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# Effects of lines and inoculants on nutritive value and production costs of triticale silages

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**ABSTRACT** - The current study was undertaken to investigate the ensilage characteristics in triticale lines treated by inoculants and their interaction on fermentation metabolites and rumen degradability. Costs were estimated for growing and feeding whole-crop triticale lines for animal production. Triticale hybrids were harvested at the dough stage of maturity (38% dry matter, DM). Plants were chopped approximately 2 cm after harvest and then treated with inoculants and were ensiled in 1.5-L mini laboratory silos. Two lactic acid bacterial inoculants with enzymes (LAB+enzymes I: *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Streptococcus faecium* with cellulase, hemicellulase, pentosanase, and amylase; LAB+enzyme II: *P. acidilactici*, *L. plantarum*, and amylase) were used as silage additives. Inoculants were applied at $1.5 \times 10^5$ cfu/g chopped fresh material. Silages with no additive served as the control. Four jars per treatment were sampled on day 60 after ensiling for chemical and microbiological analysis. At the end of the ensiling period (60 day), the silages were subjected to an aerobic stability test. The nutrient degradability of silages was determined *in situ*. Overall, there were no obvious interactions between triticale lines and the treatments for any of the parameters measured. The fermentation and nutritive value of silages were affected by treatments. LAB+enzymes increased the concentrations of lactic acid of the triticale silages and decreased the concentrations of butyric acid, total alcohols, and ammonia-N. Under aerobic conditions, LAB+enzyme treated silages had lower pH, $CO_2$ production, and number of yeasts. Fibrous fractions were decreased with the application of LAB+enzymes. The 48 h *in situ* organic matter, DM, and neutral detergent fiber digestibility of the silages were enhanced by treatments. Addition LAB+enzymes to dough stage triticale silage reduces proteolysis; the inoculant possess antimicrobial properties and improves fermentation and nutritional value. The economic results are favorable financially for growing winter triticale as an animal feed in Mediterranean-type climates.

Key Words: cost, degradability, enzymes, fermentation, lactic acid bacteria, triticale lines

## Introduction

Forage resources and their utilization will be modified in the future because of soil and climate constraints (Steenwerth et al., 2014). Triticale has been a crop of future promise. It offers several advantages including an extended growing period that is adapted well to acidic, droughty, or other extreme conditions (Kara et al., 2009; Hackett et al., 2012; Kaplan et al., 2015) with low production costs (Saade, 1995; Lozano-del Rio et al., 2004; Hackett et al., 2012). Recent advances in triticale breeding and harvesting have renewed interest in the integration of triticale as forage in animal production. This interest is particularly apparent with dairy farmers; however, triticale forage may also be suitable pasture forage for grazing beef cattle. Whole crop triticale silage can provide a good option for dairy dry cows, heifers, and beef cattle as energy and an effective fiber source (Hogg et al., 2002; Lozano del Rio et al., 2004; Myer and Lozano del Rio, 2004; Jacobs et al., 2009).

It is very important to note that the nutritional value and ensiling process of triticale vitally depend on its maturation stage. During the dough stage of maturity, the grain is comprised of a starchy endosperm, protein, some lipid in the germ, and fiber (Kennelly and Weinberg, 2003; Lozano-del Rio et al., 2010). Harvesting in the dough stage of maturity improves the chance of avoiding rainfall during the silage-making process. Harvesting in this stage can also maximize dry matter yields. However, during the dough stage of maturity, the number of epiphytic LAB as well as the level of water soluble carbohydrates (WSC) and the degree of digestibility may not be optimal (McDonald et al., 1991). Hence, multi species bacterial inoculant and enzyme treatments could be effective in mature forages (McDonald et al., 1991; Kung et al., 2003). The potential benefits of these additives include improved silage quality as measured by a reduction of the cell wall component, increased available substrate, and higher digestibility

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(McDonald et al., 1991; Kung et al., 2003). This may lead to improved livestock performance; e.g., increased feed intake, improved feed efficiency, faster daily gain, and increased milk and milk solids production (Kung et al., 2003; Adesogan, 2005; Demirel et al., 2013).

Although there is a considerable amount of information available on the growing winter triticale as a feed source (Lozano-del Rio et al., 2004; Myer and Lozano-del Rio, 2004; Nadeau, 2007; Jacobs et al., 2009; Ozduven et al., 2010; Demirel et al., 2013), little is known about harvesting at the dough stage of maturity and the levels of performance that can be achieved when grown in the subtropics. Hence, the objective of this study was to summarize the ensilage characteristics of different triticale lines by using multi species bacterial inoculants with enzymes and their interaction on fermentation metabolites and digestibility of triticale silages harvested at the dough stage of maturity. Another goal here was to understand the feasibility of using winter triticale as an economical alternative for other forages in the Mediterranean region.

## Material and Methods

The field experiment was carried out in Bursa, Turkey. The experimental station is located in the coastal zone of northwest Turkey (40°11' N, 29°04' E), 70 m above sea level. This climatic zone is characterized by a Mediterranean-type climate. The soil was a clay loam, classified as vertisol typic habloxrert, slightly alkaline (pH is 7.2), medium in P (73 kg/ha), rich in K (1130 kg/ha), and containing 1.4% organic matter. Four CIMMYT (International Maize and Wheat Improvement Center) winter triticale lines, randomly selected out of 33 triticale lines from yield trial, were evaluated for forage production in this study (Table 1).

