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Prediction of feeding value of some Tunisian hays using chemical composition and *in vitro* fermentation characteristics

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Abstract. Chemical composition, intake and *in vivo* digestibility by sheep of oat and four vetch-oat (respectively at flowering, milky, pasty and hard-grain stages), alfalfa and ray-grass hays were determined. Samples of these hays were incubated for *in vitro* fermentation studies. Correlations considering *in vivo* and *in vitro* parameters and chemical composition were established. Crude protein (CP) content was highest in alfalfa (140 g/kg DM) and ray-grass (100 g/kg DM) hays, while oat based hays were low in CP (mean value: 58 g/kg DM). All hays showed high cell wall contents (ranged from 363 to 472 g/kg DM). Lignification was highest in alfalfa (135 g/kg DM) and in hard-grain stage vetch-oat hays (103 g/kg DM) and the lowest in ray-grass hay (53 g/kg DM). *In vitro* gas production parameters were similar in all the hays (c and asymptotic gas averaged 0.053 h⁻¹ and 55.8 ml, respectively). The *in vitro* organic matter digestibility (IVOMD_{24h}) after 24 h of incubation ranged from 45.5% in pasty-stage vetch-oat hay to 55.13% in alfalfa hay. Calculated metabolisable energy (ME) ranged from 1472 kcal/kg DM in oat hay and 1813 kcal/kg DM in alfalfa hay. Voluntary dry matter intake (DMI) was highest in alfalfa hay (1912 g day⁻¹) and lowest in hard-grain vetch-oat hay (726 g day⁻¹). The digestible organic matter intake (DOMi) was highest in alfalfa hay (899 g day⁻¹) and lowest in hard-grain vetch-oat (344 g day⁻¹). Digestible crude protein intake (DCPi) averaged 54.1 g day⁻¹ and varied from 10.5 to 151.9 g day⁻¹ respectively in hard-grain vetch-oat and alfalfa hays. Significant negative correlations were found between IVOMD_{24h} and NDF (r= -0.79, P<0.05), ADF (r= -0.78, P<0.05) and ADL (r=-0.88, P<0.01) contents. Digestible OM intake (DOMi) and digestible CP intake (DCPi) were significantly (P<0.01) and positively correlated with CP content (r=0.89 and 0.97, respectively). Highly significant (P<0.01) correlations were found between DOMi and CPi and 24 h gas production (r=0.97 and 0.93, respectively) and also with IVOMD_{24h} (r=0.89 and 0.97, respectively). It was concluded that oat based hays are of low feeding value. The correlations suggest that predictive equations of intake, digestibility and feeding value from chemical composition and *in vitro* parameters could be derived considering a greater number of forages in the analysis.

Keywords. Feeding value – *In vitro* fermentation – *In vivo* digestibility – Hays.

Prédiction de la valeur alimentaire de quelques foins tunisiens à partir de la composition chimique et des caractéristiques de fermentation *in vitro*

Résumé. On a déterminé la composition chimique, l'ingestion et la digestibilité *in vivo* chez les ovins pour du foin d'avoine et quatre foins de vesce-avoine (respectivement aux stades floraison, laiteux, pâteux et grain dur), luzerne et ray-grass. Des échantillons de ces foins ont été incubés pour des études de fermentation *in vitro*. On a établi des corrélations considérant les paramètres *in vivo* et *in vitro* et la composition chimique. La teneur en protéine brute (CP) était la plus élevée chez les foins de luzerne (140 g/kg DM) et de ray-grass (100 g/kg DM), tandis que les foins d'avoine avaient une faible CP (valeur moyenne: 58 g/kg DM). Tous les foins avaient de fortes teneurs en parois cellulaires (allant de 363 à 472 g/kg DM). La lignification était la plus forte pour le foin de luzerne (135 g/kg DM) et pour le foin de vesce-avoine au stade grain dur (103 g/kg DM) et la plus faible pour le foin de ray-grass (53 g/kg DM). Les paramètres de production de gaz *in vitro* étaient semblables pour tous les foins (c et gaz asymptotique étaient en moyenne de 0,053 h⁻¹ et 55,8 ml respectivement). La digestibilité *in vitro* de la matière organique (IVOMD_{24h}) après 24 h d'incubation allait de 45,5% pour le foin de vesce-avoine au stade pâteux, à 55,13% pour le foin de luzerne. L'énergie métabolisable calculée (ME) était comprise entre 1472 kcal/kg DM pour le foin d'avoine et 1813 kcal/kg DM pour le foin de luzerne. L'ingestion volontaire de matière sèche (DMI) était la plus forte pour le foin de luzerne (1912 g/jour) et la plus faible pour le foin de vesce-avoine au stade grain dur (726 g/jour). L'ingestion de matière organique digestible (DOMi) était la plus élevée pour le foin de luzerne (899 g/jour) et la plus faible pour le foin de vesce-avoine au stade grain dur (344 g/jour).

