Comparison of the nutritive value of Madder, *Rubia tinctorum* L. and Alfalfa, *Medicago sativa*. using *in vitro* and *in situ* measurements

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Abstract This study was conducted to determine the chemical composition, digestibility, degradability and nutritive value of madder, *Rubia tinctorum* L. and alfalfa *Medicago sativa.* using *in vitro* and *in situ* measurements. The mean values for OM, CP, NDF, ADF and EE were 892.5, 172.0, 306.5, 219.4 and 16.0 g/kg DM for madder hay, and 921.3, 141.4, 482.0, 361.1 and 6.50 g/kg DM for alfalfa hay, respectively (P<0.05). The mean digestibility coefficients for DM and OM, DOMD and ME in madder hay (664.6, 556.4, 490.7 g/kg and 7.81 MJ/kg DM) were significantly higher (P<0.05) than those for alfalfa hay (510.9, 441.7, 403.6 g/kg and 6.47 MJ/kg DM, respectively). The effective degradability of dry matter (EDDM) of madder hay (63.25 %) was also significantly higher (P<0.05) than alfalfa hay (48.32 %). The average nutritive value indexes (NVI) of DM and CP for madder hay (50.59 and 70.82 %) was higher than alfalfa hay (40.60 and 65.21%, respectively). In conclusion, considering chemical composition, kinetic digestion and degradation data, madder hay has the potential to be used as a suitable forage source for small farmers during critical periods of year when feed resources are limited, especially in semiarid areas.

Keywords: madder hay, alfalfa hay, *in situ* degradability, *in vitro* digestibility

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Introduction

The high cost of animal feed in semi-arid countries, is a major limitation to sheep production which is raised predominately under extensive systems. Therefore, utilization of alternative feed resources may be a logical and suitable strategy in low input systems (Khezri et al., 2004). High quality forages are crucial for proper feeding of ruminant animals since they provide energy, protein and minerals. *Rubia tinctorum*, the common madder, is a perennial legume plant, widespread in India, Iran, Pakistan, Spain, Italy, Turkey, Syria, China and North America (Medical Economics Co., 2000).

Large amounts of madder, produced annually in semiarid and arid regions, may have the potential to be uses as forage source for small ruminants (Sepaskhah and Beirouti, Z., 2009; Nakanishi et al., 2005). There is no information on the nutritive value and ruminal digestion of madder hay; therefore, the aim of this study was to determine the nutritive value of madder hay and kinetics of its digestion in comparison to alfalfa hay, using *in vitro* and *in situ* techniques.

Material and methods

Forage sampling and chemical composition

Madder and alfalfa hays were harvested at late bloom stage in Yazd province (central-region of Iran) under similar conditions. Forage samples were dried in a forced-air oven (60° C) and ground to pass a 1-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA, USA). Nitrogen (N) content was measured by the Kjeldahl method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hogans, Sweden) and crude protein (CP) was calculated as N×6.25. Ash-free neutral detergent (NDFom) and acid detergent (ADFom) fibers were determined without sodium sulfite and alpha amylase (Udén et al., 2005), and expressed exclusive of residual ash (Van Soest et al., 1991). Ether extract (EE) was determined according to AOAC (2000, ID 7.060), and ash by the AOAC (2000, ID 942.05).

In vitro digestibility of DM (IVDMD) and OM (IVOMD)

Three ruminally fistulated Kermani rams (47±3 kg BW) fed a diet twice daily containing alfalfa hay (60%) and concentrate (40%) were used. The concentrate consisted of barley grain (73%), soybean meal (25%), calcium carbonate (0.6%), salt (0.4%) and vitamin and mineral mixture (1%). (Vitamin mixture contained: 5,000,000

IU of Vitamin A; 5,000,000 IU of vitamin D and 500,000 IU of Vitamin E per kg, and mineral mixture composition was: 82.5% Dynamad, 12.26 % Mn, 4.12 % Cu-sulfate, 0.06 % EDDI-80 and 0.044 % Na-selenide). Samples of madder and alfalfa hays were incubated with rumen fluid as described by Tilley and Terry (1963). Whole ruminal contents were collected from different parts of rumen before the morning feeding (08:00 h) by a vacuum pump and filtered through 4 layers of cheesecloth into a warmed thermos bottle that had been flushed with CO_2. The incubation inoculum was prepared by diluting the digesta inoculum with artificial saliva (Tilly and Terry, 1963) in a 1:4 (vol/vol) ratio and stirring in a water bath at 39°C with purging CO_2 until its use (10–15 min later). Samples (0.5 g dry weight) of madder and alfalfa hay were weighed into sterile plastic tubes (six replicates for each) and 20 mL of the incubation inoculum were added. Tubes were sealed with rubber stoppers, incubated for 48 h at 39 °C, and gently swirled by hand four times every 12 h. At the end of the 48 h- incubation period, the content of each tube was acidified by adding 6 M HCl to reach a final pH of 1.3–1.5. After the foam subsided, pepsin powder was added to a final concentration of 0.2% (wt/vol). The tubes were reincubated for an additional 48 h. The tubes were centrifuged at 2500 × g for 15 min, and the supernatant was discarded. To the pellet, 50 mL of H_2O was added and the tubes were recentrifuged to wash out the residual acid. The tubes containing the pellets were dried in a forced-air oven at 60°C for 48 h for determination of the weight of the residues. IVDMD and IVOMD were calculated as the DM and OM which disappeared from the weight of sample transferred into the tubes. The ME values of the forages were calculated using the equation of AFRC (1993) as ME (MJ/kg DM) = 0.016 DOMD (g/kg DM).

