TRansition paths to sUstainable legume-based systems in Europe

Best Practice Guide for legume inclusion in animal feed

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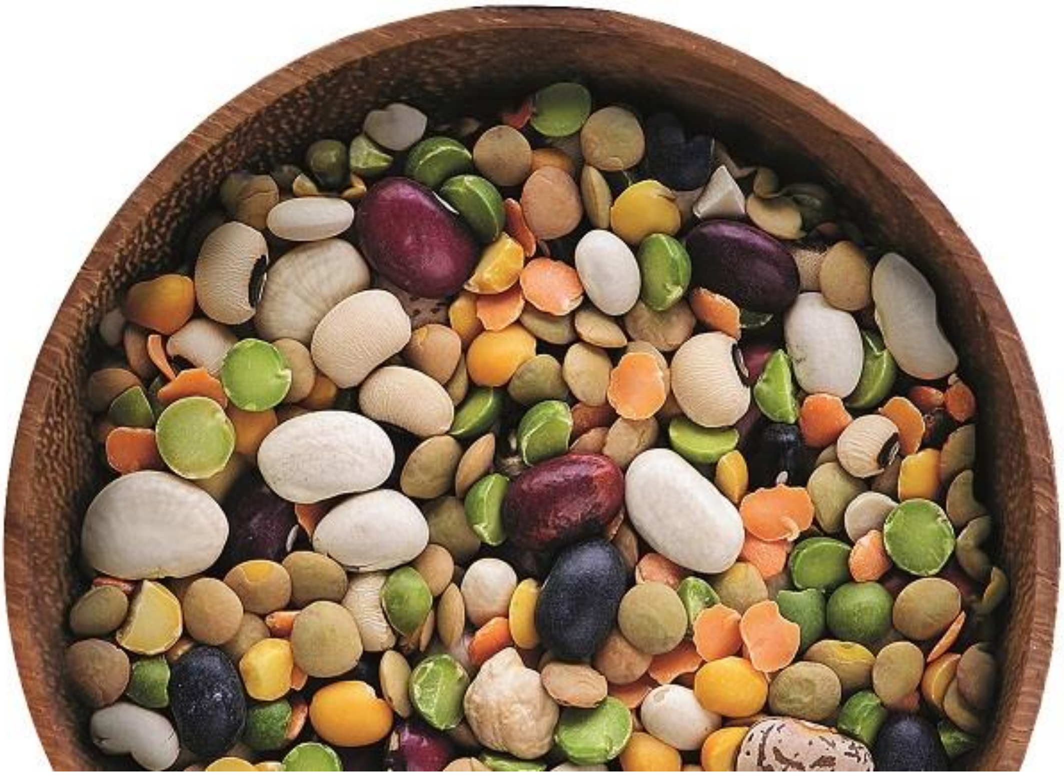

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Deliverable Description & Contributors

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- **Work package leader:** Marta Vasconcelos (UCP)
- **Deliverable Title:** Best practice guide on legume processing and inclusion across pedo-climatic regions
- **Nature of deliverable:** Report
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- **Deliverable description**: : Best practice guide on legume processing and inclusion. Publication of the 'best practice guide on legume processing and inclusion for fish and poultry feeds'. This will be based upon the five major feeds which are to be recommended to European animal feed producers based on feeding trials that balance key-nutritionals and –bioactive contents. The guide will also be applicable across the various pedoclimatic regions of the EU.

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- **Keywords**

  animal feed, fodder, aquaculture, grain-legume, pulses, feed formulation, legume processing

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Glossary

AME: apparent metabolisable energy.

Amino acids: small molecules that are the building blocks of proteins. Essential amino acids cannot be synthesised by animals; hence they must be provided in the feed.

Anti-nutritional factors: chemical substances present in the diet which, at certain doses, either by themselves, or *via* their breakdown products may reduce the effectiveness of consumed feed.

Compound feed: product made from a range of ingredients (*e.g.,* cereals, proteins, vitamins, and minerals) by a feed mill. Compound feed is often in the form of pellets and has a relatively high nutritional content *per* kg.

