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Effect of Yogurt Acid Whey on the Quality of Maize Silage

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Abstract: The increasing popularity of Greek yogurt generates large amounts of acid whey worldwide. The use of yogurt acid whey in animal nutrition is limited. The aim of this study was to determine the effect of a yogurt acid whey powder (YAWP) addition to maize forage prior to ensiling on the nutritional, microbial and fermentation quality of maize silage. Depending on the addition level of the YAWP to maize forage, there were the following four experimental treatments: YAWP 0, 2.5, 5 and 10% *w*/*w*. An increasing YAWP inclusion level linearly increased the maize silage dry matter, crude protein and ash concentrations, whereas it reduced the crude fiber, neutral-detergent fiber and acid-detergent fiber concentrations. The silage pH decreased quadratically with the increasing YAWP level, with the lower plateau noted for the YAWP 5% addition. Concentrations of total bacteria in the silage and *Lactobacillus* spp. decreased linearly with the YAWP increase. The silage acetic acid content decreased linearly, whereas propionic acid, lactic acid and the ratio of lactic to acetic acid increased linearly with the increasing YAWP level. The ammonia-N content decreased linearly with the increasing YAWP level. In conclusion, the incorporation of the 5 and 10% YAWP addition in silage preparation improved the nutritional and fermentative quality of the produced silage.

Keywords: maize silage; yogurt acid whey; ammonia-N; pH; lactic acid; volatile fatty acids

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1. Introduction

Ensiling is widely used as a fresh-forage-preservation method all around the world. Silages are commonly used as livestock feed because they are nutritious and can be available during the whole year. Silage ensures a high milk yield in ruminants [1,2], the beneficial modification of the milk fatty acid profile [3,4] and meat quality [5,6]. Moreover, silages keep the animals healthy when pasture growth is inadequate in relation to animal requirements [7]. Among the types of silages used in ruminant feeding, maize silage is the most common, especially in dairy cattle diets, since, despite its relatively low protein content, it is a low-cost fiber and energy feed [2,8].

Silage quality depends on various preharvest and postharvest factors [9,10] that can largely affect the final product both nutritionally and/or microbiologically. Due to this, microbial inoculants composed of lactic acid bacteria [11–13] or acids, such as formic, propionic or citric acid [7,14,15], are also incorporated into the forage biomass prior to the ensiling process for the preparation of the fermentation mixture, aiming to ensure the proper development of silage fermentation, the reduction of nutrient losses and the inhibition of the growth of undesirable microorganisms.

Byproducts from the food industry (e.g., molasses or cheese whey) have also served as alternative additives in ensiling processes [16,17]. Dairy byproducts, such as whey (produced by cheese), have been applied in alfalfa silage [17] and maize silage [18], improving the quality of silages, such as their chemical composition. Acid whey (produced from cheese) was used as a rehydration agent of maize grains, which resulted in the enhancement of the fermentation process and the aerobic stability of the feed after 30 days of ensiling [19].

Acid whey is a byproduct from the production of acid-coagulated cheeses, but also from strained (Greek) yogurt. In Greece, strained yogurt is a high-nutritional and consumer-desirable product with growing consumption [20]. The increased demand for strained yogurt creates a considerable volume of acid whey, as for every 100 L of milk used, 70 L of yogurt acid whey is produced [20] that must be either disposed of or repurposed. Recent research has focused on alternative uses for yogurt acid whey in food processing [21–23] and in microbial cultivation [24]. Given the main ingredients of yogurt acid whey, including lactose (40 g/kg), organic acids (lactic acid, 6.5 g/kg, citric acid 1.8 g/kg) functional proteins (e.g., β-lactoglobulin, α-lactalbumin, lactoferrin) and minerals (potassium, >1500 mg/kg, calcium, >1200 mg/kg and phosphorus, >600 mg/kg), with pHs of 3.5–4.5 [21,25], its use as a silage additive/component merits investigation.

The aim of this study was to determine the effect of a yogurt acid whey powder (YAWP) addition on the quality of maize silage. For this purpose, four YAWP addition levels (0, 2.5, 5 and 10% *w*/*w*) to maize forage material prior to ensilation were used, and the nutritional, microbiological and fermentation quality of the plant material and YAWP mix before and after ensiling were conducted.

