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***"In vivo"* and *"in vitro"* degradability of diets for Parmigiano Reggiano cheese production**

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ABSTRACT - *"In vitro"* Dry Matter (IVDMD) and fiber degradability (IVNDFD) dynamics were determined for Total Mixed Rations (TMR) typical of Parmigiano Reggiano cheese area. The same parameters were estimated on some of these ration also *"in vivo"* on a group of fresh cows. *"In vivo"* trial showed values of 62.21 and 44.82% for DMD and NDFD respectively, while average IVDMD was 67.48 and 74.33% at 24 and 48 hours respectively. At the same intervals IVNDFD was of 49.32 and 62.61%, indicating an high fiber digestibility of fresh cow. Based on the *"in vitro"* equations and the *"in vivo"* values, the ruminal retention time was estimated to be of about 21 hours for DMD and of 19 hours for NDFD.

Key words: *In Vitro* degradability, *In Vivo* degradability, Parmigiano Reggiano diet, Retention time.

Introduction – IVDMD and IVNDFD procedures are widely employed to estimate energy value of feeds and to evaluate TMR digestibility (Hoffman *et al.*, 2006). These laboratory determinations, usually based on the method of Tilley and Terry (1963) and on that of Goering and Van Soest (1970) are cheap and relatively easy to perform; if correctly standardized, they show good repeatability. A standard sample of known digestibility can be run simultaneously with the others in order to have an appropriate correction factor for the raw *"in vitro"* values. However this correction do not properly account for variations in true digestibility caused by the level of intake and by variation in passage rate caused by forage physical form. *"In vivo"* and *"in vitro"* dry matter and fiber digestibility have been studied by various authors. For postfresh cows, Hoffman *et al.* (2006) indicate as low and high respectively a TMR IVNDFD of 51 and 63 at 48 hours of incubation. *"In vivo"*, Cleale and Bull (1986) found an NDFD of 62.0% and 52.7% in diet containing early and late cut silage respectively. The same diet showed a DMD of 73.5% and 74.4%. Rate of passage has been investigated in the attempt to determine the more representative *"in vitro"* digestion time point for the estimation of *"in vivo"* feed digestion. Different *"in vitro"* digestion time points have been proposed, ranging from 4 to 48 hours, but most attention has been focused on 24, 30 and 48 hours. **30 hr incubation may better represent the amount of NDF digested by a dairy cow at maintenance levels but the results are less repeatable than 48 hours incubation that may slightly over express NDF digestibility at maintenance intakes** (Hoffman *et al.*, 2006). Oba and Allen (2005) showed that passage rate is related to milk production and that the ruminal retention time of feed is variable between 26.8 and 32 hours being lower than 27 hours in cow producing about 40 Kg per day. Based on these data, they proposed an *"in vitro"* incubation time lower than 30 hours. Van Amburgh *et al.* (2004) proposed an evaluation of forage digestibility based on a 24 hours *"in vitro"* fermentation. The aim of the present work was to study Dry Matter (DMD) and Neutral Detergent Fiber Degradability (NDFD) such as the average ruminal retention time of diets for Parmigiano Reggiano cheese production.

Material and methods – For the “*in vivo*” study, a total of 26 Holstein dairy cattle, between 30 and 60 days in milk, coming from 18 different farm located in the area of Parmigiano Reggiano cheese production were considered. Animals in those farm were fed Total Mixed Ration (TMR) with an average Forage: Concentrate (F:C) ratio of 0.88:1, containing 38.25±5.02 NDF and 27.65±4.70 peNDF. Dry matter intake (DMI) was measured for each cow and fecal samples were collected on the same animals approximatively 6 hours after the main lunch. DM, NDF and ADL content were determined on both TMR and fecal samples.

