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Protein Sources in Ruminant Nutrition

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1. Introduction

Since 2001 the European Commission banned the use of meat and bone meal and its by-products in diets for livestock animals (EC directive 999/2001) in order to assure consumer safety on animal products. Consequently, soybean meal became the most utilised protein source in the intensive livestock systems.

Moreover, the proteins of this source are low degradable in the rumen and well proportioned to the non structural carbohydrates (NSC).

Soybean meal solvent extract (s.e.) is a by-product of oil industry, where soybean seeds are treated with organic solvents (e.g. hexane) and subsequently with high temperature. For this reason soybean meal has been banned in the organic livestock (EC directive 2092/1991; EC directive 834/2007).

Even if in Europe the high part of soybean is imported, soybean solvent extract represents the less expensive protein source for its high crude protein content (44-50 % as fed). However soybean meal costs and availability are strongly related with the price development of agricultural commodities on the world market (Jezierny et al., 2010). Factors which may influence world market prices include variations in population and economic growth, changes in consumer's product preferences, but world market prices are also dependent on weather conditions (Gill, 1997; Trostle, 2008).

Finally, another factor has to be evaluated that is the genetically modification (GM) of soybean. Indeed, public concerns are increasing in GM food consumption due to the fact, even if for several years no direct evidence that it may represent a possible danger for health has been reported, recently, a number of papers have been published with controversial results.

Thus, the search for alternative protein sources has led to an increasing interest in the use of grain legumes, as they supply the important source of plant protein.

The botanical family of grain legumes is known as *Fabaceae*, also referred to as *Leguminosae*. Grain legumes are cultivated primarily for their seeds which are harvested at maturity, and which are rich in protein and energy. The mature dry seeds of grain legumes are used either as animal feed ingredient or for human consumption (Singh et al., 2007). Beans, lentils and chickpeas are utilised exclusively for human nutrition, while the other grains are used in animal feeding too.

In Italy grain legumes cultivation is progressively increased due the presence of new cultivars more hardy and productive. These new cultivars were selected principally in

France and are characterised by lower water requirements, higher production and higher resistance to the parasitic infestations and to the adverse environmental conditions.

Generally, legumes are characterised by their ability to use atmospheric nitrogen as a nutrient due to the symbiosis with nitrogen-fixing bacteria from the *Rhizobium* species (Sprent and Thomas, 1984; Zahran, 1999). Therefore, unlike other cultivated plants, legume crops need less nitrogen fertiliser for optimal growth, and the use of legumes in crop rotation systems reduces the need of nitrogen fertiliser in subsequent crops (López-Bellido et al., 2005). Nitrogen benefits in legume-cereal rotation systems have been attributed not only to the transfer of biologically fixed nitrogen (Díaz-Ambrona and Mínguez, 2001; Evans et al., 2001), but also to lower immobilisation of nitrate in the soil during the decomposition of legumes compared to cereal residues (Green and Blackmer, 1995), also termed as the nitrogen-sparing effect. Thus, nitrogen benefits may result from a combination of legume nitrogen sparing effects and the bacterial nitrogen fixation (Chalk et al., 1993; Herridge et al., 1995). In addition, crop rotation and intercropping with legumes may provide successful strategies for weed suppression (Liebman and Dyck, 1993; Bulson et al., 1997). Weed growth and development may be disrupted due to varying cultivation conditions prevailing for the different crops used (e.g. fertiliser requirements, planting or maturation dates), thereby preventing domination of only a few weed species (Froud-Williams, 1988; Liebman and Janke, 1990). Due to these crop effects, cultivation of grain legumes is an important part of crop rotation, particularly in organic farming (Badgley et al., 2007).

In animal nutrition, grain legumes are mainly used as protein supplements, but also as a valuable energy source, due to their partly high contents of starch (faba bean, peas) and lipids (lupins) (Gatel, 1994; Bach Knudsen, 1997; Salgado et al., 2002a).

However, the use of grain legumes in animal nutrition has been hampered due to partially high concentrations of secondary plant metabolites, also referred as antinutritional factors (ANFs), including condensed tannins, protease inhibitors, alkaloids, lectins, pyrimidine glycosides and saponins. Possible negative effects of these secondary plant metabolites include, for example, feed refusals (tannins, alkaloids), reduced nutrient digestibility (tannins, protease inhibitors, lectins) or even toxic effects (alkaloids) (Rubio and Brenes, 1995; Lallès and Jansman, 1998; Huisman and Tolman, 2001).

The objectives of the following chapters are the comparison of chemical composition as well as the nutritive value of soybean, soybean meal solvent extract and several legume grain (e.g.: peas, lupine, faba bean). In addition, in order to evaluate the opportunity of soybean replacement with grain, the results of *in vitro* studies are described and the influence of protein sources on meat quality are discussed.

2. Nutritional characteristics of soybean meal solvent extract and legume grains

The chemical composition of some grain legumes in comparison to soybean and soybean meals s.e are pictured in Table 1.

Soybeans (*Soja hispida*) are characterized by high protein (380 g/kg dry matter) and lipid (200 g/kg dry matter) concentrations, which provide high energy density (1.1-1.2 UFL/kg dry matter). In ruminant feeding, soybean integral seeds could represent the only source of protein supplementation for cattle fattening. However, in dairy cows it is preferable not to exceed the dry matter administration of soybean seeds as it may modify fatty acid profile of milk fat and worse butter consistency and conservation.

Soybean meal s.e. proteins, due to the heat treatment, are medium to low degradable in the rumen (Chaubility et al., 1991; Infascelli et al., 1995). In addition, soybean meal shows an elevated ratio of protein/non structural carbohydrates.

Legume grains are characterised by high energy density allowed to the high protein, starch and/or fat concentrations, as more than sufficient is their calcium concentration.

The proteins of legume grain are highly degradable in the rumen and digestible in the intestine. Notwithstanding, large part of legume grain shows anti-nutritional factors (i.e. lecithin, trypsin inhibitors, tannins, saponin, phytase), that are inactivated by the enzymes produced by the bacteria present in the rumen. Within the grain legumes, lupins have higher amounts of crude protein (324–381 g/kg dry matter), compared to faba beans (301 g/kg dry matter) and peas (246 g/kg dry matter) (Degussa, 2006). Jezierny et al. (2007) reported similar contents of crude protein in different batches of lupins, faba beans and peas averaging 387, 308 and 249 g/kg dry matter, respectively. In comparison to soybean meal, faba beans and peas contain between 45 to 55% and lupins (L. albus) even up to 70% of its crude protein content (Degussa, 2006).