The plot size was 6.0 $m^2$ (5 m × 1.2 m), consisting of eight rows spaced at 15.0 cm. Seeds were planted using a plot drill with 550 seeds/$m^2$ graining rate. The basic pre-planting fertilization rates for all plots were N 50 and P 50 kg/ha, with N top-dressing of 100 kg/ha. Irrigation was not performed and herbicide was used for broad-leaf and narrow-leaf weeds

Forage was harvested when each line reached the dough stage in June. Forage samples were harvested from a 1.2 $m^2$ center area of each plot to measure forage yields. A total of 500 g fresh sample were taken from harvested plants and dried at 70 ºC for 48 h. Then, hay yields were determined. Prior to harvest, plant height was measured from the base of the plant to the top of the spike excluding the awns in 10 randomly selected plants. Plant samples from each line were cut at ground-level and separated into leaf, spike, and stalk. These components were individually dried and weighed.

Four triticale lines were harvested by hand at the dough stage and chopped with a laboratory-type chopper (Fimaks, Turkey) to about 2.0 cm. Within 1 h of chopping, the following treatments were applied to fresh forage: a control (no additive); LAB+enzymes I (*Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Streptococcus faecium* with cellulase, hemicellulase, pentosanase, and amylase); and LAB+enzyme II (*P. acidilactici*, *L. plantarum*, and amylase). Inoculants (1.5 g) were suspended in 100 mL of tap water and the whole suspension was sprayed over 10 kg (wet weight) of the chopped forage spread over a 1 × 4 m area. The control was sprayed with the same amount of water and no additives. The amount of chopped forage for a given jar was weighed out, sprayed with the appropriate additive by a plant sprayer, mixed by hand, and then placed into the jar by hand with periodic tamping. The chopped forages were ensiled in 1.5-L anaerobic jars (Weck®, Wher-Oflingen, Germany) equipped with a lid that enables gas release only. The experiment had four different triticale lines and three treatments (untreated control and two inoculants) with four replicates (jars). In total, 48 jars were stored at ambient temperature (22-24 °C). Jars were weighed before and after filling to determine the actual amount ensiled. Each jar was filled with about 944, 919, 996, and 906 g (fresh weight) of chopped forage for C1, C11, C13, and C14 triticale lines, respectively. The packing densities were 159.4, 154.8, 166.6, and 154.3 kg of dry

Table 1 - Pedigree and origin of triticale lines

| Pedigree | Origin | Code |
|---|---|---|
| BANT-2/ RHINO-9//GIRAF/YOGUI-1 (CMT87.1891-5Y-0M-0RES-17M -1Y-0PAP-4Y-0B) | CIMMYT-Mexico | C1 |
| SUSI-2 (CMT86B.386-2Y-1M-5Y-3M-3RES-0B-2Y-0PAP) | CIMMYT-Mexico | C11 |
| PASSI-3-2 (CMT24476-1M-0Y-0H-0Y-22B-1Y-500B-502RES-0B | CIMMYT-Mexico | C13 |
| CAGUAN-3 (CTM86M.2281-5Y-2B-1Y-1B-2RES-0B-1Y-0PAP) | CIMMYT-Mexico | C14 |

matter (DM)/m$^3$ for C1, C11, C13, and C14 triticale lines, respectively. Fresh and ensiled forages were sampled for further analysis on day 60 after ensiling.

Chemical analyses of fresh forage and silages were performed in quadruplicate per treatment and presented on a DM basis. The silage pH was measured directly from the silage juice using a pH meter (Sartorius PB-20, Goettingen, Germany). The DM content of the fresh forage and silages was determined by drying at 60 °C for 48 h in a fan-assisted oven (Procedure no: 930.15; AOAC, 1990). The dry matter content of the silages was corrected ($DM_{cor}$) for the loss of volatile substances during drying, using the following equation (Weißbach, 2009): {$DM_{cor}$ = DM + 0.95 × sum of fatty acids (C2 − C6) + 0.08 × lactic acid + 0.77 × 1,2 propanediol + 1.00 × other alcohols (C2−C6 including butanediol) [g/kg]}. Fresh forage and silages were analyzed for crude protein (CP) and ash according to AOAC (984.13 and 942.05, respectively; 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by using the sodium sulfite addition method without α-amylase and expressed with residual ash (Van Soest et al., 1991). Hemicellulose (HC) was calculated as the difference between NDF and ADF. Wet samples stored at –20 °C were extracted for 3 min in a blender in water or in ethyl acetate (1:9) for WSC and fermentation products analysis. The WSC were determined by the phenol sulfuric acid method (Dubois et al., 1956). Lactic acid was determined by a spectrophotometry method (Barker and Summerson, 1941). The volatile fatty acids (VFA) and alcohol concentrations were analyzed using a gas chromatograph with a capillary column (over a temperature range 45 to 230 °C). Ammonia-N was determined by using Kjeltech auto analyzer (Gerhardt, Germany) without a digestion step, according to AOAC (ID 941.04, 1990).

Microbiological analyses of fresh forage and silages were performed in quadruplicate (per treatment for each replicate) and presented on fresh and wet silage basis. Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, U.K.), and yeast and mold on spread-plate malt extract agar (Difco, Detroit, MI) acidified with lactic acid to pH 4.0. Plates were incubated for 3 d at 30 °C. All microbiological data were transformed to $\log_{10}$.

At the end of the ensiling period (day 60), the silages were subjected to an aerobic stability test at room temperature (22 °C), which lasted 5 d, in a "polyethylene terephthalate (P.E.T.) bottle" system developed by Ashbell et al. (1991). The system was constructed from recycled soft drink bottles (polyethylene terephthalate) in two parts: the upper part (1-L) was filled with 250 g (wet weight) of loosely packed silage, and the lower part with 100 mL of 20% KOH. Gas was exchanged through 1-cm holes in the lid of the upper part to the lower part. The $CO_2$ produced during aerobic exposure was absorbed in the base and determined by titration with 1 N HCl. In addition, silage pH was measured and yeast and mold analyses were performed as the indicators of aerobic spoilage as well. The pH, yeast, and mold analysis were determined by the previously explained analysis methods. Analyses were carried out on the silage samples after 5 d of exposure to air.

