

## Health status of cows fed maize silage covered with oxo-biodegradable foil

## Status zdrowotny krów żywionych kiszonką z kukurydzy okrytą folią oksybiodegradowalną

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### Abstract

In agricultural practice, silage production uses pure, low density polyethylene foil. This foil, after use, becomes farm waste, having a negative impact on the environment. Instead of conventional foil, an environmentally safe biodegradable foil can be used, made from naturally occurring polymers or from synthetic multiparticulates, easily degradable by microorganisms. Silage covered with this type of foil should be safe for animal health. The purpose of the study was to determine whether oxo-biodegradable film could be used instead of conventional film in agricultural practice, to produce silage that is safe for the cows' health. Dairy cows were fed a partly mixed ratio (PMR), the component of which was silage made of whole maize plants, covered with oxo-biodegradable foil. The cow blood serum was marked for content of: glucose, total protein, cholesterol, triacylglycerols and enzyme activity: aspartic and alanine aminotransferase,  $\gamma$ -glutamyl transferase, alkaline phosphatase and amylase. The total protein concentration in the serum of cows analyzed at the end of the experiment was higher than the commonly accepted normal values. The content of glucose, cholesterol, triacylglycerols and the activity of aspartate and alanine aminotransferase,  $\gamma$ -glutamyl transferase, alkaline phosphatase and amylase was within reference limits. Feeding of silage from whole maize plants covered with oxo-biodegradable foil did not negatively affect the biochemical indicators of the cows' blood serum. The silage proved to be safe for the cows' health.

**Keywords:** dairy cows, health status, oxo-biodegradable foil, silage

## Streszczenie

W praktyce rolniczej przy produkcji kiszonek wykorzystuje się folię z czystego polietylenu małej gęstości. Folia ta po wykorzystaniu jest w gospodarstwie odpadem, który ma negatywny wpływ na środowisko. Zamiast folii konwencjonalnej może być stosowana bezpieczna dla środowiska folia ulegająca biodegradacji, wyprodukowana z polimerów pochodzenia naturalnego lub z syntetycznych związków wielocząsteczkowych rozkładanych przez mikroorganizmy. Kiszonka okryta taką folią powinna być bezpieczna dla zdrowia zwierząt. Celem badań było ustalenie, czy folia oksybiodegradowalna może być wykorzystana zamiast folii konwencjonalnej w warunkach praktyki rolniczej do produkcji kiszonek bezpiecznych dla zdrowia krów. Krowy mleczne żywiono dawką częściowo wymieszaną (PMR), której komponentem była kiszonka z całych roślin kukurydzy okryta folią oksybiodegradowalną. W osoczu krwi krów oznaczono zawartość glukozy, białka całkowitego, cholesterolu, triacylogliceroli i aktywność enzymów: aminotransferazy asparaginianowej i alaninowej,  $\gamma$ -glutamylotransferazy, fosfatazy alkalicznej i amylazy. Stężenie białka całkowitego w surowicy krwi krów analizowane na koniec doświadczenia, było wyższe niż ogólnie przyjęte wartości prawidłowe. Zawartość glukozy, cholesterolu, triacylogliceroli oraz aktywność aminotransferazy asparaginianowej i alaninowej,  $\gamma$ -glutamylotransferazy, fosfatazy alkalicznej i amylazy mieściły się w granicach norm referencyjnych. Skarmianie kiszonek z całych roślin kukurydzy okrytej folią oksybiodegradowalną nie wpłynęło ujemnie na wskaźniki biochemiczne surowicy krwi krów dojnych. Kiszonka była bezpieczna dla zdrowia krów.

**Słowa kluczowe:** folia oksybiodegradowalna, kiszonka, krowy mleczne, stan zdrowia

## Introduction

Silage is the basic wet forage used in dairy and meat cattle farming. The good quality of these feeds is determined by the creation of anaerobic conditions inside the silo, by correct sealing of the ensiled biomass. This allows to stun the development of undesirable oxidative native microflora, such as yeast and mold, to reduce dry matter losses, improve quality parameters, the feed quality and oxygen stability of silage during feeding (Gach et al., 2010).