L'ingestion de protéine brute digestible (DCPi) était en moyenne de 54,1 g/jour et variait de 10,5 à 151,9 g/jour respectivement pour les foins de vesce-avoine au stade grain dur et de luzerne. Des corrélations négatives significatives ont été trouvées entre IVOMD_{24h} et les teneurs en NDF ($r=-0,79$, $P<0,05$), ADF ($r=-0,78$, $P<0,05$) et ADL ($r=-0,88$, $P<0,01$). L'ingestion d'OM digestible (DOMi) et l'ingestion de CP digestible (DCPi) étaient corrélées significativement ($P<0,01$) et positivement à la teneur en CP ($r=0,89$ et $0,97$ respectivement). Des corrélations très significatives ($P<0,01$) ont été trouvées entre DOMi et CPi et production de gaz 24 h ($r=0,97$ et $0,93$ respectivement) et également avec IVOMD_{24h} ($r=0,89$ et $0,97$ respectivement). On en a conclu que les foins basés sur l'avoine ont une faible valeur alimentaire. Les corrélations suggèrent que des équations prédictives d'ingestion, digestibilité et valeur alimentaire à partir de la composition chimique et des paramètres *in vitro* pourraient être dérivées en considérant un plus grand nombre de fourrages dans l'analyse.

Mots-clés. Valeur alimentaire – Fermentation *in vitro* – Digestibilité *in vivo* – Foins.

I – Introduction

In spite of the spectacular development of grass silage in Tunisia, hays continue to be the most used basal forage for ruminant feeding. However, their nutritive value is extremely variable since it depends on the plant physiological stage, the period and weather when harvested and the conditions of conservation. This variability is well illustrated mainly by the variability of chemical composition and organic matter digestibility, frequently observed in local studies even in the same species. By another hand, *in vivo* methods to determine feeding value of forage are laborious and costly, mainly in developing countries. Consequently it is crucial to test simple and cost effective methods providing satisfactory precision to evaluate forages. In the current study, we aimed to test chemical composition and the *in vitro* cumulative gas production procedure to predict the feeding value of some common Tunisian hays.

II – Material and methods

1. Plant material

Oat and four vetch-oat (respectively at flowering, milky, pasty and hard-grain stages), alfalfa and ray-grass hays were studied. They were collected from different farms in the north of Tunisia. Samples from each hay were taken from different sites and mixed to make an overall sample. Dry matter (DM) was determined at 105°C in a forced-air oven and a part of each sample was dried at 40°C during 48h and then ground to pass through 1 mm screen and stored for chemical analysis and *in vitro* determinations.

2. Animals and measurements

Four adult local sheep housed in individual digestibility cages (average age and liveweight: 28 months and 45 kg respectively) were used to determine voluntary intake and *in vivo* digestibility of hays. Each experimental period consisted on 15 days of adaptation and 7 days of measurements.

Three adult local sheep with rumen cannula (average age and liveweight: 24 months and 48.5 kg respectively) were used for *in vitro* determinations. They were housed in individual pens and received twice daily a ration ($70 \text{ g kg}^{-1} \text{ LW}^{0.75}$) composed of 70% of oat-vetch hay and 30% of barely concentrate on dry matter (DM) basis.

