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Analytical methods to evaluate barley quality for monogastric nutrition

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SUMMARY - It has been observed that analytical parameters such as density or crude fibre content are not sufficient to evaluate the quality of barleys used in monogastric nutrition. Because of the antinutritional effects of ß-glucans in barleys, it is necessary to determine their content, directly or indirectly from the viscosity of their aqueous extracts. The addition of enzymes with ß-glucanase activity is usually made in order to improve the nutritive value of barley. Therefore, the determination of the ß-glucanase activity in enzymatic products or in mixed feed becomes necessary. In this paper, these analytical methods are reviewed. Some of them, have been applied for the evaluation of ten two- and six-row barley varieties, grown in 7 locations in Spain during 1989 (70 samples in total). In order to evaluate the nutritive value of barleys, the Apparent Metabolizable Energy of 24 of the barley samples was determined. A study was undertaken to evaluate the influence of storage on the nutritive value of Spanish barleys.

RESUME - "Méthodes analytiques d'évaluation de la qualité de l'orge pour l'alimentation des monogastriques". Il s'est avéré que des paramètres analytiques tels que la densité ou la teneur en fibre brute ne sont pas suffisants pour évaluer la qualité de l'orge utilisée pour l'alimentation des monogastriques. En raison des effets antinutritionnels des ß-glucanase des orges, il est nécessaire de déterminer leur teneur, directement ou indirectement, à partir de la viscosité de leurs extraits aqueux. L'addition d'enzymes présentant une activité ß-glucanase, sert généralement à améliorer la valeur nutritionnelle de l'orge. Par conséquent il est nécessaire de déterminer l'activité ß-glucanase chez les produits enzymatiques ou dans les aliments composés. On fait le point sur ces méthodes d'analyse. Certaines d'entre elles ont été utilisées pour évaluer une dizaine de variétés d'orge à deux et six rangs, cultivées dans sept localités de l'Espagne pendant 1989 (70 échantillons au total). Afin d'évaluer la valeur nutritionnelle des orges, l'Energie Métabolisable Apparente de 24 des échantillons a été déterminée. Un essai a été entrepris pour évaluer l'influence du stockage sur la valeur nutritionnelle des variétés espagnoles d'orge.

Introduction

In the past, the use of barley in monogastric diets was restricted because of some negative aspects. This cereal has a great variability due to variety, location and climate; in addition, barley has a lower energy value than corn. In any case, the main problem is the relatively high level of (1-3),(1-4)-ß-D-glucan, a type of polysaccharide that constitutes 70-75% of the endosperm cell wall (Fincher, 1975; Forrest and Wainwright, 1977; Jeraci and Lewis, 1989). Barley ß-glucans increase the viscosity of the intestinal content in broilers, which in turn decreases the nutritive value of the diet (Gohl, 1977; Hesselman, 1983). This polysaccharide reduces productive parameters in broilers and creates management problems, especially of the litter (Francesch *et al.*, 1989).

In order to evaluate the nutritional quality of barleys for monogastric nutrition, it is necessary to determine their proximal composition, the ß-glucan content and the viscosity of the aqueous extract of barley.

The addition of enzymes with ß-glucanase activity has the objective of improving the nutritive value of barley. Therefore, the determination of ß-glucanase activity in enzymatic products or in mixed feeds becomes necessary.

In this paper, these analytical methods are reviewed and some of them have been applied for the study of ten two- and six-row varieties, grown in 7 locations in Spain during 1989. In order to evaluate the nutritive value of barleys, the Apparent Metabolizable Energy (AME) of 24 barley samples has been determined. A study has been undertaken to evaluate the influence of storage on the nutritive value of Spanish barley.

Analytical methods for the evaluation of barley quality in monogastric nutrition

The Spanish barleys present great variability in their composition. In the years 1987 and 1988, values for crude fibre varied between 3.7 and 8.9%, and for crude protein between 8.1 and 18.2%. Density varied between 44.63 and 72.73 kg/hl. Because of this variability, the range of AME values for roosters varied by 500 kcal/kg.

In many cases, it is not possible to explain this different energy content by the differences in proximal composition (density, crude protein, crude fibre, ash, moisture and ether extract); thus, varieties with similar density may show very different energy values and varieties differing in density may show very similar AME (Table 1). This can be explained, in part, by differences in ß-glucan content. Therefore, it is necessary to determine the content of this polysaccharide in barley.

Analytical methods for ß-glucans

The ß-glucan content of barley is strongly influenced by variety (Molina-Cano and Conde, 1982), but also by location and climate.

Several methods have been developed to analyze this polysaccharide:

- Selective precipitation of ß-glucans, extracted with 20-30% ammonium sulfate (Preece and McKenzie, 1952; Bass and Meredith, 1955).
- Total acid hydrolysis of ß-glucans extracted with water and determination of free glucose (Fleming *et al.*, 1974; Palmer, 1975; Wood *et al.*, 1977).
- Enzymatic, based on the specific hydrolysis of ß-glucans by enzymes with ß-glucanase activity, and determination of free glucose (Anderson *et al.*, 1978; Martin and Bamforth, 1981; Henry, 1984; Ahluwalia and Ellis, 1984; McCleary and Glennie-Holmes, 1985; Aman and Graham, 1987; Rotter *et al.*, 1990).
- Measurement of fluorescence produced by the specific binding of high molecular weight ß-glucans to some dyes (Jensen and Aastrup, 1981). These measurements have been automated by the use of the FIA (Flow Injection Analysis) technique (Jorgensen and Aastrup, 1988; Mekis *et al.*, 1987; Sendra and Carbonell, 1989; Aastrup and Jorgensen, 1988; Toda *et al.*, 1989).

