Effect of stage of maturity at harvest for forage pea (*Pisum sativum* L.) on eating behavior, ruminal fermentation, and digestibility when fed as hay to yearling beef heifers

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ABSTRACT: The objective of this study was to evaluate the stage of maturity at harvest for pea hay (*Pisum sativum* L., c.v. CDC Horizon) on dry matter intake (DMI), eating behavior, ruminal fermentation, and digestibility when fed to beef heifers. Pea hay was cut at EARLY (defined to occur when flat pods were on one or more nodes), MID (when seeds filled the pods at one or more nodes and the leaves were changing from green to gold), and LATE (yellow dry seeds filled pods on most or all of the nodes and the pods and leaves had a yellow color) phases, and was cured in the field and baled. Six ruminally-cannulated Speckle Park heifers were used in a replicated 3 × 3 Latin square design with three 18-d periods including 12 d for adaptation, 2 d for measurement of ruminal pool sizes, and 4 d for the collection of eating behavior, ruminal pH, ruminal digesta, and feces. For all treatments, the respective pea hay was included at 40% of the dietary DM. Stage of maturity at harvest for pea hay did not affect total DMI, pea hay DMI, or the total short-chain fatty acid concentration in ruminal fluid with averages of 8.6 kg/d, 3.2 kg/d, and 96.55 mM, respectively. The duration of time spent ruminating decreased with advancing pea hay maturity when reported as min/d, min/kg DMI, and min/kg neutral detergent fiber (NDF) ($P \leq 0.01$). Mean ruminal pH also decreased with advancing pea maturity ($P < 0.01$). The ruminal DM and undigested NDF corrected for OM pools were not affected by stage of maturity ($P \geq 0.55$) nor was the rate of digestion for NDF. However, NDF passage rate decreased by 0.21%/h with advancing pea hay maturity ($P = 0.02$). Apparent total tract digestibility of NDF (average = 16.30%, $P = 0.41$) was not affected, but starch digestibility decreased from 96.10% to 93.08% with advancing pea hay maturity ($P = 0.07$). Overall, stage of maturity at harvest for pea hay does not appear to affect DMI or NDF digestibililty but decreases chewing activity, apparent total tract starch digestibility, ruminal pH, and ruminal NDF passage rate.

Key Words: eating behavior, pea hay, *Pisum sativum*, rumen turnover, ruminal fermentation, stage of maturity

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INTRODUCTION

Incorporation of legumes such as field pea into cereal crops can enhance nitrogen fixation, decrease the requirement for N fertilizer

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application, and increase protein concentration of hay (Berkenkamp and Meeres, 1987; Aasen et al., 2004; Carr et al., 2004; Strydhorst et al., 2008). As cereals and field pea mature at differing rates, the harvest timing of cereal-pea blends is currently based on cereal maturity (Uzun and Asik, 2012). However, it is not clear how maturity at harvest for pea hay affects cattle responses.

Delaying maturity at swathing for annuals used for hay has been shown to improve yield without compromising quality for small grain cereals (Rosser et al., 2013, 2016), crop mixtures (Pikul et al., 2004), and pea (Borreani et al., 2007). For cereals used as hay, harvesting at the hard dough relative to soft dough does not compromise ruminal or total tract DM digestibility (Rosser et al., 2013, 2016) or dry matter intake (DMI) in beef cattle (Rosser et al., 2016, 2017), but altering the stage of maturity for the cereal may impact pea maturity when grown in blends. Recent research evaluating the effect of pea hay harvest maturity on cattle performance is limited; however, it has been reported that allowing peas to advance in maturity increased DMI in sheep (Daniel et al., 1946). On the other hand, digestibility of pea silage was greatest when cut at EARLY and MID stages rather than when flowering or ripe (Brundage et al., 1979). Despite this information, new forage pea cultivars have been developed to improve lodging resistance (Warkentin et al., 2012), which may allow for more delayed maturity at harvest without compromising digestibility.

We hypothesized that the maturity of field pea at harvest would not affect DMI or total tract digestibility, but the greater starch concentration in advanced maturity would increase sorting and alter ruminal fermentation.

MATERIALS AND METHODS

Use of heifers and the procedures used were pre-approved by the University of Saskatchewan Animal Research Ethics Board (protocol 20100021) according to the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada).

Forage Production

CDC Horizon (Crop Development Centre, Saskatoon, SK, Canada), a semileafless forage pea cultivar (Warkentin et al., 2012), was planted May 15, 2018 in a 0.81-ha plot at the University of Saskatchewan Livestock and Forage Centre of Excellence (Clavet, SK, Canada). Seeds were treated with 0.033 mL/kg mefenoxam and 0.0219 mL/kg fludioxonil (Apron Max RTA; Syngenta, Guelph, ON, Canada) and 0.958 g/kg ethaboxam (Intego Solo; Nufarm, Calgary, AB, Canada), and inoculated with 3.33×10^{12} CFU/kg *Rhizobium leguminosarum* (TagTeam; Monsanto Company, St. Louis, MO). The pea was seeded at a rate of 112 kg/ha with a Great Plains no-till drill (Great Plains, Salina, KS) and a 23-22-0-10 (N-P-K-S) fertilizer was applied (131 kg/ha). Chemical application during growth occurred three times. On May 5, 2018, 109.8 mL/ha polyalkylenedioxide (AIM; AgPro, Big Sandy, TX) and 880.2 g acid equivalent (a.e.)/ha of glyphosate (RoundUp; Monsanto Company, St. Louis, MO) was applied. On June 5, 2018, 15.04 a.e./ha of imazamox and 15.04 a.e./ha of imazethapyr (Odyssey; BASF Canada Inc., Mississauga, ON, Canada), 166.5 g/ha of sethoxydim (Poast; BASF Canada, Mississauga, ON, Canada), and 0.25 L/ha of petroleum hydrocarbons (Merge; BASF Canada, Mississauga, ON, Canada) were applied. On June 18, 2018, 19.75 g/ha of imazamox (Viper; BASF Canada, Mississauga, ON, Canada) and 2 L/ha of liquid 28-0-0 (N-P-K) fertilizer was applied.

