EFFECT OF ORGANIC ACIDS ON NUMBERS OF YEASTS AND MOULD FUNGI: AEROBIC STABILITY IN THE SILAGE FROM ITALIAN RYEGRASS

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SUMMARY

The aim of these studies was to ascertain the influence of conservants (Amasil®99, Luprosil, Amasil®Combi) based on formic and propionic acids of the cell counts of yeasts and mould fungi in silage. The silage was prepared from Italian ryegrass (*Lolium multiflorum L.*). The effect of the applied conservants on silage oxygen stability was ascertained. The performed chemical analyses comprised the determination of: the content of dry matter, butyric and acetic acids, ethanol, water soluble sugars, crude protein and pH. The applied preparations were found to reduce numbers of yeast and mould fungi cells in all the examined silages. The growth of fungi was strongly inhibited by the Amasil®Combi preparation (containing a mixture of formic and propionic acids and ammonium ions). The yeast cell counts dropped ($P<0.05$) from $17.8x10^6$ CFU/g FM in the control to $8.4x10^6$ CFU/g FM in the combination with Amasil®Combi, whereas counts of the mould fungi cells — changed from $11.3x10^3$ CFU/g FM in the control to $4.6x10^3$ CFU/g FM in the combination with Amasil®Combi. The applied conservants increased ($P<0.05$) the content of dry matter in the silage in anaerobic phase. DM was detected in the highest concentration in combination with Amasil®Combi — 241.6 g/kg DM, the lowest in control — 236.1 g/kg DM. The conservants decreased ($P<0.05$) concentration of lactic acid in silage in anaerobic phase. The highest concentration of lactic acid was detected in control — 45.1 g/kg DM, lowest in combination with Amasil®99 — 43.2 g/kg DM. The diversifying factors decreased ($P<0.05$) acetic acid concentration in silage in anaerobic phase. The highest concentration was detected in control — 21.6 g/kg DM, a lowest in combination with Amasil®Combi — 17.5 g/kg DM. All conservants decreased ($P<0.05$) ethanol content in silage in anaerobic phase. The highest content was detected in control — 1.6 g/kg DM, the lowest with Amasil®Combi — 0.8 g/kg DM. The chemical additives increased ($P<0.05$) WSC content in silage in anaerobic phase. The highest content of WSC was in combination with Amasil®Combi — 35.1 g/kg DM, the lowest in control — 31.1 g/kg DM. The conservants decreased least of all ($P<0.05$) the crude protein content. The highest contents were detected in control — 203.1 g/kg DM, the lowest with Amasil 99 — 201.3 g/kg DM supplementation. The additives decreased the value of pH in the examined silages and improve the aerobic stability of silages in aerobic phase.

ÖSSZEFOGLALÁS

Selwet, M.: SZERVESSAV-KIEGÉSZÍTÉS HATÁSA OLASZPERJE SZILÁZSOK ÉLESZTŐ- ÉS PENÉSZGOMBA-TARTALMÁRA, VALAMINT A SZILÁZS STABILITÁSÁRA

A kísérlet célja a hangya- és propionsav (A masil®99, Luprosil, Amasil®Combi) alapú konzerváló szerek hatásának vizsgálata az élesztősejtekre és penészgombákra, valamint a szilázs oxigén stabilitásának alakulására. A szilázsok olaszperjéből (*Lolium multiflorum L.*) készültek, és szárazanyag, ecet- és butanolsav, etanol, vízben oldható cukor (WSC), nyersfehérje-tartalmuk és pH értékük került meghatározásra. Az alkalmazott kezelések mindegyike csökkentette az élesztő- és penészgombák számát. Az Amasil®Combi (hangya- és propionsav, valamint ammóniumion keverék) csökkentette legerőteljesebben a penészképződést. Az élesztősejtek száma a kontrollban levő $17,8x10^6$ CFU/g FM-ről (FM: eredeti anyag) $8,4x10^6$ CFU/g FM-re csökkent az Amasil®Combi kezelés hatására, a penészgombáké pedig $11,3x10^3$ CFU-ról $4,6x10^3$ CFU/g FM-re. A konzerválókeverékek a szárazanyag mennyiségét szignifikáns mértékben ($P<0,05$) növelték. A legnagyobb szárazanyag-tartalmat az Amasil®Combi kezelés adta, 241,6 g/kg sz.a. és 236,1 g/kg sz.a. között. Az anaerob fázisban a tejsav mennyisége csökkent ($P<0,05$) a kezelés hatására, a legtöbb tejsavat a kontroll szilázsban mérték, 45,1 g/kg a szárazanyagban, a legkevesebbet az

