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## Energy supplementation and herbage allowance effects on daily intake in lactating mares<sup>1</sup>

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**ABSTRACT:** Little is known about how to manage grazing horses, including the thresholds under which energy supplementation is required. Here we investigated the effects of daily herbage allowance (DHA) and energy supplementation (ES) on daily herbage intake in lactating mares of light breeds grazing high-quality regrowth during summer. Three contrasting DHA, low (LOW), medium (MED), and high (HIGH), that is, 35.0, 52.5, and 70.0 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>, respectively, were obtained by adjusting pasture strip width. Eighteen Anglo-Arab and French Saddle lactating mares were either supplemented with 2.6 kg DM barley/d (SUP group; *n* = 9) or left nonsupplemented (NSUP group; *n* = 9) throughout the experiment. For 3 successive 2-wk periods, 3 groups of SUP mares (*n* = 3) and 3 groups of NSUP mares (*n* = 3) grazed each DHA according to a 3 × 3 Latin square design. Pregrazing sward surface height (SSH) was similar between treatments (26.6 cm), but postgrazing SSH differed significantly between each DHA (2.9, 4.4, and 5.7 cm for LOW, MED, and HIGH, respectively;

*P* < 0.001). Herbage DMI (HDMI) increased linearly from 18.5 to 23.4 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> with increasing DHA (i.e., 0.13 kg DM eaten/kg DM of herbage offered; *P* < 0.001) independently of ES and with no significant ES × DHA interaction. This increase in HDMI resulted from an increase in grazing time between LOW (961 min/d) and MED and HIGH (1,021 min/d; *P* < 0.01) and from an increase in intake rate between LOW and MED (11.8 g DM/min) and HIGH (13.6 g DM/min; *P* < 0.01). Total digestible DMI (TDDMI) and NE intake (NEI) increased linearly from 12.3 to 15.2 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> and from 136.6 to 165.8 kJ·kg BW<sup>-1</sup>·d<sup>-1</sup> with increasing DHA (*P* < 0.001), respectively. Total digestible DMI and NEI were significantly lower for NSUP than for SUP mares: 12.5 vs. 14.9 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> (*P* < 0.01) and 134.6 vs. 166.5 kJ·kg BW<sup>-1</sup>·d<sup>-1</sup> (*P* < 0.001), respectively. Whereas SUP mares always met their energy requirements, NSUP mares no longer met theirs when DHA fell below 66 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> (i.e., 39 kg DM·mare<sup>-1</sup>·d<sup>-1</sup>).

**Key words:** concentrate, grazing, horse, nutritional requirements, sward availability

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### INTRODUCTION

Grazed pastures are known as the cheapest source of nutrients for domestic herbivores if accurately managed (e.g., horses [Micol and Martin-Rosset, 1995] and dairy cows [Peyraud and Delaby, 2001]). Under strip or rotational grazing, DMI and animal performance are primarily controlled by daily herbage allowance (DHA; Wales et al., 1998; Pérez-Prieto and Delagarde, 2013). In ruminants, the relationship is curvilinear,

with herbage intake increasing at a declining rate with increasing DHA (Dalley et al., 1999; Pérez-Prieto and Delagarde, 2013). Under low herbage allowance, concentrate supplementation increases total digestible DMI and performance (Delaby et al., 2001), whereas the response is low at high herbage allowance due to substitution between herbage and concentrate (Stockdale, 2000; Doyle et al., 2005). In spite of the increasing numbers of light horses in the last 30 to 40 yr (American Horse Council, 2005; European Horse Network, 2010), little is known about how to feed them at pasture (but see Mésochina et al. [2000], Grace et al. [2002a], and Edouard et al. [2009, 2010] for growing horses). Lactating mares are, therefore, commonly supplemented with concentrate feed at pasture to ensure their performance. Collas et al. (2014), however, observed that under unlimited herbage conditions, the adaptive capacities of mares of light breeds enabled them to meet their requirements between the first and the fifth month of lactation and to produce foals with a satisfactory growth and conformation while relying on only herbage. In the present study, we set out to determine the herbage allowance threshold under which energy supplementation (ES) is required to feed lactating mares of light breeds on high-quality regrowth.

## MATERIALS AND METHODS

The experiment was conducted in accordance to the national legislation on animal care (Certificate of Authorization to Experiment on Living Animals delivered by the Regional Ethic Committee for Animal Experimentation of Limousin, number 10-2013-10, Ministry of Agriculture, Food and Forestry, Limoges, France).

### *Treatments and Experimental Design*

The experiment was conducted from 22 June to 2 August 2013 at the experimental farm of the French Horse and Riding Institute (IFCE) in Chamberet, France (01°43'14" E, 45°35'03" N, and 440 m altitude). We used 18 lactating mares of light breeds either supplemented with 2.6 kg DM barley/d (SUP;  $n = 9$ ) or left nonsupplemented (NSUP;  $n = 9$ ). Supplemented and NSUP mares were divided into 3 groups of 3 mares and tested in a Latin square design with 3 levels of DHA measured at ground level: low (LOW), medium (MED) and high (HIGH), that is., 35.0, 52.5, and 70.0 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>, respectively. Expecting a voluntary herbage intake of 25 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> for NSUP lactating mares (i.e., mean value measured for NSUP lactating mares grazing the same pasture the previous summer; Collas et al., 2014), the high level of DHA (i.e., nonlimiting DHA) was chosen to be 2.5 to 3 times higher according to references obtained in dairy cows (Delagarde

et al., 2011). Medium and low DHA were arbitrary chosen to offer three-fourths and half of the high DHA as a gradient. Each measurement period lasted 2 wk: Period 1 from 22 June to 5 July, Period 2 from 6 July to 19 July, and Period 3 from 20 July to 2 August. Mean daily precipitation (SE) was 1.1 (0.5), 0.7 (0.7), and 8.2 mm/d (0.7) for periods 1, 2, and 3, respectively. Highs and lows for temperature were 20.0 ± 1.1 and 10.4 ± 0.7°C for period 1, 29.1 ± 0.2 and 15.5 ± 0.3°C for period 2, and 29.6 ± 0.9 and 15.1 ± 0.6°C for period 3, respectively.

