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Modeling nutrient availability of alfalfa hay harvested at three stages of maturity and in the afternoon and morning in dairy cows

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ABSTRACT

Nutritive value of alfalfa hay is influenced by stage of maturity and cutting time which affects the potential nutrient supply in dairy cows. The objective of this study was to investigate the effect of stage of maturity and cutting time of alfalfa hay grown under semi-arid climate condition on *in situ* ruminal degradation characteristics and predicted protein availability in dairy cows using two protein models based on different principles (DVE/OEB 1994 and NRC, 2001). Alfalfa was cut at early bud (June 15/16), late bud (June 26/27) and early flower (July 18/19) in the afternoon (18:00 h) and the following morning (06:00 h). Alfalfa hay at early bud and late bud contained higher *in situ* effective degradable nitrogen (ED_N) to effective degradable energy (ED organic matter (ED_{OM}) and ED carbohydrates (ED_{CHO})) ratios compared with alfalfa at early flower ($P<0.05$) and highest ED_N to ED_{OM} and ED_{CHO} ratios were reached in the first hours of ruminal incubation for all alfalfa hays. Rumen degraded protein balance decreased with advancing maturity ($P<0.05$). There was a trend towards reduction in metabolizable protein (NRC model; 78, 72 and 66 g/kg dry matter (DM), P=0.06) and truly absorbed protein (DVE value; 57, 51 and 39 g/kg DM, P=0.08) with advancing maturity. Cutting alfalfa in the afternoon increased ED_{CHO} (450 *vs.* 431 g/kg CHO; P=0.08), ruminal microbial protein synthesis based on total digestible nutrients (NRC model; MCP_E^{NRC}; 62 *vs.* 59 g/kg DM; P=0.03) and intestinally absorbable MCP_E^{NRC} (40 *vs.* 38 g/kg DM; P=0.03) compared with cutting alfalfa in the morning. In conclusion, cutting alfalfa hay at early bud stage tended to have the highest metabolizable protein content, but also the highest imbalance between rumen available protein and energy and cutting alfalfa in the afternoon increased potential ruminal microbial protein synthesis in dairy cows.

Abbreviations: aNDF, neutral detergent fiber; AMCP, truly absorbable microbial crude protein; ARUP, truly absorbable rumen undegraded protein; CT, cutting time; CP, crude protein; CHO, total carbohydrate; D, potentially degradable fraction; DM, dry matter; DVE, truly digested and absorbed protein in small intestine; ED, effective degradability; ECP, endogenous protein; ENDP, endogenous protein losses from the digestive tract; FOM, fermentable organic matter; MCP_E^{NRC}, microbial protein synthesis based on energy available; MCP_{FOM}, microbial crude protein synthesized in the rumen from FOM; MCP_{RDP}^{DVE}, synthesized MCP in the rumen from RDP; MP_{NRC}, metabolizable protein; Kd, fractional rate of degradation; NFC, non fiber carbohydrate; OM, organic matter; OEB and DPB_{NRC}, ruminal degraded protein balance; RDP, rumen degradable protein; RUP, rumen undegradable protein; TPSI, true protein supplied to the small intestine; UDM, completely undegradable DM; SM, stage of maturity; U, undegradable fraction; W, washable fraction.

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1. Introduction

Cultivated alfalfa (*Medicago sativa L.*) is the main forage ingredient for dairy rations in Iran (Kowsar et al., 2008; Yari et al., 2012) with 5.7 million metric tonnes of hay harvested during season 2009–2010 (Iranian Ministry of Agriculture, 2009–2010). Forage alone provides insufficient nutrients for the animal to achieve high milk yields and should therefore be supplemented with concentrated feed ingredients (Oba and Allen, 2005). However, cutting alfalfa hay at the optimum growth stage and time can minimize required supplement inclusion. Nutritive value of alfalfa hay is influenced by cultivar, stage of maturity (SM) (Elizalde et al., 1999; Yu et al., 2003a,b; Coblentz et al., 2008; Yari et al., 2012), climate condition (Van Soest, 1994) and cutting time (CT) (Burns et al., 2007; Brito et al., 2008, 2009; Yari et al., 2012). Nutritive value of feeds can be assessed by predicting nutrient supply of a feed to both the rumen and intestine using sophisticated protein evaluation models (ARC, 1984; Madsen, 1985; NRC, 1985,2001; Tamminga et al., 1994, 2007). Input values for these models are generated by chemical analysis and the *in situ* technique, but differences exist between principles of models (Yu et al., 2003a). Some of these models predict ruminal microbial protein synthesis (MCP) based on rumen fermentable OM (FOM) and consider endogenous protein losses (Tamminga et al., 1994, 2007), while other models predict MCP based on total digestible nutrients and consider absorption of endogenous protein (NRC, 1985,2001).

