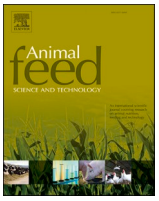
Animal Feed Science and Technology 309 (2024) 115899

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Mixed ensiling with by-products and silage additives significantly valorizes drought-impaired whole-crop corn

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ARTICLE INFO

Keywords:
Anaerobic fungi
Conservation
Forage
Ruminant
Ruminal fluid
Silage

ABSTRACT

Corn silages constitute an important roughage in diets for high-yielding dairy cows. Due to summer droughts, quantity and quality of corn silages diminish, which both can have drastic consequences on the energy and nutrient provision to dairy cows. Mixed ensiling of drought-impaired whole-crop corn with by-products may represent a promising option to valorize the nutritive value and available biomass, which has not yet received much attention. Our study analyzed the potential of mixed ensiling of drought-impaired corn with either sugar beet pulp (SBP) or wheat gluten feed (WGF), without or with the application of different silage additives, i.e., either anaerobic fungi (AF) culture supernatant, mixed ruminal fluid or lactic acid bacteria. The aim was to study the effects on the chemical composition, fermentation patterns, *in vitro* gas production (GP), as an indicator of digestibility, and aerobic stability of the silages. We observed an overall sufficient preservation in all silages as evidenced by low dry matter (DM) losses of $\leq$ 3.37%, homolactic fermentation as well as lasting aerobic stability (>336 h), while the silage pH was significantly lower with by-product inclusion. The co-ensiling with WGF predominantly increased the crude protein content to ~200 g/kg DM with still low ammonia-N levels, i.e., 17 g/kg crude protein, whereas co-ensiling with SBP increased the energy level as evidenced by the *in vitro* GP kinetics. The application of fresh AF culture supernatant further improved the preservation success, including less ammonia-N and lower silage pH, and considerably increased the energy content of pure corn silages. Remarkably, addition of fresh AF culture supernatant also improved *in vitro* GP kinetics of WGF-based silages that performed less than other silages when no additives were applied. Using fresh mixed ruminal fluid showed beneficial effects on silage quality, such as lower ammonia-N concentrations in all silages, whereas heat-inactivated mixed ruminal fluid decreased silage pH. For the application of lactic acid bacteria, our results showed their support in facilitating roughage preservation, but without influence on chemical composition or *in vitro* rumen fermentation. In conclusion, mixed ensiling with by-products is yet an overlooked option for valorizing drought-impaired corn and our data confirmed the effectiveness of this approach. Without increasing the feed-food competition, mixed ensiling represents a

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; AF, anaerobic fungi, aNDFom, neutral detergent fiber assayed with a heat-stable α-amylase and expressed exclusive of residual ash; ADL, Acid detergent lignin; CP, crude protein; DM, dry matter; GP, gas production; HGT, Hohenheim gas test; NDF, neutral detergent fiber; NEL, net energy for lactation; NFC, non-fiber carbohydrates; PGP, potential gas production; SBP, sugar beet pulp; WSC, water-soluble carbohydrates; WGF, wheat gluten feed.

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https://doi.org/10.1016/j.anifeedsci.2024.115899
Received 15 September 2023; Received in revised form 22 January 2024; Accepted 23 January 2024
Available online 26 January 2024

promising adaptation strategy to summer droughts, especially in combined use with AF culture supernatant. Future research may now investigate the impact of feeding mixed silages on performance, behavior, and health of dairy cows.

1. Introduction

Corn silages are of great importance in modern ruminant livestock production systems and constitute a common key forage in diets of dairy and beef cattle. Compared to other roughages, corn silages typically provide larger amounts of dietary energy, mainly in the form of starch, and thus are vital to meet the cattle's energy requirements for maintenance and performance. However, this logically applies only in case of a high nutritive value of corn silages. Due to climate change, pronounced drought periods have become more frequent in Europe and this trend for increased aridity is expected to maintain or even increase (Forzieri et al., 2014). As a consequence, cropland suffers from high temperatures and water stress, together resulting in reduced quantity, i.e., lower biomass production, as well as quality of harvested corn plants (Crasta and Cox, 1996; Lauer, 2012). Thereby, the quality of corn silages produced from drought-impaired plants is not only diminished by a lower proportion of starchy grains that besides as well compromises ensilability, but also in terms of overall reduced digestibility (Crasta and Cox, 1996). Without sufficient countermeasures, this is expected to have consequences, such as a decline in milk or growth performance of dairy cows.

Therefore, research needs to identify coping strategies that enable the adaptation of roughage production to extended droughts. Besides plant breeding programs (Adee et al., 2016), mixed ensiling may represent a further adaptation option that yet has not received much attention. Indeed, co-ensiling of drought-impaired corn with by-products that provide nutrients, such as easily fermentable carbohydrates, could enhance lactic acid fermentation in the silo and valorize silage stocks in terms of both quantity and quality by simultaneously maintaining low feed-food competition. In this regard, molassed sugar beet pulp (SBP) constitutes an interesting by-product due to its high availability on market (Muir and Anderson, 2022) and its nutritional profile. Apart from sugars that should promote lactic acid fermentation, SBP comprises large amounts of pectin and hemicelluloses, which are primarily fermented into acetate and propionate but with minor impact on pH in the rumen compared to starchy concentrates with a much higher acidogenic potential (Münnich et al., 2017). Moreover, wheat gluten feed (WGF) represents a by-product of industrial starch extraction that is high in rapidly fermentable starch and sugars as well as crude protein (CP), i.e., ~250 (sum of starch and sugars) and ~170 g/kg dry matter (DM), respectively (Jeroch et al., 2020). Until now, corn silage is typically used as an energy source in ruminant diets, whereas mixed silages of corn and WGF may then eventually provide both energy and CP. Although elevated CP contents could defer a rapid acidification in the silo (McDonald et al., 1991), it is worth investigating its potential as a co-substrate for ensiling. In terms of practical implementation, mixing the components at silo filling can be efficiently performed using a feed mixer or by alternately layered filling (Titterton and Maasdorp, 1997; Bundesarbeitskreis Futterkonservierung, 2011).

In addition to the increase of biomass and nutrient provision via mixed ensiling, the application of silage additives may add further value. Remarkably, our recent research revealed that, compared to control, the use of anaerobic fungi (AF) culture supernatant or mixed ruminal fluid led to improvements in the silage fermentation pattern of grass silages, such as a lower silage pH and lower DM losses (Hartinger et al., 2022). The AF culture supernatant as well significantly increased the in situ fiber degradability due to a pre-cleavage of fiber by AF enzymes in the silo. Presumably, this additive type could exert similar benefits on the ruminal degradability of corn silages. Apart from such novel silage additives, conventional additives containing lactic acid bacteria are known to improve the quality of silages prepared from difficult substrates that are low in sugars but high in buffering components like CP and minerals (Hartinger et al., 2020). Analogously, the application of lactic acid bacteria could be of particular value for the successful conservation when ensiling corn with CP-rich WGF.

Consequently, our study aimed to comprehensively evaluate mixed ensiling of drought-impaired corn and the by-products SBP or WGF by analyzing the chemical composition, fermentation pattern, *in vitro* gas production (GP) kinetics, as an indicator of digestibility, and aerobic stability of the silages. Secondly, it was tested whether the application of AF culture supernatant, mixed ruminal fluid, or lactic acid bacteria can provide further advantages. Our hypothesis was that mixed ensiling with SBP or WGF increases the concentrations of dietary energy, evidenced by *in vitro* GP kinetics, and/or CP with a sufficient conservation effect for all substrates. We further hypothesized a stronger lactic acid fermentation and therefore lower pH of silages when adding fresh AF culture supernatant, mixed ruminal fluid, or lactic acid bacteria compared to no additive. For the addition of fresh AF culture supernatant, we also expected a higher extent and rate of *in vitro* GP than for control silages because of fiber cleavage during ensiling.

2. Materials and methods

2.1. Production of silage additives

The AF culture supernatant was obtained from the fungal strain *Feramyces* sp. DF1 (GenBank accession number MW907584), isolated from deer ruminal fluid. The AF isolate was cultivated anaerobically at 39 °C on M10 medium (Caldwell and Bryant, 1966) enriched by 250 ml/l ruminal fluid with 4 g/l of xylan (Serva Electrophoresis GmbH, Heidelberg, Germany) as carbon source. After four days of incubation in 300 ml bottles, the AF culture was then centrifuged at 5000g for 20 min at 4 °C to separate mycelia from the culture broth, i.e., AF culture supernatant, which was immediately stored at 20 °C until its application. Using an aliquot, the activities of endo-1,4-β-xylanase (EC 3.2.1.8) and β-D-glucoside glucohydrolase (E.C.3.2.1.21) were determined according to methods of Lever

(1977) and Bidochka et al. (1993), respectively. The enzymatic activities of β-D-glucoside glucohydrolase and endo-1,4-β-xylanase were 214.2 nkat (139.1 μg of glucose/ml/h) and 218.4 μkat (574.2 μg of xylose/ml/h), respectively. Additionally, half of the AF culture supernatant was placed in a forced-air oven at 103 °C for 2 h to receive heat-inactivated AF culture supernatant as a control silage additive.

The mixed ruminal fluid was obtained from a dry non-pregnant rumen-cannulated Holstein cow of the University Clinic for Ruminants, University of Veterinary Medicine Vienna, fed grass hay ad libitum and 1 kg of concentrate/day (KuhKorn PLUS Energie, Garant-Tiernahrung GmbH, Pölchlarn, Austria; contained per kg DM 8.2 MJ net energy for lactation (NEL), 175 g CP, 201 g NDF, 35 g crude fat, 9 g Ca, 6 g P, 2 g Na, 3.1 g Mg, 9 g K, 52 mg Mn, 92 mg Zn, 17 mg Cu, 0.51 mg Se, 9100 IU vitamin A, 1366 IU vitamin D, 30 mg vitamin E) and kept according to the Austrian guidelines 114 of animal welfare (BGBl. II Nr. 485/2004 idF BGBl. II Nr. 151/2017). Directly before beginning with the ensiling, solid ruminal digesta was collected from the middle of the fiber mat and squeezed through three layers of gauze (Wilhelm Weisweiler GmbH & Co. KG, Münster, Germany) to obtain particle-associated mixed ruminal fluid. The mixed ruminal fluid was stored in a glass flask in a water bath at 39 °C until the ensiling of all treatments with mixed ruminal fluid was finished. Analogous to the preparation of the heat-inactivated AF culture supernatant, certain volume of the fresh mixed ruminal fluid was also placed in a forced-air oven at 103 °C for 2 h to receive heat-inactivated mixed ruminal fluid, which was allowed to cool down to ~25 °C before its application as a control silage additive.

Furthermore, a commercial biological silage additive (Bonsilage Forte, H. Wilhelm Schaumann GmbH, Pinneberg, Austria) containing the lactic acid bacteria *Pediococcus acidilactici*, *Lactobacillus paracasei* and *Lactococcus lactis*, each in a concentration of at least 1.25×10^{11}/g, was tested. The LAB additive was dissolved in tap water before application and the final solution consisted of 0.2 g/l.

2.2. Preparation of silages

The experiment was conducted with three different substrates and six different silage additives. The three substrates were drought-impaired whole-crop corn as well as the pelleted by-products WGF and SBP, both provided by a local processing company (AGRANA Beteiligungs-AG, Vienna, Austria). The chemical composition of all substrates is presented in Table 1. The cornfield was located at the research dairy farm of the University of Veterinary Medicine Vienna in Pottenstein, Austria (47°57'30.1"N 16°07'00.8"E) and exposed to drought conditions from around V14 vegetative stage on (Ciampitti et al., 2016). The presence of drought conditions during the vegetation period was also confirmed by the technical report of the European Union's Copernicus Emergency Management Service (Toreti et al., 2022). For more details, Supplementary Table 1 provides the monthly means and standard deviations of precipitation sum, duration of sunshine, and air temperature at 2 m height, all obtained from the nearest weather station (47°93'91.67"N 16°10'11.1"E) that is operated by the Austrian weather service (GeoSphere Austria, Vienna, Austria). At the end of August 2022, the corn was harvested manually at R4 dough stage (Ciampitti et al., 2016) as whole-crop corn from four random locations in the field and chopped to 25 mm using a wood chipper (UD2500, Makita, Fischamend, Austria).