### *Animals*

Throughout the entire pregnancy, the 18 mares (Anglo-Arab and French Saddle breeds; 5–23 yr old) were collectively fed, according to their mean requirements (INRA, 2015), a diet composed of 43% grass hay (DM, 856 g/kg DM; CP, 79 g/kg DM; and crude fiber [CF], 361 g/kg DM; experimental farm of the IFCE, Chamberet, France), 41% haylage (DM, 614 g/kg; CP, 60 g/kg DM; and CF, 364 g/kg DM; experimental farm of the IFCE, Chamberet, France), and 16% concentrate (61.5% barley and 35% soybean meal [Agricentre Dumas, Espartignac, France] and 3.5% minerals and vitamins [Chauveau Nutrition, Cholet, France]). As voluntary intake is affected by the mare's body condition at foaling (Doreau et al., 1993), the objective was that all mares achieved an optimal BCS of 3 at the start of the experiment, using the INRA scoring scale ranging from 0 (emaciated) to 5 (obese; Martin-Rosset et al., 2008). This optimal BCS corresponds to a BCS of 5 according to the NRC system (Henneke et al., 1983). To estimate the intake capacity of each mare, initial intake measurements were made in March indoors, with grass hay offered ad libitum for 8 d after 6 adaptation days. Intake capacity is defined as the amount of food that a horse should consume to meet its energy requirements and that is permitted by its digestive volume (INRA, 2015). In April, all the mares were treated against gastrointestinal parasites with ivermectin (Eqvalan; Merial, Lyon, France). After foaling, mares were split into 2 groups (SUP and NSUP) balanced for mare intake capacity (23.7 ± 1.4 g DM·kgBW<sup>-1</sup>·d<sup>-1</sup> for SUP and 24.1 ± 1.8 g DM·kgBW<sup>-1</sup>·d<sup>-1</sup> for NSUP), foaling date (14 April to 31 May for SUP [i.e., 47.2 ± 4.6 lactating days at the start of the experiment] and 26 April to 9 June for NSUP: [i.e., 40.3 ± 5.7 lactating days at the start of the experiment]), BCS (3.2 ± 0.2 for SUP and 3.1 ± 0.2 for NSUP [i.e., BCS between 5 and 6 according to Henneke et al., 1983]), BW at foaling (547.2 ± 14.4 kg for SUP and 538.0 ± 10.4 kg for NSUP), height at withers (163.3 ± 1.3 cm for SUP and 161.9 ± 0.9 cm for NSUP), and age (8.6 ± 1.9 yr old for SUP and 6.8 ± 0.9 yr old for NSUP). The 18 mares were, therefore, at

2, 2.5, and 3 mo of lactation, on average, during the first, second, and third experimental periods, respectively.

From 22 June to 2 August, SUP mares received a daily supplement of 2.6 kg DM of rolled barley (Table 1; Agricentre Dumas) at pasture, which accounted for 63% of energy requirements for their mean stage of lactation (i.e., 2.5 mo; INRA, 2015; i.e., 67% according to the NRC [2007] feed evaluation system). During the second week of each period, NSUP mares were fed 260 g DM of rolled barley daily; the 18 mares ate 100 g of small colored plastic balls (1 color per mare) mixed with the barley to individualize feces at pasture (see Intake Measurements). All the mares were accustomed to being individually fed with barley at pasture by the week before measurements began. They were weighed at the same time of day on the first day of each period (for SUP,  $589.3 \pm 12.0$ ,  $593.5 \pm 13.6$ , and  $588.8 \pm 12.0$  kg for LOW, MED, and HIGH, respectively, and for NSUP,  $584.5 \pm 13.2$ ,  $589.2 \pm 12.9$ , and  $576.1 \pm 10.1$  kg for LOW, MED, and HIGH, respectively).

### Pasture Composition and Grazing Management

Mares and their foals were strip grazed on a fertile permanent pasture (25 species; 60% grass cover). The most abundant species were rough bluegrass (*Poa trivialis*), perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*), and dandelion (*Taraxacum officinale*). The pasture was divided into 3 paddocks. One paddock was used for each period and was, therefore, mown beforehand at a different date to ensure identical vegetation stage for measurements. Each paddock was divided into 6 subpaddocks, and each was used by 3 mares and their foals for 1 of the treatments (SUP–LOW, NSUP–LOW, SUP–MED, NSUP–MED, SUP–HIGH, and NSUP–HIGH).

As foals graze after 2 mo of age (INRA, 2015), it was necessary to estimate the amount of herbage they would consume during the second and the third periods so that strip width could be slightly increased. We calculated that foals consumed 5 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> during the second period (mean BW = 135 kg) and 6 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> during the third period (mean BW = 145 kg) by considering foal daily gain and requirements, mares' milk yield, and sward nutritive value (Trillaud-Geyl et al., 1990; INRA, 2015).

The strips of pasture were allocated for 2 d of grazing by moving electric fences. Mares and foals were moved into a new strip of fresh herbage every 2 mornings at 0915 h. Access by mares and foals to the previously grazed strip was prevented by back fencing. This management enabled us to offer the animals identical swards and DHA during the 2-wk period by adjusting strip width to herbage mass. Strip width to be

**Table 1.** Chemical composition and nutritive value of the supplement (barley)

Item <sup>1,2</sup>	Barley
DM, g/kg	867
Chemical composition, g/kg DM	
OM	974
CP	116
CF	52
NDF	216
ADF	63
Nutritive value	
DM digestibility, g/kg DM	81
NE, MJ/kg DM	10.7
HDCP, g/kg DM	82

<sup>1</sup>CF = crude fiber; HDCP = horse digestible CP.

