EFFECT OF SILAGE MADE FROM DIFFERENT PLANT RAW MATERIALS WITH THE ADDITION OF A FERMENTATION INHIBITOR ON THE PRODUCTION RESULTS OF DAIRY COWS

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Summary. The study was conducted on three dairy farms, under production conditions. Round bale silage was produced from grass mixture (G, GI), grass and red clover mixture (GRC, GRCI), and grass and alfalfa mixture (GA, GAI), with no additives or with the addition of a fermentation inhibitor. The effect of the inhibitor on production results was evaluated on 326 Holstein-Friesian (HF) cows kept in three free-stall barns. On each farm the cows were divided into two feeding groups by the analogue method. Apart from experimental silage, all cows received a constant amount of maize silage and different amounts of concentrate. Dry matter intake, milk yield and composition, and feed conversion were analyzed in all treatments. In addition, the effect of feeding experimental silage was determined as dependent on the level of concentrate supplementation (>9, 6.1 – 9, 3.1 – 6, 0 - 3, 0 kg). The fermentation inhibitor added to high-protein components during ensiling had a positive effect on the production results of dairy cows. The effectiveness of the fermentation inhibitor was affected by the type of ensiled raw material and by the level of concentrate supplementation. The additive was found to be most effective when added to silage made from grass mixture or grass and alfalfa mixture, offered to cows fed no concentrate or a diet supplemented with less than 3 kg/head/day of concentrated feed. The inhibitor added to silage produced from grass and red clover improved the production results of cows to the lowest degree. The additive inhibiting silage fermentation enabled to increase nitrogen utilization in cows, regardless of the botanical composition of ensiled raw material.

Keywords: fermentation inhibitor, silage, dairy cows.

SILOSO IŠ SKIRTINGOS AUGALINĖS ŽALIAVOS SU FERMENTACIJOS INHIBITORIUMI POVEIKIS MELŽIAMŲ KARVIŲ PRODUKTYVUMUI

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Santrauka. Tyrimas atliktas trijuose pienininkystės ūkiuose gamybinėmis sąlygomis. Silosas ritiniuose buvo ruošiamas iš žolių mišinio, žolių ir raudonųjų dobilų mišinio bei žolių ir liucernos mišinio, be priedų, su fermentacijos inhibitoriumi. Inhibitorių poveikis gamybos rezultatams įvertintas tiriant 232 Holšteino fryzų veislės karves palaido laikymo tvartuose. Analogų principų kiekviename ūkyje karvės buvo suskirstytos į dvi grupes. Su racionu be siloso visos karvės gavo skirtingą kiekį koncentruotųjų pašarų. Buvo tiriama, kiek karvės sunaudoja sausųjų medžiagų, analizuojamas primilžis, pieno sudėtis ir pašarų pasisavinimas. Bandymo metu taip pat įvertintas šėrimo eksperimentiniu silosu poveikis priklausomai nuo gautų koncentruotųjų pašarų kiekio (> 9; 6,1–9; 3,1–6; 0–3,0 kg). Fermentacijos inhibitoriai, įmaišyti į daug baltymų turinčią žaliąją masę silosavimo metu, teigiamai veikė melžiamų karvių produktyvumą. Fermentacijos inhibitorių efektyvumui įtakos turėjo silosuojamos žaliosios masės rūšis ir koncentruotųjų pašarų kiekis. Didžiausią poveikį darė priedai, įdėti į žolių mišinį bei žolių ir liucernos mišinio silosą, duoti karvėms su racionais be koncentruotųjų pašarų arba vienam galvijui sušėrus mažiau kaip 3 kg koncentruotųjų pašarų per parą. Kai inhibitoriai buvo įmaišyti į žolių ir raudonųjų dobilų silosą, tyrimais nustatyta, kad karvių produktyvumo rodikliai pagerėjo mažiau. Siloso fermentaciją stabdantys priedai, nepriklausomai nuo silosuotos žaliavos botaninės sudėties pagerino tiriamųjų grupių karvių azoto pasisavinimą.

Raktažodžiai: fermentacijos inhibitorius, silosas, karvės.