Pure polyethylene (PE) low density foil - a conventional material that prevents spoilage of silage - is commonly used for the production of silage in tanks or prisms. Important features of standard silage foils are: air (oxygen) impermeability, UV resistance, good plasticity, pressure resistance, easy availability and relatively low price per unit. The lowest unit costs were shown for the production of silage from meadow grass in ground prisms, where the crushed green was harvested using a pickup trailer (Gach et al., 2007).

The used silage foil is difficult to dispose of and is often unlawfully removed to illegal landfills, buried or burned. The incineration of the foil causes release of toxic polymeric components (dioxins, furans, phosgene), which poses a serious threat to living organisms (Rymarz and Klecan, 2001; Majdiuk, 2002; Rutkowska et al., 2002; Denoncourt et al., 2006, 2007; Gach et al., 2010). Gach et al. (2010) report

that in Poland, the foil wrapping used in silage production is poorly handled in excess of 70% cases after being used, therefore its components should not be substances harmful to the environment. Survey research conducted by Gach et al. (2010) in Mazowieckie Voivodship showed that used up silage foil was burned in heating stoves in almost 61% of the farms surveyed, and only 7.02% of farms surveyed, located near bigger cities, performed recycling. Approximately 33% of farmers reported that they deposited the foil on municipal landfills, with some municipalities paying for the used-up foil (Gach et al., 2010).

A solution to the problem of management of pure, low-density, polyethylene (PE) waste silage film can be the introduction to the market of oxo-biodegradable foil, made of modified, low density polyethylene (PE). Polymers used in the production of biodegradable films are obtained by natural means (chitosan, cellulose, starch, soy or whey protein and others), biotechnological synthesis of macromolecular weight compounds (MMWCs) or by the manufacture of products containing biodegradable fillers or modifiers (natural polymers), possessing their own biodegradability capabilities (Majdiuk, 2002). However, the basic conditions to be met when using biodegradable materials for covering the raw material for the purposed of producing silage, is the effective protection against air (oxygen), maintaining high feed quality during feeding and producing silage that is safe for animal health (Denoncourt et al., 2006, 2007; Gach et al., 2010). The results of the study carried out by Grabowicz et al. (2015) showed that the modified, low-density, polyethylene (oxo-biodegradable) foil, similarly to standard foil, protected maize from air access during fermentation and after opening of the tank, and the time of oxo-biodegradation was adjusted to the rate of silage removal from the silo.

During the studies it was evaluated, based on selected biochemical blood serum markers, whether silage made of whole maize plants, covered with oxo-biodegradable film, was a safe feeding component for dairy cows.

## Materials and methods

The research was conducted under production conditions at the Mochełek Experimental Plant (53°12'24"N and 17°51'40"E). The silage raw material was a green crop of whole maize plants (medium early cultivar in classification of Food and Agriculture Organization of the United Nations, FAO 230), harvested with a Class 850 self-propelled chaff cutter, with a dry matter content to 348.8 g·kg<sup>-1</sup>. The maize was incubated in a covered 800 Mg (megagrams) mobile reservoir, with the addition of microbiological preparation. The inoculant contained lactic acid bacteria *Lactobacillus plantarum* and *Enterococcus faecium* (10<sup>9</sup> CFU·g<sup>-1</sup> formulation) and was dispensed during green fodder harvest. In order to create anaerobic conditions, the ensiled biomass was pressed in layers using a tractor and then sealed with silage foil and secured with tires. Half of the silage stack (400 Mg) was covered with conventional foil, made of pure, low-density polyethylene (control silage) and the second part – with oxo-biodegradable foil, made of low-density polyethylene modified with a polyethylene-based polymer, containing active metal ions (experimental silage). After 4 months of storage the silages, produced using different types of foil, were used in dairy cow feeding experiment, with a duration of 100 days.

