

SZENT ISTVÁN UNIVERSITY FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

DEVELOPMENT AND ANALYSIS OF FERMENTATION OF TOMATO POMACE AS AN ALTERNATIVE SUPPLEMENTARY FORAGE FOR GAME SPECIES

Thesis of Ph.D. dissertation

Judit Galló

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Doctoral school

Name:	Doctoral School of Animal Science	
Field of science:	Animal Science	
Head:	Dr. Miklós Mézes	
	Professor, DSc, MHAS	
	Szent István University	
	Faculty of Agricultural and Environmental Sciences	
	Institute of Basic Animal Sciences, Department of Nutrition	
Supervisor:	Dr. László Szemethy	
	Associate professor, PhD	
	University of Pécs	
	Faculty of Cultural Sciences, Education and Regional Development	
	ad of the Doctoral School Approval of the Supervisor	

1. BACKGROUND AND AIMS

Tree plantations and plantation forestry provide neither food nor hiding places for game species. However, these species find their food in adequate quantity and quality and hiding places in the shrub and herb layer (KŐHALMY 1990). A critical period for ungulates is mainly during winter (NIKODÉMUSZ et al. 1988), when forage availability and quality is low, while their energy demands are high (WHITE et al. 2009). The condition of animals declines significantly and rapidly due to the unbalanced and incomplete nutrition (ÁKOSHEGYI et al. 1989). The problem is complex, because beside the losses and impoverishment of plant species and fluctuations in food supply, the relative abundance of wild ungulates is increasing (HELTAI and SONKOLY 2009). Moreover, in fenced areas where limited space and food supply is accessible, red deer (*Cervus elaphus*), fallow deer (*Dama dama*), mouflon (*Ovis aries*), or wild boar (*Sus scrofa*) can occur in a relatively higher density than in unfenced areas (MÁTRAI et al. 2013). Fenced ungulates can degrade the vegetation, and the regeneration of the browse species becomes limited (ASNANI et al. 2006, WHITE 2012, MÁTRAI et al. 2013, SZEMETHY et al. 2013). Consequently, feeding wildlife has became necessary (HELTAI and SONKOLY 2009).

The feeding of wildlife is just supplementary feeding, because game species find the bulk of their food in the forests. Supplementary fodder is especially needed when the shrub layer is partly or totally missing from the forest, and when juicy foods are missing from food plots in winter. It should be considered that wild ruminants have different nutrition-physiological characteristics than domesticated ruminants, so their nutrient requirements are also different. In winter, when the protein content of vegetation does not meet the requirements of ungulates, providing such supplementary fodder is favourable, which protein and energy content are high, but fibre content is relatively low (crude protein/crude fibre ratio is about 1.0), so they provide the adequate nutrient content for the animals, additionally, they are relatively cheap. Fodders that are rich in digestible carbohydrates (energy) and protein and also contain enough water satisfying most of the total needs of ungulates may be fruits, cabbage and vegetables (REHBINDER and CISZUK 1985), and by-products of these, among others tomato pomace. Based on literature data, crude protein/crude fibre (CP/CF) ratio of tomato pomace is about 0.9. Its advantage is that it derives from tomato lands, so it does not require more land from forests (ecological footprint). However, production of tomato pomace does not overlap with the time of feeding wildlife, so it has to be preserved. Artificial drying increases the price of pomace substantially; however, its fermentation preserves the valuable nutrient content of pomace (carotene), moreover it provides vegetation water for the animals in wintertime. According to CHEDLY and LEE (2000), DENEK and CAN (2006), and SARGIN és DENEK (2017) it can be ensiled without additives. However, HADJIPANAYIOTOU (1994), BARROSO et

al. (2005) and GALLÓ et al. (2013a) suggested use of additives (absorbents) that increase the low dry matter content of pomace to reduce the nutrient loss in the form of effluent, moreover, using of additives can improve the conditions of the fermentation (MIRZAEI-AGHSAGHALI and MAHERI-SIS 2008; SARGIN and DENEK 2017), and the nutritive value and energy content of this by-product (GALLÓ et al. 2012).

When the aim of its use as a supplementary fodder for wildlife, use of such additives is suggested, that increase the low dry matter content of pomace, and decrease its crude protein content to a lesser degree than its crude fibre content, so it increases the CP/CF ratio, moreover it provides energy for the animals. Such additives may be primarily cereal grains.

Moreover the aspects of wildlife management should be taken into account; the storage and transport affect the quality of fodders. In the case of purchased feed, their storage and transport to feeding plots have to be solved even in extreme weather conditions. Furthermore, feed-out also affects the quality (OROSZ 2012). Taking into account the demands of wildlife management, a suitable method can be a relatively new development, the special baling technology (OROSZ et al. 2008a), that makes the large-scale ensiling technique, storage and transportation of this material possible. Chopped and bulky raw materials, which are not baleable with the conventional baling technique, can be fermented and/or storage easily with this new technique as a baled silage even in a small quantity, so the utilisation of such materials becomes possible in small and medium scales (OROSZ et al. 2008a; OROSZ et al. 2008b). One of the advantages of the baled silage compared to the conventional one is the density of the bale (700-830 kg/m³). Excellent bulky density can be achieved by this method, which provides advantegous anaerob condition for the fermentation. Further advantage of this method is the homogeneity of the raw material, furthermore baled silage does not require a permanent place to store, and the silo opening can be achieved easily (OROSZ 2007). Based on all this, this ensiling method can be integrated to the practice of wildlife management, however it is important to keep technological discipline.

1.1. AIMS

Based on considering the needs of game species, the advantages of tomato pomace and aspects of wildlife management, the main points of my thesis are as follows:

- 1. Fermentability of tomato pomace without and with additives (adsorbents) that increase the dry matter content of pomace.
 - Does the tomato pomace ferment without additives and what quality of end-product does it produce?
 - What additives should be used and how do the additives affect the fermentation?

- In what proportion should additives be added to the pomace so that the dry matter content is optimal for fermentation?
- Does use of silage inoculants improve fermentation?
- 2. Testing of large-scale ensiling technique of tomato pomace in wildlife management special baling technology.
 - Can the special baling technique be used to preserve tomato pomace?
 - Does the large-scale ensiling technique ensure the optimal conditions of fermentation and long-term storage?
 - How can storage and transportation fit into wildlife management practice?
- 3. Aerobic stability of tomato pomace silage.
 - How quickly does the silage start to deteriorate when exposed to air?
 - How does the additive affect the deterioration process?
 - Can silage inoculants improve the aerobic stability of silage?
- 4. Feeding study of consumption of tomato pomace by wild ruminants.
 - Do game species consume tomato pomace silage at all?
 - What is the consumption rate of tomato pomace silage compared to the other supplementary fodders?

