Korean Journal Of Agricultural Science

pISSN : 2466-2402
eISSN : 2466-2410

ANIMAL

# Effect of different harvesting times on the nutritive value and fermentation characteristics of late and early-maturing forage oats by rumen microbes

Yan Zhang[1,†], Ye Hyun Lee[1,†], Kim Margarette Nogoy[1], Chang Weon Choi[2], Do Hyung Kim[3], Xiang Zi Li[4,*], Seong Ho Choi[1,*]

[1] Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea
[2] Department of Animal Resources, Daegu University, Gyeongsan 38453, Korea
[3] Department of Animal Science, Gyeongbuk Provincial College, Yecheon 36830, Korea
[4] Co-Innovation Center of Beef Cattle Science and Industry Technology, Yanbian University, Yanji, Jilin, 133002, P. R. China

*Corresponding authors: seonghoc@cbnu.ac.kr, lxz@ybu.edu.cn
† These authors equally contributed to this study as first author.

## Abstract

Late-maturing Dark Horse, and early-maturing High Speed oat varieties were seeded on March 3, 2016 and harvested on three periods: May 31, June 10, and June 20 coded as early, mid, and late-harvest, respectively. Dried and ground samples were subjected to chemical analysis to determine nutritional values such as crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), organic matter (OM), and total digestible nutrient (TDN). Effective degradability (ED) of nutrients and fermentation characteristics including volatile fatty acid (VFA) composition, pH, gas production, and ammonia-N concentration were evaluated through an *in vitro* digestion method. Varieties of oat hays showed significant difference in terms of nutritional value, ED, and fermentation characteristics. Dark Horse showed higher CP and OM, and lower EE contents than High Speed. Dark Horse also showed higher EDDM (dry matter), NDF, ADF, and OM than High Speed, and although High Speed showed higher pH and ammonia-N, it had lower gas and total VFA production than Dark Horse. However, in terms of harvest period, significant difference was only observed in Dark Horse where early-harvest increased the CP, and late-harvest increased the NDF and OM contents. In addition, early-harvest of Dark Horse increased the EDDM and EDNDF of the forage. Therefore, early-harvest of late-maturing Dark Horse would give better nutrient efficiency than High Speed. Allowing Dark Horse to advance in maturity would decrease its nutrient productivity and efficiency.

**Keywords:** early-maturing, harvest time, late-maturing, nutritive value, rumen fermentation characteristics

Check for updates

**OPEN ACCESS**

**Citation:** Zhang Y, Lee YH, Kim MN, Choi CW, Kim DH, Xiang ZL, Choi SH. 2019. Effect of different harvesting time on nutritive value and fermentation characteristics of late and early-maturing forage oats by rumen microbes. Korean Journal of Agricultural Science. https://doi.org/10.7744/kjoas.20190003

**DOI:** https://doi.org/10.7744/kjoas.20190003

**Received:** October 25, 2018

**Revised:** October 25, 2018

**Accepted:** October 25, 2018

# Introduction

Oat (*Avena sativa* L.) was originally harvested as a grain crop for food but is now more often harvested as forage for ruminants, and is continuously becoming more important not only because of its highly palatability (Moreira, 1989), but also because of its high yield potential and very good feed quality (Kim et al., 2006). It makes up the primary portion of the ruminant diet that provides high cell wall contents as an energy source. Cell walls consist of cellulose, hemicellulose, pectin, and lignin. In forages, cellulose is the most abundant carbohydrate (0.2 - 0.4 kg/kg of the dry weight) for ruminant's consumption (Wilson, 1994). The energy that cell walls provide depends on their degradation in the rumen (Bergman, 1990). As is generally known, forage quality is the direct reflection of nutrients and energy that would be available for ruminants, and one of the factors affecting quality is the stage of maturity on which it is harvested. Previous works have shown that with advancing maturity, forage plants will generally increase in lignin concentrations (Kilcher and Troelsen, 1973), which can subsequently decrease the effective degradability of nutrients. However, recent experiments have suggested that harvesting at a more advanced maturity stage will not result in a reduction of dry matter (DM) degradability and that it will have no negative effects on ruminal fermentation characteristics (Rosser et al., 2016), which could potentially result in increased forage yield. Nonetheless, because of a lack of supporting studies, it is not yet well-established whether or not oat harvested at a late maturity will actually negatively affect forage nutritive value and rumen fermentation. Hence, this study aimed to assess the effect of harvesting time of Dark Horse, a late-maturing variety, and High Speed an early-maturing variety on chemical composition, fermentation characteristics, and nutrient effective degradability through *in vitro* digestion method.

# Materials and methods

## Forage production and chemical composition analysis

The cultivation of oat forages was conducted in Goesan county of North Chungcheong province, South Korea. Dark Horse, a late-maturing variety, and High Speed, an early-maturing variety, were seeded March 12, 2016. Harvest dates were May 31st (early), June 10th (mid), and June 20th (late), 2016. The Meteorological Agency of Goesan County recorded the average temperature and precipitation over the study period. Fertilizers N, P, and K were applied at 60 (130), 50 (250), and 40 (67), respectively, and compost and supplementary manures were applied at 15,000 and 60 (130), respectively.

