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In situ ruminal degradability and fermentation characteristics of novel mixtures of winter cereal and Italian ryegrass plus winter cereal silages

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Abstract: This study was conducted using three multiparous non-lactating rumen-cannulated Holstein-Friesian dairy cows, with the objective of evaluating the *in situ* ruminal degradability and fermentation characteristics of novel mixtures of winter cereal and Italian ryegrass (*Lolium multiflorum* Lam.) plus winter cereal silages (mixture A: triticale, oats, barley and wheat; mixture B: triticale, barley and wheat; mixture C: Italian ryegrass and oats; mixture D: Italian ryegrass, oats, triticale, barley and wheat). The rumen fermentation study was conducted replacing the ensiled mixtures (experimental diets) with vetch-triticale haylage in a total mixed ration (control diet). It was found that the effective protein degradability at 0.08 rumen outflow rates was 80.6% (mixture A), 66.2% (mixture B), 79.7% (mixture C) and 79.3% (mixture D). The effective neutral detergent fibre (NDF) and acid detergent fibre (ADF) effective degradability at 0.08 rumen outflow rates was 18.0% and 17.7% (mixture A), 19.7% and 20.5% (mixture B), 19.1% and 17.0% (mixture C), and 15.2% and 14.6% (mixture D), respectively. Different dietary treatments did not change ($P > 0.05$) the rumen fermentation characteristics as there was no difference ($P > 0.05$) between control and experimental diets, and the inclusion of 40–55% Italian ryegrass (mixture C and D) did not cause any difference. These results suggest that the mixture of winter cereals and Italian ryegrass plus winter cereal-based silages had good potentially degradable dry matter, effective dry matter and effective protein degradability at 0.01, 0.05 and 0.08 rumen outflow rates without affecting the rumen environment maintaining neutral pH. The ensiled mixtures had a moderate level of potentially degradable NDF and ADF fraction.

Keywords: dairy cows; effective degradability; mixture silage; rumen outflow

In many countries, maize silage is the main component of dairy cattle diet due to its high yield and energy content as well as digestibility (Keim et al. 2013). During the time of maize crop failure due to extreme weather as well as in high livestock density areas, winter cereals and/or Italian ryegrass

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(*Lolium multiflorum* Lam.) plus winter cereal mixture silage can be used as alternative forage to maize silage. However, the use of such mixtures in maize-growing regions has not been reported so far.

According to the literature data, forage from winter cereal mixtures grown and ensiled alone has outstanding yields, feed value and ruminal degradability (Kleinmans et al. 2016). For instance, the degradability of barley and winter oat in the rumen is high; as a result, it improves the dry matter intake (DMI). Winter triticale also has a high yield potential. On the other hand, Italian ryegrass both in its fresh and preserved form is frequently used as forage for dairy cows and known for its high energy value and highly digestible fibre (Tamburini et al. 1995). It has an elevated concentration of water-soluble carbohydrates (WSC, also known as high sugar grasses) (Turner et al. 2006). The high WSC improve the balance and synchrony of the nitrogen and energy supply to the rumen (Miller et al. 2001). The crude protein and net energy contents of certain varieties of Italian ryegrass (e.g. perennial Bahial hybrid, one-year Suxyl variety) are high (175–179 g/kg dry matter and 6.25–6.28 MJ/kg dry matter) (Lehel et al. 2011). High energy concentration due to good nutrient digestibility can be explained by the relatively low content of acid detergent lignin in the grass hybrid silage (20 g/kg and 27 g/kg dry matter) (Lehel et al. 2011). These characteristics could improve both the rumen degradability and fermentation characteristics of ensiled mixtures. Therefore, our hypothesis is based on how different cereal mixtures as well as the inclusion of 40–55% Italian ryegrass to the cereal mixtures affect the rumen degradability and fermentation characteristics of ensiled mixtures.

Polyculture of cereal mixtures, as well as grass plus cereal mixtures for silage-making practice, has several nutritional advantages: it makes up for the nutrient deficiency in the total mixed ration (TMR), reduces the cost of concentrate, economic and nutritional benefits to dairy farms, maintains the rumen environment and consequently increases *in situ* rumen degradation kinetics as well as the ruminal fermentation process (Lyons et al. 2016). Italian ryegrass and winter cereals can be grown together (Geren 2014) and this practice can provide feed earlier than annual ryegrass (early autumn) because they are generally more adaptable to early sowing due to higher tolerance to dry conditions (Geren 2014). The harvest window for the correct stage of maturity for high forage quality (flag-leaf) is wider for Italian ryegrass, triticale, barley, oat and wheat than for fall rye. Double cropping of winter cereals and additional Italian ryegrass with maize silage can have both environmental and economic benefits because two different forages can be obtained from an area within a year (Lyons et al. 2016; Ranck et al. 2020). Despite the benefits, little information is available on the factors that need to be considered when growing cereal grain plus Italian ryegrass mixtures as well as the composition (winter cereal grain-to-Italian ryegrass ratio), cutting (e.g., phenological phase, particle size), silage making, etc. The literature data on the rumen fermentation and *in situ* degradability of winter cereals plus Italian ryegrass mixtures are also very limited.

According to our previous findings, Italian ryegrass and winter cereal silages underwent rapid fermentation (Alemayehu et al. 2019). The high concentration of potentially degradable neutral detergent fibre (NDF) and effective protein degradability (EPD) imply that this mixture can be included successfully in high-yielding dairy cattle diets. Additionally, the proper stage of harvesting (heading stage) of all ensiled mixtures together with the high crude protein, energy and WSC (sugar) content of Italian ryegrass (Miller et al. 2001; Turner et al. 2006; Lehel et al. 2011) could boost the *in situ* ruminal degradability and ruminal fermentation characteristics.

The main objective of this study was to evaluate the *in situ* ruminal degradability of ensiled mixtures and their ruminal fermentation of substituted vetch and triticale haylage in maize silage-lucerne haylage-based TMR. A further aim of our study was to evaluate whether the 40–55% proportion of Italian ryegrass (mixtures C and D) has an advantage for the rumen degradation of nutrients and the ruminal fermentation parameters compared to the use of winter cereals (triticale, barley, wheat and/or oat) (mixtures A and B).