Subsequently, the corn was ensiled either solely [C], in mixture with 500 g/kg DM WGF pellets [W] or in mixture with 360 g/kg DM SBP pellets [S]. Those inclusion rates were chosen to either create a mixed silage moderate in energy and high in CP, i.e., ~200 g/kg DM, or a mixed silage high in energy. All silages were prepared to have a DM concentration of 350 g/kg, thus tap water was sprayed manually to treatments containing WGF and SBP pellets. The DM concentration of the fresh whole-crop corn was determined in a microwave (Oetzel et al., 1993). Then, these silages were ensiled either without a silage additive [CON], with fresh AF culture supernatant [AF], heat-inactivated AF culture supernatant [inactAF], fresh mixed ruminal fluid [RF], heat-inactivated mixed ruminal

Table 1
Chemical composition of whole-crop corn, wheat gluten feed pellets, and molassed sugar beet pulp pellets used as ensiling substrates. All values in g/kg dry matter if not stated otherwise.

	Whole-crop corn	Wheat gluten feed	Molassed sugar beet pulp
Dry matter (g/kg)	293	939	934
Ash	52.3	60.8	74.3
Crude protein	90.3	245	147
Ether extract	17.1	47.0	7.50
Starch	268	155	3.32
WSC[1]	104	89.5	120
aNDFom[2]	493	388	380
ADFom[3]	282	151	229
ADL[4]	34.7	65.8	11.6
Hemicelluloses[5]	211	237	151
Cellulose[6]	247	85.4	113
NFC[7]	347	259	391

[1] Water-soluble carbohydrates;
[2] Neutral detergent fiber assayed with a heat stable α-amylase and expressed exclusive of residual ash;
[3] Acid detergent fiber expressed exclusive of residual ash;
[4] Acid detergent lignin;
[5] Calculated as aNDFom (g/kg DM) – ADFom (g/kg DM);
[6] Calculated as ADFom (g/kg DM) – ADL (g/kg DM);
[7] Non-fiber carbohydrates, calculated as 1000 – ash (g/kg DM) – crude protein (g/kg DM) – ether extract (g/kg DM) – aNDFom (g/kg DM).

fluid [inactRF], or the lactic acid bacteria-based additive [LAB]. The AF culture supernatant and mixed ruminal fluid were added at a concentration of 10% of ensiled DM, based on previous ensiling trials (Hartinger et al., 2022), whereas the lactic acid bacteria-based additive was applied according to manufacturer's recommendation, i.e., 10 ml/kg fresh matter of substrate. Thereby, each additive accordingly replaced the tap water that was added to achieve a similar DM concentration of 350 g/kg, as described before. Consequently, 18 different silage treatments were produced in 72 individual silage bags, which are referred to as C_CON, C_AF, C_inactAF, C_RF, C_inactRF, C_LAB, W_CON, W_AF, W_inactAF, W_RF, W_inactRF, W_LAB, S_CON, S_AF, S_inactAF, S_RF, S_inactRF, S_LAB.

Each silage treatment was prepared in quadruplicate in sealed polyamide vacuum bags (400 mm × 600 mm, 90 μm; Plastar pak S.r. l., Concorezzo, Italy) using a mobile range vacuum machine (Henkovac, 's-Hertogenbosch, Netherlands) with 900 g of substrate per bag and silage bag was considered as the experimental unit (Adesogan et al., 2020). Four different batches of corn, each obtained from a different location in the field, were used for each of the four different replicates of the treatments and were mixed independently, with all equipment, such as mixing tools and buckets, being thoroughly sanitized with water before preparation of the next silage. After closing, all bags were stored at ~20 °C for 90 days as recommended for silage experiments (Bundesarbeitskreis Futterkonservierung, 2011). During this storage period, bags were checked twice daily during the first week and hereafter every second day for extend of gas production and potential damage of the bags. No bags were lost due to damages. In addition, all bags were weighed on the first and last day of storage to calculate the DM loss. Fresh samples of all substrates were also collected before ensiling and stored at -20 °C until further analysis.

2.3. Analysis of chemical composition

The fresh substrates as well as all silages were dried at 65 °C in a forced-air oven for 48 h and subsequently ground through a 1 mm screen in an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany). All analyses were performed according to the guidelines of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012). The DM concentration was determined by oven-drying the samples at 103 °C for at least 4 h (method 3.1). The DM concentration was additionally corrected for losses of volatile compounds during drying using the equation from Weißbach and Strubelt (2008). The ash concentration was analyzed by combustion in a muffle furnace overnight at 580 °C (method 8.1), ether extract was determined using the Soxhlet extraction system (method 5.1.2) and CP using the Kjeldahl method (method 4.1.1). A Fibretherm FT12 (Gerhardt GmbH & Co. KG, Königswinter, Germany) was used to obtain neutral detergent fiber assayed with a heat stable α-amylase and expressed exclusive of residual ash (aNDFom, method 6.5.1), acid detergent fiber expressed exclusive of residual ash (ADFom, 6.5.2) and acid detergent lignin (ADL, 6.5.3). These analyses have been sequentially performed on the same sample. The WSC concentration was analyzed in accordance with method 7.1.1 and the total starch concentration was determined using a commercially available kit (Megazyme, Wicklow, Ireland) in sample aliquots that were ground through a 0.5 mm screen in an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany).

In order to most precisely characterize the carbohydrate composition, hemicelluloses, cellulose, and non-fiber carbohydrates (NFC) were further calculated from the analyzed data. Thereby, hemicelluloses were calculated as aNDFom (g/kg DM) – ADFom (g/kg DM), cellulose as ADFom (g/kg DM) –ADL (g/kg DM), and NFC as 100 – ash (g/kg DM) – crude protein (g/kg DM) – ether extract (g/kg DM) – aNDFom (g/kg DM).

2.4. Analysis of silage fermentation pattern

To analyze the variables of silage fermentation, cold-water extracts were prepared from all silages according to Hartinger et al. (2022). Therefore, aliquots of 50 g were taken directly after bag opening and mixed with 100 ml of distilled water. After incubation overnight at 4 °C in the fridge, the complete content was filtered through three layers of gauze (Wilhelm Weisweiler GmbH & Co. KG, Münster, Germany). The silage pH was immediately determined by potentiometry (S40-K SevenMulti™ pH meter, Mettler Toledo, Vienna, Austria) in the liquid and aliquots were then stored at -20 °C for further analyses. The concentrations of acetic acid, propionic acid, butyric acid, and ethanol were determined by gas chromatography as described previously (Hartinger et al., 2022). The concentrations of ammonia and lactate were analyzed using the Berthelot reaction (Hinds and Lowe, 1980) and by high-performance liquid chromatography (UltiMate 3000 HPLC system, Thermo Fisher Scientific, Vienna, Austria) according to Weiß and Kaiser (1995), respectively.

2.5. Determination of in vitro gas production

The *in vitro* GP of all samples was determined using the Hohenheim gas test (HGT; Menke and Steingass, 1988). Therefore, ~200 mg of DM of each dried and ground (1 mm sieve size) sample were weighed in graduated glass syringes that were closed airtight with vaseline-greased plungers. Then, all syringes were placed in an incubator and warmed to 39 °C and the buffer solution was prepared in a water bath with 39 °C under continuous CO_2 flushing. The ruminal fluid was obtained before morning feeding from two dry non-pregnant rumen-cannulated Holstein cows of the University Clinic for Ruminants, University of Veterinary Medicine Vienna, fed grass hay ad libitum and 1 kg of concentrate/day (KuhKorn PLUS Energie, Garant-Tiernahrung GmbH, Pölchlarn, Austria) as described in Section 3.1. The ruminal fluid was immediately transported to the lab in glass flasks placed in a polystyrene box with pre-warmed water (39 °C) and directly mixed and filtered through three layers of gauze (Wilhelm Weisweiler GmbH & Co. KG, Münster, Germany). Afterwards, the ruminal fluid was added to the reduced buffer solution under constant CO_2 flushing and stirring.

30 ml of the buffered ruminal fluid solution were dispensed into each syringe, which was then immediately placed back into the incubator at 39 °C and the continuous rotation was started after all syringes were filled. In addition to the samples, three syringes with

only buffered ruminal fluid solution, i.e., blanks, as well as three syringes with concentrate standard and three syringes with hay standard, both with known GP and provided by the University of Hohenheim, were included in each run. The GP of each syringe was measured after 0, 2, 4, 8, 12, 24, 32, 48, 56 and 72 h of incubation and all samples, i.e., silage replicates, were analyzed in duplicate in two independent runs. Thereby, GP of the blanks was subtracted from the GP of the syringes filled with silages, concentrate standard, or hay standard. The GP of both replicates of each sample in each run were averaged and run was used as the replication. Therefore, eight independent observations (4 silos × 2 runs) were obtained per treatment. Although minor biological fluctuations among runs are inevitable in the HGT system, whole runs were repeated if the correction factor exceeded the range of 0.9–1.1, i.e., in case of more than 10% variance. The calculation of the correction factor was done according to the instructions of Menke and Steingass (1988), where the known GP of the standards after 24 h is divided by the actually recorded GP value of the standards for that run. Consequently, it is possible to ensure that the *in vitro* incubation followed a typical fermentation.

2.6. Determination of aerobic stability

The aerobic stability was tested in accordance with the procedure of Weiß et al. (2022). In brief, samples were loosely filled in plastic tubes of 100 mm × 200 mm and equipped with data loggers (RC-4HC, Elitech, London, UK) that were inserted into the geometric center. Then, each plastic tube was stored in an insulating polystyrene box without a lid to allow free air circulation and stored at 20 °C for 14 days. The silage and room temperatures were recorded at 1 h intervals and silages were considered aerobically instable once the silage temperature exceeded the ambient room temperature by 2.0 °C.

2.7. Calculations and statistical analysis

All calculations and statistical analyses were performed in SAS v9.4 (SAS Institute Inc., Cary, USA). To calculate the *in vitro* GP kinetic parameters of the HGT, the non-linear regression equation $Y = a + b \times e^{(c \times t)}$ from Ørskov and McDonald (1979) was used. Thereby, Y represents the GP at time t (ml/200 mg DM), a represents the initial GP from the soluble, immediately available substrate (ml/200 mg DM), b represents the GP from insoluble, fermentable substrate (ml/200 mg DM), and c represents the rate of GP (/h). The

Table 2
Effects of mixed ensiling on chemical composition and fermentation pattern of silages prepared from whole-crop corn solely or with wheat gluten feed pellets or molassed sugar beet pulp pellets. All values in g/kg dry matter (DM) if not stated otherwise.

