NUTRITIVE VALUE, AND *IN SITU* DRY MATTER AND PROTEIN DEGRADABILITY OF FRESH AND ENSILED ITALIAN RYEGRASS AT THREE STAGE OF GROWTH

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BORBA, ALFREDO E.S. & JOÃO M.C. RAMALHO RIBEIRO 1996. Nutritive value, and *in situ* dry matter and protein degradability of fresh and ensiled Italian ryegrass at three stage of growth. *Arquipélago*. Life and Marine Sciences 14A: 85-94. Ponta Delgada. ISSN 0873-4704.

The nutritive value of Italian ryegrass (*Lolium multiflorum*) cut at three stages of growth and in fresh and ensiled, was determined in this study. The highest metabolisable energy content was found at 5% ear in fresh forages, and at the fourth leaf stage in silages. *In vivo* digestibility and vonluntary intake increased from fourth leaf to the 5% ear stage of growth which was followed by a reduction at the 100% ear stage of growth. *In situ* dry matter and protein degradability decreased ($p \leq 0.05$) with advancing stage of growth. In fresh forages protein degradability was higher ($p \leq 0.05$) than in ensiled forages. It was concluded that the best harvest time for Italian ryegrass was at the 5% ear stage of growth for both fresh forage, as well as silage.

BORBA, ALFREDO E.S. & JOÃO M.C. RAMALHO RIBEIRO 1996. Variação do valor nutritivo e da degradabilidade *in situ* da matéria seca e da proteína bruta da erva castelhana com a época de corte e com o método de conservação. *Arquipélago*. Ciências Biológicas e Marinhas 14A: 85-94. Ponta Delgada. ISSN 0873-4704.

Neste trabalho determinámos o valor nutritivo da erva castelhana (*Lolium multiflorum*) em três épocas de corte, em verde e ensilada. O valor mais elevado de energia metabolizável foi determinada na fase de crescimento 5% espigado, nas forragens verdes e na fase de aparecimento da 4ª folha, para as silagens. Verificou-se um aumento da digestibilidade *in vivo* e da ingestão voluntária, da fase de 4ª folha para a fase 5% espigada, seguindo-se um decréscimo para a fase 100% espigada. A degradabilidade *in situ* da matéria seca, e da proteína bruta decresce ($p \leq 0.05$) com o avançar da fase de crescimento. A degradabilidade da proteína bruta é superior nas forragens verdes ($p \leq 0.05$) em relação às forragens ensiladas. Podemos concluir que a melhor época de corte, para a erva castelhana, verifica-se na fase de crescimento com 5% espigado.

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INTRODUCTION

Italian ryegrass (*Lolium multiflorum*) is one of the more temporary pastures utilised in the Azores. The present research was carried out to determine the nutritive value of this forage at three stages of growth, as well as the nutritive value of the forage ensiled under the climatic conditions in the Azores. TISSERAND (1984) reported that 5% ear stage of growth is the highest production of digestible dry matter.

The *in situ* technique has been used for many years to determine the dry matter digestibility of forages (NEATHERY 1969; SCALES et al. 1974).

This method represents an approach to the digestive process. MEHREZ & ØRSKOV (1977) have also used this procedure to measure protein degradability.

The present experiment was undertaken to determine the dynamically dry matter digestibility; the digestibility of fresh and ensiled Italian ryegrass. A second objective was to determine the *in situ* protein degradability of the Italian ryegrass forage.

MATERIAL AND METHODS

FORAGES

The Italian ryegrass (*Lolium multiflorum*, L.), was the Multimum variety, and was harvested at three stages of growth: appearance of the fourth leaf (L); with 5% ear (E); and 100% ear (H), and it was analysed in the fresh (F) and ensiled (E) form.

Forages were harvested with a precision-chop machine with a theoretical chopping length of 15 mm. Approximately 1.5 tones of each fresh forage was frozen at a temperature of -15°C, and other portion (also 1.5 tones approximately) was ensiled. The silos were opened after 60 days.