The ether extract content in peas and faba beans is generally rather low compared to lupins; crude fat contents of faba beans and peas range from 15 to 20 g/kg dry matter, thus being in a similar range as values for soybean meal (15–28 g/kg dry matter) (DLG, 1999; Jezierny et al., 2007). In lupins, the crude fat content varies between cultivars, with values of about 57 g/kg dry matter (*L. luteus*, *L. angustifolius*) to 88 g/kg dry matter (*L. albus*) (DLG, 1999).

The carbohydrate fraction includes the low molecular-weight sugars, starch and various non-starch-polysaccharides (NSP) (Bach Knudsen, 1997). The NSP and lignin are the principal components of cell walls and are commonly referred to as dietary fibre (Theander et al., 1989; Canibe and Bach Knudsen, 2002). Generally, faba beans and peas are rich in starch (422–451 and 478–534 g/kg dry matter, respectively) (DLG, 1999; Jezierny, 2009), whereas lupins have comparatively low levels of starch (42–101 g/kg dry matter) (DLG, 1999; Jezierny, 2010). However, it needs to be emphasized that the determination of starch in grain legumes may be confounded by the analytical method used (Hall et al., 2000).

Faba beans and peas contain rather low amounts of fibre fractions in comparison to lupins (Bach Knudsen, 1997; Jezierny, 2009), and, with regard to lignin content, faba beans and *L. angustifolius* have similar amounts of lignin (1 to 7 and 6 to 9 g/kg dry matter, respectively), whereas the lignin content in peas is of minor importance (0.4–3 g/kg dry matter) (Salgado et al., 2002a; Jezierny, 2010).

The NSP fraction of faba beans consists mainly of cellulose (89–115 g/kg dry matter), with lower levels of hemicellulose (21–57 g/kg dry matter) (Salgado et al., 2002a,b; Jezierny, 2009).

Hemicellulose contents in peas range from 23 to 95 g/kg dry matter and cellulose contents range from 52 to 77 g/kg dry matter (Salgado et al., 2002a,b; Jezierny, 2009).

Lupins contain high levels of NSP, with contents of cellulose generally being higher than hemicellulose (131 to 199 vs. 40 to 66 g/kg dry matter) (Bach Knudsen, 1997; Salgado et al., 2002a,b; Jezierny, 2009), and they also have considerable amounts of oligosaccharides (Bach Knudsen, 1997; Salgado et al., 2002a).

Comparing the different batches in each species, lupin show lower variability than faba bean and peas, probably because the genetic selection in this species was addressed principally on the reduction of secondary plant metabolites (Colombini, 2004) than to the improving of chemical characteristics.

The protein of faba beans and peas contains similar or even higher proportions of lysine (70 and 80 g/kg crude protein, respectively), when compared to protein from soybean meal s.e. (69 g/kg crude protein) or lupins (51 to 54 g/kg crude protein) (Degussa, 2006).

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Protein sources	DM	CP	EE	Ash	NDF
Soybean	93.30	33.2	25.0	5.57	14.5
Soybean meal s.e.	89.80	41.3	1.36	6.00	22.2
Lupin Lublanc	93.26	36.92	6.78	4.33	4.33
Lupin Lutteur	94.31	35.30	5.73	4.19	4.19
Lupin Multitalia	93.97	36.67	9.54	3.92	3.92
Faba bean Irena	89.22	25.62	1.05	3.99	20.94
Faba bean Lady	88.48	25.17	1.02	4.21	25.82
Faba bean Scuro di Torre Lama	90.55	26.91	0.90	4.32	21.75
Faba bean Chiaro di Torre Lama	90.80	24.69	1.01	4.19	21.23
Faba bean ProtHABAT69	90.60	28.69	1.10	4.51	18.21
Faba bean Sicania	90.29	26.52	0.95	3.89	21.43
Peas Alembo	88.49	31.27	0.73	3.94	21.60
Peas Alliance	89.20	28.47	0.56	3.82	20.40
Peas Attika	89.68	25.04	0.81	4.01	18.09
Peas Corallo	88.80	28.50	0.80	3.69	21.82
Peas Iceberg	90.08	27.30	0.78	4.12	22.75
Peas Ideal	89.94	28.28	0.88	4.17	18.37
Peas Spirale	93.00	28.74	0.55	4.30	19.05

Table 1. Mean values of chemical composition (%dry matter) of different protein sources (Calabrò et al., 2001; Calabrò et al., 2009; Calabrò et al., 2010).

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The proportion of threonine in grain legume protein (38 to 42 g/kg crude protein) is similar to that in soybean meal (45 g/kg soybean meal) (Degussa, 2006), however, there is a severe deficiency in the sulphur containing AA methionine + cystine, while tryptophan is marginally deficient to fulfill nutrient requirements for pigs (20 to 50 kg body weight) (NRC, 1998; Degussa, 2006). In fact, apart from *L. albus*, the seeds of faba beans, peas and lupins

contain less than 50% of these AA in comparison to soybean meal (Table 2), thus constraining the use of grain legumes as sole protein source in pig diets.

	Vicia faba	*Pisum sativum*	*Lupinus albus*	*Lupinus angustifolius*	*Lupinus luteus*	SBM
CP	301	246	381	324	361	541
Indispensable AA						
Arginine	26.4	21.0	39.3	33.5	38.0	39.7
Histidine	7.8	6.1	9.3	8.8	9.7	14.4
Isoleucine	11.8	10	15.3	12.7	14.2	24.3
Leucine	21.4	17.4	27.5	21.5	24.1	40.9
Lysine	18.4	17.3	18.2	15	16.3	33.1
Methionine	2.2	2.2	2.5	2.0	2.0	7.3
Phenyl-alanine	12.6	11.7	14.9	12.5	13.6	27.2
Threonine	10.5	9.1	13.3	10.9	11.9	21.3
Tryptophan	2.6	2.2	3.0	2.6	3.0	7.4
Valine	13.3	11.4	14.5	12.5	13.6	25.5
Dispensable AA						
Alanine	11.9	10.5	12.5	10.9	11.8	23.3
Aspartic acid	31.6	28.2	38.5	31.5	35.1	62.0
Cystine	3.5	3.5	6.7	4.3	4.8	8.0
Glutamic acid	46.9	40.0	79.3	65.6	72.5	97.6
Glycine	12.2	10.6	15.0	13.4	14.3	23.0
Proline	11.8	10.2	15.3	13.5	14.3	27.5
Serine	14.1	11.5	19.0	15.3	17.0	27.3

Table 2. Amino acid contents of grain legumes compared to soybean meal (g/kg dry matter) (Jezierny et al., 2010) SBM= soybean meal; CP= crude protein; AA= amino acids.