Amasil kombinációban, 43,2 g/kg sz.a. Az ecetsav-koncentráció ugyancsak csökkent (P<0,05). A kontroll szilázs tartalmazta a legtöbb ecetsavat, 21,6 g/sz.a. kg-ot, a legkevesebbet, 17,5 g/kg sz.a.-ot az Amasil®Combi kezelésű szilázs. Az összes kezelt szilázsban csökkent az etanol, a legtöbbet a kontroll tartalmazta, 1,6 g/kg sz.a., a legkevesebbet az Amasil®Combi, 0,8 g/kg a szárazanyagban. A kémiai adalékanyagok növelték (P<0,05) a vízben oldható cukor mennyiségét az aneorob fázisban, a legtöbb az Amasil®Combi kezelésű, 35,1 g/kg a szárazanyagban, a legkevesebbet 31,1 g/kg sz.a. a kontroll szilázs tartalmazta. A legkevésbé a nyersfehérje-tartalmat csökkentették (P<0,05) a konzerválószerek, ami a kontroll anyagban 203,1 g/kg sz.a. volt, az Amasil®99-es kezelésben pedig 201,3 g/kg sz.a. mutattak ki. Az adalékok csökkentették a pH-értéket és növelték a szilázsok aerob stabilitását.

INTRODUCTION

Yeasts constitute an important group of fungi occurring in silages. In aerobic conditions they are capable of lactic acid decomposition (natural conservants), whereas in anaerobic conditions, they produce ethanol. Moreover, they can serve as the main factor creating appropriate conditions for the development of mould fungi as well as other unwelcome microflora, which can deteriorate the hygienic condition of silages and lead to organic matter loss (*Selwet,* 2005).

Another important group is mould fungi, which are the main culprits responsible for the loss in foods and feeds. As pathogens, they reduce the hygiene value of silages (*Puschner,* 2002). These fungi are characterised by considerable adaptation potentials in the environment and can cause allergies in animals and produce mycotoxins (*Creppy,* 2002; *Rundberget,* 2004).

In order to eliminate harmful microflora and improve oxygen stability in silages during the ensiling process of plant material, chemical, biological and enzymatic conservants are recommended to be applied (*Kung et al.,* 2003; *Selwet,* 2006; *Avasi et al.,* 2006).

The objective of this research project was to ascertain the impact of preparations containing organic acids on yeast and mould fungi cell counts as well as on the improvement of the oxygen stability of silages manufactured from Italian ryegrass.

MATERIAL AND METHODS

Plant material: Silages were prepared from Italian ryegrass (*Lolium multiflorum L.*). Grass was harvested during the beginning flowering phase from the second cut. The plant material was wilted to the level of 30% DM and ensiled in 4 dm^3 volume laboratory micro-silos. Samples for analyses were collected on the day of silo opening (0 days), whereas the oxygen stability was determined after 7 days of sample exposure to the air reaction (7 days).

Experimental design: One silage x 4 diversifying factors (A, B, C, D*) x 6 replications=24 samples.

A: control without additives, B: Amasil®99, C: Luprosil, D: Amasil®Combi. Doses of all conservants: 4 cm^3/kg.

Characterisation of the applied conservants: Amasil®99 (BASF): active substance — formic acid, molar mass — HCOOH: 46.02 g/mol, concentration of active substance — min. 990 g/kg. Luprosil (BASF): active substance — propionic acid, molar mass — CH_3CH_2COOH: 74.1 g/mol, concentration of active substance — min. 995 g/kg. Amasil®Combi (BASF): active substance — propionic and formic acids, and ammonium ions; concentration of active substance — min. 380 g propionic acid, 340 g formic acid, 80 g ammonium ions.

Microbiological and chemical analyses: Microbiological analyses in the presence of mould fungi were conduced on the agar substrate with Bengali rouge (*Martin,* 1950), yeast cell counts — on the agar with broth (BTL Ltd. Branch of Enzymes and Peptones in Łodź). The concentration of lactic and acetic acids were determined on the Hewlett-Packard HP 1050 liquid chromatographer with a UV detector using the Supelcogel C-610H column (Supelco) and a glass column 2 m long and 0.6 cm diameter with a filler supplied by the Supelco Company type GP 10% SP-120/1% H_3PO_4 on 80/100 Chromosorb WAW. Water soluble carbohydrates (WSC) were determined according to the methodology given by *Mc Donald and Henderson* (1964). Dry matter and crude protein contents were determined by the method of *Gawęcki* (1994), ethanol — according to the method given by *Szebiotko et al.* (1973) and pH — using the ATC pH meter of Hann Instruments.