Rumen degradability characteristics of the silages were measured by the method reported by Mehrez and Ørskov (1977). Air-dried forage samples were ground through a 2.5 mm screen using a laboratory-type mill. The milled samples (5 g DM) were placed in 9 × 14 cm Dacron bags (pore size 40-60 μm), which were inserted into three rumen-cannulated male Merino sheep. The sheep were fed a diet based on wheat grain and sunflower meal plus alfalfa hay. The 48 dacron bags (16 bags/sheep) were incubated in the rumen for 48 h. In the residue after incubation, DM (ID 930.15; AOAC, 1990), OM (ID 942.05; AOAC, 1990), and NDF (Van Soest et al., 1991) were determined.

Production cost of maize (silage) and triticale was calculated based on their total fixed and variable costs. Fixed costs included land rent, permanent labor, and depreciation. Depreciation cost was calculated according to the straight line method. Miscellaneous cost was taken as 1% and interest on pre-harvest variable cost was taken as 5% of the total amount of variable costs. The exchange rate used was 1 euro = 3.14 Turkish lira.

A randomized complete block design with three replicates was used for evaluating the lines. The data obtained from silage quality were analyzed as a completely randomized design with four replications and subjected to analysis of variance by the GLM procedure of SAS (Statistical Analysis System, version 6.0). Differences among means were tested using Tukey's test and significance was declared at $P<0.05$, whereas trends were discussed at $P<0.10$, unless stated otherwise.

Statistical model:

$$Yijl = \mu + \tau i + \gamma j + \tau\gamma ij + eijl,$$

in which μ = overall mean; τi = effect of line i; γj = effect of treatment j; τγij = line × treatment interaction; and eijl = residual error.

## Results

Among the pre-ensiled triticale lines, forage yield, ear/stalk ratio, and chemical composition were not different ($P>0.05$). On the other hand, the differences between plant

height and hay yield were affected (P<0.05). Forage yield of triticale lines ranged between 44.28 and 41.61 t/ha (Table 2). The plant height and the hay yield were lowest (P<0.01) in lines C13 (91.33 cm) and C14 (13.86 t/ha), respectively. The epiphytic lactobacilli counts were low in pre-ensiled triticale, each being <4 log cfu/g.

All silages were relatively well fermented, but the extent of fermentation differed depending on the LAB+enzyme applications. The volatile corrected DM contents of the untreated and treated silages were 39.16 and 38.75, respectively. The crude protein content of the LAB+enzyme treated triticale silages tended to be higher (P = 0.06) than that of untreated silage. The use of LAB+enzyme lowered (P<0.05) the NDF and the least (P<0.05) NDF was observed in C14 silages treated with LAB+enzyme I. The LAB+enzyme I tended (P = 0.12) to reduce ADF contents in the silages from C1, C11, and C13 triticale. No interactions (P>0.05) were found between the lines and the treatments (Table 4) for fermentation metabolites except for pH and butyrate (P<0.01). The pH values for C1 and C13 control silages were greater (P<0.01) than the other silages. The butyrate for C14 control silages was greater (P<0.01) than the other groups. The concentration of lactic and acetic acid of silages ranged from 42.4 to 64.0 and 2.0 to 6.2 g/kg DM, respectively (Table 4). The acetate concentration was lower (P<0.05), whereas the lactate concentration was higher (P<0.05) in silages treated with LAB+enzymes when compared with the control. No butyric acid was detected in either LAB+enzyme treated silage. The concentrations of WSC and the total alcohols were decreased (P<0.05) by the inclusion of LAB+enzymes. The differences in the ammonia-N concentrations tended (P = 0.10) to be reduced by LAB+enzymes (Table 4).

After 60 d of ensiling, lactobacilli counts were increased (P<0.05) by both LAB+enzymes applications, whereas yeast counts were not affected (P = 0.33) by treatments (Table 5). However, there were lower amounts of yeast in C13 line triticale treated with the LAB+enzyme II than the untreated or other treated silages. Regardless of treatments, mold counts were low in all triticale silages, each being <2.

The pH and the release of $CO_2$ were lower (P<0.05) in the LAB+enzyme treated silages than the untreated silage. The most significant (P<0.05) improvement in aerobic stability was seen in C13 LAB+enzyme I treated silage because of the lower activity of yeast than the other silages. There were no obvious effects of line and treatment interactions on yeast (P = 0.80) and mold (P = 0.46) numbers of the triticale silages.

Both LAB+enzymes treatments increased (P<0.05) the *in situ* DM or NDF digestibility of triticale silages. In addition, organic matter digestibility (OMD) of triticale silage was greater (7.04%; P<0.05) after 48 h in the rumen treated by LAB+enzymes than in the untreated controls (Table 7). No interactions (P>0.05) were observed between the lines and the treatments for any of the digestibility parameters, except for DM digestibility (P<0.01). The highest (P<0.01) DM digestibility was noted in C1 LAB+enzyme I treated silages.