Post-harvest samples were dried using a forced-air drying oven at 65℃ for two days and ground using Tecator Cyclotec 1093 sample mill through a 1 mm screen. Crude protein (CP) was conducted following the Kjeldahl method; ether extract (EE), and organic matter (OM) were determined according to AOAC (1995); and neutral and acid detergent fiber (NDF and ADF) were determined according to the method outlined by Van Soest et al. (1991). The experimental design of this study involved a 2 × 3 factorial design representing two varieties of oat hays (Dark Horse and High Speed) and three harvest dates, referred to as early, mid, and late-harvest. Six treatments were triplicated per time, and the experiment was conducted three times. Replicates were averaged for every variable measured at each time, giving 18 total observations (six treatments × three repetitions).

## *In vitro* digestion method

Rumen fluid was collected from two rumen-cannulated Korean native cattle 3 h after morning feeding, and digestion medium was prepared following the formulation of McDougall (1948). The solution was then prepared by mixing rumen fluid and digestion medium in a 1 : 2 ratio. Carbon dioxide was continuously flushed into the mixed solution. Forage samples were prepared by weighing one gram of dry weight oat hays in nylon bags (pore size, 5 μm). Duplicated forage samples were placed in 250 mL Erlenmeyer flask with 150 mL mixed solution, then the flasks were capped with rubber stoppers attached with a three-way stopcock and anaerobically incubated in an orbital shaking incubator (VS-8480SR, Vison Science, Bucheon, Korea) at a speed of 120 rpm at 39℃ up to 72 h. Duplicates of each sample were used in three separate runs under similar conditions.

## Evaluation of fermentation characteristics

At 0, 3, 6, 12, 24, 48, and 72 h, incubated flasks were removed from the orbital shaking incubator in order to measure gas production by attaching a calibrated glass syringe through the three-way stopcock connected to the culture flasks. Immediately following that, the pH of the culture solution was measured. An aliquot of the solution from each flask was collected for ammonia (1.0 mL) and volatile fatty acid (VFA) (0.8 mL) analyses. Ammonia concentration was analyzed using a spectrophotometer (Optizen 3220UV, Mecasys Co., Ltd., Daejeon, Korea) according to the method of Fawcett and Scott (1960). VFA composition was analyzed by following the procedure of Li et al. (2010), where 0.2 mL 25% phosphoric acid solution was added to 0.8 mL aliquot collected and was then measured by gas chromatography (GC) using a Hewlett Packard 5890 series II GC (Hewlett Packard Co., USA) equipped with a flame ionization detector (FID).

## Nutrient effective degradability

The nylon bags containing the forage samples were washed under running tap water and dried at 65℃ for 48 h for the analysis of DM, NDF, ADF, CP, and OM degradation. The percentage disappearance of DM was calculated from the weight of the nylon bag after incubation. The disappearance rate was fitted to the equation: $Y(t) = a + b(1 - e^{-ct})$ by Ørskov and McDonald (1979), where Y (t) is the degradation rate at time t; a is the rapidly degraded fraction; b is the potentially degradable fraction; c is the degradation rate of fraction b. Estimates of the non-linear parameters a, b, and c were calculated by an iterative least square procedure. The estimated values were used for the calculation of effective degradability (ED) of dry matter, CP (EDCP), NDF (EDNDF), ADF (EDADF), and OM (EDOM) through the equation $ED = a + ((b \times c)/(c + r))$ Ørskov and McDonald (1979), where r is the fractional outflow rate and a hypothetical fractional outflow rate (kp) of 0.05/h was used.

## Statistical analysis

Data were analyzed using the general linear model procedure of SAS (2002) and the 18 observations were subjected to least squares analysis of variance according to the equation model $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, *where* $Y_{ij}$ is the observation, μ is the overall mean, $\tau_i$ is the effect of harvest time (i = 3), and $\varepsilon_{ij}$ is the error term. Statistical significance was established at $p < 0.05$.

# Results

## The chemical composition of Dark Horse and High Speed oat hays harvested at three different stages of maturity

The chemical compositions of Dark Horse and High Speed oat hays are presented in Table 1. Varieties and harvest time significantly influenced the chemical composition of oat hays. Detailed observation showed that between varieties, Dark Horse had significantly higher CP ($p < 0.021$) and OM ($p < 0.024$) as well as lower EE ($p < 0.0001$) compared to High Speed. Among harvest time, early-harvest increased CP content ($p < 0.003$) and late-harvest increased the NDF ($p < 0.010$) and OM contents ($p < 0.0001$) of Dark Horse, while no significant difference of harvest time was observed in High Speed. Varieties and harvest time showed a significant interaction in influencing the CP ($p < 0.035$) and OM ($p < 0.0001$) contents of oat hays.

