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## Comparison of Forage Quality and *in vitro* Digestibilities of Fodder Beet (*Beta vulgaris var. rapa*) Fresh Material and Silage

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**Abstract** The focus of the study is to determine the nutrient contents, forage qualities and *in vitro* truly digestibilities (IVTD) of fodder beets (*Beta vulgaris var. rapa*) and their silages. The IVTDs of feed materials were determined by using Ankom Daisy Incubator and feed materials were incubated for 48 hours. The data were analyzed by t- test. The findings showed that the contents of the crude protein of fodder beet fresh materials (10.78% dry matter) and silages (13.00% dry matter) were within the limits which do not impair microbial activities, that both fresh materials and silages were in “excellent” quality class when they classified according to relative feed value, that the IVTDs were high in fodder beet fresh materials (89.58%) and silages (92.46%). However, prior to usage as feed material in ration, some precautions should be taken, 1) soil contamination should be prevented, 2) fodder beets should be wilted prior to ensiling and 3) fodder beets should be ensiled with absorbents such as straw. In conclusion, fodder beets (fresh or silage) can be included in ruminant rations but not in excess amounts. Furthermore, more detailed *in vivo* studies are needed on this subject.

**Keywords** Fodder beet, *in vitro* digestibility, forage, relative feed value, silage

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### Introduction

Alternative forage plant production should be increased with the aim of closing the gap of forage demands of ruminants in our country. Fodder beet (FB), which is one of these alternative forages, can produce excess amounts of high-quality forages (Roots: 5-20 tons/da; leaves: 1-4 tons/da) during the periods of inadequate forage production. Sometimes this amount may exceed 20 tons/ha. Dry matter yields ranged from, 4.8 to 7.7 t/ha and 2.0 to 2.7 t/ha for fodder beets. Also, it can enter the rotation (it is planted on November and harvested in the end of April). This product can be used in fresh or ensiled form. Moreover, beneficial effects of this product during winter feeding have been reported [1-4].

In recent years, use of FB has been increased in some countries such as New Zeland, Australia and Ireland. The FB, which has nearly 8-12 % dry matter (DM), is used in winter months in European countries. This relatively cheap forage consumed in excess amounts by ruminants [5,6]. The FB is poor in crude fiber (CF) (8%), ether extract (EE) (1%) and rich in easily soluble carbohydrates (71%).The crude protein (CP) and ash contents are 10% and 10%, respectively [6, 7]. The FB is a valuable energy source due to its high sugar content (70% of DM). Excess consumption of FB can lead to digestive disorders, hypocalcemia and even death. The digestive disorders might be attributed to excessively high sugar contents of FB roots. The high sugar supply to the rumen lead to increase in lactic acid bacteria concentration and decrease in concentrations of other bacteria in rumen and consequently causes acidosis. The toxic effect of FB is related to its nitrate content [6] and this problem can



be avoided by ensilaging and wilting. Fresh materials and silages of FB can be used especially cattle, sheep and goat's nutrition. Pacheco *et al* [8] reported that feeding fresh FB should not exceed about 0.4 of their DMI with pasture for late lactation cows or about 0.6 of their intakes with silage for non-lactating cows. Waghorn *et al* [9] fed to non-lactating cows using 85% fodder beet with barley straw and 65% fodder beet with pasture silage. It is recommended to increase the dry matter of FB silage using corn husks (20%) and barley straws (10%). Thus, FB silage feed values can be increased [10] and it is reported that FB should be ensiled with grasses [11]. In this study, it was aimed to compare the feeding values, *in vitro* digestibilities and forage values of fresh FB and ensilaged FB.

## Materials and Methods

### Feed supply and silage making

The FB plants (*Beta vulgaris var. rapa*) (*Brigadier variety*) obtained from a private firm were sown at the Research and Application Farm of The University (3 parcels of 50 m<sup>2</sup>) in winter and harvested in the end of April. A portion of the FB plants was stored as fresh material and the remaining was chopped to about 2 cm, wilted for a day and then were packed into 5 replicate mini silos [12]. The silos were opened after 60 days.