The comparison of production costs for maize (silage) and triticale was calculated based on their green forage yield (Table 8). Green forage yield of maize was substantially different according to production period. While the green forage yield was between 9 and 10 t/da in April sowning, it dropped to 7 t/da in June and 5-6 t/da in July as a second crop. In our calculation, the production period was June. Maize and triticale were both produced on the same research

Table 2 - Plant height, forage yield, chemical composition, and epiphytic *lactobacilli* of fresh triticale lines before ensiling

| Parameter | Line | | | | SEM | P-value |
|---|---|---|---|---|---|---|
| | C1 | C11 | C13 | C14 | | |
| Plant height (cm) | 106.93a | 106.23a | 91.33b | 100.26a | 2.86 | <0.01 |
| Forage yield (t/ha) | 44.28 | 42.77 | 43.21 | 41.61 | 1.89 | 0.79 |
| Hay yield (t/ha) | 15.94a | 15.52a | 15.77a | 13.86b | 0.30 | <0.01 |
| Ear/stalk | 0.45 | 0.46 | 0.45 | 0.44 | 0.05 | 0.99 |
| Dry matter (g/kg) | 375.6 | 384.3 | 373.7 | 392.4 | 2.90 | 0.79 |
| pH | 6.31 | 6.33 | 6.41 | 6.38 | 0.05 | 0.50 |
| CP (g/kg DM) | 92.50 | 89.10 | 93.60 | 93.20 | 3.50 | 0.79 |
| Ash (g/kg DM) | 72.80 | 68.80 | 71.60 | 75.80 | 1.50 | 0.06 |
| NDF (g/kg DM) | 583.8 | 584.4 | 586.0 | 577.8 | 6.20 | 0.80 |
| ADF (g/kg DM) | 370.3 | 370.7 | 373.5 | 375.2 | 8.00 | 0.96 |
| HC (g/kg DM) | 226.4 | 203.5 | 212.5 | 202.6 | 1.40 | 0.39 |
| WSC (g/kg DM) | 109.43 | 108.30 | 106.63 | 110.53 | 2.22 | 0.38 |
| *Lactobacilli* (log cfu/g DM) | <2 | <2 | <2 | <2 | 0.18 | 0.85 |

SEM - standard error of the mean; DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; HC - hemicellulose (calculated as the difference between NDF and ADF); WSC - water soluble carbohydrates; log - logarithm of the numbers; cfu - colony-forming units.
Means in the same row with different letters differ significantly (P<0.05).

farm of the University. Thus, fixed costs of both were similar. Triticale was grown under dry farming conditions. The unit cost of maize was three times higher than that of triticale under the same farm conditions. In the same farm conditions, the OM degradability of maize was 60% (Filya, 2004), while the triticale OM degradability was 50%. Therefore, we calculated the cost per OMD in maize and triticale as 28.14 and 14.30 euro/t, respectively.

## Discussion

Agronomic and economic information indicates that winter sown whole crop triticale in farming operation has many advantages. The advantages of triticale for silage in semi-arid climates are that it can grow on winter rains with very high yield and could be grown for a double cropping system (Hogg et al., 2002; Jacobs et al., 2009). Good-quality whole-crop triticale silage can serve as adequate forage for lactating dry cows or beef cattle when the demand for energy is high and the need for protein is low (de Ruiter et al., 2002; Hogg et al., 2002). Stage of maturity is a key determinant of the nutritional quality of triticale. The dough stage is a good time to cut triticale for silage because most of the kernel dry matter (DM) accumulates during this stage (Kennelly and Weinberg, 2003).

In the present study, agronomical traits and production cost of triticale as well as nutritional value of triticale silages were investigated with or without LAB+enzyme. The plant height differed between triticale lines. The highest plant height was found in the C1 line (106.93 cm), whereas the lowest ($P<0.05$) plant height (91.3 cm) was observed in the C13 line (Table 2). These findings are supported by Mut et al. (2006). Forage yield of triticale for the present study was higher than the values reported by Kara et al. (2009), Lozano-del Rio et al. (2010), and Kaplan et al. (2015). Hay yield of triticale lines varied between 13.86-15.94 t/ha, with the lowest value in C14 and the highest value in C1, C13, and C11, respectively (Table 2). Hay yields of the current study were similar to those reported by Delogu et al. (2002) and Santiveri et al. (2004), but higher than the values of Lithourgidis et al. (2006). Such differences in hay yields were mainly due to differences in climate conditions and

Table 3 - Chemical composition of triticale silages and triticale silages treated with inoculants after a 60-day ensiling period