CHEMICAL ANALYSIS

Forage was dried at 65°C, and ground through a 1.0 mm screen. The following parameters were measured: dry matter (DM); ash; crude protein (CP); ether extract (EE) and crude fiber (CF) for the Weende system (AOAC 1975). Gross energy was determined in adiabatic bomb. The forages were also subject to the following analyses: NDF, ADF and ADL (GOERING & VAN SOEST 1970), MADF (CLANCY & WILSON 1966), and calcium and phosphorous (OJEC 1971).

The following were quantified in fresh silages: pH; N-NH_3 by the Conway method (CONWAY 1957); water soluble carbohydrates by the Deriaz method (DERIAZ 1961) and ethanol, lactic acid and fatty acids by gas chromatography using the method of JOUANY (1981).

IN VIVO DIGESTIBILITY

Six adult Romney-Marsh male sheep, per treatment, were used for the in vivo digestibility determinations.

Digestibility trials lasted 21 days; there was a 14 day adaptation period and a 7 day collection period. Animals were fed *ad libitum* twice daily, at 09.00 a.m. and 17.00 p.m. Quantities of offered feed were the previous days consumption amounts plus 10%.

Urines were acidified with concentrated hydrochloric acid (HCl) during collection, in order to prevent the losses of the volatile compounds.

Digestible energy (DE) and metabolisable energy (ME) has been determined from the values of crude energy (CE) ingested and faecal and urinary excretions obtained with *in vivo* assays of digestibility. To determine the metabolisable energy, we selected a value of 7% for the loss of energy as methane gas form (MCDONALD et al. 1988).

IN SITU DEGRADABILITY

Three adult Romney-Marsh male sheep, fitted with permanent 40 mm cannula were used to measure the in situ degradability. They were fed a standard mean quality hay diet *ad libitum* along with 200 concentrate and mineral supplement.

Nylon bags made of precision woven nylon cloth (Hydro-Bios- NY55 HC) measuring 17 x 9 cm and with a pore size of 55 µm were used for the *in situ* method. Approximately 5 g of the sample were placed in each bag. Three bags were prepared for each treatment. Bags were attached to a PVC structure. These were attached to the fistula cap with a 25 cm long nylon string. Bags were immersed completely in water to acquire weight and were incubated in the rumen. We

have incubated 6 bags in each sheep. Bags were incubated for 0, 1, 3, 6, 12, 24, 48 and 72 hours. The zero-hour bags were only washed in running water for 15 minutes. When taken out of the rumen, bags were washed under running water, until rinsing water was colourless and dried at 65°C in a ventilated oven. The weight and crude protein content of the residues were then determined.

We determined the fractional outflow (k) of the particles/hour from the rumen, by treating 50 g of basal diet with sodium-dichromate technique (ÚDEN et al. 1979). Following the method described by GANEV et al. (1979), the basal diet was added through a cannula to the rumen of two sheep, which received a standard hay diet *ad libitum*. Samples of fresh rumen contents were obtained through the cannula at 1, 2, 4, 6, 9, 12, 15, 18, 21, 24, 36 and 48 hours after the addition of the material. Samples were dried at 105°C for determination of dry matter. The method described by STEVENSON & LANGEN (1960) was used for chromium determination. The amount of chromium in the rumen liquor were fitted to the equation:

$$X = e\text{-}kt \qquad (1)$$

in which X represents the chromium fraction that remains in the rumen after the time t and k is the fractional outflow rate (HUNGATE 1966).

Dry matter and crude protein degradability (P) at each one of a series of incubation times (t) is exponential and can be described, following ØRSKOV & MCDONALD (1979), by the equation:

$$P = a + b(1\text{-}e^{-ct}) \qquad (2)$$

in which a is the measure of the rapidly soluble dry matter or protein fraction, b the fraction which is subjected to degradation and c the constant rate of disappearance of fraction b. The constant a, the constants c and the potential dry matter and protein degradability (a+b) were determined by the method of MCDONALD (1981).

The effective dry matter degradability (P) was calculated, following ØRSKOV & MCDONALD (1979) from the relationship:

$$P = a + \frac{bc}{(c+k)}\left(1 - e^{-(c+k)t}\right) \qquad (3).$$

STATISTICAL ANALYSIS

The experimental data were analysed as a 3x2 factorial with six repetitions, with three stages of growth, for fresh and ensiled materials using multiple comparison of the average means were computed using the t-test (STEEL & TORRIE 1980).