MATERIAL AND METHODS

Experimental site and ensiling procedures

The trial was carried out on a medium-scale experimental farm (Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Kaposvár, Hungary – 46.3666687 N, 17.7999992 E, at an al-

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titude of 153 m a.s.l. Four different cereal and Italian ryegrass mixtures (commercial products, Agroteam S.p.a., Torrimpietre, Italy) were studied: mixture A (40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat), mixture B (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat), mixture C (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat), mixture D (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat). Mixtures are used nowadays for cattle feeding in maize-based regions. The experimental field allotted 3 ha to each mixture. Deep loosening and disc + cylinder cultivation were executed as stubble tillage. Before sowing on sandy soil, 351 kg/ha of artificial fertilizer (NPK: 16 : 16 : 16) was applied. The seedbed was prepared by a Kongskilde VibroFlex 7400 cultivator (lifted; Kongskilde Agriculture, Hudson, IL, USA). The four different forage mixtures were sown on 29 September 2018 (sowing rate of 75 kg seed/ha for each mixture) at a depth of 3 cm by a John Deere 740A seed drill (John Deere, Moline, IL, USA). No plant protection treatment was applied during the growing season. The annual precipitation was 425 mm in 2018. Cutting was carried out on the heading stage of wheat based on the existing extended BBCH-scale (Meier 2001) (BBCH 51–58, Italian ryegrass: BBCH 51; oat: BBCH 51; triticale: BBCH 53; winter wheat: BBCH 52; winter barley: BBCH 58) at a 10-cm stubble height. The fresh mixture A [dry matter (DM): 186 g/kg; crude protein (CP): 125 g/kg DM; total sugar: 168 g/kg DM], mixture B (DM: 184 g/kg; CP: 117 g/kg DM; total sugar: 166 g/kg DM), mixture C (DM: 168 g/kg; CP: 108 g/kg DM; total sugar: 168 g/kg DM) and mixture D (DM: 173 g/kg; CP: 95 g/kg DM; total sugar: 140 g/kg DM) were wilted to ~35% DM (24 h) without any movement on the windrow. The wilted forage was chopped by a forage harvester (John Deere 7300; John Deere, Moline, IL, USA) on the concrete surface with theoretical chop length (TCL) of 9 mm (weight: 800 kg). Wilted and chopped material (510 g) was packed by hand into glass jars (0.000 72 m^3 volume). Additionally, the remaining harvested and wilted forage was used for baleage, with a capacity of 578–675 kg, and ensiled for 90 days for the ruminal fermentation study.

Chemical analysis of mixture silages

At the end of 90 days of fermentation, five laboratory silos per experimental mixture were opened. The DM, CP, crude fibre (CF), NDF, acid detergent fibre (ADF), ether extract (EE) and total sugar content of all mixtures were determined following specific procedures with identification numbers 37 (nitrogen), 39 (fat), 44 (fibre), 55 (sugars). The chemical analyses of fresh mixtures and mixture silages as well as samples from baleage (rumen fermentation study) were done following the AOAC (2006) protocol and Van Soest et al. (1991) for fibre fraction analysis (ADF, NDF, acid detergent lignin) using the sodium sulphite assay. The nitrogen-free extract (NFE) was calculated as 100% – (%EE + %CP + %Ash + %CF).

Ruminal degradability

After 90 days of fermentation, the ensiled mixtures (laboratory silos) were subjected to the ruminal degradability study. The ruminal degradability trial was carried out with three multiparous non-lactating Holstein-Friesian dairy cows (600 ± 35 kg body weight) previously surgically fitted (ethical permission No. SOI/31/01044-3/2017) with a ruminal cannula (10 cm inner diameter, Bar-Diamond Inc., Parma, ID, USA) on the experimental dairy farm of Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Hungary. Cows were fed TMR formulated according to the dairy nutrient requirement and feeding standard (NRC 2001) in equal portions at 8:00 and 14:00. The baseline/control diet (6.32 MJ net energy for lactation/kg DM; 14.4% DM CP; 39.06% DM NDF; 23.66% DM ADF, and 35.71% DM non-fibrous carbohydrate) consisted of 5.5 kg/day of maize silage, 3.5 kg/day of lucerne haylage, 3.5 kg/day of vetch-triticale haylage, 3 kg/day of concentrate, 1 kg/day of grass hay and 0.75 kg/day of liquid molasses (Table 1). Water and licking salt were available *ad libitum*. Rumen incubations were carried out according to Herrera-Saldana et al. (1990). The silage was dried in a forced oven (60 °C for 48 h) and ground using a 1-mm screen and retained in a sealed bag. Then the dried and ground sample was measured and filled to Ankom nylon bags of 5 × 10 cm with a pore size of 53 µm (Ankom Technology, Macedon, NY, USA) with a sample

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Table 1. Composition and calculated values of the baseline diet used in the rumen degradability study and in the ruminal fermentation study as a control diet

Parameter	Baseline/control diet
Ingredient (kg)	
Maize silage	5.50
Lucerne haylage	3.50
Vetch-triticale haylage	3.50
Concentrate[1]	3.00
Grass hay	1.00
Molasses (liquid)	0.75
Calculated nutrients	
Dry matter (%)	47.42
Crude protein (%DM)	14.40
Neutral detergent fibre (%DM)	39.06
Acid detergent fibre (%DM)	23.66
Acid detergent lignin (%DM)	4.68
Ether extract (%DM)	2.83
Non-fibrous carbohydrate (%DM)	35.71
Starch (%DM)	25.60
Sugar (%DM)	6.06
Calcium (%DM)	1.08
Phosphorus (%DM)	0.40
Sodium (%DM)	0.23
Vitamin A (IU/kg)	8 725
Vitamin D (IU/kg)	1 722
Vitamin E (mg/kg)	43
Net energy for lactation (MJ/kg DM)	6.32

DM = dry matter

[1]Vitafort Co., Dabas, Hungary, dry matter: 88.00%, crude protein: 16.00%, net energy for lactation MJ/kg: 6.74, crude fibre: 5.00%, ether extract: 2.90%, ash: 8.30%, starch: 42.71%, sugar: 2.34%, calcium: 1.71%, phosphorus: 0.57%, sodium: 0.66%, magnesium: 0.37%, vitamin A: 22 800 IU/kg, vitamin D: 4 500 IU/kg, vitamin E: 128 mg/kg

weight of 5.0 g and sealed with electric sealer. Then the bags (two hours after morning feeding) were incubated for 0 h, 2 h, 4 h, 8 h, 16 h, 24 h, 48 h and 72 h incubation time sequentially for each incubation hour and recovered for each hour. In each incubation 60 bags per sample were used (five bags × four replications per sample × three cows; Table 2).