2. Materials and Methods

2.1. Preparation of Crop Maize Silage In Silo Bags and Sampling

Maize (Zea mays) forage material was provided by the Research Institute of Animal Science, ELGO 'DIMITRA' (Paralimni Giannitsa, 58100 Pella, Greece, 40°44′ N, 22°27′ E), in early September 2022. The maize hybrid Leonidas (700 FAO; 135 days maturation; KWS, Italia S.p.A.) was sown in late April in the experimental fields of the Institute according to the soil type, which were characterized as clay to sandy clay and well-drained. The applied fertilization program included 400 kg/ha of N-P-K (20-10-10) and 400 kg/ha of urea (40-0-0), and an irrigation program of 6 waterings, 7 min per meter at each watering, using a hose reel traveler. The fresh maize biomass was harvested at the one-half milk-line of kernel maturity using a farm-scale maize-forage harvester (Jaguar 900, CLAAS KGaA mbH, Harsewinkel, Germany) 20 cm above the ground level, which was simultaneously chopped to an approximate particle size of 2 cm. On the same day as the harvest, the maize forage was transported to the facilities of the Department of Nutritional Physiology and Feeding at the Agricultural University of Athens (Greece).

After receiving the maize raw material in the laboratory, the experimental treatments (0, 2.5, 5 and 10% *w*/*w*) were applied to separate batches, and 7 replicates per treatment were prepared from each batch. Then, the material of the replicates was placed in appropriate vacuum-tight airbags, thus producing 7 replicate bags per experimental treatment. For each experimental treatment, prior to ensiling, from each bag (7.5 kg of the fresh maize forage batch), a 0.5 kg sample was taken and stored at −30 °C until further analysis. Therefore, all 28 bags (7.0 kg of the fresh maize forage batch) were then stored at an ambient temperature (18 ± 2 °C) for 10 weeks. The yogurt acid whey powder composition (g/kg) is presented in Table 1. At the end of the ensiling experiment (70 days), 800 g of each ensiled batch sample was taken from the center of the bag in an aseptic way, and subsequently placed in a sterile bag and immediately stored at −30 °C until further analysis.

Table 1. Yogurt acid whey powder composition (g/kg).

Lactose	720
Ash	110
Protein	50
Lactic acid	60
Dry matter	960

2.2. Chemical Composition of the Maize Forage Samples

All maize forage samples were analyzed for dry matter (DM) using the oven-drying method (65 °C for 24 h until constant weight) according to Acosta Aragón et al. [26]. Subsequently, samples were analyzed for crude fiber (CF), ether extract (EE), crude protein (CP; determined as 6.25 × Kjeldahl nitrogen) and ash using the routine procedures [27]. In addition, neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were determined according to Mertens [28] and Mollers [29] using an Ankom 2000 fiber analyzer (Ankom Technology Ltd., Macedon, NY, USA).

2.3. Microbial Analyses of the Maize Forage Samples

For the microbial analyses, 5 g samples of the maize forage samples were diluted 1:10 in sterile ice-cold anoxic PBS (0.1 M; pH of 7.0) and subsequently homogenized for 3 min in a stomacher (Bagmixer 100 Minimix, Interscience, Arpents, France). The suspension was serially diluted (10^{-1} to 10^{-5}) in buffered peptone water (Neogen Heywood, Bury, UK), and each dilution was spread in duplicate onto plate count agar (PCA, Neogen Heywood, UK) for the total bacteria enumeration; de Man, Rogosa and Sharpe (MRS, Neogen Heywood, UK) for the enumeration of *Lactobacillus* spp. and reinforced clostridial agar (RCA, Neogen Heywood, UK) with the addition of neutral red (0.005%) and D-cycloserine (200 ppm) for the enumeration of *Clostridium* spp. (Jonsson 1989); violet red bile agar (Oxoid Basingstoke, Basingstoke, UK) for coliforms; Sabouraud dextrose agar (SDA, Neogen Heywood, UK) with the addition of chloramphenicol supplements (Oxoid Basingstoke, UK) for the yeast and molds. PCA and SDA were incubated at 30 °C, while MRS was incubated at 37 °C, aerobically. RCA was incubated at 30 °C anaerobically. Results were expressed as log10 colony-forming units per gram silage DM.