The disadvantage of the two first methods is that only soluble ß-glucans can be determined, and they are not very specific since glucose is the end product measured. Thus, compounds yielding glucose would be measured as ß-glucans.

Table 1. Relationship between density and metabolizable energy of three varieties of barley (IRTA-Dept. Animal Nutrition, unpublished data).

Variety	Density (kg/h)	E.M.A. (kcal/kg)
Alpha	54.6	2.988
Alpha	66.4	2.989
Dobla	55.2	2.931
Dobla	55.7	3.112
Barbarrosa	55.4	3.066
Barbarrosa	69.2	3.079

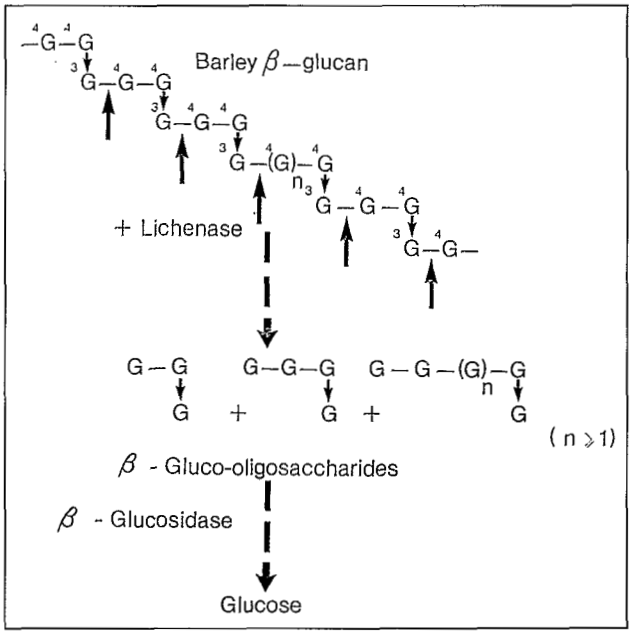

Fig. 1. Enzymatic method for the determination of ß-glucans (McCleary and Glennie-Holmes, 1985).

The enzymatic method of McCleary and Glennie-Holmes is the most commonly used (Fig. 1). In order to obtain glucose from ß-glucans, two enzymes are used: Lichenase (from *Bacillus subtilis*) and ß-glucosidase. The glucose obtained is measured by the color produced in the reaction with the glucose oxidase/peroxidase reagent. This is the method used in the determination of total ß-glucan content of barleys grown in Spain in 1987, 1988 and 1989. The results of these determinations and some European values are shown in Table 2.

The FIA-Calcofluor method developed by Jorgensen (1988) is a two-step method consisting of a mild hydrolysis of ß-glucan with sulfuric acid in order to

Table 2. ß-Glucan content of barleys from several European countries.

Country	Crop	ß-glucans (% on dry matter)				Reference
		Winter	Spring	Global	Range	
England	1983	3.49	4.14	3.82	3.24-5.03	Alexander and Fish (1984)
Scotland	1983	5.00	4.08	4.54	3.58-5.16	Alexander and Fish (1984)
Sweden	1981	–	–	3.69	2.9 -4.2	Aman (1986)
Finland	1986	4.54	3.91	4.22	2.8 -5.6	Lehtonen and Aikasalo (1987)
Spain	1987	2.89	2.90	2.90	1.32-4.33	Francesch *et al.* (1989)
Spain	1988	2.93	2.61	2.77	1.46-4.56	Francesch *et al.* (1989)
Spain	1989	2.13	2.02	2.07	1.00-3.47	IRTA (unpublished)

solubilize all ß-glucans and subsequent reaction of the Calcofluor dye with high molecular weight ß-glucan.

The enzymatic method is more time-consuming than the FIA-Calcofluor method, but specific equipment is not required. Both methods, accepted as EBC (European Brewery Convention) (Munck *et al.*, 1989) methods, show a good correlation coefficient.

Methods for determination of viscosity of aqueous extract of barley

As indicated by Aastrup (1979), the viscosity of acid extracts of barley is closely related to the soluble ß-glucan content of this cereal. Therefore, the viscosity measurement can be used to estimate the soluble ß-glucan fraction. Several methods have been developed for the measurement of viscosity, the difference between them is the composition of the extracting solution.

The extraction can be performed at acidic pH (HCl/KCl pH = 1.5) (Greenberg and Whitmore, 1974; Aastrup, 1979; De Silva *et al.*, 1983) or at more neutral pH (pH = 5-6.5) (Aastrup, 1979; Henry, 1984). The interference of endogenous ß-glucanases is avoided with acidic solutions; depending on the interest in this phenomenon, a neutral or acidic solution has to be chosen.

The effect of enzymatic compounds with ß-glucanase activity on barley can be monitored by the measurement of viscosity of the aqueous extract of this cereal; in this case it is necessary to work at the adequate pH for each enzyme (between 4 and 8).

The viscosity of the aqueous extract of barley based feeds is useful to estimate the level of barley inclusion in feed (Table 3), and the level of added ß-glucanase (Table 4). Therefore, viscosity of the aqueous extract of barley is useful for feed quality control (Fengler *et al.*, 1990).