	Treatment[1]				
	C_CON	W_CON	S_CON	SEM[2]	*P*-value
DM (g/kg)	341	323	356	6.28	0.08
Ash	60.4^{b}	67.5^{a}	61.9^{ab}	0.92	0.02
Crude protein	119^{b}	210^{a}	116^{b}	1.93	< 0.01
Ether extract	17.3^{b}	37.2^{a}	13.6^{b}	4.70	< 0.01
Starch	221^{a}	158^{b}	179^{b}	16.6	0.04
WSC[3]	30.0^{b}	54.0^{a}	47.0^{a}	1.44	< 0.01
aNDFom[4]	492^{a}	415^{b}	424^{b}	6.96	< 0.01
ADFom[5]	296^{a}	221^{b}	243^{b}	7.83	< 0.01
ADL[6]	35.8^{a}	27.5^{b}	33.6^{a}	0.12	0.01
Hemicelluloses[7]	195	194	181	10.7	0.69
Cellulose[8]	260^{a}	187^{b}	215^{b}	7.92	< 0.01
NFC[9]	312^{b}	270^{b}	385^{a}	7.51	< 0.01
DM loss (%)	2.67	3.37	2.32	2.11	0.12
pH	4.12^{a}	3.95^{b}	3.95^{b}	0.23	< 0.01
Lactic acid	38.7^{b}	55.3^{a}	41.0^{b}	1.20	< 0.01
Acetic acid	10.3	8.10	7.51	0.43	0.06
Propionic acid	n.d.[10]	n.d.	n.d.	-	-
Butyric acid	n.d.	n.d.	n.d.	-	-
Ethanol	8.13^{b}	22.1^{a}	3.24^{b}	0.81	< 0.01
Ammonia-N (g/kg CP[11])	25.7^{a}	17.1^{b}	22.1^{ab}	1.56	0.05

In each row, superscript letters indicate difference between least square means ($P \leq 0.05$).
[1] C_CON = Whole-crop corn without a silage additive, W_CON = Whole-crop corn and wheat gluten feed without a silage additive, S_CON = Whole-crop corn and molassed sugar beet pulp without a silage additive;
[2] Standard error of the mean;
[3] Water-soluble carbohydrates
[4] Neutral detergent fiber assayed with a heat stable α-amylase and expressed exclusive of residual ash
[5] Acid detergent fiber expressed exclusive of residual ash
[6] Acid detergent lignin;
[7] Calculated as aNDFom (g/kg DM) – ADFom (g/kg DM)
[8] Calculated as ADFom (g/kg DM) – ADL (g/kg DM)
[9] Non-fiber carbohydrates, calculated as 1000 – ash (g/kg DM) – crude protein (g/kg DM) – ether extract (g/kg DM) – aNDFom (g/kg DM);
[10] Not detected;
[11] Crude protein.

Table 3
Effects of fresh or heat-inactivated anaerobic fungi culture supernatant on chemical composition, energy concentration, and fermentation pattern of silages prepared from whole-crop corn solely or with wheat gluten feed pellets or molassed sugar beet pulp pellets. All values in g/kg dry matter (DM) if not stated otherwise.

	Treatment[1]										*P*-values	
	C_CON	C_inactAF	C_AF	W_CON	W_inactAF	W_AF	S_CON	S_inactAF	S_AF	SEM[2]	Additive	Additive × Substrate
Dry matter (g/kg)	341[a]	277[b]	309[ab]	323	321	351	356	362	34.0	7.30	0.01	< 0.01
Ash	60.4[b]	70.0[a]	59.2[b]	67.5[a]	71.1[a]	59.9[b]	61.9	59.2	64.1	1.12	< 0.01	< 0.01
Crude protein	119[b]	143[a]	128[b]	210	219	205	116	118	123	2.18	< 0.01	< 0.01
Ether extract	17.3	17.2	19.5	37.2	34.5	37.7	13.6	14.3	15.4	5.54	0.41	0.93
Starch	221[ab]	156[b]	261[a]	158[a]	111[b]	163[a]	179	201	201	17.8	< 0.01	< 0.01
WSC[3]	30.0	36.8	33.1	54.0	59.1	59.4	47.0	42.3	38.1	3.61	0.60	0.24
aNDFom[4]	492	496	447	415	427	369	424	384	414	11.5	0.01	0.01
ADFom[5]	296	288	253	221	216	184	243	228	256	7.88	0.03	0.01
ADL[6]	35.8	44.5	33.5	33.6[b]	64.4[a]	58.3[a]	27.5	30.0	34.6	2.72	< 0.01	< 0.01
Hemicelluloses[7]	195	208	194	194	211	184	181	156	158	10.5	0.19	0.17
Cellulose[8]	260	244	220	187[a]	152[ab]	126[b]	215	198	222	7.93	< 0.01	< 0.01
NFC[9]	312[ab]	273[b]	345[a]	270[ab]	249[b]	329[a]	385	425	383	11.8	0.01	< 0.01
NEL[10] (MJ/kg DM)	5.76[b]	5.57[b]	6.05[a]	-	-	-	-	-	-	0.09	0.02	-
Dry matter loss (%)	2.67	2.70	2.74	3.37	3.23	3.15	2.32	1.91	2.66	2.33	0.64	0.70
pH	4.12[a]	3.93[b]	3.93[b]	3.95	3.99	3.99	3.95[ab]	3.96[a]	3.87[b]	0.24	< 0.01	< 0.01
Lactic acid	38.7[b]	53.1[a]	49.3[a]	55.3	55.8	52.6	41.0[b]	41.3[b]	50.5[a]	1.37	< 0.01	< 0.01
Acetic acid	10.3	7.07	6.62	8.19	4.50	3.24	7.53	10.4	7.56	1.17	0.01	0.05
Propionic acid	n.d.[11]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-
Butyric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-
Ethanol	8.12	9.65	8.86	22.1	14.7	13.6	3.24	4.49	0.98	1.55	0.58	< 0.01
Ammonia-N (g/kg CP[12])	25.7	14.9	17.0	17.1	5.67	6.20	22.1	23.0	20.3	2.03	< 0.01	0.10

In each row, superscript letters indicate difference between least square means within a type of silage substrate ($P \leq 0.05$).

[1] C_CON = Whole-crop corn without a silage additive, C_inactAF = Whole-crop corn with heat-inactivated anaerobic fungi culture supernatant, C_AF = Whole-crop corn with fresh anaerobic fungi culture supernatant, W_CON = Whole-crop corn and wheat gluten feed without a silage additive, W_inactAF = Whole-crop corn and wheat gluten feed with heat-inactivated anaerobic fungi culture supernatant, W_AF = Whole-crop corn and wheat gluten feed with fresh anaerobic fungi culture supernatant, S_CON = Whole-crop corn and molassed sugar beet pulp without a silage additive, S_inactAF = Whole-crop corn and molassed sugar beet pulp with heat-inactivated anaerobic fungi culture supernatant, S_AF = Whole-crop corn and molassed sugar beet pulp with fresh anaerobic fungi culture supernatant;

[2] Standard error of the mean;

[3] Water-soluble carbohydrates;

[4] Neutral detergent fiber assayed with a heat stable α-amylase and expressed exclusive of residual ash;

[5] Acid detergent fiber expressed exclusive of residual ash;

[6] Acid detergent lignin;

[7] Calculated as aNDFom (g/kg DM) – ADFom (g/kg DM);

[8] Calculated as ADFom (g/kg DM) – ADL (g/kg DM);

[9] Non-fiber carbohydrates, calculated as 1000 – ash (g/kg DM) – crude protein (g/kg DM) – ether extract (g/kg DM) – aNDFom (g/kg DM);

[10] Net energy for lactation, only estimated for pure corn silages.

[11] Not detected;

[12] Crude protein.

potential GP (ml/200 mg DM; PGP) was defined as the sum of the parameters a and b. Besides, the NEL concentration was estimated from 24 h GP values and proximate nutrients for the pure corn silages only using the equation of Menke and Steingass (1988): NEL (MJ/kg DM) = 0.096 × GP + 0.0038 × CP + 0.000173 × ether extract^2 + 0.54 with GP expressed in ml/200 mg DM and CP and ether extract in g/kg DM. As this equation was developed for roughages only, we decided to not use it for mixed silages as the derived estimates may be not reliable.

For the statistical analysis, data were first assessed for normal distribution using the Shapiro-Wilk's normality method of the UNIVARIATE procedure in SAS v9.4 (SAS Institute Inc., Cary, USA). If the data of a certain variable were not normally distributed, they were logarithmically or in a second step square root transformed. In specific, data of CP, WSC, pH, acetic acid, and aerobic stability were logarithmically transformed, while ammonia-N and ethanol concentrations were square root transformed. Afterwards, the data were separated in subsets. To analyze the effect of ensiling the corn alone or mixed with a by-product, only data of control silages were considered. Therefore, data were analyzed with the GLM procedure using the model:

$$Y = \mu + c_i + e_{ij}$$

where μ is the mean, c_i is the main effect of silage substrate and e_{ij} is the residual error. The effects of the applied silage additives were analyzed per silage additive type, i.e., AF culture supernatant, mixed ruminal fluid, and lactic acid bacteria. This resulted in three subsets including the following silage additives: (i) control, inact_AF, and AF, (ii) control, inact_RF, and RF, as well as (iii) control and LAB. These subsets were again analyzed using the GLM procedure of SAS in the following model:

$$Y = \mu + c_i + a_j + (c \times a)_{ij} + e_{ij}$$

where μ is the mean, c_i is the main effect of silage substrate, a_j is the main effect of silage additive, $(c \times a)_{ij}$ is the two-way interaction between the main effects, and e_{ij} is the residual error. For all analyses, the differences between least square means were analyzed by Tukey-Kramer post-hoc test to determine the impact of silage additives within each silage type. All results are reported as least square means and logarithmically or square root transformed data were back-transformed after analysis by raising the power to the 10 and squaring, respectively. The significance level was defined at $P \leq 0.05$ and a trend was declared at $0.05 < P < 0.10$ for all analyses. The boxplot figures were created in RStudio v14.1717 using the package ggplot2 v3.3.3 (Wickham, 2016).

3. Results

3.1. Chemical composition and fermentation pattern of silages

3.1.1. Mixed silages

The W_CON silages had higher concentrations of CP, ether extract, lactic acid, and ethanol than C_CON and S_CON silages (each $P < 0.01$; Table 2). The C_CON silages had a higher pH ($P < 0.01$) as well as different fiber compositions with higher concentrations of aNDFom, ADFom, and cellulose than other silages (each $P < 0.01$), whereas ADL was lower in W_CON than in C_CON or S_CON ($P = 0.01$). The ash concentration was 0.71% points higher in W_CON than in C_CON with S_CON as intermediate ($P = 0.02$), whereas WSC concentrations were ~2% points higher in W_CON and S_CON than in C_CON ($P < 0.01$). The ammonia-N concentrations were higher in C_CON silages than in W_CON silages, while S_CON silages did not differ from others ($P = 0.05$). Furthermore, the DM concentration tended to be lower in W_CON than S_CON with C_CON as intermediate ($P = 0.08$), while acetic acid tended to be more abundant in C_CON than in others ($P = 0.06$). The NFC concentration was higher in S_CON than in C_CON and W_CON, which did not differ ($P < 0.01$). The DM loss was on average 2.79% and not different between silages ($P = 0.12$), which was also true for hemicelluloses ($P = 0.69$), while propionic acid and butyric acid were not detectable in any silage.

3.1.2. Anaerobic fungi culture supernatant

The effects of fresh or heat-inactivated AF culture supernatant on silage composition and fermentation quality are presented in Table 3. We observed an interaction of AF culture supernatant and silage substrate on DM concentration with lower values in C_inactAF than in C_CON and C_AF as intermediate ($P < 0.01$). The CP and ash concentrations were higher in C_inactAF than in C_CON and C_AF (both $P < 0.01$), while for WGF-based silages, ash concentration was lower in W_AF than in W_CON or W_inactAF ($P < 0.01$). Also, W_CON silages had lower concentrations of ADL than W_inactAF or W_AF ($P < 0.01$), whereas cellulose concentration was lower for W_AF than for W_CON with W_inactAF as intermediate ($P < 0.01$). The starch concentrations were higher in C_AF than C_inactAF with C_CON as intermediate, while W_AF and W_CON were higher in starch than W_inactAF and no differences were found between SBP-based silages ($P < 0.01$). Similarly, for pure corn and WGF-based silages, the NFC concentrations were higher with fresh than with heat-inactivated AF culture supernatant and control silages as intermediate, while no differences were found in the SBP-based silages ($P < 0.01$). For variables of the fermentation pattern, interaction effects of additive and silage substrate were also present for silage pH and lactic acid (both $P < 0.01$). Thereby, C_CON silages had a higher pH but lower lactic acid concentration than C_inactAF or C_AF silages. The S_AF silages showed lower pH than S_inactAF silages, which was reflected inversely in lactic acid concentration (both $P < 0.01$). Other interactions were statistically significant, i.e., $P \leq 0.05$, but multiple comparisons of least square means did not reveal different manifestation of the effect of silage additive within different silage substrates.