Statistical analysis: Data concerning numbers of mould fungi, yeast cells and chemical parameters in individual silages were subjected to statistical verification. The applied calculations employed the GLM procedure from the SAS package (1999). The significance of differences was verified using the Duncan's and t-Student test (*Table 1–8*).

Table 1.

Number of yeast cells (10^6 CFU/g FM) and mould fungi (10^3 CFU/g FM) in the silage from Italian ryegrass

Treatment(1)	Yeasts(2)		Muld fungi(3)	
	0 day(4)	7 day(4)	0 day(4)	7 day(4)
A	17.8^{aA}	37.1^{aB}	11.3^{aA}	28.1^{aB}
B	12.2^{bA}	14.5^{bA}	6.4^{bA}	8.0^{bA}
C	10.6^{cA}	12.7^{cA}	7.4^{bA}	10.8^{bB}
D	8.4^{cA}	9.8^{dA}	4.6^{cA}	5.6^{cA}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(5)

1. táblázat: Az élesztősejtek (10^6 CFU/g eredeti anyag) és a penészgombák száma (10^3 CFU/g eredeti anyag) olaszperjéből készült szilázsban

kezelés(1), élesztő(2), penészgomba(3), nap(4), ^{abcd}: azonos betűjelzés esetén, az oszlopon belül; ^{AB}: azonos betűjelzés esetén a sorok között nincs szignifikáns eltérés P<0,05 szinten(5)

RESULTS

Cell counts of yeasts and mould fungi in the silages from Italian ryegrass (*Lolium multiflorum L.*). The applied preparations were found to reduce the cell

counts (P<0.05) of yeasts and mould fungi in comparison with the control. The employed Amasil®Combi preparation (D) acted as the strongest growth inhibitor for fungi with Luprosil (C) and Amasil®99 (B) preparations exhibiting weaker activities. The applied diversifying factors verified the improvement of silage oxygen stability.

Table 2.

Dry matter content (g/kg DM) in the silage

Treatment(1)	Value(2)	
	0 day(3)	7 day(3)
A	236.1^{aA}	188.2^{aB}
B	238.2^{aA}	220.1^{bB}
C	240.1^{bA}	218.2^{bB}
D	241.6^{bA}	235.4^{cA}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(4)

2. *táblázat: A szilázs szárazanyag-tartalma (g/kg sz.a.)*
kezelés(1), érték(2), nap(3), ^{abcd}: azonos betűjelzés esetén az oszlopon belül; ^{AB}: azonos betűjelzés esetén a sorok között nincs szignifikáns eltérés P<0,05 szinten(4)

Table 3.

Crude protein content (g/kg DM) in the silage

Treatment(1)	Value(2)	
	0 day(3)	7 day(3)
A	203.1^{aA}	180.2^{aB}
B	201.3^{aA}	198.5^{bA}
C	201.8^{aA}	194.2^{aA}
D	202.4^{aA}	199.9^{bA}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(4)

3. *táblázat: A szilázs nyersfehérje-tartalma (g/kg sz.a.)*
lásd 2. táblázat(1–4)

Table 4.

WSC content (g/kg DM) in the silage

Treatment(1)	Value(2)	
	0 day(3)	7 day(3)
A	31.1^{aA}	27.6^{aB}
B	33.2^{aA}	31.2^{bA}
C	34.1^{aA}	32.0^{bA}
D	35.1^{bA}	33.9^{bA}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(4)

4. *táblázat: A szilázs WSC (vízoldható szénhidrát)-tartalma (g/kg sz.a.)*
lásd 2. táblázat(1–4)

Fungal cell counts increased subsequent to the opening of the experimental silos in individual combinations. In case of the control, the yeasts counts (P<0.05) increased by 43% and mould fungi by 60% ; in case of Amasil®99, the yeasts counts (P<0.05) increased by 16%, and mould fungi by 20% ; Luprosil

the yeasts counts increased (P<0.05) by 17%, and mould fungi by 31%; Amasil®Combi the yeasts counts increased (P<0.05) by 14%, and mould fungi by 17% (*Table 1*).

Table 5.