2.4. Determination of the Fermentation Quality

Firstly, the pH was determined by direct sample measurement. Samples were diluted (1:2 *w*/*v*) in sterile deionized water and subsequently homogenized in a stomacher (Bagmixer 100 Minimix, Interscience, Arpents, France). The pH was measured by a glass electrode pH/ATC electrode #300729.1 (Denver Instrument GmbH, Göttingen, Germany).

For the determination of the ammonia-N and lactic acid contents, a fresh silage sample was diluted in sterile deionized water and subsequently homogenized in a stomacher (Bagmixer 100 Minimix, Interscience, Arpents, France). The resulting supernatants were used for the enzymatic determination of the ammonia-N and lactic acid contents using commercial kits, (kit K-AMIAR and K-DLATE, respectively; Megazyme, Bray, Ireland).

For the determination of the volatile fatty acid (VFA) concentration, samples were homogenized following a 2-fold dilution (i.e., 1:2 *w*/*v*) in sterile ice-cold phosphate-buffered saline (0.1 mol/L, pH of 7.0). Homogenates were subsequently centrifuged at 12,000× *g* for 10 min at 4 °C, and the resulting supernatants were stored at 80 °C until their analysis by capillary gas chromatography (GC) using an Agilent 6890 GC system, equipped with a 30 m 0.25 mm i.d. Nukol column (Supelco, Sigma-Adrich, St Louis, MO, USA) and a flame ionization detector (FID). The analysis was isothermal (185 °C), and the temperatures of the injector and FID were set at 185 and 200 °C, according to Mountzouris et al. [30]. The VFAs determined were acetic, propionic, isobutyric, butyric and heptanoic acids. Results were expressed as g/kg of the silage DM.

2.5. Statistical Analysis

Data for the nutritional, microbiological and fermentation quality were analyzed using the SPSS statistical package (version 17.0). Prior to analysis, data were tested for normality using Kolmogorov–Smirnov's test. Dependent variables that were not normally distributed were analyzed following appropriate transformation. The effect of four YAWP addition levels (0, 2.5, 5 and 10% *w*/*w*) to the quality of maize forage material ensilation was evaluated by one-way ANOVA, comparing the means of the 4 independent groups. The statistical model of the above described analysis is as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} is observation j in treatment i, μ is the overall mean, T_i is the fixed effect of treatment (YAWP addition), and ε_{ij} is the residual error. The vacuum-tight airbag was considered as the experimental unit. Statistically significant effects were further analyzed, and means were compared using Tukey's honestly significant difference (HSD) multiple comparison procedure. Statistical significance was determined at $p < 0.05$. The linear and quadratic effects of the dietary YAWP inclusion level were studied using polynomial contrasts.

3. Results

3.1. Chemical Composition and Epiphytic Microflora of the Maize Forage before Ensiling

The chemical composition and epiphytic microflora of the freshly chopped maize forage are shown in Table 2. Before ensiling, the counts of epiphytic microbes in the fresh maize, such as total bacteria, *Lactobacillus* spp., *Clostridium* spp. and yeasts–molds, were 7.27, 7.29, 3.90, 5.64 and 2.65 log cfu/g DM, respectively.

Table 2. Analyzed chemical and microbial composition of the fresh maize forage.

Nutritional Quality	**Average ± Std**
Dry matter (g/kg)	261.7 ± 4.87
Crude protein (g/kg DM)	81.0 ± 1.61
Ether Extract (g/kg DM)	22.5 ± 2.30
Crude fiber (g/kg DM)	211.0 ± 9.93
Neutral-detergent fiber (g/kg DM)	429.1 ± 19.36
Acid-detergent fiber (g/kg DM)	244.6 ± 1.41
Acid-detergent lignin (g/kg DM)	14.5 ± 1.11
Microbial Quality (log cfu/g DM)	
Total bacteria	7.27 ± 0.612
Lactobacillus spp.	7.29 ± 0.680
Clostridium sp.	3.90 ± 0.286
Yeast, Molds	5.64 ± 1.084
Coliforms	2.65 ± 1.169

DM: Dry matter.