### Nutrient content analysis

The fresh material and ensilaged forms of FB were dried in a forced-air oven at 65 °C for 48 hours and then ground to 1mm mesh size. The ground samples were analyzed for Kjeldahl N and CP was calculated by multiplying N by 6.25. Dry matter (DM) and ash were determined according to AOAC [13]. The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude fiber (CF) analysis were done according to the method of Van Soest *et al* [14] using ANKOM 2000 semi-automated fiber analyzer (Ankom Technology, Macedon, NY). The EE content was determined using ANKOM<sup>XT15</sup> analyzer (AOCS 2005 Am-5-04). The contents of organic material (OM), nitrogen free extract (NFE), cellulose and hemicellulose were determined by calculation.

### Determination of *in vitro* truly digestibilities (Ankom Technology)

In *in vitro* study, the rumen content was obtained from 3 healthy Karayaka rams (about 45 kg) slaughtered at a private slaughterhouse and then mixed. Rumen content was taken under CO<sub>2</sub> atmosphere, strained through two layers of cheesecloth and was put into a thermos (39 °C) with 2 handful rumen content and was transported to the laboratory within 15-20 minutes.

The AnkomDaisy<sup>II</sup> incubator (filter bag system digestibility) makes *in vitro* dry matter disappearance study easy and efficient because it uses an equipment which was designed with four rotating digestion jar and maintains constant, uniform heat and agitation within a controlled (39.5°C) chamber [14, 15]. Daisy incubator instrument contains 4-cylinder incubators which 1 cylinder need 1600 ml buffer solution and 400 ml rumen fluid as inoculums and bag filter. Filter bags (25 pieces F57) could be placed inside each other cylinder with solution. The cylinder was bubbled with CO<sub>2</sub> immediately before closed with lid of cylinder well and placed into incubator box for 48h incubation keep in 38°C. After incubation, filter bags were cleaned under water flow and dried. The bags were analyzed for NDF digestibility with semi-automated ANKOM 2000 fibre analyzer. *In vitro* truly digestibilities of the samples were estimated as follows;

$$\% \text{ In Vitro Truly Digestibility (IVTD)} = 100 - ((W3 - (W1 \times C1)) * 100) / W2$$

Where: W1: Weight of filter bag, W2: Weight of sample, W3: Final weight after NDF analysis, C1: The bag without sample was prepared also for correction.

### Determining pH, volatile fatty acid (VFA) and ammonia nitrogen (NH<sub>3</sub>-N) analysis in rumen fluid

The rumen pH values were determined using in rumen fluids obtained from slaughtered rams at slaughterhouse immediately using digital pH-meter (HANNA INSTRUMENTS 1332 model) in three replicates. The total volatile fatty acid (TVFA) and NH<sub>3</sub>-N analysis were done according to Markham [16] in 3 replicates.



**Determining pH and VFA analysis in silages**

The pH values of silages were determined by the samples obtained from different parts of silages. With this aim, 25 g silage sample was put in a mixer, 100 ml distil water added and mixed for 5-10 minutes. The fluid part of the mix was filtered to a beaker via a filter paper and after 15-20 minutes the pH was measured using a digital pH-meter (HANNA INSTRUMENTS 1332 model PH metre) in 3 replicates. The lactic acid (LA) and acetic acid (AA) contents were determined spectrophotometrically [17].

**Determination of forage quality and silage quality**

The relative feed value (RFV) was used as indices of the nutritive value of FB. The RFV was calculated as follows [18];

Dry matter digestibility (DMD, %) = 88.9- (0.779 x ADF%)

Dry matter intake (DMI, liveweight, %) = 120/(NDF%)

Relative feed value (RFV, %) = (DMDxDMI)/1.29

The quality class of the silages were determined by using Flieg score (FS) [19].