| Factor | DM | $DM_{cor}$ | CP | Ash | NDF | ADF | HC |
|---|---|---|---|---|---|---|---|
| | | | (g/kg DM) | | | | |
| Line (L) | | | | | | | |
| C1 | 38.04a | 38.87a | 9.02 | 7.44 | 56.97 | 35.49 | 21.48 |
| C11 | 37.94ab | 38.77ab | 8.97 | 7.38 | 56.84 | 36.14 | 20.71 |
| C13 | 37.67b | 38.53b | 9.14 | 7.55 | 57.61 | 35.95 | 21.65 |
| C14 | 38.38a | 39.24a | 8.92 | 7.41 | 56.41 | 36.36 | 20.05 |
| SEM | 0.21 | 0.22 | 0.16 | 0.12 | 0.38 | 0.4 | 0.5 |
| Treatment (T) | | | | | | | |
| Control | 38.15 | 39.14 | 8.73 | 7.47 | 57.62a | 36.6 | 21.02 |
| LAB+E I | 37.95 | 38.75 | 9.12 | 7.43 | 56.89ab | 35.84 | 20.85 |
| LAB+E II | 37.93 | 38.68 | 9.18 | 7.44 | 56.36b | 35.5 | 21.04 |
| SEM | 0.19 | 0.18 | 0.14 | 0.11 | 0.32 | 0.34 | 0.48 |
| L × T | | | | | | | |
| C1 Control | 392.4a | 395.1cd | 87.4 | 74.2 | 577.0abc | 356.2 | 220.9 |
| LAB+E I | 384.3b | 392.1bc | 90.4 | 75.2 | 566.0bcd | 350.0 | 216.0 |
| LAB+E II | 383.9b | 388.9bcd | 92.8 | 73.7 | 566.1bcd | 358.5 | 207.6 |
| C11 Control | 381.8bc | 394.1b | 87.4 | 74.9 | 574.8abc | 372.8 | 202.1 |
| LAB+E I | 381.1bcd | 386.9bcd | 88.8 | 71.6 | 562.9bcd | 354.5 | 208.3 |
| LAB+E II | 379.6bcd | 382.1d | 93.0 | 75.0 | 567.7bcd | 356.8 | 210.9 |
| C13 Control | 379.5bcd | 383.4d | 89.1 | 74.9 | 577.4ab | 363.2 | 214.2 |
| LAB+E 1 | 379.0bcd | 383.4d | 92.0 | 74.5 | 561.2cd | 355.6 | 205.7 |
| LAB+E II | 375.6cd | 389.4bcd | 93.0 | 77.0 | 589.6a | 359.9 | 229.7 |
| C14 Control | 375.6cd | 402.9a | 85.3 | 74.7 | 575.5abc | 371.9 | 203.7 |
| LAB+E I | 374.7cd | 387.4bcd | 93.7 | 75.8 | 564.3bcd | 360.2 | 204.1 |
| LAB+E II | 373.7d | 386.9bcd | 88.7 | 71.7 | 552.4d | 358.7 | 193.8 |
| SEM | 0.13 | 0.16 | 0.16 | 0.12 | 0.32 | 0.43 | 0.56 |
| Line (L) | <0.01 | <0.01 | 0.79 | 0.79 | 0.09 | 0.53 | 0.18 |
| Treatment (T) | 0.44 | 0.06 | 0.06 | 0.96 | <0.01 | 0.12 | 0.95 |
| L × T | <0.01 | <0.01 | 0.78 | 0.61 | <0.01 | 0.89 | 0.53 |

DM - dry matter; $DM_{cor}$ - dry matter corrected for loss of volatiles; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; HC - hemicellulose (calculated as the difference between NDF and ADF); LAB+E I - *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Streptococcus faecium* with cellulase, hemicellulase, pentosanase, and amylase; LAB+E II - *P. acidilactici*, *L. plantarum*, and amylase; SEM - standard error of the mean.
Means in the same column with different letters differ significantly ($P<0.05$).

different responses of genotypes against different conditions (Kaplan et al., 2015). These differences may also result from higher nutrient accumulation levels of early-spiking plants (Delogu et al., 2002). Ear/stalk ratio was 0.44-0.46, averaging 0.45 (Table 2). Contrary to the result in this study, Nadeau (2007) determined this ratio as 0.85. In this study, we found that the DM content (373.7-392.4 g/kg fresh matter), and ADF (372.7 g/kg DM) values were optimal to ensure maximum quality silage and dry matter intake, whilst protein content was moderate, between 89.1 and 93.6 g/kg DM (Table 2), which is characteristic of the dough stage whole-crop triticale (Kennelly and Weinberg, 2003). Available soluble carbohydrates for primary fermentation were sufficient (106.6-110.5 g/kg DM; Table 2) in all triticale lines (McDonald et al., 1991). Nadeau (2007) reported that whole crop triticale harvested in the early dough stage of maturity contained 350 g/kg DM, 770.0 g/kg CP, and 334.3 g/kg ADF compared with 292 g/kg DM, 103.1 g/kg CP, and 229.9 g/kg ADF for triticale silage harvested in the early milk stage of maturity.

The CP content of the untreated (87.3 g/kg DM) triticale silages was slightly affected by the storage period in relation to the fresh material (92.1 g/kg DM; Tables 2 and 3). The CP content of untreated silage was lower than that of both fresh forage (5.21%) and LAB+enzyme treated (4.7%) silages. This indicates a controlled fermentation with less proteolysis in LAB+enzyme treated silages than in untreated silages. Similar results were also reported by Jacobs et al. (2009), who demonstrated that LAB+enzyme increased the CP content of the early dough triticale silage relative to either fresh forage or untreated control silage. The ammonia-N in silages shows the degree of protein degradation. Extensive proteolysis adversely affects the utilization of nitrogen by ruminants (McDonald et al., 1991). It was observed that ammonia-N was lower in LAB+enzyme treated silages relative to untreated silage. On the other hand, the level of ammonia-N formation in all silages in the present experiment was below the threshold level of 80 g/kg of total nitrogen for good quality silages (McDonald et al., 1991). The LAB+enzymes treatment influenced (P<0.05) the fibrous fractions of the triticale silages (Table 3). Decomposition of cell walls in enzyme treated silages with or without LAB is supposedly a result of hydrolysis of hemicellulose (Kung et al., 2003), which