3.2. Nutritional Quality of the Maize Forage Silages Prepared with the YAWP

The nutritional quality of the maize silage was affected significantly by the YAWP inclusion (Table 3). The DM content was significantly higher ($P_{anova} < 0.001$) in the 10% YAWP addition compared with the 0% and 2.5% YAWP additions, whereas the 5% YAWP addition received intermediate values. The CP content was significantly higher ($P_{anova} \leq 0.05$) in the 10% YAWP addition in comparison with the 0% addition, whereas the 2.5 and 5% additions were intermediate. Ether extract content was not affected ($P_{anova} > 0.05$) by the addition of the YAWP. Addition of the 10% YAWP significantly reduced ($P_{anova} > 0.01$) the silage CF and NDF contents compared with the other treatments, whereas the ADF content was significantly lower ($P_{anova} > 0.05$) in the 10% YAWP addition compared with the 0% and 2.5% YAWP additions, with the 5% addition being intermediate. The YAWP addition did not affect the ADL content ($P_{anova} > 0.05$). The ash content was significantly higher ($P_{anova} < 0.01$) in the 10% YAWP addition, followed by the 5% addition, compared with the 0% addition, while the 2.5% YAWP-addition silage received intermediate values and differed only from the 10% YAWP addition.

Table 3. Nutritional quality of the maize silages prepared with the yogurt acid whey powder (YAWP).

	Inclusion Level of YAWP [a]				**Statistics**			
	0%	**2.5%**	**5%**	**10%**	**SEM** [b]	P_{anova}	**Polynomial Contrast** [c]	
							P_{linear}	$P_{quadratic}$
Dry matter (DM) %	26.09 [a]	26.62 [ab]	27.30 [b]	28.73 [c]	0.388	***	***	NS
Crude protein (% DM)	7.85 [a]	7.96 [ab]	7.96 [ab]	8.08 [b]	0.769	*	*	NS
Ether extract (% DM)	2.29	2.27	2.30	2.07	0.144	NS	NS	NS
Crude fiber (% DM)	21.93 [b]	22.32 [b]	22.77 [b]	19.81 [a]	0.837	**	*	**
Neutral-detergent fiber (% DM)	44.53 [b]	42.84 [b]	42.14 [b]	38.31 [a]	1.478	**	***	NS
Acid-detergent fiber (% DM)	24.43 [b]	24.23 [b]	23.38 [ab]	21.13 [a]	1.148	*	**	NS
Acid-detergent lignin (% DM)	1.74	1.80	1.75	1.68	0.205	NS	NS	NS
Ash (% DM)	4.66 [a]	5.62 [ab]	6.29 [bc]	7.53 [c]	0.532	**	***	NS

[a] Data represent treatment means from seven replicates per treatment. Means within a row followed by the same letter are not significantly different from each other ($p > 0.05$). *** $p < 0.001$; ** $p < 0.01$; * $p \leq 0.05$; NS, not significant. [b] SEM, pooled standard error of means. [c] Polynomial contrasts test the linear and quadratic effects of the YAWP inclusion levels in the silage preparation.

Polynomial contrast results showed that the DM, CP and ash increased linearly ($P_{linear} < 0.001$, $P_{linear} < 0.05$ and $P_{linear} < 0.001$, respectively) and the NDF and ADF decreased linearly ($P_{linear} < 0.001$ and $P_{linear} < 0.01$, respectively) with the increasing YAWP addition level in the maize forage material. In addition, the CF decreased in both a linear and quadratic manner ($P_{linear} < 0.05$ and $P_{quadratic} < 0.01$, respectively) with the increasing YAWP level in the silage.