RESULTS AND DISCUSSION

The chemical composition of the Italian ryegrass forage is give in tables 1 and 2. Dry matter content increased with advancing of the stage of growth, with a high reduction of crude protein content. The crude protein content of the Italian ryegrass (134 to 271 g/kg) was much higher than found in literature.

The fiber, or the cell wall fraction, represented by crude fiber, NDF, ADF, MADF and ADL, increased with advancing of stage of growth.

Based on the pH values and the classification used by VANBELLE et al. (1981), the LS and ES silage can be classified as average quality silage whereas the HS was a poor silage. Based in N-NH_3 and followed the same authors, the LS silage can be considered acceptable, the ES good, the HS a poor silage.

Based on other observations, the LS silage could be classified as an acetate silage, the ES as a lactate silage and the HS as an acetate silage, with higher content in butyric acid.

The results obtained were a surprising; the HS forage had a high content of water soluble carbohydrates when compared with other forages and ones reported by MCGRATH (1992). MULLER (1981) obtained good results with an Italian ryegrass silage in an advanced stage of maturity (i.e. 27% of DM). The lower quality of the LS silage can be explained by its lower content in water soluble carbohydrates of the fresh forage.

Table 1

Mean chemical composition of the green and ensiled Italian ryegrass (n=3): all values are presented in g/kg DM except DM which represents g/kg material.

Forages	DM	CP	CF	NDF	ADF	MADF	ADL	EE	Ash	WSC	Ca	P
LF	119.0	256.3	204.1	498.6	264.1	252.7	9.5	15.5	168.2	9.5	49.6	3.3
LS	107.4	271.9	244.6	454.1	309.1	274.8	12.1	55.9	156.0	1.5	48.1	2.8
EF	145.8	188.1	260.9	540.7	335.1	322.3	12.8	32.3	131.8	21.0	45.2	2.6
ES	140.3	186.3	273.2	530.4	334.6	305.2	14.7	44.0	121.9	2.2	38.5	2.3
HF	216.3	163.1	316.8	617.0	367.4	348.9	25.3	20.6	77.0	29.5	46.7	2.3
HS	142.0	134.4	278.4	636.3	406.5	387.2	28.3	17.9	103.6	0.6	38.1	2.0

DM= Dry matter; CP= Crude protein; CF= Crude fiber; ADF= Acid detergent fiber; NDF= Neutral detergent fiber; ADL= Acid detergent lignin; MADF= Modified acid detergent fiber; WASC= water soluble carbohydrate; EE= Ether extract.

Table 2

Additional mean chemical data for Italian ryegrass silages (n=3).

		NH_3/	Fatty acids- all in 100g DM				
Forages	pH	%TN	lactic	acetic	propionic	butyric	ethanol
LS	5.00	13.31	0.85	5.74	0.29	0.47	0.78
ES	4.67	8.47	7.02	2.94	0.23	0.20	0.58
HS	5.03	46.51	0.03	4.72	1.23	2.28	0.59

The digestible and metabolisable energy contents of Italian ryegrass (Table 3) decreased ($p \leq 0.05$) with increasing plant age. An exception to the forages in the stage 100% ear was observed, in which the content in energy of the ensiled forages was higher than in fresh forages.

The *in vivo* digestibility of Italian ryegrass (Table 4) decreased with advance of the stage of growth. We observed a significantly decrease ($p \leq 0.05$) between the nutritive value of the fresh forages and silages at all stages, for the measured parameters. MUNRO & WALTERS (1985) observed that the Italian ryegrass is more digestible that other gramineae because they have a higher cell content. Same authors have reported digestibility values closed to what we have determined (WILMAN & OMALIKO 1978; DULPHY & MICHALET-DUREAU 1983; O'KIELY et al. 1989). Others, however have reported higher digestibilities (LIPPKE & BARTON 1988; CULLETON & MURPHY 1989; KEATING et al. 1989).

Voluntary intake of fresh forage was highest in the 5% ear stage of growth. We observed an increase in intake for EF forage and then a reduction in the HF forage. The same tendency was also observed with the silages.