## *In vitro* gas production and pH of Dark Horse and High Speed oat hays harvested at three different stages of maturity

Showed in Table 2, the pH of all treatments decreased over progressing incubation time. Varieties significantly affected pH values from 24 h to72 h, ($p < 0.009$ - $0.028$) in which Dark Horse had lower pH values than High Speed. While pH dropped, *in vitro* gas production increased with increasing incubation time, as shown in Table 3. Varieties significantly affected the gas production of oat hays where gas production of Dark Horse was found to be higher than High Speed from 3 h to 72 h ($p <$ 0.006 - 0.041), whilst harvesting time did not influence the gas production of either variety at any incubation time. In addition, no significant interaction was observed on gas production between varieties and harvest time of oat hays.

## The ammonia-N concentration (mg 100/mL) of Dark Horse and High Speed oat hays harvested at three different stages of maturity

The ammonia-N concentration of culture solution with Dark Horse and High Speed oat forage increased with progressing incubation time, as presented in Table 4. Between varieties, High Speed had significantly higher ammonia-N concentration

**Table 1.** The chemical composition of Dark Horse and High Speed oat hays harvested at three different stages of maturity.

| Breed (B) | Harvest time (HT) | Chemical Composition (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | CP | EE | NDF | ADF | OM | TDN |
| Dark Horse | Early | 11.75a | 2.82 | 61.69b | 37.59 | 92.26c | 53.13 |
| | Mid | 8.78b | 2.85 | 61.41b | 37.67 | 93.62b | 53.02 |
| | Late | 9.44b | 2.63 | 65.41a | 38.30 | 94.73a | 52.30 |
| High Speed | Early | 9.49b | 5.17 | 62.39ab | 35.91 | 93.82b | 55.06 |
| | Mid | 9.04b | 5.48 | 61.63b | 35.55 | 93.74b | 55.47 |
| | Late | 8.69b | 5.33 | 64.40ab | 38.13 | 93.77b | 52.49 |
| SEM | | 0.289 | 0.109 | 0.747 | 0.882 | 0.087 | 1.014 |
| p-value | B | 0.021 | < 0.0001 | 0.975 | 0.187 | 0.024 | 0.186 |
| | HT | 0.003 | 0.378 | 0.010 | 0.339 | < 0.0001 | 0.338 |
| | B*HT | 0.035 | 0.446 | 0.675 | 0.684 | < 0.0001 | 0.684 |

CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; OM, organic matter ; TDN, total digestible nutrient; SEM, standard error of means; p-value, probability values .
a - c: Means in the same line with different superscripts significantly differed ($p < 0.05$).

than Dark Horse at 48 h and 72 h ($p < 0.004 - 0.011$), while different harvest time did not influence the ammonia-N concentration of Dark Horse and High Speed at any incubation time. Furthermore, no interaction was observed on the ammonia-N concentration between varieties and harvest time of oat hays.

## The VFA composition of Dark Horse and High Speed oat hays in culture solution harvested at three different stages of maturity

Varieties of oat hays significantly affected the VFA composition from 3 h to 72 h ($p < 0.05$) as shown in Table 5. Detailed observation showed that Dark Horse had significantly higher total VFA concentration ($p < 0.010$), acetate ($C_2$) proportion ($p < 0.025$), and $C_2$ to $C_3$ ratio ($p < 0.0009$), as well as lower propionate ($C_3$) ($p < 0.011$) and butyrate ($C_4$) ($p < 0.009$) than High Speed. At 3, 12, and 24 h, the molar proportions of VFA were significantly affected by harvest time. Early-harvest of Dark Horse increased $C_4$ proportion ($p < 0.040$) at 3 h; early and late-harvest increased $C_2$ proportion ($p < 0.010$) and $C_2/C_3$ ratio ($p < 0.017$), and mid- harvest increased $C_4$ proportion at 12 h ($p < 0.002$); late-harvest decreased $C_3$ proportion ($p < 0.016$) but increased $C_2/C_3$ ratio ($p < 0.039$) at 24 h. Nonetheless, the total VFA concentration of both varieties at all incubation times remained unaffected. Breed of oat and harvest time showed significant interactions in influencing $C_2$ ($p < 0.026$) and $C_4$ ($p <$

**Table 2.** Effect of different harvest time of Dark Horse and High Speed on pH of culture solution.

| Breed (B) | Harvest time (HT) | pH | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 h | 3 h | 6 h | 12 h | 24 h | 48 h | 72 h |
| Dark Horse | Early | 6.64 | 6.39 | 6.30 | 6.15 | 6.02 | 5.85 | 5.80 |
| | Mid | 6.63 | 6.31 | 6.20 | 6.02 | 5.86 | 5.71 | 5.68 |
| | Late | 6.61 | 6.23 | 6.06 | 5.94 | 5.80 | 5.71 | 5.63 |
| High Speed | Early | 6.61 | 6.37 | 6.24 | 6.19 | 6.11 | 5.91 | 5.89 |
| | Mid | 6.59 | 6.27 | 6.23 | 6.09 | 6.01 | 5.87 | 5.80 |
| | Late | 6.60 | 6.32 | 6.24 | 6.11 | 6.02 | 5.83 | 5.82 |
| SEM | | 0.063 | 0.041 | 0.049 | 0.053 | 0.046 | 0.041 | 0.042 |
| p-value | B | 0.670 | 0.864 | 0.326 | 0.132 | 0.009 | 0.028 | 0.010 |
| | HT | 0.980 | 0.173 | 0.212 | 0.126 | 0.055 | 0.146 | 0.086 |
| | B*HT | 0.980 | 0.445 | 0.206 | 0.649 | 0.595 | 0.671 | 0.662 |

SEM, standard error of means; p-value, probability values.