Introduction. In the moderate climate zone, the feed ration for dairy cows is usually composed of maize silage supplemented with silage made from grasses, legumes or grass-legume mixtures as an additional source of nitrogen compounds. The production efficiency of silage containing high-protein components is largely determined by the degree of protein protection against hydrolysis and by the degree of water-soluble carbohydrate (WSC) protection against excessive loss during fermentation. In ruminants. the conversion of protein contained in silage is often accompanied by a considerable loss of nitrogen compounds and high emissions of nitrogen into the natural environment (Jones, 2000; Driehuis and VanWikselaar, 2001; Frank et al., 2002; Slottner and Bertilsson, 2006; Nadeau et al., 2007; Guo et al., 2008). Microbial protein production increases proportionally to the supply of readily digestible energy, thus improving the synchronization of solubility of both nitrogen compounds and carbohydrates available to bacteria. The simplest way to increase the energy content of the diet for ruminants is to protect the WSC fraction in silage. The WSC content of silage may vary widely depending on the growth stage, season of the year and time of the day, but primarily on the ensiled crop (Winters et al., 2002). Another element that contributes to improving protein utilization in ruminants is preventing protein degradation during the production of grass and legume silage. It influences microbial protein synthesis and the level of rumen undegradable protein (Jones, 2000; Huhtanen et al., 2003; Givens and Ruelquin, 2004). Protein and WSC degradation in silage can be reduced by decreasing fermentation rate through the use of pre-wilted raw materials or through the addition of fermentation inhibitors (Winters et al., 2001). The most effective inhibitors are bactericidal compounds like formaldehyde or hexamine (Henderson, 1993). However, due to their negative impact on humans and animals, the above compounds are not widely applied in practice. Additives inhibiting the activity of epiphytic microflora via the acidification of the surrounding medium, such as low-molecular-weight organic acids, mineral acids and their salts, are used most often (Brzóska et al., 1999; Randby, 2000). They have a restrictive effect on the fermentation process, as manifested by low concentrations of lactic acid and acetic acid, the absence of butyric acid, a low content of ammonium nitrogen and biogenic amines as well as a high proportion of WSC, which positively affects silage palatability and intake (Henderson, 1993; Brzóska, 1995). Moreover, the addition of inhibitors modifies the composition of silage microflora, thus improving the hygienic quality and storage stability of silage (Henderson, 1993). Formic acid is widely used as an additive inhibiting silage fermentation due to low production costs, high acidification capacity and a broad-spectrum activity against bacterial pathogens and selected fungal pathogens (McDonald et al., 1991; Randby, 2000). The high tolerance of yeasts for formic acid, combined with considerable amounts of WSC, pose a risk of reducing the aerobic stability of silage (Henderson, 1993). That is why apart from formic acid fermentation inhibitors contain also propionic acid, sorbic acid, acetic acid and benzoic acid known for its anti-proteolytic activity (McDonald et al., 1991; Randby, 2000). A positive effect of fermentation inhibitors, sorbents, and the pre-wilting of grass prior to ensiling on the level and rate of protein degradation has been demonstrated by (Brzóska et al.,1999); Randby, 2000).

The effectiveness of fermentation inhibitors is dependent on such technological factors as the dose and method of application, the degree of pre-wilting and bulk density of raw materials (Davies et al., 1998; Han et al., 2006), and primarily on the ensiled crop. Crop species determines the content of water-soluble carbohydrates, the concentration and amino acid composition of protein, and hydrolyzability during the ensilage process. These factors affect the degree of bacterial protein synthesis and, in consequence, milk protein production (Hedqvist and Uden, 2006). Beever and Thorp (1996) have found that red clover silage and alfalfa silage fed to cattle are a more efficient source of nitrogen for bacterial protein synthesis than grass silage. Davies et al. (1998) have concluded, based on *in vitro* studies, that the efficiency of microbial nitrogen synthesis is 34% higher in red clover silage, compared with alfalfa silage. Dewhurst et al. (2003) observed higher intake of silage made from white clover, red clover or alfalfa, in comparison with grass silage, which resulted in higher milk production. Therefore, the objective of the present study was to determine the effect of silage produced from selected vegetable raw materials, with no additives or with the addition of a fermentation inhibitor, on milk yield and composition as well as on feed conversion in dairy cows.