Crude protein: the protein content of a feed, for example faba beans have ca. 25-30 % crude protein and soybean meal 44-48 %, assessed using the Kjeldahl method (% N x 6·25).

Digestible protein: only part of the protein in feed can be digested by the animal, this is the digestible protein.

Farming system: the way the farm production is organised. A farming system may be intensive *i.e.,* based on intensive animal and crop production, high-input high-yield approach such as modern indoor pig production. Extensive farming uses a lower-input approach by for example using native livestock breeds, grazing on marginal lands.

Fodder: animal feed comprising green vegetative material during the vegetative and/or late-reproductive life history stages. Used for domesticated livestock, fodder is typically of high fibre content, in addition to protein and carbohydrate provisions. The green material may be cut (and processed into pellets or bales) to feed contained livestock or, may be foraged by livestock directly in the fields.

GMO: genetic modified organism.

Grain legume: dried grains from leguminous crops such as faba bean, pea, lupin, soybean.

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Legume: family of plants with biological nitrogen fixing capabilities, including species with relevance as feed crops such as alfalfa, clover, vetches, peas, and faba beans. In this report lupins and soybeans are regarded as 'leguminous plants' or 'grain legumes', including pulses and oleaginous grains, and their green vegetative material used as fodder.

Non-GMO: a term used for crops that are not made from genetic modified plants.

Pedo-climatic zone: climatic zones defined by climatic regime, the soil properties and water run of.

Protein ingredient: protein source that is used to provide the protein in the feed, for example faba beans, soybean meal, fish meal, or skimmed milk powder.

Pulses: non-oleaginous dried grains from crops such as peas, faba beans, chickpea, lentils, and common bean. Their characterisation as 'pulses' is mainly on the basis that their main carbohydrate reserve is starch. Here in this report the dominant feed crops are discussed, specifically peas and faba beans in a feed context.

Ruminants: group of animals with four stomachs *i.e.,* cattle, sheep, goats.

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Executive Summary

The scope of this Deliverable, in the following referred to as the *report*, is to provide best practice guidelines – in the form of fully formulated, tested, and optimised diets for target species - for legume inclusion in compound feeds used for animal husbandry. This report is interlinked with **D4.3 Facilitating the EU market demand for legume-grain and -fodder as feeds** (Hamann, 2020) and is centred around protein rich ingredients for compound feeds, that is why grain-legumes are of central importance to this report. The report builds on desk research, Case Studies with practitioners, extensive research in controlled feed formulation and controlled feeding experiments, as well as stakeholder consultations. The results reported in this best practice guide are numerous, highly successful diet formulations, which can form the basis of economic analysis and pilot commercial production of diet for selected high-value aquaculture and poultry species. The results of the work show that legumes can be used to: 1) replace high-cost protein sources at very high inclusion levels; and 2) produce animals feed using diets formulated from purely locally sourced or home-grown ingredients. The success of direct testing and the analysis of diet-potential indicate that, with increasing scale along the value chain, whilst overcoming of basic production limitations, the standardisation of product quality and exploitation of key advantages of home-grown legumes in formulated diets could lead to sustainable EU-produced legumes being a highly successful component in future EU animal feeds.

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1. The importance of sustainable legume inclusion in feed

Global economic growth since the 1960's increased the disposable income of people and thus increased the demand for meat and dairy products (Billen *et al.*, 2012; Lassaletta *et al.*, 2014). Technical changes in livestock production enabled an increase of production and consumption of these products of about 395% from 1961 – 2011 (Watson *et al.*, 2017). This intensification in production, particularly for pigs and poultry, was decoupled from the local agricultural-land resource usually used to provide feed. Compound feed, mainly based on European-grown cereals had to be supplemented with a suitable protein source to reach a satisfying amino acid profile. Changes in trade policies provided farmers access to imported low-cost soybean meal (SBM). To control bovine spongiform encephalopathy (BSE) and prevent the spread of similar diseases, the use of animal protein sources in feed was severely restricted in Europe in the 1990s (Vicenti *et al.*, 2009), leading to a further increase in the use of soybean.