Table 3. Viscosity of aqueous extract of broiler feed. Effect of barley inclusion and enzyme addition[1].

Treatment	Enzyme	Barley level	Viscosity (cSt)
T-1	0	Corn	1.58
T-2	0	15 % barley	1.93
T-3	0	30 % barley	2.68
T-4	0	45 % barley	3.70
T-1	1	Corn	1.56
T-2	1	15 % barley	1.81
T-3	1	30 % barley	2.32
T-4	1	45 % barley	2.92

[1] % ß-glucan of barleys = 4.2 (s/DM), enzyme = 1 g/kg

Methods for determination of ß-glucanase activity

The ß-glucanase activity of germinated barleys is determined in the brewing industry, because it is necessary to have malts with high ß-glucanase activity.

Table 4. Effect of enzyme level on viscosity of the aqueous extract (pH=5) of broiler diets.

Treatment	Composition	Viscosity (cSt)
T-1	Corn	1.49
T-2	30 % barley	2.55
T-3	30 % barley 0.75 g enzyme/kg	2.23
T-4	30 % barley 1.1 g enzyme/kg	2.18
T-5	30 % barley 1.5 g enzyme/kg	2.07

Because of the use of enzymatic compounds with ß-glucanase activity in monogastric diets, the evaluation of this activity becomes necessary both in the enzymatic products and in mixed feeds, specially, after pelleting. Several methods have been used for the determination of this ß-glucanase activity:

- Viscommetric methods, in which the enzymatic activity is evaluated by the reduction of viscosity of a ß-glucan solution over time (Fig. 2) (Wood and Weisz, 1987; Stuart *et al.*, 1988; Buckee, 1985; Buckee *et al.*, 1988).
- Radial Gel Diffusion. In these methods, ß-glucan is incorporated into agar gel, and the enzyme diffuses from a solution placed in wells cut in the gel. Enzyme activity may then be assessed visually by the breakdown of a colored ß-glucan-dye complex, resulting in decolorated halos surrounding the well. The decolorated area is proportional to enzyme concentration (Wood and Weisz, 1987; Edney *et al.*, 1986). This technique has been used to monitor the stability of the enzyme added to mixed feeds, after pelleting (Table 5).
- Dye-labelling methods. The complex formed by precipitation of ß-glucans from barley with Congo Red or Remazolbrilliant Blue may be used as an insoluble subtract to assay ß-glucanase activity. These subtracts remain essentially insoluble in the absence of enzyme, but in the presence of enzyme release soluble dyed products linearly over time. The rate of release of dye is proportional to enzyme activity (Gill and Haslemore, 1987; McCleary and Shameer, 1987; Wood and Jorgensen, 1988).
- Measurement of the rate of increase of reducing sugar equivalents. The enzyme activity is evaluated by measuring the reducing sugars liberated in defined conditions of pH and temperature, with the Nelson/Somogyi reagent.

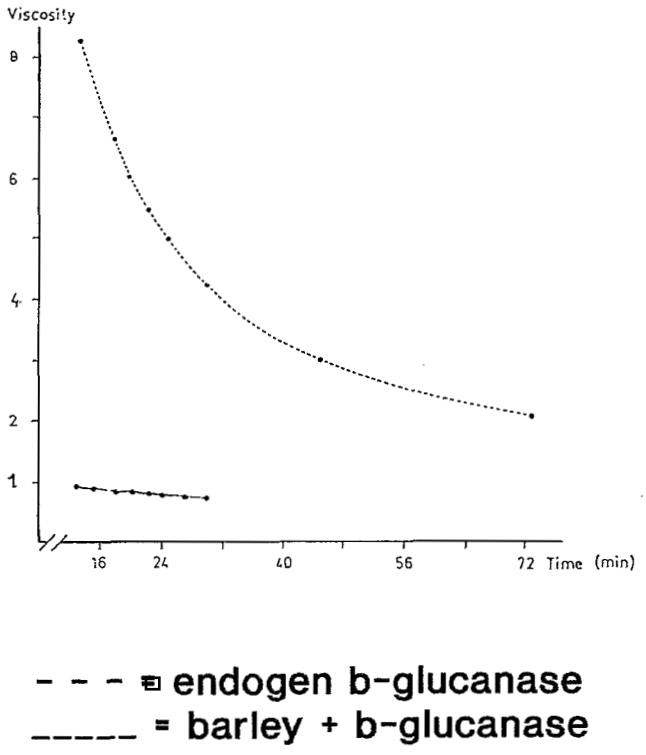

Fig. 2. Variation of the viscosity of a barley ß-glucan solution over time (Hesselman, 1983).

Application of NIRS for the determination of nutrient content of barley

Near Infrared Spectroscopy (NIRS) is a rapid and versatile technique, because it only requires grinding of the sample. Some instruments even work with grain samples (NITS, Near Infrared Transmittance Spectroscopy).

This technique is only useful if good correlations are obtained between the measurements from the NIR spectrophotometer and the values of the other determination from which the calibration is made.

In barley, for parameters such as moisture and crude protein good calibrations are obtained (Fig. 3), while starch and crude fibre give lower correlation coefficients (Table 6) (Carnielo *et al.*, 1985).

