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Chemical Characterization and *in Vitro* Digestibility of Faba Bean Seed Cultivars and Varieties Grown in Albania

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Abstract **– The nutritional value of faba bean seed Albanian cultivars (Cekin, Belshi, and Skrapari) and foreign varieties (Aguadulce, Jogeva, Bauska, and Lielplatones) cultivated in Albania for ruminant feeding, were characterized for their chemical composition, and in vitro digestibility according to Tilley and Terry and pepsin-cellulase methodologies. The chemical composition of the seven faba seed varied greatly with and within cultivars and varieties. Cultivars of faba bean seeds presented (dry matter basis) 29.4-30.2% crude protein (CP), 0.70-1.30% ether extract (EE), 16.3-20.9% neutral detergent fiber (NDF), 12.3-12.8% acid detergent fiber (ADF), 2.00-3.50% acid detergent lignin (ADL), and 41.1-47.8% starch., whereas faba seed varieties presented 28.3-36.% CP, 0.60-1.10% EE, 16.4-39.9% NDF, 11.1-11.9% ADF, 1.90-3.70% ADL and 38.6-49.9% starch.. The DMD estimated according to Tilley and Terry method was higher than that estimated according to pepsin-cellulase, average values being, respectively, 94.0% vs. 84.8%. Overall, the present study confirmed the high nutritive value of seven faba bean seed cultivars and varieties grown in Albania. Although variable, the chemical composition of faba seed cultivars and varieties suggested to be good locally produced alternative feed ingredients for use in ruminant nutrition. Nevertheless, further studies should evaluate the characterization of other relevant components with impact in nutrition as secondary plant metabolites and anti-nutritional compounds.**

Keywords **– Chemical Composition; Cultivars and Varieties; Faba Bean Seeds; In Vitro Digestibility; Ruminant Feeding.**

I. Introduction

Legumes (family Fabaceae) have been recognized to be the second most valuable plant source for human and animal nutrition [1] Among legume seeds, faba bean (*Vicia faba* L.) is a multipurpose crop used for both food and fodder (hay, silage and straw) [2] due to their high protein, starch and energy content [3]-[5]. The use of faba as foodstuff has been increased during recent years in several regions (e.g., Mediterranean region) [6] and countries (e.g., China, Ethiopia, and Egypt). Furthermore, faba is a key ingredient to meet the protein requirements in regions of Africa, Asia and Latin America [8], [9], or to replace meat consumption in Western countries.

A renewed interest on faba bean has also emerged in animal nutrition as a locally produced alternative protein source particularly in Europe [9], [10], thus contributing to the reduction of soybean meal imports from non-European countries. This is aligned with an increased interest on legume value chain in order to promote the use of local cultivars to enable sustainable cropping systems and to enhance the supply of new protein food products and new feeds (e.g., EUROLEGUME, FP7 funded) [11]. In this context, different cultivars and varieties of faba bean seeds are cultivated in Albania both for food and feed consumption. Although mainly used for human consumption, the evaluation of the nutritive value of faba bean seeds for animal feeding is still an open topic of interest due to the lack of reference values for local varieties and/or cultivars. Within livestock, ruminant production is of particular importance and interest in Albania due to the economic impact of red meat, milk and dairy products in the local economy. The introduction of locally produced feeds that can meet ruminal nutrition requirements will certainly contribute for a more efficient and sustainable livestock industry in Albania. Nevertheless, this will be only possible by knowing the nutritive value of these feeds. Therefore, the present study aimed to evaluate the nutritional value of faba bean cultivars and varieties cultivated in Albania for ruminant feeding, by characterizing their chemical composition, and *in vitro* digestibility.

II. Materials and Methods

A. Samples

Different cultivars and varieties of *V. faba major* (broad bean) and *V. faba minor* (horse bean), grown in Albania, were evaluated in terms of chemical composition and *in vitro* digestibility. The traditional cultivars were Cekin, Belshi and Skrapari, all broad beans originated from Albania. Varieties included Aguadulce (broad bean, Italy), Jögeva (broad bean, Latvia), Bauska (horse bean, Latvia) and Lielplatones (horse bean, Latvia). Faba seeds were air-dried, preserved under vacuum before being ground through a 1-mm screen.

B. Chemical Analysis

Faba seeds were analyzed according to [12] for dry matter (DM), ash, ether extract (EE), and Kjeldahl for N. Crude protein (CP) was calculated as N × 6.25. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to [13] and [14]. Neutral detergent fiber (NDF) and acid detergent lignin (ADL) were expressed exclusive of residual ash and ADF was expressed inclusive of residual ash. Starch content was determined according to Salomonsson et al. (1984) in finely ground (0.5 mm) samples. All chemical analyses were performed in duplicate.