There are several negative aspects related to SBM imports to Europe and inclusion in feed, which are not detailed in this best practice guide – instead we direct reader to other TRUE Deliverables, and articles such as Rajão *et al.* (2020). Such ecopolitical considerations aside, it is important to note that European sourced grain legumes do not only have promising amino acid profiles and can serve as an alternative to imported (GMO) soy, legume-based products also offer significant benefits in terms of carbon footprint, ecological impact, and local / short value chains over soy. In addition to the question of sustainability of these vast amounts of livestock production, modalities of feed production need to be reconsidered. To meet sustainability in compound feeds, the most important step is an accurate demand-oriented feed formulation to optimise growth and health, whilst at the same time avoid wastage of nutrients. Livestock producers demand the best nutrition at the best price, so the animals stay healthy and perform well. Animal diets are formulated according to availability of feed sources and nutritional requirements. Special requirements prevail for diets targeted at organic and non-GMO farming systems, encouraging local value chains for fodder- and grain-legumes.

A feed industry using European legumes as a protein base would increase agro-biodiversity in European fields, make the use of South American rainforest areas for soybean cultivation less

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attractive, shorten transport distances (thereby promoting regional sourcing of raw materials), and provide an ecologically beneficial alternative to the use of transgenic soybean and fish meal (FM). Several initiatives funded by the EU or national governments of the member states, such as "GL-Pro", Lupinennetzwerk, or BMEL - Eiweißpflanzenstrategie aim to promote the cultivation of legumes in the EU.

2. Legumes across the Pedo-climatic regions of Europe

Pedo-climatic regions are defined on the one hand by climatic regime, and on the other hand by soil properties and water run of (Tóth, Song & Hermann, 2017). The terminology used is not consistent, hence in this report, the terms used in other EU reports (EU-Commission, 2011) for the biogeographical stratification of Europe was adopted, that is: Alpine North, Boreal, Nemoral, Atlantic North, Alpine South, Continental, Atlantic Central, Pannonian, Lusitanian, Anatolian, Mediterranean, Mountains, Mediterranean North, and Mediterranean South (Figure 1 and Figure 2).