The NIR technique has also been applied for the determination of barley ß-glucans (Henry, 1985), with a correlation coefficient of r = 0.880 and a standard error of calibration 0.261.

Table 5. Effect of pelleting on ß-glucanase activity of *Bacillus subtilis* (Edney *et al.*, 1986)[1].

	Enzyme units/g material	
	Whole barley	Hull-less barley
Starter diets		
Mash	0.035	0.038
Pelleted	0.028	0.028
Activity lost	- 20%	- 20%
Finishing diets		
Mash	0.044	0.053
Pelleted	0.028	0.038
Activity lost	- 36%	- 28%

[1] Activity of enzyme product = 264 u/g material

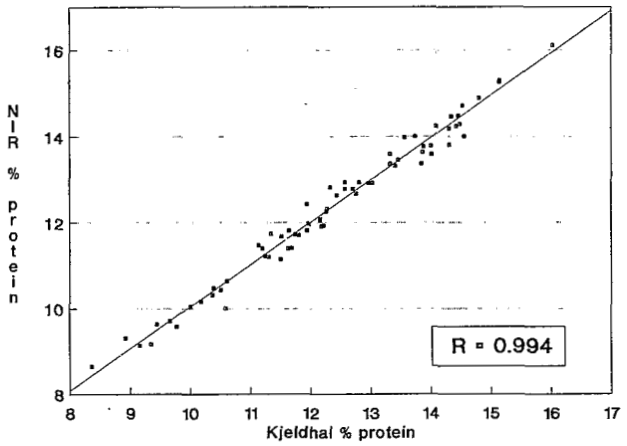

Fig. 3. Correlation plot of NIR calibration for barley ß-glucans (IRTA-Dept. Animal Nutrition, unpublished data).

Table 6. Determination of several analytical parameters of barley by NIRS technique.

Parameter	Multiple correlation coefficient	Calibration standard error	Reference
Crude protein	0.997	0.035	Henry (1985)
	0.920	0.360	Downey (1986)
	0.988	0.316	Szalanczy (1987)
Moisture	0.998	0.125	Henry (1985)
	0.980	0.270	Downey (1986)
	0.708	0.123	Szalanczy (1987)
Crude fiber	0.852	0.380	Szalanczy (1987)
Starch	0.857	1.506	Szalanczy (1987)
ß-glucans	0.880	0.261	Henry (1985)

The NIR determination has at best the same degree of error as the analytical methods with which it is calibrated, but the results can be obtained very quickly, which is important in quality control of raw materials.

Evaluation of nutritive quality of barleys grown in Spain

In order to evaluate the nutritive value of Spanish barleys for monogastrics, ten two- and six-row barley varieties were chosen, grown in 7 locations of Spain, in 1989 (Fig. 4). The varieties studied and the parameters evaluated are shown in Table 7, with the maximum and minimum values obtained for each parameter.

Statistical analysis of the results showed that the variability of moisture and protein was mainly explained by location (82% and 69%, respectively); otherwise the variability of crude fibre (96%) and total ß-glucan content (61%) was explained by variety. The influences of location and variety on density (variety: 54%, location: 46%) and viscosity (variety: 40%, location: 60%) were similar.

The density of spring varieties (mean = 66.94 kg/hl) was higher than that of winter varieties (mean = 62.32 kg/hl); 'Albacete' had the lowest density and 'Zaida' had the highest.

The values of moisture were very similar within location, but statistical differences were found between locations. The highest values were registered in Galicia (mean = 12%), and the lowest in Albacete (mean = 9.57%).

The same behavior was observed for protein. The values for spring were similar to those of winter (13.43% and 13.38%, respectively), but location was important. The highest values were found in Lleida (15.26%), while Granada had the lowest.

The varieties showed very different viscosities and ß-glucan content (Fig. 5 and 6). There was great variability for viscosity, both in spring and winter barleys. The viscosity of spring varieties was lower than that of winter varieties (6.33 cSt versus 8.28 cSt); 'Beka' was the least viscous of the spring varieties, 'Plaisant' the least viscous of winter varieties and 'Hatif de Grignon' was the winter variety with highest viscosity (12.95 cSt) (Fig. 4). Therefore, this parameter is strongly influenced by variety.

The ß-glucan content of barleys grown during 1989, was lower (total mean = 2.07) than that for 1987 and 1988 barleys. The highest values were those of 'Barbarrosa' (2.66% /DM), and the lowest 'Plaisant' and 'Beka' (1.69% and 1.72% /DM).

The nutritive value of these barleys was studied with the varieties 'Dobla', 'Beka', 'Albacete' and 'Barbarrosa', grown in six different locations (Girona, Lleida, Valladolid, Albacete, Navarra and La Coruña). 'Barbarrosa' was chosen because of the high ß-glucan content, and 'Albacete' because of the high crude fibre content. As shown in Fig. 7, the spring varieties had a higher energy value. 'Beka' had 200 kcal/kg more of AME (3.228 kcal/kg) than 'Barbarrosa' and 'Albacete' (3.031 and 3.016 kcal/kg, respectively); thus, better animal performance could be obtained using 'Beka' and 'Dobla'. This effect is independent of location, although location accounted for 20% of the variation of metabolizable energy.