### **The feeding of cows**

Of a herd of 50 cows, of the Polish Holstein-Friesian black and white breed, 16 were selected, with an average body weight of 650 kg and a daily milk yield of 32.33 kg. Cows of similar age and lactation stage (4-5 month of III lactation) were randomly assigned to two food groups, 8 cows per each. The animals were fed partially mixed rations (PMR) (Table 1), which was balanced on the basis of the new feed evaluation systems (Institut National de la Recherche Agronomique, INRA) for the production of 25 kg of milk (Strzetelski et al., 2009). Cows with higher yields received 1 kg of concentrate per 1-2 liters of milk produced over 25 kg. The control (C) group was fed PMR containing maize silage covered with standard foil ( $324.8 \pm 1.5$  g dry matter). Experimental animals (E) - received a dose of PMR with silage covered with oxo-biodegradable film ( $322.2 \pm 1.22$  g dry matter). The PMR dose was administered twice daily *ad libitum*. The animals had unlimited access to water. The flock was under the control of the Polish Federation of Cattle Breeders and Producers of Milk. PMR samples were subjected to chemical analysis to determine the nutritional value. During the experiment, the changes in chemical composition and quality parameters of silages between 4 and 7 months of storage were additionally evaluated and changes in the properties of the elastomeric biodegradable foil were compared with the initial parameters, based on the relative elongation at break-point, along and across the direction of extrusion. The results of this study were the subject of a separate publication (Grabowicz et al., 2015).

### **Chemical composition of PMR**

In PMR trials, the content of basic nutrients was determined (Commission Regulation EC, 2009) and the content of unit for lactation (UFL) and protein digested in the small intestine when rumen-fermentable nitrogen is limiting (PDIN) was calculated (Strzetelski et al., 2009) (Table 1).

### **Biochemical indicators of blood serum**

In order to determine the health status of the cows, two samples (at the beginning and at the end of the experiment) were collected from each specimen, from the jugular vein, using tubes without anticoagulant. The blood was centrifuged (10 minutes,  $3,000 \text{ rotations} \cdot \text{min}^{-1}$ ) and sterile needle syringes were used to take serum samples, which were then transferred to sterile Eppendorf test-tubes and analyzed.

The selected serum biochemical markers were determined using the Olympus AU 640 apparatus, applying the following methods: glucose (GLU) - ultraviolet photometry using hexokinase catalyzed enzyme reactions; total protein (TP) - quantitative colorimetric determination; total cholesterol (CHOL) - colorimetric enzymatic assay; triacylglycerols (TG) - color enzyme test; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - kinetic quantitative determination with ultraviolet detection;  $\gamma$ -glutamyl transferase ( $\gamma$ -GT), alkaline phosphatase (ALP) and amylase (AMY) - kinetic quantitative colorimetric determination.

### Statistical analyses

Results were statistically calculated using the Statistica computer program. The t-Student test was used to evaluate the differences between the two means.

Table 1. Composition and nutritive value of PMR and silages

Feeds	Participation (%)		
Corn silage	64.3		
Grass-legume mixture silage	18.4		
Fresh brewer's grain	9.2		
Silage with triticale grains	1.8		
Rapeseed cake	1.8		
Extracted rapeseed meal	4		
Supplementary mineral mix	0.3		
Feed chalk	0.2		
Nutritional value (in 1 kg):	PMR	C	E
Net energy (UFL)	16.3	0.98±0.02	0.98±0.01
Protein (PDIN) (g)	1,620	61.15±3.22	60.80±2.11
FV (UEL)	17.5	1.27±0.01	1.27±0.01
Calcium (g)	133	nd	nd
Phosphorus (g)	73.5	nd	nd

C – control silage, E – experimental silage, UFL – unit for lactation, PDIN – protein digested in the small intestine when rumen-fermentable nitrogen is limiting, FV – fill values, UEL – fill unit for cows, nd – not determined.