C. Tilley and Terry Method

Briefly, 250 mg DM of each seed were incubated with 50 mL of buffered rumen inocula according to the

methodology as in [16], modified by [17]. The rumen contents were obtained from two adult non-pregnant and non-lactating Holstein cows fitted with rumen cannulae. Cows were housed at Vairao Agricultural Campus of ICBAS (Vila do Conde, Portugal) and were handled in accordance with good animal practices defined by the Portuguese authorities and European Union Directive 2010/63/EU. Two total mixed rations (TMR) were used to feed the cows. One TMR comprised 10 kg hay silage (55 g/ kg CP, 710 g /kg NDF, DM basis) and 3.5 kg concentrate (230 g/kg CP, 187 g/kg starch, 294 g/kg NDF, DM basis) while the other TMR comprised 12 kg corn silage (77 g/kg CP, 369 g/kg starch, 392 g/kg NDF, DM basis), 2 kg wheat straw (28 g/kg CP, 812 g/kg NDF, DM basis) and 3 kg concentrate (230 g/kg CP, 187 g/kg starch, 294 g/kg NDF, DM basis). Both TMR were supplemented daily with 40 g calcium carbonate, 20 g dicalcium phosphate, 10 g salt and 8 g of a mineral-vitamin mixture (Premix; Viana do Castelo, Portugal). Cows were fed twice a day, at 0930 and 1730 h, and had continuous access to fresh drinking water. After two weeks adaptation to the diet, the rumen contents were collected to a pre-warmed (39 ◦C) thermos container and transported to the laboratory. After the rumen inocula collection, diets were exchanged between cows and a new two weeks adaptation period started. The rumen contents were filtered through cheesecloth and one part of the strained rumen fluid mixed with two parts of [18] buffer. Seeds and blanks (only buffered rumen inocula) were incubated in a water-bath at 39 ºC for 48 h. After incubation, contents were filtered through fritted glass crucibles under vacuum, dried at 103 ºC for 12 h, and weighed for determination of the dry content and calculation of DMD. Blanks and samples were incubated in duplicate per inoculum and per incubation, incubations being replicated in two separate runs, resulting in eight replicates for each faba bean seed.

D. Pepsin-Cellulase Method

The pepsin-cellulase digestibility was evaluated according to the method described by [19]. Briefly, 300 mg DM of each seed were incubated with 50 mL pepsin solution (0.2% pepsin in 0.1 N hydrochloric acid; Pepsin 1:10.000, Biotechnology, VWR Portugal, Carnaxide, Portugal) in a water bath at 39 ºC for 24 h, followed by an incubation at 80 ºC for 30 min. Blanks were run along with samples. Contents were filtered through fritted glass crucibles under vacuum and the residue washed with distilled water at 40 ºC. Residues were then incubated with 50 mL cellulase from *Trichoderma viride* (Onozuka R-10, Yakult Pharmaceutical, Japan) solution for another 24 h at 39 ºC. At the end of this second incubation, residues were washed with distilled water at 40 ºC, vacuum filtered, and dried for 48 h at 103 ºC to determine the dry residue and estimate the DMD. Blanks and samples were incubated in triplicate.

III. Results and Discussions

A. Chemical Composition of Faba Bean Seeds

Chemical parameters (see Table I) of the seven faba bean varied with and within cultivar and variety. Ash content of faba bean cultivars ranged from 3.10 to 3.90% (DM basis), respectively in Cekin and Belshi. Faba bean varieties presented ash content with the range of cultivars (3.20-3.60%, DM basis). These results are in accordance with the ash content earlier reported for faba bean seeds of cultivars and varieties [20]-[22], [24], [25]. CP content ranged from 28.3% to 36.5% (DM basis), Lielplatones and Aguadulce varieties presenting, respectively, the highest and the lowest protein content. A wide range of CP content is reported in the literature (22.4-40.0% DM basis, [26]-[29] similar to those herein determined, except for Lielplatones that presented a higher CP content (36.5% DM basis). Variations on CP content may be explained by different genotype [7], [8] and environmental (*e.g.*, soil, climate) origins [30]. Legume seeds, particularly faba seeds, are characterized for their low-fat content [22]. In our study, EE content was low, ranging from 0.60% DM in Aguadulce variety to 1.30% DM in Belshi cultivar. Although EE contents of 2.30-3.20% as it has been reported by [31], most studies reported faba bean seed EE content between 0.70–2.00% DM [4], [8], [22], [24], [25], which are similar to those herein reported. Faba bean seeds with white flower typically present considerable fiber contents, namely *ca.* 16.1% NDF, 10.7% ADF and 0.8% ADL (DM basis), slightly lower than those of colored flower beans [32]. The cultivars and varieties herein evaluated were spring-sown white flower faba bean seeds, yet fiber constituents' content greatly differed from the tabled ones. Apart from Belshi cultivar and Jogeva variety that presented similar NDF contents (16.3 and 16.4%, respectively), NDF varied from 17.2% in Lielplatones variety to 39.9% in Aguadulce variety, whereas ADF ranged from 11.1% in Lielplatones to 12.8% in Belshi cultivar. ADL content was considerably high across cultivar and varieties, ranging from 1.9-3.7% DM, respectively in Jogeva and Bauska varieties. The variability on ADL content among faba beans were may be attributed to cultivar/varieties and the year of growth that significantly affected the content of ADL [33]. Starch was the major seed component, yet starch content was found to vary among faba bean seeds, varieties Jögeva presented the highest (49.9% DM basis) and Lielplatones the lowest (38.6% DM basis) starch content, and cultivars intermediate ones. A similar wide variety of starch content was reported in faba bean seeds, ranging from 39.8 to 48.5% DM [34], [35]. Reference [36] reported a negative correlation between starch and CP content. A similar negative correlation was observed in cultivars but not in varieties. It is consistent with findings reported by [37] and [38] that starch content is significantly affected by cultivar.