The ultimate goal is the rational utilization of tomato pomace produced in large quantities but in a short period as a by-product of the canning industry.

2. MATERIAL AND METHODS

Analyses presented in my dissertation were carried out in four experiments. Following the second experiment, a feeding study was carried out.

2.1. ANALYSIS OF FERMENTATION PARAMETERS OF TOMATO POMACE ENSILED ALONE AND MIXED WITH DRIED WHOLE SEED WHEAT, TYPE OF SILO: METAL BARREL

Fresh tomato pomace was used; the ensiling was carried out in metal barrels. The bulk fresh material was put into metal barrels with a capacity of 200 litres with partial pre-compaction and the barrels were closed with air-tight lids. Experimental treatments were designed as follows:

- Tomato pomace without any additive as control, marked as **TP**.
- Tomato pomace without any additive covered with 1 kg/barrel salt (NaCl), marked as **STP** (salted tomato pomace).
- Mixture of tomato pomace and dried whole seed wheat at 20% (w/w) on a fresh matter basis and covered with 1 kg/barrel salt (NaCl), marked as **STPWM** (salted tomato pomace-wheat mixture).
- Mixture of tomato pomace and dried whole seed wheat at 20% (w/w) on a fresh matter basis, treated with silage inoculant (LAB) and covered with 1 kg/barrel salt (NaCl), marked as **ISTPWM** (inoculated and salted tomato pomace-wheat mixture).

The purpose of the whole seed wheat addition was to reduce the risks of effluent production and undesirable fermentation processes, moreover to increase the energy content (easily fermentable carbohydrates) of tomato pomace. The purpose of the salt (NaCl) covering was to reduce aerobic spoilage on the top surface of the pomace.

The silage inoculant contained lactobacillus species (*Lactobacillus acidophylus* and *Enterococcus faecium*). The number of colony forming units in inoculant was 10⁷ CFU/g; dose 10 kg/ton fresh material, the carrier was wheat bran. The silage inoculant had been developed and was owned by Hungaromix Ltd. (Komárom, Hungary). On the 100th day of fermentation samples were taken from the barrels. Laboratory analyses were made in accordance with Hungarian Standards.

Physical evaluation: subsidence, thickness of spoiled layer, sensory evaluation

Chemical analyses:

- Raw materials: nutrient content, fibre fraction, sugar-content
- *Silage*: pH, nutrient content, sugar-content, lactic and volatile fatty acids, aerobic mesophyl bacteria (AEMB) and moulds

2.2. EXPERIMENT 2: ANALYSIS OF FERMENTATION PARAMETERS OF TOMATO POMACE MIXED WITH DRIED GROUND MAIZE GRAINS, TYPE OF SILO: BALED SILAGE

Fresh tomato pomace was mixed with dried ground maize grains at 20% (w/w) on a fresh matter basis. Baling was carried out using a Göweil LT Master fixed-chamber baler-wrapper machine. Experimental treatments were as follows:

- Mixture of tomato pomace and dried ground maize grains in 20% (w/w) on a fresh matter basis as control (6 bales), marked as **TP+GMG** (tomato pomace+ground maize grains)
- Mixture of tomato pomace and dried ground maize grains (20% (w/w) on a fresh matter basis) treated with 0.5% salt (feed salt for animals; S; 8 bales), marked as **TP+GMG+S**
- Mixture of tomato pomace and dried ground maize grains (20% (w/w) on a fresh matter basis) treated with enzyme-supplemented silage inoculant (SI; 9 bales), marked as **TP+GMG+SI**.

The salt was feed salt for animals. The purpose of salt addition was to increase the mineral content of the silage fed during wintertime, and to determine the possible antibacterial and antifungal effect of the salt. The silage inoculant (Sil-All 4x4TM) had been distributed by Alltech Hungary Inc., it contained *Enterococcus faecium, Pediococcus acidilactici, Lactobacillus plantarum, Lactobacillus salivarius* as well as enzymes (amylase, hemicellulase, cellulase and pentosanase). The number of colony forming units in inoculant was 10¹¹ CFU/g. The inoculant was applied at 5 g/tonne of fresh material to provide 10⁵ CFU/g of fresh material. On the 70th day of fermentation the baled silages were opened and samples were taken for laboratory analyses. Laboratory analyses were made in accordance with Hungarian Standards.

Physical evaluation: sensory evaluation, amount of effluent

Chemical analyses:

- *Raw materials*: crude protein, crude fibre, starch, aerobic mesophyl bacteria (AEMB) and moulds
- *Silage*: crude protein, crude fibre, starch, carotene, pH, lactic and volatile fatty acids composition

Feeding study

The feeding study was conducted in a hunting preserve at Baláta Hunting Company in Bodony, Hungary. The hunting preserve located in the North Hungarian Mountains. This fenced area was 275 ha. There were red deer (*Cervus elaphus*), fallow deer (*Dama dama*), mouflon (*Ovis aries*) and wild boar (*Sus scrofa*).

All treatments for tomato pomace baled silage were placed in three feeding plots during the feeding period. Maize silage was also placed in the same feeding plots in order to assess which

forage was preferred by the game species. Maize grain was scattered on the ground for wild boars several times. A week after placing the two different types of silage (tomato pomace vs. maize silage) in the feeding plots, ten individual fresh faecal pellet groups were collected from each game species from each feeding plots along 8 transect lines, which were 1 m wide and 50 m long and starting at the feeding plot, from which we determined the attendance of the feeding plots. Only one sample of the close-fitting (2-3 m) faecal groups was taken, reducing the chance of collecting more samples from one individual. The samples collected from each species were treated as a composite sample, from which three sub-samples were taken and analysed. Five categories were determined: tomato, maize (silage and grain), monocotyledonous (grasses), dicotyledonous (herbs) and woody plants.

The diet composition was determined by microhistological faecal analysis (KATONA and ALTBÄCKER 2002) using a microhistological anatomical key (MÁTRAI et al. 1986). According to ALIPAYO et al. (1992) faecal analysis can be a useful tool to estimate diet composition of ruminants, accuracy of faecal analysis is little influenced by digestibility. KATONA et al. (2014) reported that microscopic analysis could be more accurate than the macroscopic one to reveal the consumption of supplementary feed by ungulates.