To date there is no information available on the effect of SM and CT on nutrient supply of alfalfa hay grown under semi-arid condition in dairy cows. The objectives of the current study were to investigate the effect of SM (early and late bud and early flower stage) and CT (afternoon *versus* morning) of alfalfa hay grown under semi-arid condition on *in situ* rumen degradation kinetics and predicted protein supply in dairy cows with two models with different basic principles (Tamminga et al., 1994; NRC, 2001).

2. Materials and methods

2.1. Alfalfa plots management

A second year alfalfa field (20 m × 24 m) seeded with cv. Ranger at the Research Farm of Ferdowsi University of Mashhad (Mashhad, Iran; 36 17′52.8″N, 59 36′20.52″E) was used in this study. The whole field was harvested before the experiment at April 6, 2010 and irrigated every 10 days during experiment. Alfalfa was cut at early bud (June 15/16), late bud (June 26/27) and early flower (July 18/19) both in the afternoon (18:00 h) and the following morning (06:00 h).

Six plots (4 m × 4 m each) within 5 replicate blocks within the field were randomly assigned to 6 treatments in a factorial arrangement (3 SM × 2 CT). The SM was determined according to Kalu and Fick (1981) as described by Yari et al. (2012). At each harvest, an area of 3 m × 3 m was manually clipped using a small scythe at *ca.* 5 cm above the soil surface.

Fresh alfalfa harvested from each plot was air dried in the shade for 10 to 15 days. After air drying, alfalfa hay samples were chopped using a hay chopper with 20 mm screen (Agri-Equip, Nasr Co., Isfahan, Iran). The hay from the first, second and third blocks were pooled to one sample and hay from the fourth and fifth blocks were pooled to another sample to generate sufficient material for chemical analysis and *in situ* incubations.

2.2. Rumen incubation procedure

For *in situ* incubations, three rumen fistulated non-pregnant dry Holstein Frisian cows were used which had been reviewed and approved by the Animal Care Committee of the University of Saskatchewan (Animal use protocol # 19910012). Cows were individually housed in pens at the experimental farm of the University of Saskatchewan (Saskatoon, SK, Canada) and were cared for according the Canadian Council on Animal Care guidelines (1993). The cows had free access to water and were fed 15 kg dry matter (DM) per day of a total mixed ration twice daily in equal portions at 08:00 h and 16:00 h. The total mixed ration consisted in g/kg DM of 550 g barley silage, 125 g alfalfa hay, 50 g dehydrated alfalfa and 275 g concentrate as described in more detail by Yu et al. (2009).

Prior to the *in situ* incubations, chopped alfalfa samples were ground to pass through a 2 mm screen using a cyclonic mill (Retsch SM-3000, Brinkmann Instruments, ON, Canada). *In situ* ruminal degradation kinetics were determined as described by Yu et al. (2004), using number-coded nylon bags (10 cm × 20 cm, pore size 40 μm, Nitex 03-41/31 monofilament open mesh fabric, Screen Tech, Mississagua, ON, Canada). Approximately 7 g of alfalfa hay samples was placed into each bag, resulting in a sample-to-bag surface ratio of ~17.5 mg/cm^2. Filled bags were randomly assigned to the three cows and incubated in the rumen for 72, 36, 12, 8 and 4 h (4, 4, 3, 2 and 2 bag per alfalfa hay sample respectively) by the "all out method". Immediately after retrieval from the rumen, all bags were placed in a bucket with cold tap water and then washed ten times manually followed by oven drying at 55 °C for 48 h. Two bags of zero h alfalfa hay samples were washed in the same way. Rumen incubations were carried out in one run. The two-pooled blocks were used as replicates. Incubation residues from the treatment bags were combined within time per block.