<sup>2</sup>From INRA feed tables (INRA, 2015).

offered for 2 d was determined using the mean sward surface height (SSH) measured every 2 d in the next area to be grazed and a SSH–herbage mass regression updated once weekly (see Vegetation Characteristics).

### Vegetation Characteristics

Sward surface height and herbage mass were simultaneously measured once weekly in twelve 0.49-m<sup>2</sup> (70 by 70 cm) quadrats (i.e., 2 per subpaddock), randomly selected among areas of short (11 to 19 cm on average according to period), medium (14 to 24 cm on average according to period), and tall (16 to 30 cm on average according to period) SSH, on the next area to be grazed. Twelve SSH measurements were made before herbage cut in each quadrat by recording the first contact of a stick with the undisturbed sward surface. Herbage cut at ground level with hand shears was weighed and then divided into 2 samples. One sample per quadrat was dried for 24 h at 103°C to determine herbage DM content and then calculate herbage mass and establish the SSH–herbage mass regression. The other sample was dried for 72 h at 60°C and analyzed for CP (Dumas method NF V18-120; AFNOR, 1997), cellulose (CF; Weende method NF V03-040; AFNOR, 1993), and NDF and ADF (Goering and Van Soest, 1970). Thirty pregrazing SSH measurements were made in each subpaddock before the animals entered so that strip width could be determined using the SSH–herbage mass regression according to the targeted low, medium, and high DHA. Postgrazing SSH was also measured every 2 d with 15 random points per strip.

### Daily Intake and Energy and Protein Balances

Daily herbage DMI (HDMI) was measured for each mare during the last 4 d of each period as

**Table 2.** Daily offered area and characteristics of herbage on offer for grazing according to energy supplementation (ES; supplemented with 2.6 kg DM barley/d [SUP] and nonsupplemented [NSUP]) level and daily herbage allowance (DHA; low [LOW], medium [MED], and high [HIGH])

Item <sup>1</sup>	SUP			NSUP			RMSE <sup>2</sup>	P-value				
	LOW	MED	HIGH	LOW	MED	HIGH		ES	DHA	ES × DHA	Lin <sup>3</sup>	Quad <sup>3</sup>
DHA, g DM·kg BW <sup>-1</sup> ·d <sup>-1</sup>	35.0	52.5	70.0	35.0	52.5	70.0	–	–	–	–	–	–
DHA, kg DM·mare <sup>-1</sup> ·d <sup>-1</sup>	20.6	30.9	41.2	20.6	30.9	41.2	–	–	–	–	–	–
DOA, m <sup>2</sup> ·mare <sup>-1</sup> ·d <sup>-1</sup>	59.2	90.2	122.3	65.1	86.1	121.2	19.0	0.972	<0.001	0.438	<0.001	0.477
Sward surface height, cm												
Pregrazing	27.6	25.9	28.2	23.7	27.0	27.3	6.7	0.295	0.377	0.225	0.560	0.460
Postgrazing	2.8	4.6	5.8	3.1	4.1	5.6	1.5	0.699	<0.001	0.422	<0.001	<0.001
Pregrazing HM, t DM/ha	3.86	3.62	3.86	3.38	3.82	3.86	0.70	0.448	0.281	0.077	0.334	0.933
DM, g/kg	236	259	249	264	255	232	0.05	0.865	0.687	0.459	0.792	0.607
Sward quality, g/kg DM												
OM	899	897	876	889	908	896	0.04	0.368	0.193	0.245	0.430	0.123
CP	144	156	163	145	147	154	0.03	0.443	0.237	0.837	0.176	0.960
CF	268	245	230	248	256	265	0.05	0.509	0.800	0.274	0.505	0.908
NDF	571	540	545	555	577	566	0.04	0.340	0.911	0.289	0.670	0.939
ADF	321	298	301	311	324	319	0.03	0.221	0.841	0.235	0.617	0.876

<sup>1</sup>DOA = daily offered area; HM = herbage mass; CF = crude fiber.

<sup>2</sup>RMSE = root mean square error.

<sup>3</sup>Lin = linearity of DHA effect; Quad = quadraticity of DHA effect.

$$\text{HDMI} = \text{FO} / (1 - \text{HDMD}),$$

in which **FO** is the dry weight of fecal output over 24 h attributable to herbage and **HDMD** is the DM digestibility of ingested herbage expressed as a decimal value.

In horses, feces are dry enough to collect them without losses or contamination. Fecal output was, therefore, determined by total fecal collection, which is the reference method (Penning, 2004) previously used in a large number of horse studies (e.g., Duncan, 1992; Mésochina et al., 2000; Grace et al., 2002a,b; Edouard et al., 2009, 2010; Collas et al., 2014). Feces were collected once a day over 4 successive days after the paddock had been cleaned of feces. Individualization of feces was made possible identifying the small plastic balls of different colors (1 color per mare) that were mixed with the barley (see Animals). Total daily fecal outputs were individually weighed, and a subsample was dried for 72 h at 80°C to determine fecal DM and CP contents. Fecal DM output attributable to herbage was then calculated by subtracting the indigestible DM attributable to barley (INRA, 2015; Table 1) from the total fecal DM output (Delagarde et al., 1999; Collas et al., 2014).

Dry matter digestibility of ingested herbage was estimated from fecal CP content attributable to herbage according to the equation of Mésochina et al. (1998):

$$\text{HDMD} = 0.734 - (17.872 / \text{fecal CP content}),$$

in which fecal CP content is expressed in grams per kilogram DM.