Regarding the main effect of AF culture supernatant, we found higher concentrations of ash and CP with heat-inactivated than with fresh AF culture supernatant or in control silages (both $P < 0.01$). The ammonia-N concentrations were higher in control silages than in silages with fresh or heat-inactivated AF culture supernatant ($P < 0.01$). The concentrations of ether extract, WSC, hemicelluloses, and

Table 4
Effects of fresh or heat-inactivated mixed ruminal fluid on chemical composition, energy concentration, and fermentation pattern of silages prepared from whole-crop corn solely or with wheat gluten feed pellets or molassed sugar beet pulp pellets. All values in g/kg dry matter (DM) if not stated otherwise.

	Treatment[1]										*P*-values	
	C_CON	C_inactRF	C_RF	W_CON	W_inactRF	W_RF	S_CON	S_inactRF	S_RF	SEM[2]	Additive	Additive × Substrate
Dry matter (g/kg)	341	335	365	323^{b}	375^{a}	339^{ab}	356	366	362	10.1	0.10	0.04
Ash	60.4	50.6	50.9	67.5	59.3	66.4	61.9	58.6	63.7	2.46	0.01	0.23
Crude protein	119	110	115	210^{a}	181^{b}	204^{a}	116	111	112	3.14	< 0.01	0.01
Ether extract	17.3	25.2	18.8	37.2	30.7	33.6	13.6	20.8	13.6	5.92	0.20	0.33
Starch	221	246	216	158	208	142	179	212	182	18.9	0.01	0.90
WSC[3]	30.0	31.3	32.7	54.0	61.8	66.5	47.0^{a}	30.4^{b}	38.2^{ab}	3.47	0.24	0.01
aNDFom[4]	492	474	436	415	387	395	424	432	426	20.0	0.34	0.58
ADFom[5]	296	264	245	221	200	215	243	247	256	13.1	0.28	0.21
ADL[6]	35.8^{b}	91.0^{a}	29.3^{b}	33.6^{b}	62.0^{a}	60.1^{a}	27.5	36.1	33.3	5.58	< 0.01	< 0.01
Hemicelluloses[7]	195	210	191	194	187	180	181	185	171	10.5	0.33	0.92
Cellulose[8]	260	173	216	187	138	154	215	211	223	12.1	< 0.01	0.12
NFC[9]	312	340	379	270	342	302	385	378	381	22.7	0.16	0.23
NEL[10] (MJ/kg DM)	5.76	6.02	5.79	-	-	-	-	-	-	0.11	0.10	-
Dry matter loss (%)	2.67	2.07	2.76	3.37	2.69	2.49	2.32	2.54	2.38	2.24	0.31	0.19
pH	4.12^{a}	3.95^{b}	4.05^{ab}	3.95	3.93	3.95	3.95^{ab}	3.90^{b}	4.02^{a}	0.25	< 0.01	0.01
Lactic acid	38.7	42.9	38.4	55.3	48.1	53.5	41.0	41.9	38.6	1.69	0.71	0.09
Acetic acid	10.3^{ab}	12.4^{a}	7.20^{b}	8.18	5.18	5.31	7.57^{b}	12.2^{a}	11.1^{ab}	0.60	0.02	< 0.01
Propionic acid	n.d.[11]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-
Butyric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-
Ethanol	8.14	6.63	5.41	22.1^{a}	11.1^{b}	5.19^{c}	3.29	8.59	5.83	1.77	< 0.01	< 0.01
Ammonia-N (g/kg CP[12])	25.7	18.6	19.0	17.1	15.9	4.48	22.1	17.3	12.9	1.91	< 0.01	0.19

In each row, superscript letters indicate difference between least square means within a type of silage substrate ($P \leq 0.05$).

[1] C_CON = Whole-crop corn without a silage additive, C_inactRF = Whole-crop corn with heat-inactivated mixed ruminal fluid, C_RF = Whole-crop corn with fresh mixed ruminal fluid, W_CON = Whole-crop corn and wheat gluten feed without a silage additive, W_inactRF = Whole-crop corn and wheat gluten feed with heat-inactivated mixed ruminal fluid, W_RF = Whole-crop corn and wheat gluten feed with fresh mixed ruminal fluid, S_CON = Whole-crop corn and molassed sugar beet pulp without a silage additive, S_inactRF = Whole-crop corn and molassed sugar beet pulp with heat-inactivated mixed ruminal fluid, S_RF = Whole-crop corn and molassed sugar beet pulp with fresh mixed ruminal fluid;

[2] Standard error of the mean;

[3] Water-soluble carbohydrates;

[4] Neutral detergent fiber assayed with a heat stable α-amylase and expressed exclusive of residual ash;

[5] Acid detergent fiber expressed exclusive of residual ash;

[6] Acid detergent lignin;

[7] Calculated as aNDFom (g/kg DM) – ADFom (g/kg DM);

[8] Calculated as ADFom (g/kg DM) – ADL (g/kg DM);

[9] Non-fiber carbohydrates, calculated as 1000 – ash (g/kg DM) – crude protein (g/kg DM) – ether extract (g/kg DM) – aNDFom (g/kg DM);

[10] Net energy for lactation, only estimated for pure corn silages;

[11] Not detected;

[12] Crude protein.

the DM losses were not affected by the additive or its interaction with silage substrate (each $P > 0.10$), while propionic acid and butyric acid were not detectable in any silage. Additionally, the NEL concentration, which was only estimated for pure corn silages, was higher in C_AF than in C_inactAF or C_CON ($P = 0.02$).

3.1.3. Mixed ruminal fluid

The effects of using fresh or heat-inactivated mixed ruminal fluid as a silage additive on silage composition and fermentation quality are summarized in Table 4. The interaction of additive and silage substrate affected the DM concentration with higher values for W_inactRF than W_CON and W_RF as intermediate ($P = 0.04$), while the CP concentration was lower in W_inactRF than W_CON and W_AF ($P = 0.01$). The WSC concentration was only affected in SBP-based silages with higher concentrations in S_CON than S_inactRF, while S_RF did not differ ($P = 0.01$). The ADL concentrations were higher in C_inactRF than in C_CON and C_AF, while for WGF-based silages, W_inactRF and W_RF had higher ADL values than W_CON ($P < 0.01$). Regarding the silage fermentation pattern, pH was higher in C_CON than in C_inactRF and higher in S_RF than S_inactRF ($P = 0.01$). The acetic acid concentration was higher in C_inactRF and S_inactRF when compared to C_RF and C_CON or S_RF and S_CON, respectively ($P < 0.01$). The W_RF silages had lower ethanol concentrations than W_inactRF silages, which was again lower than W_CON silages ($P < 0.01$). As stated before, other interactions were statistically significant, i.e., $P \leq 0.05$, but multiple comparisons of least square means did not reveal different manifestation of the effect of silage additive within different silage substrates.

As a main effect, the addition of heat-inactivated mixed ruminal fluid resulted in lower concentrations of ash ($P < 0.01$) and cellulose ($P = 0.01$) compared to control silages with fresh mixed ruminal fluid as intermediate, while the starch concentration was lower with the addition of heat-inactivated mixed ruminal fluid than for other silages ($P < 0.01$). The ammonia-N concentrations were lower in silages prepared with fresh mixed ruminal fluid than other silages ($P < 0.01$). The concentrations of ether extract, aNDFom, ADFom, hemicelluloses, NFC, and the DM losses were not affected by the additive or its interaction with silage substrate (each $P >$

Table 5
Effects of a lactic acid bacteria-based silage additive on chemical composition, energy concentration, and fermentation pattern of silages prepared from whole-crop corn solely or with wheat gluten feed pellets or molassed sugar beet pulp pellets. All values in g/kg dry matter if not stated otherwise.

	Treatment[1]							*P*-values	
	C_CON	C_LAB	W_CON	W_LAB	S_CON	S_LAB	SEM[2]	Additive	Additive × Substrate
Dry matter (g/kg)	341[a]	301[b]	323	336	356	347	6.37	0.12	0.03
Ash	60.4	65.4	66.4	67.5	61.9	62.4	2.11	0.47	0.45
Crude protein	119[b]	141[a]	210	214	116	116	2.30	< 0.01	0.01
Ether extract	17.3	20.2	37.2	37.1	13.6	16.3	5.18	0.28	0.71
Starch	221	236	158	158	179	159	27.7	0.57	0.88
WSC[3]	30.0	28.6	54.0	62.4	47.0[a]	28.3[b]	3.04	0.18	< 0.01
aNDFom[4]	492	471	415	375	424	430	17.4	0.23	0.47
ADFom[5]	296	275	221	201	243	266	10.5	0.58	0.19
ADL[6]	35.8	42.4	33.6[b]	55.2[a]	27.5	26.7	1.63	< 0.01	< 0.01
Hemicelluloses[7]	195	196	194	174	181	164	10.5	0.20	0.63
Cellulose[8]	260	233	187	146	215	239	10.4	0.16	0.05
NFC[9]	312	303	270	307	385	375	19.4	0.72	0.44
NEL[10] (MJ/kg DM)	5.76	5.97	-	-	-	-	0.82	0.16	-
Dry matter loss (%)	2.67	2.17	3.37	2.25	2.32	2.21	2.11	0.04	0.29
pH	4.12	4.02	3.95	3.90	3.95	3.90	0.20	< 0.01	0.43
Lactic acid	38.7	43.0	55.3	55.4	41.0	42.9	1.23	0.14	0.47
Acetic acid	10.3	16.7	8.10	10.7	7.58	14.8	2.71	0.02	0.61
Propionic acid	n.d.[11]	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-
Butyric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-
Ethanol	8.17	3.24	22.1[a]	3.84[b]	3.26	2.39	0.85	< 0.01	< 0.01
Ammonia-N (g/kg CP[12])	25.7	17.4	17.1	9.11	22.1	39.4	6.18	0.94	0.05

In each row, superscript letters indicate difference between least square means within a type of silage substrate ($P \leq 0.05$).

[1] C_CON = Whole-crop corn without a silage additive, C_LAB = Whole-crop corn with lactic acid bacteria-based silage additive, W_CON = Whole-crop corn and wheat gluten feed without a silage additive, W_LAB = Whole-crop corn and wheat gluten feed with lactic acid bacteria-based silage additive, S_CON = Whole-crop corn and molassed sugar beet pulp without a silage additive, S_LAB = Whole-crop corn and molassed sugar beet pulp with lactic acid bacteria-based silage additive;

[2] Standard error of the mean;

[3] Water-soluble carbohydrates;

[4] Neutral detergent fiber assayed with a heat stable α-amylase and expressed exclusive of residual ash;

[5] Acid detergent fiber expressed exclusive of residual ash;

[6] Acid detergent lignin;

[7] Calculated as aNDFom (g/kg DM) – ADFom (g/kg DM);

[8] Calculated as ADFom (g/kg DM) – ADL (g/kg DM);

[9] Non-fiber carbohydrates, calculated as 1000 – ash (g/kg DM) – crude protein (g/kg DM) – ether extract (g/kg DM) – aNDFom (g/kg DM);

[10] Net energy for lactation, only estimated for pure corn silages;

[11] Not detected;

[12] Crude protein.

0.10), while propionic acid and butyric acid were not detectable in any silage. Likewise, the NEL concentration that was only estimated for pure corn silages was not different between treatments ($P = 0.10$).