In situ ruminal degradability of DM and CP

Three ruminally fistulated Kermani rams, weighting 47 ± 3 kg and consuming 1.2 ± 0.2 kg DM per day, were used. The sheep were fed a mixed ration containing alfalfa hay (60%) and concentrate (40%) twice daily at 08:00 and 17:00 h. The *in situ* technique (Orskov and McDonald, 1979) was used to measure the kinetics of DM and CP degradation of forages. Dried samples (2 g) were weighed into 5 × 13- cm nylon bags (50 µm pore size), 9 bags for each sample and each incubation time, and incubated in the rumen for 3, 6, 12, 24, 48, 72 or 96 h. The bags were removed after incubation in the rumen and washed in cold running tap water until the washing was clear. Zero time disappearance was obtained by washing the unincubated bags in a similar way. All washed bags were dried in an air-forced oven (60 °C, 48 h) and the protein content of the remaining matter was determined. The *in situ* DM or CP degradation was fitted to the exponential equation "$y= a+b\ (1-e^{-ct})$" (Orskov and McDonald, 1979), where "y" is the disappearance rate at time "t", "a" the soluble DM or CP fraction which is rapidly washed out of the bags and assumed to be completely degradable, "b" the proportion of insoluble DM or CP which is potentially degradable by the ruminal microorganisms, and "c" is the degradation rate of fraction "b" per hour. The effective degradability (ED) was calculated by the equation ED (%) = a + (b×c) / (c+k); assuming the passage rate of the digesta from the rumen (K) at 0.02 h^{-1}, which is an average value for animals fed at approximately the maintenance level (AFRC, 1993). The nutritive value index (NVI) of each nutrient was calculated using the equation NVI=a+0.4b+200c, with "a", "b" and "c" being explained earlier (Orskov and McDonald, 1979).

In situ ruminal disappearance of OM and NDF

Dried samples (2 g) were weighed into 5 cm ×13 cm bags (50 µm pore size), and 9 bags for each sample were incubated in the rumen for 24 h (Riasi et al., 2008). After incubation, the bags were rinsed with cold tap water until the rinse water was clear, and then dried for 48 h in a 60°C forced-air oven. Dried samples were analyzed for OM (AOAC, 2000, ID 942.05) and NDF (Van Soest et al., 1991). The OM and NDF disappearance were calculated in a similar way to DM and CP disappearance.

Statistical analysis

For both experiments, data were analyzed using the general linear model procedure (SAS, 2005) according to a completely randomized design as $Y_{ij}= \mu + T_i + \varepsilon_{ij}$, where μ is mean, T_i is the experimental treatment effect and ε_{ij} is the random error. Mean differences were considered significant at $P<0.05$.

Results

The chemical analysis of madder and alfalfa hays is shown in Table 1. The OM, CP, NDF, ADF and EE contents of madder hay was different ($P<0.05$) from alfalfa hay. Table 2 shows the mean digestibility of DM, OM, DOMD and ME of madder and alfalfa hay samples by the *in vitro* method. The digestibility coefficients of DM and OM, DOMD and ME for madder hay were higher than those for alfalfa hay. In the present study, DM and CP disappearance of madder and alfalfa hay samples increased with increasing incubation time (Tables 3 and 4). At all incubation times (Tables 3 and 4), DM and CP disappearance of madder hay was significantly higher

Table 1. Chemical composition (DM basis) of madder and alfalfa hays

Constituent	Madder hay	Alfalfa hay	SEM	*P value*
DM (g/kg)	923.1	951.2	10.3	Ns
OM (g/kg DM)	892.5	921.3	6.4	*
CP (g/kg DM)	172.0	141.4	5.3	**
NDF (g/kg DM)	306.5	482.0	12.6	**
ADF (g/kg DM)	219.4	361.1	11.8	**
EE (g/kg DM)	16.0	6.50	1.2	**

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber exclusive of residual ash, ADF: acid detergent fiber exclusive of residual ash, EE: ether extract, SEM: standard error of the mean, *: P <0.05, **: P <0.01, ns: non-significant.