### Results and discussion

The analysis of the test cows' serum selected biochemical indicators (Table 2) showed that mean glucose (GLU) concentrations ranged from 3.1 (control) to 3.27 (experimental group) mmol·dm<sup>-3</sup> and had not exceeded the reference range (Mordak, 2008; Winnicka, 2015). In the research by other authors (Kupczyński and Chudoba-Drozdowska, 2002; Mordak and Nicpoń, 2006; Mikuła et al., 2008; Adamski, 2010; Klebaniuk and Rocki, 2011; Bjerre-Harpøth et al., 2014; Kafilzadeh et al., 2015; Klebaniuk et al., 2015) the values of this parameter for lactating cows

ranged from 2.65 to 4.11 mmol·dm<sup>-3</sup> and, as in own studies, were within physiological norms.

The concentration of total protein in serum of all the animals, both at the beginning and at the end of the experiment, was increased and exceeded the reference value (Winnicka, 2015) on average by 14% in the control group and by 22% in the experimental group. The highest values of this parameter (87.62 g·dm<sup>-3</sup>) were found at the end of the experiment, in cows fed with maize silage covered with oxo-biodegradable foil. As in own studies, other authors (Mordak and Nicpoń, 2006; De Cardoso et al., 2008; Adamski, 2010) observed exceeding of the reference range (Winnicka, 2015) for the evaluated parameter by 18, 23 and 19%, respectively. Adamski et al. (2010) showed that the serum total protein level in cows at 8 weeks of lactation decreased as the condition of these animals increased from 84.52 g·dm<sup>-3</sup> at BCS (Body Condition Score) 1-3 to 72.38 g·dm<sup>-3</sup> of the fatted cows (BCS≥4). Kupczyński and Chudoba-Drozdowska (2002) report that total serum protein levels are one of the indicators of nitrogen metabolism in the body and are dependent on protein content in feed, lactation phase, cows' age and season of the year. The above-quoted authors have shown that total protein concentration in serum, in the third week after birth, has exceeded the reference value (Winnicka, 2015) by 5% and by 6% in the 6th week. Similar results are quoted by Bjerre-Harpøth et al. (2014). Klebaniuk and Rocki (2011) have shown that the concentration of total protein in blood serum during lactation in winter was between 62.19 and 63.08 g·dm<sup>-3</sup> and was lower than in cows tested in winter, which had shown an increase in the value of this parameter by 5%, as compared to physiological standards. The results presented by the above-quoted authors were lower than in own studies. Wasilieva (1982), taking into account the physiological state, lactation period, season of the year and the yield of cows, gives a wider range of physiological norms for this parameter, reaching 88.4 g·dm<sup>-3</sup>.

In the group fed maize silage covered with standard foil (C), growth tendencies were recorded for liver enzymes (AST, ALT, γ-GT) and amylase (AMY) during the experiment. Reverse dependencies were demonstrated with respect to cows (E) receiving dosages using silage coated with oxo-biodegradable foil. The activity of AST, ALT, γ-GT and ALP in the blood serum of cows from the experimental group at the end of the experiment was lower than in the serum of the control group, respectively by 19; 10.5; 20 and 26.5%, with a statistically significant difference only for ALP activity. Increased ALP activity was also reported in cows with liver damage, resultant from acidosis or alkalosis (Filar, 2004; Mordak and Nicpoń, 2006). Lower levels may indicate correct animal health status. The amylase activity in the serum of the control group at the end of the experiment (37.25 U·dm<sup>-3</sup>) has reached the upper limit of reference values (Winnicka, 2015) and was 9% higher than in the experimental group. It should be emphasized that the values of the enzymes analyzed during the whole experiment period were within physiological norms (Winnicka, 2015). Adamski et al. (2010) showed that AST activity increased along with the increase in condition of cows from 65.75 to 76.81 U·dm<sup>-3</sup>. Klebaniuk and Rocki (2011) have shown that AST concentration was significantly higher in the blood serum of cows in winter (67.1-79 U·dm<sup>-3</sup>), than in cows in summer (49.5-56 U·dm<sup>-3</sup>). In contrast, De Cardoso et al. (2008) and Mikuła et al. (2008) cite higher values for this parameter (over 93 U·dm<sup>-3</sup>), compared with the results of own research and other