3.1.4. Lactic acid bacteria

The results of the effect of a lactic acid bacteria-based silage additive are presented in Table 5. We found an interaction of additive and silage substrate for DM concentration ($P = 0.03$) with lower values in C_LAB than in C_CON. For WSC, concentration was lower in S_LAB than S_CON ($P < 0.01$). In WGF-based silages, the ADL concentration was higher in W_LAB than W_CON ($P < 0.01$), whereas the ethanol concentration was higher in W_CON than in W_LAB ($P < 0.01$). Again, other interactions were statistically significant, i.e., $P \leq 0.05$, but multiple comparisons of least square means did not reveal different manifestation of the effect of silage additive within different silage substrates. Regarding the main effect of additive, compared to control silages, the addition of a lactic acid bacteria-based additive resulted in higher concentration of acetic acid ($P = 0.02$), whereas DM losses ($P = 0.04$) and silage pH ($P < 0.01$) were reduced when adding a lactic acid bacteria-based additive. The concentrations of ash, ether extract, starch, aNDFom, ADFom, hemicelluloses, NFC, and lactic acid were not affected by the additive or its interaction with silage substrate (each $P > 0.10$), while

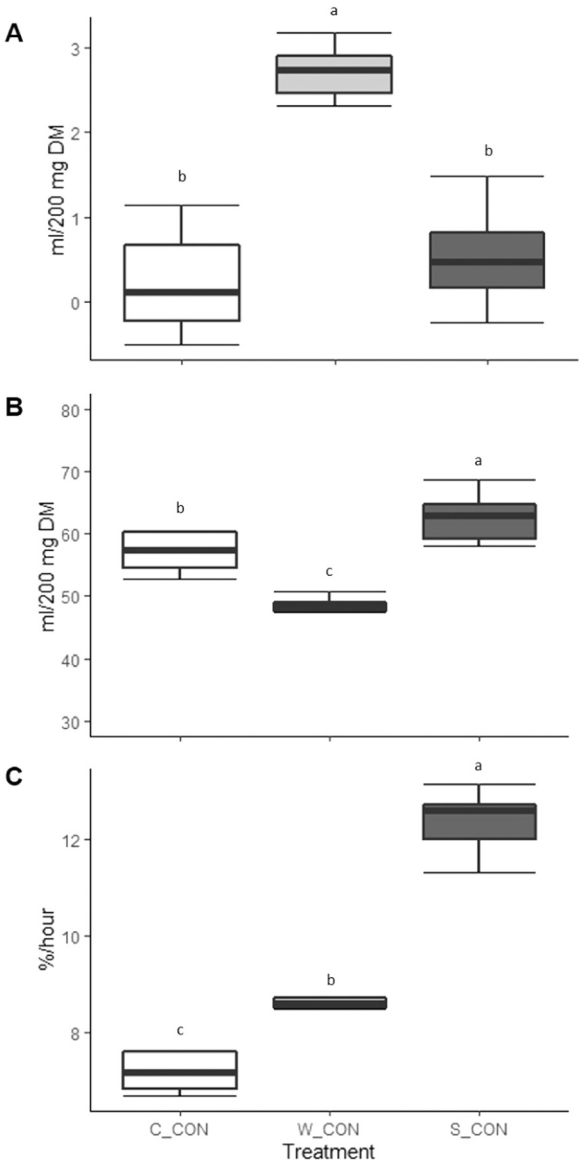

Fig. 1. Effects of mixed ensiling on *in vitro* gas production kinetics of silages prepared from whole-crop corn (C_CON) solely or with wheat gluten feed pellets (W_CON) or molassed sugar beet pulp pellets (S_CON). Boxplots represent the initial gas production from the soluble, immediately available substrate [A], the gas production from insoluble, fermentable substrate [B], and the rate of gas production [C]. Different superscript letters indicate significant difference ($P \leq 0.05$).

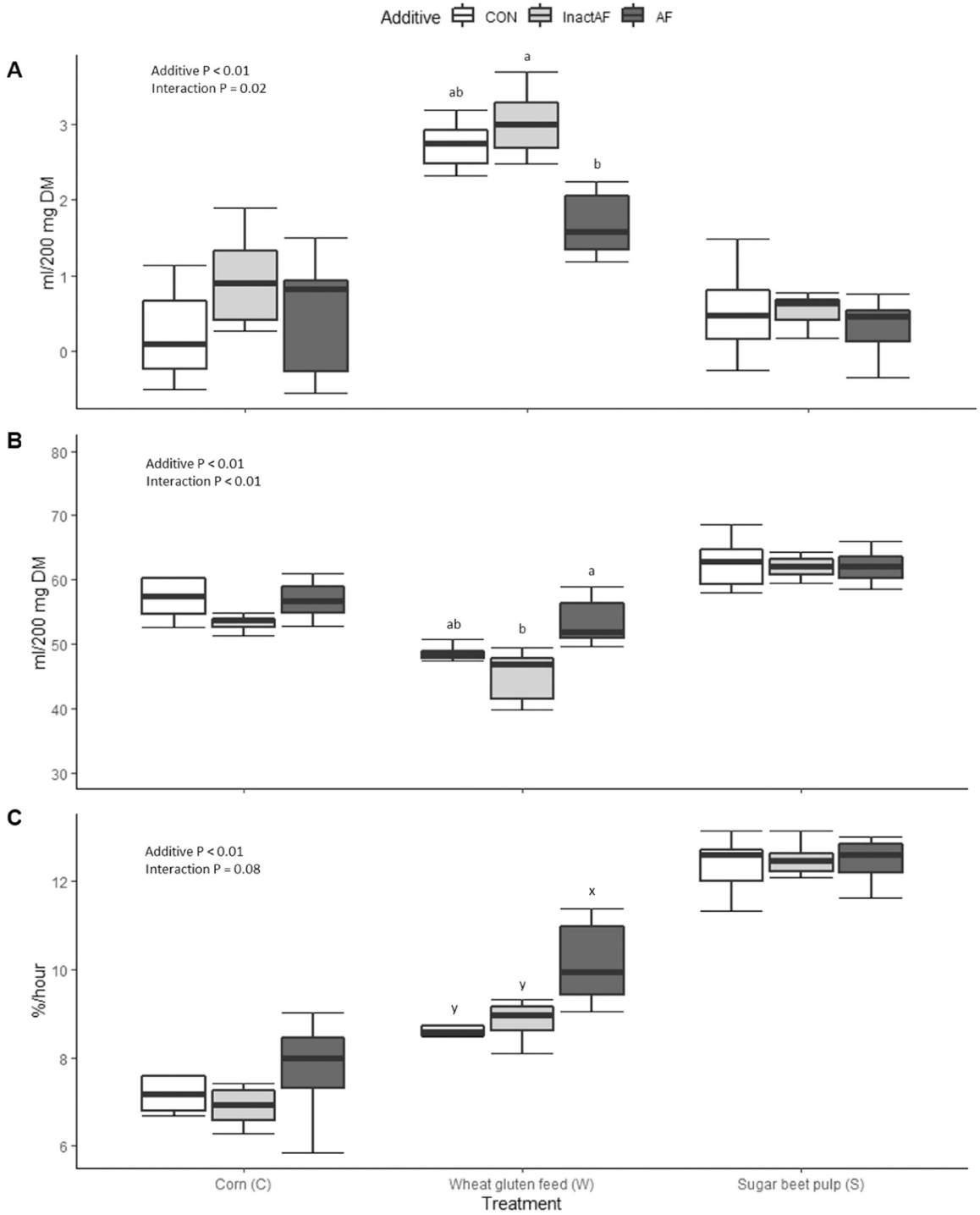

Fig. 2. Effects of no additive (CON), fresh (AF) or heat-inactivated (inactAF) anaerobic fungi culture supernatant on *in vitro* gas production kinetics of silages prepared from whole-crop corn solely or with wheat gluten feed pellets or molassed sugar beet pulp pellets. Boxplots represent the initial gas production from the soluble, immediately available substrate [A], the gas production from insoluble, fermentable substrate [B], and the rate of gas production [C]. Different superscript letters indicate significant difference ($P \leq 0.05$).

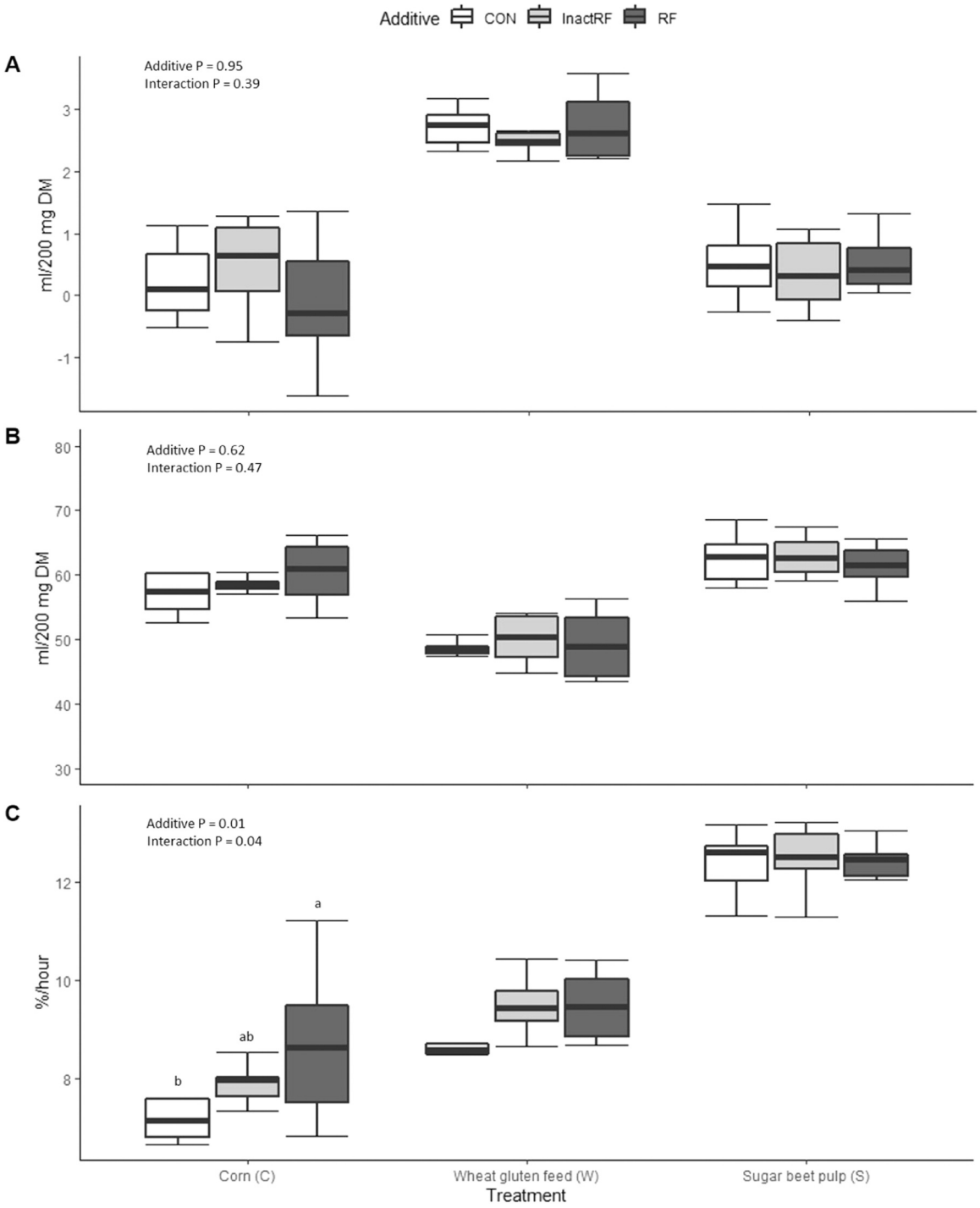

Fig. 3. Effects of no additive (CON), fresh (RF) or heat-inactivated (inactRF) mixed ruminal fluid on *in vitro* gas production kinetics of silages prepared from whole-crop corn solely or with wheat gluten feed pellets or molassed sugar beet pulp pellets. Boxplots represent the initial gas production from the soluble, immediately available substrate [A], the gas production from insoluble, fermentable substrate [B], and the rate of gas production [C]. Different superscript letters (a, b) indicate significant difference ($P \leq 0.05$), different superscript letters (x, y) indicate a trend ($0.05 < P < 0.10$).

propionic acid and butyric acid were not detectable in any silage. Likewise, the NEL concentration, which was only estimated for pure corn silages, was not different between LAB-treated silages and controls ($P = 0.16$).