Peas were harvested at three stages of maturity based upon those outlined by Knott (1987). Briefly, the early harvested pea (**EARLY**) was defined as the reproductive 204 and 205 stages when pea plants had flat unfilled pods at one or more nodes. The mid stage harvested pea (**MID**) was defined as reproductive stages 207 to 209, which occurred when seeds filled the pods at one or more nodes and leaves were changing from green to gold. The late harvest maturity (**LATE**) was defined as senescent stages 301 to 302 defined to occur when yellow dry seeds filled the pods on most or all of the nodes and the leaves and pods were yellow. Forage was swathed using a Case IH 8825 swather (CIH, Racine, WI) on July 15, July 25, and August 9, 2018 for the EARLY, MID, and LATE stages, respectively. All stages of pea hay were baled using a Massey Ferguson 1839 baler (AGCO, Duluth, GA) on August 10, 2018, when DM was ≥ 85%. However, the EARLY and MID pea swaths had precipitation (33.8 mm and 32 mm, respectively) fall on them during the curing process (Table 1) which may have further decreased soluble components within the hay. Bales were stored under shelter until use for the feeding study.

Experimental Design

Six Speckle Park heifers previously fit with a ruminal cannula (model 9C; Bar Diamond Inc., Parma, ID) were used in a replicated 3 × 3 Latin square design. Heifers were housed indoors in individual 3 × 3

Table 1. Environmental conditions prior to and after swathing for pea (*Pisum sativum* L.; c.v. CDC Horizon) harvested for hay at EARLY, MID, and LATE maturity

	Stage of maturity[4]		
Agronomic management	EARLY	MID	LATE
Environmental conditions[1], preswathing			
Days from seeding	62	72	87
Growing degree days[2]	761	887	1115
Mean ambient temperature, °C	17.6	17.7	18.1
Precipitation, mm	109.8	111.6	141.8
Date of swathing (2018)	15-Jul	25-Jul	9-Aug
Environmental conditions, postswathing			
Mean ambient temperature, °C	19.4	19.8	19.6
Precipitation, mm	33.8	32.0	1.8
Date of baling	10-Aug	10-Aug	10-Aug
Time required for curing, d	26	16	1
Forage DM at baling, %	87.28	87.88	87.14
Yield[3], T/ha	1.869	4.350	4.421

[1]Data derived from University of Saskatchewan Department of Civil and Geological Engineering.

[2]Growing degree days were calculated as (maximum temperature + minimum temperature) / 2 – base temperature, with the base temperature being 5°C.

[3]Actual DM yield calculated as (number of bales harvested per harvest maturity × average bale weight of that harvest maturity × DM coefficient of bales) / (ha used per harvest).

[4]Harvest maturities consisted of EARLY (plants with flat pods at one or more nodes), MID (filled pods at one or more nodes and leaves that were turning from green to gold), and LATE (yellow dry seeds filled pods on most nodes, and the leaves and pods were yellow).

Table 2. Inclusion rates and nutrient composition of treatment diets consisting of pea hay (*Pisum sativum* L.; c.v. CDC Horizon) harvested at EARLY, MID, and LATE maturity for beef cattle diets

	Treatment[1]		
Variable	EARLY	MID	LATE
Ingredient inclusion rate, % DM			
Early pea	40.00	–	–
Mid pea	–	40.00	–
Late pea	–	–	40.00
Mineral[2]	16.44	16.44	16.44
Oat pellet[3]	43.56	43.56	43.56
Chemical composition, % DM			
DM, %	89.1	90.2	89.4
OM	95.26	95.52	95.71
CP	15.32	14.15	14.20
$aNDF_{OM}$	41.98	42.15	37.67
ADF	29.59	29.54	25.49
Ethanol soluble carbohydrates	3.71	3.54	3.77
Starch	13.15	15.10	18.40
Ether extract	1.85	1.83	1.81
Ca	0.68	0.72	0.62
P	0.30	0.28	0.30

[1]Harvest maturities consisted of EARLY (plants with flat pods at one or more nodes), MID (filled pods at one or more nodes and leaves that were turning from green to gold), and LATE (yellow dry seeds filled pods on most nodes, and the leaves and pods were yellow).

[2]Mineral was composed of ground wheat (69.3%), porcine tallow (9.3%), molasses (8.3%), and urea (5.0%). The supplement provided 2.1% Ca, 1.1% P, 0.2% Mg, 0.6% K, 0.4% Na, 0.8% Cl, 0.2% S, 86.2 ppm Mn, 182.2 ppm Cu, 555.5 ppm Fe, 282.7 ppm Zn, 2.9 ppm I, 0.6 ppm Co, 0.6 ppm Se, 14,543.6 IU/kg Vitamin A, 1,872.5 IU/kg Vitamin D3, 424.4 IU/kg Vitamin E, 0.10% monensin (Elanco Division of Eli Lilly Canada Inc., Guelph, ON, Canada), and 0.11% melengestrol acetate (MGA; Federated Co-operatives Limited; Saskatoon, SK, Canada).

[3]Pellet was composed of oat hulls (50%), wheat middlings (40%), and molasses (10%).

m pens with rubber mat flooring. Pens were cleaned twice daily. Water was provided ad libitum and heifers were fed once daily at 0900 h. Diets (DM basis) consisted of 40.00% pea hay, 43.56% of a high-fiber oat pellet, and 16.44% of a vitamin and mineral supplement (Table 2). Pelleted feeds and the pea hay were provided in separate feed bunks to allow for accurate determination of forage intake.