3.3. Microbial Quality of the Maize Silages Prepared with the YAWP

The effects of the YAWP addition on the microbial populations of the silage are presented in Table 4. Total bacteria were significantly higher in the 0% and 2.5% YAWP additions compared with the 5% and 10% YAWP additions ($P_{anova} < 0.001$). The addition of 5% and 10% YAWP in the silage decreased the *Lactobacillus* spp. concentration in comparison with the 0% and 2.5% YAWP. The *Clostridium* spp. concentration was not affected by the YAWP addition in the silage. Yeast and mold values were not affected ($P_{anova} > 0.05$) either by the YAWP addition.

Table 4. Microbial quality (log cfu/g dry matter) of the maize silages prepared with the yogurt acid whey powder (YAWP).

	Inclusion Level of YAWP [a]				**Statistics**			
	0%	**2.5%**	**5%**	**10%**	**SEM** [b]	P_{anova}	**Polynomial Contrast** [c]	
							P_{linear}	$P_{quadratic}$
Total bacteria	7.95 [b]	7.71 [b]	6.93 [a]	6.73 [a]	0.160	***	***	NS
Lactobacillus spp.	7.90 [b]	7.90 [b]	7.23 [a]	7.09 [a]	0.191	***	***	NS
Clostridium spp.	3.36	3.56	3.47	3.42	0.241	NS	NS	NS
Yeast, Molds	4.03	3.60	3.80	4.98	0.863	NS	NS	*
Coliforms	ND	ND	ND	ND				

[a] Data represent treatment means from seven replicates per treatment. Means within a row followed by the same letter are not significantly different from each other ($p > 0.05$). ND, not detected, i.e., below detection limits. *** $p < 0.001$; * $p \leq 0.05$; NS, not significant. [b] SEM, pooled standard error of means. [c] Polynomial contrasts test the linear and quadratic effects of the YAWP inclusion levels in the silage preparation.

Polynomial contrast results showed that the total bacteria and *Lactobacillus* spp. counts decreased linearly ($P_{linear} < 0.001$ and $P_{linear} < 0.001$, respectively) with the increasing YAWP in the silage. Yeast and mold concentrations increased quadratically ($P_{quadratic} \leq 0.05$)

with the increasing YAWP addition in the maize forage prior to ensiling, with the 2.5% YAWP addition having the lowest concentration. In addition, coliform counts were below detection limit in all treatments.

3.4. Fermentation Quality of the Maize Silage Prepared with the YAWP

The pH values displayed a quadratic pattern of decline ($P_{quadratic} \leq 0.05$) with the increasing YAWP level, with the YAWP 5% addition having the lowest value (trend, $P_{anova} = 0.083$).

The ammonia-N content was significantly affected ($P_{anova} < 0.001$) by the YAWP inclusion (Table 5). The ammonia-N content was significantly lower in the 5% YAWP addition compared with the 0% and 2.5% YAWP additions, whereas the 10% YAWP addition received intermediate values.

Table 5. Fermentation quality of the maize silages prepared with the yogurt acid whey powder (YAWP).

	Inclusion Level of YAWP [a]				Statistics			
							Polynomial Contrast [c]	
	0%	2.5%	5%	10%	SEM [b]	P_{anova}	P_{linear}	$P_{quadratic}$
pH direct	3.74	3.60	3.59	3.71	0.069	NS	NS	*
pH diluted	3.74	3.64	3.65	3.76	0.071	NS	NS	NS
Ammonia-N % DM [d]	4.03 [b]	4.02 [b]	3.71 [a]	3.81 [ab]	0.087	***	**	NS
D/L Lactic acid (g/kg DM)	22.68 [a]	30.24 [b]	34.51 [bc]	37.45 [c]	1.650	***	***	NS
Lactic acid/acetic acid ratio	2.05 [a]	3.32 [b]	4.91 [c]	4.97 [bc]	0.425	***	***	NS
Total VFAs (g/kg DM) [e]	20.75 [b]	21.56 [ab]	14.06 [a]	17.98 [ab]	2.370	**	*	NS
Acetic acid (g/kg DM)	11.18 [b]	10.23 [ab]	7.10 [a]	7.66 [a]	1.218	***	**	NS
Propionic acid (g/kg DM)	1.36 [a]	1.96 [ab]	1.52 [ab]	2.15 [b]	0.234	**	*	NS
Isobutyric acid (g/kg DM)	1.36	2.06	1.46	1.66	0.244	NS	NS	NS
Butyric acid (g/kg DM)	4.62	4.29	ND	ND				
Heptanoic acid (g/kg DM)	2.23 [a]	3.03 [ab]	3.98 [bc]	6.50 [c]	0.736	**	***	NS

