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Fermentation characteristics of different grain legumes cultivars with the *in vitro* gas production technique

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ABSTRACT - In the present trial the fermentation characteristics of some grain legumes were studied using the *in vitro* gas production technique with a view to using them as an alternative protein source to soybean in animal feeding. Three cultivars of lupine, six cultivars of faba bean and seven cultivars of peas were incubated at 39°C with buffalo rumen fluid for 96h. OM degradability and fermentation kinetics were studied. Few differences in fermentation characteristics were observed among the cultivars for each legumes grains. "Scuro di Torre Lama" showed significantly (P<0.01) lower values of dOM and OMCV than the other 5 faba bean cultivars; "Lublanc" had lower (P<0.01) OMCV than the other 2 lupine cultivars and "Spirale" produced less gas and showed a faster kinetics than the other 6 peas cultivars. *In vitro* fermentation characteristics of the tested grain legumes were comparable to that obtained from soybean meal in our previous *in vitro* study. The pooled peas showed the significantly (P<0.01) higher gas production (OMCV: 394 ml/g) and faster fermentation kinetics (Rmax: 12.6 ml/h); the pooled lupine showed the lowest gas production (OMCV: 284 ml/g) and the slowest fermentation process (Rmax: 7.42 ml/h).

Key words: Grain legumes, *In vitro* gas production, Fermentation kinetics.

Introduction – Grain legumes are attracting increasing attention as a protein source alternative to soybean meal in animal feeding due to the potential risk involved in the use of GMOs and the impossibility of using meal extraction in the organic system production. In addition, these legumes, cultivated in some area of Southern of Italy, improve soil fertility and reduce the need for nitrogen fertilization. Finally, according to Martini *et al.* (2005) and Cutrignelli *et al.* (2008) replacing soybean, either totally or partially, with grain legumes should not affect livestock performance. The aim of the present trial was to study the fermentation characteristics of grain legumes using the *in vitro* gas production technique (IVGPT).

Material and Methods – Three grain legumes were tested: lupine (*Lupinus* spp.) (var. Lublanc, Luteur, Multitalia), faba bean (*Vicia faba* L.) (var. Chiaro di Torre Lama, Irena, Lady, ProtHABAT69, Scuro di Torre Lama, Sicania) and peas (*Pisum sativum*) (var. Alembo, Alliance, Attika, Corallo, Iceberg, Ideal, Spirale). They were sown between October and December 2006 at the CRA Research Centre for Industrial Crops, Battipaglia (SA). All the 16 samples, ground to pass a 1 mm screen were analysed for chemical composition (AOAC, 1999) and structural carbohydrate fractions (Van Soest *et al.*, 1991). The *in vitro* fermentation characteristics and kinetics were evaluated using the IVGPT according to Calabrò *et al.* (2006). Each cultivar and a pool for each grain legume, was incubated (1.0214±0.0324 g) in triplicate at 39°C in 120 ml serum bottles under anaerobic conditions. Rumen liquor for the inoculum was collected at the slaughterhouse from 2 buffaloes fed the same diet, and immediately transported to the laboratory. The gas measurements were made at 2-24 time intervals using a manual system consisting of a pressure

transducer. The cumulative gas produced at each time was fitted to the Groot *et al.* (1996) model which estimates the asymptotic value (A, ml/g), the time after incubation at which A/2 is formed (B, h), the time to reach the maximum rate (tmax, h) and the maximum rate (Rmax, ml/h). At the end of incubation (96 h), final gas production was related to incubated OM (OMCV, ml/g); degraded OM (dOM, %) was determined by filtration and ashing at 550°C. The Tuckey test (SAS, 2000) was used to assess differences among grain legume cultivars and among the three species. In order to compare the data obtained from these legumes grain with soyabean meal, in vitro fermentation data from our previous trial were considered.

Results and Conclusions – Lupine samples showed a higher crude protein (36.3±0.87 % DM), and lignin (ADL: 2.27±1.74 % DM) content and a lower hemicellulose content (7.09±1.22 % DM) than peas (CP: 28.2±1.85, ADL: 0.94±0.72, hemicellulose: 24.8±8.32 % DM) and faba bean (CP: 26.3±1.45, ADL: 0.82±0.74, hemicellulose: 22.7±4.7 % DM). The chemical composition of peas and faba bean were quite similar. The fermentation characteristics are reported in table 1. For reference purposes, at the bottom of table 1, data from our previous trial performed on soybean meal are reported. The values of pH ranged between 6.35 and 6.72, indicating a normal pattern of fermentation, and were in line with the crude protein content.

As regards faba bean, the "Scuro di Torre Lama" showed significantly (P<0.01) lower values of dOM and OMCV than the other 5 cultivars; in the case of lupine the "Lublanc" had lower (P<0.01) OMCV than the other 2 cultivars and for peas the "Spirale" produced less gas and showed a faster

Table 1. Table 1. Fermentation characteristics of the different grain legume cultivars and soybean meal.