As concerns the fatty acid profile, the rather high proportion of essential unsaturated fatty acids of some grain legumes, e.g. some *Vicia* species (Akpinar et al., 2001) or *L. albus* (Erbas¸ et al., 2005) may be attractive both from the human and animal nutrition perspective (Bézard et al., 1994), while adverse effects of unsaturated fatty acids on meat quality should be taken into account (Wood et al., 2003). For example, in faba beans a ratio of saturated to unsaturated fatty acids of 40–60 has been reported (Akpinar et al., 2001), whereas in *L. albus*, a ratio of saturated, monounsaturated and polyunsaturated fatty acids of 13.5 to 55.4 to 31.1 has been established (Erbas¸ et al., 2005).

As concerns the mineral composition of soybean and grain legumes, only few data are reported in literature.

The calcium concentration ranges between 1.0 g/kg (faba beans) and 1.9 g/kg (*L. angustifolius*). Phosphorus concentration varies between 4.2 g/kg (*L. angustifolius*) and 7.6 g/kg (*L. luteus*). No extreme differences in trace mineral concentrations occurred except for the manganese concentration of *L. albus*, which containes approximately 10 times more manganese than the other legume grains (Brand et al., 2004).

Concerning the trace elements, Cabrera et al (2003) reported that in legumes their levels ranged from 1.5–5.0 µg Cu/g, 0.05–0.60 µg Cr/g, 18.8–82.4 µg Fe/g, 32.6–70.2 µg Zn/g, 2.7–45.8 µg Al/g, 0.02–0.35 µg Ni/g, 0.32–0.70 µg Pb/g and not detectable—0.018 µg Cd/g. In nuts, the levels ranged from 4.0–25.6 µg Cu/g, 0.25–1.05 µg Cr/g, 7.3–75.6 µg Fe/g, 25.6–69.0 µg Zn/g, 1.2–20.1 µg Al/g, 0.10–0.64 µg Ni/g, 0.14–0.39 µg Pb/g, and not detectable—0.018 µg Cd/g. The authors found a direct statistical correlation between Cu–Cr, Zn–Al and Cr–Ni ($P<0.05$), and Al–Pb ($P<0.001$).

Sankara Rao and Deosthale (2006) comparing for their total ash, calcium, phosphorus, iron, magnesium, zinc, manganese, copper, and chromium contents five grain legumes (indian legumes, chick pea, pigeon pea, green gram, and black gram), found significant varietal differences only for chromium content in black gram. The cotyledons of these legumes were significantly lower in calcium content as compared to the whole grains. The authors concluded that for human nutrition, differences in mineral composition of whole grain and cotyledons were marginal except for calcium. These legumes as whole grain and cotyledons, appeared to be significant contributors to the daily requirements of magnesium, manganese, and copper in the diet.

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3. *In vitro* evaluation of soybean and legume grains

The nutritive value of a feed is assessed by its chemical composition, digestibility and level of voluntary intake. Feed evaluation methods are use to express nutritive value of feed. It is basically description of feeds interns that allow for a prediction of the performance of animals offered the feeds (Medsen et al., 1997).

Several methods are used in feed evaluation such as chemical analysis, rumen degradability measurement using the nylon bag technique, digestibility measurement and feed intake

prediction. However, the tables supply mean values, which cannot be used for individual lots, and all the *in vivo* techniques are very expensive and time consuming while accuracy may be low.

The *in vitro* gas production technique (IVGPT, Theodorou et al., 1994) has proved to be a potentially useful technique for ruminant feed evaluation (Herrero et al., 1996; Getachew et al., 2004), as it is capable of measuring rate and extent of nutrient degradation (Groot et al., 1996; Cone et al., 1996).

To evaluate a feedstuff by IVGPT, it is incubated at 39°C and under anaerobiosis condition with buffered rumen fluid and gas produced is measured as an indirect indicator of fermentation kinetics. During the incubation the feedstuff is first degraded and the degraded fraction may either be fermented to produce gas (CO_2 and methane) and fermentation acids, or incorporated into microbial biomass.

The IVGPT is considered the most complete *in vitro* technique, because it allows to estimate the fermentation kinetics and contemporary gives information on the fermentation products (degradability of dry matter, volatile fatty acids).

The IVGPT has been used by our research group for many years in order to investigate feed fermentation kinetics.

In particular, in this chapter we report some results obtained incubating different protein sources (i.e. soybean and legumes grains) in order to compare their fermentation kinetics (Calabrò et al., 2001a), to assess the effect of some technological treatments (i.e. crushing and flaking) on the carbohydrates fermentation kinetics (Calabrò et al., 2001b) and to test different legumes grain cultivars (Calabrò et al., 2009).

Calabrò et al. (2001a), in order to study the *in vitro* fermentation characteristics and kinetics proposed the following protocol: the samples (about 1.00 g), ground to pass a 1 mm screen, were incubated in triplicate at 39°C in 120 ml serum bottles under anaerobic conditions. Rumen liquor for the *inoculum* was collected from four buffaloes fed a standard diet, and immediately transported to the laboratory where it was homogenised and filtered.

The gas measurements was made at 2-24 time intervals using a manual a pressure transducer (figure 1).

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, the degraded organic matter (dOM, %) was calculated as a difference between incubated and residual OM (filtering the bottle content through pre-weighed glass crucibles and burning at 550°C for 3 hours), and pH and volatile fatty acid concentration (VFA, mM/g) were determined, using a pH-meter and a gas chromatography, respectively.

Several concentrate ingredients such as cereals and grain legumes, used in ruminant diets in order to increase production levels, were evaluated. In particular barley, maize, hard wheat, soft wheat, oats, faba bean and pea were used as test substrates.

Carbohydrates fractionation was carried out according to Cornell Net Carbohydrate and Protein System (CNCPS, Sniffen *et al.,* 1992).

The Cornell Net Carbohydrate and Protein System was developed to predict requirements, feed utilization, animal performance and nutrient excretion for dairy and beef cattle and sheep, using accumulated knowledge about feed composition, digestion, and metabolism in supplying nutrients to meet requirements.

Fig. 1. Pressure transducer.

The CNCPS partitions crude protein into fractions A, B, and C, depending on their rate and extent of degradability in the rumen (NRC, 2001). Fraction A represents the non-protein N (NPN) (ammonia, peptides, amino acids) and is considered to be completely soluble; fraction B, subdivided into B1, B2, and B3, consists of true protein with progressively declining ruminal degradability. Fraction C is unavailable true protein. Broadly, these crude protein fractions are categorized into rumen degradable protein (RDP) and rumen undegradable protein (RUP). The rumen degradable protein meets protein requirements for ruminal microbial growth and protein synthesis.

Once reaching the rumen, feed and protein degradation is a function of microbial activity. Rumen microbial activity, growth and protein synthesis is primarily limited by the rate and extent of carbohydrate fermentation in the rumen. Consequently, dietary fiber fractions in the forage determine the animal response to feed.