Materials and Methods. The study was conducted on three dairy farms located in north-eastern Poland, under production conditions. Experimental silage was produced from grass mixture – G (meadow fescue - 35%, timothy grass - 30%, perennial ryegrass - 15%, italian ryegrass - 20%), grass and red clover mixture – GRC (red clover - 40%, timothy grass - 60%), and grass and alfalfa mixture – GA (alfalfa - 30%, timothy grass - 45%, perennial ryegrass - 25%), under identical soil and climatic conditions, and by the standardized technology.

Silage making. Two types of round bale silage were produced on each farm: with no additives (G, GRC, GA) or with the addition of a commercial fermentation inhibitor (GI, GRCI, GAI) containing: formic acid – 55%, ammonium formate – 24%, propionic acid – 5%, benzoic acid – 1% and ethyl benzoate – 1%. Experimental silage was made from plants in the second year of vegetation, following two-phase harvesting. Slurry (30 m^3/ha - half rate in the winter, half rate in the spring) and mineral fertilizers (160 kg N - half rate in the spring, one-third after the first cut, one-sixth after the second cut; 70 kg P – full rate in the spring; 160 kg K – full rate in the spring) were applied in each treatment. Grassland was mown at the budding stage of legumes and at the ear formation stage of grass (13 – 15 May), with a drum mower equipped with a mulching attachment. Green forage was wilted for 24 hours. The swath was turned over once during that period. The raw material was harvested with a SIPMA roll baler equipped with the MP–5 applicator (Junkkari). Only up to 1.5 ha of grassland was mown at a time, to closely monitor the wilting process. In order to ensure the same dry matter content of all silage types, they were produced in cycles, five bales per treatment. The chemical preservative was applied at a dose of 3.5 l per ton of ensiled material. In order to protect them from adverse weather conditions, round bales were tightly wrapped using a SIPMA bale wrapping machine. The time between the formation and wrapping of respective bales did not exceed 2 hours. The bales were wrapped with four layers of white stretch film, 30 micrometers in thickness. Silage bales were stored in vertical position, in a single layer. In each treatment silage was also made from medium-early maize hybrids harvested at the dough stage (5 -10 October) with a Claas Jaguar series self-propelled forage harvester equipped with a forage crusher (theoretical straw length - 8 mm). All silage piles were protected with a polyethylene film and a net.

Animals and feeding. The study involved a total of 326 Holstein-Friesian (HF) cows kept in three free-stall barns. All-year indoor feeding was based on preserved feed. Cows on each farm were divided into two feeding groups by the analogue method, taking into account lactation stage and productivity. Roughage was supplied from a feed cart with a horizontal working unit. Concentrated feed was dosed individually from automatic feed stations, using the animal identification system. Particular types of experimental silage were offered in turn, over 30-day proper periods preceded by 15-day adaptation periods. Apart from experimental silage, cows in all treatments were also given a constant amount of maize silage (15 kg/head/day) and the same type of concentrated feed. Maize silage was administered in the morning, together with experimental silage. In the afternoon cows received experimental silage only. The amount of experimental silage fed to cows was recorded daily, with the use of automatic balances on feed carts. Cows whose milk yield exceeded 16 kg received concentrate in the amount of 1 kg per 2 kg milk. Starting from day 100 of lactation, the daily concentrate ration determined at the beginning of the feeding trial remained unchanged. In earlier phases of lactation the quantity of concentrated feed varied subject to milk yield. Feed intake was estimated as a sum of the average daily intake of experimental silage and maize silage, and individual consumption of concentrate (Andersen et al., 2003). The results regarding experimental silage are presented as average dry matter (DM) intake and energy-corrected milk (ECM) yield. The effect of feeding experimental silage on ECM yield was also determined as dependent on the level of concentrate supplementation (>9, 6.1 – 9, 3.1 – 6, 0 - 3, 0 kg). The milk yield of cows was monitored twice daily in milking parlors, and converted to kg of standardized milk relative to the energy value of ECM, as follows: ECM (kg) = milk (kg) [38.3 fat (g/kg) + 24.2 protein (g/kg) + 783.2]/3140 (Huhtanen et al., 2003).