2.3. EXPERIMENT 3: ANALYSIS OF FERMENTATION PARAMETERS OF TOMATO POMACE MIXED WITH DRIED GROUND MAIZE GRAINS IN DIFFERENT PROPORTIONS (TYPE OF SILO: MINI-SILO: PLASTIC BUCKET)

The aim of the mini-silo experiments was to refine the ensiling methods of tomato pomace, which proved to be an appropriate wildlife feed. Fresh tomato pomace was mixed with dried ground maize grains. The ensiling was carried out in snap-lock plastic buckets with a capacity of 11.5 litres and sealed with plastic lids. The aim of the study was to determine the optimal proportion of dried ground cereal (maize grains) within tomato pomace for ensilage. Experimental treatments were designed as follows:

- Tomato pomace without any additive as control, marked as **TP**.
- Mixture of tomato pomace and dried ground maize grains (20% (w/w) on a fresh matter basis), marked as **TP+GMG 80:20**.
- Mixture of tomato pomace and dried ground maize grains (30% (w/w) on a fresh matter basis), marked as **TP+GMG 70:30**.

On the 5th, 19th and 264th days of fermentation samples were taken from the buckets (5 buckets/treatment). Laboratory analyses were made in accordance with Hungarian Standards.

Physical evaluation: thickness of spoiled layer on the top surface of the silage, subsidence, sensory evaluation

Chemical analyses:

- Raw materials: crude nutrients, fibre fractions, starch-, carotene and sugar-content
- Silage samples from different silo opening: pH, lactic and volatile fatty acids composition, aerobic mesophyl bacteria (AEMB) and moulds

2.4. EXPERIMENT 4: ANALYSIS OF EFFECT OF DRIED GROUND WHEAT GRAINS OR MAIZE GRAINS AND SILAGE INOCULANT ON FERMENTATION PARAMETERS OF TOMATO POMACE (TYPE OF SILO: MINI-SILO: GLASS JAR)

Fresh material was manually compacted into screw cap glass jars with a capacity of 1.7 litres and sealed with metal lids ensuring airtight closure. Inside each lid a rubber ring seal was placed to eliminate oxygen. Silos were packed to a density of about 1000 kg/m³ (fresh material). Experimental treatments were designed as follows:

- Tomato pomace (TP) without any additive as control, marked as **TP**.
- Tomato pomace treated with silage inoculant (SI), marked as **TP+SI**.
- Mixture of tomato pomace and dried ground wheat grains (GWG) at 20% (w/w) on a fresh matter basis, marked as **TP+GWG**.
- Mixture of tomato pomace and dried ground wheat grains (GWG) at 20% (w/w) on a fresh matter basis treated with silage inoculant (SI), marked as **TP+GWG+SI**.
- Mixture of tomato pomace and dried ground maize grains (GMG) at 20% (w/w) on a fresh matter basis, marked as **TP+GMG**.
- Mixture of tomato pomace and dried ground maize grains (GMG) at 20% (w/w) on a fresh matter basis treated with silage inoculant (SI), marked as **TP+GMG+SI**.

The silage inoculant contained homofermentative lactic acid bacteria strains. The number of colony forming units in inoculant was 10^{11} CFU/g. The inoculant was applied at 5 g/tonne of fresh material to provide 10^5 CFU/g of fresh material. The carrier was maltodextrin, the distributor was ChemSystAnt Ltd.. On the 119^{th} day of fermentation the jars were opened and samples were taken for laboratory analyses. The laboratory analyses were made in accordance with Hungarian Standards.

Physical evaluation: thickness of spoiled layer on the top surface of the silage, subsidence, sensory evaluation

Chemical analyses:

- *Raw materials*: crude nutrients, fibre fractions, starch- and sugar-content, buffering capacity
- *Silage*: crude nutrients, fibre fractions, starch- and sugar-content, pH, lactic and volatile fatty acids composition, NH3-N, ADIN, aerobic mesophyl bacteria (AEMB) and moulds

During the fermentation process, gross weight of the jars was measured twice a week. During fermentation losses appeared in the forms of effluent and gases, fermentation loss was calculated at the end of the experiment. On the 119th day the silos were opened and samples were taken from jars (5 jars/treatment) for laboratory analyses and aerobic stability. Aerobic stability test was carried out on ambient temperature (20°C) for 10 days to know how quick the silages spoil under aerobic conditions. During aerobic stability test, changes of temperature were recorded and pH value of samples on a daily basis was determined.

2.5. STATISTICAL EVALUATION OF THE RESULTS

Data processing, tables and diagrams were created using Microsoft Office 2010 Excel. Statistical evaluation of the data was carried out using IBM SPSS Statistics 22.0 software. Variance analysis (ANOVA) was used to compare the results. Based on the results of the homogeneity analysis, Tukey or Dunnett tests were used for comparing the means. Correlation analysis (Pearson-correlation) was used to prove the correlation of given parameters. In the case of correlation analysis, the value of 'r' and its significance level are presented. To evaluate correlation, categories written by SVAB (1981) were used:

<0,4	weak relationship
0,4-0,7	moderate relationship
0,7-0,9	strong relationship
>0,9	very strong relationship

Variance analysis was also used for statistical evaluation of the feeding study.

3.RESULTS

3.1. FERMENTATION RESULTS OF TOMATO POMACE ENSILED ALONE AND MIXED WITH DRIED WHOLE SEED WHEAT – THE FIRST EXPERIMENTAL RESULTS

The pH was adequate for all treatments (p>0.05). Fermentation intensity of TP and STP was moderate with moderate lactic acid and slightly higher acetic acid concentration (*Figure 1*.). Salt on the top had no significant effect on fermentation (lower lactic acid and higher acetic acid content, p>0.05); however, it decreased numerically the number of moulds compared to control (p>0.05). Butyric acid was found in the case of TP and STP. Moderate negative and significant correlation was found between the dry matter and butyric acid content (r=-0.511; p=0.043).

Mixing of 20% dried whole seed wheat significantly reduced the volatile fatty acid concentration (p<0.05), including acetic acid concentration. The silage inoculant had a negative effect on fermentation (ISTPWM). Mould count and acetic acid content were significantly higher (p<0.05), in addition numerically higher propionic acid content was also found in ISTPWM compared to STPWM (p>0.05). A strong positive and significant correlation was found between pH value and propionic acid content (r=0.803, p=0.000). Microbial status of all treatments (based on numbers of aerobic mesophilic bacteria and moulds number) was good.