Microbial protein and rumen undegradable protein reaching the small intestine are absorbed to meet the ruminant's protein requirement. When rumen degradable protein exceeds the capacity of the rumen microbes to assimilate it, ammonia builds up in the rumen. This is followed by absorption of ammonia into the blood, conversion into urea by the liver, and excretion in the urine. The conversion of ammonia to urea costs the dairy cow energy that could otherwise be used for milk production. This loss of dietary crude protein and energy reduces the utilization efficiency of rumen degradable protein and therefore, reduced ruminant production (NRC, 2001). It also causes a negative energy balance that leads to a reduced fertility.

In the study of Calabrò et al. (2001a), the CNCPS carbohydrate fractions (table 3) was consistent with the values reported by Sniffen *et al.* (1992).

Oats (table 4) had the lowest potential gas production (A: 251 *ml/g,* P<0.0) and a very fast fermentation process (evidenced by low B and high RM). This result was probably due to its high soluble sugars contents (A fraction). Interestingly, oat values were very high compared to *in situ* observations. As reported by Van Soest *et al., (*1992), maize proved to be the slowest because of its high starch content (B_1 fraction), which degraded slowly. Hard and soft wheat showed very similar fermentation characteristics according to their chemical composition. Barley has fermentation kinetics between those of wheat and maize. Results related to these last three grains were similar to the results obtained *in vitro* using the IGPT (Mould *et al.*, 2005).

	Barley	Maize	Oats	Hard wheat	Soft wheat	Faba bean	Pea
CP (% DM)	11.0	8.79	12.2	14.4	13.5	29.8	23.9
A	3.80	0.49	12.6	2.40	5.00	6.00	18.0
B1	66.4	75.4	45.6	67.6	65.7	43.6	40.8
B2	11.4	6.14	16.0	9.86	9.64	9.99	7.92
C	3.60	2.57	6.77	2.64	2.16	6.41	4.18

Table 3. Chemical composition and CNCPS fraction of the tested grains.

Legumes were characterised by high gas production alongside a rather slow fermentative process. However, interpretation of faba bean and pea data was complicated by their high protein content, whose degradation can interact with gas production (Schofield., 2000).
Organic matter degradability for all tested grains was always higher than 95%.
At 72 h the pH always remains good to guarantee microbial activity.
Oats reached the maximum rate before all the other grains (figure 2) and remained the highest until 12 incubation hours, subsequently decreasing below the soft wheat level. Until 6 hours of incubation barley was faster than the two wheat, later becoming slower until the end; up to this time the three sharp curves were practically overlapping, suggesting that the fermentation trend was similar for the three grains. Maize, faba bean and pea always showed the slowest fermentation rate with overlapping trends: fermentation slowly reaches its maximum rate and slowly decreases. The results of this investigation evidenced the validity of the IVGPT in describing the kinetics fermentation of the examined grains.
The range of rates obtained with the GPT (oats>wheat>barley>maize>legumes) approximately reflects that reported by other authors cited by Sniffen *et al.* (1992): wheat>oats>barley>maize.
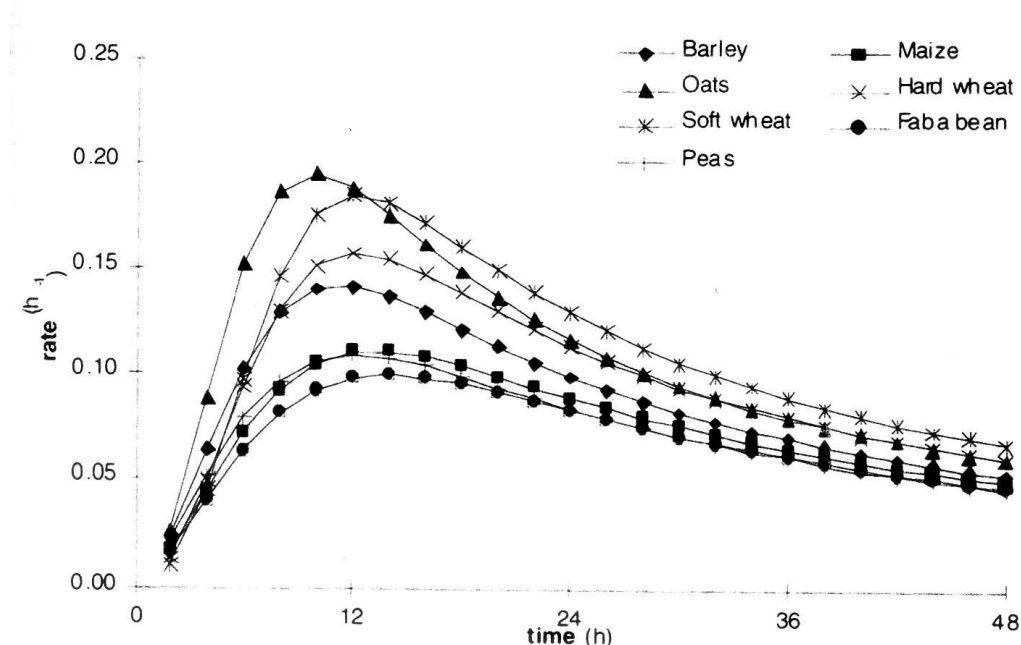

Fig. 2. Gas production fractional rate plots of different grains.

In the second investigation Calabrò et al. (2001b) evaluated the effects of flaking and crushing on some cereal and legume grains, using the *in vitro* cumulative gas production technique (Theodorou *et al.*, 1994).

The grains used in animal nutrition are commonly subjected to different technological treatments (i.e.: grounding, crushing, flaking, extrusion, cooking, micronization). Flaking is a hydrothermal-mechanical process which causes starch gelatinization and increases the enzymatic hydrolysis rate of the polysaccharide, which in turn favours the rumen microbial attack. Crushing differs from flaking in the shorter exposure to vapour. Also in this case, the obtained product presents a larger surface area compared to the primary grain, and a modified starch, which promotes faster fermentation kinetics.

Several investigations to evaluate the effect of flaking on organic matter degradability in the rumen, indicated the considerable influence of the primary grain (Arieli *et al.*, 1995; Bittante *et al.*, 1989); by contrast, little was known about the effects of crushing.