Sampling and chemical analysis. Samples of silage and mixed feed were collected three times during a feeding trial in each treatment. The proximate chemical composition of feed was determined by standard methods (AOAC 1990). Water-soluble carbohydrates (WSC) were determined by the Dreywood method (1946), as described by Rutkowska et al. (1981). The dry matter content of silage was corrected relative to volatile substances, according to the Haigh equation (1995). Fiber fractions (NDF, ADF and ADL) were determined as described by Goering and Van Soest (1970), with the use of ANKOM 220 (silage) or Foss Tecator Fibertec 2010 (concentrated feed). The pH of all silage types was measured with a HI 8314 pH-meter. The separation of nitrogen fractions in silage was carried out according to the procedure proposed by Brzóska et al. (1999). The content of protein nitrogen and ammonium nitrogen was estimated by the Bernstein method and by the Conway method respectively (Skulmowski, 1974). The nutritive value of feed was determined according to INRA 1988 (2001), based on chemical composition, usin,g WinWar software (Table 1). The content of fat, protein and urea, and somatic cell count (SCC) were determined in bulk milk samples, three times during feeding each type of silage (Milkoscan 605, Foss Electric, Denmark). Feed conversion was estimated based on the quantity of ECM produced per kg DM of the ration, concentrate consumption, UFL and PDI per kg ECM. Nitrogen utilization (%) was measured as a ratio between the average daily milk nitrogen efficiency and the average nitrogen intake (milk N was calculated dividing daily protein efficiency by 6.38, while N intake was calculated dividing daily protein intake by 6.25).

The results of tests on animals were verified by a one-factor analysis of variance, to determine the effect of the fermentation inhibitor in plant raw materials on ECM yield. Statistical calculations were performed using Statistica7 software.

Results and Discussion. Regardless of the type of forage and the proportion of concentrate in the diet, the intake of silage with an additive inhibiting fermentation was slightly higher than the intake of control silage (Table 2). This affected higher intake of net energy (UFL) and protein digested in the intestine (PDI) (non-significant differences). The fermentation inhibitor had a positive effect on the average herd productivity expressed as kg of milk and ECM. Such a trend was also observed with respect to protein and fat yield. The greatest differences were noted in cows fed diets containing grass and alfalfa silage (GA, GAI). The increase in ECM yield ($p < 0.05$), milk fat yield ($p<0.05$) and milk protein yield ($p<0.01$) reached 2.05, 0.07 and 0.08 kg/day respectively. The fermentation inhibitor added to silage had no influence on the mean content of fat and protein, and SCC in milk. Milk urea content decreased ($p<0.01$) in cows fed grass and alfalfa silage with an additive inhibiting fermentation (GAI), and increased ($p<0.05$) in cows given grass silage with a fermentation inhibitor (GI). No significant differences were found in the amount of ECM produced per kg DM intake and in concentrate consumption to produce 1 kg ECM. There was also a tendency towards reduced net energy intake (UFL) for the production of 1 kg ECM. Cows fed GAI were characterized by lower average PDI intake required to produce 1 kg ECM ($p<0.05$) and by higher nitrogen utilization efficiency ($p<0.01$). A statistically non-significant improvement in nitrogen utilization was also noted in feeding trials involving cows fed GRCI and GI silage.

An analysis of cow productivity in particular treatments, at various levels of concentrate supplementation of the ration, is presented in Tables 3 - 5. The greatest differences ($p< 0.05$) in ECM yield were reported in cows fed G vs. GI silage and GA vs. GAI silage, with no concentrate supplementation (Tables 3, 5). The highest increase in protein content and in protein yield (GI; GAI; $p<0.05$) was also noted in those groups. In cows receiving GRCI silage, without concentrate supplementation or with <3 kg of concentrated feed in the diet, neither the yield nor the concentration of milk components increased (Table 4). A beneficial influence of GRCI silage was recorded in cows receiving >3 kg of concentrated feed. Irrespective of the proportion of concentrate in the diet, milk urea content was lower (non-significant differences) in cows fed grass and legume silage containing a fermentation inhibitor (GAI, GRCI) than in cows given control silage (Tables 4, 5). This trend was confirmed by a statistical analysis ($p<0.01$) only in cows fed GAI silage with the highest concentrate portion (Table 5). The opposite trend, statistically significant for the levels of concentrate supplementation of 3 - 6 kg ($p<0.05$) and 6 - 9 kg ($p<0.01$), was reported in cows fed diets containing GI silage (Table 3).