**Table 3.** Effect of different harvest time of Dark Horse and High Speed on in vitro gas production.

| Breed (B) | Harvest time (HT) | Gas production | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3 h | 6 h | 12 h | 24 h | 48 h | 72 h |
| Dark Horse | Early | 21 | 34 | 51 | 76 | 103 | 120 |
| | Mid | 19 | 30 | 52 | 83 | 111 | 126 |
| | Late | 23 | 37 | 59 | 84 | 121 | 135 |
| High Speed | Early | 13 | 26 | 42 | 67 | 98 | 111 |
| | Mid | 17 | 27 | 53 | 73 | 111 | 122 |
| | Late | 15 | 26 | 46 | 73 | 102 | 113 |
| SEM | | 2.070 | 2.781 | 2.315 | 2.883 | 3.351 | 3.318 |
| p-value | B | 0.023 | 0.028 | 0.016 | 0.008 | 0.041 | 0.006 |
| | HT | 0.685 | 0.743 | 0.094 | 0.148 | 0.058 | 0.116 |
| | B*HT | 0.501 | 0.535 | 0.118 | 0.983 | 0.169 | 0.158 |

SEM, standard error of means; p-value, probability values.

0.014) proportions at 12 h and 24 h, but showed no significant influence on the total VFA concentration of oat hays at any incubation times.

### Nutrient effective degradability of Dark Horse and High Speed oat hays harvested at three different stages of maturity

Presented in Table 6 are the degradation parameters and nutrient ED of Dark Horse and High Speed. Varieties markedly influenced the ED of all nutrients, except for EDCP, as Dark Horse had significantly higher EDDM ($p < 0.0002$), EDNDF ($p < 0.001$), EDADF ($p < 0.0001$), and EDOM ($p < 0.027$) than High Speed. In terms of harvest time, early-harvest of Dark Horse increased EDDM and EDNDF compared to mid and late-harvest ($p < 0.05$), while no significant effect was observed on the nutrients ED of High Speed.

## Discussion

Changes in the nutritional composition of forage are affected by numerous factors, and one of the major factors is the choice of the stage of maturity at which the forage is harvested (Hwangbo and Oh, 2017; Rosser et al., 2017). In the present study, increased CP content was observed in the early-harvest of Dark Horse, which is consistent with other studies stating that a reduction in protein content occurs with advancing maturity (Cherney and Marten, 1982; Contreras-Govea and Albrecht, 2006). It has also been reported that as plants mature, there are changes in the forage composition such as the increased concentration of lignin (Kilcher and Troelsen, 1973; Jung and Allen, 1995; Khorasani et al., 1997) and OM (Erickson et al., 1977). Similarly, in this study, high NDF and OM contents were found on the late-harvest of Dark Horse. NDF values represent the total fiber fraction (cellulose, hemicellulose, and lignin); thus, a high NDF content indicates high overall fiber (Newman et al., 2006) affecting the digestibility of hays. However, it has been reported that CP content improves fiber digestibility because the population of cellulolytic bacteria is increased (Currier et al., 2004). Hence, the increased EDDM observed in early-harvest Dark Horse compared to mid and late-harvest could be due to its low NDF and high CP content. Furthermore, EDNDF in late-harvest Dark Horse could best be explained by the pH and ammonia-N measured. The lowest pH values of 5.68 and 5.63 were gathered from mid and late-harvest Dark Horse, and it has been reported that pH

**Table 4.** Effect of different harvest time of Dark Horse and High Speed on ammonia-N concentration (mg/100 mL) of culture solution.

| Breed (B) | Harvest Time (HT) | Ammonia-N concentration | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 h | 3 h | 6 h | 12 h | 24 h | 48 h | 72 h |
| Dark Horse | Early | 7.22 | 12.56 | 13.91 | 16.91 | 19.79 | 22.31 | 28.24 |
| | Mid | 7.43 | 11.33 | 11.73 | 15.43 | 18.31 | 21.59 | 27.28 |
| | Late | 7.43 | 11.16 | 12.19 | 13.87 | 17.39 | 19.63 | 26.36 |
| High Speed | Early | 6.55 | 11.49 | 12.80 | 16.86 | 18.90 | 26.25 | 31.52 |
| | Mid | 7.65 | 11.56 | 12.87 | 17.18 | 18.93 | 27.42 | 31.69 |
| | Late | 7.71 | 11.84 | 12.30 | 15.86 | 17.32 | 25.36 | 30.73 |
| SEM | | 0.378 | 0.442 | 0.428 | 1.213 | 1.942 | 1.601 | 1.039 |
| p-value | B | 0.891 | 0.912 | 0.917 | 0.362 | 0.956 | 0.011 | 0.004 |
| | HT | 0.331 | 0.554 | 0.124 | 0.451 | 0.737 | 0.591 | 0.617 |
| | B*HT | 0.577 | 0.326 | 0.175 | 0.787 | 0.957 | 0.881 | 0.897 |

SEM, standard error of means; p-value, probability values.