2.3. Degradation characteristics

The rumen degradation characteristics were calculated for organic matter (OM), crude protein (CP), neutral detergent fiber (aNDF), non fiber carbohydrate (NFC) and total carbohydrate (CHO). Three fractions were determined for each component: a

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rapidly degradable washable fraction (W) which consists of material that escapes from the bag after manually washing in cold tap water; a truly undegradable fraction (U) which was determined as the asymptote of the degradation curve at infinite incubation time; and a potentially degradable fraction (D) calculated as $100 - W - U$. The first order kinetic degradation model including a lag time, $R(t) = U + D \times e^{-K_d \times (t - \text{lag})}$, was used to calculate the fractional rate of degradation (K_d) and lag time of D fraction and U fraction with $R(t)$ is residue of the amount of incubated material after t h of rumen incubation (Robinson et al., 1986). The first order kinetics model parameters were calculated using the NLIN (nonlinear) procedure of SAS (2003) using iterative least-squares regression (Gauss–Newton method). In the original sample and incubation residues, NFC was calculated as NFC (g/kg DM) = 1000 − (CP + ash + aNDF + EE) and CHO was calculated as CHO (g/kg DM) = 1000 − (CP + ash + EE) (NRC, 2001). A correction factor for disappearance of ether extract (EE) at different incubation times was used as described by Tamminga et al. (2007).

The effective degradability (ED) of CP, OM, aNDF, NFC and CHO were calculated as $ED = W + (D \times K_d)/(K_d + K_p)$ (Ørskov and McDonald, 1979), assuming a passage rate of 0.045/h (Tamminga et al., 1994). The hourly effective degradability was calculated as $ED = W[(D \times K_d)/(K_d + K_p)] \times [1 - e^{-t(K_d + K_p)}]$ (Sinclair et al., 1993). The difference in cumulative amounts degraded at successive hours was regarded as the amount degraded each hour.

2.4. Modeling nutrient supply with the DVE/OEB 1994 system and NRC 2001 model

Protein supply in dairy cows was predicted with the DVE/OEB system (Tamminga et al., 1994) and NRC model (2001). Detailed equations and comparison between these two models are described by Yu et al. (2003a). Equations in the DVE/OEB system are used to calculate rumen degraded protein balance (OEB) and true intestinal absorbable protein (DVE). The DVE value comprises of intestinal absorbable rumen undegradable feed protein (ARUP), absorbable microbial protein (AMCP), and endogenous protein losses into the feces (ENDP). The OEB value was calculated based on the difference between MCP based on rumen degradable feed protein (MCP_{RDP}) and MCP based on FOM (MCP_{FOM}). The MCP_{RDP} was calculated as $MCP_{RDP} = CP \times [1 - (1.11 \times RUP(CP)/100)]$ and MCP_{FOM} was calculated as $MCP_{FOM} = FOM \times 0.15$ (Tamminga et al., 1994).

Metabolizable protein (MP; equivalent to DVE) in the NRC model (2001) comprises of ARUP, AMCP and absorbable endogenous protein (AECP). The MCP in this model is calculated based on total digestible nutrients (NRC, 2001). Total digestible nutrients was calculated based on a combination of *in situ* NDF degradability at 36 h of incubation and summative equations from NRC (2001) as described by Yari et al. (2012).

2.5. Statistical analysis

Data was analyzed using proc mixed of SAS 9.2 (2003) with the following statistical model:

$$Y_{ijk} = \mu + CT_i + SM_j + CT_i \times SM_j + B_k + e_{ijk}$$

where Y_{ijk} is the observation of the dependent variable ijk; μ is the fixed effect of population mean for the variable; CT_i is the fixed effect of cutting time ($i = 2$; 18:00 and 06:00 h); SM_j is the fixed effect of stage of maturity ($j = 3$; early bud, late bud and early flower); $CT_i \times SM_j$ is the fixed effect of interaction between factor CT at level i and the factor SM at level j; B_k is the random effect of block ($k = 2$) and e_{ijk} is the random error associated with the observation ijk. Experimental replicates were block and CT for SM ($n = 4$) and block and SM for CT ($n = 6$).

The effect of $CT_i \times SM_j$ was not significant and was therefore excluded from the model. The Fisher's protected least significant difference test was used for multiple treatment comparisons using the LSMEAN statement of SAS 9.2 (SAS, 2003) with letter groupings obtained using the SAS pdmix800 macro (Saxton, 1998). For the different statistical tests, significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$, unless otherwise stated.

3. Results

3.1. Degradation kinetics

The detailed results and discussion on the effect of SM and CT on botanical traits, chemical composition, CP and CHO factions and energy content of current samples are published in Yari et al. (2012). Alfalfa at early and late bud had lower U_{CP} and higher ED_{CP} than alfalfa at early flower ($P<0.05$; Table 1). The ED_{OM} of alfalfa at early bud was higher than at early flower ($P<0.05$), with late bud intermediate and U_{OM} tended to increase with advancing maturity ($P=0.08$; Table 1). The ED_{aNDF} of alfalfa at early bud was higher than at late bud and early flower ($P<0.05$; Table 2). Alfalfa cut in the afternoon had higher Kd_{aNDF} ($P=0.03$) and tended to have higher ED_{CHO} ($P=0.08$) than alfalfa cut in the morning (Table 2). Other *in situ* CP, OM, aNDF, NFC and CHO degradation characteristics were similar among SM and between CT (Tables 1 and 2).