Each Latin square had a unique treatment sequence and, when considering both squares they were designed to balance for carryover effects. Periods were 18 d in duration. Within each period, day 1 through day 12 were used to adapt heifers to their respective treatment. Rumen evacuations were conducted on day 13 and day 14, in which a subsample of the solid fraction of digesta was collected for chemical analysis. Behavior monitoring and sample collection of feed, refusals, feces, ruminal fluid, and ruminal pH occurred from day 15 through day 18.

Heifer Body Weight and Dry Matter Intake

Heifers were weighed on day 1 and day 2 of each period, as well as the 2 d following completion of the experiment. The amount of feed offered and refused (pea hay and concentrate were measured individually) were weighed daily to determine feed intake. During sampling periods, 500 g of each feed ingredient and a representative sample equating to 20% of the feed refusals from each heifer were collected daily and dried to constant weight at 55 °C to determine the DM content. The amount of feed offered and refused was corrected for DM and used to determine DMI. Feed and refusal samples were composited by heifer within period for chemical analysis.

Ruminal Pool Size and Turnover Rate

The reticulo-ruminal digesta was completely evacuated 0300 h postfeeding on day 13 and at 0300 h prior to feeding on day 14 to evaluate ruminal neutral detergent fiber (NDF) turnover (Dado

and Allen, 1995). The weight of the digesta was recorded, mixed, and a 4-L sample was collected from each heifer. The digesta sample was weighed and separated into liquid and solid fractions using a wine press (Harvest Bounty Wine Press, Pleasant Hill Grain LLC, Hampton, NE; Karnati et al., 2007). The arising solid and liquid fractions were weighed and the representative 500-g samples from each fraction were collected. Samples were dried in a forced-air oven at 55 °C until a constant weight was achieved for determination of DM.

Eating Behavior

The particle size distribution of the forage and forage refusals were measured in duplicate using Penn State Particle Size Separator with 19, 8, and 4-mm sieves, and a bottom pan (Nasco, Newmarket, ON, Canada). From these data, the sorting index was determined according to Leonardi and Armentano (2003). Briefly, the sorting index was calculated using the quantity of each fraction consumed relative to the amount that would have been consumed if no sorting had occurred. Values greater than 100 were considered to indicate selective consumption, whereas values less than 100 indicate selective avoidance.

Eating behavior was evaluated using RumiWatch halters (ITIN + HOCH GmbH, Liestal, Switzerland) as validated by Zehner et al., 2017 and Ruuska et al. (2016). The noseband of the halter included an oil-filled flexible silicone tube. Jaw movements were recorded by a pressure sensor on the noseband of the halter and halters were programmed to record the time spent eating, drinking, and ruminating from 0900 h on day 15 and to extend for 96 h (Zehner et al., 2017). Differentiation between eating, ruminating, and drinking was derived based upon the pressure patterns and length of time of behavioral bouts.

Ruminal Fermentation

Ruminal pH, in the ventral sac, was recorded every 5 min for 96 h, from 0900 h on day 15 until 0855 h on day 1 of the following period using the Lethbridge Research Centre Ruminal pH Measurement System (Dascor Inc., Escondido, CA) as described by Penner et al. (2006). The pH system was standardized in pH buffers 7 and 4 before insertion into the rumen and upon removal. The starting and ending regressions were used to convert the mV data to pH assuming a linear drift over time while accounting for temperature effects on pH.

Ruminal digesta samples were collected every 12-h with a 3-h offset among the 4 d of collection. This approach resulted in eight samples representing every 0300 h of a 24-h cycle. During collection, three 250-mL samples of digesta were collected from the central portion of the caudal, cranial, and ventral sacs of the rumen. Digesta were strained through two layers of cheesecloth and a 10-mL sample of the strained ruminal fluid was added to 2 mL of 25% (w/v) metaphosphoric acid. Samples were stored at −20 °C. Ruminal fluid samples were used to measure the concentration of short-chain fatty acid (SCFA) using gas chromatography (Agilent 6890; Agilent Technologies Canada Inc., Mississauga, ON, Canada) according to Khorasani et al. (1996).

Apparent Total Tract Digestibility

Fecal samples (100 g per sample) were collected directly from the rectum at the same time as ruminal digesta collection and samples were composited for each heifer by period. Fecal composite samples were dried at 55 °C in a forced-air oven until achieving a constant weight. Fecal output was determined using undigested $aNDF_{OM}$ ($uNDF_{OM}$) as a marker. Digestibility (% DM) was then determined by expressing the difference between nutrient intake and nutrient output when divided by nutrient intake.

Chemical Analysis

All dried feed ingredient, refusals, solid ruminal digesta, and fecal samples were ground through a 1-mm screen using a hammer mill (Christy and Norris Ltd, Chelmsford, UK) prior to being sent to Cumberland Valley Analytical Services (Waynesboro, PA) for analysis. Samples were analyzed for DM, OM, crude protein (CP), NDF, ash-free NDF ($aNDF_{OM}$), undigested NDF ($uNDF_{OM}$), acid detergent fiber (ADF), starch, ethanol-soluble carbohydrate, ether extract, ash, Ca, and P.