Table 1. **Chemical composition and nutritive value of experimental silage, maize silage and concentrate**

Item	Silage type												Concentrate
	G	GI	SE	MS	GRC	GRCI	SE	MS	GA	GAI	SE	MS	
DM, g/kg	392.2	397.7	12.44	341.2	385.0	385.4	4.51	301.9	382.1	394.9	6.33	320.4	890.0
pH	5.02	5.45	0.33	3.97	4.64	4.90	0.32	3.80	4.35	4.65	0.11	3.78	-
In g/kg DM:													
OM	886.2	903.8	5.16	955.3	899.3	909.2	4.89	9.55	899.7	869.6	5.12	967.0	915.0
CP	137.5	138.8	1.11	85.3	153.4	151.2	1.78	87.7	167.9	166.9	1.76	84.4	233.3
WSC	38.0	78.1	2.34	13.3	54.6	97.0	5.22	17.4	25.4	43.6	4.76	31.8	-
NDF	580.8	581.6	7.98	477.0	546.7	574.7	17.12	476.4	585.6	547.6	12.88	420.8	157.9
ADF	318,6	317.9	2.65	346.2	308.2	297.0	12.76	291.6	286.5	266.8	11.44	245.8	76.8
ADL	33.8	32.9	0.66	42.2	32.0	30.3	1.99	31.1	33.2	35.0	1.32	27.8	-
NP/NT, g/kg	507.0	547.7	9.78	-	516.7	551.3	9.87	-	466.1	538.6	7.34	-	-
$N\text{-}NH_3$/NT, g/kg	60.5	32.9	4.77	-	77.7	57.7	8.12	-	46.1	42.7	2.12	-	-
Nutritive value													
UFL	0.85	0.87	0.02	0.89	0.86	0.86	0.01	0.88	0.86	0.85	0.01	0.90	1.18
PDIN	79.9	80.9	2.11	53.2	89.9	92.1	2.67	56.9	9.13	102.3	1.77	51.8	133.0
PDIE	66.1	69.0	3.12	65.9	63.0	65.2	1.66	63.7	68.1	68.5	1.68	62.4	148.5

DM –dry matter, OM – organic matter, CP – crude protein, WSC – water sololuble carbohydrates, NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin, NP – protein nitrogen, $N\text{-}NH_3$ – ammonia nitrogen, NT – total nitrogen, UFL – units for milk production (net energy), PDIN – protein truly digestible in the small intensine dependent on the amount of nitrogen, PDIE - protein truly digestible in the small intensine dependent on the amount of energy

G – grass without additive, GI – grass inhibitor, GA – grass alfalfa mixture without additive, GAI - grass alfalfa mixture with inhibitor, GRC – grass red clover mixture without additive, GRCI – grass red clover mixture with inhibitor, MS – maize silage

The increase in ECM yield in dairy cows fed experimental silage resulted most probably not only from a higher supply of the absorbed silage components (Moorby et al., 2002), but also from the modification of silage composition caused by the addition of a fermentation inhibitor (Huhtanen et. al., 2003; Givens and Rulquin, 2004). This suggestion was confirmed by the results of a chemical analysis of silage containing the tested additive, which in the majority of cases was characterized by a higher content of protein nitrogen and WSC. A decrease in acidity and a higher WSC content (Table 1) of silage with the inhibitor are indicative of restrictive fermentation and of reduced protein degradation during the ensilage process. An increase in the concentration of protein nitrogen is accompanied by an improvement in bacterial protein synthesis efficiency (Winters et al., 2002), while a decrease in the amount of protein nitrogen during ensiling is accompanied by an increase in the content of proteolysis products, i.e. free amino acids and ammonia, as well as by the formation of biogenic amines in silage. The level of amines is negatively correlated with the rate of pH reduction during ensilage, and with DM intake. Acidifying additives inhibiting fermentation could positively affect silage intake due to a decrease in amine content (Steidlová and Kalač, 2004).