**Table 5.** Effect of different harvest time of Dark Horse and High Speed on total VFA concentration and molar proportion of VFAs in the culture solution.

| Items | Breeds (B) | | | | | | SEM | p-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dark Horse | | | High Speed | | | | B | HT | B*HT |
| | Early | Mid | Late | Early | Mid | Late | | | | |
| 0 h | | | | | | | | | | |
| Total VFA (mmoles $100mL^{-1}$) | 20.08 | 19.79 | 19.21 | 19.18 | 20.64 | 19.41 | 0.721 | 0.950 | 0.854 | 0.829 |
| Molar proportion (moles $100moles^{-1}$) | | | | | | | | | | |
| Acetate (C2) | 62.49 | 62.37 | 61.71 | 61.40 | 61.33 | 61.75 | 0.697 | 0.400 | 0.978 | 0.821 |
| Propionate (C3) | 19.49 | 19.69 | 19.94 | 19.87 | 20.10 | 20.03 | 0.267 | 0.364 | 0.711 | 0.902 |
| Butyrate (C4) | 12.84 | 12.88 | 13.25 | 13.25 | 13.26 | 13.21 | 0.352 | 0.550 | 0.932 | 0.887 |
| C2/C3 | 3.22 | 3.19 | 3.11 | 3.10 | 3.06 | 3.09 | 0.070 | 0.276 | 0.833 | 0.830 |
| 3 h | | | | | | | | | | |
| Total VFA (mmoles $100mL^{-1}$) | 34.79 | 36.3 | 50.9 | 29.04 | 32.56 | 30.3 | 2.951 | 0.004 | 0.089 | 0.068 |
| Molar proportion (moles $100moles^{-1}$) | | | | | | | | | | |
| Acetate (C2) | 62.02 | 63.23 | 66.1 | 59.32 | 59.79 | 59.94 | 0.800 | < 0.0001 | 0.098 | 0.236 |
| Propionate (C3) | 19.63 | 19.64 | 18.92 | 21.75 | 21.54 | 21.54 | 0.420 | < 0.0001 | 0.688 | 0.797 |
| Butyrate (C4) | 13.29a | 12.39ab | 10.77b | 13.98a | 13.43a | 13.22a | 0.445 | 0.009 | 0.040 | 0.271 |
| C2/C3 | 3.16 | 3.25 | 3.53 | 2.73 | 2.78 | 2.78 | 0.104 | < 0.0001 | 0.318 | 0.470 |
| 6 h | | | | | | | | | | |
| Total VFA(mmoles $100mL^{-1}$) | 63.5 | 63.52 | 60.11 | 40.00 | 36.33 | 35.73 | 4.364 | < 0.0001 | 0.789 | 0.947 |
| Molar proportion (moles $100moles^{-1}$) | | | | | | | | | | |
| Acetate (C2) | 64.94 | 62.62 | 64.72 | 61.58 | 58.53 | 57.49 | 1.017 | 0.0001 | 0.111 | 0.312 |
| Propionate (C3) | 19.37 | 20.3 | 19.04 | 20.82 | 22.38 | 22.25 | 0.468 | 0.0001 | 0.149 | 0.344 |
| Butyrate (C4) | 11.07 | 12.79 | 11.81 | 13.07 | 14.14 | 15.19 | 0.624 | 0.003 | 0.139 | 0.478 |
| C2/C3 | 3.39 | 3.11 | 3.45 | 2.96 | 2.62 | 2.59 | 0.127 | 0.0002 | 0.197 | 0.367 |
| 12 h | | | | | | | | | | |
| Total VFA(mmoles $100mL^{-1}$) | 65.82 | 64.35 | 77.63 | 42.55 | 44.27 | 43.43 | 2.263 | < 0.0001 | 0.155 | 0.164 |
| Molar proportion (moles $100moles^{-1}$) | | | | | | | | | | |
| Acetate (C2) | 66.85a | 59.47b | 68.38a | 60.92b | 58.67b | 58.4b | 0.910 | < 0.0001 | 0.010 | 0.026 |
| Propionate (C3) | 19.18 | 20.30 | 19.4 | 21.12 | 23.08 | 22.36 | 0.388 | < 0.0001 | 0.054 | 0.625 |
| Butyrate (C4) | 10.03b | 15.32a | 8.21b | 13.63a | 13.91a | 14.58a | 0.501 | 0.0002 | 0.002 | 0.001 |
| C2/C3 | 3.48a | 2.93b | 3.53a | 2.91b | 2.55bc | 2.63b | 0.089 | < 0.0001 | 0.017 | 0.237 |
| 24 h | | | | | | | | | | |
| Total VFA(mmoles $100mL^{-1}$) | 72.58 | 76.66 | 81.05 | 47.74 | 57.82 | 50.01 | 2.752 | < 0.0001 | 0.247 | 0.349 |
| Molar proportion (moles $100moles^{-1}$) | | | | | | | | | | |
| Acetate (C2) | 59.36 | 58.53 | 64.17 | 57.17 | 56.05 | 55.4 | 0.991 | 0.0003 | 0.212 | 0.032 |
| Propionate (C3) | 21.05bc | 22.49ab | 20.18c | 22.94a | 23.8a | 22.87ab | 0.366 | < 0.0001 | 0.016 | 0.416 |
| Butyrate (C4) | 14.01 | 13.96 | 11.25 | 14.47 | 14.85 | 16.04 | 0.564 | 0.005 | 0.590 | 0.014 |
| C2/C3 | 2.84ab | 2.61b | 3.20a | 2.50b | 2.36b | 2.43b | 0.085 | 0.0001 | 0.039 | 0.077 |
| 48 h | | | | | | | | | | |
| Total VFA(mmoles $100mL^{-1}$) | 82.16 | 87.32 | 89.95 | 60.89 | 66.38 | 62.91 | 6.780 | 0.010 | 0.869 | 0.931 |
| Molar proportion (moles $100moles^{-1}$) | | | | | | | | | | |
| Acetate (C2) | 59.19 | 57.18 | 58.76 | 54.78 | 53.13 | 54.44 | 1.475 | 0.025 | 0.666 | 0.996 |
| Propionate (C3) | 20.62 | 22.24 | 22.71 | 23.74 | 24.87 | 24.80 | 0.784 | 0.011 | 0.419 | 0.907 |
| Butyrate(C4) | 14.02 | 14.34 | 12.76 | 15.64 | 16.03 | 15.02 | 0.775 | 0.003 | 0.425 | 0.949 |
| C2/C3 | 2.88 | 2.60 | 2.62 | 2.33 | 2.15 | 2.25 | 0.132 | 0.009 | 0.556 | 0.917 |
| 72 h | | | | | | | | | | |
| Total VFA (mmoles $100mL^{-1}$) | 85.58 | 89.58 | 98.89 | 63.22 | 74.34 | 63.23 | 4.552 | < 0.0001 | 0.372 | 0.297 |
| Molar proportion (moles $100moles^{-1}$) | | | | | | | | | | |
| Acetate (C2) | 61.62 | 57.89 | 61.30 | 54.8 | 52.31 | 52.01 | 1.401 | < 0.0001 | 0.252 | 0.646 |
| Propionate (C3) | 21.15 | 22.04 | 21.27 | 24.02 | 23.15 | 24.44 | 0.591 | 0.001 | 0.934 | 0.419 |
| Butyrate (C4) | 11.22 | 13.68 | 11.69 | 14.7 | 16.82 | 16.38 | 0.822 | 0.0003 | 0.121 | 0.786 |
| C2/C3 | 2.95 | 2.65 | 2.92 | 2.30 | 2.27 | 2.14 | 0.116 | < 0.0001 | 0.569 | 0.467 |