The DM content was determined by heating samples at 105 °C for 0300 h according to method 2.1.4 (Shreve et al., 2006). Ash was determined according to AOAC method 942.05 (AOAC, 2000), with the modification, that a 1.5-g sample was ashed for 0400 h at 600 °C and was subtracted from 100 to determine OM. Crude protein was analyzed using a Leco FP-528 Nitrogen Combustion Analyzer (LECO Corp., St. Joseph,

MI). NDF was determined according to Van Soest et al. (1991) using Whatman 934-AH glass microfiber filters with 1.5-µm particle retention (GE Healthcare Life Sciences, Piscataway, NJ) utilizing α-amylase and sodium sulfite. The residue remaining from the NDF analysis was also used to determine $aNDF_{OM}$ by ashing the residue at 535 °C for 0200 h. Acid detergent fiber was determined according to AOAC method 973.18 (AOAC, 2000) using Whatman 934-AH glass microfiber filters with a 1.5-µm particle retention instead of glass crucibles. Starch was determined according to Hall (2009) and ether extract was determined according to AOAC method 2003.05 (AOAC, 2006). Ethanol-soluble carbohydrate was analyzed according to Dubois et al. (1956). Calcium and P concentrations were determined according to AOAC method 985.01 (AOAC, 2000) by ashing samples for 0100 h at 535 °C followed by a digestion using 15% nitric acid. Samples were then adjusted to 50 mL and analyzed on an inductively-coupled plasma optical emission spectrometer (model 5300, PerkinElmer, Shelton, CT).

Calculations and Statistical Analysis

The degradation rate of $aNDF_{OM}$ (k_d) was calculated according to Dado and Allen (1995). The rate of degradation for digestible $aNDF_{OM}$ was calculated as k_d = (hourly digestible $aNDF_{OM}$ intake) / (rumen pool size of digestible $aNDF_{OM}$) – k_p, with k_p being the fractional ruminal passage rate of digestible $aNDF_{OM}$. This model is based on the assumption that passage rate for digestible and indigestible $aNDF_{OM}$ fractions are equivalent and that the ruminal pool size of $aNDF_{OM}$ was constant.

Data were analyzed using the Mixed Model procedure in SAS (version 9.4, SAS Inst. Inc., Cary, NC). The model included the fixed effect of treatment, with the random effect of period and cow nested within square. Mean separation was conducted using the Bonferroni means separation test. Sorting behavior was also evaluated using a two-tailed *t*-test to determine if individual treatment means differed from 100. In all analyses, significance was declared when $P < 0.05$.

RESULTS

Forage Production and Treatments

EARLY, MID, and LATE pea hay received 761, 887, and 1,115 growing degree days between seeding and swathing, respectively (Table 1). The mean ambient temperature for during the growing season was 17.8 ˚C, and precipitation ranged from 109.8 mm to 141.8 mm among treatments. To ensure adequate DM concentration for baling, EARLY hay required 26 d of curing in the swath, MID hay required 16 d, and LATE hay only required 1 d. While statistical analysis could not be conducted for yield or composition data, harvest yield numerically increased with maturity relative to the EARLY cut hay, with the MID and LATE stages yielding similarly. Starch concentration numerically increased with advancing pea maturity at the expense of CP and fibrous components ($aNDF_{OM}$ and ADF; Table 3).

Body Weight, DMI, and Eating Behavior

No effect of pea hay maturity was observed on body weight, pea hay DMI, or total DMI ($P > 0.54$; Table 4). There were no differences among treatments for time spent eating or drinking ($P > 0.095$). However, rumination time (min/d) decreased with advancing pea hay maturity ($P < 0.001$) and decreased relative to EARLY maturity when expressed as min/kg DM and min/kg $aNDF_{OM}$ ($P \leq 0.016$). Heifers fed the EARLY harvested pea hay sorted more against particles retained on the 4-mm sieve and those on the pan ($P \leq 0.024$; Table 4) than those fed the MID and LATE pea treatments.

Ruminal Fermentation, NDF Turnover, and Apparent Total Tract Digestibility

Maturity of pea hay at swathing did not affect minimum or maximum ruminal pH ($P \geq 0.074$; Table 5), but mean pH decreased from 6.59 for EARLY to 6.30 for LATE pea hay ($P = 0.005$). Total SCFA concentration was not affected by pea hay maturity, nor were the molar proportions of individual SCFA ($P \geq 0.24$) with the exception of caproate that was greater for LATE than for EARLY or MID ($P < 0.001$).

The ruminal pool sizes of DM, $aNDF_{OM}$, $uNDF_{OM}$, and potentially degradable NDF were not different ($P \geq 0.32$; Table 6) among treatments. However, the rate of passage (k_p) was slowest for the LATE maturity pea hay treatment (1.89%/h) and fastest for the MID maturity pea hay (2.24%/h, $P = 0.022$) with the EARLY maturity being intermediate but not different from the other treatments (2.10%/h). While passage rate differed, the k_d for $aNDF_{OM}$ did not differ among treatments ($P = 0.15$). Likewise, apparent total

Table 3. Nutrient composition of ingredients used in treatment diets of consisting of forage pea (*Pisum sativum* L.; c.v. CDC Horizon) harvested for hay at EARLY, MID, and LATE maturities, mineral supplement, and pellet supplement for beef cattle

Nutrient composition[1], % DM	EARLY pea[2]	MID pea	LATE pea	Mineral[3]	Pellet[4]
DM	87.28 ± 0.72	87.88 ± 1.97	87.14 ± 1.07	91.98 ± 1.29	88.11 ± 0.78
OM	94.11 ± 2.80	94.57 ± 2.68	95.12 ± 2.32	94.07 ± 4.65	96.78 ± 2.11
CP	16.30 ± 1.39	13.37 ± 5.20	13.50 ± 0.46	27.75 ± 0.81	9.80 ± 0.80
$aNDF_{OM}$	49.97 ± 1.46	50.40 ± 2.49	39.20 ± 1.87	11.53 ± 0.70	46.13 ± 2.05
ADF	42.50 ± 0.20	42.37 ± 2.12	32.23 ± 2.71	5.77 ± 0.25	26.73 ± 1.38
ESC	3.90 ± 0.35	3.47 ± 0.75	4.03 ± 0.76	3.53 ± 2.19	3.73 ± 2.19
Starch	2.00 ± 1.23	6.87 ± 5.59	15.12 ± 12.59	27.64 ± 23.14	17.92 ± 14.96
Ether extract	1.11 ± 0.70	1.07 ± 0.64	1.02 ± 0.54	5.29 ± 4.00	1.22 ± 0.71
Ca	0.83 ± 0.52	0.93 ± 0.64	0.67 ± 0.43	1.63 ± 1.21	0.19 ± 0.02
P	0.18 ± 0.14	0.12 ± 0.08	0.17 ± 0.12	0.89 ± 0.43	0.19 ± 0.06

[1]Values stated as mean ± SD based on the chemical analysis of feed ingredients from each period of the 3 × 3 Latin square design.