		VC$_{SOI}$	A	B	RM	dOM	Y	VFA
		ml/g	ml/g	h	h^{-1}	%	ml/g	mM/g
Maize	wholegrain	340	362	14.9	0.078	92.9	363	99.1
	flaked grain	338	347	13.2	0.096	96.6	343	83.3
	whole + flaked	339[A]	355[A]	14.1[B]	0.087[B]	94.8	353[A]	91.2[A]
Barley	wholegrain	304	323	10.2	0.125	90.6	341	92.4
	flaked grain	346	369	11.7	0.104	97.9	359	94.8
	whole + flaked	325[B]	346[A]	11.0[C]	0.115[A]	92.6	350[A]	93.6[A]
Soybean	wholegrain	204	236	15.5	0.051	96.7	209	71.6
	flaked grain	225	280	19.8	0.039	86.8	257	76.0
	whole + flaked	215[C]	258[B]	17.6[A]	0.045[C]	91.8	233[B]	73.8[B]
Main effect								
	grain	***	***	***	***	n.s.	***	**
	treatment	***	**	**	n.s.	n.s.	**	n.s.
Interaction								
	grain. x treat.	***	***	***	***	***	***	n.s.
Var.er.		87.9	135	1.21	0.00009	10.5	206	103

OMCV, cumulative gas production related to incubated OM at 120 h; A, potential gas production; B, time at which A/2 is produced; Rmax, maximum fermentation rate; Y, yield related to the degraded OM; VFA, volatile fatty acids related to degraded OM.
*a,b,c: P<0.05; A,B: P<0.001ns, not significant;***: p < 0.001.*

Table 4. *In vitro* fermentation characteristics of whole and flaked grains.

In this experiment three whole and flaked grains (barley, maize, soybean) and four whole and crushed grains (barley, oats, maize, faba bean) were used as test substrates.

Fermentation characteristics (Table 4 and 5) were affected by the grain type and treatment; in addition, the two factors interacted with each other.

		OMCV	A	B	Rmax	dOM	Y	VFA
		ml/g		*H*	*h⁻¹*	*%*	*ml/g*	*mM/g*
Oats	whole grain	321	334	10.6	0.107	87.8	362	90.6
	crushed grain	272	311	10.3	0.106	86.9	332	90.5
	whole +crushed	296^B	322^B	10.4^C	0.0106^{Aa}	87.3^{aCD}	347^B	90.5^{AB}
Faba bean	whole grain	335	360	14.4	0.071	96.7	344	94.3
	crushed grain	325	367	14.8	0.062	89.9	359	79.3
	whole +crushed	330^A	364^A	14.6^A	0.066^B	93.3^B	352^B	86.8^{AbB}
Maize	whole grain	324	348	12.2	0.099	97.6	331	90.6
	crushed grain	327	350	13.4	0.092	96.7	338	100
	whole +crushed	326^{Ab}	349^A	12.8^B	0.096^{AbC}	97.2^A	335^B	95.5^{aA}
Barley	whole grain	310	327	8.68	0.138	81.5	398	77.3
	crushed grain	370	386	9.59	0.128	90.1	395	91.7
	whole +crushed	340^{Aa}	357^A	9.13^D	0.133^D	85.8^{bD}	397^A	84.5^B
Main effect								
	Grain	***	***	***	***	***	***	***
	Treatment	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Interaction								
	gran x treat	***	***	n.s.	n.s.	***	***	n.s.
	Var. er	204	398	0.73	1•10⁻¹	1.85	437	48.19

OMCV, cumulative gas production related to incubated OM at 120 h; A, potential gas production; B, time at which A/2 is produced; Rmax, maximum fermentation rate; Y, yield related to the degraded OM; VFA, volatile fatty acids related to degraded OM.
a,b,c: P<0.05; A,B: P<0.001ns, not significant;***: p < 0.001.

Table 5. *In vitro* fermentation characteristics of whole and crushed.

Flaking increased the potential gas production (A) for soybean and barley (P< 0.01), and slowed their fermentation kinetics (increased t/1/2 and decreased RM values). By contrast, maize flakes showed lower gas production (P<0.05) and the fermentation process was faster (P<0.01), compared to whole grain.
Organic matter degradability decreased after the treatment for soybean (P<0.01), while it increased for barley (P<0.01) and, not significantly, for maize. The slower fermentative process for barley flakes (increased t/1/2and decreased RM values) agrees with the results of other authors on treated cereals for both *in vitro* (Bittante et al., 1989) and *in situ* experiments (Arieli et al., 1995).
Comparing each grain with the respective flakes, OM degradability did not strictly follow the trend of potential gas production, confirming that not all the degraded OM is fermented. This result holds mainly for soybean flakes, which had a higher A (P<0.01) and a lower dOM (P<0.01) compared to the whole grain.
Such decreased OM degradability was probably due to the thermal treatment causing a decrease in the rapidly degradable fraction (Sarubbi, 1999).
Overall, the two cereals showed higher gas and VFA production, as well as a faster fermentative process.

Crushing showed slighter effects compared to flaking, and also the results of the grain x treatment interaction were less important.

The different technological condition of the two treatments (less drastic for crushing) may well have contributed to this trend. In particular, crushed barley presents, with respect to the whole grain, the same behaviour as the flakes (higher A and dOM, P<0.001; higher *t/1/2* and lower RM, not significant).

Interestingly, there was a decrease in faba bean degradability due to the treatment (P<0.01), which agrees with the results of a contemporaneous *in situ* trial (Sarubbi, 1999). Besides, faba bean also showed a lower VFA concentration, which was probably also caused by the considerable decrease in their protein degradability (P<0.001) as found *in situ* (Sarubbi, 1999). As usually observed *in vivo* in young bulls fed a cereal-rich diet, pH values were always quite low.

In the third study Calabrò *et al*. (2009) evaluated the fermentation characteristics of different cultivars of grain legumes using IVGPT.

Three grain legumes were tested: lupine (*Lupinus* spp.) (Lublanc, Luteur, Multitalia), faba bean (*Vicia faba* L.) (Chiaro di Torre Lama, Irena, Lady, ProtHABAT69, Scuro di Torre Lama, Sicania) and peas (*Pisum sativum*) (Alembo, Alliance, Attika, Corallo, Iceberg, Ideal, Spirale).

The fermentation characteristics are reported in table 6.

The values of pH ranged between 6.35 and 6.72, indicating a normal pattern of fermentation, and were consistent to the crude protein content.

As regards faba bean, the cultivar "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 cultivar "Lublanc" had lower (P<0.01) OMCV than the other 2 cultivars and for peas the cultivar "Spirale" produced less gas and showed a faster 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 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 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, the authors concluded 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.

The reported results are of particular importance as well as highlight the differences in dietary and nutritional characteristics of soybean and legume grains, provide data on the effects of the treatments of these feedstuffs and the differences among the cultivars present on the European market.

With this information, the nutritionist may from time to time choose the most suitable protein source in order to satisfy animal requirements ensuring the simultaneous availability of nitrogen and energy for the bacteria present in the rumen with beneficial effects on livestock production and environmental impact.

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.

Table 6. Fermentation characteristics of the different grain legume cultivars and soybean meal.

