases compared with the untreated silage. Fredeen and McQueen (1993) reported an increase in the apparent DM digestibility of the lucerne/grass silages preserved by enzymes (cellulases, amylases, glucose oxidases, including inoculants of lactobacillus) compared with the sample ensiled without additives. The present study did not compare the effect of the enzyme additives on the nutritive characteristics of the forage plants with the untreated silage as did the above mentioned authors. However, in the present experiment the OM apparent digestibility of red clover treated with the preparations containing enzymes (Bactozyme, Bactensil 2000) did not differ ($P > 0.05$) from that of red clover treated with the chemical agents. On the other hand, the enzyme treatment increased ($P < 0.01$) apparent CP digestibility of the clover samples compared with direct acidifiers.

Hristov (1993) compared the in situ CP degradability of lucerne treated with the enzyme preparation based on cellulases, hemicellulases and glucose oxidases (Clampzyme) and with formic acid. The author observed no protective effect of the formic acid on the silage protein degradability. Results concerning lucerne are similar (Table 4): CP effective degradability of lucerne treated with formic acid or with the mixture of preparations Clampzyme + Pioneer did not differ ($P > 0.05$). However, as far as red clover is concerned, results of this study showed the protective effect of formic acid on the CP effective degradability (67.9%) compared with the enzyme preparations (72.5%, $P < 0.05$, Table 4).

## 5. Conclusions

The in situ method is sufficiently sensitive to be able to discriminate (by a reasonable number of repetitions) the differences in the effect of the various ensiling preparations on the nutritive value (effective degradability) of forages (lucerne, red clover). On the other hand, the differences in the UDP intestinal digestibility of lucerne or red clover ensiled with chemical and enzymatic agents were not proven.

The mean of the PDI values of clover samples ensiled with phytogenic preparations did not differ ($P > 0.05$) from that of PDI values of the samples conserved with the direct acidifiers. This is, in the opinion of the authors, a positive finding from the environmental viewpoint.

From the viewpoint of PDI and NEL content formic acid is recommended as the best from tested preparations for the conservation of lucerne and red clover.

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# Utilization of different fats and oils by adult chickens as a source of energy, lipid and fatty acids

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**Abstract**

Sixty adult Warren roosters approximately 1 year old, randomized in individual cages, were fed a basal diet with or without 4% added fat. Nine fats and oils were evaluated: tallow (T1 and T2); tallow + soybean acid oil (50:50, TSAO); palm oil (PO); palm oil + soybean acid oil (50:50, PSAO); soybean acid oil (SAO); lard (L); soybean oil (SO); and linseed oil (LO). Soybean lecithin was included at 50 g $kg^{-1}$ in T2. Experimental diets were evaluated for apparent fat availability (AFA), apparent availability of individual fatty acid (AAFA) and apparent metabolizable energy (AME). The AFA and AAFA values for added fats and oils were derived from those obtained for the basal diet and those obtained for the fat-supplemented diets, by difference, assuming the utilization of fat and fatty acid in the basal diet to be constant. The AME values of added fats and oils were calculated as the product of their AFA values and their gross energy values. In addition, the AME of added fats was calculated by substitution from the AME values of the basal diet and the fat-supplemented diets. The AFA and AME of added fats and oils ranged from 873 to 1013 g $kg^{-1}$ and 33.5 to 40.2 MJ $kg^{-1}$, respectively. The addition of soybean lecithin to tallow did not affect the nutritive value of this animal fat. The nutritive value of added fats was more influenced by their free fatty acid content (FFA) and non-nutritive fraction than by their degree of saturation. SAO, with high free fatty acids and unsaponifiable contents, resulted in the lowest values for AME and AFA, but both measurements increased significantly when it was blended with T1 or PO. The AME values of experimental diets supplemented with different fats and oils were equivalent.

*Keywords:* Chicken, cockerel; Fats; Fatty acids; Energy metabolism

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