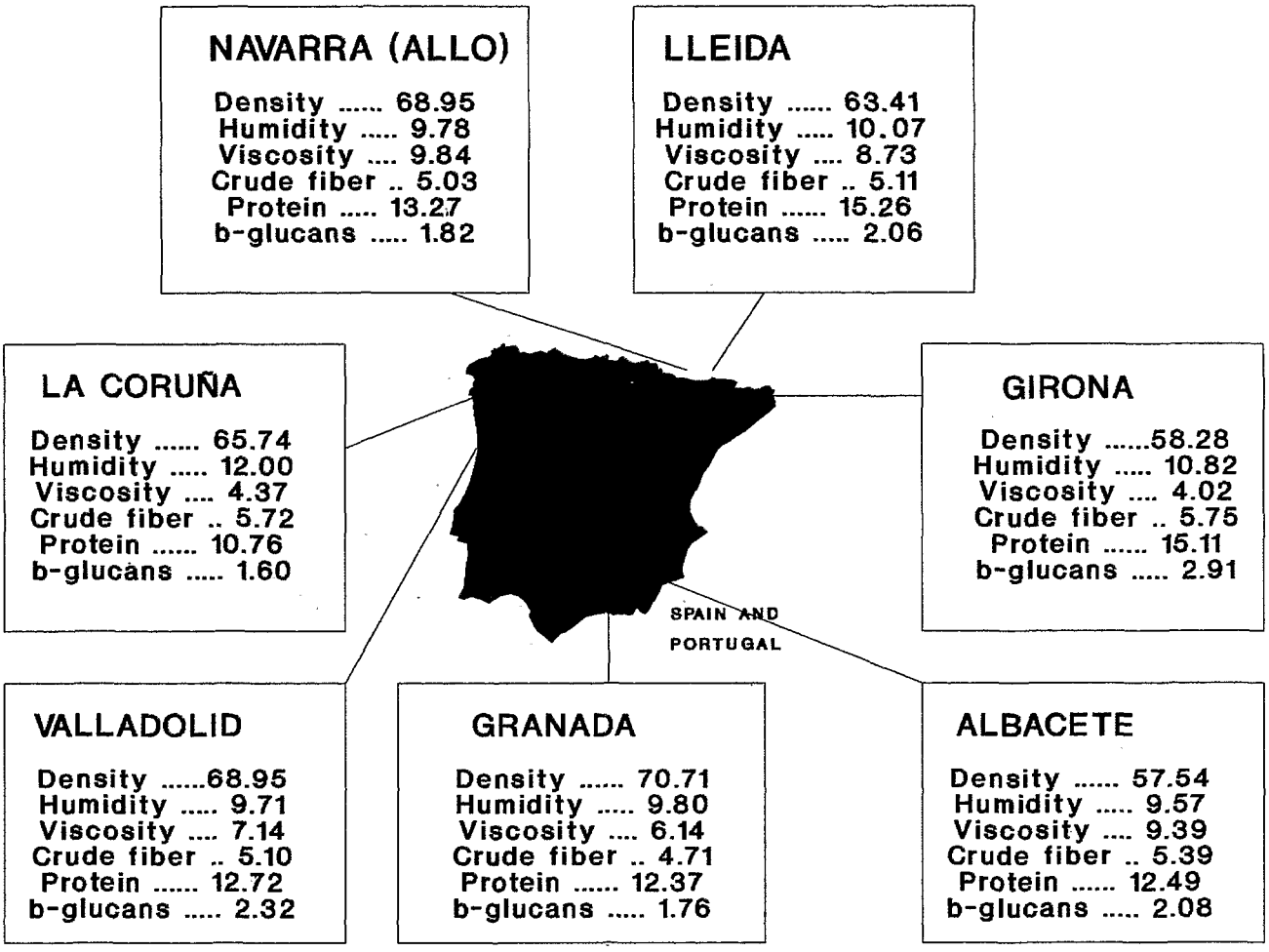

Fig. 4. Barley composition. 1989 crop.

The effect of storage on barley composition

The effect of storage on dormancy and grain germination is well known (Gothard, 1984). The effect of type of storage was evaluated by storing some varieties during 6 months of two different temperatures: room temperature in the feed mill, and low temperature in a cold chamber (4 °C). The germination of barley increased with storage time, but this increase was higher at room temperature (Fig. 8). ß-glucans form the cell walls of the endosperm, and a variation in content or solubility of these polysaccharides could influence germination. In the study of our Department, the content and solubility of ß-glucans of three barleys stored in the conditions described were modified (Table 8) by the different storage conditions. This is in contrast with the observations of Aman *et al.* (1989), who stated that storage did not change either content or solubility of ß-glucans.

The ß-glucan content of barley stored at room temperature and at cold chamber decreased, and at the same time, the proportion of insoluble ß-glucan fraction increased. This reduction of soluble ß-glucans was the reason for the decrease of viscosity. These effects were higher at room temperature. The differences between these results and those of Aman *et al.* (1989) could be due to the use of different varieties, or to different conditions of storage (temperatures, etc.).

Conclusions

Because of the great variability of barleys grown in Spain, it is not recommended to use average values for composition, but the analysis of each sample is required. Moreover, the estimation of ß-glucan content, directly or indirectly by means of viscosity, is necessary in order to predict the nutritive value for poultry. A rapid and accurate analysis of these polysaccharides can be made

Table 7. Composition of Spanish barleys. Crop 1989[1].