Samples (300 mg DM) of hays were incubated in triplicate in 100 ml glass syringes according to the technique of Menke and Steingass (1988). Incubation medium (30 ml) was composed of rumen fluid diluted 1:2 with a Menke buffer solution. Gas production was read for 1, 2, 4, 6, 12, 24, 36, 48, 72 and 96 h. Diets were incubated in triplicate and two successive incubations were carried out.

3. Chemical analysis

Feeds were analysed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cell wall composition (NDF, ADF and ADL) in feeds were analyzed as described by Van Soest *et al.*, (1991).

4. Calculation and statistical analysis

Gas productions were fitted using the non-linear model of Ørskov and McDonald (1979): $p=a+b(1-e^{-ct})$, where: (i) "p" is the gas production at time t; (ii) "a" is the immediate gas production; (iii) "b" is the slowly fraction of gas production; (iv) "c" is the rate of gas production; and (vi) "a+b" is the total gas production. Parameters were calculated using the Non Linear procedure (SAS, 1996). *In vitro* organic matter digestibility at 24h (IVOMD_{24h}) and metabolisable energy (ME) were calculated according to the specific equation of Menke and Steingass (1988). Predictive equations of intake, digestibility and feeding value from chemical composition were established using GLM procedure (SAS, 1996).

III – Results and discussion

Chemical compositions of the studied hays are presented in Table 1. Ash content ranged from 79 to 112 g/kg respectively in alfalfa hay and ray-grass hay. Crude protein was the highest in alfalfa hay (140 g/kg DM) and the lowest in vetch-oat hay at milky stage (40 g/kg DM). The crude protein content decreased with increased maturity stage. These results are in concordance with those found by Kamalak *et al.* (2004) who studied the effect of maturity stage on chemical composition of tumbleweed hay in Turkey. All hays have approximately equivalent CF content (averaged 354.4 g/kg DM). On the contrary, lignification level was the lowest in ray-grass hay (ADL: 53.3 g/kg DM) and the highest in alfalfa hay (ADL: 135 g/kg DM).

Table 1. Chemical composition of hays

Hays	Ash	CP	CF	NDF	ADF	ADL
Alfalfa hay	79	140	374	573	462	135
Ray-grass hay	112	100	314	689	362.8	53.3
Oat hay	84	55.1	383	757	481	83
Vetch-oat hay (flowering stage)	91	65	372	680	454	74
Vetch-oat hay (milky stage)	94	68	382	670	478	78
Vetch-oat hay (pasty stage)	80	63	392	700	481	81
Vetch-oat hay (hard grain stage)	82	40	364	687	474	103

In vitro fermentation parameters of the studied hays are presented in Table 2. *In vitro* gas production parameters were similar in all the hays (c and asymptotic gas a+b averaged respectively 0.053 h⁻¹ and 55.8 ml). The *in vitro* organic matter digestibility after 24 h of incubation (IVOMD_{24h}) ranged from 45.5% in pasty-stage vetch-oat hay to 55.1% in alfalfa hay. Calculated metabolisable energy (ME) ranged between 1472 kcal/kg DM in oat hay and 1813 kcal/kg DM in alfalfa hay.

In vivo digestibility parameters are presented in table 3. Voluntary dry matter intake (DMI) was the highest in alfalfa hay (1912 g day⁻¹) and the lowest in hard-grain vetch-oat hay (726 g day⁻¹), Vetch-oat hay DMI decreased with harvesting stage from 944 g day⁻¹ at flowering stage to 726 g day⁻¹ at hard grain stage. The digestible organic matter intake (DOMI) was the highest in alfalfa hay (899 g day⁻¹) and the lowest in hard-grain vetch-oat (344 g day⁻¹). Digestible crude protein intake (DCPI) averaged 54.1 g day⁻¹ and varied from 10.5 to 151.9 g day⁻¹ respectively in hard-grain vetch-oat and alfalfa hays.