3.2. Ruminal protein to energy ratios

The ED nitrogen (ED_N):ED_{OM} (ED_N:ED_{OM}) and ED_N:ED_{CHO} ratios were higher in alfalfa at early and late bud than at early flower ($P<0.05$; Tables 1 and 2). Alfalfa at late bud and early flower had higher U_N:U_{OM} and U_N:U_{CHO} ratios

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Table 1
In situ degradation kinetics of crude protein (CP) and organic matter (OM) of alfalfa hay cut at three stages of maturity and in the afternoon and next morning.

Items[e]	Stage of maturity (SM)			SED	Cutting time (CT)		SED	Level of significance[d]	
	Early bud	Late bud	Early flower		06:00 h	18:00 h		SM	CT
DM (g/kg)	936[a]	937[a]	932[b]	1.1	935	934	1.6	0.02	0.90
CP (g/kg DM)	220[a]	195[b]	162[c]	4.8	192	192	4.0	<0.01	0.96
In situ CP fractions and degradation (g/kg CP)									
W_{CP}	176	219	170	38.8	188	189	31.6	0.43	0.98
D_{CP}	575	524	501	36.8	536	531	30.0	0.18	0.87
U_{CP}	248[b]	257[b]	329[a]	25.2	276	280	20.0	0.03	0.83
Kd_{CP} (/h)	0.14	0.12	0.12	0.011	0.12	0.13	0.020	0.37	0.75
lag (h)	0.0	0.0	0.3	0.20	0.0	0.2	0.20	0.41	0.35
ED_{CP}	613[a]	596[a]	529[b]	19.0	581	578	17.5	<0.01	0.80
OM (g/kg DM)	893[b]	898[b]	919[a]	2.6	901	905	2.1	<0.01	0.18
In situ OM fractions and degradation (g/kg OM)									
W_{OM}	130	140	129	17.0	129	137	13.8	0.76	0.60
D_{OM}	522	497	473	25.6	501	494	20.9	0.22	0.73
U_{OM}	348	363	398	19.1	370	369	15.6	0.08	0.99
Kd_{OM} (/h)	0.09	0.08	0.08	0.010	0.08	0.08	0.010	0.36	0.33
lag (h)	0.0	0.0	0.6	0.40	0.1	0.3	0.31	0.28	0.43
ED_{OM}	475[a]	452[ab]	425[b]	13.3	444	457	11.2	0.02	0.26
Ruminal available and un-available nitrogen (N) to ruminal available and un-available OM ratios (g/kg)									
W_N:W_{OM}	61	58	42	5.4	55	52	6.4	0.06	0.60
ED_N:ED_{OM}	56[a]	51[a]	40[b]	1.3	42	42	1.5	<0.01	0.29
U_N:U_{OM}	31[a]	27[b]	26[b]	0.7	28	28	0.8	<0.01	0.80

[d] There was no interaction between SM and CT; SED, standard error of difference; Means with different letters (a, b and c for SM) within the same row differ ($P<0.05$).

[e] DM, dry matter content in alfalfa samples in storage; *W*, washable fraction; *D*, potentially degradable fraction; *U*, undegradable fraction; K_d, fractional degradation rate; ED, effective degradability; $R(t) = U + D \times e^{-K_d \times (t-\text{lag})}$ was used to calculate the K_d in/h and lag time of *D* fraction where $R(t)$ = residue of the amount of incubated material after *t* h of rumen incubation (Robinson et al., 1986); $ED = W + (D \times K_d)/(K_d + K_p)$, as described by Ørskov and McDonald (1979), assuming a passage rate of 0.045/h (Tamminga et al., 1994).

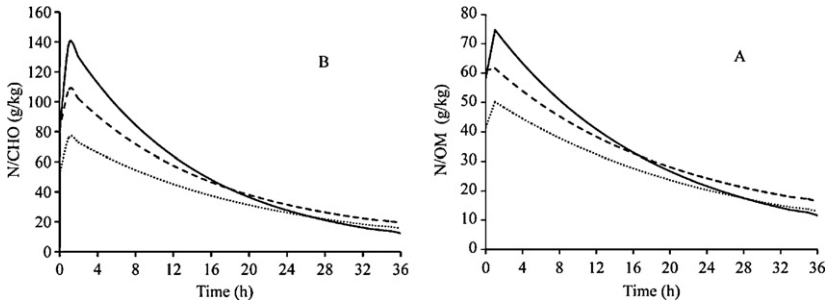

Fig. 1. The mean hourly ratio between rumen effectively degraded nitrogen (ED_N) and organic matter (ED_{OM}, A) and between ED_N and ED carbohydrate (ED_{CHO}, B) of alfalfa hay cut off at early bud (—), late bud (- - -) and early flower (· · ·) stage.