Table 3

Mean energy value of green Italian rygrass and ensiled forages.

forages	MJ/ Kg DM		
	CE*	DE**	ME**
LF	18.66	12.36 (±1.00)	10.10 (±1.00)
LS	20.61	13.82 (±1.10)	11.32 (± 0.20)
EF	18.70	12.52 (±0.50)	10.75 (±0.40)
ES	19.99	12.88 (±0.40)	10.82 (±0.40)
HF	19.23	10.51 (±0.90)	8.86 (±1.00)
HS	18.94	9.62 (±0.60)	7.47 (±0.40)

CE= Crude energy; DE= Digestible energy; ME= Metabolizable energy; * n=3; ** n=6

Table 4

Mean values (n=6) of *in vivo* digestibility (g/kg DM) and dry matter voluntary intake (g DM/kg live weight$^{0.75}$)of green and ensiled Italian rygrass. Standard deviations are given in brackets.

forages	DM	OM	D value	CP	NDF	ADF	DM Intake g/Kg $^{0.75}$
LF	682 c	728 d	606 c	695 d	850 d	796 d	49 ab
	(± 18)	(±17)	(± 15)	(± 29)	(± 25)	(± 31)	(±10)
LS	664 bc	714 d	604 c	740 e	842 d	821 e	43 a
	(± 56)	(± 47)	(± 42)	(± 44)	(± 32)	(± 37)	(± 6)
EF	712 d	714d	620 c	601 b	782 c	769 c	68 d
	(± 37)	(± 36)	(± 31)	(± 29)	(± 24)	(± 17)	(± 6)
ES	649 b	683 c	600 c	624 bc	770c	760 c	64 cd
	(± 20)	(±17)	(± 15)	(± 31)	(± 15)	(± 13)	(± 8)
HF	572 a	592 b	551 b	651 c	602 a	532 a	62c
	(± 28)	(± 27)	(± 25)	(± 34)	(± 33)	(± 35)	(±11)
HS	564 a	570 a	511 a	490 a	662 b	604b	52 b
	(±22)	(± 21)	(± 18)	(±58)	(± 15)	(± 31)	(± 7)
S							
Growth	*	*	*	*	*	*	*
Conservation	*	*	*	NS	*	*	*

OM= Organic matter; S= level of significance ≤0.05; a,b,c,d and e- Where similar letters on the same line are indicated, there are not significant differences between means.

The lower intake of the young forage was probably due to a higher content of water. Influence of the water content on voluntary intake has been reported by different authors (CAMPLING 1964; HOLMES & LANG 1963). HOLMES & LANG (1963) had the opinion that it is unlikely that the intake of fresh grass is reduced by the higher internal water content or by the water from the rain in grass surface. DEMARQUILLY (1966) reported that dry matter intake significantly increased with the content in dry matter.

Constants of dry matter degradability are presented in Table 5. We observed a decrease of the fraction b with the advancing stage of growth.

Dry matter degradability decreased ($p \leq 0.05$) with advancing plant development, specially in ensiled forages ($p \leq 0.05$). The effective dry matter degradability estimated by equations, when k determined was 0.058/hour, also was reduced with advancing plant maturity (Fig. 1).

In situ dry matter degradability was higher in fresh than in ensiled forages (Fig. 2). These are similar to those observed *in vivo* digestibility.

In relation to crude protein degradability, the constants showed that the rapidly soluble fraction (a) was higher in fresh forages than in silages. We also observed a decrease in the fraction which was subjected to degradation (b), with the development of the plant (Table 5).

Table 5

Constants of dry matter and protein degradability from the fresh and ensiled Italian ryegrass forages.