Five bags refer to total number/batch of bags incubated for each mixture to have a precise mean degradable value. Then the five bags are repeated twice (which means we have 10 bags for each incubation hour for each cow). Therefore, for the three cows we have 30 bags/cows/mixture/each incubation hour. Again, the same procedure was repeated in the second phase of incubation for each hour.

Table 2. Incubation procedure of nylon bags for rumen degradability study

	Batch	Bags	Cow 1	Cow 2	Cow 3
Incubation phase I	1	5	10 bags	10 bags	10 bags
	2	5			
Incubation phase II	3	5	10 bags	10 bags	10 bags
	4	5			

The 0 h samples were not placed in the rumen, but soaked and rinsed as described below. Removed bags were placed in cold tap water immediately after removal from the rumen, and washed by hand until the water was clear. After washing, the bags were dried in a forced air oven at 60 °C for 48 h, air equilibrated and weighed. Residues from the bags were pooled within time and animal (n = 96/mixture), finely ground using a mortar and pestle to the particle size which passed through a 1-mm screen to determine the DM (%), CP (%DM), NDF (%DM) and ADF (%DM). The NDF (%DM) and ADF (%DM) contents were residual portions after rinsing according to Van Soest et al. (1991).

Ruminal fermentation

The ruminal fermentation trial was carried out with three multiparous non-lactating, rumen-cannulated Holstein-Friesian dairy cows. The ruminal fermentation trial was conducted with mixtures of silage following 90 days' fermentation in the form of baleage capacity (578–675 kg) for all ensiled mixtures. Cows consumed TMR (control diet) as described in the ruminal degradability study, plus the substitution of 3.5 kg/day of ensiled mixtures (experimental diets 1, 2, 3 and 4) for vetch-triticale haylage (Tables 1 and 3). The daily ration of both the control and experimental diets was given in two instalments (8:00 and 14:00). The pre-feeding (adaptation) period lasted two weeks, followed by the two-week experimental phase (experimental diets 1–4). The control and experimental diets (four mixtures) in a TMR were allocated for each cow sequentially, all the three cows received both the con-

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Table 3. Control and experimental diets and their compositions for the ruminal fermentation study

Diets	Components
Control diet (CT)	5.5 kg/day of maize silage, 3.5 kg/day of lucerne haylage, 3.5 kg/day of VTH, 3 kg/day of concentrate, 1 kg/day of grass hay and 0.75 kg/day of liquid molasses
Experimental diet 1	CT + mixture A silage (3.5 kg/day, replacing VTH)
Experimental diet 2	CT + mixture B silage (3.5 kg/day, replacing VTH)
Experimental diet 3	CT + mixture C silage (3.5 kg/day, replacing VTH)
Experimental diet 4	CT + mixture D silage (3.5 kg/day, replacing VTH)

Control diet = 6.32 MJ net energy for lactation/kg dry matter; 14.4% crude protein; 39.06% neutral detergent fibre; 23.66% acid detergent fibre and 35.71% non-fibrous carbohydrate (see Table 1); mixture A = 40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat; mixture B = 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C = 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D = 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat; VTH = vetch-triticale haylage

trol and experimental diets one by one (Figure 1). On sampling days, approximately 150 ml of rumen fluid samples were collected three times a day (immediately before the morning feeding and 3 h and 6 h thereafter, n = 36/mixture) through the cannula using a sample tube with pump for rumen fluid collection (Bar-Diamond Inc., Parma, ID, USA). The pH was measured immediately using a digital pH meter (Metrohm 744; Metrohn AG, Herisau, Switzerland). Ammonia was determined by the Berthelot method (Chaney and Marbach 1962). Thereafter, samples were centrifuged to analyse the volatile fatty acids (VFA) and lactic acid. The concentrations of volatile fatty acids (acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid or n-valeric acid, iso-caproic acid, caproic acid) of rumen fluid and silages were measured by gas chromatography (Model CP 9002;

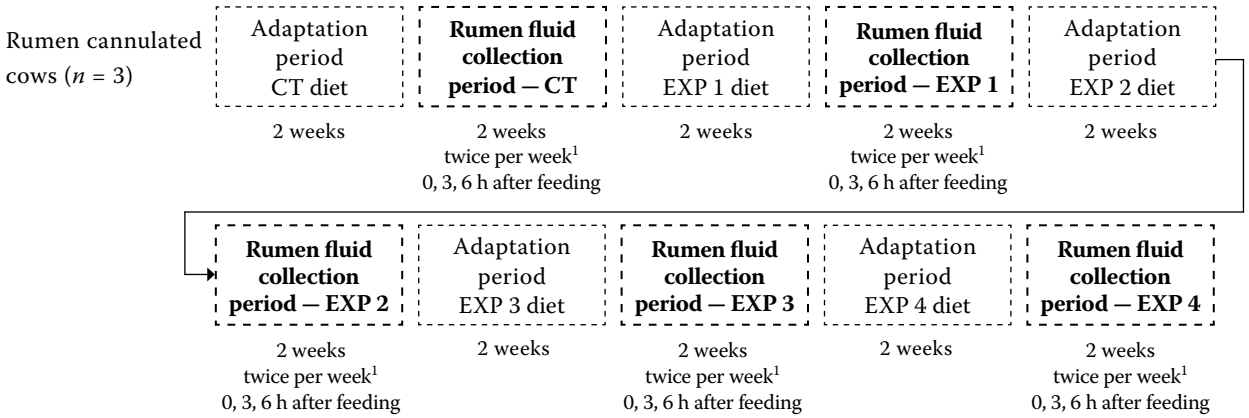

Figure 1. The experimental design for rumen fermentation study

CT = control diet: 5.5 kg/day of maize silage, 3.5 kg/day of lucerne haylage, 3.5 kg/day of vetch-triticale haylage, 3 kg/day of concentrate, 1 kg/day of grass hay and 0.75 kg/day of liquid molasses; EXP 1 = experimental diet 1: control + mixture A silage (40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat) 3.5 kg/day, replacing vetch-triticale haylage; EXP 2 = experimental diet 2: control + mixture B silage (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) 3.5 kg/day, replacing vetch-triticale haylage; EXP 3 = experimental diet 3: control + mixture C silage (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) 3.5 kg/day, replacing vetch-triticale haylage; EXP 4 = experimental diet 4: control + mixture D silage (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat)

[1]Rumen fluid collection twice (on Monday and on Wednesday) for each week and a total of four collections per two weeks

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Chrompack, Goes, The Netherlands), as described by Playne (1985). The lactate was analysed by the high-performance liquid chromatography method developed by Playne (1985).