[2]Harvest maturities consisted of EARLY (plants with flat pods at one or more nodes), MID (filled pods at one or more nodes and leaves that were turning from green to gold), and LATE (yellow dry seeds filled pods on most nodes, and the leaves and pods were yellow).

[3]Mineral was composed of ground wheat (69.3%), porcine tallow (9.3%), molasses (8.3%), and urea (5.0%). The supplement provided 2.1% Ca, 1.1% P, 0.2% Mg, 0.6% K, 0.4% Na, 0.8% Cl, 0.2% S, 86.2 ppm Mn, 182.2 ppm Cu, 555.5 ppm Fe, 282.7 ppm Zn, 2.9 ppm I, 0.6 ppm Co, 0.6 ppm Se, 14,543.6 IU/kg Vitamin A, 1,872.5 IU/kg Vitamin D3, 424.4 IU/kg Vitamin E, 0.10% monensin, and 0.11% melengestrol acetate (MGA; Federated Co-operatives Limited; Saskatoon, SK, Canada).

[4]Pellet was composed of oat hulls (50%), wheat middlings (40%), and molasses (10%).

Table 4. Effect of harvesting pea hay (*Pisum sativum* L.; c.v. CDC Horizon) at EARLY, MID, and LATE maturities on body weight, DMI, and eating behavior for yearling beef heifers

Variable	EARLY[1]	MID	LATE	SEM	*P*-value
Body weight, kg					
Initial	346	343	343	17.2	0.66
Final	368	366	366	19.3	0.91
DMI (total diet), kg/d	8.5	8.3	9.1	0.52	0.59
DMI (pea hay only), kg/d	3.0	3.3	3.4	0.22	0.54
Behavior[1]					
Eating time, min/d	442	479	484	15.4	0.17
Eating time, min/kg DM	53.9	58.2	53.3	4.65	0.69
Eating time, min/kg $aNDF_{OM}$	127.7	130.5	148.0	11.04	0.095
Ruminating time, min/d	403[a]	366[b]	321[c]	7.7	<0.001
Ruminating time, min/kg DM	49.3[a]	44.3[ab]	36.4[b]	3.63	0.016
Ruminating time, min/kg $aNDF_{OM}$	116.6[a]	98.8[b]	97.4[b]	7.77	0.006
Drinking time, min/d	8	10	11	21.3	0.91
Sorting index[2] %					
> 19 mm	114.85	101.24	99.52	4.616	0.089
< 19, > 8 mm	101.80	100.38	98.80	5.480	0.62
< 8, > 4 mm	61.25[b]	99.8[a]	101.89[a]	9.249	0.024
< 4	28.54[b]	95.30[a]	94.27[a]	14.34	0.017

[1]Harvest maturities consisted of EARLY (plants with flat pods at one or more nodes), MID (filled pods at one or more nodes and leaves that were turning from green to gold), and LATE (yellow dry seeds filled pods on most nodes, and the leaves and pods were yellow).

[2]Sorting index was calculated as (actual consumed)/(theoretical consumed) × 100, as described by Leonardi and Armentano (2003).

[abc]Means within a row with uncommon superscripts are different ($P < 0.05$).

tract digestibility of OM, CP, NDF, $aNDF_{OM}$, ADF, starch, and ether extract were not affected by stage of pea hay maturity ($P \geq 0.051$; Table 7). However, ethanol soluble carbohydrate digestibility was greater for EARLY maturity when compared to MID and LATE ($P = 0.013$).

DISCUSSION

It is important to note that the pea hay treatment consisted of 40% of the dietary DM due to limited forage availability. In an applied beef cow production setting, forage would ideally consist of the majority of the diet and be offered ad libitum

Table 5. Effect of harvesting pea hay (*Pisum sativum* L.; c.v. CDC Horizon) at EARLY, MID, and LATE maturities on ruminal pH and SCFA concentrations in beef cattle

Variable	EARLY[1]	MID	LATE	SEM	*P*-value
pH					
Minimum	5.87	5.72	5.67	0.126	0.074
Maximum	7.06	6.99	6.87	0.062	0.11
Mean	6.59[a]	6.40[b]	6.30[c]	0.118	0.005
Total SCFA, mM	91.2	101.2	97.3	9.97	0.24
SCFA, mol/100 mol					
Acetate	63.68	63.65	62.28	1.545	0.78
Propionate	19.88	21.32	21.67	2.721	0.87
Isobutyrate	0.88	0.85	0.77	0.093	0.73
Butyrate	11.85	11.08	11.35	1.353	0.89
Isovalerate	1.77	1.85	1.45	0.236	0.42
Valerate	1.50	1.30	1.70	0.690	0.53
Caproate	0.23[b]	0.15[b]	0.27[a]	0.127	<0.001

[1]Harvest maturities consisted of EARLY (plants with flat pods at one or more nodes), MID (filled pods at one or more nodes and leaves that were turning from green to gold), and LATE (yellow dry seeds filled pods on most nodes, and the leaves and pods were yellow).