**Flieg score (FS)= 220+(2 x dry matter % – 15) –40 x pH**

The required pH value in a silage is related to DM content. In other words, each silage should have a pH value which is determined according to its DM content. The “required pH values” were determined by using the following formula [20]. This pH value prevents the proliferation of clostridia and enterobacteria.

**Required pH (RpH)= 0.00359 x DM (g/kg) + 3.44**

Fermentation loss (gas losses) (% of the starting material)was determined by the decreases in silo weight from the beginning of the fermentation up to the opening of the silos. After opening the silos, high amounts of oxygen enter the silo caps and consequently silages begin to deteriorate. Aerobic stability test was performed with the aim of determining the aerobic stability of silages [21].

**Statistical Analysis:** Observations of fresh and ensilaged forms of FB were subjected to t-test.

**Results**

The pH, ammonia-N (NH<sub>3</sub>-N) and total volatile fatty acids (TVFA) contents of the rumen fluid used for determining the truly digestibilities of silages were found as 6.62 (5.90-6.76), 312 mg/l (264-402 mg/l) and 113 mmol/l (88-134 mmol/l). These values are within the normal ranges [22, 23].

The nutrient contents of FB and some other for ages (fresh and silage) were given in Table 1 (as fed basis) and Table 2 (as dry matter basis). The fresh form FB had lower DM content than its ensilaged form (P<0.001).

**Table 1:** Nutrient contents of fresh and ensilaged forms of FB (as fed basis, %)

Forage type	DM	Ash	EE	CP	CF	OM	NFE	NDF	ADF	ADL	HCel	Cel
Fresh	10.66±0.01	1.29±0.01	0.08±0.01	1.15±0.02	0.80±0.02	9.37±0.01	7.34±0.02	2.85±0.04	1.94±0.02	1.20±0.02	0.90±0.06	0.74±0.02
Silage	14.48±0.01	1.91±0.01	0.13±0.01	1.88±0.02	2.67±0.09	12.57±0.01	7.89±0.11	4.15±0.02	2.42±0.02	0.98±0.05	1.73±0.04	1.44±0.06
Sig.	<0.001	<0.001	0.032	<0.001	0.002	<0.001	0.031	<0.001	<0.001	0.010	<0.001	<0.001

DM: Dry matter, EE: Ether extract. CP: Crude protein. CF: Crude fiber. OM: organic material, NFE: Nitrogen free extract, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid detergent lignin, HSel: Hemicellulose, Cel: Cellulose

**Table 2:** Nutrient contents of fresh and ensilaged forms of FB (as dry matter basis, %)

Forage type	Ash	EE	CP	CF	OM	NFE	NDF	ADF	ADL	HCel	Cel
Fresh	12.11±0.07	0.77±0.05	10.78±0.23	7.48±0.14	87.89±0.07	68.86±0.19	26.70±0.40	18.22±0.18	11.26±0.16	8.48±0.56	6.96±0.21
Silage	13.21±0.02	0.87±0.09	13.00±0.16	18.42±0.64	86.79±0.02	54.50±0.73	28.65±0.15	16.68±0.17	6.76±0.31	11.97±0.29	9.92±0.41
Sig.	<0.001	0.364	0.001	0.002	<0.001	0.001	0.010	0.003	<0.001	0.005	0.003

EE: Ether extract. CP: Crude protein. CF: Crude fiber. OM: organic material, NFE: Nitrogen free extract, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid detergent lignin, HSel: Hemicellulose, Cel: Cellulose

The FB silages had higher CP, CF, NDF, hemicellulose and cellulose contents and lower NFE, OM and ADF contents compared to FB forages (fresh form) (P<0.01). The EE content was found similar in fresh and ensilage forms of FB (P>0.05).