	Dry matter				Crude protein			
Forage	a	b	c	rsd	a	b	c	rsd
LF	28.18	58.96	0.0347	2.23	26.41	71.49	0.0260	3.80
LS	23.63	62.36	0.0374	8.95	39.42	54.66	0.0363	7.84
EF	35.04	62.56	0.0168	2.55	48.00	42.47	0.0292	1.83
ES	24.60	45.63	0.0288	4.24	37.86	37.69	0.0559	3.82
HF	25.69	37.57	0.0299	6.22	52.79	37.17	0.0164	5.40
HS	25.37	35.67	0.0151	1.27	43.53	12.53	0.0848	1.60

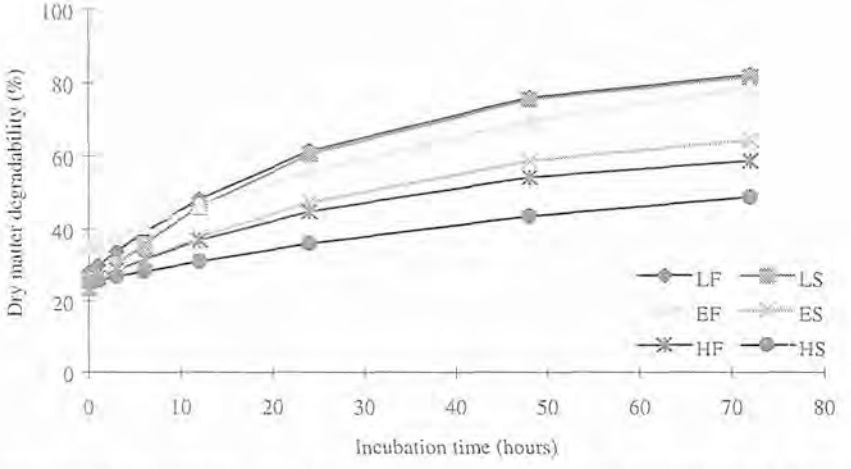

Fig. 1. Dry matter degradability, as a function of time, calculated by the ØRSKOV & MCDONALD (1979) equation, $P = a + b\,(1-e^{-ct})$, for fresh and ensiled Italian ryegrass forages.

Crude protein degradability determined by the equation 2 was significantly higher ($p \leq 0.05$) in fresh than in silage forages (Fig. 3), and decreased significantly ($p \leq 0.05$) with advancing growth stage. The effective crude protein degradability found by the equation 3 (Fig. 4), when k was 0.058/hour, had a similar conduct.

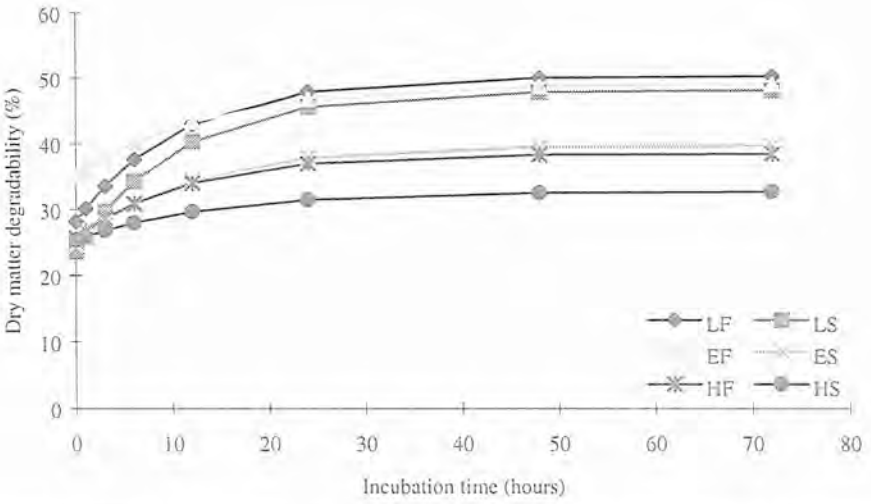

Fig. 2. Effective dry matter degradability, as a function of time, calculated by the ØRSKOV & MCDONALD (1979) equation, $P = a + \frac{bc}{(c+k)} (1-e^{-(c+k)t})$, for fresh and ensiled Italian ryegrass forages.