4. Effect of different protein sources on animal performance

Several researches have been carried out in order to compare the nutritional characteristics of soybean solvent extract and legume grains, as faba bean, peas and lupine, for ruminant feeding.

Di Francia et al. (2007) evaluated the effect of partial replacement of soybean cake with extruded peas in the diet of lactating buffalo cows on milk yield and quality over the first 100 days of lactation. Their results showed that peas could represent an attractive GMO free

protein feed when approaching the problem of the choice of a protein source alternative to soybean in diet formulation for buffalo cows raised in organic farms.

On the other hand Morbidini et al (2005) investigating on the effect of two different fattening diets with different protein sources - soybean meal and flaked faba bean - on slaughtering performance and carcass quality in light Apennine and Italian Merino lambs, observed that the use of faba beans lightly depressed growth performance (Morbidini et al., 2004) and slaughtering weight, even if did not affect the carcass quality.

In order to make a contribution on this issue, our research group conducted a trial on the effect of protein source on growth performance and meat quality of Marchigiana young bulls (Cutrignelli et al 2008 a, b).

The trial was carried out on a farm situated at 700 m a.s.l. in Campania Region (Southern Italy), where 12 weaned young bulls (129 d of age) were equally divided into two groups. Each animal was placed in individual box up to the slaughtering weight (620 kg).

The groups were fed diets with the same protein and energy concentrations and the same forage/concentrate ratios (F/C), but differing in protein source: faba bean (*Vicia faba minor* L.) vs soybean meal (*Soja hispida*).

All the animals were regularly weighed until the body weight (BW) of 620 kg fixed in advance as slaughter weight, was reached. All animals were slaughtered in an authorized slaughterhouse according to EU legislation (EU Regulation EC No 882/2004).

Live animals and carcasses were weighed and measured according to ASPA (1991). After 9 days of refrigeration at 4 ± 1 °C, dissection of the carcasses was carried out.

Samples of *Longissimus thoracis* (LT), *Semitendinosus* (ST), *Iliopsoas* plus *Psoas minor* (IP) muscles, perirenal (PF) and subcutaneous (SF) adipose tissues were collected and rapidly transported, upon refrigeration temperature, to the laboratories for the chemical analysis in order to evaluate the rheological (water holding capacity) and nutritional (chemical composition fatty acid profile, cholesterol and hydrossiproline contents) characteristics of meat.

The protein source did not affect any *infra vitam* parameters except the body weight (BW) at 180 d of age (173 vs. 186 kg, for group FB and SB, respectively; P<0.01).

The difference was probably due to the higher non-protein nitrogen (NPN) concentration of the faba bean than the soybean meal (about 12 vs 1.3% of crude protein, respectively). Indeed, in the months immediately following the weaning it should be preferable to administer diets with higher rumen undegradable protein content because at this age the rumen is not yet perfectly functional and microbial protein synthesis is less efficient. Furthermore, for the same reason, the animals in this period are probably unable to neutralize possible anti-nutritional factors of faba beans.

As respects the influence of the replacement of soybean meal solvent extract with legumes seeds, the literature results are contrasting. Moss et al. (1997) found no significant effects on weight gain and feed intake when soybean meal was replaced on an iso-protein basis by lupin seeds in diets for growing bulls (BW from 182 to 243 kg); similar results are reported by Kwak and Kim (2001) on Korean native bulls (BW from 247 to 427 kg) utilising two different concentrations (15 and 30%) of flaked lupin. Instead, according to our results, Murphy and McNiven (1994) found significantly higher weight gain in growing steers (BW from 235.2 to 343.7 kg) fed soybean meal vs raw or roasted lupin although the differences were not significant in the finishing phase (final BW 503.4 kg).

No significant differences between the groups were found for carcass measurements. In each case the carcass measurements of this trial ranged into the interval indicated by Keane (2003) for European/North American breeds.

No differences were found in dressing out, organs and tare incidence on net weight.

Protein source influenced neither body and carcasses conformations nor dressing out with the exception of the incidence of long bones showed a significant difference (6.2 vs 6.7 for faba and soybean, respectively; P<0.05).

The first quality meat cuts were acceptable in both groups (58.1 and 57.8%, for faba and soybean, respectively). Concerning the comparison between the two protein sources, only the incidence of long bones showed a significant difference (6.2 vs 6.7 for faba and soybean, respectively; P<0.05). It is important to underline that the sample cut measurements were contradictory and conflicted with the data obtained from total carcass dissection. The results of the sample cut dissection indicated a significant (P<0.05) difference between groups faba and soybean in meat incidence (69.4 vs 66.9, for faba and soybean, respectively); while no differences were found between groups at carcass dissection. Moreover, sample cut of soybean group showed in the meantime the smallest meat incidence and the highest LT area (88.4 vs. 84 for soybean and faba group, respectively).

The animals fed faba bean showed significantly higher water losses, measured with the compression method (WHC 7.6 vs. 5.7% for faba and soybean group, respectively; P<0.01).

Our grilling loss data (31.7 vs. 28.3 % for faba and soybean group, respectively; P>0.01) were higher than those reported by Sami *et al.* (2004) in Simmental young bulls but they were in agreement with those reported by Pen *et al.* (2005) cooking ST samples of Holstein steers in an oven, on the contrary our drip loss data resulted higher than that reported by these authors.

The chemical composition of LT was not statistically different between groups. Meat from both groups showed a very low fat content (<3%) and higher protein concentrations than the Holstein steers (Pen *et al.*, 2005), confirming the high quality of the Marchigiana meat.

Hydroxyproline (60.0 vs. 62.6 mg 100g^{-1} of meat, in group faba and soybean, respectively) contents were not influenced by protein sources. Regarding the differences registered for this parameter among the texted muscles (LT: *Longissimus thoracis*; ST: *Semitendinosus*; IP: *Iliopsoas* plus *Psoas minor*) the IP samples, which correspond to the tenderloin, showed in all the groups significantly (P<0.01) lower hydroxyproline concentrations than the other two muscles; also between the LT and ST muscles the differences were statistically significant (P<0.05) being lower for the former.

Cholesterol content (56.3 vs.55.1 mg 100g^{-1} of meat, in group faba and soybean, respectively) was not influenced by protein sources according to the observations of Cutrignelli (2000) on Podolian young bulls and by Poli *et al.* (1996) on Chianina young bulls.