The different effect of the applied additive on the rate of synthesis of individual milk components in feeding trials (Table 2) could result from the fact that proteins in various crop species show different susceptibility to proteolytic degradation. As demonstrated by Winters et al. (2002), the protein nitrogen content of red clover silage and alfalfa silage is 57% and 33% respectively. In the present study red clover and alfalfa were mixed with grasses, so the ultimate differences between particular silage types were smaller. The most significant effect of a fermentation inhibitor on an increase in the yield of milk and milk components was observed in cows fed diets containing GA and GAI silage. This is associated with higher susceptibility of alfalfa protein to degradation during ensiling, as compared with grass or alfalfa protein, as well as with the lowest proportion of concentrated feed in the above ration. According to Ferris et al. (2001), the fermentative quality of silage has a considerable effect on milk yield in high-producing cows only if their diet contains a low amount of concentrate. The above authors have also found that the influence of silage quality diminishes along with an increase in the proportion of concentrated feed in the ration. The fact that the fermentation inhibitor added to grass and red clover silage (GRC) had no effect on milk yield could result from a higher level of concentrate in the diet and from lower protein loss during red clover ensilage (Davies et al., 1998). Red clover has high levels of the enzyme polyphenol oxidase (PPO) which interacts with proteases to form insoluble complexes (Sullivan et al., 2006; Lee et al., 2008).

Table 2. **Mean treatment effects on intake, milk production and nutrient utilization**

Item	Silage type								
	GA	GAI	SE	GRC	GRCI	SE	G	GI	SE
n	47	47		61	61		55	55	
Intake/day									
Dry matter, kg									
Experimental silage	8.97	9.39	0.62	8.43	9.07	0.49	8.53	8.92	0.44
Forage	14.09	14.51	0.48	12.96	13.60	0.36	13.34	13.73	0.51
Total	18.88	19.33	0.70	19.47	20.04	0.69	20.24	20.79	0.66
UFL	17.93	18.23	0.58	18.92	19.39	0.47	19.73	20.43	0.60
PDIN, g	1723.7	1868.8	42.1	1874.9	1937.8	61.2	1842.2	1902.7	42.3
PDIE, g	1658.6	1695.4	36.1	1786.2	1841.4	42.1	1888.7	1964.9	39.1
Protein, g/kg DM	162.1	161.2	-	165.1	163.2	-	157.5	158.3	-
Yield, kg/day									
Milk	22.70	24.39	1.09	26.78	27.96	1.00	29.09	30.30	0.84
ECM	23.73a	25.78b	1.13	27.14	28.51	0.89	28.44	29.87	0.72
Fat	1.01a	1.08b	0.05	1.13	1.20	0.04	1.12	1.17	0.03
Protein	0.76A	0.84B	0.03	0.87	0.91	0.03	0.98	1.02	0.03
Milk composition									
Fat, g/kg	44.45	44.32	0.65	41.46	42.15	0.72	39.78	39.30	0.66
Protein, g/kg	33.48	34.45	0.55	33.52	33.42	0.52	33.87	33.92	0.43
Urea, mg/l	218.8A	200.9B	5.13	248.2	232.1	7.45	210.3a	248.9b	7.43
SCC, LN	11.61	11.65	0.16	12.5	11.86	0.15	12.13	12.20	0.15
Nutrient utilization									
ECM/DM, kg/kg	1.22	1.27	0.02	1.40	1.42	0.03	1.41	1.44	0.01
Concentrate/ 1kg ECM	0.23	0.21	0.01	0.27	0.26	0.01	0.27	0.27	0.01
UFL/1kg ECM	0.78	0.75	0.02	0.70	0.68	0.02	0.69	0.68	0.01
PDI/ECM, g/kg	72.17a	68.79b	1.05	65.8	64.57	1.46	64.67	63.99	0.72
Milk protein/PDI intake, g/g	0.47	0.48	0.01	0.49	0.49	0.01	0.54	0.55	0.01
Nmilk/N intake, %	22.9A	25.4B	0.34	26.5	27.3	1.54	30.1	31.1	1.21