Variety	Density (kg/hl)	Moisture (%)	Crude fibre (%)	Crude protein (%)	Germination (% germinated seeds)		Viscosity (cSt)	ß-glucans (% dry matter)
					3 days	5 days		
Albacete	56.53 48.93-62.96	9.91 8.86-11.4	5.96 5.2-6.64	12.62 10.47-14.8	75 42-99	96 85-100	8.81 4.48-12.04	2.08 1.54-2.72
Alpha	64.76 52.30-72.19	10.03 9.52-12.32	5.04 4.58-5.76	12.14 10.01-14.1	94 80.99	99 98-100	6.25 3.85-8.57	2.01 1.13-2.67
Barbarrosa	63.02 53.96-68.87	9.89 9.32-10.65	5.31 4.62-5.78	11.21 9.44-12.97	85 52-99	93 84-99	7.80 3.94-15.27	2.66 2.00-3.26
Beka	67.94 57.71-75.29	10.60 9.44-13.13	3.86 3.32-4.61	12.46 9.77-15.14	94 74.99	99 97-100	5.14 2.46-8.69	1.72 1.17-3.12
Dobla	64.24 56.95-71.32	10.28 9.48-11.64	4.38 4.1-4.70	11.66 9.66-12.26	88 77-100	97 82-100	7.24 4.88-10.19	2.04 1.27-3.47
Hatif	62.87 57.55-67.93	10.11 9.24-11.70	5.30 4.85-5.82	12.31 9.44-13.12	94 88-98	98 92-99	12.95 5.47-24.84	2.23 1.29-3.18
Kym	67.33 60.53-72.71	10.49 9.45-12.01	4.17 3.18-5.08	11.28 8.36-14.42	90 68-99	99 95-100	5.14 2.74-7.25	1.85 1.24-3.18
Pallas	66.87 57.39-13.11	10.25 9.42-11.36	4.32 3.72-5.15	12.24 10.5-14.93	97 90-99	99 95-100	7.62 3.56-14.52	2.40 1.58-3.23
Plaisant	64.40 57.10-70.93	10.32 9.69-12.85	4.69 4.18-5.18	11.90 8.92-14.30	93 78-99	98 93-100	5.53 3.11-9.04	1.69 1.12-3.11
Zaida	68.30 39.50-74.63	10.39 8.9-12.12	4.03 3.64-4.08	12.52 9.16-14.46	97 97-99	98 97-100	6.50 2.41-10.99	2.08 1.00-3.38

with modern analytical equipment (FIA, NIRS). In Mediterranean conditions, storage modified grain germination and also the content and solubility of ß-glucans and, consequently, viscosity. Therefore, animal performance would change depending on whether barley was used immediately after collection or stored.

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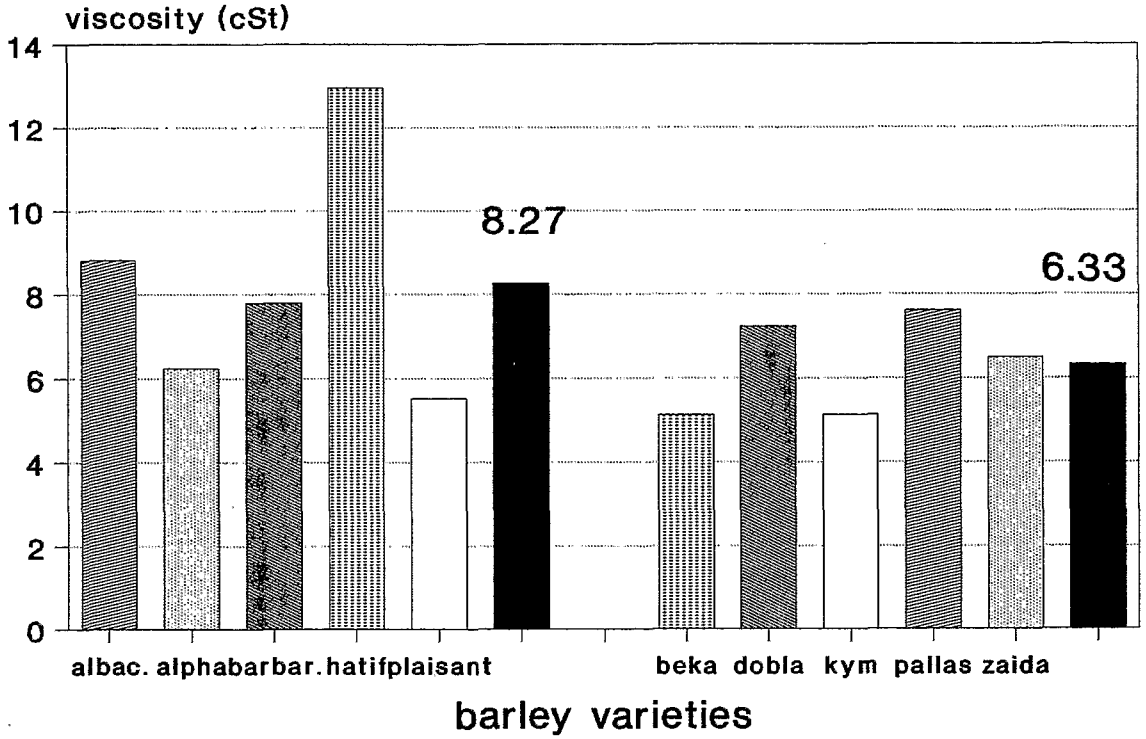

Fig. 5. Viscosity. 1989 barleys. Variety means (IRTA-Dept. Animal Nutrition, unpublished data).

