

## Nutritive value of ensiled Italian ryegrass and winter cereal mixture

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The study was conducted with the objective of evaluating nutrient digestibility and energy concentrations of wilted and ensiled mixture composed of 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley. After 90 days of fermentation digestibility experiments were carried out on six Hungarian Merino wethers (4 years of age, initial body weight  $84.56 \pm 5.53$  kg). The net energy for lactation ( $NE_l$ ), maintenance ( $NE_m$ ) and growth ( $NE_g$ ) was computed following NRC (2001) equation to estimate energy values of feed. The apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were 67.90%, 72.12%, 73.44%, 70.90% and 70.76%, respectively. The  $NE_l$ ,  $NE_m$  and  $NE_g$  values were 5.37, 5.74 and 3.32 MJ/kg DM, respectively. The nitrogen and energy dependent metabolizable protein values were 97.03 and 88.87 g/kg DM, respectively. These results suggest that the high NDF and CP digestibility and energy concentration implies that this mixture can be included successfully in dairy cattle diets.

**Keywords:** digestibility, energy, metabolizable protein, Italian ryegrass, silage, winter cereals

### 1 Introduction

Identifying viable alternative forage crops to fill intensive corn silage demands of high producing dairy cows is currently urgent. However, information is not frequent, even though interest is increasing in recent years due to climate change effect on corn crop production (David & Gary, 2018; Tigchelaar et al., 2018). The use of drought resistance corn breed (Lathrop & Namuth, 2011), whole crop sorghum silage (Colombini et al., 2015; Cattani et al., 2017; Khosravi et al., 2018), high sugar forage sorghum silage (Su-jiange et al., 2016) as well as sorghum and Sudan grass in a drought prone region (Getachew et al., 2016) is an indicator for imminent need of alternative forage. The use of cereals silage is also popular now (Van Duinkerken et al., 1999; Bernard et al., 2002; Baldinger et al., 2011, 2014; Harper et al., 2017; Orosz et al., 2019). However, the use of Italian ryegrass and winter cereals for silage is not reported until today. Italian ryegrass (*Lolium multiflorum*) is one of the fastest growing grass species with excellent nutritional qualities particularly high fiber digestibility (NDFd), CP and sugar content (Baldinger et al., 2011, 2014; Field crop news, 2014; DLF seeds, UK, 2018; Byron Seeds, LLC, 2019). Field crop news (2014) reported that the yield of Italian ryegrass is not as high as winter cereals such as oats, but nutrient quality and palatability is greater which makes it more suitable for high producing dairy cow feed. Therefore the use of these mixtures complements each other properties for the benefits of nutritionally excellent ensiled biomass. For instance, the digestibility of barley, winter oats and Italian ryegrass is excellent, while wheat and triticale gives high yields. The high energy content in rye, barley and triticale silage (NRC, 2001) together with Italian ryegrass could

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boost the energy content of ensiled mixtures. Additionally the high NDF digestibility, crude protein and sugar content as well as palatability of Italian ryegrass (Baldinger et al., 2011, 2014) could improve the intake and digestibility of cereals. High fiber digestibility can improve the dry matter intake (DMI) and milk production of the dairy cow (Raffrenato & Van Amburgh, 2010; Grant, 2012). The high sugar content of Italian ryegrass together with proper stage of harvesting of winter cereals improve the fermentation process of ensiled mixtures. Legumes are not included in the mix because they have a lower fiber digestibility than winter cereals or Italian ryegrass. The aim of the present study was to evaluate nutrient digestibility and energy concentrations of wilted and ensiled Italian ryegrass and winter cereals mixture in order to utilize it in the nutrition of lactating dairy cows.