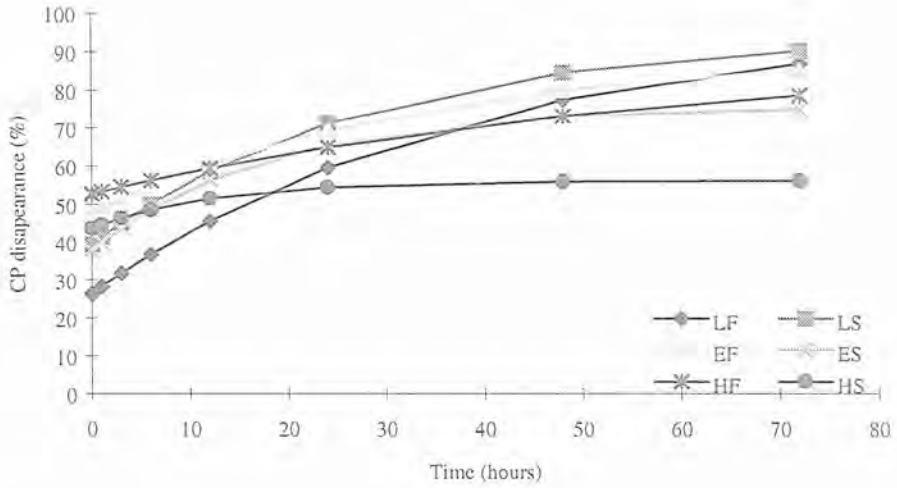

Fig. 3. Crude protein degradability, as a function of time, calculated by the ØRSKOV & MCDONALD (1979) equation, $P = a + b (1-e^{-ct})$, for fresh and ensiled Italian ryegrass forages.

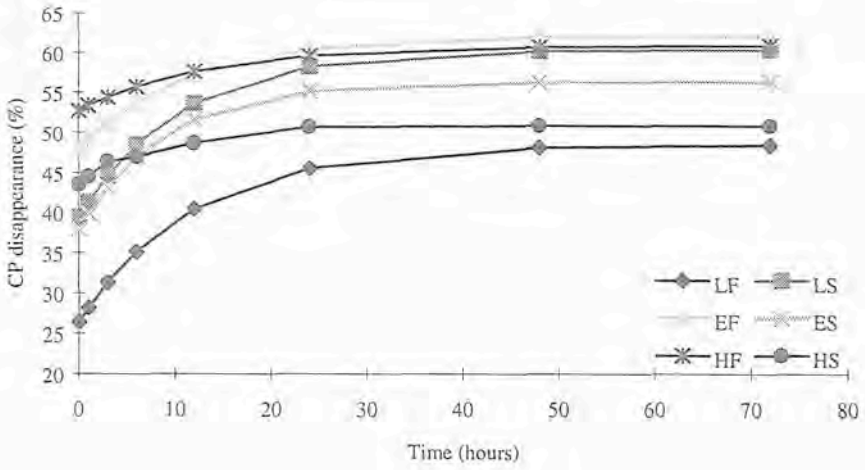

Fig. 4. Effective crude protein degradability, as a function of time, calculated by the ØRSKOV & MCDONALD (1979) equation, $P = a + \frac{bc}{(c+k)} (1-e^{-(c+k)t})$, for fresh and ensiled Italian ryegrass forages.

The effective crude protein degradability, estimated by equation 3, was higher in fresh than in ensiled forages. HOFFMAN et al. (1993) reported that mature grasses were lower in ruminally degradable CP. VIK-MO (1989) and PETIT & TREMBLAY (1992) found a decrease of the nitrogen degradability, in silages, parallel with an increased DM content.

CONCLUSION

In this study we observed a higher digestibility and voluntary intake, for the Italian ryegrass at the 5% ear stage of growth and a reduction in the nutritive value when with ensiled matter than fresh forages.

The best harvesting time is in the stage of growth 5% ear, either to be utilised as fresh forage, or as silage.

We can assume that the *in situ* dry matter degradability of the fresh and ensiled Italian ryegrass forages decreases with the advance of the plant stage of growth and is higher in fresh than in ensiled forages.

In situ crude protein degradability of Italian ryegrass is higher in ensiled than in fresh forages, and decreasing with the advance of the stage of growth.

ACKNOWLEDGEMENTS

The authors express their appreciation to Goretti Fagundes and Gilberta Goulart for their excellent technical assistance in the laboratory.

We would like to thank Engº J.F. Moreira da Silva for advice and valuable discussions and for the revision of the English text.

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Accepted 12 July 1996