Considering the differences among the muscles, cholesterol values were significantly (P<0.05) lower for IP. Cifuni *et al.* (2004) found no differences in cholesterol contents among muscles, while Rusman *et al.* (2003) found significant differences. This contradiction is probably due to the different muscles analysed in each experiment. As theorised by Wheeler *et al.* (1987) the cholesterol content may be affected by the different physiological function of the muscles. In both groups, and especially for LT and ST muscles, the cholesterol contents were slightly higher than the value (less than 50 mg100g^{-1} of muscle) indicated by the Protected Geographical Indication (PGI) of the "Vitellone Bianco dell'Appennino Centrale" (Council Regulation EEC No 2081/92; Floroni, 2002). Nevertheless, our results are very close

to those reported for Italian meat breeds (Poli *et al.* 1996; Cifuni *et al.,* 2004) and lower than those from other breeds (Migdal *et al.,* 2004).

Regarding the fatty acids profile in both groups and in each analysed tissues (intramuscular, perirenal and subcutaneous adipose tissues) palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids were the most widely represented fatty acids. In particular, in intramuscular fat, the sum of oleic, stearic and palmitic acids represents over 50% of total fatty acids, according to the observations of Cifuni *et al.* (2004) and Migdal *et al.* (2004).

Comparing the data of groups faba and soybean, the only significant (P<0.01) difference was for stearic acid being higher for bulls fed faba bean than for those fed soybean meal solvent extract. However this result did not significantly affect the SFA concentration, or AI and TI indexes (table 7).

Protein source did not affected the fatty acids composition of the analysed adipose tissues. This is probably due to the very low concentration of phospholipids in subcutaneous and perirenal fat tissue.

Comparing the fatty acid composition of the three adipose tissues the following differences were noted:

- the erucic (C22:1), docosahexaenoic (DHA, C22:6, ω-3) eicosapentaenoic (C 20:5, ω-3) and docosapentaenoic (C 22:5, ω-3) acids, were present only in intramuscular fat, due to the lower level of intramuscular fat compared to the two adipose tissues. Lower fat content is associated with fewer and smaller adipocytes containing fewer triglycerides, accompanied by a relative increase in the proportion of phospholipids in total lipids and an increased PUFA content (Scollan *et al.,* 2006);
- the proportion of total SFA was minimal in LT and maximal in SF (mean values 46.56 *vs* 54.50 and 59.35% of total fatty acids; in LT, PF and SF, respectively, Figure 1); the single saturated fatty acids amounts also showed a similar trend. These data are partly in contrast with those of Kim *et al.* (2004) who in Hanwoo cattle found a higher SFA proportion in perirenal than in subcutaneous fat. Even if the differences in the fatty acids profile between internal and external fat deposits have not been fully elucidated, Eguinoa *et al.* (2003) suggested that the lipogenic enzyme activities per cell could be influenced by several factors such as adipocite size, nutrient supply, etc.;
- the proportion of total polyunsaturated fatty acids (PUFA) was higher in LT and lower in the perirenal adipose tissue (mean values 22.23 *vs* 11.36 *vs* 11.02% of total fatty acids, respectively in LT, SF and PF, Figure 1). This difference may be primarily ascribed to the lower concentration of ω-6 PUFA and to the absence DHA in SF and PF adipose tissues;
- the atherogenic and thrombogenic indexes were considerably higher in SF and PF tissues than in LT. This is due to the high SFA concentration of SF and PF adipose tissues as well as to the high PUFA concentration of LT.

The LT fatty acid profile was similar to those reported by Raes *et al.* (2003) for *Longissimus lumborum* of Belgian Blue and Limousin beef (SFA: 338 and 506 mg 100g[-1] edible portion; MUFA: 323 and 554 mg 100g[-1] edible portion; PUFA: 195 and 195 mg 100g[-1] edible portion; in Belgian Blue and Limousin bulls, respectively) and different from values found in Irish and Argentine beef. The latter showed significantly higher total intramuscular fatty acid content compared to the former, probably due to genetic selection.

This observation confirms that of Carnovale and Nicoli (2000) who concluded that Italian meat showed a favourable intramuscular fatty acid composition with high PUFA content. Muscle with a high percentage of unsaturated fatty acids (UFA) generally scored higher in

taste panel evaluation (Westerling and Hedrick, 1979) and food with high UFA, especially PUFA, is good for human health (Rusman *et al.*, 2003). The ω-6/ω-3 ratio was higher than the value (less than 3) reported by Scollan *et al.* (2006) but lower than that registered by Warren *et al.* (2003) for steers fed corn silage and concentrates (8.9).

It has to be underlined that the fatty acid profile o the food arouse high interest in human medicine due to their influence on the functionality of the cardio-circulatory apparatus. A number of epidemiological researches put in evidence that diets with high content of saturated fatty acids (SFA) were associated with high levels of serum cholesterol (especially of low density lipoprotein, LDL) which appear important in atheroma. Successively, Ulbricht and Southgate (1991) reported that:

1. diets high in C18:0, stearic acid, do not raise serum cholesterol;
2. short-chain SFA (C 10 and below) likewise do not raise blood cholesterol, so the putative atherogenic SFA are C12:0 (lauric), C14:0 (myristic) and C16:0 (palmitic). Myristic acid is the most atherogenic, with about four times the cholestrol-raising potential of palmitic acid.

PUFA are considered protective factors: ω-6 fatty acids show mainly anti-atherogenic activity while ω-3 fatty acids have anti-thrombogenic activity. More recently, high prominence is attributed to the role developed by the MUFA, and particularly by the oleic acid that, reducing the oxidation of the cholesterol LDL, may slow the progression of atherosclerosis.

As was pointed out above, the P/S (poliunsaturated/saturated) ratio is not suitable measure of the atherogenicity or thrombogenicity of a diet or foods. Currently they are expressed as follows:

Index of atherogenicity: C12:0 + (4 x C14:0) + C16:0/ ω-3 + ω-6 + MUFA

Index of thrombogenicity: C14:0 + C16:0 +C18:0/(0,5 x C18:1) + (0,5 other MUFA) + 0,5(ω-6) + 3(ω-3) + (ω-3/ ω-6).

The AI of the meat in this trial was particularly interesting, rather lower than the data reported by Ulbricht and Southgate (1991) for raw minced beef and than those reported by Badiani *et al.* (2002) for cooked beef (0.72 and 0.77, respectively); our data were similar to those of Poli *et al.* (1996) on Chianina young bulls (AI: 0.58).

	FB	SB	Significance
SFA	365.1 ± 39.1	330.6 ± 26.9	Ns
MUFA	244.3 ± 39.5	271.7 ± 29.4	Ns
PUFA	186.6 ± 20.5	201.9 ± 15.7	Ns
ω-6	174.2 ± 19.9	188.3 ± 12.9	Ns
ω-3	12.39 ± 1.5	13.57 ± 0.8	Ns
AI	0.54 ± 0.04	0.49 ± 0.07	Ns
TI	1.46 ± 0.12	1.20 ± 0.09	Ns

FB: faba bean; SB: soybean meal solvent extract;AI: atherogenic index; TI thrombogenic index.
ns: not significant.