Calculations

Residues from the nylon bags at each incubation time were analysed for DM (%), CP (%DM), NDF (%DM) and ADF (%DM) as described above. Ruminal nutrient disappearance data (DM, CP, ADF) were used to determine nutrient degradation parameters using the Orskov and McDonald (1979) model (Equations 1 and 2). The model for the kinetics of NDF digestion was a simple first-order kinetic equation with the addition of a discrete lag time (Mertens and Loften 1980).

Equation 1 (Orskov and McDonald 1979):

$$P = a + b\,(1 - e^{-ct}) \quad (1)$$

where:

P – the dry matter, crude protein, neutral detergent fibre or ADF disappearance (%) at time t;
a – the soluble fraction (%);
b – the potentially degradable fraction (%);
c – the rate of degradation of the b fraction (%/h).

Effective degradability (ED) of DM, CP, NDF and ADF was then calculated according to Equation 2 (Orskov and McDonald 1979):

$$ED = a + [(b \times c)/(k + c)] \quad (2)$$

where:

a – the soluble fraction (%);
b – the potentially degradable fraction (%);
c – the rate of degradation of the b fraction (%/h);
k – the rumen outflow rate assumed to be 1%/h, 5%/h and 8%/hour.

NLIN program in SAS v9.4 (SAS Institute, Inc., Cary, NC, USA) was used to calculate the values of a, b and c.

Statistical Analysis

Data were analysed using the GLM procedure for ANOVA in SAS v9.4 (SAS Inst. Inc., Cary, NC, USA). Significant mean value differences were evaluated by Tukey's test followed by a post hoc pairwise comparison of means. A significance level of $P < 0.05$ was used. Comparison of means for degradability components was performed following the model:

$$Y_i = \mu + \alpha_i + \varepsilon_i \quad (3)$$

where:

Y_i – the observation in the i^{th} silage type;
μ – the overall mean;
α_i – the i^{th} silage type effect;
ε_i – the random error.

Comparison of means for effective nutrient degradability was computed for 1%, 5% and 8% rumen outflow rates. Additionally, comparison of the means of variables between treatments for rumen fermentation characteristics was computed using two-way ANOVA and the following model:

$$Y_i = \mu + \alpha_i + \varepsilon_i \quad (4)$$
$$Y_j = \mu + \beta_j + \varepsilon_j \quad (5)$$

where:

Y_i and Y_j – the observations in the i^{th} treatment and j^{th} rumen fluid sampling period;
μ – the overall mean;
α_i and β_j – the i^{th} treatment and the j^{th} rumen fluid sampling period effects;
ε_i and ε_j – the random errors.

RESULTS

Chemical composition and fermentation characteristics

Table 4 shows the chemical composition and fermentation characteristics (baleage and laboratory silos) of the ensiled mixtures after 90 days of fermentation. The chemical composition results showed the DM (30.32–33.94%), CP (9.36–12.56% DM), NDF (57.54–66.66% DM) and ADF (33.18–38.16% DM) for ensiled mixtures. The study of fermentation characteristics showed pH (4.47–5.30), lactic acid (3.44–5.32% DM), NH_3–N (3.99–5.35 g/100 g total N) and lactic acid per total fermentable acid (74.28–88.54) for silages. Additionally, the VFAs were limited to acetic acid and propionic

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Table 4. Nutritional compositions and fermentation characteristics of silage at the end of 90 days of fermentation (n = 15/mixture)

Components	Silage types			
	mixture A	mixture B	mixture C	mixture D
Nutritional composition				
DM (%)	33.06	30.32	33.94	32.38
CP (%DM)	11.70	9.36	11.18	12.56
EE (%DM)	2.96	3.56	2.78	3.74
CF (%DM)	35.10	30.32	34.20	34.10
NDF (%DM)	66.66	57.54	61.14	61.92
ADF (%DM)	38.16	33.18	36.78	34.48
Total sugar (%DM)	13.30	5.28	6.18	10.28
NFE (%DM)	41.76	50.74	39.06	40.68
Fermentation characteristics				
pH	5.03	4.47	5.04	5.30
Acetate (%DM)	0.42	0.70	0.73	0.41
Butyrate (%DM)	0.19	1.10	0.02	0.10
Propionate (%DM)	nd	0.05	nd	nd
Lactate (%DM)	4.35	5.32	3.44	4.08
TFA	4.97	7.23	4.20	4.59
LA/AA	10.39	8.00	5.80	10.27
LA (%TFA)	87.46	74.28	82.74	88.54
NH_3–N (g/100 g total N)	3.99	5.35	4.22	4.42

AA = acetic acid; ADF = acid detergent fibre; CF = crude fibre; CP = crude protein; DM = dry matter; EE = ether extract; LA = lactic acid; mixture A = 40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat; mixture B = 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C = 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D = 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat; nd = not detected; NDF = neutral detergent fibre; NFE = nitrogen free extract; TFA =total fermentable acid

acid and their values were low (< 1% DM) in all ensiled mixtures. Other examined VFAs like caproic, valeric and butyric acid (except mixture B) were not detected. The lactic acid content for the Italian ryegrass plus winter cereal grain-based ensiled mixtures (mixtures C and D) was lower than in the winter cereal-based mixtures (mixtures A and B).

Ruminal degradability

Table 5 shows ruminal degradability of DM, CP, NDF and ADF and effective degradability (ED). There was a difference ($P < 0.05$) in the soluble fraction, potentially degradable DM fraction, and effective DM degradability 1 (ED_1), degradability 5 (ED_5) and degradability 8 (ED_8) between the mixtures; however, the rate of DM degradation was similar ($P > 0.05$) between the mixtures. Mixture D had the highest soluble DM fraction (31.9% of DM) and the lowest potentially degradable DM fraction (42.5% of DM) compared to the other mixture silages. The effective degradable DM at the three rumen outflow rates (ED_1, ED_5, and ED_8) for all ensiled mixtures was higher — above 66%. The effective DM degradability at 1% rumen outflow rate (ED_1) was in the range of 71–75%. Italian ryegrass plus winter cereal grain-based silage (mixtures C and D) had higher ($P < 0.05$) effective DM degradability-8 (ED_8) than cereal-based silage (mixtures A and B).