Using lignin (ADL) as internal marker, “*in vivo*” DM Degradability was calculated as reported by Lippke (2002):

$$DDM=1-[(\text{Marker concentration in feed}) \times (\text{Marker recovery rate}) \times (\text{Marker concentration in feces})^{-1}]$$

Again, using ADL (lignin) as a marker, Fecal Output was calculated as reported by Lippke (2002):

$$\text{Fecal output} = \text{Dose of marker} \times (\text{Marker concentration in feces})^{-1} \times \text{Recovery rate.}$$

“*In vivo*” NDFD was then calculated as follows:

$$\text{In vivo NDFD} = (\text{Fecal NDF}(\%) \times \text{Fecal Output}) / (\text{TMR NDF}(\%) \times \text{DMI})$$

The “*in vitro*” data were obtained from a study conducted on a total of 10 fresh cow TMR ration samples with an average Forage: Concentrate (F:C) ratio of 0.93:1, containing 36.42±3.47 NDF and 24.15±5.37 peNDF, collected from farms located in the Parmigiano Reggiano cheese area. Samples were analyzed for DMD and NDFD. NDFD was determined as described by Goering and Van Soest (1970) while, after the digestion phase, samples were filtered through crucibles (Por. 2) and rinsed twice with warm water for residual dry matter DM determination. DMD and NDFD were determined at 4, 12, 24 and 48 hours and digestion curves were obtained.

The estimation of the ruminal retention time was obtained introducing the “*in vivo*” values in the “*in vitro*” digestion curves equations.

Results and conclusions – Data regarding “*in vivo*” and “*in vitro*” DM and NDF degradability are reported in table 1. Data from “*in vivo*” trial showed values of 62.21 (ranging between 50.95 and 72.17%) and 44.82% (ranging between 29,90 and 62,40%) for DMD and NDFD respectively. Both “*In vivo*” results appears on average lower in comparison to data from other authors, which reported DMD over 70 % and NDFD between 52 and 62 for silage based diet (Cleale and Bull, 1986); this could be related to a lower digestibility of hay forages in Parmigiano Reggiano cheese rations in comparison to silages.

Average IVDMD was 67.48 and 74.33% at 24 and 48 hours respectively. At the same intervals IVNDFD was of 49.32 and 62.61%; this 48 hours fermentation result is consistent with high NDFD

Table 1. Data of “*in vivo*” and “*in vitro*” DM and NDF degradability.

Parameter	<i>In vivo</i>	<i>In vitro</i> Time (h)					
		0	4	12	24	48	
DMD (%)	Mean ±sd	62.21±5.85	0	34.06±3.24	50.96±6.52	67.48±9.79	74.33±5.22
	Min.	50.95	0	29.71	42.54	54.2	67.02
	Max	72.17	0	37.95	59.48	76.79	80.04
NDFD (%)	Mean ±sd	44.82±9.75	0	18.67±5.56	34.67±3.05	49.32±5.36	62.61±11.26
	Min.	29.90	0	12.01	30.73	41.41	51.69
	Max	62.40	0	24.71	38.32	56.11	83.01

fresh cow TMR data reported by Hoffman *et al.* (2006). At 12 hours of incubation an IVDMD of 50.96% and an IVNDFD of 34.67% were observed. The equation describing IVDMD was $y=16.637\ln(x)+11.424$. The dynamic of IVNDF digestion was described by the equation $y=17.797\ln(x)-7.2687$.

Based on the “*in vitro*” equations and the “*in vivo*” values, the ruminal retention time was estimated for both DM and NDF as follows:

- 1) $62.21=16.637\ln(x)+11.424$; $x=21.170$ (estimated ruminal retention time of DM);
- 2) $44.82=17.797\ln(x)-7.2687$; $x=18.668$ (estimated ruminal retention time of NDF).

From these calculations, it appears that the ruminal retention time was of about 21 hours for DMD and of 19 hours for NDFD. These retention time half of the retention time observed by other authors for lactating cow entire gastro-enteric tract (Hartnell and Satter, 1979). It must be observed that because of the possible “*in vivo*” intestinal digestion of DM and fermentation of NDF, estimated ruminal retention time could be slightly over estimated. Based on these data, the average ruminal retention time of diets for Parmigiano Reggiano cheese production could be slightly lower in comparison to that estimated by Oba and Allen (2005) for high producing dairy cows. However, considering the small dimension of rations and cows samples, at the moment the “*in vitro*” DMD and NDFD 24 hours “*in vitro*” fermentation time seems to be the most representative procedure to estimate “*in vivo*” OM and NDF digestibility.

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