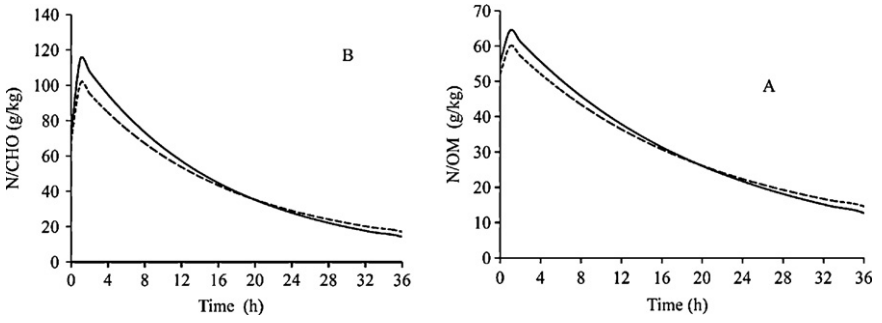

Fig. 2. The mean hourly ratio between rumen effectively degraded nitrogen (ED_N) and organic matter (ED_{OM}, A) and between ED_N and ED carbohydrate (ED_{CHO}, B) of alfalfa hay cut off in the morning (—) and afternoon (- - -).

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Table 2
In situ degradation kinetics of neutral detergent fiber (aNDF), non fiber carbohydrate (NFC) and total carbohydrate (CHO) of alfalfa hay cut at three stages of maturity and in the afternoon and next morning.

Items[e]	Stage of maturity (SM)			SED	Cutting time (CT)		SED	Level of significance[d]	
	Early bud	Late bud	Early flower		06:00 h	18:00 h		SM	CT
EE (g/kg DM)	24	25	23	1.0	24	24	0.8	0.65	0.86
aNDF (g/kg DM)	425[b]	444[b]	491[a]	14.0	456	449	11.4	0.03	0.67
In situ aNDF fractions and degradation (g/kg aNDF)									
D_{aNDF}	478	475	419	27.0	472	443	22.0	0.11	0.23
U_{aNDF}	522	525	581	27.0	528	557	22.0	0.11	0.23
Kd_{aNDF} (/h)	0.04	0.03	0.05	0.007	0.03[y]	0.05[x]	0.004	0.10	0.03
lag_{aNDF} (h)	2.0	3.0	3.0	1.01	2.0	3.0	1.01	0.89	0.79
ED_{aNDF}	260[a]	226[b]	222[b]	11.0	232	241	9.0	0.02	0.34
NFC (g/kg DM)	226	235	243	8.6	229	240	7.0	0.37	0.28
In situ NFC fractions and degradation (g/kg NFC)									
W_{NFC}	299	320	375	46.0	314	348	38.0	0.30	0.40
D_{NFC}	670	648	595	42.0	662	613	34.0	0.26	0.20
U_{NFC}	32	32	30	13.0	24	39	11.0	0.98	0.21
Kd_{NFC} (/h)	0.15	0.14	0.16	0.021	0.14	0.15	0.020	0.65	0.32
lag_{NFC} (h)	0.5	0.2	2.0	0.80	0.7	0.8	0.71	0.30	0.96
ED_{NFC}	808	806	832	18.0	810	820	15.0	0.50	0.25
CHO (g/kg DM)	650[c]	679[b]	734[a]	7.0	685	689	5.7	<0.01	0.60
In situ CHO fractions and degradation (g/kg CHO)									
W_{CHO}	164	170	176	11.0	166	175	9.0	0.58	0.37
D_{CHO}	471	457	430	22.0	457	448	18.0	0.26	0.64
U_{CHO}	366	373	393	17.0	378	377	14.0	0.31	0.99
Kd_{CHO} (/h)	0.07	0.06	0.07	0.007	0.06	0.07	0.006	0.49	0.14
lag_{CHO} (h)	0.2	0.0	1.0	0.51	0.2	0.5	0.40	0.22	0.48
ED_{CHO}	452	435	435	11.0	431	450	9.0	0.29	0.08
Ruminal available and un-available nitrogen (N) to ruminal available and un-available carbohydrate (CHO) ratio (g/kg)									
W_N:W_{CHO}	64	67	38	10.0	58	54	9.0	0.06	0.69
ED_N:ED_{CHO}	80[a]	71[a]	49[b]	4.0	69	65	3.0	<0.01	0.27
U_N:U_{CHO}	40[a]	36[b]	33[b]	1.0	36	36	1.0	<0.01	0.90