Mésochina et al. (1998) stressed that the conditions of applicability of this equation are for herbage CP contents greater than 70 g/kg DM, which limits N recycling by horses. This was always the case in the present experiment (Table 2). The fecal CP content attributable to herbage was calculated by dividing the amount of fecal CP attributable to herbage by the fecal DM output attributable to herbage. The amount of fecal CP attributable to herbage was calculated by subtracting the amount of fecal CP attributable to barley from the total CP amount excreted from feces (Delagarde et al., 1999; Collas et al., 2014). The amount of fecal CP attributable to barley was calculated from the CP content of barley and from the apparent CP digestibility of barley (INRA, 2015; Table 1).

Total DMI (**TDMI**) was calculated as the sum of HDMI and barley DMI (**BDMI**) recorded daily when mares were supplemented. Daily total digestible DMI (**TDDMI**) was then calculated as the sum of herbage digestible DMI and barley digestible DMI:

$$\text{TDDMI} = \text{HDMI} \times \text{HDMD} + \text{BDMI} \times \text{BDMD},$$

in which **BDMD** is the DM digestibility of barley (INRA, 2015; Table 1).

Net energy intake was estimated from herbage and barley NE contents according to the INRA (INRA, 2015) feed evaluation system:

$$\text{herbage NE content} = [(0.825 - 0.0011 \times \text{CF} + 0.0006 \times \text{CP}) \times 2,250] \times 4.18,$$

in which herbage NE content is expressed in kilojoules per kilogram DM, CF and CP are expressed in grams per kilogram DM, and 2,250 is the NE content (kcal) of 1 kg of fresh standard barley (INRA, 2015).

Horse digestible CP (**HDCP**) intake was estimated from herbage and barley digestible CP (**DCP**) contents according to the INRA (INRA, 2015) feed evaluation system:

$$\text{herbage DCP content} = (-74.52 + 0.9568 \times \text{CP} + 0.1167 \times \text{CF}) \times 0.9,$$

in which herbage DCP content is expressed as grams DCP per kilogram DM and CP and CF are expressed in grams per kilogram DM.

Individual consumptions of energy and protein were then expressed in relation to the mares' requirements at each period. Mares' requirements were estimated using their reference BW at foaling, their stage of lactation, and INRA nutrient requirements tabulated per BW classes (INRA, 2015).

Individual energy and protein intakes were also calculated according to the NRC (2007) feed evaluation system and were expressed in relation to the mares' requirements using tabulated NRC requirements and the same procedure as previously described.

Digestible energy intake was estimated from herbage and barley DE contents according to the NRC (2007) feed evaluation system:

$$\text{herbage DE content} = (4.22 - 0.11 \times \text{ADF} + 0.0332 \times \text{CP} + 0.00112 \times \text{ADF}^2) \times 4.18 \times 10^3 \text{ and}$$

$$\text{barley DE content} = (4.07 - 0.055 \times \text{ADF}) \times 4.18 \times 10^3,$$

in which herbage DE content and barley DE content are expressed in kilojoules per kilogram DM and ADF and CP are expressed as a percentage of DM.

Crude protein intake was estimated from herbage and barley CP contents according to the NRC (2007) feed evaluation system.

### **Grazing Behavior**

Daily grazing time of the mares was recorded over 48 h in the middle of the first week of each period using Ethosys collars (Greenway System GmbH, Frankfurt, Germany) to record head position and movements (1 recording every 5 min; Scheibe et al., 1998). Animals were accustomed to wearing the collars by the week before measurements began. We also evaluated grazing time during the first 12 h (from 0915 to 2115 h),

after the animals entered a new strip, and during the last 12 h (from 2115 to 0915 h), before they left it. Herbage intake rate was estimated by dividing daily herbage intake by daily grazing time.

### **Statistical Analyses**

Data were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Sward and grazing management data were analyzed in a model that included the effects of period, DHA, ES, and the ES  $\times$  DHA interaction. Animal data were analyzed in a model that included the effects of period, DHA, ES, mare nested within ES (as mares were either supplemented or not for all the experiment), and the ES  $\times$  DHA interaction. Effect of ES was tested separately using the mare effect as residual term. Orthogonal contrasts were used to test whether the DHA effect was linear or quadratic. Differences between DHA were investigated using the Tukey correction for multiple comparisons; the significance threshold was  $P < 0.05$ .

## **RESULTS**

### **Vegetation Characteristics**

Pregrazing SSH (26.6 cm) and pregrazing herbage mass (3.73 t DM/ha) were similar across all the treatments (Table 2). The 3 contrasting DHA chosen were obtained by linearly increasing the area offered daily (Table 2). Postgrazing SSH was not affected by ES and increased by 0.13 cm/kg of dry herbage offered (linear and quadratic effects,  $P < 0.001$ ; Table 2). Herbage DM (249 g/kg), OM (895 g/kg DM), CP (151 g/kg DM), CF (252 g/kg DM), NDF (559 g/kg DM), and ADF (312 g/kg DM) contents were independent of ES and DHA levels (Table 2).