Table 2. Mean digestibility (DM basis) of dry matter (DM), orgnic matter (OM), organic dry matter digestibility (OMD) and metabolizable (ME) of madder and alfalfa hays by *in vitro* method

	Madder hay	Alfalfa hay	SEM	*P* value
Digestibility of DM (g/kg)	664.6	510.9	23.6	**
Digestibility of OM (g/kg DM)	556.4	441.7	24.9	*
DOMD (g/kg DM)	490.7	403.6	14.4	*
ME (MJ/kg DM)	7.81	6.47	0.25	*

DM: dry matter, OM: organic matter, DOM: digestible organic matter, DOMD: digestible organic matter in dry matter, ME: metabolizable energy, SEM: standard error of the mean, *: P <0.05, **: P <0.01.

(P<0.05) than that for alfalfa hay. Table 4 shows the rapidly degradable CP fraction (%) of madder and alfalfa hays. Although no significant difference (P>0.05) was observed between madder and alfalfa hays for the slowly degraded CP fraction, there was a significant difference (P < 0.05) between the disappearance rate of CP (%) for madder and alfalfa hays. The NVICP values were similar to NVIDM values, and higher (P<0.01) for madder hay in comparison to alfalfa hay (Table 4). Ruminal OM and NDF disappearance data (Table 5) indicated that madder hay had higher values than alfalfa hay.

Discussion

Significant differences were recorded between the chemical composition of madder and alfalfa hays, including OM, CP, NDF, ADF and EE but not DM. At authors' knowledge, there is no information considering the chemical composition of madder hay, but the current findings on alfalfa hay are in agreement with those of Mirzaei-Aghasghali et al. (2008) and Karabulut et al. (2006). Factors such as maturity, location and plant growing conditions have great impacts on the nutritive value of forages (Flachowsky et al., 1993; Long et al.,

Table 3. Dry matter disappearance (%) of madder and alfalfa hays in the rumen by *in situ* method

	Madder hay	Alfalfa hay	SEM	*P* value
Incubation times (h)				
3	42.57	33.39	0.85	**
6	45.16	37.78	0.91	**
12	57.52	40.05	0.52	**
24	68.45	46.31	0.69	**
48	79.981	58.47	0.43	**
72	82.23	61.29	0.36	**
96	83.66	62.50	0.81	**
Estimated parameters				
a (%)	10.75	8.77	0.66	**
b (%)	73.4	62.03	0.78	**
c (h^{-1})	0.05	0.035	0.005	**
EDDM (%)	63.25	48.32	0.94	**
NVIDM (%)	50.59	40.60	0.35	*

A: rapidly degradable fraction, b: slowly degradable fraction, c: rate constant of degradation of the b fraction, EDDM: effective degradability of dry matter calculated at ruminal outflow rate of 0.02 h^{-1}, NVIDM: nutritive value index of dry matter, SEM: standard error of the mean, *: P <0.05, **: P <0.01.

Table 4. Crude protein disappearance (%) of madder and alfalfa hays in the rumen by *in situ* method

	Madder hay	Alfalfa hay	SEM	*P* value
Incubation times (h)				
3	64.82	61.48	0.72	ns
6	72.89	64.32	0.58	*
12	74.95	65.26	0.61	*
24	80.76	74.42	0.47	ns
48	92.26	82.11	0.28	**
72	93.33	83.25	0.42	**
96	94.13	84.28	0.26	**
Estimated parameters				
a (%)	41.19	39.09	0.53	*
b (%)	53.99	50.33	0.46	ns
c (h^{-1})	0.04	0.03	0.003	*
EDCP (%)	77.21	69.24	0.48	*
NVICP (%)	70.82	65.21	0.62	*

a: rapidly degradable fraction, b: slowly degradable fraction, c: rate constant of degradation of the b fraction, EDCP: effective degradability of crude protein calculated at ruminal outflow rate of 0.02 h^{-1}, NVICP: nutritive value index of crude protein, SEM: standard error of the mean, *: P <0.05, **: P <0.01, ns: non-significant.

Table 5. Organic matter and NDF disappearance (%) of madder and alfalfa hays in the rumen using an in situ procedure

	Madder hay	Alfalfa hay	SEM	P value
OM	82.23	68.29	0.86	**
NDF	55.72	49.01	0.93	**

OM: organic matter, NDF: neutral detergent fiber, SEM: standard error of the mean, **: P <0.01.