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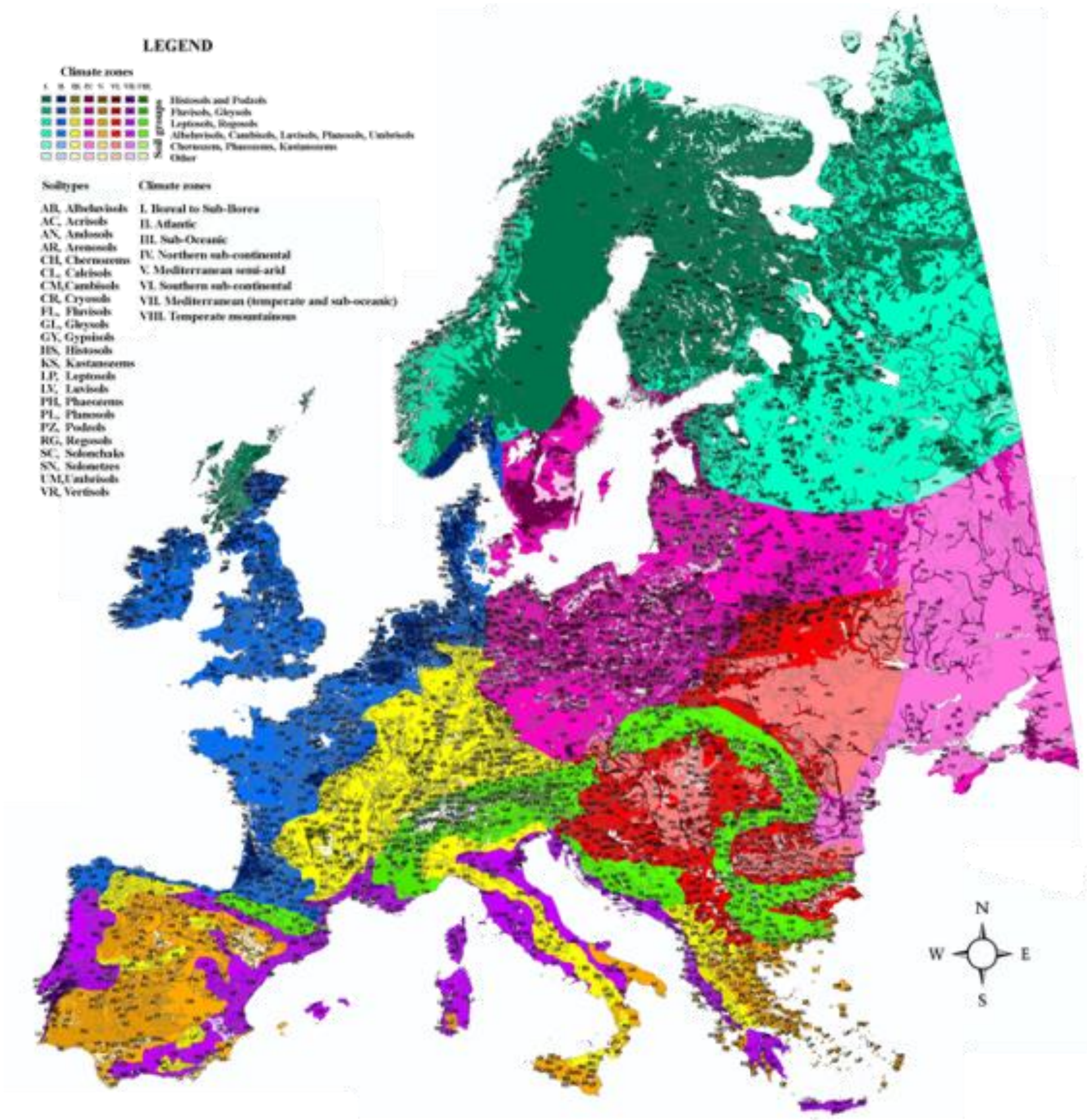

Figure 1. Biogeographical regions of Europe from Tóth 2016.

From the climatic point of view (Figure 2), some countries can be assigned to more than one climate regimes. For instance, Germany and Poland can be assigned to the Continental region, while the German south is Alpine South. The Alpine South also includes Austria and the Italian North. Hungary and Romania on the other hand are mainly Pannonian. The northern German part as well as Denmark, Ireland, the northern part of Great Britain can be allocated to the Atlantic North. Large parts of France as well as the South of GB belong to the Atlantic Central region, while the Westcoast of France as well as the North- and Westcoast of Spain belong the Lusitanian. The Southern parts of Spain and Italy as well as Greece and the coastlines of other Mediterranean countries on the Adriatic Sea can be assigned to the Mediterranean Mountains, Mediterranean North and South.