There was a difference ($P < 0.05$) in all degradable CP components between the mixture silages. Mixture A had a higher ($P < 0.05$) *in situ* soluble CP fraction (68.3% of DM) and a lower ($P < 0.05$)

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Table 5. Ruminal degradability of nutrients in different silage mixtures (n = 96/mixture)

	Mixture A	Mixture B	Mixture C	Mixture D	SEM	Significance level
Dry matter (DM)						
Soluble fraction (%DM)	10.2^{c}	7.4^{d}	13.3^{b}	31.9^{a}	0.201	***
Potentially degradable fraction (%DM)	63.7^{a}	64.9^{a}	62.7^{a}	42.5^{b}	1.257	***
Degradation rate (%/h)	0.78	0.78	1.00	0.69	0.120	ns
Effective degradability 1 (%)	73.2^{ab}	71.5^{b}	75.4^{a}	73.8^{ab}	1.312	*
Effective degradability 5 (%)	70.0^{bc}	68.4^{c}	73.0^{a}	71.5^{b}	0.882	**
Effective degradability 8 (%)	67.9^{b}	66.2^{b}	71.4^{a}	69.9^{a}	0.650	***
Crude protein						
Soluble fraction (%DM)	68.3^{a}	7.4^{d}	18.3^{c}	45.2^{b}	0.358	***
Potentially degradable fraction (%DM)	16.9^{c}	64.9^{a}	65.1^{a}	36.5^{b}	0.860	***
Degradation rate (%/h)	0.22^{d}	0.78^{c}	1.27^{a}	1.08^{b}	0.071	***
Effective degradability 1 (%)	84.5^{a}	71.5^{c}	82.9^{ab}	81.4^{b}	0.798	***
Effective degradability 5 (%)	82.0^{a}	68.4^{c}	81.0^{ab}	80.1^{b}	0.668	***
Effective degradability 8 (%)	80.6^{a}	66.2^{b}	79.7^{a}	79.3^{a}	0.631	***
Neutral detergent fibre						
Soluble fraction (%DM)	6.9^{b}	7.6^{b}	9.5^{a}	7.5^{b}	0.536	**
Potentially degradable fraction (%DM)	42.0	34.3	31.9	37.0	0.188	ns
Degradation rate (%/h)	0.02	0.044	0.03	0.02	0.009	ns
Effective degradability 1 (%)	38.0	32.5	34.2	31.7	0.467	ns
Effective degradability 5 (%)	22.2^{a}	23.5^{a}	22.5^{a}	18.4^{b}	0.295	**
Effective degradability 8 (%)	18.0^{a}	19.7^{a}	19.1^{a}	15.2^{b}	0.788	***
Acid detergent fibre						
Soluble fraction (%DM)	6.2	7.4	8.0	7.5	0.887	ns
Potentially degradable fraction (%DM)	39.0	29.7	33.3	41.2	4.725	ns
Degradation rate (%/h)	0.03	0.06	0.03	0.02	0.017	ns
Effective degradability 1 (%)	36.2	32.9	32.0	30.6	0.992	ns
Effective degradability 5 (%)	21.9^{ab}	23.9^{a}	20.1^{bc}	17.4^{c}	0.348	**
Effective degradability 8 (%)	17.7^{b}	20.5^{a}	17.0^{bc}	14.6^{c}	0.918	***

Mixture A = 40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat; Mixture B = 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mixture C = 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mixture D = 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat; ns = not significant

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

$^{a–d}$Values in rows with different letters differ significantly ($P \leq 0.05$)

potentially degradable CP fraction (16.9% of DM) than the other mixture silages. The effective protein degradability (at 8% rumen outflow rate/h) was 80.6% (mixture A), 66.2% (mixture B), 79.7% (mixture C) and 79.3% (mixture D).

The soluble NDF and ADF fractions of all ensiled mixtures were low. Mixture C had a higher ($P < 0.05$) *in situ* soluble NDF fraction than the other mixture silages; however, there was no difference ($P > 0.05$) in the *in situ* soluble ADF fraction or in the potentially degradable NDF and ADF fraction between the mixture silages. The effective NDF degradability at 0.08/h rumen outflow rate was low ($P < 0.05$) for mixture D but similar

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($P > 0.05$) between mixture A, B and C silages. Mixture B had higher ($P < 0.05$) effective ADF degradability at 0.08/h rumen outflow rate than the other mixture silages. There was no difference ($P > 0.05$) in effective NDF and ADF degradability at 0.01/h rumen outflow rate between mixture A, B, C and D silages. The potentially degradable as well as effective degradable NDF and ADF at 0.01 and 0.08/h rumen outflow rates were low (Table 5).

Ruminal fermentation

The replacement of ensiled mixtures with vetch-triticale haylage in TMR did not modify the rumen fermentation characteristics (Table 6); there was no difference ($P > 0.05$) between control and experimental diets, even the inclusion of 40–55% Italian ryegrass (mixture C and D) did not cause any difference. This result also indicated that the inclusion level can be increased beyond this level in a total mixed ration formulation as far as an appropriate forage to concentrate ratio as well as energy requirement of dairy cows is maintained depending on its production status. However, the rumen ammonia nitrogen (NH_3–N) and butyric acid (BA) concentrations were affected ($P < 0.05$) by sampling time (3 h) (Table 7). The mean ruminal pH ranged between 6.93 and 7.06 and the mean ruminal NH_3–N concentration ranged from 12.37 to 19.25 mg/l (Table 6). The rumen NH_3–N concentration was higher ($P < 0.05$) in experimental diet 1 as compared to experimental diet 4, and the ruminal BA concentration was higher ($P < 0.05$) in experimental diet 4 than in the other dietary treatments (Table 7, sampling time 3 h). The mean total VFA, AA, PA and BA ranges were 99.39–108.32 mmol/l, 74.73–76.97 mmol/l, 16.96–21.60 mmol/l and 7.72–10.49 mmol/l, respectively (Table 6). The VFA concentration of the rumen was limited to acetic acid, propionic acid and butyric acid. Other investigated VFAs such as caproic and valeric acid concentration were below the detectable level (< 0.1 mmol/l). Neither was lactic acid detected.