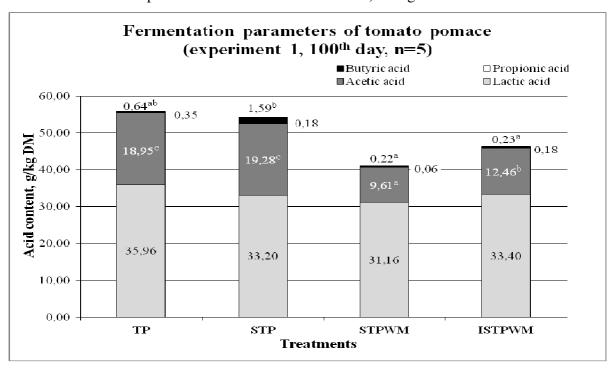


Figure 1: Fermentation profile of tomato pomace silage and mixed silages containing tomato pomace and dried wheat seeds

a, b, c – the different letters indicate significant difference (p<0.05)

TP – tomato pomace; STP – salted tomato pomace; STPWM – salted tomato pomace wheat mixture; ISTPWM – inoculated, salted tomato pomace wheat mixture

3.2. EXPERIMENT 2: FERMENTATION RESULTS OF TOMATO POMACE MIXED WITH DRIED GROUND MAIZE GRAINS, TYPE OF SILO: BALED SILAGE

High bale weight (1120±12.6 kg/bale), high density (355±4.0 kg DM/m³) and low density deviation were achieved with the new technology due to the high pressurization (130 bars) and small particle size. Low fermentation intensity was found in the treatments with low lactic acid and acetic acid concentrations (*Figure 2*.). Treatment with salt increased the butyric and propionic acid concentration of the silage compared to control, therefore the application of salt is not recommended. A strong negative and significant correlation was found between lactic acid and the pH value (r=-0.750, p=0.020), as well as strong positive and significant correlation was found between butyric acid and pH value (r=0.808, p=0.008). Based on the pH value, the absence of butyric acid and the total acid content of inoculated treatment (TP+GMG+SI), its fermentation was the most favourable.

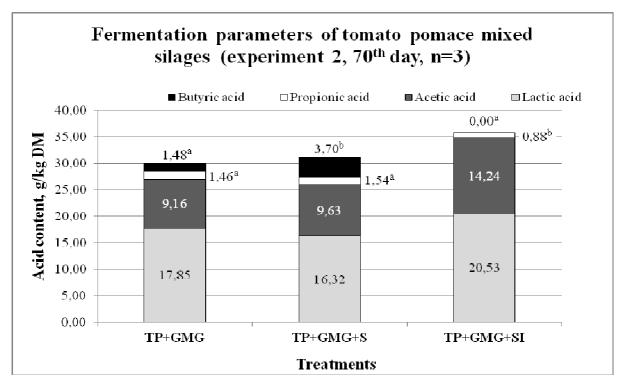


Figure 2: Fermentation profile of tomato pomace silage and mixed silages containing tomato pomace and dried ground maize grains

 $a,\,b-the\,\,different\,\,letters\,\,indicate\,\,significant\,\,difference\,\,(p{<}0.05)$

TP+GMG – tomato pomace+ground maize grains; TP+GMG+S – tomato pomace+ ground maize grains+salt; TP+GMG+S –tomato pomace+ ground maize grains+silage inoculant

3.2.3. Results of the feeding study

Tomato pomace ensiled with 20% ground maize grains was a new type of feed for game species. Despite that, tomato pomace silage was consumed almost immediately, its total amount disappeared within two days after feeding, while maize silage remained there even for one week. In

the case of red deer and wild boar, consumption of tomato pomace dominated over the whole period, since the proportion of tomato items was significantly higher compared to the other plant fragments in the faeces (p<0.01 in the case of red deer and p<0.05 in the case of wild boar). With the exception of March, tomato pomace mixed silage was the most common component in the diet of red deer and fallow deer (*Figure 3*.). It was also important in the diet of wild boar. Tomato fragments occurred rarely in the faeces of mouflon between November and January, but were frequent in February and March. The most common component in its diet was monocot and woody plant material. In the case of red deer and wild boar, the proportion of tomato items was significantly higher compared to the other plant fragments in the faeces. The proportion of maize silage in the faeces was low in almost all cases (red deer 0.9-8.4%; fallow deer: 0.6-7.7%; mouflon: 2.6-6.6%). Its presence was the highest in case of wild boar (5.7-33.3%), although it did not exceed the proportion of any other fragments in the faeces.

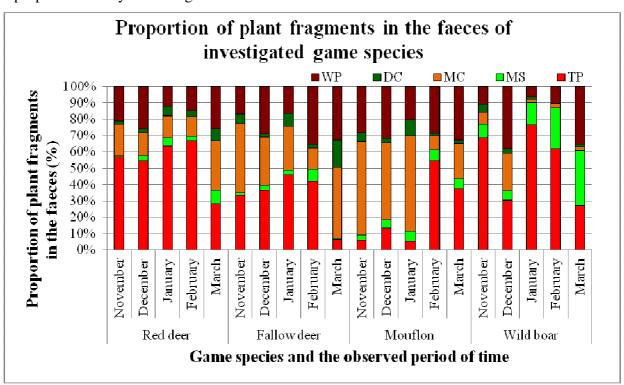


Figure 3: Proportion of different food categories in the faeces of ungulate species in the study period

TP – tomato pomace silage, MS – maize silage, MC – monocotyledon, DC – dicotyledon, WP – woody plants

3.3. EXPERIMENT 3: FERMENTATION RESULTS OF TOMATO POMACE MIXED WITH DRIED GROUND MAIZE GRAINS IN DIFFERENT PROPORTIONS (MINI-SILO: PLASTIC BUCKET)

On the 5th day of fermentation, the pH values of all treatments were high at the dry matter content of the silage. The pH values increased with the increasing proportion of dried ground maize grains in pomace. A strong positive and significant correlation was found between the proportion of maize in pomace and the pH value (r=0.770, p=0.015). Total acid content was low in all treatments,

with low lactic acid and almost the same amount of acetic acid content (*Figure 4*.). Butyric acid was found in TP+GMG 80:20. Initial fermentation of TP+GMG 70:30 was slow; butyric acid was not detectable on the 5th day of fermentation (1st silo opening). Dry matter content of this treatment was above 40%, this was not optimal for butyric acid bacteria due to osmotic conditions.

On the 19th day of fermentation (2nd silo-opening), the pH value of control treatment (TP) slightly increased indicating undesirable fermentation processes. The pH values of the two mixtures decreased, it was adequate in TP+GMG 80:20; however it was not significant because of the high variance. The pH value of TP+GMG 70:30 also decreased, but it was still high. Total acid content of TP and TP+GMG 70:30 did not change significantly compared to the previous silo opening, however volatile fatty acid composition deteriorated (increased acetic acid content). Lactic acid significantly increased in TP+GMG 80:20 compared to the previous silo opening (p<0.01), so changes in total acid content was favourable. However, it contained a considerable amount of butyric acid (21% of the total acid content).