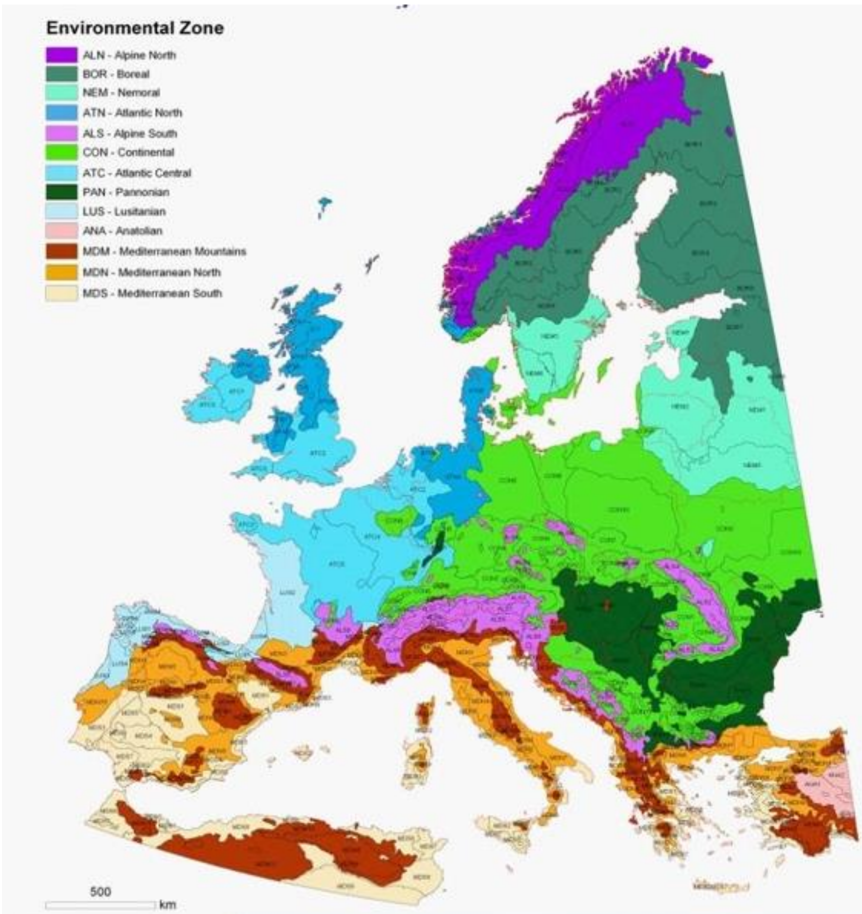

Figure 2. Environmental stratification of Europe (EU-Commission 2011).

Statistic values for the yields of legumes are available *per* country (not *per* biogeographical region), and Figure 3 shows the crop composition *per* country for the largest legume producers in 2019. The main grain legumes produced in Europe are broad beans, soy, peas, and lupin. Moreover, lentils are grown in larger quantities are included in ‘pulses not elsewhere specified’ or “pulses nes” (Figure 3).

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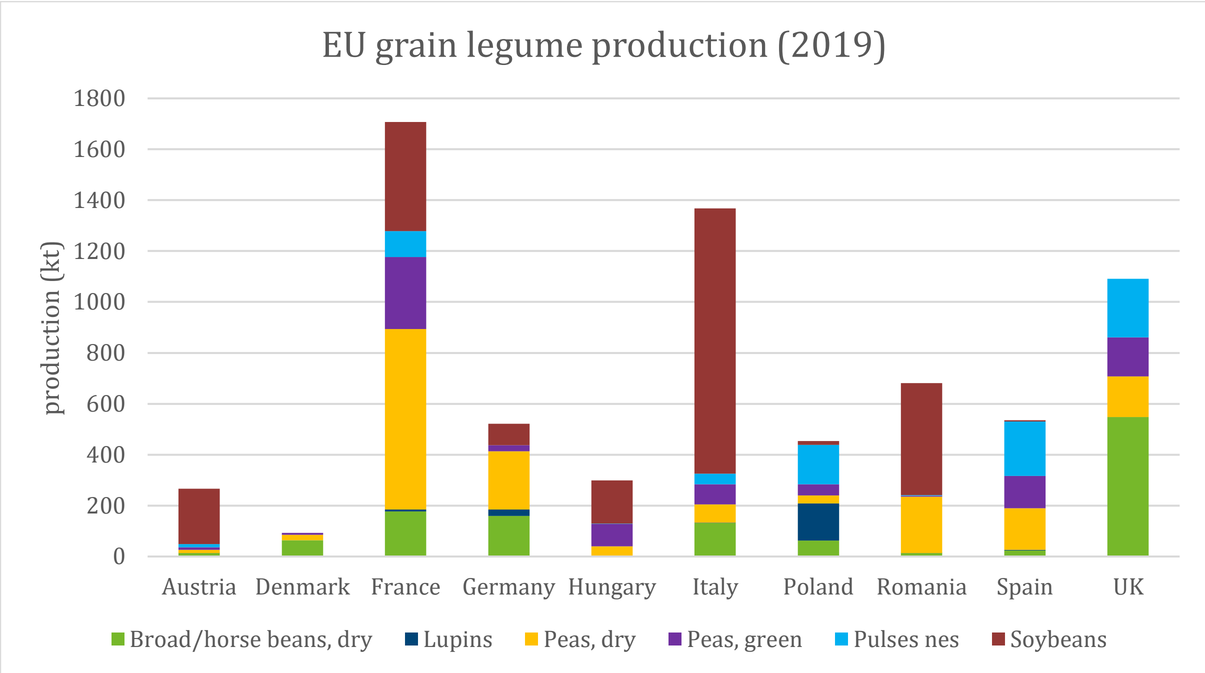