Table 2. *In vitro* parameters of incubated hays

Hays	a%	b%	c (h ⁻¹)	a+b (ml)	IVOMD _{24h} (%)	ME (kcal/kg DM)
Lucerne hay	-6.1	60.4	0.0781	54.2	55.1	1812.8
Ray-grass hay	-3.9	66.4	0.0537	62.5	51.8	1648.1
Oat hay	-7.3	68.5	0.0445	61.2	45.7	1471.9
Vetch-oat hay (flowering stage)	-1.2	58.4	0.043	57.2	47.1	1760.8
Vetch-oat hay (milky stage)	-2	55.6	0.046	53.6	46.1	1722.7
Vetch-oat hay (pasty stage)	-1.5	52.1	0.049	50.6	45.5	1702.3
Vetch-oat hay (hard grain stage)	-2.3	53.5	0.054	51.2	47	1761.1

Table 3. *In vivo* parameters of hays

Hays	DMi (g day ⁻¹)	DMD (%)	OMD (%)	CPD (%)	DOMi (g day ⁻¹)	DCPi (g day ⁻¹)
Alfalfa hay	1912	47.9	50.8	56.8	899.4	151.9
Ray-grass hay	1428	63.4	64.9	59.7	823	84.2
Oat hay	1118	55.9	57.6	47.4	589.7	31.4
Vetch-oat hay (flowering stage)	944	56.3	59.5	60.8	483	37.4
Vetch-oat hay (milky stage)	825	53.7	56.1	63.4	401.4	35.6
Vetch-oat hay (pasty stage)	733	51.9	54.1	59.6	350	27.5
Vetch-oat hay (hard grain stage)	726	51.6	54.4	36.2	343.8	10.5

Main correlations between *in vivo*, *in vitro* parameters and chemical composition are presented in Table 4. Significant negative correlations were found between DMD and ADF ($r=-0.78$, $P<0.05$) and ADL ($r=-0.882$, $P<0.01$) and significant positive correlations with total gas production a+b ($r=0.79$, $P<0.05$). Digestible OM intake (DOMi) and digestible CP intake (DCPi) were significantly ($P<0.01$) and positively correlated with CP content ($r=0.89$ and 0.97 , respectively) and with 24 h gas production ($r=0.97$ and 0.93 , respectively) and also with IVOMD_{24h} (respectively $r=0.89$ and $r=0.972$ $P<0.05$).

Table 4. Main correlation coefficients between chemical composition, *in vitro* parameters and feeding value

	Ash	CP	NDF	ADF	ADL	c	a+b	Gas 24h	Gas 48h	IVOMD _{24h}
DMi		0.958**						0.964**		0.926**
DMD				-0.783*	-0.882**		0.789*			
OMD	0.9**			-0.785*	-0.876**		0.772*			
DOMi		0.888**						0.975***	0.869*	0.89**
DCPi		0.972***	-0.774*			0.908**		0.935**	0.774*	0.972**

** : $P<0.01$. * : $P<0.05$.

Predictive equations of intake, digestibility and feeding value from chemical composition and *in vitro* parameters are shown in Table 5. All of them presented high determination coefficients, suggesting the possibility of establishing precise predictive equations among increasing the number of hays. This alternative could result in reducing time and cost feeding value determinations of hays.

Table 5. Predictive equations of intake, digestibility and feeding value from chemical composition and gas parameters

Equations	R2 (%)
OMD = 71.0 - 0.237 NDF - 0.368 ADF - 0.710 ADL - 0.497 CP + 0.517 (a+b)	99.9
OMD= 86 + 0.0187 MSi - 1.9 MAT - 315 NDF + 0.0751 ADF - 1.89 ADL	99.9
DMD = 74.4 + 0.0149 MSi - 1.39 CP - 0.0878 NDF - 0.0977 ADF - 1.69 ADL	99.9
DOMi = -2955.43+82.1 MM+70.94 CP+33.32 NDF-13.294 ADF+67.54 ADL	97
DOMi = -1763.07+42.5 G24+13.52 G48	96
DCPi = -189.76+4.1 MM+13.7 CP+1.389 NDF- 0.936 ADF+ 5.973 ADL	99
DCPi = -203.242 + 14.797 G24 - 6.249 G48	88

IV – Conclusions

It was concluded that oat based hays are of low feeding value. The correlations suggest that predictive equations of intake, digestibility and feeding value from chemical composition and *in vitro* parameters could be derived considering a greater number of forages in the analysis.

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