On the 264th day of fermentation (3rd silo-opening), the pH value of control treatment significantly increased compared to the previous silo-opening (p<0.01), indicating the instability of this treatment. TP (control) treatment had presumably undergone a clostridial fermentation based on the continually increasing pH value, acetic and butyric acid content as well as decreasing lactic acid content. Consequently, almost all the lactic acid content disappeared for the 264th day. The pH value of the mixtures decreased compared to the second silo opening (19th day), it was significant in the case of TP+GMG 70:30 (p<0.01). The slow decrease of the pH value could be explained by the slow production of lactic acid. Lactic acid bacteria require an adequate content of fermentable substrate in the form of WSC, but in this case its amount was limiting. On the 264th day (last silo opening) lactic acid content increased in a higher proportion than its acetic acid content, and butyric acid content decreased, so changes in acid composition were favourable; however total acid content was still low.

Mixing of 20% ground maize grains improved the fermentation, but the fermentation process was not favourable. Although total acid content exceeded the value of 100 g/kg DM, it contained considerable amount of butyric acid (8.21% of the total acid content). Due to the slowly decreasing pH value and the slow production of lactic acid it could be assumed that the conditions of fermentation were not optimal, which could be caused by inadequate density

The microbial status of the different treatments was adequate. The total number of anaerobic bacteria and mold number were under the critic value at each silo openings. Number of aerobic mesophyl bacteria and moulds changed with the increasing proportion of maize in pomace (correlation between AEMB and the proportion of maize grains r=0.872, p=0.002; correlation between mould numbers and the proportion of maize grains r=0.714, p=0.031).

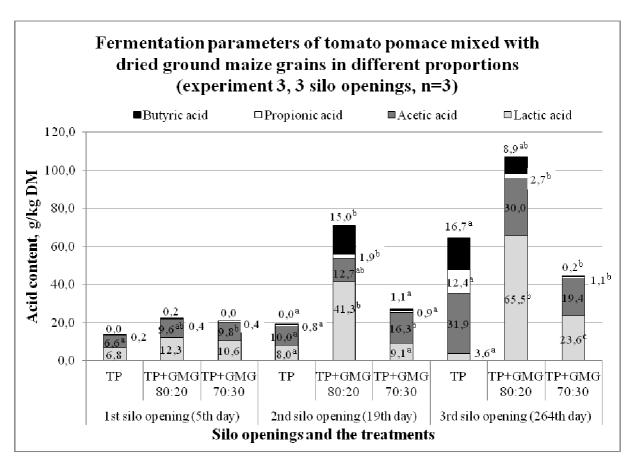


Figure 4: Fermentation profile of tomato pomace silage and mixed silages containing tomato pomace and dried ground maize grains

a, b, c – the different letters indicate significant difference (p<0.05)

TP – tomato pomace as control, TP+GMG 80:20 – tomato pomace+20% ground maize grains; TP+GMG 70:30 – tomato pomace+30% ground maize grains

3.4. EXPERIMENT 4: ANALYSIS OF EFFECT OF DRIED GROUND WHEAT GRAINS OR MAIZE GRAINS AND SILAGE INOCULANT ON FERMENTATION PARAMETERS OF TOMATO POMACE (MINI-SILO: GLASS JAR)

High density was achieved; it was similar to that of the baled silage (355±4.0 kg DM/m³). Changes in nutrient content originated from fermentation and respiration losses, in addition effluent may have caused changes in nutrient content. A strong negative and significant correlation was found between dry matter content and fermentation losses (r=-0.961; p=0.000).

The pH value of the mixtures (TP+GWG, TP+GWG+SI; TP+GMG, TP+GMG+SI) was good, while that of control (TP) and TP+SA was a little bit high with this dry matter content. Similarly to previous experiments, low fermentation intensity was found in all treatments except in TP+GWG. Low lactic acid, high acetic acid and considerable amount of butyric acid contents were found in control TP and its pair, TP+SI. A strong negative and significant correlation was found between dry matter and butyric acid content (r=-0.869, p=0.000).

Total acid content of TP+GMG was the lowest with low lactic acid and high acetic acid

concentration; however, butyric acid was present only in traces (0.25% of the total acid content). Similarly to control, silage inoculant in TP+GMG+SA increased the acetic acid and propionic acid concentration (p>0.05).

Fermentation intensity of TP+GWG was similar to that of maize silage, total acid content of this treatment was about 103 g/kg DM. Mixing of ground wheat grains significantly increased the lactic acid and decreased the acetic acid, propionic and butyric acid concentration (p<0.01, butyric acid was 0.03% in total acid content). Silage inoculant in TP+GWG+SI significantly decreased the lactic acid and increased the acetic acid and propionic acid content (p<0.01), while butyric acid content did not change (0.02% of the total acid content) compared to its control pair (TP+GWG). The silage inoculant did not improve fermentation parameters of either of the treatments. Ammonia-N content (of total N) was under 10% in all treatments, and ethanol-content did not exceed the recommended value (15 g/kg DM). Microbial status of treatments was good.

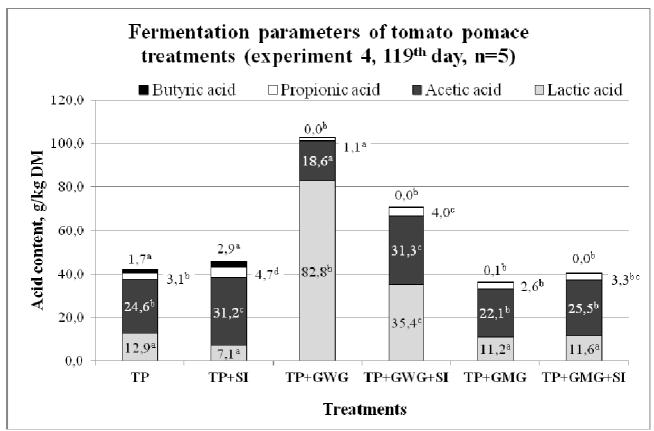


Figure 5: Fermentation profile of tomato pomace silage and mixed silages containing tomato pomace and dried ground wheat grains or dried ground maize grains

a, b, c, d – the different letters indicate significant difference (p<0.05) TP – tomato pomace as control; TP+SI – tomato pomace + silage inoculant; TP+GWG – tomato pomace + ground wheat grains; TP+GWG+SI – tomato pomace + ground wheat grains + silage inoculant; TP+GMG – tomato pomace + ground maize grains; TP+GMG+SI – tomato pomace + ground maize grains + silage inoculant

3.4.3. Aerobic stability test

The material was considered to be stable for a relatively long period of time in all of the

treatments. Mixing tomato pomace with ground cereals significantly increased the hours of aerobic stability (p<0.05). All mixture silages treated with silage inoculant improved significantly aerobic stability as indicated by increased stable hours compared to their control pairs. Changes in pH value followed the same pattern of changes as with the temperature, a strong positive and significant correlation was found between these two parameters (r=0.805; p=0.000).