## 2 Material and methods

### 2.1 Diets, animals and chemical analysis

A mixture composed of 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley was sown on September 2017 (75 kg seed/ha) then the cutting was carried out in heading stage of wheat on April 2018. After cutting the mixture forage (DM 18.9%; CP: 16.1% DM; NDF: 48.5% DM, and total sugar: 13.7% DM) was wilted (24h) without any movement on the windrow. The wilted forage was chopped by a forage harvester with theoretical chop length (TCL) of 9 mm (weight: 800 kg). Wilted and chopped materials were ensiled in separate minisilo (0.2 m<sup>3</sup>, laminated container) the applied density was 780 kg wilted material/m<sup>3</sup> (218 kg DM/m<sup>3</sup>). At the end of 90 days fermentation the silo was opened for chemical composition and fermentation quality analysis (Table 1 and 2). Dry matter (DM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), ash, and total sugar content of ensiled mixture were determined. Nitrogen free extract (NFE) was calculated as 100% - (% EE + % CP + % Ash + % CF). Chemical analyses of the silage followed AOAC (2006) protocol. Approximately 25 g composite sample was taken from the silo immediately after opening. The sample silage was mixed with 100 ml of distilled water. After hydration for 10 min using blender, the diluted material was then filtered through cheese cloth and then pH was determined by using a digital pH meter (Metrohm 744, Switzerland). Lactate was analyzed by high-performance liquid chromatography (HPLC) method developed by Megias et al. (1993). Acetic acid, butyric acid, propionic acid and ethanol were measured by gas chromatography (Crompak, Model CP 9002, The Netherlands) as described by Playne (1985). Ammonia concentration was determined by a modified Berthelot method (Robinson et al., 1986).

**Table 1** Nutritional composition of ensiled mixture after 90 days of fermentation (n = 5) (mean±sd)

	(% DM)								
DM (%)	CP	EE	CF	NDF	ADF	ADL	Ash	Total Sugar	NFE
27.46 ±0.49	15.24 ±0.43	4.00 ±0.12	27.06 ±0.32	48.10 ±0.60	32.04 ±0.39	9.82 ±5.39	15.58 ±0.50	0.12 ±0.18	38.12 ±0.90

DM – dry matter, CP – crude protein, EE – ether extract, CF – crude fiber, NDF – neutral detergent fiber, ADF – acid detergent fiber, NFE – nitrogen free extract.

**Table 2** Fermentation characteristics of ensiled mixture after 90 days of fermentation (n = 5) (mean±sd)

pH	Ethanol (% DM)	Acetate (% DM)	Lactate (% DM)	LA/AA	LA (% TFA)	NH <sub>3</sub> -N (g/100 g total N)
4.26±0.02	0.16±0.01	2.25±0.23	8.92±0.82	3.97±0.16	79.84±0.68	8.8±0.01

LA – lactic acid, AA – acetic acid, TFA – total fermentable acid

Digestibility was studied at the National Agricultural Research and Innovation Centre Research, Hecseghalom, Hungary. Six wethers Hungarian Merino sheep (4 years of age) with an average body weight of 84.56±5.53 kg were housed in individual metabolic cages with slatted floors with *ad libitum* access to water. The trial consisted of a 10-days adjustment period followed by 5-days of complete faeces collection. The experimental ensiled mixture was offered as the sole feed to the sheep and fed two equal meals per day (0700 and 1500 h). A daily ration was determined on the basis of live weight

(above maintenance DM requirement, approximately 2% of the body weight). The sheep received daily 30 g mineral and vitamin premix (producer: Bábolna Takarmányipari Kft., Nagyigmánd, Hungary) plus 10 g NaCl. Feed intake, feed refusal and faecal output were recorded daily during the collection period. A 25% sample of faeces was sub-sampled daily from each animal, dried in a forced air oven at 60°C for 24 h and ground through a 1 mm screen to determine the DM percentage. Feed samples were taken at the beginning of adaptation period, and at the beginning and the end of collection period. Feed and faecal samples were analysed for DM, CP, CF, EE, ash, NDF, ADF were determined according to the official methods in Hungary (Hungarian Feed Codex, 2004).

## 2.2 Calculation of digestibility, energy and protein values and statistical analysis

### 2.2.1 Digestibility

The digestibility coefficient (DC, %) for nutrients was calculated for each animal on the basis of quantitative data for intake and output according to the classical formula:  $DC (\%) = 100 \times (NI - NE) / NI$ , where, NI represented the nutrient intake and NE expressed the nutrient excreted.