[d] There was no interaction between SM and CT; SED, standard error of difference; Means with different letters (a, b and c for SM) and (x and y for CT) within the same row differ (P<0.05).
[e] See footnote of Table 1. EE, ether extract; NFC, non fiber carbohydrate calculated as NFC = 1000 – (CP + ash + aNDF + EE); CHO, total carbohydrate calculated as CHO = 1000 – (CP + ash + EE) (NRC, 2001) with correction factor for disappearance of EE at different times of incubation obtained from Tamminga et al. (2007).

than at early bud (P<0.05; Tables 1 and 2). *In situ* W_N:W_{OM} and W_N:W_{CHO} ratios tended to decrease with advancing maturity (P=0.06; Tables 1 and 2). Cutting time had no impact on *in situ* ruminal N to energy (OM and CHO) ratios (Tables 1 and 2).

The largest differences in hourly ED_N:ED_{OM} and ED_N:ED_{CHO} ratios among treatments were found during the first hours of ruminal incubation for both CT and SM (Figs. 1 and 2). For all alfalfa hays, hourly ED_N:ED_{OM} and ED_N:ED_{CHO} ratios peaked after 2 h of incubation after which the ratios rapidly decreased.

3.3. Modeling nutrient supply to dairy cows

Using both the DVE/OEB system and NRC (2001), nutrient supply in dairy cows was unaffected by alfalfa hay CT, except for MCP_E^{NRC} and $AMCP_E^{NRC}$ which were higher in the afternoon cut than the morning cut (P<0.05; Tables 3–4). The RDP^{DVE}, RDP^{NRC}, $MCP_{RDP}{}^{DVE}$, $MCP_{RDP}{}^{NRC}$, OEB and DPB^{NRC} decreased with advancing maturity (P<0.05; Tables 3–4). Alfalfa at early flower and late bud stage had lower RUP^{DVE} than alfalfa at early bud (P<0.05; Table 3). Alfalfa cut at early bud had higher TPSI compared with alfalfa at early flower (P<0.05), with alfalfa at late bud intermediate (Table 3). With advancing maturity, MCP_E^{NRC}, $AMCP_E^{NRC}$, $ARUP^{NRC}$, $ARUP^{DVE}$, ENDP, DVE and MP^{NRC} tended to decrease (P<0.10) and UDM tended to increase (P=0.07; Tables 3–4). According to NRC (2001), alfalfa at early bud and late bud stage had higher RUP^{NRC}, endogenous CP (ECP) and absorbable ECP (AECP) than alfalfa at early flower (P<0.05; Table 4).

4. Discussion

4.1. Ruminal protein to energy ratios

Alfalfa at early and late bud had higher ED_N:ED_{OM}, ED_N:ED_{CHO} ratios and OEB than alfalfa at early flower and all were above the optimum ratio of 25 g N/kg OM, 32 g N/kg CHO and 0, respectively (Czerkawski, 1986; Tamminga et al., 1994).

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Table 3
Predicted rumen degraded protein balance (OEB) and protein supply to the small intestine in dairy cows of alfalfa hay cut at three stages of maturity and in the afternoon and next morning.

Items[e]	Stage of maturity (SM)			SED	Cutting time (CT)		SED	Level of significance[d]	
	Early bud	Late bud	Early flower		06:00 h	18:00 h		SM	CT
Ruminal phase (g/kg DM)									
FOM	474	468	446	21.0	463	462	18.0	0.33	0.96
MCP_{FOM}	71	70	67	3.0	69	69	3.0	0.33	0.96
RDP^{DVE}	125[a]	108[b]	77[c]	6.0	104	103	5.0	<0.01	0.88
$MCP_{RDP}{}^{DVE}$	125[a]	108[b]	77[c]	6.0	104	103	5.0	<0.01	0.88
OEB	54[a]	38[b]	10[c]	4.0	34	34	4.0	<0.01	0.86
Intestinal phase (g/kg DM)									
AMCP	45	45	43	2.0	44	44	2.0	0.33	0.96
RUP^{DVE}	94[a]	88[b]	84[b]	5.0	89	89	4.0	<0.01	0.81
ARUP	37	32	25	4.0	31	33	3.0	0.08	0.61
UDM	339	354	394	21.0	361	364	17.0	0.07	0.86
ENDP	30	27	25	2.0	27	27	1.0	0.07	0.86
TSPI	148[a]	140[ab]	134[b]	4.0	141	141	3.0	0.04	0.91
DVE	57	51	39	7.0	48	49	5.0	0.08	0.79

[d] There was no interaction between SM and CT; SED, standard error of difference; Means with different letters (a, b and c for SM) within the same row differ ($P<0.05$).