[abc]Means within a row with uncommon superscripts are different ($P < 0.05$).

Table 6. Effect of harvesting pea hay (*Pisum sativum* L.; c.v. CDC Horizon) at EARLY, MID, and LATE maturities on ruminal pool sizes of DM, $aNDF_{OM}$, and $uNDF_{OM}$, and the passage and reticulo-ruminal degradation rates of $aNDF_{OM}$ for yearling beef heifers

Variable	EARLY[1]	MID	LATE	SEM	*P*-value
Reticulo-ruminal pool, kg					
Total	49.1	47.5	47.9	2.06	0.78
DM	9.2	10.2	9.0	3.29	0.55
$aNDF_{OM}$	6.4	7.0	6.1	2.20	0.57
$uNDF_{OM}$	4.8	5.0	4.4	1.55	0.61
Potentially degradable $aNDF_{OM}$[2]	1.7	2.0	1.8	0.66	0.32
$aNDF_{OM}$ k_p, %/h	2.10[ab]	2.24[a]	1.89[b]	0.459	0.022
$aNDF_{OM}$ k_d, %/h	2.55	1.83	2.10	0.514	0.15

[1]Harvest maturities consisted of EARLY (plants with flat pods at one or more nodes), MID (filled pods at one or more nodes and leaves that were turning from green to gold), and LATE (yellow dry seeds filled pods on most nodes, and the leaves and pods were yellow).

[2]Potentially degradable NDF was calculated as ($aNDF_{OM} - uNDF_{OM}$).

[ab]Means within a row with uncommon superscripts are different ($P < 0.05$).

Table 7. Effect of harvesting pea hay (*Pisum sativum* L.; c.v. CDC Horizon) at EARLY, MID, and LATE maturities on apparent total tract digestibility when fed to beef heifers

Digestibility, %	EARLY[1]	MID	LATE	SEM	*P*-value
OM	49.56	46.74	52.53	1.706	0.051
CP	63.50	60.06	62.72	4.213	0.58
$aNDF_{OM}$	15.45	18.58	14.89	2.237	0.41
ADF	21.19	19.98	17.14	3.185	0.63
Starch	96.10	92.23	93.08	1.286	0.071
Ether extract	71.09	60.70	66.01	7.460	0.36

[1]Harvest maturities consisted of EARLY (plants with flat pods at one or more nodes), MID (filled pods at one or more nodes and leaves that were turning from green to gold), and LATE (yellow dry seeds filled pods on most nodes, and the leaves and pods were yellow).

with minimal supplementation to meet vitamin and mineral requirements (McCartney et al., 2004). However, Beck et al. (2009) were able to detect differences in DM and NDF total tract digestibility between wheat forage harvested at the boot and hard dough stages with only 40% dietary DM derived from forage. Rosser et al. (2016) utilized a similar model and was able to detect differences in DMI, ruminal pH and SCFA concentrations, and apparent total tract digestibility when oat and barley

were harvested at differing stages of maturity and fed as hay to beef heifers. Moreover, the supplemented pellet in the present study was comprised of mostly oat hulls, thereby mitigating negative associative effects that could be observed should a grain-based pellet have been used. Therefore, while our experimental model included a high inclusion of supplemental pellet, it was presumed that the supplement level would not mask treatment differences in response to pea hay maturity.

When grown as a forage, pea is often included in a mixture with other crops such as oat or barley (Aasen et al., 2004; Bastida Garcia et al., 2011; Uzun and Asik, 2012). When grown with other crops, harvest maturity is based upon the maturity of the cereal crop rather than for pea. This practice ignores potential effects of pea maturity on yield, palatability, or animal performance. When grown as a monoculture, the recommendation is to harvest pea for hay when the bottom pods are filling. While we did not have replicated field plots and could not compare yields statistically, we observed a numerical increase in yield with advancing maturity supporting the work of Rosser et al. (2013) that evaluated harvest maturity in cereal crop yield. Moreover, in our study, starch and CP concentration numerically increased which in turn decreased the concentrations of NDF and ADF with advancing stages of maturity. Similar nutrient composition changes for cereals resulted in a greater yield of potentially digestible nutrients (Rosser et al., 2013). Greater yield and starch concentrations are in agreement with previous work showing that delaying the stage of maturity at harvest for cereal hay can increase forage yields (Rosser et al., 2013) without affecting consumption or digestibility (Rosser et al., 2016).

Starch digestibility tended to decrease with advancing maturity, but remained above 90% for all stages of maturity. It is important to note, that the bulk of the dietary starch was delivered by the supplement (17.92% starch) and mineral pellets (27.64% starch) with pea hay contributing more dietary starch as the maturity advanced. In fact, the EARLY, MID, and LATE pea hay contained 2.00, 6.87, and 15.12% starch with dietary starch concentrations of 13.15, 15.10, and 18.40%, respectively. Thus, the tendency for decreasing starch digestibility with advancing pea maturity suggests that the whole-pea within the hay may have been the causative factor. That said, pea starch is highly digestible even without processing (Anderson et al., 2007). Thus, while a tendency for a reduction in starch digestibility was observed, data including a reduction in ruminal pH suggests that the starch was available in the rumen. However, the reduced pH may also result from a combination of increased starch intake, reduced rumination time, and less selective avoidance of small (passed through a 4-mm sieve) and medium size (passed through a 8-mm sieve) particles by heifers when fed pea hay with more advanced maturity. Rumination is thought to stimulate ruminal buffering due to increased saliva production rates (Allen, 1997) and greater ruminal mixing allowing SCFA to contact the ruminal epithelium for absorption (Storm and Kristensen, 2010). It is important to note that while advancing maturity of pea hay may contribute to a decreased mean ruminal pH, the mean and minimum pH were well within normal ranges and the reduction does not imply greater risk for ruminal acidosis.