Table 4 - Fermentation metabolites of triticale silages and triticale silages treated with inoculants after a 60-day ensiling period

| Factor | pH | Lactate | Acetate | Butyrate | WSC | Alcohol | Ammonia-N |
|---|---|---|---|---|---|---|---|
| | | (g/kg DM) | | | | (mg/kg DM) | (g/kg of TN DM) |
| Line (L) | | | | | | | |
| C1 | 3.89 | 56.31 | 3.51 | 0.11b | 38.73 | 334.22 | 57.30 |
| C11 | 3.84 | 56.93 | 3.48 | 0.16ab | 40.87 | 322.89 | 64.02 |
| C13 | 3.82 | 64.40 | 4.05 | 0.07c | 41.1 | 336.89 | 56.44 |
| C14 | 3.81 | 55.94 | 3.74 | 0.24a | 41.23 | 330.11 | 60.55 |
| SEM | 0.10 | 0.19 | 0.27 | 0.04 | 9.64 | 10.19 | 6.14 |
| Treatment (T) | | | | | | | |
| Control | 3.90b | 44.93a | 5.82a | 0.4a | 44.65a | 348.75a | 69.95 |
| LAB+E I | 3.76a | 61.53b | 2.89b | 0b | 39.03b | 329.42ab | 55.31 |
| LAB+E II | 3.75a | 61.08b | 3.39b | 0b | 37.76b | 314.92b | 53.48 |
| SEM | 0.10 | 0.17 | 0.24 | 0.03 | 9.70 | 8.83 | 5.32 |
| L × T | | | | | | | |
| C1 Control | 4.03a | 44.4 | 5.6 | 0.30bc | 46.7 | 357.0 | 63.55 |
| LAB+E I | 3.77c | 64.0 | 3.0 | 0d | 39.4 | 308.3 | 60.08 |
| LAB+E II | 3.74c | 60.5 | 2.0 | 0d | 30.1 | 337.3 | 48.26 |
| C11 Control | 3.95b | 47.6 | 5.5 | 0.50b | 44.3 | 315.3 | 79.99 |
| LAB+E I | 3.78c | 63.5 | 2.6 | 0d | 38.9 | 323.3 | 51.16 |
| LAB+E II | 3.74c | 59.7 | 2.3 | 0d | 39.4 | 330.0 | 60.91 |
| C13 Control | 4.04a | 42.4 | 6.0 | 0.20c | 43.7 | 384.0 | 63.98 |
| LAB+E I | 3.75c | 59.0 | 3.0 | 0d | 39.1 | 304.7 | 50.61 |
| LAB+E II | 3.74c | 61.2 | 3.2 | 0d | 40.5 | 322.0 | 54.73 |
| C14 Control | 3.94b | 45.3 | 6.2 | 0.70a | 43.9 | 338.7 | 72.27 |
| LAB+E I | 3.74c | 59.7 | 3.0 | 0d | 38.7 | 323.3 | 59.37 |
| LAB+E II | 3.75c | 62.8 | 2.0 | 0d | 41.1 | 328.3 | 50.00 |
| SEM | 0.10 | 2.10 | 0.30 | 0.03 | 9.69 | 10.06 | 6.61 |
| Line (L) | <0.01 | 0.82 | 0.49 | <0.01 | 0.79 | 0.57 | 0.84 |
| Treatment (T) | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.10 |
| L × T | <0.01 | 0.91 | 0.77 | <0.01 | 0.21 | 0.63 | 0.92 |

WSC - water soluble carbohydrates; DM - dry matter; TN - total nitrogen; LAB+E I - *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Streptococcus faecium* with cellulase, hemicellulase, pentosanase, and amylase; LAB+E II - *P. acidilactici*, *L. plantarum*, and amylase; SEM - standard error of the mean.
Means in the same column with different letters differ significantly (P<0.05).

can improve the availability of energy for fermentation in the silo and in the rumen proven by the increase in OM digestibility (7.2%; Table 7) in our LAB+enzyme treated silages. Ozduven et al. (2010) and Demirel et al. (2013) similarly reported that LAB with enzymes disturbed cell membranes and released soluble cell contents in triticale and barley/triticale silages. However, a study (Zahiroddini et al., 2004) with barley silage yielded conflicting results. In that study, it was reported that enzyme with LAB or alone increased the silage NDF and ADF content, showing that the enzyme-mediated release of soluble sugars from cell wall was lower (Zahiroddini et al., 2004).

In this case, using a LAB+enzyme mixture promoted a more satisfactory fermentation, which improved conservation of dry matter, reduced solubilization of nitrogen, and gave greater stability to triticale silages (Tables 3-6). Resulting from lactobacilli activity, LAB+enzyme treated silages contained significant levels of lactic acid. The production of volatile fatty acids, other than acetic, was very small and no butyric acid was detected in LAB+enzyme treated silages. The disappearance of soluble carbohydrates (WSC) was measured in concert with the process of acidification in treated silages. These results are in accordance with those with cereal silages (Zahiroddini et al., 2004; Sucu and Filya, 2006; Nadeau, 2007; Xie et al., 2012) and can be related to better preservation.

Temperature, DM and WSC content, microbial population, and concentration of fermentation products in interaction with pH have the greatest direct effects on aerobic stability. The consumption of sugar and acids raises the pH and accelerates the process of aerobic instability with high dry matter losses after opening the silo (McDonald et al., 1991). In the current study, after exposing the silages for five days, the number of yeasts was increased when compared with the number of yeasts that are not exposed to air (Table 6). As a result, the pH increased (4.3) during air exposure compared with final silage pH (3.9). This suggests that, regardless of using additives, silages sooner or later

