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The influence of additives facilitating ensiling on the quality of quinoa (*Chenopodium quinoa* Willd.) silage

Wpływ dodatków ułatwiających kiszenie na jakość kiszonki z komosy ryżowej (*Chenopodium quinoa* Willd.)

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Abstract

Chenopodium quinoa Willd. is a new plant in the conditions of Poland. At harvest, it has a low content of dry matter, which makes it difficult to ensile. For this reason, a study was undertaken on the effect of microbial and chemical additives on selected quality characteristics of quinoa silage. Traits determining the suitability of the green forage and the influence of silage additives were assessed. A microbial additive and a chemical additive were used. The microbial additive contained bacterial strains of *Enterococcus faecium*, *Lactobacillus plantarum* and *Pediococcus acidilactici* (concentration $1.25 \cdot 10^{11}$ CFU·g^{-1}) and was applied in the amount of 1 g·t^{-1} of fresh material. The chemical additive contained formic acid, propionic acid and ammonium formate, and was added in the amount of 5 l·t^{-1} of fresh material. The quality of the quinoa silage depending on the additive used was evaluated. The fresh material of *Chenopodium quinoa* Willd. contained only 6.42% water-soluble carbohydrates (WSC) in dry matter (DM) and its fermentability coefficient was 29.2. Lactic acid was predominant in the silage, while the content of acetic acid was average. In the control silage (without additives), small amounts of butyric acid (0.04% DM) were noted, so its quality according to the Flieg-Zimmer scale was good. No butyric acid was found in the silage prepared with additives, and their quality was very good. The control silage contained more N-NH_3 than the silage prepared with additives ($P \leq 0.01$). This indicated that the preservatives (silage additives) limited the process of protein degradation in the quinoa silage.

Keywords: chemical additives, microbial additives, quality, quinoa, silage

Streszczenie

W warunkach Polski komosa ryżowa *Chenopodium quinoa* Willd. jest rośliną nową. W chwili zbioru cechuje się niską zawartością suchej masy, co utrudnia jej zakiszanie. Dlatego podjęto badania nad wpływem dodatku mikrobiologicznego i chemicznego na wybrane elementy jakości kiszonki. Określono cechy przydatności do zakiszania zielonki oraz oceniono wpływ dodatków kiszonkowych. Zastosowano dodatek mikrobiologiczny i chemiczny. Dodatek mikrobiologiczny zawierał szczepy bakteryjne: *Enterococcus faecium, Lactobacillus plantarum* i *Pediococcus acidilactici* (koncentracja $1{,}25 \cdot 10^{11}$ jtk·g^{-1}), zastosowano dawkę 1 g·t^{-1} zakiszanej masy. Dodatek chemiczny zawierał: kwas mrówkowy, kwas propionowy i mrówczan amonu, dawka 5 l·t^{-1} zielonki. Oceniono jakość kiszonek z komosy w zależności od dodatku. W zielonce z *Chenopodium quinoa* Willd. znajdowało się tylko 6,42% cukrów rozpuszczalnych (WSC) w suchej masie (SM), a jej współczynnik fermentacji wynosił 29,2. W kiszonkach przeważał kwas mlekowy, przy średniej zawartości kwasu octowego. W kiszonce kontrolnej (bez dodatków) stwierdzono niewielkie ilości kwasu masłowego (0,04% SM), dlatego jej jakość według skali Flieg-Zimmera była dobra. W kiszonkach sporządzonych z dodatkami nie stwierdzono obecności kwasu masłowego, a ich jakość była bardzo dobra. W kiszonce kontrolnej było więcej N-NH_3 niż w kiszonkach sporządzonych z dodatkami ($P \leq 0{,}01$). Świadczyło to o tym, że konserwanty (dodatki kiszonkowe) ograniczyły proces rozkładu białka w kiszonce z komosy ryżowej.