[a] Data represent treatment means from seven replicates per treatment. Means within a row followed by the same letter are not significantly different from each other ($p > 0.05$). *** $p < 0.001$; ** $p < 0.01$; * $p \leq 0.05$; NS, not significant. ND, not detected, i.e., below detection limits. [b] SEM, pooled standard error of means. [c] Polynomial contrasts test the linear and quadratic effects of the YAWP inclusion levels in the silage preparation. [d] DM: dry matter. [e] Total VFAs; acetic + propionic + butyric + isobutyric + eptanoic.

The lactic acid concentration was significantly higher ($P_{anova} < 0.001$) in the 10% and 5% YAWP additions compared with the 0% addition, whereas the 2.5% addition was intermediate and differed from the 10% YAWP addition. The lactic acid/acetic acid ratio was significantly higher ($P_{anova} < 0.001$) in the 5 and 10% YAWP additions compared with the control. Furthermore, the 2.5% YAWP addition was intermediate and differed from the control and 5% YAWP addition.

Polynomial contrast results showed that the lactic acid content and lactic acid/acetic ratio increased linearly ($P_{linear} < 0.001$ and $P_{linear} < 0.001$, respectively) with the increasing YAWP in the silage. On the other hand, the ammonia-N content decreased linearly ($P_{linear} < 0.01$) with the increasing YAWP-incorporation level.

Total VFAs, acetic, propionic, butyric and heptanoic contents in the silages prepared with the YAWP addition are presented in Table 5. Total VFA content was significantly lower ($P_{anova} < 0.01$) in the 5% YAWP addition compared with the 0% YAWP addition, whereas the 2.5 and 10% YAWP additions received intermediate values. Acetic acid content significantly decreased ($P_{anova} < 0.001$) with the 5% and 10% YAWP additions in comparison with the 0% YAWP addition, with the 2.5% addition being intermediate. The incorporation of 10% YAWP in the silage significantly increased ($P_{anova} < 0.01$) the propionic content compared with the 0% YAWP, whereas the YAWP 2.5 and 5% treatments received intermediate values. The butyric acid content was detected only in the 0 and 2.5% YAWP additions. The addition of 5 and 10% YAWP in silage significantly increased ($P_{anova} < 0.01$) the heptanoic acid content compared with the 0% YAWP, whereas the 2.5% YAWP differed only from the 10% YAWP additions.

Polynomial contrast results showed that, of the total VFAs, acetic acid decreased linearly ($P_{linear} < 0.05$, $P_{linear} < 0.01$) by increasing the YAWP level. By increasing the YAWP level, the propionic and heptanoic acids increased linearly ($P_{linear} < 0.01$ and $P_{linear} < 0.001$, respectively).

4. Discussion

Usage of the food industry's byproducts in animal nutrition can play an important role in converting low-value materials into high-quality products, while at the same time reducing their environmental footprint, thus contributing to the circular economy [31,32]. In the last decade, strained (Greek) yogurt production has increased significantly due to the growing demand from consumers for its organoleptic characteristics and its health-promoting profile [21]. In the United States, approximately 771,000 metric tons of Greek yogurt was manufactured in 2015, representing nearly 40% of the yogurt market [33]. However, the production process results in yogurt acid whey, a byproduct of strained yogurt, which poses a serious environmental problem if left untreated. In particular, acid whey has a high biological oxygen demand, making it difficult to dispose of into the environment without costly effects on the surrounding ecosystems [20]. Given that yogurt acid whey is rich in lactose and organic acids (e.g., lactic acid and citric acid), its use as a silage additive component merits investigation. Lactose is a fermentable carbohydrate, and its addition to the incorporation of plant matter accelerates the fermentation phenomena and produces a better silage quality [18,19]. Lactic acid is classified among the alpha hydroxy acids and it is also known for its antibacterial activity [34].