Ethanol content (g/kg DM) in the silage

Treatment(1)	Value(2)	
	0 day(3)	7 day(3)
A	1.6^{aA}	1.0^{aB}
B	0.9^{bA}	0.7^{bA}
C	0.9^{bA}	0.9^{aA}
D	0.8^{bA}	0.5^{cB}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(4)

5. táblázat: A szilázs etanoltartalma (g/kg sz.a.)
lásd 2. táblázat(1–4)

Table 6.

Lactic acid concentration (g/kg DM) in the silage

Treatment(1)	Value(2)	
	0 day(3)	7 day(3)
A	45.1^{aA}	39.2^{aB}
B	43.2^{aA}	41.2^{aA}
C	44.0^{aA}	41.6^{aA}
D	44.2^{aA}	43.2^{bA}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(4)

6. táblázat: Tejsav-koncentráció a szilázsban (g/kg sz.a.)
lásd 2. táblázat(1–4)

Table 7.

Acetic acid concentration (g/kg DM) in the silage

Treatment(1)	Value(2)	
	0 day(3)	7 day(3)
A	21.6^{aA}	28.3^{aB}
B	18.6^{bA}	21.2^{bA}
C	19.0^{bA}	22.4^{bB}
D	17.5^{bA}	19.0^{cA}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(4)

7. táblázat: Ecetsav-koncentráció a szilázsban (g/kg sz.a.)
lásd 2. táblázat(1–4)

Chemical analysis: The applied conservants improved aerobic stability of silages in aerobic phase. The DM content decreased by 20% (P<0.05) in control, with Amasil99 by 8%, Luprosil by 9% and Amasil®Combi by 3% (*Fig. 1*). The crude protein concentration decreased by 11% (P<0.05) in control, with Amasil 99 by 1%, Luprosil by 4% and Amasil®Combi by 1% (*Fig. 2*).

Table 8.

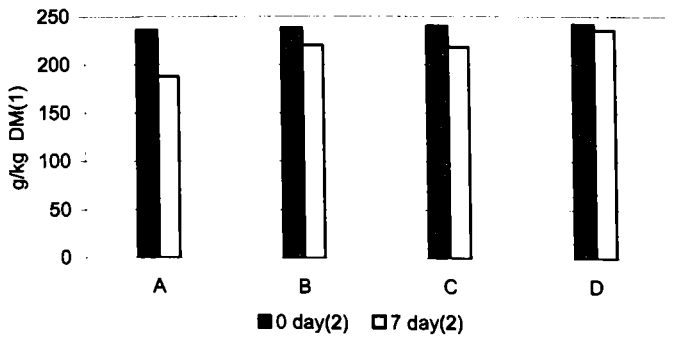
Value pH in the silage

Treatment(1)	Value(2)	
	0 day(3)	7 day(3)
A	4.8^{aA}	5.1^{aA}
B	4.2^{bA}	4.6^{bA}
C	4.3^{bA}	4.6^{bA}
D	4.1^{bA}	4.5^{bA}

^{abcd}: means in columns; ^{AB}: means in lines designated with the same letters do not differ significantly at the level of P<0.05(4)

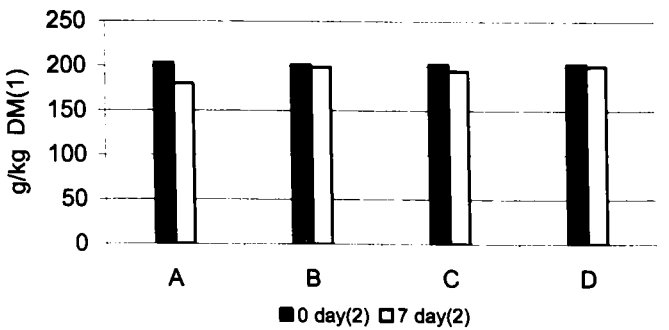
8. táblázat: A szilázs pH-ja
lásd 2. táblázat(1–4)

Fig. 1.: **Dry matter content in the silage**



1. ábra: A szilázs szárazanyag-tartalma
g/kg sz.a.(1), nap(2)