The organic acid contents, pH values, aerobic stabilities and Flieg scores of FB silages are given in Table 3. The LA and AA contents (4.06 and 1.57%, respectively) were found like those in high quality silages. The FB silage was a medium quality with a Flieg's score of 36.22 due to its low dry matter content.

The fermentation gas lost that occurred during fermentation was determined as weight difference between before and after fermentation weights. Fermentation gas lost of the silage of FB determined as 1.41% (Table 3).

**Table 3:** Fermentation quality, aerobic stability and Flieg score in FB silages

LA	AA	RpH	pH	FS	Silage quality	AS	GL
3.84-4.28 (4.06)	1.61-1.52 (1.57)	3.96	4.86-5.01 (4.94)	33.6-39.6 (36.22)	21-40 Medium	30.1-29.9 (30.0)	0.76-1.98 (1.41)

LA: lactic acid (%DM), AA: Acetic acid (%DM), RpH: required pH, FS: Flieg score, AS: Aerobic stability (CO<sub>2</sub>: g/kg DM), GL: gas loses during fermentation (%). Mean values are given in parantheses.

The DMD, DMI, RFVs and *in vitro* truly digestibilities (IVTD) of fresh and ensilaged forms of FB are given in Table 4. While the DMD (P<0.01) and IVTD (P<0.05) were higher in FB silage, the DMI and RFV were higher in fresh form (P<0.05). The fresh and ensilaged forms of FB were considered as "Excellent" according to the RFV classification.

**Table 4:** Forage quality and IVTD values of FB silages

	DMD %	DMI, %LW	RFV	RFV Quality class*	IVTD%
Fresh FB	74.71±0.14	4.5±0.066	260.34±3.434	Excellent	89.58±0.519
FB silage	75.9±0.126	4.19±0.022	246.47±1.047	Excellent	92.46±0.48
Sig.	0.003	0.012	0.018		0.015

DMD: dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed value, IVTD: *in vitro* truly digestibility According to the Quality Grading Standard assigned by The Hay Marketing Task Force of the American Forage and Grassland Council, the RFV were assessed as roughages based on 5 (<75) or "reject" and prime "excellent" (>151).

## Discussion

As show in Table 1, the DM contents of both fresh and ensilaged forms of FB were found to be extremely low to be used in ruminant nutrition. The DM content of FB (fresh form) in present study was found to be lower compared to margin values [24] and like one found in another study [25]. Dry matter content of silages of FB in this study were found to be lower compared to values (17.4%) declared by Seiden and Pfander [26]. Besides, the values were found to be higher compared to other study values [25]. The OM (74.9%) and CP (11.5%) values reported by Moloney and O'Kiely [27] were lower than those found in our study. The lower ash content found in our study compared to this study (25.1%) might be due to the fact that the FB materials were thoroughly cleaned (no soil contamination). ADF and NDF contents were found like ones (26.4 and 17.1%) reported by Moloney and O'Kiely [27].

In present study, the CP values were above 8.0%, indicating that this alternative forage source (FB) positively affects the microbial protein digestibility. Indeed, it is reported that the feeds containing less than 8% CP cannot provide the minimum ruminal ammonia levels required for normal rumen functions [28]. The CP contents found in present study were higher than margin values (5.1-9.0%) reported in literature [24]. The CF and EE contents found in our study were found like values (4.3-7.4% and 0.1-2.8%, respectively) reported in literature [24]. The ash (4.2-9.6%), NDF (10.2-17.2%), ADF (5.4-8.4%) and ADL (1.0%) contents reported in literature [24] are lower than those found in our study. These discrepancies might be sourced from the different variety in ratios of leaf/root.

The higher NFE contents in fresh form FB lead to increase in desired lactic acid production and decrease in pH value during silage fermentation [12]. Furthermore, the higher NFE contents increase voluntary intake of FB in fresh form. The lower NDF and ADF contents in ensilaged and fresh forms of FB decrease the intake and digestibility of this feed material. However, the lower ADL content of FB silage compared to fresh FB indicate the advantage of silage in terms of intake and digestibility.