Słowa kluczowe: dodatki chemiczne, dodatki mikrobiologiczne, jakość, kiszonka, komosa

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a plant that has been cultivated in South America for 5,000 years. In Europe it is little known and not widely grown, although it has recently been gaining popularity, as it has numerous beneficial traits as a crop plant and as animal feed. It tolerates frosts even up to −5 °C, produces high yields at rainfall levels of over 400 mm, and shows high resistance to drought and salinity. In addition, it is highly competitive with most weeds, so that the use of chemical weed control is unnecessary (Gęsiński, 2012). Owing to its low soil requirements, it can be used successfully for reclamation of post-industrial areas (Vega-Galvez et al., 2010). Quinoa is a plant with strong growth and abundant foliage and produces high yields of green matter. It develops particularly vigorously in the climatic conditions of Poland. Quinoa can be used as feed for cattle and poultry. Other uses of this plant are also known: its flour is used to produce bread, pasta, breakfast cereals, muesli and polenta. The raw grains and sprouts can be added to soups or salads. The young leaves are suitable for salads (Gęsiński, 2012).

Owing to the valuable properties of quinoa, its unusual resistance and ecological plasticity, and its high nutritional value, the Food and Agriculture Organization (FAO) declared the year 2013 the 'International Year of Quinoa' (Kakabouki et al., 2014; Papastylianou et al., 2014).

Quinoa has a long growing season and even European varieties do not mature in Poland until late September (Gęsiński, 2012). Due to weather conditions and the plant's thick stalk, harvesting at this time prevents the bulb from drying out before ensilage, and at the time of harvest the green forage contains less than 20% dry matter. It is difficult to obtain good silage from material with such high moisture content because of its high levels of acetic acid and ammonia, and often also the presence of undesirable *Clostridium* bacteria (McDonald et al., 1991). One of the basic methods of improving silage characteristics is the use of various additives to accelerate and direct the fermentation process, improve the quality and stability of the silage, and reduce nutrient and energy losses (Wilkins et al., 1999; Filya, 2003, Filya et al., 2006; Knický and Spörndly, 2009).

The aim of the study was to determine the suitability of green forage from quinoa for ensilage and to evaluate the effect of silage additives (microbial and chemical) on selected quality characteristics of silage prepared from quinoa (*Chenopodium quinoa* Willd.).

Materials and methods

To achieve these objectives, to determine the potential for production of silage from quinoa (*Chenopodium quinoa* Willd.) and to obtain green matter for the study, a field experiment was carried out in 2015 at the Experimental Cultivar Testing Station in Chrząstowo (53° 11' N, 17° 35' E), located in Nakło County in the Kuyavian-Pomeranian Voivodeship. The research plots were located on grade IVa soil – sandy loam on sandy clay loam. The soil contained 68.17 mg·kg^{-1} P, 150.77 K and 36.18 Mg, and the pH was 6.1. Mineral fertilizers were applied at rates of 120 kg·ha^{-1} N, 42 kg·ha^{-1} P and 120 kg·ha^{-1} K in the form of triple superphosphate and 60% potassium chloride, and nitrogen in the form of ammonium nitrate. Quinoa seeds of the Faro cultivar were sown at 9 kg seeds per hectare with row spacing of 40 cm and seed depth of 1 - 2 cm. Green matter for the experiment was harvested at the full flowering stage, chopped, and ensiled in polyethylene 'micro-silos' (⌀ 15 cm, height 49 cm). When the forage had been thoroughly compacted in the micro-silos, they were sealed with rubber stoppers. A fermentation tube was placed in each stopper for release of gases. The tubes were filled with glycerol to protect the containers from access to air. The following silage treatments were prepared: control; with the microbial additive Polmasil, containing *Enterococcus faecium*, *Lactobacillus plantarum* and *Pediococcus acidilactici* (concentration 1.25·10^{11} CFU·g^{-1}, at 1 g·t^{-1} of ensilaged green matter); and with a chemical additive (formic acid, propionic acid and ammonium formate, at 5 l·t^{-1} green matter). Each treatment was prepared in four replicates. After 6 weeks, the micro-silos were opened and samples were taken for analysis.