A,B $P \leq 0,01$ a,b $P \leq 0,05$

Nitrogen utilization efficiency was generally high, indicating that the diets were well-balanced, primarily due to the presence of maize silage (Groff and Wu, 2005). Maize silage is a rich source of starch and rumen-digestible NDF – one of the main factors determining nitrogen utilization efficiency in cattle (Nadeau et al., 2007). Differences in nitrogen utilization between treatments depended on the botanical composition of high-protein silage. In our study nitrogen utilization efficiency improved even when milk yield increased. This is consistent with the findings of Dewhurst et al. (2003) who reported that dairy cows fed exclusively grass, clover or alfalfa silage supplemented with concentrate were characterized by nitrogen utilization efficiency of 25.6, 21.0 and 18.2% respectively. In our study an additional factor improving nitrogen utilization could be also a lower concentration of protein in the diet given to cows fed grass silage (G and GI). According to Gustafsson and Palmquist (1993), nitrogen utilization efficiency in dairy cows improves by 1.5 to 2 percentage units as protein concentration in the ration decreases. The improvement in nitrogen utilization resulting from the addition of a fermentation inhibitor, observed in the present study, was also reported by Wu and Satter (2000). The cited authors found that the supply of rumen-undegradable protein in the amount of 60 - 70 g/ kg DM of the diet and 20 - 40 g/ kg DM of the diet during early and late lactation, respectively, improves nitrogen utilization efficiency and enables to maintain or increase milk yield. The increase in the proportion of protein nitrogen in total nitrogen in three types of silage containing an additive inhibiting fermentation, by 35, 40 and 72 g/kg N respectively (Table 1), ensured such a supply of rumen-undegradable protein. A positive effect of the applied additive on silage protein utilization could be also associated with a limited extent of qualitative changes in the amino acid composition of silage protein resulting from decarboxylation and deamination which involve an increase in the levels of alanine, methionine and branched-chain amino acids, and a decrease in the levels of such amino acids as arginine and histidine (Jones, 2000; Givens and Rulquin, 2004). According to Winters et al. (2001), enhanced protein synthesis in ruminants fed grass silage with an additive inhibiting fermentation is related to higher levels of arginine and lysine, i.e. amino acids limiting milk production. However, the conversion ratio of protein digested in the intestine (PDI/ECM) indicates that the increased supply of PDI originating from silage with the inhibitor was not accompanied by an improvement in its amino acid composition.

Table 3. **Milk yield at various levels of concentrate supplementation of the diet - G i GI ($\bar{x}$)**

Item	Concentrate level										SE
	> 9 kg		6-9 kg		3-6 kg		0-3 kg		0 kg		
	G	GI	G	GI	G	GI	G	GI	G	GI	
n	13	13	13	12	11	10	12	13	6	7	
Milk, kg/d	42.20	43.21	33.60	35.11	27.47	28.56	18.76	21.31	14.52	17.11	1.31
ECM, kg/d	38.77	39.96	32.09	33.50	27.87	28.67	20.25	22.48	15.22a	18.49b	1.74
Fat, g/kg	35.25	34.93	37.67	37.21	40.70	40.10	44.78	43.79	42.46	43.68	2.72
Fat, kg/d	1.48	1.51	1.22	1.31	1.18	1.15	0.82	0.91	0.62	0.74	0.21
Protein, g/kg	31.05	30.70	31.94	32.55	34.88	34.55	36.78	36.81	36.28a	38.78b	1.42
Protein, kg/d	1.32	1.33	1.08	1.14	0.96	0.98	0.70	0.78	0.53a	0.67 b	0.16
SCC, LN	12.02	11.96	11.72	12.25	12.34	12.34	12.53	12.03	13.11	12.25	0.64
Urea, mg/l	252.41	265.95	202.88A	269.14B	194.48a	215.15b	213.03	243.62	176.17	193.50	25.44

Table 4. **Milk yield at various levels of concentrate supplementation of the diet. GRC i GRCI ($\bar{x}$)**