[e] AMCP, truly absorbable microbial protein (MCP); ARUP, truly absorbable rumen undegraded protein (RUP); ENDP, endogenous protein losses for the digestive tract; FOM, fermentable organic matter; MCP_{FOM} and $MCP_{RDP}{}^{DVE}$, microbial crude protein synthesized in the rumen from FOM and RDP, respectively; OEB, degraded protein balance; TPSI, true protein supplied to the small intestine; UDM, completely undegradable DM; DVE, truly digested and absorbed protein in the small intestine calculated as DVE = ARUP + AMCP − ENDP (Tamminga et al., 1994).

Table 4
Predicted rumen degraded protein balance (DPB) and metabolizable protein in dairy cows of alfalfa hay cut at three stages of maturity and in the afternoon and next morning.

Items[e]	Stage of maturity (SM)			SED	Cutting time (CT)		SED	Level of significance[d]	
	Early bud	Late bud	Early flower		06:00 h	18:00 h		SM	CT
Ruminal phase (g/kg DM)									
Total digestible nutrients	524	502	499	11.0	497[y]	520[x]	8.7	0.10	0.03
$MCP_E{}^{NRC}$	63	60	60	1.0	59[y]	62[x]	1.0	0.10	0.03
RDP^{NRC}	135[a]	116[b]	86[c]	6.0	113	112	4.9	<0.01	0.89
$MCP_{RDP}{}^{NRC}$	114[a]	99[b]	73[c]	5.1	96	95	4.1	<0.01	0.89
DPB^{NRC}	61[a]	46[b]	15[c]	5.0	38	42	4.1	<0.01	0.36
Intestinal phase (g/kg DM)									
ECP	11.11[a]	11.12[a]	11.07[b]	0.014	11.11	11.07	0.013	0.01	0.24
AECP	4.44[a]	4.45[a]	4.43[b]	0.006	4.44	4.44	0.005	0.01	0.24
$AMCP_E{}^{NRC}$	40	38	38	0.8	38[y]	40[x]	0.7	0.10	0.03
RUP^{NRC}	85[a]	79[a]	76[b]	4.1	80	80	4.0	<0.01	0.81
ARUP	33	29	23	3.8	28	29	3.1	0.08	0.61
MP^{NRC}	78	72	66	4.1	70	74	3.4	0.06	0.34

[d] There was no interaction between SM and CT; SED, standard error of difference; Means with different letters (a, b and c for SM) and (x and y for CT) within the same row differ ($P<0.05$).

[e] AECP, absorbed endogenous protein; $AMCP^{NRC}$, absorbable microbial CP (MCP) derived from energy substrate; $ARUP^{NRC}$, absorbed ruminaly undegraded feed CP (RUP); DPB^{NRC}, degraded protein balance reflecting the difference between the potential MCP based on ruminaly degraded protein ($MCP_{RDP}{}^{NRC}$) and that based on energy ($MCP_E{}^{NRC}$); AECP, absorbable endogenous CP (ECP); MP_{NRC}, metabolizable protein calculated as: MP = ARUP + AMCP + AECP (NRC, 2001).

Higher ruminal N to energy ratios results in N losses from the rumen when feeding alfalfa alone which is in agreement with reported literature (Yu et al., 2004; Jonker et al., 2011). The ED_N and ED_{OM} decreased and ED_{CHO} stayed similar with advancing SM. With advancing SM, ED_N (g/kg DM) decreased because of decreased forage N content and increased U_{CP} fraction; ED_{OM} (g/kg OM) decreased but forage OM increased which result in similar ED_{OM} (g/kg DM); ED_{CHO} (g/kg DM) increased because of increased forage CHO content and ED_{CHO} (g/kg CHO).