HT, harvest time; SEM, standard error of means; p-value, probability values.
a - c: Means in the same row with different superscripts indicate a statistically significant difference ($p < 0.05$).

lower than 5.8 negatively affects NDF degradability (Grant, 1994). Low pH values could have caused a shift in the microbial population, which can lead to low ammonia-N concentration, and could have extended NDF digestion time and subsequently decreasing degradability of NDF (Calsamiglia et al., 2002). While harvest time affected the EDDM and EDNDF of Dark Horse, it did not influence the total VFA concentration, which directly reflects the energy content of forage (Qin et al., 2013). This might be due to the low OM content of early-harvest containing low carbohydrate sugar and starch, which could have resulted in a decreased rate of carbohydrate digestion, thereby decreasing the VFA production.

The influence of harvest time on nutrient ED was only observed on Dark Horse and was not observed on any nutrients ED of High Speed, which may be due to the innate characteristics of an early-maturing oat variety.

As plants mature, there is an increase in the proportion of stem present in the plant (Cherney and Marten, 1982; McCartney et al., 2006). Due to the early maturation of High Speed, it might be at the peak of its maturity at the time of the first period of harvest, resulting in its high NDF and low CP content at all harvest times, as observed on the chemical composition, thus affecting its degradability. Furthermore, lower gas production was observed in High Speed compared to Dark Horse, which could indicate that there was no effective degradability occurring on any nutrients (DM, NDF, ADF, CP, and OM) of High Speed oat hays. As other studies have reported, the generation of gas is an indication of feed degradation (Menke et al., 1979; Getachew et al., 2004).