Fig. 2.: **Protein content in the silage**

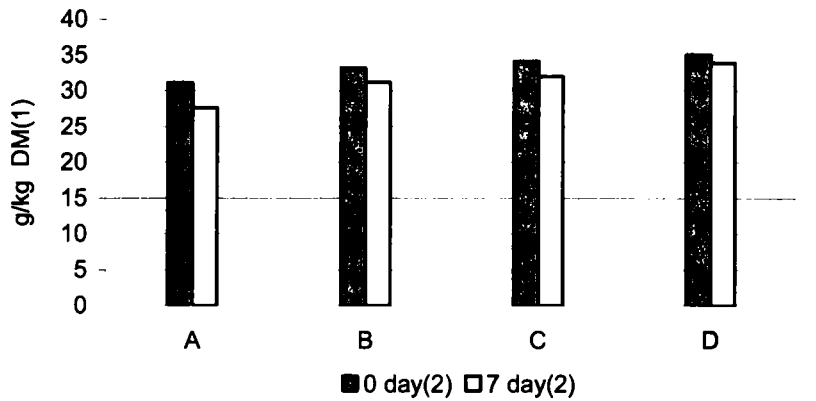


2. ábra: A szilázs nyersfehérje-tartalma
lásd 1. ábra(1–2)

The WSC content decreased in control by 11% in control, with Amasil99 by 6%, Luprosil by 6% and Amasil®Combi by 3% (*Fig. 3*). The ethanol concentration decreased (P<0.05) in control by 36% in control, with Amasil99 by 22%, Luprosil by 22% and Amasil®Combi by 31% (*Fig. 4*). The lactic acid concentration decreased (P<0.05) by 13% in control, with Amasil99 by 5%, Luprosil by

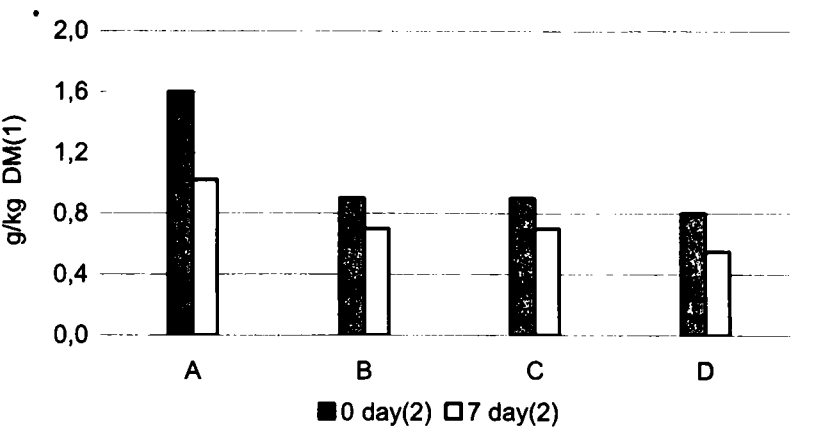
6% and Amasil®Combi by 2% (*Fig. 5*). The acetic acid concentration increased (P<0.05) by 24% in control, with Amasil®99 by 12%, Luprosil by 15% and Amasil®Combi by 8% (*Fig. 6*). The determined pH values decreased in all samples with conservant preparations. The highest pH values were observed in the control sample (*Fig. 7*).

Fig. 3.: **WSC content in the silage**



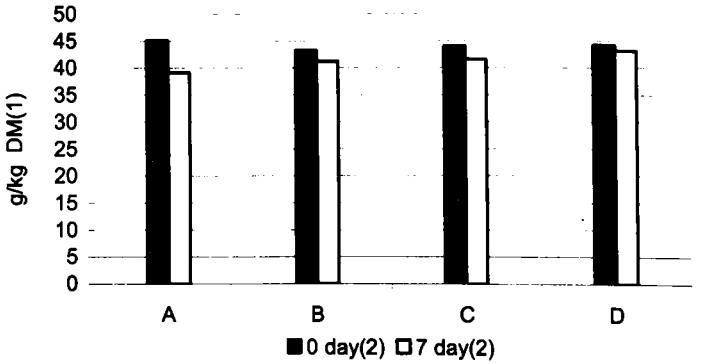
3. ábra: A szilázs vízben oldható szénhidráttartalma
lásd 1. ábra(1–2)

Fig. 4.: **Ethanol content in the silage**



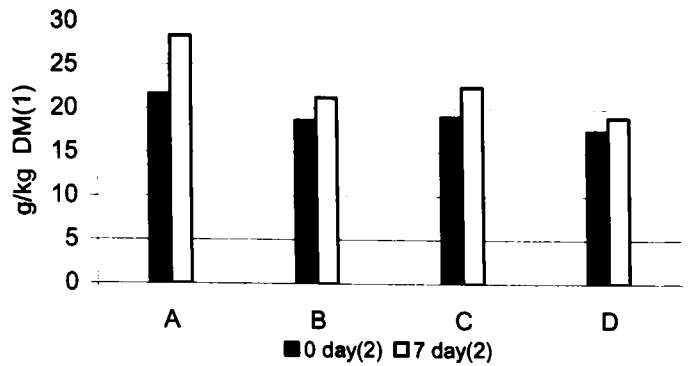
4. ábra: A szilázs etanoltartalma
lásd 1. ábra(1–2)