DISCUSSION

Ruminal degradability

The ensiled mixtures (novel mixtures of winter cereals and Italian ryegrass plus winter cereals silages, cutting at the heading stage of wheat) had high effective degradable DM and CP at the three rumen outflow rates (ED_1, ED_5 and ED_8) and mod-

Table 6. The rumen fermentation characteristics of control and experimental diets (n = 36/treatment)*

Components	Treatments					SEM	P-value
	control	experimental diet 1	experimental diet 2	experimental diet 3	experimental diet 4		
pH	6.98	6.94	7.06	6.98	6.93	0.27	ns
NH_3–N (mg/l)	16.82	19.25	15.21	14.22	12.37	12.50	ns
Total VFA (mmol/l)	103.70	103.12	99.39	103.10	108.32	17.10	ns
Acetate (mmol/l)	76.97	76.08	74.73	76.88	76.23	10.83	ns
Propionate (mmol/l)	17.36	17.45	16.96	17.14	21.60	4.50	ns
Butyrate (mmol/l)	9.36	9.61	7.72	9.08	10.49	2.29	ns

Control = 5.5 kg/day of maize silage, 3.5 kg/day of lucerne haylage, 3.5 kg/day of vetch-triticale haylage, 3 kg/day of concentrate, 1 kg/day of grass hay and 0.75 kg/day of liquid molasses; experimental diet 1 = control + mixture A silage (40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat) 3.5 kg/day, replacing vetch-triticale haylage; experimental diet 2 = control + mixture B silage (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) 3.5 kg/day, replacing vetch-triticale haylage; experimental diet 3 = control + mixture C silage (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) 3.5 kg/day, replacing vetch-triticale haylage; experimental diet 4 = control + mixture D silage (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat); ns = not significant; VFA = volatile fatty acids

*All the 36 measurements per treatment were considered as repeated measures in the model rather than independent measures in statistical analysis

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Table 7. The rumen fermentation characteristics of control and experimental diet at different sampling times (n = 36/treatment)

	Treatments					SEM	P-value
	control	experimental diet 1	experimental diet 2	experimental diet 3	experimental diet 4		
0 h							
pH	7.33	7.31	7.28	7.26	7.23	0.107	ns
NH_3–N (mg/l)	11.68	12.23	9.69	10.64	11.95	2.443	ns
Total VFA (mmol/l)	88.86	85.54	83.82	84.75	83.03	5.852	ns
Acetate (mmol/l)	66.92	64.32	64.18	64.25	62.85	5.126	ns
Propionate (mmol/l)	14.33	14.01	13.59	13.51	13.27	0.830	ns
Butyrate (mmol/l)	7.10	7.20	5.88	6.91	6.89	0.774	ns
3 h (after feeding)							
pH	6.78	6.75	6.90	6.78	6.81	0.106	ns
NH_3–N (mg/l)	29.73^{ab}	36.93^{a}	30.80^{ab}	27.06^{ab}	22.50^{b}	5.925	*
Total VFA (mmol/l)	114.62	113.74	116.63	117.19	123.99	7.458	ns
Acetate (mmol/l)	83.85	81.07	86.34	86.27	87.16	6.401	ns
Propionate (mmol/l)	19.93	20.05	20.99	20.33	23.26	1.737	ns
Butyrate (mmol/l)	10.83^{b}	11.12^{b}	9.29^{b}	10.59^{b}	13.58^{a}	0.850	***
6 h (after feeding)							
pH	6.83	6.77	6.94	6.91	7.41	0.707	ns
NH_3–N (mg/l)	9.06	8.66	5.13	4.95	2.70	3.964	ns
Total VFA (mmol/l)	108.12	110.18	97.72	107.40	117.95	12.682	ns
Acetate (mmol/l)	80.12	81.34	73.49	80.11	78.66	7.766	ns
Propionate (mmol/l)	17.84	18.30	16.22	17.55	28.29	8.880	ns
Butyrate (mmol/l)	10.15	10.53	8.00	9.76	10.99	1.409	ns

Control = 5.5 kg/day of maize silage, 3.5 kg/day of lucerne haylage, 3.5 kg/day of vetch-triticale haylage, 3 kg/day of concentrate, 1 kg/day of grass hay and 0.75 kg/day of liquid molasses; experimental diet 1 = control + mixture A silage (40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat) 3.5 kg/day, replacing vetch-triticale haylage; experimental diet 2 = control + mixture B silage (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) 3.5 kg/day, replacing vetch-triticale haylage; experimental diet 3 = control + mixture C silage (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) 3.5 kg/day, replacing vetch-triticale haylage; experimental diet 4 = control + mixture D silage (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat); ns = not significant; VFA = volatile fatty acids

*$P \leq 0.05$; ***$P \leq 0.001$; ^{a,b}values in rows with different letters differ significantly ($P \leq 0.05$)

erate potentially degradable DM and CP. The *in situ* degradability of the examined nutrient content (DM, CP, NDF, ADF) of the mixtures varied greatly depending on the proportion of cereals (mixtures A and B) and Italian ryegrass (mixtures C and D). The degradable fraction of DM and CP in the novel mixtures showed significantly different degradation values depending on whether 45% of oats were associated with 40% of Italian ryegrass (mixture C) or other cereals (15% triticale, 30% oats, 10% barley, 10% wheat) with 55% of Italian ryegrass (mixture D). A significant difference was found in the effective degradability (ED_5, ED_8) of the NDF content of the two Italian ryegrass plus winter cereal silages (mixture C versus mixture D).

Ruminal degradability of DM

The effective ruminal DM degradability at 8% rumen outflow rate was 67.9%, 66.2%, 71.4% and 69.9%, respectively. These values were higher than the DM degradability of Italian ryegrass (60.7%) reported by Andrighetto et al. (1993). The effec-

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tive DM degradability at 1% rumen outflow rate, which defines the maintenance DM requirement, was 73.2% (mixture A), 71.5% (mixture B), 75.4% (mixture C) and 73.8% (mixture D) by far better than in the report of Andrighetto et al. (1993).