Schnieder [25] reported that the ash, CP, CF, EE and NFE contents as 0.9, 0.8-1.5, 0.8, 0.1 and 8.2-10.3% in FB roots and 1.0-2.9, 0.8-1.3, 0.7-0.9, 0.1 and 5.5-7.3% in FB silages, respectively. Dalley et al. [29] stated that for fodder beet (roots and leaves) concludes CP 7.6% (1.9-11.1), soluble sugar and starch 48.6% (23.7-73.8), ADF 19.1% (6.8-31.1) and NDF 30.9% (12.0-45.0). As seen, these values are generally like those found in present study as fresh material. The desired NDF contents in ruminant rations for maintaining rumen function are



reported as 27-30% [30]. In present study, the NDF contents are within these values. However, as the higher NFE content is an important risk factor for acidosis, it is not recommended to use both fresh and ensilaged forms of FB alone in ruminant rations.

In present study, the true pH value (3.96) was found lower than RpH value (4.96). As it is known, the RpH, which is calculated using the DM content of silage, is only important in the determination of fermentation quality. This indicates that addition of some materials with high DM content might be an efficient application for preventing the undesirable fermentation in silage. An alternative application for increasing the silage DM content is wilting process. These two applications might aid in decreasing pH in a short time and consequently the desired silage fermentation might be attained [31].

The fermentation gas lost showed that, some ammonia was gone away from protein structures of the silage of FB. It occurs protein losses from FB silages. In the aerobic stability test (Table 3), the amount of CO<sub>2</sub> produced at 5<sup>th</sup> day (30 g/kg DM) was higher than that (16.3 g/kg DM) reported by Ashbell *et al.* [21]. This indicates that FB silage deteriorates quickly during aerobic exposure and consequently loses its forage value for ruminants [12, 19].

Moloney and O'Kiely [27] reported lower pH (3.67) and higher lactic acid (17.0%) and acetic acid (6.7%) values compared to ones determined in present study. These differences might be attributed to differences in DM contents determined in these studies.

In a previous study [32], the FB silages prepared with straw addition (10% straw+90% FB) had higher DM content (31.63%) compared to our study. This indicates that the DM content of FB silage can be increased to appropriate levels and consequently the silage effluents can be decreased. In this way, the nutrient losses and environmental pollution can be reduced.

In this study, root and leaves of FB were used together. Therefore, this fact should be taken into consideration. The *in vitro* DMD value (70.0%) reported in a previous study [27] was found like value (74.71%) found in present study. In this study, IVTD of fresh and silages of FB were determined as 89.58% and 92.46%. No significant difference between fresh form and silages of FB. Besides, a previous study [27] reported that the cows fed on FB silage had lower rumen pH and acetic acid contents (6.44 and 628 mmol/mol vs 6.75 and 638 mmol/mol) and higher propionic acid content (238 mmol/mol vs 193 mmol/mol) than cows fed on grass silage. These values indicate that FB silage can not be used alone in dairy cow nutrition, but it is more appropriate to be used in beef cow nutrition. Besides, it is known that the addition of fodder beet 20% reduces 18-20% methane production (approximately 18-20%) in dairy cows [3].

## Conclusion

Both ensilaged and fresh forms of FB are considered as valuable feed sources in ruminant nutrition due to their high nutritive values. But soil contamination should be prevented prior the use of FB due to the risk of listeriosis. Furthermore, it is recommended to increase the dry matter content of FB prior to ensilaging process by wilting or mixing with some materials such as straw. Also, fresh and ensilaged forms of FB silages should not be given in excess amounts and they should be used with other high-quality forages. Furthermore, *in vivo* experiments are needed for determining the effects of FB on performances of animals.

## Conflict Interest

Authors state no conflict of interest.

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