### 2.2.2 Energy evaluation

The net energy for lactation, maintenance and growth was calculated on the basis of digestible nutrients as suggested by the NRC (2001). The energy concentration was calculated as follows:

DE (Digestible Energy) of feeds using equation 2-8 (NRC, 2001)

$$DE_{1X} (\text{Mcal/kg}) = (\text{tdNFC}/100) * 4.2 + (\text{tdNDF}/100) * 4.2 + (\text{tdCPf}/100) * 5.6 + (\text{FA}/100) * 9.4 - 0.3$$

Where,

Truly digestible NFC (tdNFC) =  $0.98 (100 - [(NDF - NDICP) + CP + EE + \text{Ash}]) * \text{PAF}$  (NRC, 2001, equation 2-4a)

Truly digestible CP for forage (tdcpf),  $\text{tdcpf} = CP * \exp(-1.2 * (\text{ADICP}/CP))$  (NRC 2001, equation 2-4b)

Truly digestible FA (tdFA) = FA, If  $EE < 1$ , then  $FA = 0$  (NRC 2001, equation 2-4d)

Truly digestible NDF (tdNDF),  $\text{tdNDF} = 0.75 * (\text{NDF}_n - L) * (1 - (L/\text{NDF}_n)^{0.667})$  (NRC 2001, equation 2-4e)

Processing Adjustment Factors (PAF) is 1 for all other feeds (NRC, 2001, Table 2-1)

Fatty acid (FA) =  $EE - 1$

Assume an 8% discount factor (i.e., multiply value from step 1 by 0.92)

$$DE_P = DE_{1X} * 0.92$$

ME (Metabolizable Energy) of feeds using equation 2-10 (NRC, 2001)

$$ME_P (\text{Mcal/kg}) = [1.01 * (DE_P) - 0.45] + 0.0046 * (EE - 3)$$

Where  $DE_P$  is Mcal/kg and EE in percent DM

$NE_l$  (Net Energy for Lactation) of feeds using equation 2-12 (NRC, 2001)

$$NE_l (\text{Mcal/kg}) = 0.703 * ME_P - 0.19 + [(0.097 * ME_P + 0.19)/97] * [EE - 3]$$

where  $ME_P$  is expressed as Mcal/kg and EE in percent DM, EE – Ether extract, 1 mega calorie (Mcal) is equal to 4.184 mega joules (MJ).

### Estimating Net Energy of Feeds for Maintenance and Gain

ME (Metabolizable Energy) of feeds (NRC, 1996)

$$ME = DE_{1X} * 0.82$$

where  $DE_{1X}$  = Digestible energy

$NE_m$  (Net Energy for maintenance) of feeds using equation 2-13 (NRC, 2001)

$$NE_m = 1.37 ME - 0.138 ME^2 + 0.0105 ME^3 - 1.12 \text{ (Garrett, 1980)}$$

where ME – Metabolizable energy

$NE_g$  (Net Energy for growth) of feeds using equation 2-14 (NRC, 2001)

$$NE_g = 1.42 ME - 0.174 ME^2 + 0.0122 ME^3 - 1.65 \text{ (Garrett, 1980)}$$

where ME – Metabolizable energy

### 2.2.3 Protein evaluation

The protein evaluation was done following the Hungarian metabolizable protein system for ruminants (Schmidt et al., 1998). The formulas proposed for the calculation of protein values of feed was the follows:

$$MPE \text{ g/kg DM} = 0.9 (\text{UDP} - \text{ADIN} \times 6.25) + 160\text{FOM} \times 0.8 \times 0.8$$

$$\text{MPN g/kg DM} = 0.9 (\text{UDP} - \text{ADIN} \times 6.25) + \text{RDP} \times 0.9 \times 0.8 \times 0.8.$$

where MPE energy dependent metabolizable protein and MPN – Nitrogen dependent metabolizable protein UDP – Rumen undegradable protein, ADIN – acid detergent insoluble nitrogen, RDP – Rumen degradable protein, FOM – Fermentable organic matter, FOM = DOM – (UDP + digestible fat + fermentation products + bypass starch), where DOM – Digestible organic matter

### 2.2.4 Statistical analysis

Data obtained in the experiment were analyzed using proc means procedure in SAS 9.1 software (SAS Inst. Inc., Cary, North Carolina, USA). Comparison of means of variables for digestibility of nutrients was carried out using the following model:  $Y_i = \mu + \alpha_i + \varepsilon_i$ , where  $Y_i$  is the digestibility of nutrients in the  $i^{\text{th}}$  sheep effect,  $\mu$  is the overall mean,  $\alpha_i$  is the  $i^{\text{th}}$  sheep effect and  $\varepsilon_i$  is the random error.