It is generally considered that forage quality and digestibility decrease with advancing maturity (NASEM, 2016). However, for annual cereal grains, the starch concentration increases with advancing maturity offsetting and causing a decrease in the NDF concentration (Rosser et al., 2013, 2016). While NDF digestibility generally decreases with advancing maturity (Beck et al., 2009; Rosser et al., 2013), we did not observe differences in apparent total tract NDF digestibility or the ruminal NDF turnover rate with advancing maturity. However, in our study, NDF digestibility was quite low (ranging from 14.9% to 18.6%) and it is possible that the use of oat hulls (Thompson et al., 2000) as a major component of the supplement pellet may have masked treatment differences for NDF digestibility. That said, previous studies (Baron et al., 1992; Rosser et al., 2013, 2016) and the present study have reported that OM and DM digestibility are not reduced with advancing maturity suggesting that the reduction in NDF digestibility is offset by the reduction in NDF concentration and the increased starch concentration.

The reduction in rumination time with advancing pea hay maturity may be a result of increased starch concentration and decreased NDF concentration observed with increasing maturity. However, ruminating time in min/kg NDF also decreased with advancing maturity, suggesting that the physical effectiveness of NDF decreased (Mertens, 1997). Decreased physically effective NDF, along with the decreased ruminal pH, can reduce ruminal motility (Allen, 1997) thereby affecting particulate passage out of the rumen and Okine et al. (1989) reported that k_p can be altered without differences in digestibility (Okine et al., 1989). Indeed, we observed a reduction in rumination time and reduced NDF k_p

with advancing pea maturity, without differences in nutrient digestibility. The reduction in peNDF with advancing maturity may be partially explained through sorting of the diet given that cattle fed EARLY pea hay selectively avoided short particles and cattle fed LATE pea hay tended to selectively avoid long particles. However, it is not clear why k_p for NDF decreased as the increased consumption of small and medium particles for the EARLY and reduction in consumption of large particles for the LATE treatments would be expected to increase k_p. It is plausible that the selective eating behavior may have altered rumen motility thereby decreasing both rumination time and k_p. However, further research is needed to confirm this speculation.

As indicated above, cattle fed EARLY pea hay selectively avoided small and medium size particles and cattle fed LATE pea hay selectively avoided long particles. These data support previous work reporting similar sorting trends based on the stage of maturity for cereal hay (Rosser et al., 2016, 2017) where harvesting at a less mature stage increased selective avoidance against fine particles. It is important to note that the selection index against small particles may be, in part, due to increased fragility for EARLY pea hay vs. MID and LATE. Particle fragility can result in the production of fine particles from longer particles and would appear as selective consumption of the larger particles and selective avoidance of the smaller particles. However, we did not specifically evaluate forage fragility, so whether differences in fragility with advancing maturity occurs remains speculative.

CONCLUSION

Overall, the stage of pea maturity at harvest when used for hay does not affect DMI for beef cattle but decreases rumination time, the rate of ruminal NDF passage, mean ruminal pH, and may reduce total tract starch digestibility without affecting the digestibility of other nutrients. These results suggest harvesting for field pea can be delayed to reduce curing time and maximize yield.

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LITERATURE CITED

Aasen, A., V. Baron, G. W. Clayton, D. A. Campbell, and D. H. McCartney. 2004. Swath grazing potential of spring cereals, field pea and mixtures with other species. Can. J. Plant Sci. 84:1051–1058. doi:10.4141/p03-143

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447–1462. doi:10.3168/jds.s0022-0302(97)76074-0

Anderson, V. L., G. P. Lardy, and B. R. Ilse. 2007. Review: field pea grain for beef cattle. Prof. Anim. Sci. 23:1–7. doi:10.1532/s1080-7446(15)30931-1

AOAC. 2000. Official methods of analysis of AOAC International. 17th ed. Gaithersburgm, MD: Association of Official Analytical Chemists.

AOAC. 2006. Official Methods of Analysis. 18th ed. Gaithersburgs, MD: Association of Official Analytical Chemists.

Baron, V. S., A. C. Dick, and M. S. Wolynetz. 1992. Characterization of barley silage-maturity relationships for central Alberta. J. Plant Sci. 72:1009–1020. doi:10.4141/cjps92-127

Bastida Garcia, J. L., M. González-Ronquillo, I. A. Domínguez Vara, J. Romero-Bernal, and O. Castelán Ortega. 2011. Effect of field pea (*Pisum sativum* L.) level on intake, digestion, ruminal fermentation and in vitro gas production in sheep fed maintenance diets. J. Anim. Sci. 82:654–662. doi:10.1111/j.1740-0929.2011.00884.x

Beck, P. A., C. B. Stewart, H. C. Gray, J. L. Smith, and S. A. Gunter. 2009. Effect of wheat forage maturity and preservation method on forage chemical composition and performance of growing calves fed mixed diets. J. Anim. Sci. 87:4133–4142. doi:10.2527/jas.2009-2184

Berkenkamp, B., and J. Meeres. 1987. Mixtures of annual crops for forage in central Alberta. Can. J. Plant Sci. 67:175–183. doi:10.4141/cjps87-021

Borreani, G., P. G. Peiretti, and E. Tabacco. 2007. Effect of harvest time on yield and pre-harvest quality of semi-leafless grain peas (*Pisum sativum* L.) as whole-crop forage. F. Crop. Res. 100:1–9. doi:10.1016/j.fcr.2006.04.007

Brundage, A. L., R. L. Taylor, and V. L. Burton. 1979. Relative yields and nutritive values of barley, oats, and peas harvested at four successive dates for forage. J. Dairy Sci. 62:740–745. doi:10.3168/jds.s0022-0302(79)83317-2

Carr, P. M., R. D. Horsley, and W. W. Poland. 2004. Barley, oat, and cereal-pea mixtures as dryland forages in the Northern Great Plains. Agron. J. 677–684. doi:10.2134/agronj2004.0677

Dado, R. G., and M. S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J. Dairy Sci. 78:118–133. doi:10.3168/jds.S0022-0302(95)76622-X

Daniel, T. W., F. B. Wolberg, V. L. Miller, J. H. Alswager, M. E. Ensminger, and A. A. Spielman. 1946. Chemical

composition and digestibility of flat pea forage in three stages of maturity. J. Anim. Sci. 6:559–565.