Table 5 - Microbiological composition of triticale silages and triticale silages treated with inoculants after a 60-day ensiling period

| Factor | *Lactobacilli* | Yeasts | Molds |
|---|---|---|---|
| | | (log cfu/g DM) | |
| Line (L) | | | |
| C1 | 6.56 | 2.37 | 1.71 |
| C11 | 6.84 | 2.43 | 1.37 |
| C13 | 7.00 | 2.00 | 1.51 |
| C14 | 7.05 | 1.98 | 1.48 |
| SEM | 0.43 | 0.25 | 0.20 |
| Treatment (T) | | | |
| Control | 5.71b | 2.45 | 1.70 |
| LAB+E I | 7.46a | 2.10 | 1.36 |
| LAB+E II | 7.42a | 2.05 | 1.49 |
| SEM | 0.37 | 0.22 | 0.18 |
| L × T | | | |
| C1 Control | 5.32c | 2.71 | 2.26 |
| LAB+E I | 7.15abc | 1.67 | <2 |
| LAB+E II | 7.21ab | 2.74 | <2 |
| C11 Control | 5.71bc | 2.65 | <1 |
| LAB+E I | 7.39ab | 2.49 | <2 |
| LAB+E II | 7.42ab | 2.16 | <2 |
| C13 Control | 6.00abc | 1.93 | <2 |
| LAB+E I | 7.50ab | 2.65 | <2 |
| LAB+E II | 7.51ab | 1.43 | <2 |
| C14 Control | 5.81bc | 2.49 | <2 |
| LAB+E I | 7.80a | 1.57 | <2 |
| LAB+E II | 7.53ab | 1.87 | <2 |
| SEM | 0.31 | 0.23 | 0.20 |
| Line (L) | 0.77 | 0.38 | 0.69 |
| Treatment (T) | <0.01 | 0.33 | 0.40 |
| L × T | <0.01 | 0.15 | 0.45 |

log - logarithm of the numbers; cfu - colony-forming units; DM - dry matter; LAB+E I - *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Streptococcus faecium* with cellulase, hemicellulase, pentosanase, and amylase; LAB+E II - *P. acidilactici*, *L. plantarum*, and amylase; SEM - standard error of the mean.
Means in the same column with different letters differ significantly (P<0.05).

Table 6 - Results of aerobic stability test (5 d) of triticale silages and triticale silages treated with inoculants after a 60-day ensiling period

| Treatment | pH | $CO_2$ (g/kg DM) | Yeasts (log cfu/g DM) | Molds (log cfu/g DM) |
|---|---|---|---|---|
| Line (L) | | | | |
| C1 | 4.25 | 4.82 | 5.67a | 2.28 |
| C11 | 4.31 | 5.27 | 4.69ab | 2.15 |
| C13 | 4.26 | 5.23 | 4.20b | 2.20 |
| C14 | 4.24 | 5.25 | 4.10b | 2.18 |
| SEM | 0.03 | 0.21 | 0.36 | 0.06 |
| Treatment (T) | | | | |
| Control | 4.50a | 5.96 | 5.82a | 2.26 |
| LAB+E I | 4.20b | 4.99 | 4.53b | 2.14 |
| LAB+E II | 4.11b | 4.48 | 4.63b | 2.19 |
| SEM | 0.03 | 0.18 | 0.30 | 0.06 |
| L × T | | | | |
| C1 Control | 4.48 | 5.60 | 5.73 | 2.46 |
| LAB+E I | 4.13 | 4.82 | 5.55 | 2.19 |
| LAB+E II | 4.13 | 4.05 | 5.73 | 2.29 |
| C11 Control | 4.51 | 6.00 | 4.62 | 2.04 |
| LAB+E I | 4.25 | 5.18 | 5.92 | 2.13 |
| LAB+E II | 4.17 | 4.63 | 4.34 | 2.27 |
| C13 Control | 4.55 | 6.16 | 4.72 | 2.37 |
| LAB+E I | 4.11 | 5.06 | 3.41 | 2.23 |
| LAB+E II | 4.21 | 4.46 | 4.46 | 2.19 |
| C14 Control | 4.49 | 6.07 | 4.23 | 2.27 |
| LAB+E I | 4.23 | 4.88 | 4.05 | 2.13 |
| LAB+E II | 4.01 | 4.79 | 4.02 | 2.03 |
| SEM | 0.03 | 0.22 | 0.36 | 0.06 |
| Line (L) | 0.46 | 0.47 | <0.01 | 0.53 |
| Treatment (T) | <0.01 | <0.01 | 0.02 | 0.33 |
| L × T | 0.36 | 0.98 | 0.81 | 0.46 |

DM - dry matter; log - logarithm of the numbers; cfu - colony-forming units; LAB+E I - *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Streptococcus faecium* with cellulase, hemicellulase, pentosanase, and amylase; LAB+E II - *P. acidilactici*, *L. plantarum*, and amylase;SEM - standard error of the mean.
Means in the same column with different letters differ significantly (P<0.05).

deteriorate. In this study, using LAB+enzymes helped to reduce the negative signs of aerobiosis in triticale silages proven by the decrease (P<0.05) in pH, $CO_2$ production, and the number of yeasts in comparison with untreated silage. Addah et al. (2011) similarly demonstrated the same trend for barley silages. However, in some cases, the addition of LAB with or without enzymes adversely affected aerobic stability of triticale (Ozduven et al., 2010) or other cereal (Sucu and Filya, 2006; Xie et al., 2012) silages.

In the current study, LAB+enzymes inclusion increased (P<0.05) the amount of degradable OM as well as the degradable DM and NDF in the rumen (Table 7). This indicated that the LAB+enzymes complement caused enough amount of fiber breakdown due to the adequate application rate ($1.5 \times 10^5$). These findings agree with other authors (Sucu and Filya, 2006; Ozduven et al., 2010) who demonstrated that LAB inoculation with or without enzymes increased the DM and NDF digestibilities of cereal silages. However, the lack of an effect of additives on degradable DM and NDF in the rumen was reported by Zahiroddini et al., (2004). These authors demonstrated that there was no impact of using LAB ($1.25 \times 10^5$) with cellulolytic and amylolytic enzymes on effective degradability of DM and NDF of whole crop barley silages.