3.2. *In vitro gas production kinetics of silages*

3.2.1. *Mixed silages*

Incubation of W_CON silages resulted in a higher initial GP from the soluble, immediately available substrate than C_CON or S_CON silages ($P < 0.01$; Fig. 1A). The GP from insoluble, fermentable substrate was also affected by silage substrate ($P < 0.01$) and highest for S_CON, followed by C_CON, and lowest in W_CON (Fig. 1B). Therefore, the PGP followed the same pattern ($P < 0.01$). The rate of GP was highest in S_CON, followed by W_CON and then C_CON ($P < 0.01$; Fig. 1C). The related GP regression curves used to calculate GP kinetic parameters are given in Supplementary Fig. 1.

3.2.2. *Anaerobic fungi culture supernatant*

An interaction of additive and silage substrate was observed for the initial GP from soluble, immediately available substrate ($P = 0.02$) with lower values for W_AF than W_inactAF and W_CON as intermediate (Fig. 2A). Moreover, the GP from insoluble, fermentable substrate was also affected by the interaction ($P < 0.01$) with higher values for W_AF than W_inactAF and W_CON as intermediate (Fig. 2B), which was also true for PGP ($P = 0.03$). The interaction of additive and silage substrate further tended to affect higher GP rates in W_AF than W_inactAF and W_CON ($P = 0.08$; Fig. 2C). In contrast, addition of AF culture supernatant did not affect

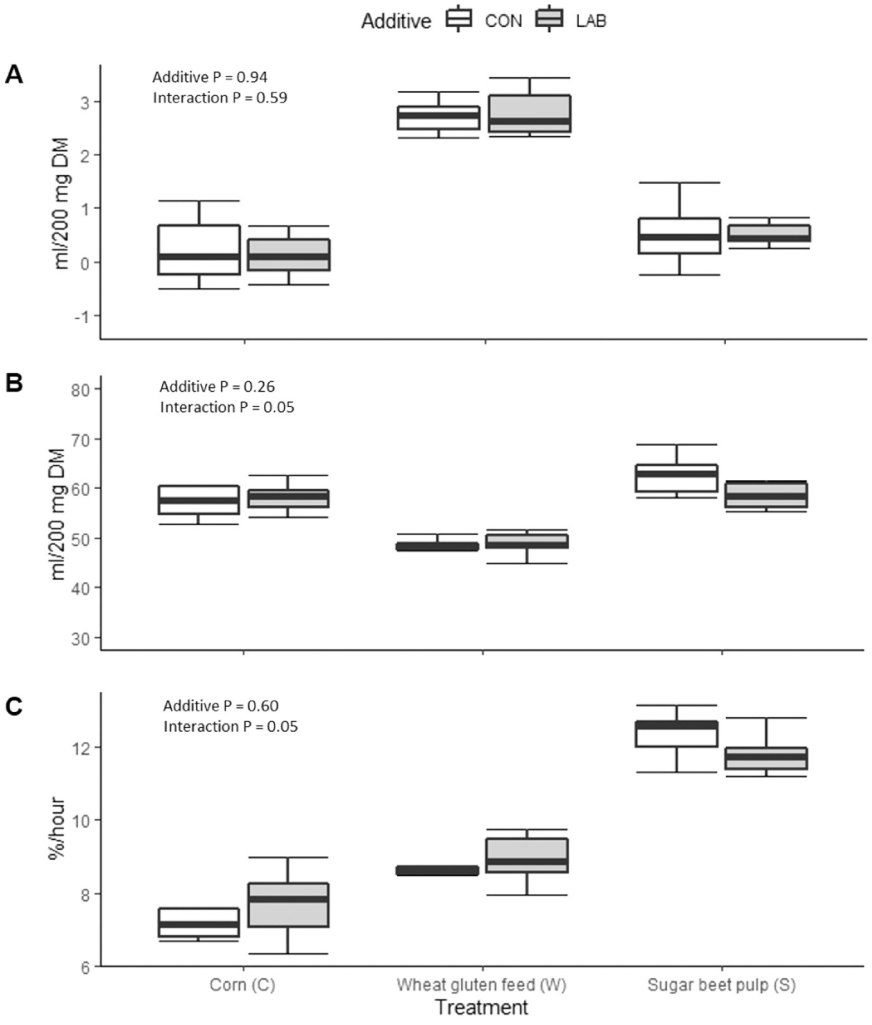

Fig. 4. Effects of no additive (CON) or a lactic acid bacteria-based silage (LAB) on *in vitro* gas production kinetics of silages prepared from whole-crop corn solely or with wheat gluten feed pellets or molassed sugar beet pulp pellets. Boxplots represent the initial gas production from the soluble, immediately available substrate [A], the gas production from insoluble, fermentable substrate [B], and the rate of gas production [C]. Different superscript letters indicate significant difference ($P \leq 0.05$).

GP parameters in pure corn or SBP-based silages. The related GP regression curves used to calculate GP kinetic parameters are given in Supplementary Fig. 2.

3.2.3. Mixed ruminal fluid

The rate of GP was affected by the interaction of additive and silage substrate ($P = 0.04$) with higher rates for C_RF than C_CON and C_inactRF as intermediate, while GP rate was not influenced by additive in WGF and SBP silages (Fig. 3C). The initial GP from soluble, immediately available substrate, the GP from insoluble, fermentable substrate, and the PGP were influenced by neither additive nor the interaction with silage substrate (Figs. 3A and 3B; each $P > 0.10$). The related GP regression curves used to calculate GP kinetic parameters are given in Supplementary Fig. 3.

3.2.4. Lactic acid bacteria

We found interactions of additive and silage substrate for the GP from insoluble, fermentable substrate (Fig. 4B; $P = 0.05$) and the GP rate (Fig. 4C; $P = 0.05$). However, the multiple comparisons of least square means of these interactions did not reveal different manifestation of the effect of silage additive within different silage substrates. The initial GP from soluble, immediately available substrate (Fig. 4A) as well as the PGP were not influenced by the interaction of additive and silage substrate, and we further observed no main effects of additive on *in vitro* GP kinetics (each $P > 0.10$). The related GP regression curves used to calculate GP kinetic parameters are given in Supplementary Fig. 4.

3.3. Aerobic stability of silages

The silage substrate had no effect on the aerobic stability as all control silages remained stable for > 336 h. Regarding the impact of AF culture supernatant, we found no statistical effect of additive or its interaction with silage substrate (both $P > 0.10$), but W_AF and W_inactAF silages had an aerobic stability of 309 h and 240 h, respectively. Silages with other substrates, i.e., pure corn or mixed with SBP, were aerobically stable for > 336 h. Likewise, the addition of mixed ruminal fluid showed no main effect or interaction with silage substrate (both $P > 0.10$). However, W_inactRF and W_RF showed a reduced aerobic stability of 292 h and 277 h, respectively. All silages treated with lactic acid bacteria showed an aerobic stability of > 336 h ($P > 0.10$) and were not different to control silages.

4. Discussion

The present study investigated the chemical composition, silage fermentation pattern, *in vitro* GP kinetics, and aerobic stability of silages prepared from drought-impaired corn solely and with SBP or WGF, and without or with the application of three distinct silage additives.

4.1. Mixed ensiling of whole-crop corn with by-products

The silages prepared from drought-impaired whole-crop corn solely or with WGF or SBP showed a generally sufficient fermentation pattern in the silo as evidenced by a strongly homolactic fermentation, the complete absence of butyric acid and a lasting aerobic stability. Likewise, the DM losses were not different between silages and on an overall low level when compared to the data pool assembled by Borreani et al. (2018). However, results for silage pH may be seen more critical: The pure corn silages had a pH of 4.12 that is higher than the recommended final pH ≤ 4.0 (Kung et al., 2018), which probably resulted from the lower NFC content compared to corn silage grown in Austria under favorable climatic conditions (347 vs. 458 g/kg DM; Gruber et al., 2018). This outlines the benefit of mixed ensiling with by-products, which resulted in adequately low silage pH and so also confirmed our hypothesis. Noteworthy, the inclusion of WGF and therefore the introduction of rather high CP amounts into the silo did not hamper silage fermentation quality and the low ammonia-N levels indicated no extensive CP degradation during ensiling, meaning an appropriate provision with high-quality CP from this mixed silage.

Despite a mostly satisfying conservation effect, our data unambiguously showed that corn silages produced from drought-impaired substrate were clearly deficient in starch and eventually in energy, which is typical for crops exposed to drought conditions (Crasta and Cox, 1996; Lauer, 2012). The dimension becomes particularly obvious when comparing the present starch and estimated energy concentrations of C_CON, i.e., 221 g/kg DM and 5.76 MJ NEL/kg DM, with the data collected for corn silages from 2009 to 2020 across Austria, showing the all-time minimums of 212 g/kg of DM and 6.23 MJ NEL/kg DM for starch and energy, respectively (Resch, 2021). Therefore, the development of strategies to improve the nutritive value of such drought-impaired roughages is urgently needed. Indeed, our findings show that the mixed ensiling of drought-impaired corn with by-products resulted in higher nutrient and energy density as shown by both chemical composition and *in vitro* GP kinetics. The inclusion of WGF substantially elevated the CP concentration to ~210 g/kg DM, which for roughages is achieved almost only when ensiling forage legumes (Kenneth and Beauchemin, 2015) that, however, are difficult to ferment and pose a considerable risk for butyric acid fermentation (Bundesarbeitskreis Futterkonservierung, 2011; Kung et al., 2018). The substantive WSC and starch amounts of in sum > 240 g/kg DM in WGF should have been the key for the strong acidification in the silo and hence outweighed CP-mediated buffering. Consequently, mixed ensiling of corn with WGF could indeed be a promising way to deliver high protein in particular, but still sufficient quantities of energy from roughages. Hereby, it may be kept in mind that the present WGF had a higher CP concentration than typically described, 245 vs. 170 g/kg DM (Jeroch et al., 2020), meaning that CP supply may vary between WGF batches and needs consideration in silage preparation and later diet formulation.

Surprisingly, WGF-based silages had the lowest PGP among all silage types. Admittedly, the WGF-based silages had lower NFC contents than other silages, which also held true for starch if comparing C_CON vs. W_CON. However, we suppose that this was not the sole reason for the low PGP or indicated a per se low ruminal degradability of WGF-based silages. Especially because the GP rate for W_CON was still higher than for C_CON. Instead, the lower PGP may also be reasoned in the stoichiometry of fermentation. The WGF-based silages were substantially higher in CP and fat than other silages and compared to carbohydrates, protein and fat fermentation yields only small or marginal gas amounts, respectively (Wolin, 1960; Menke and Steingass, 1988). Consequently, *in vitro* incubation of WGF-based silages showed a minor PGP than other silages, which, however, may not necessarily stand for a lower provision of energy and microbial biomass.