The target of silage production is to preserve as much of the fresh plant material nutrients as possible. In this sense, the YAWP in maize forage improved the nutritional quality of the maize silage by increasing the DM and CP, and reducing the CF, NDF and ADF, in a dose-dependent manner. The increase in the DM could be linked with the high-DM content of the YAWP (960 g/kg, Table 1), but also the ability of the YAWP to improve silage fermentation by the promotion of lactic acid fermentation, the decrease in clostridial fermentation and proteolysis (discussed below). Similar results have been previously mentioned by the addition of whey powder in maize silage or alfalfa silage [17,18]. The increase in the DM content has been linked in higher feed intake by cattle [35,36]. The reduction of crude fiber and its fractions (NDF and ADF) was more or less expected due to the dilution effect of the YAWP. Although not directly comparable, other studies have also reported decreases in the NDF and ADF contents of silage prepared using whey powder [16]. Nevertheless, the decrease in fiber is expected to increase the nutritional value of the maize silage.

The addition of the YAWP increased the CP content of silages in a dose-dependent manner. Moreover, the linear reduction of the ammonia-N content by the YAWP level of addition highlights the YAWP properties to reduce protein degradation. Protein degradation in silage is attributed either to the action of enzymes, which are released from the plant cells at harvest, or to proteolytic epiphytic microorganisms present on the plant [37]. Regarding the latter, organic acid additives (e.g., citric acid, formic acid, maleic acid) via direct acidification and antimicrobial effects have been shown to limit proteolysis and to improve the fermentation quality [38–40]. Given that the YAWP not only contains high levels of lactic acid, but also reduced the concentration of total bacteria in the silages in a dose-dependent manner, it could be postulated that the YAWP modified beneficially the proteolytic processes during ensiling via its antimicrobial activity. Since maize silage is low in protein, the increase in the CP and the reduction of the ammonia-N content in YAWP-treated silages could be regarded as an enhancement of the silage dietary value.

In this study, using vacuum ensiling bags in a laboratory setting, the low-pH values (below 4) of the untreated silages indicated good fermentation. This can be attributed either to the low-buffering capacity of maize [41] and/or due to the good experimental ensiling conditions. In large-scale silos, it is harder to pack well and silages are more prone to air infiltration, and thus result in ensiling deterioration [42]. The addition of the YAWP in the silage preparation resulted in a drop in the pH (trend) when added up to 5% (3.59), while a further addition (10% YAWP) caused a numeric increase in the pH value. The high mineral composition of the YAWP (e.g., calcium, phosphorus, magnesium, potassium) [20] may have conferred a buffering capacity. Mineral addition by the YAWP could also enhance the dietary value of the silage [43].

The epiphytic microflora of forage significantly affects the quality of natural silage fermentation [44,45]. Therefore, an additional purpose of this study was to assess the concentration of various microbial populations, such as total bacteria, *Lactobacillus* spp., *Clostridium* spp., yeast and molds and coliforms. It appeared that increased addition levels of the YAWP acted more as an acidifier and silage fermentation inhibitor, due to its concentration of lactic and citric acid and other antimicrobial components, by lowering the concentration of total bacteria and *Lactobacillus* spp. in the maize silage. In general, the use of organic acids as silage additives decreases the concentration of bacterial populations due to their antibacterial activity, as has been seen in experiments adding formic acid, malic acid or citric acid to forages for silage [38,46,47]. Reducing the concentration of bacterial populations limits unwanted fermentations and nutrient losses [47]. Although the presence of *Lactobacillus* spp. is important for forage fermentation and, by extension, for the drop in its pH value, studies have shown that the reduction in the concentration of lactic acid bacteria does not negatively affect the quality of the produced product when an organic acidifier has been added to the forage [38,46,47].