**Table 6.** Effect of different harvest time of Dark Horse and High Speed on degradation parameters (a, b, and c) and effective degradability (ED) of forage nutrients.

| Items | Breeds (B) | | | | | | SEM | p-values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dark Horse | | | High Speed | | | | | | |
| | Early | Mid | Late | Early | Mid | Late | | B | HT | B*HT |
| a | 31.10 | 30.45 | 27.69 | 29.90 | 29.47 | 28.53 | 0.855 | 0.623 | 0.083 | 0.588 |
| b | 23.99a | 16.01b | 21.64a | 11.55b | 11.07b | 12.72b | 1.379 | < 0.0001 | 0.047 | 0.133 |
| c | 0.027c | 0.038bc | 0.032bc | 0.04b | 00.06a | 0.04b | 0.004 | 0.0001 | 0.002 | 0.329 |
| EDDM | 39.63a | 37.00b | 35.59bc | 34.84bc | 35.64bc | 34.39c | 0.551 | 0.0002 | 0.011 | 0.028 |
| a | 10.43a | 5.22bc | 4.87bc | 6.79b | 3.86c | 4.98bc | 0.623 | 0.026 | 0.0001 | 0.137 |
| b | 16.34b | 15.12b | 43.69a | 8.21b | 14.13b | 12.62b | 2.485 | 0.0001 | 0.0002 | 0.0003 |
| c | 0.029 | 0.037 | 0.011 | 0.048 | 00.04 | 0.04 | 0.006 | 0.023 | 0.187 | 0.218 |
| EDNDF | 15.70a | 11.59bc | 12.02b | 10.58bc | 08.94c | 10.50bc | 0.667 | 0.001 | 0.019 | 0.203 |
| a | 10.26a | 5.60b | 1.95c | 3.35c | 03.40c | 2.85c | 0.402 | < 0.0001 | < 0.0001 | < 0.0001 |
| b | 16.04b | 15.73b | 45.24a | 7.18c | 11.38bc | 11.81bc | 1.381 | < 0.0001 | < 0.0001 | < 0.0001 |
| c | 0.024 | 0.033 | 0.013 | 0.049 | 0.051 | 0.035 | 0.009 | 0.056 | 0.777 | 0.376 |
| EDADF | 14.98 | 11.48 | 11.01 | 6.80 | 07.29 | 7.95 | 0.505 | < 0.0001 | 0.089 | 0.007 |
| a | 66.44 | 63.43 | 61.07 | 68.08 | 67.57 | 64.55 | 2.199 | 0.220 | 0.325 | 0.909 |
| b | 21.65 | 21.35 | 24.21 | 14.08 | 17.20 | 17.42 | 1.951 | 0.009 | 0.529 | 0.807 |
| c | 0.125 | 0.095 | 0.089 | 0.114 | 0.203 | 0.126 | 0.095 | 0.051 | 0.308 | 0.104 |
| EDCP | 81.24 | 76.47 | 75.97 | 78.14 | 80.97 | 77.12 | 0.951 | 0.431 | 0.056 | 0.024 |
| a | 26.34 | 25.26 | 25.31 | 28.48 | 26.99 | 25.16 | 0.935 | 0.231 | 0.271 | 0.623 |
| b | 30.49 | 15.37 | 23.36 | 8.04 | 11.32 | 11.25 | 2.457 | < 0.0001 | 0.196 | 0.030 |
| c | 0.022 | 0.051 | 0.039 | 0.058 | 0.063 | 0.077 | 0.007 | 0.001 | 0.093 | 0.130 |
| EDOM | 34.99 | 33.05 | 33.73 | 32.77 | 33.14 | 31.32 | 0.599 | 0.027 | 0.291 | 0.187 |

HT, harvest time; a, rapidly soluble fraction; b, degradable fraction in the culture solution; c, degradation rate of b per time; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; OM, organic matter; SEM, standard error of means; p-value, probability values.
a - c: Means in the same row with different superscripts differ ($p < 0.05$).

## Conclusions

Based on the experimental data of this study, Dark Horse had higher gas and VFA production; lower pH and ammonia-N concentration; and higher EDDM, EDNDF, EDADF, and EDOM than High Speed. In addition, its ED was also influenced by harvest time, as early-harvest increased EDDM and EDNDF. Allowing the late-maturing Dark Horse variety to advance in maturity would decrease the effective degradability of oat hays. Therefore, the study suggests early-harvest for Dark Horse variety in order to result in high EDDM and EDNDF of oat hays. For the early-maturing variety, the study demonstrated that High Speed had higher pH and ammonia-N concentration but lower gas and VFA production as well as ED of DM, NDF, ADF, and OM than Dark Horse, suggesting that High Speed might have already passed its peak maturation. Hence, the ED of nutrients was not influenced by harvest time.

## Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

## Acknowledgements

This work was supported by the research grant of the Chungbuk National University in 2016.