Ruminal Degradability of CP

The soluble fraction of mixture A (68.3%) silage was higher than the soluble CP fraction of ryegrass silage (49.0%) at its vegetative stage (Valderrama and Anrique 2011) and different forage cereals (Hadjipanayiotou et al. 1996). On the other hand, the soluble fraction of mixture A (68.3%) was comparable with the values in oat forages (68.47%) (Hadjipanayiotou et al. 1996). Soluble CP fractions of mixture B (7.4%) and mixture C (18.3%) silages were lower than in Italian ryegrass forage at the first (20.6%) and second cut (19.2%) of the leaf stage (Amrane and Michelet-Doreau 1993). Both the potential and effective degradability of CP in present silage mixtures was lower than that of Italian ryegrass forage at the first (81.4%) and second cut (82.3%) of the leaf stage and grazing (81.8%) and heading (82%) stages. The effective CP degradability at 8% rumen outflow rate, except mixture B, was higher than the CP degradability of Italian ryegrass forage at the end of its heading stage (76.9%). The high effective CP degradability at 8% rumen outflow rate could be attributed to the early harvest (heading) of all ensiled mixtures as well as the inclusion of more Italian ryegrass in Italian ryegrass plus winter cereal-based fermented mixtures (55% in mixture C and 40% in mixture D). Italian ryegrass has higher CP at the appropriate stage of harvesting, i.e. at the second cut (Baldinger et al. 2011), which is similar to the CP values at the end of the 90-day fermentation period in the present study. The potentially degradable CP fraction of all ensiled mixtures was lower than the slowly degradable CP fraction of Italian ryegrass forage at the first (74.8%) and second cut (76.8%) of the leaf stage (Amrane and Michelet-Doreau 1993). The degradability rate of all ensiled mixtures was substantially higher than that of Italian ryegrass forage at the first (0.14/h) and second cut (0.14/h) of the leaf stage, grazing stage (0.11/h) and heading stage (0.10/h) (Amrane and Michelet-Doreau 1993). This higher rate of CP degradability would make the current silage mixture attractive to combine with other higher fibre crops for better forage utilization in the nutrition of dairy cows. As compared to effective protein degradability (EPD) values at 0.05/h and 0.08/h in the present ensiled mixtures, Valderrama and Anrique (2011) reported higher EPD values at 0.05/h and 0.08/h for lucerne forage (88.25% and 85.16%) and oat forage (90.80%) at the vegetative stage. The EPD at 0.08/h rumen outflow rate of ryegrass forage (80.6%) was comparable with mixture A (80.6%), and higher than mixtures B, C and D (66.2%, 79.7% and 79.3%). The EPD values at 0.05/h rumen outflow rate of all ensiled mixtures were better than in barley (69%, 6% and 56.0%) and oats (66%, 60% and 56%) at flowering, pod formation and early maturity, respectively (Hadjipanayiotou et al. 1996).

Ruminal degradability of NDF and ADF

The amount and ruminal degradability of NDF are very important factors in dairy cow nutrition because forage NDF varies widely in its degradability in the rumen and NDF digestibility influences animal performance (Bender et al. 2016). The low potential and effective ruminal degradability of NDF and ADF in our trial could be associated with the high NDF and ADF contents of ensiled mixtures (see Table 3). The potential ruminal NDF degradability of all ensiled mixtures was lower than the NDF degradability of Italian ryegrass (59.8%) reported by Andrighetto et al. (1993). Ali et al. (2014) also reported higher NDF degradability of grass silage (76.4%) as compared to the present silage mixtures. The rate of ruminal NDF degradation (k_p) for all ensiled mixtures was higher than in grass/grass-clover silage (0.01%/h) as well as whole-crop cereal silage (0.01%/h) reported by Weisbjerg et al. (2007).

Ruminal fermentation

The observed ruminal pH values of cows fed different dietary treatments were closer to neutral and ideal values for all rumen microbes (Wales et al. 2004). Castillo-Gonzalez et al. (2014) reported that, within the ruminal ecosystem, the microorganisms coexist in a reduced environment and the pH remains close to neutral. All the pH values (except experimental diet 2) were in the normal range of ruminal pH 6.0–7.0 (Wales et al. 2004) and pH 5.5–7.0

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(Krause and Oetzel 2006) depending on the diet and buffering capacity of saliva. The lack of difference in pH between control and experimental diets implies that the dietary treatment did not alter the rumen environment for efficient fermentation; as a result, the rumen microbes were able to adapt to the given diet. This result was similar to the report of Nur Atikah et al. (2018). The observed NH_3–N values for experimental diets 2, 3 and 4 were lower than the range of an optimum NH_3–N level (16.5–37.9 mg/l) that favours the ruminal microbial activity in animals fed materials rich in lignocellulose (Nur Atikah et al. 2018). The observed VFA, AA, and PA values were higher than the total VFA (86.6 mmol/l), AA (58.7 mmol/l) and PA (16.9 mmol/l) of the normal dairy diet (Sutton et al. 2003). The total VFA and AA values were also higher than the total VFA (92.8 mmol/l) and AA (48.6 mmol/l) of low-roughage diets reported by the same authors. PA values were lower than the PA (36.6 mmol/l) value of low-roughage diets; additionally, the BA values of experimental diets were lower than BA (11.0 mmol/l) and BA (8.8 mmol/l) of normal and low-roughage diets, respectively.

CONCLUSION

The ensiled mixtures had high effective degradable DM and CP at the three rumen outflow rates (ED_1, ED_5 and ED_8) and moderate potentially degradable DM and CP. The 40–55% inclusion of Italian ryegrass in cereal grain (mixture C and D) caused higher effective degradability (ED_8) of DM and CP (except mixture A) and lower NDF and ADF (except mixture C) degradability (ED_8) over winter cereal mixture silages (mixture A and B). The replacement of ensiled mixtures with vetch-triticale haylage in TMR did not cause any deleterious effect on rumen environment as pH remained similar to control diets. Due to the high effective degradable DM and CP of the silages (A, B, C, D), it could be successfully included in the diet of high-producing lactating cows; however, the low potential and effective ruminal degradable NDF and ADF should be considered in the proper ration formulation, particularly for high-producing lactating cows. Additionally, the inclusion of a moderate dose of some exogenous enzymes that improve the rumen degradability of NDF and ADF will be considered in future use.

Therefore, when formulating cattle feed, special attention should be paid to the tested nutrients and ruminal degradability data available for the mixture used. Further experiments should be performed to improve the practical use of the novel mixtures of winter cereals and Italian ryegrass plus winter cereal-based silages. The optimal proportions of winter cereals plus Italian ryegrass mixtures should be established in these experiments, besides determining the effect of the phenological phase at cutting on the nutrient content of the mixtures and their ruminal degradability. Feeding those field trials should be performed to determine the effects of the mixtures on the production parameters.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Alemayehu W, Tothi R, Orosz S, Febel H, Kacsala L, Bazar G, Toth T. Nutrient content and fermentation characteristics of ensiled Italian ryegrass and winter cereals mixtures for dairy cows. Krmiva. 2019;61(1):3-10.