Fig. 5.: **Lactic acid concentration in the silage**



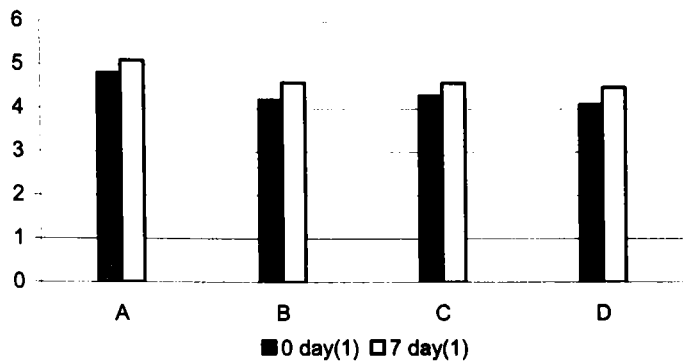
5. ábra: Tejsav-koncentráció a szilázsban
lásd 1. ábra(1–2)

Fig. 6.: **Acetic acid concentration in the silage**



6. ábra: Ecetsav-koncentráció a szilázsban
lásd 1. ábra(1–2)

Fig. 7.: **Value pH in the silage**



7. ábra: A szilázs pH-ja
nap(1)

DISCUSSION

The applied conservants reduced the cell counts of yeasts and mould fungi in the examined silages. Investigations show different effect of organic acids on the development of fungi in silages. *Nishino et al.* (2004) and *Kleinschmit et al.* (2005) failed to show any influence of chemical conservants leading to the reduction of fungi in silages. The application of formic and propionic acids may decrease cell counts of yeasts and mould fungi (*Kung et al.,* 2004; *Selwet,* 2005; *Selwet,* 2006) and, in addition, it improves the oxygen stability of silages (*Kung et al.,* 2004; *Selwet,* 2006).

In anaerobic phase in silages treated with Amasil®99, Luprosil and Amasil®Combi preparations, a higher dry matter content was determined (*Driehuis,* 2000; *Kung et al.,* 2000), and concentration of lactic acid was lower than in the control. *Steidlova and Kalai* (2002) and *Selwet* (2006) demonstrated that conservants increase the concentration of lactic acid in silages. The inclusion of conservants in anaerobic phase reduced the concentration of acetic acid. Similar results were obtained by *Haigh* (1998) and *Kleinschmit et al.* (2005), although *Kung et al.* (2004) in their experiments do not confirm this fact. The application of preparations based on organic acids reduces the content of ethanol in the silage (*Kung et al.,* 2004; *Selwet,* 2006) and the content reduction of this alcohol is very advantageous as it improves the stability of silages. Yeast cell counts usually increase usually in aerobic phase (gemmation cells) whereas ethanol is being oxidised to acetic acid. The high level of acetic acid results in reduced silage intake by animals. However, this fact is not corroborated by studies conducted by *Kleinschmit et al.* (2005). The supplementation of silages (in anaerobic phase) with formic and propionic acids increases concentrations of water soluble sugars in silage (*Kung et al.,* 2004; *Selwet,* 2005) and, in addition, reduces the content of crude protein, although this fact was not confirmed in experiments carried out by *Haigh* (1998) and *Kleinschmit et al.* (2005). In comparison with the control, the pH of experimental silages was reduced under the influence of diversifying factors. Similar results were reported by *Kung et al.* (2004), *Nadeau et al.* (2000) and *Selwet* (2006), although *Kleinschmit et al.* (2005) failed to observe such an influence.

CONCLUSIONS

In addition to this chemical conservants containing propionic and formic acid improved the aerobic stability and DM recovery of Italian ryegrass silage and a decrease of the concentration of acetic acid and ethanol in silage was observed. Amasil®Combi was the most effective in WSC recovery of silage in aerobic phase. All the conservants decreased lactic acid concentration in silage in anaerobic phase. In comparison with untreated silage, formic and propionic acids treatment significantly reduced silage pH in anaerobic phase.

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