### **Daily Intake and Energy and Protein Balances**

None of the intake variables were affected by ES  $\times$  DHA (Table 3; Fig. 1), which means that the mares responded similarly to DHA variations whether supplemented or not. Fecal output attributable to herbage (FO), HDMD, HDMI, herbage digestible DMI (**HDDMI**), TDMI, and HDCP intake did not significantly differ between SUP and NSUP mares, despite a tendency for FO to be greater in NSUP mares and for TDMI to be greater in SUP mares (Table 3; Fig. 1). Mares ate all the barley offered during the experiment. Consequently, SUP mares achieved greater TDDMI (14.9 g digestible DM·kg BW<sup>-1</sup>·d<sup>-1</sup> for SUP and 12.5 g DDM·kg BW<sup>-1</sup>·d<sup>-1</sup> for NSUP;  $P < 0.01$ ) and NE intake (**NEI**; 166.5 kJ·kg BW<sup>-1</sup>·d<sup>-1</sup> for SUP and 134.6 kJ·kg BW<sup>-1</sup>·d<sup>-1</sup> for NSUP;  $P < 0.001$ ) than NSUP mares (Table 3; Fig. 1). Dry matter digestibility of ingested herbage increased linearly

**Table 3.** Mares' daily intake according to energy supplementation (ES; supplemented with 2.6 kg DM barley/d [SUP] and nonsupplemented [NSUP]) level and daily herbage allowance (DHA; low [LOW], medium [MED], and high [HIGH])

Item <sup>1</sup>	SUP			NSUP			RMSE <sup>2</sup>	P-value				
	LOW	MED	HIGH	LOW	MED	HIGH		ES	DHA	ES × DHA	Lin <sup>3</sup>	Quad <sup>3</sup>
FO, g DM·kg BW <sup>-1</sup> ·d <sup>-1</sup>	7.8	8.4	9.7	8.5	9.8	10.7	0.6	0.064	<0.001	0.635	<0.001	0.669
HDMD, g/kg DM	560	566	570	551	556	557	0.5	0.146	<0.05	0.754	<0.01	0.599
HDMI, g DM·kg BW <sup>-1</sup> ·d <sup>-1</sup>	17.9	19.2	22.5	19.0	22.0	24.3	2.4	0.148	<0.001	0.590	<0.001	0.655
HDMI, kg DM·mare <sup>-1</sup> ·d <sup>-1</sup>	10.6	11.4	13.2	11.1	12.9	14.0	1.4	0.241	<0.001	0.568	<0.001	0.976
HDDMI, g DDM·kg BW <sup>-1</sup> ·d <sup>-1</sup>	10.1	10.9	12.8	10.5	12.3	13.6	1.3	0.281	<0.001	0.561	<0.001	0.661
HDDMI, kg DM·mare <sup>-1</sup> ·d <sup>-1</sup>	5.9	6.5	7.5	6.1	7.2	7.8	0.8	0.390	<0.001	0.543	<0.001	0.945
TDMI, g DM·kg BW <sup>-1</sup> ·d <sup>-1</sup>	22.4	23.7	27.0	19.5	22.5	24.8	2.4	0.118	<0.001	0.585	<0.001	0.634
TDMI, kg DM·mare <sup>-1</sup> ·d <sup>-1</sup>	13.2	14.1	15.8	11.4	13.2	14.2	1.4	0.083	<0.001	0.568	<0.001	0.976
TDDMI, g DDM·kg BW <sup>-1</sup> ·d <sup>-1</sup>	13.7	14.5	16.5	10.9	12.6	14.0	1.4	<0.01	<0.001	0.542	<0.001	0.614
TDDMI, kg DM·mare <sup>-1</sup> ·d <sup>-1</sup>	8.1	8.6	9.7	6.3	7.4	8.0	0.8	<0.01	<0.001	0.543	<0.01	0.973

<sup>1</sup>FO = fecal output attributable to herbage; HDMD = DM digestibility of ingested herbage; HDMI = herbage DMI; HDDMI = herbage digestible DMI; HDMD = digestible DM; TDMI = total DMI; TDDMI = total digestible DMI.

<sup>2</sup>RMSE = root mean square error.

<sup>3</sup>Lin = linearity of DHA effect; Quad = quadraticity of DHA effect.

with increasing DHA and was significantly lower on LOW than on HIGH (556 vs. 564 g/kg DM). Fecal output attributable to herbage (8.2, 9.1, and 10.2 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively), HDMI (18.4, 20.6, and 23.4 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively), HDDMI (10.3, 11.6, and 13.2 g DDM·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively), TDMI (20.9, 23.1, and 25.9 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively), TDDMI (12.3, 13.6, and 15.2 g DDM·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively), NEI (136.6, 149.3, and 165.8 kJ·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively), and HDCP intake (1.9, 2.1, and 2.3 g HDCP·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively) increased linearly with increasing DHA ( $P < 0.001$ ), with significant differences between each DHA (Table 3; Fig. 1). Mares increased their HDMI and their TDMI by 0.13 kg DM/kg dry herbage offered ( $P < 0.001$ ). Mares' consumption of energy and protein in relation to their requirements increased linearly with increasing DHA ( $P < 0.001$ ), although no significant difference was observed for protein requirements between LOW and MED (Fig. 2). Supplemented mares met a higher proportion of both their energy ( $P < 0.001$ ) and protein ( $P = 0.051$ ) requirements than NSUP mares (Fig. 2). Supplemented mares always met their energy requirements, but NSUP mares fell short at the low and medium DHA (Fig. 2). All the mares met their protein requirements irrespective of DHA and ES (Fig. 2). The same conclusions were obtained when expressing our results according to the NRC system: SUP and NSUP mares increased their DE intakes with DHA (for SUP mares, 247.6 ± 12.3, 260.2 ± 8.6, and 293.0 ± 14.4 kJ·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively, and for