authors (Kupczyński and Chudoba-Drozdowska, 2002; Klebaniuk et al., 2009, 2015). The GT activity in tested cows was comparable to that of Adamski et al. (2010), who showed that, along with the increase of condition in cows, the value of this parameter increased from 28.33 to 30.71 U·dm<sup>-3</sup>. A wider range of γ-GT activity was obtained by Kupczyński and Chudoba-Drozdowska (2002). The results of ALT activity were comparable with data cited by Kupczyński and Chudoba-Drozdowska (2002) and Mordak and Nicpoń (2006), but almost twice lower than in research by Klebaniuk et al. (2009, 2015). The increase in enzymatic activity is accompanied by disturbances in energy metabolism, so lowered levels are more beneficial to the animals (Filar, 2004).

Table 2. Serum biochemical indicators for dairy cows

Items	Study period	Feeding group		Reference values <sup>1</sup>
		C	E	
Glucose GLU (mmol·dm <sup>-3</sup> )	Beginning	3.31±0.45	3±0.24	2.2 - 4.5
	End	3.24±0.31	3.21±0.39	
Total protein TP (g·dm <sup>-3</sup> )	Beginning	81.07±3.64	85.41±5.8	51 - 71
	End	80.7*±3.04	87.62**±8.04	
Cholesterol CHOL (mmol·dm <sup>-3</sup> )	Beginning	4.98±0.97	4.26±0.83	1.8 - 5.2
	End	5.39±1.17	4.47±0.9	
Triacylglycerols TG (mmol·dm <sup>-3</sup> )	Beginning	0.13 <sup>***</sup> ±0.02	0.18 <sup>**</sup> ±0.04	0.1 - 0.3
	End	0.19 <sup>a</sup> ±0.03	0.18±0.02	
AST AST (U·dm <sup>-3</sup> )	Beginning	79.62±16.38	68.25±11.01	58 - 100
	End	82.37 <sup>**</sup> ±10.1	66.37 <sup>**</sup> ±8.54	
ALT ALT (U·dm <sup>-3</sup> )	Beginning	26.77±4.79	24.26±5.68	25 - 74
	End	28.16±4.71	25.19±5.5	
γ-GT γ-GT (U·dm <sup>-3</sup> )	Beginning	30.12±8.1	29.25±7.9	22 - 64
	End	32±8.41	25.50±3.74	
ALP ALP (U·dm <sup>-3</sup> )	Beginning	61.62±31.05	48.50 <sup>a</sup> ±15.07	41 - 116
	End	57±17.04	41.88 <sup>a</sup> ±10.3	
AMY AMY (U·dm <sup>-3</sup> )	Beginning	34.62 <sup>a</sup> ±7.55	35.63±9.33	30 - 38
	End	37.25 <sup>b</sup> ±8.09	34.25±6.8	

<sup>1</sup>Winnicka, 2015; <sup>ab</sup>P≤0.05 – statistically significant differences within the feeding groups; \*P≤0.05; \*\*P≤0.01 – statistically significant differences between feeding groups.

## Conclusions

Regardless of the type of silage fed in dosages to cows, selected blood serum biochemical indicators, excluding total protein and triacylglycerols, were within the reference ranges reported by Winnicka (2015). The upper limit for the reference value for total protein concentration, both at the beginning and at the end of the experiment, was exceeded for all cows tested.

In the group fed with corn silage coated with biodegradable foil, tendencies for decreased serum enzyme activity were noted.

Analysis of selected biochemical blood serum indicators indicates that maize silage coated with biodegradable foil can be fed in doses to dairy cows without any adverse effect on their health.

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