The most important metabolic products of silage bacterial populations are lactic, acetic and propionic acid. Nevertheless, unwanted metabolic products can also be produced, such as butyric acid, ethanol and ammonia, and, depending on their concentration, the quality of the silage and its acceptance by the animals can be affected [48]. In this study, the addition of the YAWP in the silage preparation contributed to the increase in lactic acid as well as the decrease in acetic acid in a dose-dependent manner, therefore resulting in an increased lactic acid/acetic acid ratio. It has been demonstrated that the lactic acid/acetic acid ratio is an efficient indicator of homo- or heterofermentation. As the ratio increases, the fermentation leads to homofermentation, since homolactic acid bacteria use water-soluble carbohydrates for their growth and produce only lactic acid [49]. This indicates that the YAWP favored the homofermentation of the silage, and thus resulted in higher concentration of lactic acid. In general, lactic acid should be at least 65 to 70% of the total silage acids in a good silage [50].

In our study, the percentage of lactic acid out of the total silage acids was 52% in the YAWP 0% addition, 58% in the YAWP 2.5% addition, 71% in the YAWP 5% addition and 67% in the YAWP 10% addition. The latter showed that sufficient lactic acid was determined when the 5% and 10% YAWP additions were added in the silage preparation. On the other hand, the ratio of lactic acid/acetic acid is also used as a qualitative indicator of fermentation; with a good silage fermentation, the ratio should be between 2.5 and 3.0. In the present study, the addition of the YAWP 2.5% to the silage preparation led to this range, whereas the addition of the YAWP 5 and 10% resulted in higher values (4.91 and 4.97, respectively). Silages with very high levels of the lactic acid: acetic acid ratio may sometimes be more aerobically unstable than those with lower ratios, because low concentrations of acetic acid may not be sufficient to inhibit lactate-assimilating yeasts [41]. However, considering that the overall yeast–mold concentration in this work was not affected, it could be an indicator that aerobic stability was not compromised. In addition, given the vacuum application, the assessment of the silage aerobic stability was not specifically addressed by this study and merits research. The increase in the lactic acid: acetic acid ratio below 3 has been previously reported by the addition of 300 g/kg fresh Scotta whey in alfalfa silage [51].

Propionic acid was enhanced by the YAWP supplementation in the silage preparation in a dose-dependent manner. Propionic acid is a fermentation product resulting from the metabolism of bacteria, such as propionibacteria, *Lactobacillus bifermentans*, *Clostridium* *propionicum* and *Selenomonas ruminantium* [52,53]. Typically, its content in maize silages is low, <0.25% DM [12,54], which can also be confirmed by this study. Consequently, due to its low concentration (i.e., the higher value of 0.22% DM by the YAWP 10% addition), it may not be of practical significance. While, non-directly comparable, increasing levels of propionic acid have been reported by the increasing addition level of sour yogurt in maize silage preparation [54]. However, it is worth noting that the addition of propionic acid in maize silage preparation has been used to improve its aerobic stability by inhibiting the growth of molds and yeasts after the silage was exposed to air [14].

The butyric acid content is critical for evaluating the silage quality, as it can affect the silage intake and can induce severe ketosis when presented at a critical level in the diet of cows in early lactation [55,56]. In this experiment, butyric acid was detected in the control (0.46% of the DM) and in the 2.5% YAWP addition (0.42% of the DM), whereas, in the YAWP 5% and 10% treatments, it was not detected. The latter could highlight that the YAWP 5 and 10% addition properties inhibit the clostridial metabolic activity, further supporting the beneficial role of YAWP in silage preparation. The decrease in the butyric acid concentration has been previously reported by the addition of whey at 2 and 4% [17].

5. Conclusions

The YAWP addition in the maize silage preparation beneficially modified the silage nutritional and fermentative qualities. The YAWP 5 and 10% levels of addition in the maize forage were the most effective. In particular, the inclusion of the 10% YAWP positively modified the nutritional value of the maize silage by increasing the DM and CP, and by decreasing the CF, NDF and ADF contents. The incorporation of the 5 and 10% YAWP favored the homofermentation of the silage, and thus resulted in a higher concentration of lactic acid. The YAWP 5 and 10% levels of addition inhibited the clostridial metabolic activity, which was depicted by the absence of butyric acid in the silages prepared with these levels. The addition of the 5% YAWP in the silage preparation modified beneficially the proteolytic processes by lowering the ammonia-N concentration. Further research is recommended for the evaluation of the YAWP addition in other forages, as well as in large-scale silage-production units.

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