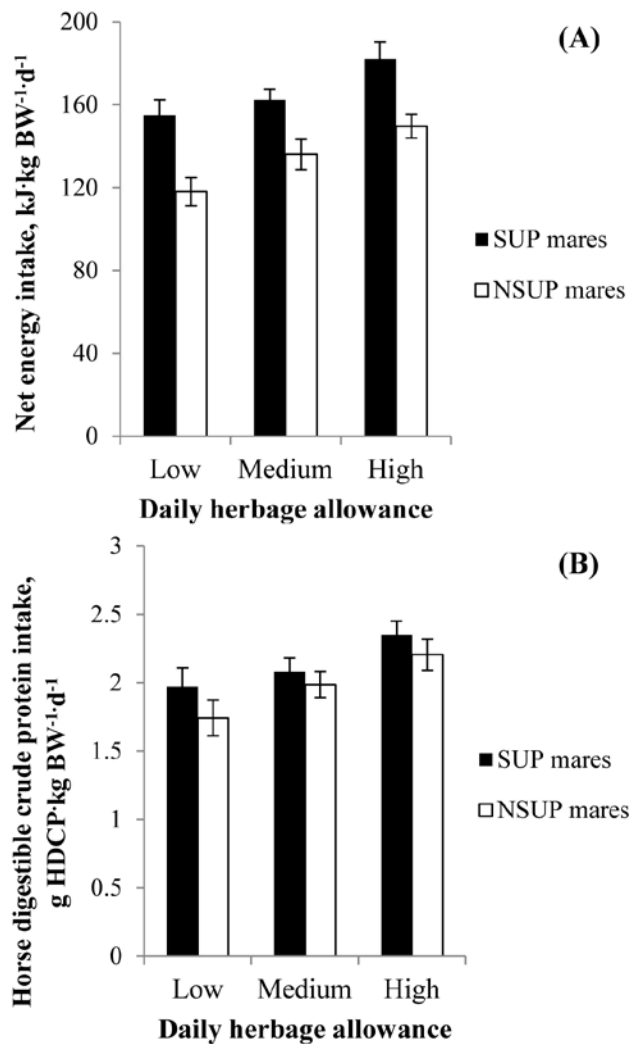
NSUP mares, 196.4 ± 11.1, 226.7 ± 12.6, and 249.4 ± 9.8 kJ·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively) but NSUP mares did not meet their energy requirements on low and medium DHA (for SUP mares, 104, 110, and 123% for LOW, MED, and HIGH, respectively, and for NSUP mares, 82, 95, and 102% for LOW, MED, and HIGH, respectively). Consumption of protein (for SUP mares, 3.3 ± 0.2, 3.4 ± 0.1, and 3.9 ± 0.2 g CP·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively, and for NSUP mares, 2.9 ± 0.2, 3.4 ± 0.2, and 3.7 ± 0.2 g CP·kg BW<sup>-1</sup>·d<sup>-1</sup> for LOW, MED, and HIGH, respectively) always met the mares' requirements whatever the treatment (119 to 142% for SUP mares according to DHA and 106 to 133% for NSUP mares according to DHA).

### Grazing Behavior

Mares' grazing behavior was affected by neither ES nor ES × DHA (Table 4). Both SUP and NSUP mares grazed significantly longer on medium and high than on low DHA (1,021 vs. 962 min/d;  $P < 0.01$ ; Table 4). The lower daily grazing time spent by mares on LOW resulted from a significantly lower activity from 2115 to 0915 h (359 vs. 420 min;  $P < 0.01$ ; Table 4), with DHA mainly influencing grazing activity on the second day on a strip (Fig. 3). All the mares achieved a significantly greater herbage intake rate when DHA was highest than with MED and LOW (13.6 vs. 11.8 g DM/min;  $P < 0.01$ ; Table 4).

### DISCUSSION

The aim of this experiment was to assess the effect of herbage allowance and ES on herbage intake by

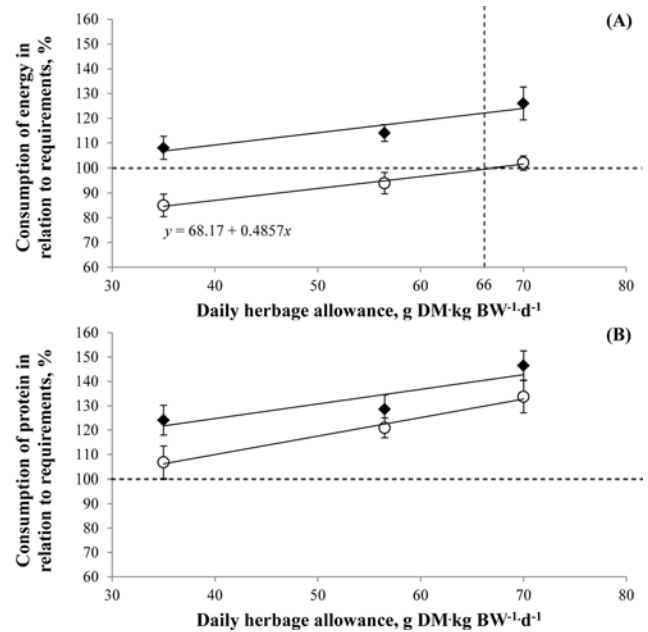


**Figure 1.** (A) Net energy intake according to energy supplementation (ES;  $P < 0.001$ ) and daily herbage allowance (DHA;  $P < 0.001$ ; mean  $\pm$  SE; ES  $\times$  DHA interaction,  $P > 0.05$ ; linearity of DHA effect,  $P < 0.01$ ; and quadraticity of DHA effect,  $P > 0.05$ ). (B) Horse digestible CP intake according to ES ( $P > 0.05$ ) and DHA ( $P < 0.001$ ; mean  $\pm$  SE; ES  $\times$  DHA interaction,  $P > 0.05$ ; linearity of DHA effect,  $P < 0.001$ ; and quadraticity of DHA effect,  $P > 0.05$ ). SUP = supplemented with 2.6 kg DM barley/d; NSUP = nonsupplemented; HDCP = horse digestible CP.

lactating mares. Most of the work showing that these factors are major determinants of DMI, grazing time, and animal performance has, so far, been performed with ruminants (mainly dairy cows), and therefore, it is not known whether the same rules apply to horses.