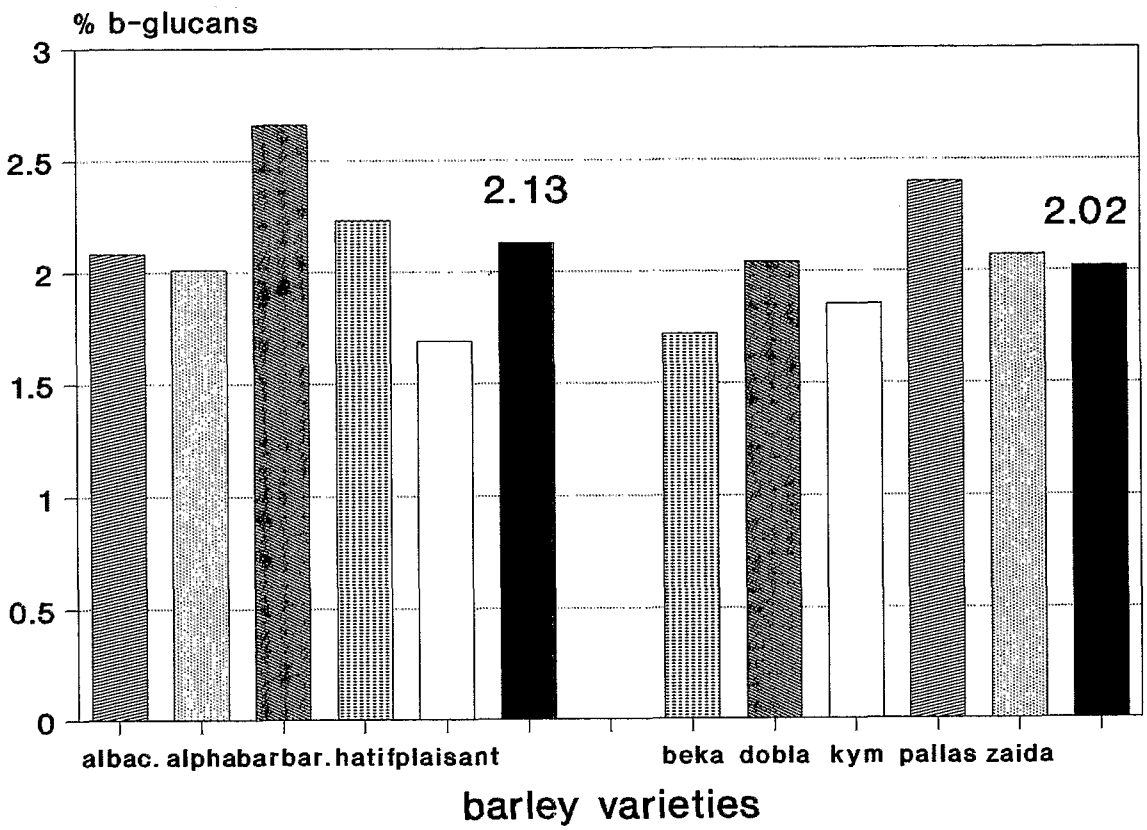

Fig. 6. ß-glucans. 1989 barleys. Variety means (IRTA-Dept. Animal Nutrition, unpublished data).