3.5. NEW SCIENTIFIC RESULTS

- 1. Based on the results I determined that fermentation intensity of mixing tomato pomace with dried ground maize grains was low, volatile fatty acid composition was not favourable. However, butyric acid can be excluded from fermentation by achieving high density.
- 2. My analyses confirmed that silage inoculant applied in the experiments did not improve the fermentation. In the baled silage experiment the silage inoculant decreased the pH value and the butyric acid content, however it increased the acetic acid content of the silage similarly to the results of the other two treatments that is advantageous from the aerobic stability point of view, but not from the aspect of feed intake of animals.
- 3. I determined that tomato pomace silage was consumed by big game species. Consumption of tomato pomace by wild ruminants was above 35% in the examined period, while consumption of maize silage was barely above 4%.
- 4. I have justified that despite the high moisture content (75-80%), extremely low sugar content and heterogeneity of wet tomato pomace, it could be ensiled without any additive (absorbents). However, its pH value did not reach the critical value, the organic acid profile was not ideal, low lactic and high acetic acid content were typical for its fermentation, additionally butyric acid could also be detected even in high density; therefore an additive (absorbents) is recommended for ensiling.
- 5. I have found that fermentation intensity of mixed tomato pomace with dried whole wheat grains was low, but the volatile fatty acid composition was favourable. However, due to the softening of wheat grains, spoilage was found in the top layer of the silage, therefore adding whole seeds to the tomato pomace should be avoided. I have found that adding dried ground wheat grains to the pomace could be favourable for the fermentation.
- 6. I determined that tomato pomace ensiled alone (without absorbents) can be stable for a long period of time. However, adding dried ground cereals to the pomace significantly increased the aerobic stability of pomace. It had a significant role in wildlife management.

4. CONCLUSIONS AND FUTURE RECOMMENDATIONS

4.1. EXPERIMENT 1: EVALUATION OF THE FERMENTATION RESULTS OF TOMATO POMACE ENSILED ALONE AND MIXED WITH DRIED WHOLE SEED WHEAT (TYPE OF SILO: METAL BARREL)

4.1.1. Fermentation

Fermentation intensity of fresh tomato pomace was low, under anaerobic conditions its long term storage can only be solved by providing adequate conditions (GALLÓ et al. 2013b, 2013c) with a good microbial status, so the silage can be fed. Total acid content of control treatment (TP) was low (55.91 g/kg DM). HADJIPANAYIOTOU (1994) and BARTOCCI et al. (1980) also found low total acid content in tomato pomace silage; lactic acid/acetic acid (LA/AA) ratio was unfavourable in both cases. SARGIN and DENEK (2017) measured more favourable values; pH value and LA/AA were good presumably due to the adequate amount of water soluble carbohydrates, sugar content of their pomace was 16.92% in DM. In our study, total sugar content was only 2.65% in DM; it probably caused the low fermentation intensity, in addition the high protein content increased the buffering of the ensiling process and thus increased the risk of a poorer fermentation. In the experiment of WEISS et al. (1997) tomato pomace did not ferment, they concluded that it could be stored for at least two months without spoilage. We did not confirm this result, tomato pomace ensiled alone did ferment not only in the 1st experiment, but also in the other two experiments (3rd and 4th). Fermentation of STPWM and ISTPWM was adequate; their dry matter content was good for fermentation. The pH values of the mixtures were good, they reached the critical pH, and they could be considered stable. However, spoilage was found in the top 20 cm of the mixed silages compared to the control, it was presumably caused by mixing tomato pomace with wheat in the form of seed, therefore it is not recommended to add whole seed to the pomace due to the negative effect on the top layer (20 cm). The addition of dried ground cereal is suggested as an alternative (GALLÓ et al. 2012; 2013b). The silage inoculant decreased the butyric acid content, but it significantly increased the acetic acid content compared to STPWM, so it did not improve the fermentation. Many of the homofermentative inoculant bacteria use pentose sugars and produce among others acetic acid, so if there is a large proportion of pentose sugar in tomato pomace then the fermentation will be heterofermentative with a lot of acetic acid. That may have been one of the causes of the high proportion of acetic acid. The other cause may have been that the silage inoculant contained not only homofermentative but heterofermentative bacteria, which species produce not only lactic acid, but among others acetic acid.

4.2. EXPERIMENT 2: EVALUATION OF THE FERMENTATION RESULTS OF TOMATO POMACE MIXED WITH DRIED GROUND MAIZE GRAINS AND THE FEEDING STUDY (TYPE OF SILO: BALED SILAGE)

4.2.1. Fermentation

Similarly to the previous experiment, fermentation intensity was low in all treatments. Mixing feed salt to the mixture did not improve the fermentation (higher pH and butyric acid content). I do not confirm WOOLFORD's (1978) statement, that salt inhibited the growth of microbes, decreased the rate of pH decline and resulted in lower acid content in silages. In our study, the salt treatment (TP+GMG+S) contained more acids than control. I cannot confirm the statements of CAI et al (1997), who found that salt inhibits the growth of undesirable microorganisms, but not the growth of useful microorganisms, so it inhibits the proteolytic activity and growth of butyric acid, but not the growth of LAB, so salt improve fermentation. The lack of those favourable effects on fermentation may be the low salt concentration applied in our study (0.5%), which was not sufficient to inhibit the growth of butyric acid. The other reason may be that salt was feed salt (so had a different composition than common salt). Despite the high pH value it did not spoil presumably due to the high bale weight and density (355±4 kg DM/m³).

The third generation silage inoculant improved the fermentation; however, it was not as effective as expected presumably due to the lack of soluble carbohydrates. The inoculated silage had higher acetic acid content and LA/AA was the most unfavourable, however, the silage inoculant significantly decreased the pH value, butyric and propionic acid content, so it had a positive effect on fermentation.

Based on the results wet tomato pomace can be baled, under anaerobic conditions it was possible to store it for a long period with a good microbial status. The new baling system was able to form well-shaped and stable bales (130 bars) such a wet by-product as fresh tomato pomace with a small particle size (GALLÓ et al. 2017). High density was the reason of its palatability and good quality, so it is a practically applicable method for fermentation tomato pomace. The low fermentation intensity could be caused by the characteristics of the raw material and not technological reasons. Advantage of this baling system is quick wrapping and high density.

4.2.2. Feeding study – hunting preserves

The most important results of the feeding study was that tomato pomace silage was consumed almost immediately, its total amount disappeared within two days after feeding, while maize silage remained there for several days (even for a week). Despite the fact that game species in the hunting preserve (red deer, roe deer, mouflon and wild boar) had never eaten such silage before, the individuals investigated consumed it in a relatively high proportion, and they preferred it to the most common supplementary feed i.e. maize silage. No obvious impact of ensiling treatments on

consumption of tomato pomace silage or on health status of ungulates was recognised. I confirmed the statement of ROOSENDAAL (1992), DUBOIS and FRASER (2013), as well as FERNYE et al. (2013) who found that supplementary winter feeding could be very important for big game species in intensively managed hunting reserves with dense game populations.