DuBois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chem. 28:350–356. doi:10.1021/ac60111a017

Hall, M. B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92:42–49.

Karnati, S. K., J. T. Sylvester, S. M. Noftsger, Z. Yu, N. R. St-Pierre, and J. L. Firkins. 2007. Assessment of ruminal bacterial populations and protozoal generation time in cows fed different methionine sources. J. Dairy Sci. 90:798–809. doi:10.3168/jds.S0022-0302(07)71564-3

Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 1996. Forage source alters nutrient supply to the intestine without influencing milk yield. J. Dairy Sci. 79:862–872. doi:10.3168/jds.S0022-0302(96)76435-4

Knott, C. M. 1987. A key for stages of development of pea. Ann. Appl. Biol. 116:391–404. doi:10.1111/j.1744–7348.1990.tb06621.x

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557–564. doi:10.3168/jds.s0022-0302(03)73634-0

McCartney, D., J. A. Basarab, E. K. Okine, V. S. Baron, and A. J. Depalme. 2004. Alternative fall and winter feeding systems for spring calving beef cows. Can. J. Anim. Sci. 84:511–522. doi:10.4141/a03-069

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463–1481. doi:10.3168/jds.S0022-0302(97)76075-2

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient requirements of beef cattle, 8th rev. edn. Washington (DC): The National Academies Press. doi:10.17226/19014

Okine, E. K., G. W. Mathison, and R. T. Hardin. 1989. Effects of changes in frequency of reticular contractions on fluid and particulate passage rates in cattle. J. Anim. Sci. 67:3388–3396. doi:10.2527/jas1989.67123388x

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a standalone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132–2140. doi:10.3168/jds.S0022-0302(06)72284-6

Pikul, J. L., J. K. Aase, and V. L. Cochran. 2004. Water use and biomass production of oat-pea hay and lentil in a semiarid climate. Agron. J. 96:298–304. doi:10.2134/agronj2004.0298

Rosser, C. L., A. D. Beattie, H. C. Block, J. J. McKinnon, H. A. Lardner, P. Górka, and G. B. Penner. 2016. Effect of maturity at harvest for whole-crop barley and oat on dry matter intake, sorting, and digestibility when fed to beef cattle. J. Anim. Sci. 94:697–708. doi:10.2527/jas.2015-0063

Rosser, C. L., P. Górka, A. D. Beattie, H. C. Block, J. J. McKinnon, H. A. Lardner, and G. B. Penner. 2013. Effect of maturity at harvest on yield, chemical composition, and in situ degradability for annual cereals used for swath grazing. J. Anim. Sci. 91:3815–3826. doi:10.2527/jas.2012-5677

Rosser, C. L., A. D Beattie, H. C. Block, J. J. McKinnon, H. A. Lardner, P. Górka, and G. B. Penner. 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. Prof. Anim. Sci. 33:85–91. doi:10.15232/pas.2016-01556

Ruuska, S., S. Kajava, M. Mughal, N. Zehner, and J. Mononen. 2016. Validation of a pressure sensor-based system for measuring eating, rumination and drinking behavior of dairy cattle. Appl. Anim. Behav. Sci. 174:19–23. doi:10.1016/j.applanim.2015.11.005

Shreve, B., N. Thiex, and M. Wolf. 2006. National Forage Testing Association Reference Method: Dry Matter by Oven Drying for 3 Hours at 105o C. Omaha, NB: NFTA Reference Methods. National Forage Testing Association.

Storm, A. C., and N. B. Kristensen. 2010. A model of ruminal volatile fatty acid absorption kinetics and rumen epithelial blood flow in lactating Holstein cows. J. Dairy Sci. 95:2919–2934. doi:10.3168/jds.2011–4239

Strydhorst, S. M., J. R. King, K. J. Lopetinsky, and K. N. Harker. 2008. Forage potential of intercropping barley with faba bean, lupin, or field pea. Agron. J. 100:182–190. doi:10.2134/agronj2007.0197

Thompson, R. K., A. F. Mustafa, J. J. McKinnon, D. Maenz, and B. Rossnagel. 2000. Genotypic differences in chemical composition and ruminal degradability of oat hulls. Can. J. Anim. Sci. 377–379. doi:10.4141/cjps92-127

Uzun, A., and F. F. Asik. 2012. The effect of mixture rates and cutting stages on some yield and quality characters of pea (*Pisum sativum* L.)+oat (*Avena sativa* L.) mixture. Turkish J. F. Crop. 17:62–66.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Warkentin, T., J. Kostuik, E. Klassen, B. Barlow, S. Ife, B. Tar'an, and A. Vandenberg. 2012. CDC Horizon forage pea. Can. J. Plant Sci. 92:207–209. doi:10.4141/cjps2011-081

Zehner, N., C. Umstätter, J. J. Niederhauser, and M. Schick. 2012. System specification and validation of a noseband pressure sensor for measurement of ruminating and eating behavior in stable-fed cows. Comp. Electronics Agric. 136:31–41. doi:10.1016/j.compag.2017.02.021 0168-1699