Ali M, Cone JW, Van Duinkerken G. Relationship between chemical composition and in situ rumen degradation characteristics of grass silages in dairy cows. NJAS-Wagen J Life Sci. 2014 Dec 6;70:9-15.

Amrane R, Michelet-Doreau B. Effect of maturity stage of Italian ryegrass and lucerne on ruminal nitrogen degradability. Ann Zootech. 1993;42(1):31-7.

Andrighetto I, Bailoni L, Cozzi G, Tolosa HF. Observations on in situ degradation of forage cell components in alfalfa and Italian ryegrass. J Dairy Sci. 1993 Sep 1;76(9):2624-31.

AOAC – Association of Official Agricultural Chemists. Official methods of analysis. 18th ed. Washington, DC: AOAC International; 2006.

Baldinger L, Baumung R, Zollitsch W, Knaus WF. Italian ryegrass silage in winter feeding of organic dairy cows:

https://doi.org/10.17221/12/2021-CJAS

Forage intake, milk yield and composition. J Sci Food Agri. 2011 Feb;91(3):435-42.

Bender RW, Lopes F, Cook DE, Combs DK. Effects of partial replacement of corn and alfalfa silage with tall fescue hay on total-tract digestibility and lactation performance in dairy cows. J Dairy Sci. 2016 Jul 1;99(7):5436-44.

Castillo-Gonzalez AR, Burrola-Barraza ME, Dominguez-Viveros J, Chavez-Martinez A. Rumen microorganisms and fermentation. Arch Med Vet. 2014;46(3):349-61.

Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem. 1962 Apr 1;8(2):130-2.

Geren H. Dry matter yield and silage quality of some winter cereals harvested at different stages under Mediterranean climate condition. Turk J Field Crops. 2014 Jan 1; 19(2):197-202.

Hadjipanayiotou M, Antoniou I, Theodoridou M, Photiou A. In situ degradability of forages cut at different stages of growth. Liv Prod Sci. 1996 Jan 1;45:49-53.

Herrera-Saldana RE, Huber JT, Poore MH. Dry matter, crude protein, and starch degradability of five cereal grains. J Dairy Sci. 1990 Sep 1;73(9):2386-93.

Keim J, Valderrama X, Alomar D, Lopez I. In situ rumen degradation kinetics as affected by type of pasture and date of harvest. Sci Agric. 2013 Dec;70(6):405-14.

Kleinmans JJ, Densley RJ, Hurley T, Williamsa ID, Calvert F. Brief communication: Feed value of maize silage in New Zealand - a review. Proc New Zeal Soc Anim Prod. 2016 Jul 4;76:100-2.

Krause KM, Oetzel GR. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim Feed Sci Technol. 2006 Mar 9;126(3-4):215-36.

Lehel L, Orosz S, Tothne PA, Sumeghy L, Hajda Z, Sipiczki B, Varhegyi J, Febel H. The rye grass silage apparent digestibility, rumen protein degradability, content of metabolizable protein and net energy. AWETH. 2011;7:149-57.

Lyons SE, Kilcer TF, Ort S, Swink S, Godwin G, Hanshar J, Czymmek KJ, Ketterings QM. Double crop rotations with winter cereals and corn silage or forage sorghum. 2016: 6 p.

Meier U. Growth stages of mono- and dicotyledonous plants. BBCH monograph. 2nd ed. Berlin, Germany: Federal Biological Research Centre for Agriculture and Forestry; 2001. 158 p.

Mertens DR, Loften JR. The effect of starch on forage fiber digestion kinetics in vitro. J Dairy Sci. 1980 Sep 1;63 (9):1437-46.

Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, Theodorou MK. Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.). Milk production from late-lactation dairy cows. Grass Forage Sci. 2001 Dec;56(4):383-94.

NRC – National Research Council. Nutrient requirements of dairy cattle. 7th ed. Washington, DC: National Academies Press; 2001.

Nur Atikah I, Alimon AR, Yaakub H, Abdullah N, Jahromi MF, Ivan M, Samsudin AA. Profiling of rumen fermentation, microbial population and digestibility in goats fed with dietary oils containing different fatty acids. BMC Vet Res. 2018 Dec;14(1): 9 p.

Orskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci. 1979 Apr;92(2):499-503.

Playne MJ. Determination of ethanol, volatile fatty acids, lactic and succinic acids in fermentation liquids by gas chromatography. J Sci Food Agric. 1985 Aug;36(8):638-44.

Ranck EJ, Holden LA, Dillon JA, Rotz CA, Soder KJ. Economic and environmental effects of double cropping winter annuals and corn using the Integrated Farm System Model. J Dairy Sci. 2020 Apr;103(4):3804-15.

Sutton JD, Dhanoa MS, Morant SV, France J, Napper DJ, Schuller E. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J Dairy Sci. 2003 Nov 1; 86(11):3620-33.

Tamburini A, Rapetti L, Crovetto GM, Succi G. Rumen degradability of dry matter, NDF and ADF in Italian ryegrass (Lolium multiflorum) and winter cereal silages. Zootec Nutr Anim. 1995;21(Suppl):75-80.

Turner LB, Cairns AJ, Armstead P, Ashton J, Skot K, Whittaker D, Humphreys MO. Dissecting the regulation of fructan metabolism in perennial ryegrass (Lolium perenne) with quantitative trait locus mapping. New Phytol. 2006 Jan;169(1):45-58.

Valderrama XL, Anrique RG. In situ rumen degradation kinetics of high-protein forage crops in temperate climates. Chil J Agric Res. 2011 Oct 1;71(4):572-7.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991 Oct 1;74(10):3583-97.

Wales WJ, Kolver ES, Thorne PL, Egan AR. Diurnal variation in ruminal pH on the digestibility of highly digestible perennial ryegrass during continuous culture fermentation. J Dairy Sci. 2004 Jun 1;87(6):1864-71.

Weisbjerg MR, Koukolova V, Lund P. Rate of NDF degradation. J Anim Feed Sci. 2007 Sep 17;16(2):151-5.

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