The silage from the micro-silos was separated and samples were taken for wet analysis. The rest of the silage samples and green matter samples were dried to a constant weight at 55 °C and then ground in a mill (SM 100, Retsch) to a particle size of 1 mm. The dry matter content (DM) of the dried samples was determined by the oven-drying method and crude ash by combustion in a muffle furnace at 600 °C. The total protein content was determined by the Kjeldahl method on a 2200 Kjeltec Auto Distillation unit (FossTecator AB); crude fat by the Soxhlet

method using the Soxtec System HT 1043 extraction unit (FossTecator AB); and crude fibre according to Henneberg and Stohmann in a Fibertec 1010 Heat Extractor System (FossTecator AB) (AOAC, 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using an Ankom 220 Fiber Analyzer (ANKOM Technology) according to Van Soest et al. (1991). The level of water-soluble carbohydrates (WSC) was analysed by the Luff-Schoorl titration method (EU Regulation, 2009). The buffering capacity (BC) of the green matter was determined according to Weissbach (1992). The fermentability coefficient (FC) was calculated according to the formula given by Pahlow et al. (2002):

$$FC = DM\ (\%) + 8\ WSC \cdot BC^{-1}$$

Samples of wet silage were analysed for quality. The silage was analysed for the content of basic acids using standard procedures. Lactic acid content was determined using a HP6890 gas chromatograph with a flame ionization detector (FID), JW Scientific DB-FFAP column, length 30 m, diameter 0.53 mm, argon as the carrier gas, injector temperature 200 °C, detector temperature 240 °C, column temperature 60-210 °C. The content of acetic and butyric acids was determined using a Shimadzu LC-2010 liquid chromatograph with an 8 × 250 mm steel column packed with OSTION LG KS 0800 H+ from Tessek, mobile phase 5 mM H_2SO_4. The pH of the silage was determined using an N 5172 pH meter (TELEKO Wrocław). The quality of the silages was assessed according to the Flieg-Zimmer scale (Zimmer, 1966).

All results were statistically analysed by one-way analysis of variance, and the significance of differences between means was verified by the F test (SAS/STAT, 1995).

Results and discussion

The chemical composition of the quinoa and its suitability for silage are presented in Table 1. Gęsiński (2012) reports that crude protein accounts for 23.1% of the dry weight of quinoa green forage, classifying it among high-protein feed components. In this study, crude protein accounted for only 11.21% of the dry weight of the green forage. Papastylianou et al. (2014) also reported crude protein levels of 11.1 - 14.7% DM in green matter from this plant. In the samples of quinoa green forage analysed in this study, the concentrations of crude ash, crude fat, crude fibre and nitrogen-free extract were higher than those reported by Gęsiński (2012).

For fermentation to proceed properly, the content of water-soluble carbohydrates in the dry weight of the material should be at least 7.5% (Cruz et al., 2011). The content of WSC in the green matter of *Chenopodium quinoa* Willd. is only 6.42% DM, which is insufficient to ensure that the silage obtained will be of good quality. The fermentability coefficient of quinoa is 29.2. Pahlow et al. (2002) report that when the fermentability coefficient is so low there is a risk of a clostridium fermentation. It is therefore necessary to use additives to stimulate the fermentation process.

Table 1. Chemical composition and suitability of quinoa forage for ensiling

Trait	Content
Dry matter (%)	18.91 ± 0.19
Crude ash (% DM)	14.7 ± 0.59
Crude protein (% DM)	11.21 ± 1
Crude fat (% DM)	4.44 ± 0.25
Crude fibre (% DM)	29.03 ± 1.25
NFE (% DM)	40.37 ± 0.6
NDF (% DM)	46.65 ± 1.1
ADF (% DM)	32.08 ± 0.94
WSC (% DM)	6.42 ± 0.13
Buffering capacity (g lactic acid·kg^{-1})	49.9 ± 1.2
Fermentability coefficient	29.2