## 3 Results and discussion

### 3.1 Dry matter and nutrient digestibility

Some end-products of fermentation associated with poor fermentation, such as AA and BA and ammonia are associated with the decrease in the intake of silages and some changes resulting from the ensiling process influence the digestibility of silages (de Oliveira et al., 2016). However, the overall apparent digestibility of nutrients in the present study was better and above 67% attributed to absence of those undesirable fermentation end products like BA and ammonia (Table 2). On the other hand the complement effect of ensiled materials could also be a reason for high digestibility as digestibility of barley, winter oats and Italian ryegrass is excellent. The apparent DM digestibility was 67.92%, (Table 3) which is lower as compared to CP, NDF and ADF digestibility. The low DM digestibility could be associated with the inclusion of more winter cereals (60%) which has lower DM digestibility due to its high fiber content as compared to Italian ryegrass. On the other hand grasses typically have higher NDF content and lower DM digestibility as compared to corn and alfalfa silage. The observed DM digestibility in the present study was higher than DM digestibility of alfalfa silage (Hassanet et al., 2014) and sorghum silage with different tannin content (Teixeira et al., 2014), but lower than DM digestibility of corn silage (Hassanet et al., 2014) and grass silage (Yan & Agnew, 2004). The apparent fiber digestibility and NDF or ADF were high (>69%). This could be attributed to lower lignification of cell wall contents (NDF and ADF) (Table 1) of ensiled mixtures. This high fiber digestibility particularly NDF digestibility is very important because NDF digestibility influences animal performance. Study reports reveal that for effective rumen function sufficient NDF should be included in the diets of dairy cows and large proportion of dietary NDF should come from forages and at least 25% of dairy ration should be composed of NDF (Allen & Oba, 1996). The apparent digestibility of OM, CP and NDF was better than grass silage reported by Yan and Agnew (2004). On the other hand OM, CP, NDF and ADF digestibility was higher than the OM (68.5%, 62.8% and 63.3%), CP (72.2%, 72.2% and 68.5%), NDF (69.8%, 59.7% and 50.7%) and ADF (69.1%, 58.3% and 48.3%) digestibility of oat silage at heading, early milk and early dough stages respectively (Wallsten et al., 2009). It was better than OM (69.4 %, 66.7%), CP (69.3%, 66.6%), NDF (68.3%, 57.3%) and ADF (64.2%, 51.6%) digestibility of oat silage at early milk and early dough stages respectively (Wallsten et al., 2009). The observed OM and NDF digestibility was better than 48 hours incubation in vitro OM digestibility, NDF digestibility and digestible NDF of corn silage, alfalfa (silage, haylage and hay), ryegrass silage and grass silage reported by Orosz et al. (2019). However, the OM digestibility of the current study was lower than OM digestibility of corn silage (dough stage) and ryegrass silage reported by the same author. Additionally

the digestibility of OM and NDF value was comparable with ryegrass silage (before and in heading stage) as reported by Orosz et al. (2019).

**Table 3** Digestibility coefficients (%) determined with sheep for the ensiled mixture (n = 6) (mean ± S.D.)

Nutrient	Digestibility coefficient (%)	SD
Dry matter	67.92	1.48
Organic matter	72.12	1.12
Crude protein	73.44	1.42
Crude fat	70.47	3.03
Crude fiber	75.06	2.31
N-free extracts	69.76	1.21
Neutral detergent fiber	70.90	2.39
Acid detergent fiber	70.76	2.17

### 3.2 Energy concentration in ensiled mixture

Energy content of ensiled mixture and other silages are given in Table 4. The overall energy values of the current silage mixture were better than all conserved forages including alfalfa silage reported by the NRC (2001). The value for net energy for lactation (NE<sub>l</sub>), net energy for maintenance (NE<sub>m</sub>) and net energy for growth (NE<sub>g</sub>) are all better than the values for alfalfa silage and also exceeds the values of good quality grass silage. Thus, early heading harvest of the mixture was not accompanied by a decline in energy content. Ensiled mixture silage contains very low amount of starch, but the digestibility of the fiber was so favourable that its net energy content averaged 0.9 MJ/kg DM still exceeded the average energy content of cereal silages and appears to be similar to rye silage; however the value was lower than the energy content of corn silage.