The increase of the nutritive value by the inclusion of SBP became most obvious from the *in vitro* GP data. The highest extent and rate of GP with SBP as co-substrate vividly suggested a high ruminal degradability and energy provision from the SBP-based silages, therefore confirming our hypothesis and showing the possibility to produce energy-dense roughages with drought-impaired whole-crop corn. A high ruminal degradability is in line with previous findings on SBP-based diets (e.g. Münnich et al., 2018) and should be related to the efficient microbial fermentation of hemicelluloses and especially pectin, both being highly abundant in SBP and the latter considered as the most rapidly fermentable complex carbohydrate (Van Soest, 1994). Notably, such a fermentation may be beneficial for rumen health as the milieu is less challenged (Münnich et al., 2017), particularly when compared to the scenario of providing the same amount of energy via grains (Mojtahedi and Danesh Mesgaran, 2011). Besides the impact of high pectin fermentability, the lignin content could have been a contributing factor as this fraction is assumed to be the main inhibitor of ruminal degradation processes (Jung et al., 1997) and higher lignin contents are common in corn grown under high temperatures (Bernardes et al., 2018). Accordingly, the present ADL concentrations for pure corn silages, i.e., 36 g/kg DM, were deviating upwards from typical concentrations for corn silages (e.g. ~26 g/kg DM in the aforementioned data set of Resch, 2021). But interestingly, ADL levels were not different between C_CON and S_CON and so likely, qualitative differences in lignin, at least in part, could have contributed to the different GP rates. Indeed, growing evidence suggests that as well the composition rather than merely the concentration of lignin has a strong influence on the fermentability in the rumen (Grabber, 2019; Kärkönen et al., 2014; Zhong et al., 2021).

4.2. Application of silage additives

4.2.1. Anaerobic fungi culture supernatant

One of our most important findings was the clearly positive effect of the application of fresh AF culture supernatant on both silage quality and *in vitro* rumen fermentation, therefore confirming our hypothesis. Indeed, fresh AF culture supernatant led to a stronger homolactic fermentation and lower silage pH in pure corn silages and corn co-ensiled with SBP. As mentioned before, silage pH of pure corn silages without additives was slightly above the critical value of 4.0 (Kung et al., 2018), but fell below this threshold with the addition of fresh AF culture supernatant. Similarly, the positive impact of fresh AF culture supernatant was also expressed in higher NEL concentrations for pure corn silages. Therefore, both conservation of and energy provision from drought-impaired whole-crop corn may be enhanced via AF treatments and hence also when mixed ensiling is either not desired or not feasible. For illustration purpose of the AF treatment potential: C_AF had 0.29 MJ NEL/kg DM more than C_CON. Assuming a dairy cow with 22.0 kg DM intake of a diet with 70% roughage proportion, this would mathematically mean a daily surplus of 4.47 MJ NEL that is equivalent to a plus of 1.36 kg fat-corrected milk (GfE, 2001) for each day from the identical ensiled whole-crop corn.

Moreover, fungal enzymes present in the supernatant pre-cleaved the fiber during the silo storage period as evidenced by substantial reductions of cellulose when compared to control, which was most distinct in WGF-based silages, i.e., 187 vs. 126 g/kg DM. This observation matches prior findings on the usage of AF culture supernatant as a novel additive in grass silages (Hartinger et al., 2022). It remains unclear why this cellulolytic impact of AF culture supernatant was not found in SBP-based silages and since a substrate specificity has been observed before (Hartinger et al., 2022), this phenomenon deserves further attention in research. At the same time, fresh AF culture supernatant also enabled highest preservation of starch and NFC in pure corn silages and corn co-ensiled with WGF. In fact, almost complete starch of fresh whole-crop corn was recovered in C_AF. For WGF-based silages, preservation of NFC was disproportionately higher than of starch. Since cellulose decreased with the AF treatment, it can be speculated whether part of the preserved NFC might actually derive from fungal fibrolysis as also WSC and DM losses remained similar between treatments. Therefore, the fate of AF-related fibrolysis products has to be explored in follow-up investigations.

Worth of remark are also the *in vitro* GP kinetics in WGF-based silages when fresh AF culture supernatant was added as the GP from insoluble, fermentable substrate, the PGP as well as the GP rate were higher than for other WGF-based silages. Therefore, AF treatment improved ruminal degradability of WGF-based silages that otherwise performed less than silages prepared from pure corn or corn with SBP. Presumably, the increased starch and NFC concentrations or changes in fiber fractions could explain those improvements in *in vitro* GP kinetics. But, even if the exact mode of action is yet not fully understood, our hypothesis of a higher extent and rate of *in vitro* GP in response to fresh AF culture supernatant was confirmed for WGF-based silages. In this context, we emphasize that the fresh AF treatment indeed showed an overall benefit on the present silages, but not each single effect was present in all silage types and so substrate specificity has to be considered, as well. Likewise, it became also clear from our data that those benefits were indeed associated with the fresh AF treatment and not the heat-inactivated AF culture supernatant. As an additional note, albeit not statistically significant, we observed a slightly reduced aerobic stability in WGF-based silages with fresh AF culture supernatant that may need attention. This incident could be associated with the decrease in acetic acid (Danner et al., 2003) and especially with high temperatures at feed out, a greater risk for spoilage may then be present (Bernardes et al., 2018).

4.2.2. Mixed ruminal fluid

In contrast to the laborious procedure of AF cultivation (Dollhofer et al., 2015) necessary to produce AF culture supernatant, silage inoculation directly with ruminal fluid would be another option. Also, the rumen microbiome harbors a diverse enzymatic repertoire, including AF enzymes (Puniya et al., 2015) and mixed ruminal fluid has been recently explored as a silage additive for grass and straw silages, demonstrating positive impact on silage preservation but not in situ fiber degradability (Hartinger et al., 2022). Due to these still encouraging findings, we as well evaluated mixed ruminal fluid as a silage additive in the present silages. Compared to the controls, the preservation effect was in parts improved by adding fresh mixed ruminal fluid as ethanol and ammonia-N decreased, especially in WGF-based silages. In contrast, prior research showed an increase of ammonia-N in grass and straw silages with fresh mixed ruminal fluid, presumably by proteolytic rumen microbes that were brought into the silo (Hartinger et al., 2022). The present data on ammonia-N, however, showed a sufficient preservation of CP in all silos. A certain degree of substrate specificity and variation in mixed ruminal fluid composition may be causative.

Interestingly, not fresh but heat-inactivated mixed ruminal fluid lowered silage pH, also when compared to control silages. Since DM losses and lactic acid concentrations remained unaffected, our hypothesis of a stronger lactic acid fermentation in response to the application of mixed ruminal fluid was not confirmed and also contrasting previous findings on lactic acid in grass and straw silages (Hartinger et al., 2022). Still, a lower silage pH can be interpreted positively and together with the higher starch levels in silages treated with heat-inactivated mixed ruminal fluid, it may be pursued as an additive that would actually be more convenient in terms of provisioning and handling than fresh mixed ruminal fluid. Nevertheless, the slightly reduced aerobic stability of WGF-based silages treated with mixed ruminal fluid may be kept in mind.

In pure corn silages, fresh mixed ruminal fluid increased the *in vitro* GP rate when compared to controls. At first sight, this may be related to numerically reduced lignin and increased NFC contents in C_RF because lignin predominantly inhibits ruminal degradation (Jung et al., 1997). However, we observed the reverse pattern in WGF-based silages, i.e., significantly more lignin with fresh mixed ruminal fluid than in controls, but still found a similar *in vitro* GP kinetics. Therefore, we could not identify a consistent effect and the causes remain yet unknown. Apart from the discussed parameters, silages were rather marginally influenced by both fresh and heat-inactivated mixed ruminal fluid, which was further reflected in widely invariable *in vitro* GP kinetics.

4.2.3. Lactic acid bacteria

The addition of lactic acid bacteria increased the conservation success as DM losses and silage pH were both lower than in control silages and thus confirmed our hypothesis. Interestingly, these beneficial effects were not reflected in the lactic acid concentration. Although lactic acid was dominant in all silages, the addition of lactic acid bacteria increased its concentration only numerically and reduced WSC in SBP-based but not in other silages. This was rather unexpected since the conversion of WSC into lactic acid represents the main mode of action of lactic acid bacteria-based silage additives (Hartinger et al., 2020). Presumably, the reduction of ethanol and slight increase of acetic acid with the LAB treatment may have contributed to the higher conservation success compared to the control. The additive raised the acetic acid contents by ~7 g to maximum 15 g/kg DM, which may be related to the activity of *Lactobacillus paracasei* that is a constituent of the LAB treatment and shifts between homolactic and heterolactic fermentation (Makras et al., 2005). The present acetic acid concentrations, however, should not adversely affect feed intake (Gerlach et al., 2021), but may improve aerobic stability due to the antimicrobial character of acetic acid (Danner et al., 2003). Although the aerobic stability of LAB-treated and control silages was equally sufficient, slightly higher acetic acid concentrations should indeed aid stabilizing silages during warm feed-out periods when there is an increased spoilage risk (Bernardes et al., 2018). We only found marginal effects on the chemical composition of silages when applying lactic acid bacteria, indicating no further functions of this additive, such as fiber pre-cleavage during the storage period. Similarly, the *in vitro* GP kinetics were not influenced by the LAB treatment.

Taken together, our results of the different silage additives clearly showed that lactic acid bacteria fulfill the ‘classical’ purpose of silage additives, i.e., facilitating roughage preservation (Muck et al., 2018). In contrast, mixed ruminal fluid and fresh AF culture supernatant may each represent a novel type of silage additive with multipurpose character, meaning they as well support substrate preservation and further improve the nutritive value by pre-cleaving fibrous structures that eventually promote ruminal degradability. Yet, differences in their effectiveness between silage substrates have to be considered. From a practical point of view, we already point out that these novel silage additives can be equally applied as commercial silage additives, e.g. lactic acid bacteria, and hence no additional or customized equipment is necessary. Still, production and provisioning of AF culture supernatant as well as mixed ruminal fluid for large-scale application is not yet feasible and needs further efforts, besides the clarification of legal frameworks.

5. Conclusions

Our study showed that mixed ensiling of drought-impaired whole-crop corn with WGF or SBP substantially improved the nutrient composition without restrictions in silage fermentation quality, while lowering silage pH, and a sufficient stability after silo opening. In specific, inclusion of WGF predominantly increased the supply with high-quality CP, while SBP inclusion increased the NFC concentration and boosted the ruminal fermentability. The application of fresh AF culture supernatant improved the preservation effect and considerably increased the energy content of pure corn silages. Noteworthy, this AF treatment as well improved *in vitro* GP kinetics of WGF-based silages that otherwise performed less than other silages. The use of fresh mixed ruminal fluid also showed beneficial effects on silage quality, such as lower ammonia-N concentrations in all silages, whereas heat-inactivated mixed ruminal fluid decreased silage pH. The application of lactic acid bacteria showed no impact on chemical composition or *in vitro* rumen fermentation, whereas substrate preservation was still positively influenced. To date, mixed ensiling with by-products is an unnoticed option for valorizing drought-impaired corn without increasing the feed-food competition, thus deserving more attention as an adaptation

strategy to summer droughts. This may be especially true in combination with AF culture supernatant, representing a promising novel silage additive. Prospective research should explore the impact of feeding such mixed silages on performance, behavior, and health of dairy cows.

Funding

This study was funded by the Austrian Federal Ministry of Agriculture, Forestry, Regions and Water Management, Grant Number 101623.

CRediT authorship contribution statement

Hartinger Thomas: Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fliegerová Katerina:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Gruber Theresa:** Validation, Investigation, Data curation. **Terler Georg:** Writing – review & editing, Methodology, Funding acquisition. **Zebeli Qendrim:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

The authors thank AGRANA Beteiligungs-AG, Vienna, Austria, for providing the by-products and H. Wilhelm Schaumann GmbH, Pinneberg, Germany for the provision of the lactic acid bacteria-based silage additive. The authors further acknowledge the work of A. Dockner, S. Sharma, S. Leiner, and M. Hollmann from the Institute of Animal Nutrition and Functional Plant Compounds at the University of Veterinary Medicine Vienna during the chemical analyses of samples, as well as L. Štrosová from the Institute of Animal Physiology and Genetics, Czech Academy of Sciences, for the AF supernatant preparation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2024.115899.