Table I. [1]Chemical composition of faba beans cultivars and varieties (% dry matter)

Samples	Moisture (%)	Ash	CP	EE	NDF	ADF	ADL	Starch
Cultivars								
Cekin	`	3.1	29	0.8	20.9	12.6	2	47.8
Belshi	9.3	3.9	30	1.3	16.3	12.8	3.5	43.5
Skrapari	10	3.5	30	0.7	17.3	12.3	2.6	41.1
Varieties								
Jögeva	9.7	3.6	34	1.1	16.4	11.9	1.9	49.9
Aguadulce	9.7	3.4	28	0.6	39.9	11.7	3.1	39.2
Bauska	9.3	3.2	35	1	21.6	11.4	3.7	41.7
Lielplatones	9.9	3.6	37	0.9	17.2	11.1	2.4	38.6

B. Digestibility of Faba Bean Seeds

The *in vitro* DMD of faba bean cultivars and varieties (see Table II) determined according to the Tilley and Terry and pepsin-cellulase methodologies. Tilley and Terry DMD of cultivars ranged from 91.6-96.1%, respectively for Belshi and Skrapari, whereas pepsin-cellulase DMD ranged from 79.9% in Skrapari to 88.2% in Cekin. Regarding faba bean varieties, Tilley and Terry DMD ranged from 92.0% to 95.2%, respectively for Bauska and Aguadulce, whereas pepsin-cellulase DMD ranged from 89.8% in Bauska to 79.2% in Jögeva. Overall, DMD estimated according to Tilley and Terry method was higher than that estimated according to pepsin-cellulase, average values being, respectively, 94.0% vs. 84.8%. The consistently highest DMD determined by Tilley and Terry when compared to pepsin-cellulase method are in agreement with previous reports [39]-[41]. Nevertheless, other studies found pepsin-cellulase DMD to be higher than Tilley and Terry DMD [42], [43]. Reference [44] suggested that such differences in DMD estimated by rumen inocula or purified enzymes may be related to rumen-liquor from different fistulated animal and the ingredients of the animal diet.

Table II. *In vitro* dry matter digestibility of faba bean cultivars and varieties according to Tilley and Terry and pepsin-cellulase methods (% dry matter).

Samples	Tilley and Terry	Pepsin-cellulase
Cultivars		
Cekin	95.0	88.2
Belshi	91.5	88.1
Skrapari	95.0	79.9
Varieties		
Jögeva	94.0	79.2
Aguadulce	95.2	88.7
Bauska	92.0	89.8
Lielplatones	94.0	79.5

IV. Conclusion

The present study confirmed the high nutritive value of seven faba bean seed cultivars and varieties grown in Albania. The major chemical components were starch and protein content. Although variable, the chemical omposition of faba seed cultivars and varieties suggested to be good locally produced alternative feed ingredients for use in ruminant nutrition. All faba bean cultivars and varieties presented high DMD, although Tilley and Terry method estimated higher digestibility than that of pepsin-cellulase, suggesting that purified enzymatic methods might not replace the use of rumen inocula to predict digestibility of faba bean seeds by ruminants. Overall, the seven faba bean seed cultivars and varieties suggest being interesting underexploited feeds for ruminant nutrition, yet the evaluation of other important components, as secondary metabolites including phenolic compounds, must be conducted in further.

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[1]CP (crude protein), EE (ether extract), NDF (neutral detergent fiber), ADF (acid detergent fiber), ADL (acid detergent lignin).

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