The ratio of rumen available N to available energy should not only be optimal, but also synchronized to achieve efficient microbial growth and minimize N losses from the rumen (Sinclair et al., 1993). The ruminal N to energy synchronization improved with advancing SM and when cutting alfalfa in the afternoon in our study. However, SM had bigger impact on hourly ED_N:ED_{OM} and ED_N:ED_{CHO} ratios than CT. The pattern of hourly ED_N:ED_{CHO} ratio for our alfalfa hays was similar to fresh forages, but the imbalance was much smaller compared with fresh vegetative alfalfa (Jonker et al., 2011) and slightly higher compared with fresh perennial ryegrass (Tas et al., 2006). The excessive N supplied above microbial requirements occurred mainly during the first few incubation hours peaking at 2 h after which the oversupply rapidly decreased in a

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curvilinear fashion. Current findings are similar to previous results (Yu et al., 2004; Jonker et al., 2011) which indicate that alfalfa hay needs to be supplemented with feeds that supply ruminal energy in the diet of dairy cows.

4.2. Modeling nutrient supply to dairy cows

Metabolizable protein content (MP and DVE) tended to decrease with advancing SM, while CT had no effect. This trend was mainly the result of decreasing forage CP and RUP and ARUP with advancing SM. Current alfalfa samples had lower predicted nutrient supply in terms of DVE and MP compared with alfalfa grown under western Canadian conditions (Yu et al., 2003a). This seems to be the result of higher U_{CP}, U_{DM} and U_{OM} content in current samples, since overall CP, DM and OM content were similar between our study and the study of Yu et al. (2003a,b). These higher U fractions resulted in lower FOM, MCP and AMCP, and higher ENDP, while ARUP and ECP were similar compared with Yu et al. (2003a). The U fractions in our study were also higher compared with Balde et al. (1993). In current study we dried alfalfa samples outdoors in the shade for 10 to 15 days, while Balde et al. (1993) and Yu et al. (2003a) directly oven dried alfalfa samples after harvest. Easily degradable material might have disappeared and/or changed into undegradable material in alfalfa samples during the outdoor drying period which increases the U fractions. Our metabolizable protein values (MP and DVE) give probably a better representation of outdoor dried alfalfa hay and the MP and DVE values of Yu et al. (2003a) for artificially dried alfalfa hay or cubicles.

Additionally, environmental factors and geological location might affect nutritive value of alfalfa as well. Lignification of alfalfa stems and consequent reduced digestibility occurs with high temperatures (Van Soest, 1994). Temperatures in our study were higher (Yari et al., 2012) compared with temperature data reported by Yu et al. (2003b). Latitude may influence nutrient supply of forages as well (Van Soest, 1994). Deinum et al. (1981) found that digestibility of timothy grass harvested at the same morphological stage was higher at higher latitudes (69°N *vs.* 51°N), mainly as a result of lower weather temperatures and longer day length. The current study was conducted at lower latitude (36°N) than the study of Yu et al. (2003a,b; 55°N). However, more research is required to understand the effect of temperature and latitude on nutrient supply of alfalfa hay.

Alfalfa cut in the afternoon had higher MCP_E^{NRC} compared with alfalfa cut in the morning which was a result of increased total digestible nutrients during the day. Brito et al. (2008, 2009) found increased MCP in cows fed alfalfa baleage cut in the afternoon compared with alfalfa baleage cut in the morning, likely due to higher soluble carbohydrate content and digestibility in the afternoon cut. Soluble carbohydrate content of samples used in the current study was ~10 g/kg DM higher in the afternoon than the morning cut (Yari et al., 2012). We previously found that including alfalfa hay cut in the afternoon at 200 g/kg DM in the total mixed ration of dairy cows increased total tract nutrient digestibility and digestible nutrient intake compared with including alfalfa hay cut in the morning (Yari et al., 2011). However, FOM was similar between CT and therefore MCP based on FOM was similar between CT as well.

5. Conclusion

With advancing maturity of alfalfa hay *in situ* degradability of crude protein, organic matter and neutral detergent fiber, predicted nutrient supply in dairy cows in terms of intestinally absorbed protein and ruminal degraded protein balance decreased and ruminal nitrogen to energy (organic matter and total carbohydrate) synchronization improved. Cutting alfalfa in the afternoon increased ruminally derived microbial protein from energy substrate (MCP_E; NRC basis) and intestinally absorbable MCP_E and tended to increase total ruminal degradability of carbohydrate and improved nitrogen to energy synchronization compared with cutting alfalfa in the morning. Both models (DVE/OEB and NRC) detected that alfalfa had a positive ruminal degraded protein balance which indicates a potential imbalance between feed nitrogen degradation and utilization if alfalfa is fed alone.

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