1999). Higher mean digestibility coefficients of DM, OM, DOMD and ME for madder hay are consistent with its lower NDF and ADF contents (306.5, 219.4 g/kg DM) in comparison to alfalfa hay (482.0, 361.1 g/kg DM, respectively). Generally, as cell wall (NDF and ADF) increases, the digestibility and in turn the energy content of the feed are negatively affected (Dayani et al., 2012).

The "a" fraction represents that part of the DM that is soluble or the fine particles that escape through the bag pores, and is affected by plant physical structure, and NDF and ADF content (Griffin et al, 1994). This may explain the higher "a" fraction for madder hay. The *in situ* parameters, EDDM and NVIDM, were also significantly higher for madder than alfalfa hay, which might be due to more rapidly degradable DM fraction "a" in madder hay (Table 3). Similar results were reported by Mansouri et al (2004) who studied the alfalfa hay, grass hay and wheat straw. Regarding the *in situ* degradability of protein, "a" fraction, EDCP and NVICP values were higher for madder hay in comparison to alfalfa hay. For comparative purposes, there is no information available on the digestion kinetics and disappearance of madder hay in the rumen. The data on rapidly degradable fractions of DM in the present study showed that alfalfa hay was low in rapidly degradable fractions of DM in comparison to madder hay, which can reduce voluntary DM intake (Van Soest, 1994). Furthermore low rapidly degradable DM fractions of alfalfa hay could be due to its high NDF and ADF contents as shown in Table 1. The higher ruminal disappearance of NDF in madder hay may be related to plant anatomy of the cell wall and also Cell-wall concentration (phenolic acids and lignin) which may have positively influenced its digestibility. Jung and Engels (2002) reported that the proportion of soluble DM, lignin and hemicellulose in feeds may account for differences in their cell wall digestibility.

Conclusions

Based on the results of the current study, madder hay appears to have more beneficial chemical nutrient components and nutritional values than alfalfa hay. In regard to the results of our experiment and the high production level of this forage, madder hay may have the potential to be used as forage for small farmers during critical periods of year when feed resources are limited, especially in semiarid areas. This needs to be verified by studying the performance of animals being fed with this forage.

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مقایسه ارزش غذایی علوفه روناس (*Rubia tinctorum* L.) و علوفه یونجه (*Medicago sativa*) با استفاده از روش های *in vitro* و *in situ*

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چکیده هدف از این مطالعه تعیین ترکیب شیمیایی، قابلیت هضم، تجزیه پذیری و ارزش غذایی علوفه روناس و یونجه بااستفاده از روش های *in vitro* و *in situ* بود. در مطالعه حاضر میانگین ماده آلی، پروتئین خام، الیاف نامحلول در شوینده خنثی، الیاف نامحلول در شوینده اسیدی و عصاره اتری به ترتیب برای علوفه روناس ۸۹۲/۵، ۱۷۲/۰، ۳۰۶/۵، ۲۱۹/۴ و ۱۶ گرم در کیلوگرم ماده خشک و برای علوفه یونجه ۹۲۱/۳، ۱۴۱/۴، ۴۸۲/۰، ۳۶۱/۱ و ۶/۵ گرم در کیلوگرم ماده خشک بوده و اختلاف معنی داری را نشان داد (p<۰/۰۵). میانگین ضرائب قابلیت هضم برای ماده خشک، ماده آلی، ماده آلی قابل هضم در ماده خشک و انرژی متابولیسمی برای علوفه روناس (به ترتیب ۶۶۴/۶، ۵۵۶/۴، ۴۹۰/۷ گرم در کیلوگرم ماده خشک و ۷/۸۱ مگاژول در کیلوگرم ماده خشک) به طور معنی دار بالاتر از علوفه یونجه (به ترتیب ۵۱۰/۹، ۴۴۱/۷، ۴۰۳/۶ گرم در کیلوگرم ماده خشک و ۶/۴۷ مگاژول در کیلوگرم ماده خشک) بود (p<۰/۰۵). تجزیه پذیری موثر ماده خشک علوفه روناس (۶۳/۲۵) به طور معنی دار بالاتر از علوفه یونجه (۴۸/۳۲ درصد) بود. همچنین در مطالعه حاضر شاخص های ارزش تغذیه ای برای ماده خشک و پروتئین خام علوفه روناس (به ترتیب ۵۰/۵۹ و ۷۰/۸۲ درصد) بالاتر از علوفه یونجه (به ترتیب ۴۰/۶ و ۶۵/۲۱ درصد) بود (p<۰/۰۵). به طور کلی نتایج بدست آمده از ترکیب شیمیایی و داده های هضم و تجزیه پذیری علوفه روناس در مقایسه با یونجه نشان می دهد که علوفه روناس قابلیت استفاده به عنوان منبع علوفه ای در دامداری های کوچک و در شرایط محدود منابع خوراکی خصوصا در مناطق نیمه خشک را دارد.