**Table 4** Energy content (MJ kg<sup>-1</sup>) of ensiled mixture and silages (NRC, 2001)

Forage types	Energy value				
	DE	ME	NE <sub>l</sub>	NE <sub>m</sub>	NE <sub>g</sub>
<b>Ensiled mixture*</b>	<b>11.42</b>	<b>9.36</b>	<b>5.37</b>	<b>5.74</b>	<b>3.32</b>
Alfalfa silage	10.96	8.28	5.02	5.39	3.01
Rye, annual, vegetative	11.40	8.70	5.36	5.73	3.31
Corn silage, normal (32-38%DM)	12.50	9.75	6.07	6.57	4.06
Grass silage, mid maturity(56-60%NDF)	10.70	8.03	4.85	5.23	2.85
Italian ryegrass silage	10.40	7.78	4.69	4.98	2.64
Sorghum silage	10.40	7.74	4.64	4.94	2.59
Barley silage, headed	11.20	8.49	5.19	5.56	3.18
Oat silage, headed	10.60	7.99	4.81	5.15	2.76
Triticale silage, headed	10.80	8.12	4.94	5.23	2.89
Wheat silage, early headed	10.70	7.99	4.85	5.19	2.80

DE – Digestible Energy, ME – Metabolisable Energy, NE<sub>l</sub> – Net energy for lactation, NE<sub>m</sub> – Net energy for maintenance, NE<sub>g</sub> – Net energy for growth.

\* ensiled mixture – 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley

### 3.3 Protein evaluation

In ruminant nutrition accurate estimation of microbial protein is very essential as metabolizable protein synthesis is governed by microbial protein synthesis (Castillo-Lopez and Domínguez-Ordóñez, 2019). Availability of dietary carbohydrate, ruminally degradable protein and dietary fat are the main factors which influence ruminal microbial protein synthesis (Fernando et al., 2010). On the other hand Abdulkarim and Kedir (2019) reported that the availability of energy generated by the fermentation of carbohydrates largely influence microbial protein synthesis. It further noted that on average, 20 grams of bacterial protein is synthesized per 100 grams of organic matter fermented in the rumen. Therefore it seems to be appropriate that all feed should be given two metabolizable protein values namely the nitrogen-dependent metabolizable protein (MPN) and energy dependent metabolizable protein (MPE) which are the quantity of protein originated from the true digestible protein proportion of UDP and the digestible true microbial protein potentially synthesized from RDP and the fermentable organic matter (FOM) content of the feed respectively (Schmidt and Zsédely, 2011). Both metabolizable protein values should be calculated for ration because the production of animals is always limited by the lower value. Table 5 shows the protein evaluation values of ensiled mixtures. Both the nitrogen and energy dependent metabolizable protein values are higher than 88 g/kg DM. When a ration is formulated a protein balance (MPN-MPE, g) in the rumen should be also calculated (Schmidt and Zsédely, 2011).

**Table 5** Protein evaluation values of ensiled mixtures

Silage	MPN (g/kg DM)	MPE (g/kg DM)
ensiled mixture*	97.03	88.87

\*ensiled mixture – 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley

MPN – Nitrogen dependent metabolizable energy; MPE – Energy dependent metabolizable energy

The high nitrogen dependent metabolizable protein concentration as compared to energy dependent metabolizable protein attributed to digestibility of CP as well as proper stage of harvesting (early heading) of the ensiled mixtures.

## 4 Conclusions

The apparent nutrient digestibility of all nutrients was better and above 67% attributed to absence of undesirable fermentation products such as butyric acid and ammonia and proper stage of harvest. The higher fiber digestibility particularly NDF could improve the performance of ruminants. The good apparent nutrient digestibility improved the energy concentration ( $NE_i$ ,  $NE_m$  and  $NE_g$ ) of ensiled mixture. This result implies that due to good apparent nutrient digestibility particularly NDF as well as high energy concentration; the ensiled mixture can be included in the nutrition of high producing dairy cows and complete the forage sources of a country. However, further studies needed to confirm the effect of the ensiled mixture on the performance of dairy cows and on the composition of milk.

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