Table 2. Chemical composition of silages

Constituent	Control silage	Silage with microbial additive	Silage with chemical additive
Dry matter (%)	20.93 ± 0.48	20 ± 1.07	18.62 ± 1.97
Crude ash (% DM)	14.76 ± 0.55	14.94 ± 0.34	15.53 ± 1.15
Crude protein (% DM)	10.31^{Aa} ± 1.15	11.82^{b} ± 0.53	12.88^{B} ± 0.93
Crude fat (% DM)	4.54 ± 0.13	4.66 ± 0.31	4.69 ± 0.38
Crude fibre (% DM)	30.79 ± 2.18	30.89 ± 1.03	29.36 ± 2.85
NFE (% DM)	39.61 ± 2.28	37.70 ± 0.47	37.54 ± 1.04
NDF (% DM)	45.31 ± 1.7	45.08 ± 2.35	42.90 ± 3.31
ADF (% DM)	34.24 ± 2.53	32.76 ± 1.63	31.94 ± 1.56
WSC (% DM)	1.49 ± 0.09	1.39 ± 0.08	1.6 ± 0.26

$^{a,b}P\leq0.05$; $^{A,B}P\leq0.01$

The chemical composition of the silage is shown in Table 2. Silages prepared with additives showed a higher concentration of crude protein than the control silage. The content of the remaining nutrients in the control silage and the silage prepared with additives was similar.

In the fermentation process, sugar is converted to lactic acid. Haigh (1998) reports that water-soluble carbohydrates remaining in the silage should account for more than 5% of its dry weight, as they are a valuable source of energy for ruminal microflora. In carbohydrate-poor silages, less than 0.5% DM remains. In the quinoa silages, the average content of WSC was 1.49% DM.

Lactic acid was predominant in the silages, while its acetic acid content was moderate (Table 3). According to Haigh (1998), high concentration of lactic acid in silage (over 6.5% DM) indicates that fermentation has proceeded correctly. When the fermentation process is flawed, the silage contains less than 3% DM lactic acid. In this study, the content of lactic acid in the silages ranged from 1.83% to 1.92% DM. The low content of lactic acid in the silages may be due to the low carbohydrate content of the ensiled green matter, which is the substrate for the production of this acid. If lactic acid accounts for more than 80% of all acids, the silage should be considered to be of very good quality (Haigh, 1998). All the silages prepared from *Chenopodium quinoa* Willd. had a high proportion of lactic acid in the total acids.

Small amounts of butyric acid (0.04% SM) were found in the control silage. This reduced the quality of the silage to 'good', as compared to the very good quality of the silages made with additives (Table 3). According to Haigh (1998), the dry matter of good quality silage contains less than 0.5% butyric acid. According to this measure, all the quinoa silage tested was of good quality.

Table 3. Quality traits of silages

Trait	Control silage	Silage with additive	
		microbial	chemical
pH	4.13 ± 0.1	4.07 ± 0.06	3.99 ± 0.14
Lactic acid (% DM)	1.92 ± 0.25	1.91 ± 0.15	1.83 ± 0.17
Acetic acid (% DM)	0.37 ± 0.08	0.33 ± 0.03	0.41 ± 0.05
Butyric acid (% DM)	0.04 ± 0.05	0 ± 0	0 ± 0
Percentage of lactic acid in total acids (%)	82.4	85.3	81.7
Silage quality score	78 good	100 very good	98 very good
$N\text{-}NH_3$ ($g \cdot 100\ g^{-1}\ N_{total}$)	$8.02^{A} \pm 1.05$	$6.91^{B} \pm 0.93$	$6.75^{B} \pm 0.89$

$^{A, B}P \leq 0.01$

The content of ammonia nitrogen in silage is another parameter indicative of the course of the fermentation process. In properly prepared silage it should not exceed 10% of total N (Haigh, 1996, 1998). In this study, the level of ammoniacal nitrogen in the silages was lower. The addition of preservatives to the green forage reduced the amount of N-NH_3 in them, which indicates a reduction in the protein degradation process. This is consistent with results obtained by other authors (Pahlow et al., 2002).

Conclusions

Quinoa (*Chenopodium quinoa* Willd.) has a low fermentability coefficient, which means that to produce silage additives must be used to properly direct the fermentation process. The presence of butyric acid was detected in the control silage, which reduced its quality. The use of additives improved the quality of the silage and reduced the degradation of protein in them.

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