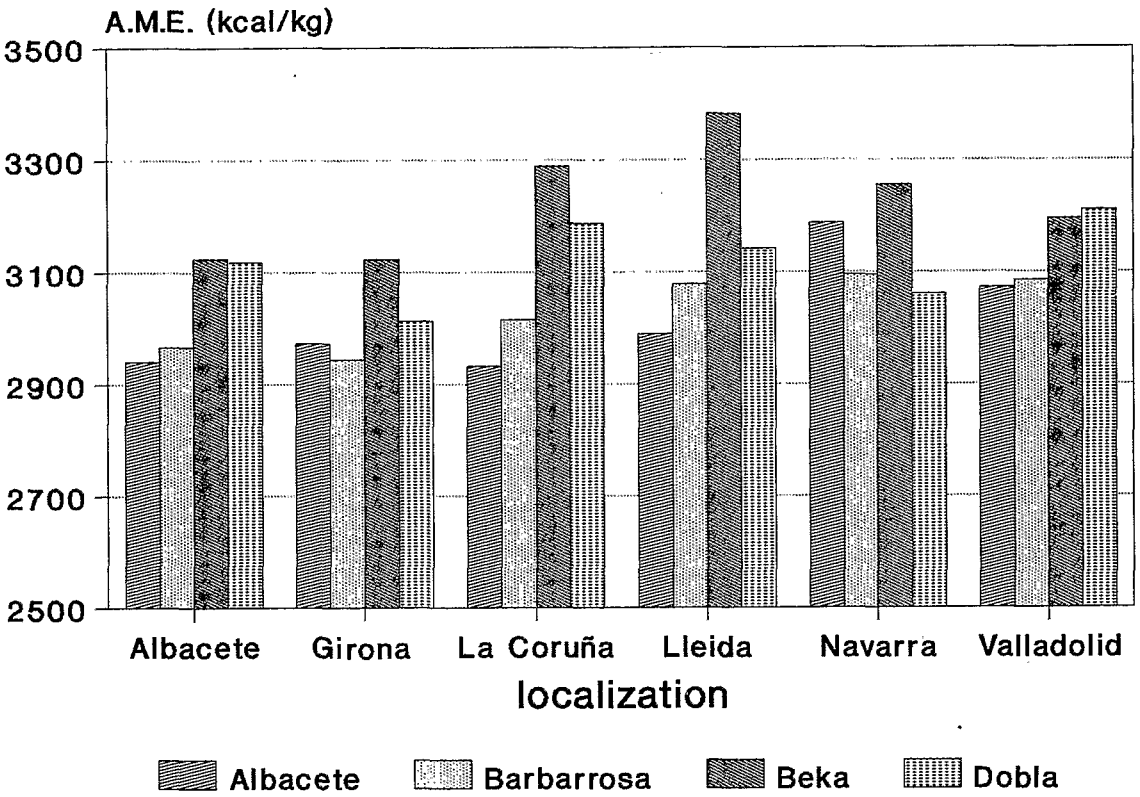

Fig. 7. Metabolizable energy of barleys. 1989 crop (IRTA-Dept. Animal Nutrition, unpublished data).

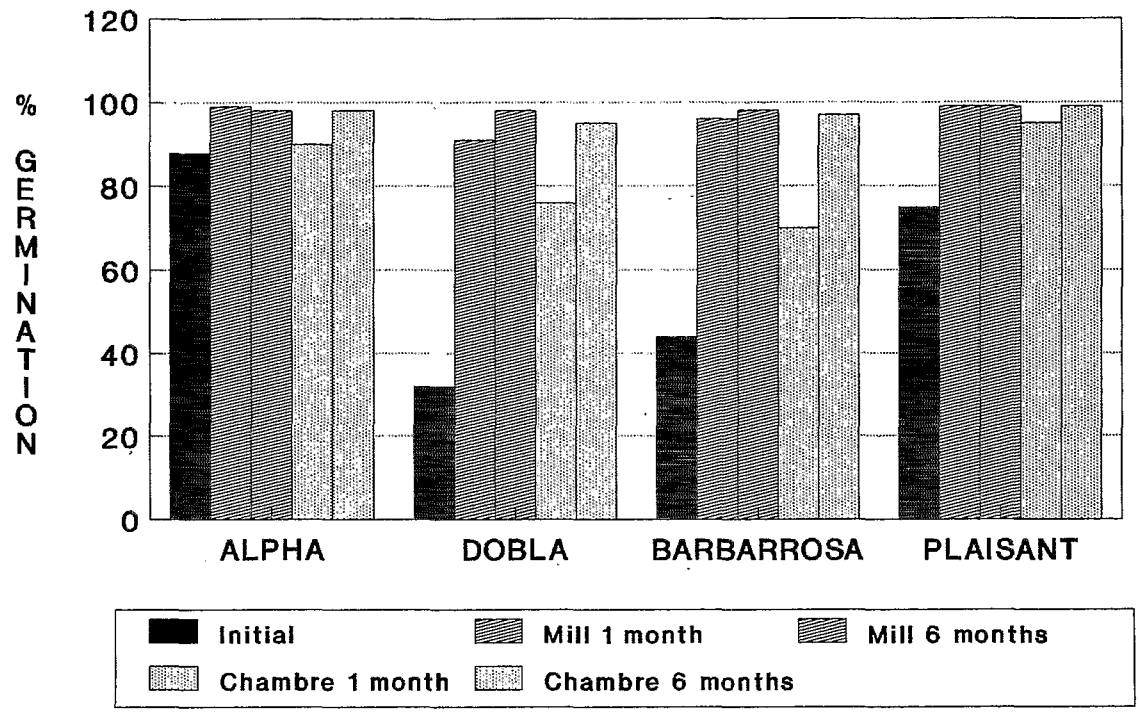

Fig. 8. Germination of barleys. Storage in mill and cold chamber (IRTA-Dept. Animal Nutrition, unpublished data).

Table 8. Effect of storage (1 year) on barley composition. Crop 1989.

	Start	Feed mill	Cold room
		Barbarrosa	
Density (kg/hl)	60.75	58.48	60.70
Crude protein (%)	14.76	15.59	15.30
Crude fiber (%)	6.10	6.97	6.11
Viscosity (cSt)	23.55	14.20	18.29
Total glucans (% DM[1])	3.80	3.70	3.85
Insoluble ß-glucans (% DM)	1.31	1.43	1.29
Soluble ß-glucans (% DM)	2.49	2.27	2.56
		Albacete	
Density (kg/hl)	57.32	55.72	57.95
Crude protein (%)	14.11	13.89	12.32
Crude fiber (%)	6.84	6.74	6.37
Viscosity (cSt)	10.37	9.72	7.08
Total glucans (% DM)	2.86	2.04	2.70
Insoluble ß-glucans (% DM)	0.96	0.99	1.33
Soluble ß-glucans (% DM)	1.90	1.05	1.37
		Beka	
Density (kg/hl)	62.26	62.31	63.72
Crude protein (%)	16.13	16.90	16.74
Crude fiber (%)	4.22	4.41	4.46
Viscosity (cSt)	13.58	11.38	8.05
Total glucans (% DM)	2.94	2.11	2.51
Insoluble ß-glucans (% DM)	1.19	1.15	1.40
Soluble ß-glucans (% DM)	1.75	0.96	1.11

[1]DM = Dry matter

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