The consumption rate of the supplementary fodder was much higher than it was found earlier in free-living deer populations (less than 10%, KATONA et al. 2010b; KATONA et al. 2014). It verified that we have to make distinctions between fenced and unfenced areas in terms of the importance of supplementary winter feeding. The food selection of wild ruminants corresponded to HOFMANN's categorisation (1989), who described red and fallow deer as intermediate feeders, meanwhile mouflon as a grass/roughage eater. Our results were in accordance with his classification, since red and fallow deer consumed the mixed diet with better quality with higher protein content and higher CP/CF ratio, while mouflon consumed the diets containing mostly monocot (fibrous) plant material. Another potential reason for those dietary differences might have been that red and fallow deer used the feeding plots more intensively than mouflon due to their potentially different strategies for avoidance for competition and predators. The low consumption rate of natural forage could be due to the fact that the dominant shrub species on the investigated area are blackthorn and hawthorn. These species are not preferred by game species. The low consumption of maize silage could indicate that this may not be an optimal supplementary feed for game species. Maize silage is rich in fibre, but its protein content is low. Despite the fact that game species consumed tomato pomace mixed silage almost immediately, however, it did not necessarily mean that it is an optimal supplementary food for them. It could be possible that it was the food, which was available in a greater amount in the hunting preserve in the observed period of time. However, if we accept that supplementary feeding is necessary in hunting reserves, then we have to feed such supplemental fodders, which meet the requirements of the animals. Tomato pomace may be a good solution; however, not all of the parameters of tomato pomace silage meet the nutrient requirements of ungulates. I emphasize the fundamental importance of the natural food resources of the habitat, such as the understory food supply, food plots or forest edge vegetation.

4.3. EVALUATION OF THE FERMENTATION RESULTS OF TOMATO POMACE MIXED WITH DRIED GROUND MAIZE GRAINS IN DIFFERENT PROPORTIONS (MINI-SILO: PLASTIC BUCKET)

This experiment was not very successful presumably due to inadequate density and imperfect fit of the lids on the buckets. Due to this latter fact, closure was not hermetic; oxygen continuously entered the silage. Although silage density of the treatments reached the desired DM density (240 kg DM/m³; OELBERG et al. 2006; HOLMES and MUCK 2007; CHARLEY 2008) with the exception of the control; however, based on our previous results higher density could be achieved in

the case of tomato pomace mixtures, so the DM density achieved was not adequate for good quality silage production. According to LEURS et al. (2004), secondary fermentation processes are closely correlated to insufficient compaction of the material. The sign of the deterioration process might have been the appearance of a white 'ring' on the surface of the material. It could be seen at the first silo-opening (5th day). Based on the microbial results, the materials were not mouldy; samples were taken from the core not from the surface of the silage.

On the 5th day of fermentation, the pH value of control treatment was under 5.0; however, it was high at the given dry matter content of the forage. It can be assumed, that this relatively low pH was not the results of fermentation, but the acid characteristics of tomato pomace. Based on the experimental results, fermentation intensity of tomato pomace ensiled alone was low, however, based on the total acid content measured on the last silo opening (264th day), it could be able to ferment as intensively as alfalfa silage.

Fermentation was intensive in TP+GMG 80:20 compared to the other two treatments; however, the pH did not reach the critical value fast enough presumably due to insufficient compaction and aeration of the material, consequently secondary fermentation processes began. Despite the secondary fermentation, the total acid content of this treatment was high. We did not experience such a high total acid content in the previous experiments. Based on the high total acid content of TP+GMG 80:20, tomato pomace mixed with 20% dried ground maize grains can be able to ferment as intensively as maize silage.

The initial slow fermentation of TP+GMG 70:30 was presumably due to the higher dry matter content (>40%). Silage fermentation is slower with increasing DM content because the low water activity (WHITER and KUNG 2001; HRISTOV and McALLISTER 2002; RIZK et al. 2005). Its lower total acid content compared to the other mixture confirmed the slow initial fermentation. WHITER and KUNG (2001) found that numbers of lactic acid bacteria (LAB) did not increase and pH did not decrease as rapidly in silages with a higher DM content compared to silages with a lower DM content.

In addition the plus 10% maize grains added to pomace compared to TP+GMG 80:20 did not increase the energy content to such an extent to justify mixing tomato pomace with grains in a larger proportion than 20%.

4.4. EVALUATION OF EFFECT OF DIFFERENT ADDITIVES AND SILAGE INOCULANT ON FERMENTATION PARAMETERS AND AEROBIC STABILITY OF TOMATO POMACE (MINI-SILO: GLASS JAR)

4.4.1. Fermentation

The pH of the control treatment (TP) and its pair (TP+SI) is a little bit high at the dry matter content of the silage. Despite the high density, the critical pH could not be achieved, and the pH was not as low as it was in the study of WEISS et al. (1997), DENEK and CAN (2006), as well as SARGIN and DENEK (2017). However, the pH in our study was more favourable than the pH in the study of HADJIPANAYIOTIOU (1994). According to WARD and ONDARZA (2008), wet silages that have undergone a long fermentation process sometimes contain higher levels of acetic acid (>3% DM). In addition butyric acid appeared in TP and TP+SI, presumably due to the low dry matter content (<30%). According to WARD and ONDARZA (2008), below 32% dry matter content, there is a probability of 55% or less that the material ferment without butyric acid. Above 32%, the probability of success can reach or exceed 74%. This is confirmed by the strong negative and significant correlation between dry matter and butyric acid content (r=-0.869, p=0.000). Low lactic acid and high acetic acid contents were found by BARTOCCI et al. (1980) and HADJIPANAYIOUTOU (1994), but not WU et al. (2014) and SARGIN and DENEK (2017), who experienced an intensive fermentation with a high lactic acid and low acetic acid content (LA/AA was more than 3.0).

The pH of the mixtures was adequate for the given dry matter content. With the exception of TP+GWG, fermentation intensity was low and similar to previous experiments with unfavourable lactic and acetic acid contents. The cause of the low fermentation intensity may have been the extremely low sugar content of the fresh materials. However, butyric acid content could be detected in traces presumably due to the high density.