Table 7. Fatty acid profile of Longissimus thoracis muscle (mg 100g^{-1} of edible part).

However, the index of thrombogenicity in this trial was higher than the findings of the above-cited authors (1.27 and 1.30 for Ulbricht and Southgate, 1991 and Poli *et al.*, 1996, respectively); only the TI (1.77) reported by Badiani *et al.* (2002) was similar to our data.

These results show that the faba bean could be used as an alternative protein source to soybean meal solvent extract as it did not affect the growth rate (body weight, daily weight gain and biological efficiency of growth) or the feed conversion indexes during the whole experimental period, and offers decided agronomical, economical and healthy advantages.

Nevertheless, in the first period after weaning the faba bean reduced the growth rate, probably due to the higher concentrations of NPN and anti-nutritional factors. It might be useful in this period to use this protein source associated with other richer in rumen undegradable.

Our results contribute to show that both protein sources (soybean meal and faba bean) could be utilised in the diet for young Marchigiana bulls ensuring the high quality of the meat obtained by this breed.

Although the meat of group fed faba bean had significantly higher concentrations of stearic acid compared to the level found in soybean group, neither the atherogenic and thrombogenic indexes, nor the cholesterol content were influenced.

As regards the effect of protein source on organolepitc characteristics, our results on hydroxyproline content and water holding capacity were conflicting. While the meat of the group fed soybean meal solvent extract showed a potential low tenderness (higher level of hydroxiproline) the water holding capacity measured by compression was lower for the group receiving faba bean.

From our results it is also possible to formulate a favourable assessment of the nutritional characteristics of the of meat Marchigiana young bulls. Indeed, the cholesterol values were very close to those indicated by the PGI of the "Vitellone Bianco dell'Appennino Centrale" and lower than those found in other breeds. Moreover, the fatty acids profile of LT confirms that the meat of the Italian breed specialised in meat production has higher unsaturated fatty acids concentration and lower saturated fatty acids levels, which in turn ensures medium-low atherogenic and thrombogenic indexes.

5. Conclusions

The protein source largely used in ruminant nutrition (soybean, soybean meal solvent extract and grain legumes) are characterised by several differences in chemical composition, in particular as concerns crude protein, ether extract and carbohydrates concentrations. Also the technological treatments as flaking and crushing could affect these parameters. In addition, mainly concerning the grain legumes, the contents of different nutrients is affected by the cultivar.

In order to better estimate the nutritive value of protein source, the *in vitro* gas production technique seems to be the most useful methods, as it allow to study also the kinetics of degradation in the rumen. The knowledge of the latter phenomenon is particularly useful, since the nutritionist may choose to treat a particular protein source in order to synchronize the availability of nitrogen and energy for rumen bacteria.

From our *in vitro* data, faba bean, lupin and peas, even if from different cultivars, may be considered in replacing, totally or partially, soybean as only few differences were found among these feeds.

In addition, our *in vivo* studies concerning the *infra vitam* and *post mortem* performances of young bulls fed either faba bean or soybean showed:

- no influence of protein source on growth rate (body weight, daily weight gain and biological efficiency of growth) and feed conversion indexes;
- no influence of protein source on the nutritional characteristics of meat. Indeed, even if the meat of group fed faba bean had significantly higher concentrations of stearic acid compared to the level found in soybean group, neither the atherogenic and thrombogenic indexes, nor the cholesterol content were influenced;
- conflicting results on hydroxyproline content and on water holding capacity. Indeed, while the meat of the group fed soybean showed a potential low tenderness (higher level of hydroxiproline), the water holding capacity measured by compression was lower for the group receiving faba bean.

From these results, it can be concluded that the use of grain legumes as a protein source in ruminant diets could be used as alternative to soybean meal. However, mainly in the intensive livestock system the soybean meal solvent extract represents the protein source par excellence for the high protein content (in particular undegradable fraction), the absence of anti-nutritional factors and the extremely favourable biological value of its proteins. Not insignificant, then is the economic assessment, soybean is, especially in the areas where the ruminants breeding has been particularly developed, the protein source with the quality/price ratio more favourable.

By contrast, it has to be underlined that biotech herbicide tolerant soybean continued to be the principal biotech crop in 2010, occupying 73.3 million hectares or 50% of global biotech area. Farm animals are currently fed soybean and soybean meal developed from genetic transformation. Europe is strongly dependent upon the American continent for its protein requirements amounting up to 90 to 95%.

Thus, the possible risk connected to genetically modified organisms use in animal breeding has led to the reconsideration of animal production processes with special reference to the use of alternative protein sources (e.g. faba beans, dried peas, lupine seeds, chickpeas) able to replace soybean. These legumes have agronomic importance because they improve soil fertility and reduce nitrogenous dressing, with positive effects on environmental pollution. Moreover, they need a limited initial investment for their modest requirements of chemical and energetic inputs and their short culture cycle.

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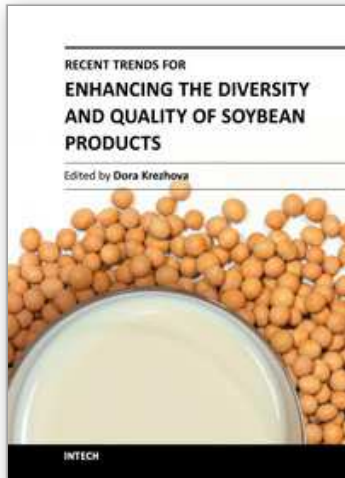
Recent Trends for Enhancing the Diversity and Quality of Soybean Products

Edited by Prof. Dora Krezhova

ISBN 978-953-307-533-4

Hard cover, 536 pages

Publisher InTech

Published online 28, October, 2011

Published in print edition October, 2011

This book presents new aspects and technologies for the applicability of soybean and soybean products in industry (human food, livestock feed, oil and biodiesel production, textile, medicine) as well as for future uses of some soybean sub-products. The contributions are organized in two sections considering soybean in aspects of food, nutrition and health and modern processing technologies. Each of the sections covers a wide range of topics. The authors are from many countries all over the world and this clearly shows that the soybean research and applications are of global significance.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Monica I. Cutrignelli, Serena Calabrò, Raffaella Tudisco, Federico Infascelli and Vincenzo Piccolo (2011). Protein Sources in Ruminant Nutrition, Recent Trends for Enhancing the Diversity and Quality of Soybean Products, Prof. Dora Krezhova (Ed.), ISBN: 978-953-307-533-4, InTech, Available from: http://www.intechopen.com/books/recent-trends-for-enhancing-the-diversity-and-quality-of-soybean-products/protein-sources-in-ruminant-nutrition