References

Adee, E., Roozeboom, K., Balboa, G.R., Schlegel, A., Ciampitti, I.A., 2016. Drought-tolerant corn hybrids yield more in drought-stressed environments with no penalty in non-stressed environments. Front. Plant Sci. 7, 1534. https://doi.org/10.3389/fpls.2016.01534.

Adesogan, A.T., Auerbach, H., Bernardes, T.F., Bolsen, K.K., Borreani, G., Cai, Y., Coblentz, W.K., Daniel, J.L.P., Davies, D.R., Driehuis, F., Ferraretto, L.F., Grant, R.J., Huhtanen, P., Kung, L., McAllister, T.A., Muck, R.E., Nadeau, E.M.G., Nishino, N., Nussio, L.G., Rinne, M., Shaver, R.D., Südekum, K.H., Tabacco, E., Vyas, D., Weinberg, Z., Weiß, K., 2020. Letter to the Editor: silage manuscripts in the Journal of Dairy Science. J. Dairy Sci. 103, 6737–6738. https://doi.org/10.3168/jds.2020-18359.

Bernardes, T.F., Daniel, J.L.P., Adesogan, A.T., McAllister, T.A., Drouin, P., Nussio, L.G., Huhtanen, P., Tremblay, G.F., Bélanger, G., Cai, Y., 2018. Silage review: unique challenges of silages made in hot and cold regions. J. Dairy Sci. 101, 4001–4019. https://doi.org/10.3168/jds.2017-13703.

Bidochka, M.J., Tong, K.I., Khachatourians, G.G., 1993. Partial purification and characterization of two extracellular N-acetyl-D-glucosaminidases produced by the entomopathogenic fungus *Beauveria bassiana*. Can. J. Microbiol. 39, 40–45. https://doi.org/10.1139/m93-006.

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.E., 2018. Silage review: factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101, 3952–3979. https://doi.org/10.3168/jds.2017-13837.

Bundesarbeitskreis Futterkonservierung, 2011. Praxishandbuch Futter- und Substratkonservierung, eighth ed. DLG-Verl., Frankfurt am Main.

Caldwell, D.R., Bryant, M.P., 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. Appl. Microbiol. 14, 794–801. https://doi.org/10.1128/am.14.5.794-801.1966.

Ciampitti, I.A., Elmore, R.W., Lauer, J., 2016. Corn Growth and Development, Kansas State University Agricultural Experiment Station and Cooperative Extension Service. (https://bookstore.ksre.ksu.edu/pubs/MF3305.pdf). (Accessed on 15 November 2023).

Crasta, O.R., Cox, W.J., 1996. Temperature and soil water effects on maize growth, development yield, and forage quality. Crop Sci. 36, 341–348. https://doi.org/10.2135/cropsci1996.0011183×003600020022x.

Danner, H., Holzer, M., Mayrhuber, E., Braun, R., 2003. Acetic acid increases stability of silage under aerobic conditions. Appl. Environ. Microbiol. 69, 562–567. https://doi.org/10.1128/AEM.69.1.562-567.2003.

Dollhofer, V., Podmirseg, S.M., Callaghan, T.M., Griffith, G.W., Fliegerová, K., 2015. Anaerobic fungi and their potential for biogas production. In: Gübitz, G.M., Bauer, A., Bochmann, G., Gronauer, A., Weiss, S. (Eds.), Biogas Science and Technology. Springer, Cham.

Forzieri, G., Feyen, L., Rojas, R., Flörke, M., Wimmer, F., Bianchi, A., 2014. Ensemble projections of future streamflow droughts in Europe. Hydrol. Earth Syst. Sci. 18, 85–108. https://doi.org/10.5194/hess-18-85-2014.

Gerlach, K., Daniel, J.L.P., Jobim, C.C., Nussio, L.G., 2021. A data analysis on the effect of acetic acid on dry matter intake in dairy cattle. Anim. Feed Sci. Technol. 272, 114782 https://doi.org/10.1016/j.anifeedsci.2020.114782.

Grabber, J.H., 2019. Relationships between cell wall digestibility and lignin content as influenced by lignin type and analysis method. Crop Sci. 59, 1122–1132. https://doi.org/10.2135/cropsci2018.09.0563.

Gruber, L., Terler, G., Knaus, W., 2018. Nutrient composition, ruminal degradability and whole tract digestibility of whole crop maize silage from nine current varieties. Arch. Anim. Nutr. 72, 121–137. https://doi.org/10.1080/1745039X.2018.1436665.

Hartinger, T., Fliegerová, K., Zebeli, Q., 2022. Suitability of anaerobic fungi culture supernatant or mixed ruminal fluid as novel silage additives. Appl. Microbiol Biotechnol. 106, 6819–6832. https://doi.org/10.1007/s00253-022-12157-w.
Hartinger, T., Kube, K., Gresner, N., Südekum, K.-H., 2020. Varying ensiling conditions affect the fermentation quality and abundance of bacterial key players in lucerne silages. J. Agric. Sci. 158, 297–303. https://doi.org/10.1017/S002185962000057X.
Hinds, A.A., Lowe, L.E., 1980. Application of the Berthelot reaction to the determination of ammonium-N in soil extracts and soil digests. Commun. Soil Sci. Plant Anal. 11, 469–475. https://doi.org/10.1080/00103628009367054.
Jeroch, H., Drochner, W., Rodehutscord, M., Simon, A., Simon, O., Zentek, J., 2020. Ernährung landwirtschaftlicher Nutztiere. utb GmbH, Stuttgart, Deutschland.
Jung, H.G., Mertens, D.R., Payne, A.J., 1997. Correlation of acid detergent lignin and Klason lignin with digestibility of forage dry matter and neutral detergent fiber. J. Dairy Sci. 80, 1622–1628. https://doi.org/10.3168/jds.S0022-0302(97)76093-4.
Kärkönen, A., Tapanila, T., Laakso, T., Seppänen, M.M., Isolahti, M., Hyrkäs, M., Virkajärvi, P., Saranpää, P., 2014. Effect of lignin content and subunit composition on digestibility in clones of timothy (*Phleum pratense* L.). J. Agric. Food Chem. 62, 6091–6099. https://doi.org/10.1021/jf5016494.
Kenneth, A.A., Beauchemin, K.A., 2015. Alfalfa and other perennial legume silage. In: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Silage Science and Technology. John Wiley & Sons, Ltd, pp. 633–664.
Kung, L., Shaver, R.D., Grant, R.J., Schmidt, R.J., 2018. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci. 101, 4020–4033. https://doi.org/10.3168/jds.2017-13909.
Lauer, J., 2012. The effects of drought and poor corn pollination on corn. Field Crops 28, 493–495.
Lever, M., 1977. Carbohydrate determination with 4-hydroxybenzoic acid hydrazide (PAHBAH): effect of bismuth on the reaction. Anal. Biochem 81, 21–27. https://doi.org/10.1016/0003-2697(77)90594-2.
Makras, L., van Acker, G., Vuyst, L. de, 2005. *Lactobacillus paracasei* subsp. *paracasei* 8700:2 degrades inulin-type fructans exhibiting different degrees of polymerization. Appl. Environ. Microbiol 71, 6531–6537. https://doi.org/10.1128/AEM.71.11.6531-6537.2005.
McDonald, P., Henderson, N., Heron, S., 1991. The Biochemistry of Silage, second ed. Chalcombe Publications, Marlow, p. 340.
Menke, K.-H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res Dev. 28, 7–55.
Mojtahedi, M., Danesh Mesgaran, M., 2011. Effects of the inclusion of dried molassed sugar beet pulp in a low-forage diet on the digestive process and blood biochemical parameters of Holstein steers. Livest. Sci. 141, 95–103. https://doi.org/10.1016/j.livsci.2011.05.009.
Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung, L., 2018. Silage review: recent advances and future uses of silage additives. J. Dairy Sci. 101, 3980–4000. https://doi.org/10.3168/jds.2017-13839.
Muir, B.M., Anderson, A.R., 2022. Development and diversification of sugar beet in Europe. Sugar Tech. 24, 992–1009. https://doi.org/10.1007/s12355-021-01036-9.
Münnich, M., Khiaosa-Ard, R., Klevenhusen, F., Hilpold, A., Khol-Parisini, A., Zebeli, Q., 2017. A meta-analysis of feeding sugar beet pulp in dairy cows: effects on feed intake, ruminal fermentation, performance, and net food production. Anim. Feed Sci. Technol. 224, 78–89. https://doi.org/10.1016/j.anifeedsci.2016.12.015.
Münnich, M., Khol-Parisini, A., Klevenhusen, F., Metzler-Zebeli, B.U., Zebeli, Q., 2018. Graded replacement of maize grain with molassed sugar beet pulp modulated ruminal microbial community and fermentation profile in vitro. J. Sci. Food Agric. 98, 991–997. https://doi.org/10.1002/jsfa.8547.
Oetzel, G.R., Villalba, F.P., Goodger, W.J., Nordlund, K.V., 1993. A comparison of on-farm methods for estimating the dry matter content of feed ingredients. J. Dairy Sci. 76, 293–299. https://doi.org/10.3168/jds.S0022-0302(93)77349-X.
Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92, 499–503. https://doi.org/10.1017/s0021859600063048.
Puniya, A.K., Singh, R., Kamra, D.N. (Eds.), 2015. Rumen Microbiology: from Evolution to Revolution. Springer India, New Delhi, India.
Resch, R., 2021. Quality potentials of grass and maize silages in Austria Findings from the LK-Silageproject 2020 [Qualitätspotenziale bei Gras- und Maissilagen in Österreich – Erkenntnisse aus dem LK-Silageprojekt 2020]. In: Alternative Ansätze im Milchvieh-Management Grundfutterqualität: Aktuelle Forschungsergebnisse aus dem Institut Milchrassekälber - Herausforderungen und Lösungen, 48. Viehwirtschaftliche Fachtagung. HBLFA, Raumberg-Gumpenstein, 33–67.
Titterton, M., Maasdorp, B.V., 1997. Nutritional improvement of maize silage for dairying: mixed crop silages from sole and intercropped legumes and a long season variety of maize. 2. Ensilage. Anim. Feed Sci. Technol. 69, 263–270. https://doi.org/10.1016/S0377-8401(97)81640-9.
Toreti, A., Bavera, D., Acosta Navarro, J., Cammalleri, C., de Jager, A., Di Ciollo, C., Hrast Essenfelder, A., Maetens, W., Magni, D., Masante, D., Mazzeschi, M., Niemeyer, S., Spinoni, J., 2022. Drought in Europe August 2022. Publ. Off. Eur. Union. https://doi.org/10.2760/264241.
Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, second ed. Cornell University Press, Ithaca, NY.
VDLUFA, 2012. VDLUFA-Methodenbuch Bd. III Die chemische Untersuchung von Futtermitteln, third ed., VDLUFA-Verlag, Darmstadt.
Weiß, K., Kaiser, E., 1995. Milchsäurebestimmung in Silageextrakten mit Hilfe der HPLC. Wirtschaftseig. Futter 41, 69–80.
Weiß, K., Kroschewski, B., Auerbach, H.U., 2022. The influence of delayed sealing and repeated air ingress during the storage of maize silage on fermentation patterns, yeast development and aerobic stability. Fermentation 8, 48. https://doi.org/10.3390/fermentation8020048.
Weißbach, F., Strubelt, C., 2008. Correcting the dry matter content of maize silages as a substrate for biogas production. Landtechnik 63, 82–83.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer, New York, USA, p. 260.
Wolin, M.J., 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43, 1452–1459. https://doi.org/10.3168/jds.S0022-0302(60)90348-9.
Zhong, H., Zhou, J., Abdelrahman, M., Xu, H., Wu, Z., Cui, L., Ma, Z., Yang, L., Li, X., 2021. The effect of lignin composition on ruminal fiber fractions degradation from different roughage sources in water buffalo (*Bubalus bubalis*). Agriculture 11, 1015. https://doi.org/10.3390/agriculture11101015.