Tomato pomace had a limited fermentation capacity; the homofermentative LAB inoculants used in my experiments did not improve the fermentation parameters of either low or high moisture tomato pomace silages. Inoculation of forages with homofermentative lactic acid bacteria can improve silage fermentation if sufficient fermentable substrate (water soluble carbohydrate) is available (KOC et al. 2009). Acetic content increased in inoculated silages, consequently decreased the LA/AA ratio. The reason of that may be that not only homofermentative, but heterofermentative bacterial strains were in the inoculant, concretely *Lactobacillus buchneri*, which produces a lot of acetic acid among others. It is necessary to keep in mind that the success of microbial additives depends on many factors, such as the type and properties of the crops to be ensiled, epiphytic

microflora, ensiling skill, the properties and inoculation level of the inoculants, climatic conditions and moisture content of the forage (SADEGHI et al. 2012).

The fermentation was very intensive in TP+GWG compared to the other treatments, with high lactic and relatively low acetic acid content. The reason for this may be that the sugar content, which was also extremely low in this treatment, was a little bit higher than that of the control TP and TP+GMG. Changes in nutrient content during fermentation, especially hydrolysis of fibre content can produce additional sugar for lactobacilli (HUISDEN et al. 2009). This can explain the more intensive fermentation. Tomato pomace mixed with dried ground wheat grains at 20% (w/w on a fresh matter basis) was able to ferment as intensively as maize silage, and the result is well preserved silage with low pH, low ammonia-N and low concentration of butyric acid.

The ammonia-N concentration in silage reflects the degree of protein degradation. The ammonia-N concentration was higher in inoculated treatments compared to their control pairs, however, it did not exceed the limit value of 11% (of total N) recommended by UMANA et al. (1991) or 9% (of total N) suggested by PHIRI et al. (2007).

4.4.2. Aerobic stability of tomato pomace silage

The changes occurring during the aerobic feed-out phase are at least as important as those taking place in the anaerobic storage phase from the preserving nutrients and maintaining good quality point of view until fed to the animal. A target for potential silage aerobic stability is 7 days including time in the feed trough (WILKINSON and DAVIES 2013). Tomato pomace silage could be considered a relatively aerobically stable material. Aerobic stability was improved by mixing tomato pomace with dried ground wheat grains or dried ground maize grains. Based on the results it seemed that the silage inoculant improved the aerobic stability, since the temperature and also the pH increased at a slower rate compared to TP and the control pairs of the treatments. However, this presumably was not due to the effect of the inoculant, but the fact, that the inoculant increased the acetic acid content in all treatments, which is an inhibitor of the growth of yeasts and moulds (MOON 1983). The amount of undissociated acetic acid has been identified as the most important factor to consider when attempting to inhibit the growth of yeasts (COURTIN and SPOELSTRA 1990; WEISSBACH 1996). In TP and TP+SI, the presence of butyric and propionic acid are additional factors, which contributed to the long stability during exposure to air. Many silages that contain higher concentrations of acetic, propionic and butyric acids tend to be more stable when exposed to air (KUNG 2010b). Further reason for the long-term stability of tomato pomace silages (control and mixtures) were the absence of moulds and the relatively low number of bacteria, since unspoiled conditions are affected by temperature and the microorganisms in silages (yeasts, moulds and bacteria) (SZŰCSNÉ 2007). Therefore, silage deterioration indicators are pH, temperature change and increase in yeast and mould numbers (KOC et al. 2009).

The pH is an indicator of aerobic deterioration of the silage because the lactic acid is consumed by yeasts during aerobic exposure, and the silage becomes favourable to the growth of other undesirable microorganisms such as moulds and bacteria (BASSO et al. 2012). It should be kept in mind that the aerobic stability test was conducted at room temperature (20±0.5°C), while the supplementary feeding is typically in winter at colder temperatures during wildlife management, and the intensity of the deterioration process in silages is strongly influenced by temperature (KAKUK and SCHMIDT 1988), so longer stability of tomato pomace silage can be expected in winter. However, if results of the feeding study are considered, the tomato pomace silage was consumed almost immediately, its total amount disappeared within two days after feeding; the aerobic stability results of tomato pomace silage can be considered very favourable.

4.5. PRACTICAL SUGGESTIONS

Based on the results, tomato pomace could be ensiled, and the end-product silages could be fed, it did not cause any health problems to animals. The ensiling process and technology are crucial in terms of nutrition. Baled silage presented a relatively small risk; but my opinion is that silage bags may be another potential method for fermentation and storage of tomato pomace. High density and airtight closure are also critical points in this case.

Tomato pomace can be ensiled alone, but it is not advisable. Because of its high moisture content appearance of butyric acid is almost inevitable, it is advisable to use such additives that increase the low dry matter content of pomace. If the aim is to use it as feed for game species, grains can be used as additives; the recommended mixing ratio is 20% (w/w on a fresh matter basis).

Feed salt added at a concentration of 0.5% to the mixture of tomato pomace and ground maize grain affected the fermentation negatively, so mixing tomato pomace with feed salt is not recommended either in a smaller or a higher concentration. However, common salt can improve the palatability of the forage, it can be attractive for the animals, moreover, it can increase the mineral content of the feed. For this reason, I consider it necessary to analyse the effect of salt on fermentation and its utilisation in different concentrations.

The extremely low sugar content of tomato pomace was not increased to such an extent by adding dried ground grains at 20% that it would produce sufficient lactic acid required to achieve stable silage. Therefore, it is recommended to add such an additive that increases water soluble carbohydrates content of the mixture. There are more additives, which can increase the WSC content of a material, e.g. malt, molasses or dried sugar beet pulp. The necessary amount of these material is not available presently on the market for ensiling with them, in addition they are very

expensive. The price is an important factor in game management. Another solution may be the using of hydrolyzed maize grains, RIGÓ et al. (2010) experienced favourable experimental results with the using of that. However, it is very expensive, because it has to be produced at first. My opinion is that dried apple pomace may be a potential solution. The price of apple pomace is higher than that of grains, but it is cheaper than molasses or malt. Using apple pomace increases the cost of the fodder, however, the end-product would be good-quality silage with relatively high protein content that can satisfy the needs of game species and increase the likelihood of a stable fermentation process.

Based on the results of the treatments, I do not recommend using a silage inoculant. The inoculants studied here improved the fermentation on one occasion, in the baled silage treatment (lower pH, absence of butyric acid); however, good quality silage could be made without inoculation by achieving high density and airtight closure. However, it may be that the inoculants we tested were not the best for tomato pomace ensiling and other inoculants on the market may have a more successful ensiling capability with tomato pomace.

Tomato pomace cannot be inserted in the category of "traditional forage", its fermentation is affected by such factors, which factors have not been known yet.

4. PUBLICATIONS

5.1. PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

Publications in scientific journals with impact factor:

<u>GALLÓ J.</u>, FERNYE CS., OROSZ SZ., KATONA K., SZEMETHY L. (2017): Tomato pomace silage as a potential new supplementary food for game species. *Agricultural and Food Science* 26 (2): 80-90. p.

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