### Daily Herbage Allowance

The 3 DHA used in this experiment resulted in contrasting HDMI by lactating mares ranging from 77 to 98% of their intake capacity (i.e., intake measurements performed in March indoors, with grass hay offered ad libitum; see Materials and Methods). The effect of DHA on HDMI was linear, and we could calculate an increase of 0.13 kg DM eaten by mares/kg DM of herbage offered at ground level. This value is close to that



**Figure 2.** (A) Consumption of energy in relation to requirements according to energy supplementation (ES;  $\blacklozenge$  = supplemented with 2.6 kg DM barley/d [SUP] mares and  $\circ$  = nonsupplemented [NSUP] mares;  $P < 0.001$ ) and daily herbage allowance (DHA;  $P < 0.001$ ) using INRA (INRA, 2015) recommendations (ES  $\times$  DHA interaction,  $P > 0.05$ ; linearity of DHA effect,  $P < 0.01$ ; and quadraticity of DHA effect,  $P > 0.05$ ). From the linear effect of DHA on herbage DMI we estimate at 66 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> (i.e., 39 kg DM·mare<sup>-1</sup>·d<sup>-1</sup>) the DHA threshold under which ES is needed for lactating mares to meet their energy requirements on high-quality regrowth. (B) Consumption of protein in relation to requirements according to ES ( $\blacklozenge$  = SUP mares and  $\circ$  = NSUP mares;  $P = 0.051$ ) and DHA ( $P < 0.001$ ) using INRA (INRA, 2015) recommendations (ES  $\times$  DHA interaction,  $P > 0.05$ ; linearity of DHA effect,  $P < 0.001$ ; and quadraticity of DHA effect,  $P > 0.05$ ).

reported by Peyraud and Delagarde (2013) for dairy cows (i.e., 0.15 kg DM eaten/kg DM of herbage offered) within the typical range of herbage allowance for dairy systems. Herbage intake by growing horses (1 and 2 yr old) was unaffected by DHA in the range of 130 to 200 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> (Mésochina et al., 2000), which suggests that herbage intake reaches a plateau for high DHA. No study on grazing horses has been reported with the same range of DHA as that tested in this experiment. Conversely, under low DHA conditions, mares grazed closer to the ground (2.9 cm on LOW vs. 5.7 cm on HIGH) and probably ingested a greater proportion of fiber, which decreased herbage digestibility by 10 g/kg DM from high to low DHA, that is, a 2% relative variation. However, the decrease in HDDMI observed between HIGH and LOW (-22%) was mostly explained by a decrease in HDMI (-21%), with regrowth quality remaining high (15% DM for CP and 56% DM for NDF). Our results are entirely consistent with those previously reported for dairy cows grazing at 3 DHA (Peyraud et al., 1996): herbage OM digestibility linearly decreased by 2% from high to low DHA, with a simultaneous curvilinear decrease in herbage intake (-4% between high and medium DHA



**Table 4.** Grazing time and herbage intake rate according to energy supplementation (ES; supplemented with 2.6 kg DM barley/d [SUP] and nonsupplemented [NSUP]) level and daily herbage allowance (DHA; low [LOW], medium [MED], and high [HIGH])

Item	SUP			NSUP			RMSE <sup>1</sup>	P-value				
	LOW	MED	HIGH	LOW	MED	HIGH		ES	DHA	ES × DHA	Lin <sup>2</sup>	Quad <sup>2</sup>
Grazing time												
Total, min/d	956	1,018	1,050	965	1,028	989	52.7	0.673	<0.01	0.115	<0.05	<0.05
First 12 h, <sup>3</sup> min	661	656	654	631	635	632	12.4	0.182	0.848	0.597	0.599	0.832
Last 12 h, <sup>4</sup> min	359	438	439	357	397	406	46.5	0.317	<0.01	0.508	<0.01	0.073
Herbage IR, <sup>5</sup> g DM/min	11.4	11.3	12.9	11.7	12.6	14.3	1.6	0.149	<0.01	0.682	<0.01	0.249

<sup>1</sup>RMSE = root mean square error.

<sup>2</sup>Lin = linearity of DHA effect; Quad = quadraticity of DHA effect.

<sup>3</sup>First 12 h in a strip from 0915 to 2115 h.

<sup>4</sup>Last 12 h in a strip from 2115 to 0915 h.

<sup>5</sup>IR = intake rate.

vs. -15% between medium and low DHA). In the present experiment, mares first decreased their intake rate from high to medium DHA (-12%) and then their daily grazing time from medium to low DHA (-6%). At a low DHA, the mares' grazing activity was mainly reduced during the last hours of their presence on a strip, probably as a result of limiting grazing conditions such as low postgrazing sward height. Here, a reduction in DHA of 50% decreased pasture intake per mare by 21% and increased pasture intake per unit area by 54%. This reveals that grazing management designed to maximize individual animal performance is unlikely to maximize pasture utilization rate. Additional studies, therefore, are needed to establish the relationship between herbage allowance, animal intake, and pasture utilization by lactating mares to reach the best equilibrium between individual performance and efficient pasture utilization (Peyraud and Delagarde, 2013).