## Authors Information

Yan Zhang, https://orcid.org/0000-0003-2752-0578
Ye Hyun Lee, https://orcid.org/0000-0003-2681-8022
Kim Margarette Nogoy, https://orcid.org/0000-0002-0958-7632
Chang Weon Choi, https://orcid.org/0000-0001-7681-5335
Do Hyung Kim, https://orcid.org/0000-0002-0726-8531
Xiang Zi Li, https://orcid.org/0000-0003-3061-3847
Seong Ho Choi, https://orcid.org/0000-0001-8869-0218

## References

AOAC (Association of official analytical chemists). 1995. Official methods of analysis. 13th ed. AOAC, Washington, D.C., USA.

Bergman EN. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews 70:567-590.

Calsamiglia S, Ferret A, Devant M. 2002. Effects of pH and pH fluctuations on microbial fermentation and nutrient flow from a dual-flow continuous culture system. Journal of Dairy Science 85:574-579.

Cherney J, Marten G. 1982. Small grain crop forage potential: II. Interrelationships among biological, chemical, morphological, and anatomical determinants of quality1. Crop Science 22:240-245.

Contreras-Govea FE, Albrecht KA. 2006. Forage production and nutritive value of oat in autumn and early summer. Crop Science 46:2382-2386.

Currier T, Bohnert D, Falck S, Schauer C, Bartle S. 2004. Daily and alternate-day supplementation of urea or biuret to ruminants consuming low-quality forage: II. Effects on the site of digestion and microbial efficiency in steers. Journal of animal science 82:1518-1527.

Erickson D, Riveland N, French E. 1977. The nutritional value of oat hay harvested at several stages of maturity. North Dakota Farm Research, USA.

Fawcett J, Scott J. 1960. A rapid and precise method for the determination of urea. Journal of clinical pathology 13:156-159.

Getachew G, Robinson P, Depeters E, Taylor S. 2004. Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. Animal Feed Science and Technology 111:57-71.

Grant R. 1994. Influence of corn and sorghum starch on the *in vitro* kinetics of forage fiber digestion. Journal of Dairy Science 77:1563-1569.

Hwangbo S, Oh MG. 2017. Effects of seeding and harvest dates on the productivity, nutritive values, and livestock carrying capacity of spring-seeded oats (*Avena sative* L.) in the northern Gyeongbuk province. Korean Journal of Agricultural Science 44:400-408. [in Korean]

Jung H, Allen M. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of animal science 73:2774-2790.

Khorasani G, Jedel P, Helm J, Kennelly J. 1997. Influence of stage of maturity on yield components and chemical composition of cereal grain silages. Canadian Journal of Animal Science 77:259-267.

Kilcher M, Troelsen J. 1973. Contribution and nutritive value of the major plant components of oats through progressive stages of development. Canadian Journal of plant science 53:251-256.

Kim J, Kim S, Abuel S, Kwon C, Shin C, Ko K, Park B. 2006. Effect of location, season, and variety on yield and quality of forage oat. Asian Australasian Journal of Animal Sciences 19:970-977.

Li X, Long R, Yan C, Choi S, Jin G, Song M. 2010. Rumen microbial responses in fermentation characteristics and production of CLA and methane to linoleic acid is associated with malate or fumarate. Animal feed science and technology 155:132-139.

Mccartney D, Block H, Dubeski P, Ohama A. 2006. The composition and availability of straw and chaff from small grain cereals for beef cattle in western Canada. Canadian journal of animal science 86:443-455.

McDougall E. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochemical Journal 43:99-109.

Menke K, Raab L, Salewski A, Steingass H, Fritz D, Schneider W. 1979. The estimate ion of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. The Journal of Agricultural Science 93:217-222.

Moreira N. 1989. The effect of seed rate and nitrogen fertilizer on the yield and nutritive value of oat-vetch mixtures. The Journal of Agricultural Science 112:57-66.

Newman YC, Lambert B, Muir JP. 2006. Defining forage quality. The Texas A&M University System, Texas, USA.

Ørskov E, McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. The Journal of Agricultural Science 92:499-503.

Qin WZ, Choi SH, Lee SU, Lee SS, Song MK. 2013. Effect of defaunation on *in vitro* fermentation characteristics and methane emission when incubated with forages. Journal of the Korean Society of Grassland and Forage Science 33:197-205. [in Korean]

Rosser C, Beattie A, Block H, Mckinnon J, Lardner H, Górka P, Penner G. 2016. Effect of maturity at harvest for whole-crop barley and oat on dry matter intake, sorting, and digestibility when fed to beef cattle. Journal of animal science 94:697-708.

Rosser C, Beattie A, Block H, Mckinnon J, Lardner H, Górka P, Penner G. 2017. Effects of the frequency of forage allocation and harvest maturity of whole-crop oat forage on dry matter intake and ruminal fermentation for beef heifers. The Professional Animal Scientist 33:85-91.

SAS (Statistical Analysis System). 2002 'SAS User's guide: Statistical analysis system institute. SAS Inc., Cary, USA.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science 74:3583- 3597.

Wilson J. 1994. Cell wall characteristics in relation to forage digestion by ruminants. The Journal of Agricultural Science. 122:173-182.