We found that winter sown triticale has the potential to produce significantly higher forage and grain yields compared with winter wheat (10.4-13.0 t/ha; Keady, 2005; Walsh et al., 2008) and might have the potential to reduce unit production costs of forage when compared with corn as a silage. Likewise, Hackett et al. (2012) obtained the same findings with winter sown triticale under Irish conditions. They also stated that triticale has greater disease resistance than wheat and is also thought to be more suited than wheat to lighter and more marginal soils and to take-all prone sites (Hackett et al., 2012). According to Lozano-del Rio et al. (2004), the high forage biomass production and forage quality of triticale increase animal performance, reduce feeding costs, and result in increased return to farmers. In Alberta (Canada), feeding research with triticale, barley, and corn suggested that swath-grazing triticale could reduce winter feeding costs by over $100 per cow compared with wintering cows for 100 days in a corral. Savings were lower for swath-grazing barley ($89) due to lower yields,

Table 7 - *In situ* 48 h organic matter, dry matter, and fiber digestibility (OMD, DMD, and NDFD) of triticale silages and triticale silages treated with inoculants after a 60-day ensiling period

| Factor | OMD | DMD | NDFD |
|---|---|---|---|
| | | (g/kg) | |
| Line (L) | | | |
| C1 | 500.3 | 484.7 | 357.2 |
| C11 | 503.6 | 489.5 | 359.0 |
| C13 | 496.7 | 484.9 | 370.2 |
| C14 | 500.8 | 483.9 | 364.8 |
| SEM | 0.43 | 0.50 | 0.50 |
| Treatment (T) | | | |
| Control | 477.2b | 473.9b | 354.5b |
| LAB+E I | 510.7a | 495.3a | 364.5a |
| LAB+E II | 513.0a | 488.1a | 369.8a |
| SEM | 0.37 | 0.43 | 0.43 |
| L × T | | | |
| C1 Control | 487.3 | 477.9abc | 345.0 |
| LAB+E I | 496.5 | 498.9a | 357.8 |
| LAB+E II | 516.3 | 477.2abc | 368.9 |
| C11 Control | 481.7 | 477.5abc | 356.9 |
| LAB+E I | 516.9 | 497.8ab | 349.6 |
| LAB+E II | 512.3 | 493.3abc | 370.5 |
| C13 Control | 460.0 | 469.1c | 361.3 |
| LAB+E I | 517.6 | 495.6abc | 369.4 |
| LAB+E II | 512.6 | 490.0abc | 379.8 |
| C14 Control | 479.7 | 470.9bc | 355.0 |
| LAB+E I | 511.8 | 488.9abc | 379.3 |
| LAB+E II | 510.9 | 491.7abc | 360.2 |
| SEM | 0.53 | 0.37 | 0.48 |
| Line (L) | 0.87 | 0.63 | 0.24 |
| Treatment (T) | <0.01 | <0.01 | <0.01 |
| L × T | 0.84 | <0.01 | 0.30 |

LAB+E I - *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Streptococcus faecium* with cellulase, hemicellulase, pentosanase, and amylase; LAB+E II - *P. acidilactici*, *L. plantarum*, and amylase; SEM - standard error of the mean.
Means in the same column with different letters differ significantly (P<0.05).

Table 8 - Production cost of maize (silage) and triticale

| | Unit | Maize | Triticale |
|---|---|---|---|
| Production area | ha | 9 | 58 |
| A - Fixed production costs | | | |
| Land rent | euro/ha | 2,388.54 | 2,388.54 |
| Permanent labor | euro | 12,184.06 | 12,184.06 |
| Depreciation | euro | 7,643.31 | 7,643.31 |
| Subtotal fixed cost | | 20,066.23 | 20,066.23 |
| B - Variable costs | | | |
| Seed | euro | 1,019.11 | 7,536.31 |
| Fertilizer | euro | 2,420.38 | 6,753.12 |
| Pesticides | euro | 350.32 | 2,898.15 |
| Irrigation water | euro | 1,003.18 | 0.00 |
| Fuel | euro | 4,777.07 | 5,606.05 |
| Labor | euro | 6,369.43 | 10,796.50 |
| Transport | euro | 159.24 | 159.24 |
| Miscellaneous (1% of variable costs) | euro | 160.99 | 337.49 |
| Interest on pre-harvest variable cost (5%) | euro | 812.99 | 1,704.34 |
| Subtotal variable cost | euro | 17,072.70 | 35,791.20 |
| C - (A+B) total production costs | euro | 37,138.93 | 55,857.43 |
| D - Co-product return | euro | 18,691.56 | 20,236.62 |
| Yield (green forage) | t/ha | 72.85 | 42.96 |
| E - Total production | t/ha | 655.65 | 2.491.68 |
| OMD yield | t/ha | 38.46 | 18.26 |
| Unit cost | euro/t | 56.64 | 22.42 |
| Price | euro/t | 54.14 | 19.10 |
| Unit cost of OMD (C-D/E) | euro/t | 28.14 | 14.30 |

OMD - organic matter digestibility.

and for corn ($83) due to higher input costs. Saade (1995) found that the cost of production of triticale was lower than that of durum and bread wheat, and incorporating 20% triticale in feed concentrates for ruminants would result in 3.6 Tunisian dinar cost savings per ton of concentrate in Tunisian conditions.

## Conclusions

Triticale improves the profitability of animal production systems. Inoculation of triticale with lactic acid bacteria and enzymes before ensiling improves the quality of the resulting silage. Several interacting factors are involved, including the preservation of more forage protein, the improvement of organic matter digestion in the rumen, and possible probiotic effects of inoculant bacteria and enzymes. These all are linked to increased production response in animals.

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