Item	Concentrate level										SE
	> 9 kg		6-9 kg		3-6 kg		0-3 kg		0 kg		
	GRC	GRCI	GRC	GRCI	GRC	GRCI	GRC	GRCI	GRC	GRCI	
n	11	12	14	16	13	13	14	12	9	8	
Milk, kg/d	38.47	38.06	31.22 a	32.54 b	24.98	25.36	17.30	17.08	11.73	11.09	1.47
ECM, kg/d	36.21	35.48	31.80	32.68	25.04	25.85	18.32	18.41	13.06	12.41	1.78
Fat, g/kg	37.44	36.87	42.48	41.61	41.40	42.64	44.24	45.34	46.48	46.26	2.80
Fat, kg/d	1.44	1.40	1.33	1.36	1.03	1.08	0.75	0.77	0.53	0.51	0.08
Protein, g/kg	30.47	30.43	32.69	32.04	32.36	32.57	36.96	36.77	41.01	39.86	1.92
Protein, kg/d	1.17	1.16	1.02	1.04	0.81	0.82	0.63	0.62	0.48	0.44	0.06
SCC, LN	12.02	11.69	12.35	11.99	11.81	11.62	12.23	12.15	12.61	11.72	0.72
Urea, mg/l	290.24	262.50	252.62	236.82	272.21	256.13	224.68	204.00	190.67	180.13	32.58

A beneficial influence of the tested fermentation inhibitor on nitrogen utilization efficiency was reflected in the urea content of milk from cows fed GAI and GRCI silage. There was a negative correlation between urea level and the rate of milk protein synthesis. A reverse relationship was observed with respect to GI silage. A positive correlation between milk urea content and the content and yield of milk protein, and a negative correlation between milk urea content and nitrogen utilization efficiency, reported by other authors (Khalili et al., 2005; Nadeau et al., 2007), were not fully confirmed by our data. This does not, however, change the fact that average milk urea content in all treatments shows that the diets were well-balanced with regard to the amount of nitrogen compounds and carbohydrates, and the rate of their degradation.

An analysis of the production results of dairy cows, taking into account various levels of concentrate supplementation of the ration (Tables 3 - 5), points to a growing tendency in ECM yield in cows fed silage produced with the addition of a fermentation inhibitor (GAI, GI) with no concentrate supplementation or supplemented with less than 3 kg/head/day of concentrated feed. It was found that GRCI silage could exhibit a positive effect on cow productivity also at a higher level of concentrate supplementation. Dewhurst et al. (2003) have demonstrated that milk yield is affected by feeding silage made from different legume and grass species at both a lower (4 kg/head/day) and a higher (8 kg/head/day) proportion of concentrate in the diet.

Table 5. **Milk yield at various levels of concentrate supplementation of the diet - GA i GAI ($\overline{x}$)**

Item	Concentrate level										SE
	> 9 kg		6-9 kg		3-6 kg		0-3 kg		0 kg		
	GA	GAI	G	GAI	GA	GAI	GA	GAI	GA	GAI	
n	5	6	7	7	13	14	12	11	10	9	
Milk, kg/d	36.77	40.54	31.24	29.80	26.43	25.65	16.49	18.39	12.30	14.79	1.53
ECM, kg/d	35.80 a	41.84 b	31.58	29.99	27.26	26.13	17.84	20.25	13.56a	16.69b	1.97
Fat, g/kg	41.13	43.05	42.54	42.24	43.93	42.63	46.15	46.99	46.23	47.41	2.84
Fat, kg/d	1.51	1.74	1.33	1.26	1.16	1.11	0.76	0.87	0.57	0.70	0.11
Protein, g/kg	28.9	33.41	31.48	31.34	31.93	32.28	34.95	36.09	37.42a	38.98b	1.84
Protein, kg/d	1.06 a	1.36 b	0.99	0.94	0.85	0.84	0.58	0.67	0.46a	0.58b	0.06
SCC, LN	10.52 a	11.40 b	11.60	11.77	11.40	11.54	11.79	11.54	11.89	11.83	0.581
Urea, mg/l	311.33A	286.88B	204.92	181.22	214.08	195.79	193.93	180.18	218.21	192.53	25.22

Conclusion. The obtained results testify to a positive effect of a fermentation inhibitor (in the form of a mixture of short-chain carboxylic acids) added to ensiled high-protein forages on the production results of dairy cows. The effectiveness of the fermentation inhibitor was affected by the type of ensiled raw material and by the level of concentrate supplementation. The additive was found to be least effective when added to silage made from grass and red clover. When silage was produced from high-protein components with the addition of a fermentation inhibitor, milk urea content was not a reliable indicator for assessing feeding strategies, nitrogen utilization efficiency and nitrogen emission levels.

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