Cultivar	pH	dOM	OMCV	Yield	A	B	tmax	Rmax
		%	ml/g	ml/g	ml/g	h	h	ml/h
Faba bean								
Irene	6.46	92.9	370	397	328	22.9	12.42	9.14
Lady	6.35	93.3	354	363	333	24.5	15.67	9.26
Scuro di Torre Lama	6.49	87.8	308	351	269	22.0	15.39	8.84
Chiaro di Torre Lama	6.41	91.8	348	379	310	23.1	13.20	8.69
ProtHABAT69	6.47	93.8	359	383	303	20.2	12.49	10.02
Sicania	6.40	92.9	324	349	299	21.0	12.83	9.71
MSD	0.135	3.19	60.1	49.3	60.1	3.90	3.65	1.89
Lupine								
Lublanc	6.63	93.4	256	279	283	26.1	10.25	6.73
Luteur	6.69	92.4	275	298	309	25.6	5.58	7.83
Multitalia	6.72	91.2	273	297	303	27.0	8.48	7.08
MSD	0.219	5.40	26.6	91.5	13.9	45.8	15.6	4.74
Peas								
Alembo	6.57	99.0	406	410	361	20.6	12.52	11.73
Alliance	6.49	99.3	397	396	358	20.1	11.99	11.72
Attika	6.57	98.4	397	404	360	20.5	11.82	11.46
Corallo	6.53	98.9	393	394	365	22.3	11.42	10.38
Iceberg	6.55	98.8	381	385	347	21.0	12.45	10.86
Ideal	6.58	97.0	371	383	336	20.7	13.24	11.06
Spirale	6.58	98.8	344	343	310	17.1	10.66	12.14
MSD	0.188	2.81	52.5	53.5	3.68	74.1	5.22	2.47
Faba bean[1]	6.52Ab	90.9b	368B	405A	321a	21.1ab	12.4	10.0Ab
Lupine[1]	6.64B	91.8ab	284C	309B	293b	24.4a	9.03	7.42B
Peas[1]	6.60a	95.1a	394A	413A	336a	18.2b	11.4	12.6Aa
Soybean meal[2]	6.73	96.5	295	306	323	18.7	6.01	10.67
MSE	0.001	2.31	56.2	30.5	101	4.40	2.99	0.56

MSD: Minimum Significant Differences for P<0.01. MSE: Mean Square Error.,In the column A,B,C: P<0.01; a,b,c: P<0.05. [1]Data obtained from the grain legumes incubated in vitro as a pool. [2]Data not statistically assessed.

Figure 1. Gas production over time.

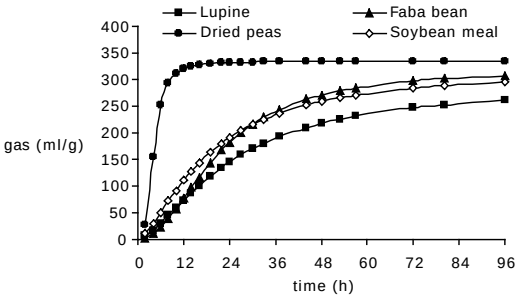
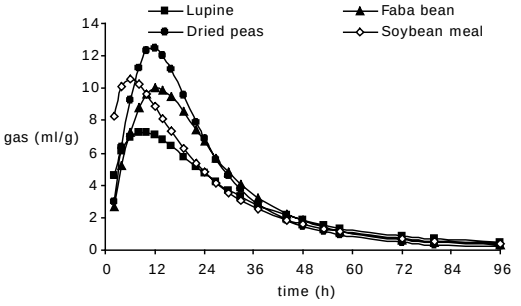


Figure 2. Fermentation rate over time.



kinetics than the other 6 cultivars.

As expected, the OM degradability resulted very high in any case. However, comparing the pools of the grain legumes, dOM was in each case lower than that of soybean meal. OMCV was significantly ($P<0.01$) higher for pea than faba bean (330 vs. 316 ml/g, $P<0.05$) and lupine (330 vs. 258 ml/g, $P<0.01$). Gas production of peas (Figure 1) was always higher than that of lupine, faba bean and also soybean meal according to the results of Buccioni *et al.* (2007) who studied the *in vitro* fermentation of soybean meal, faba bean and pea, and found in the latter the best balance between energy and nitrogen inputs. The slower fermentation kinetics (Figure 2) of faba bean may be due to the content in polyphenols while that of lupine may be caused by the very low starch content (INRA, 1988). From the data obtained, we can conclude that the tested grain legumes show only few differences compared to soybean meal (higher dOM and lower OMCV), consequently they may be considered in replacing, totally or partially, soybean. However, some of their characteristics (i.e. anti-nutritional factors, high protein degradability) need to be taken into account.

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