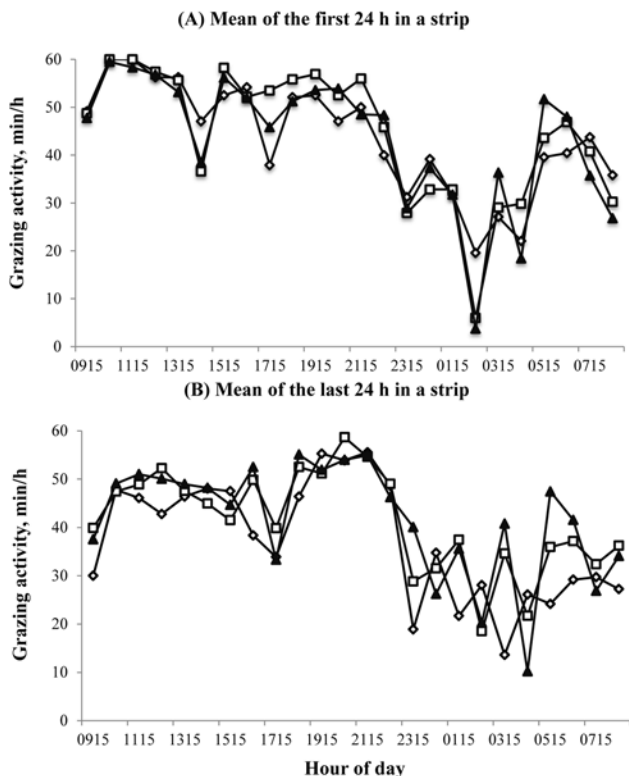
### Energy Supplementation

Energy supplementation affected neither mares' herbage intake nor their grazing behavior. The substitution rate (i.e., kg DM reduction in herbage intake/kg DM of concentrate eaten) was, therefore, close to zero ( $0.36 \pm 0.12$ ), which could be explained by the relatively restricted pasture conditions. Substitution rates have been reported to be higher in dairy cows when DHA is high (Meijs and Hoekstra, 1984; Grainger and Mathews, 1989; Bargo et al., 2002; McEvoy et al., 2008). Literature is scarce on substitution between forages and concentrate in horses. However, substitution has been observed at the trough when horses received forages ad libitum, which may indicate a general trend (Agabriel et al., 1982; Martin-Rosset and Doreau, 1984; Martin-Rosset and Dulphy 1987; Winsco et al., 2013; INRA, 2015). The substitution rate calculated in the present experiment (i.e., 0.36 on average) is, therefore,

logically among the lowest values reported for horses fed indoors, which are between 0.3 and 2.4 according to forage type and quality (INRA, 2015). Here, mares receiving barley thus achieved a greater total daily intake (i.e., +19% TDDMI and +24% NEI) than NSUP mares. Supplemented mares met their energy requirements whatever the DHA, whereas NSUP mares fell short of their requirements at low or medium DHA: 85 and 94%, respectively, using the INRA system or 82 and 95%, respectively, using the NRC system. From the linear effect of DHA on HDMI, we estimate at  $66 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  (i.e.,  $39 \text{ kg DM} \cdot \text{mare}^{-1} \cdot \text{d}^{-1}$ ) the DHA threshold under which ES is needed for lactating mares to meet their energy requirements on high-quality regrowth (Fig. 2). This DHA threshold corresponds to a postgrazing sward height of 5.4 cm considering the positive linear relationship between DHA and postgrazing SSH. A postgrazing SSH of 5 cm could, therefore, be considered a rough reference indicator for this type of pasture and grazing management. However, because postgrazing SSH is usually linked to the pregrazing SSH under rotational grazing, at least in dairy cows (Pérez-Prieto et al., 2013), it would be worthwhile extending this analysis to a wider range of pregrazing sward structures.

### Energy Supplementation × Daily Herbage Allowance Interaction

We did not find any effect of the ES × DHA interaction on herbage intake using an amount of barley representative of what is observed in commercial farms. However, on the high-DHA treatment, mares may have decreased herbage intake if they had received more concentrate. Increasing the proportion of concentrate from 15 to 64% of TDMI in growing horses fed maize silage offered ad libitum decreased silage intake (Agabriel et al., 1982). Also, in a previous grazing study in which supplemented mares received 2.6 kg DM barley, their herbage intake was



**Figure 3.** Daily pattern of grazing activity of mares during the 2 d of grazing in a strip according to daily herbage allowance (○ = low, □ = medium, and ▲ = high). (A) Mean of the first 24 h in a strip and (B) mean of the last 24 h in a strip (mean grazing time in a strip: low vs. medium,  $P < 0.05$ ; low vs. high,  $P < 0.05$ ; and medium vs. high,  $P > 0.05$ ).

significantly lower than that of NSUP mares when DHA was greater than in this study ( $124 \text{ g DM}\cdot\text{kg BW}^{-1}\cdot\text{d}^{-1}$ ; Collas et al., 2014). These different results suggest possible ES  $\times$  DHA interactions outside the range tested in the present experiment, with substitution rate being positively related to DHA as observed in ruminant studies.

## Conclusion

For lactating mares grazing high-quality regrowth during the summer, increasing DHA from 35 to 70  $\text{g DM}\cdot\text{kg BW}^{-1}\cdot\text{d}^{-1}$  linearly increased HDMI ( $0.13 \text{ kg DM eaten/kg DM of herbage offered}$ ), whether or not mares were supplemented with barley. The effects of DHA and ES were additive, so that supplemented mares always met their energy requirements, whereas NSUP mares were underfed at low or medium DHA. The positive linear relationship we established between DHA and HDMI enables us to estimate the herbage allowance threshold ( $66 \text{ g DM}\cdot\text{kg BW}^{-1}\cdot\text{d}^{-1}$  or  $39 \text{ kg DM}\cdot\text{mare}^{-1}\cdot\text{d}^{-1}$ ) under which lactating mares should be supplemented with barley to meet their energy requirements. Further investigation of the interactions between concentrate supplementation level, herbage allowance, and herbage nutritive value are needed to improve the efficiency of mare nutrition and maintain a high pasture utilization rate.

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