Figure 3. Legume production in Europe. Crop composition per country for the largest legume producers in 2019. Source FAO.

Comparing the biogeographical zones and the legume production of the assigned countries the following prevailing legumes stand out (Table 1).

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Table 1. Prevailing grain legume in the different biogeographical regions of Europe.

Biogeographical region	Prevailing grain legume	Preferred soil properties
Atlantic North	Broad beans	medium soils, high humus content
Atlantic Central	Peas	deep humus-rich soils
Continental	Lupin	light to medium (sandy) soils
Alpine South	Soy	Warm, deep, medium to heavy soils ideal pH 6.5 to 7.0
Pannonian	Soy, Peas	as already defined above for these crop species
Lusitanian	Peas	
Mediterranean	Soy, Peas	

The GL-Pro (2005) Guidelines for growing grain legumes in Europe gives explicit recommendations on potential growing areas, in terms of climate and soil, of a variety of legumes.

3. Legume products

3.1 Conventional products and results from questionnaire

Grain legumes are available as conventional products. Those might be used as whole grain or as the dehulled grains (kernels), either whole grain or kernel may be milled (or kibbled) to a flour or 'meal' of specification. Since the hulls of legumes present significant amounts of anti-nutritional substances it is recommended that hulls are removed, and only kernels are included in feed diets. The need for prior processing of legumes strongly depends on the type (and variety) of legume (see section 5.4), but also on the nutritional demand and susceptibility to antinutritional substances of the target organism. Diverse protein- or starch-enriched products (*e.g.,* as achieved by air classified, or as hydrolysate) are available (Muschiolik & Schmandke, 2000). However, many of such processing methodologies are too costly in terms of equipment capital costs and/or labour to use these products for conventional feeds for animal.

In a non-representative online survey sent to the members of the VDT (German feed association), 80% of respondents reported the use of grain legumes in feed (60% faba bean, 60% pea, 50% lupin and 50% non-GMO or GMO soy). Forty percent stated that the grain is milled before use and (for example) comprises 15% of feed for fattening pigs. Twenty percent of respondents expressed

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concerns regarding the low usage of local legumes due to uncertainties regarding consistent availability and price instability.

The suitability of various legumes and the safe inclusion rates for pig, poultry and ruminants are presented in GL-Pro (2005), "Guidelines for growing grain legumes in Europe".

3.2 Processing

Processing of grain legumes can be used to reduce the content of anti-nutritional factors (ANFs) and / or to improve the protein content before preparation of compound feed due to high protein requirements of the target species.

3.2.1 Flakes production from legumes

The process of flake production can be used to enhance the protein content of a product. To produce flakes from legumes like pea, chickpea or lupin, legume protein isolate, legume flour, water and a sweetener like xylitol is needed. At first, the dry components are weighed, mixed, and conveyed into an extruder. At IGV, a planetary roller extruder is used for that. This type of extruder enables a relatively gentle handling of the raw materials. Then, specific amounts of water are added to the mixture and the extrusion process begins. In extrusion, material is continuously pressed out of a shaping opening (die) under high pressures. Within the extruder, one or two screws rotate and thereby convey the material from the filler to the die. By combining different screw elements, kneading, shearing, and crosslinking of the material is achieved. In this way, the legume proteins undergo shear stress, which under the combined effect of the high pressure and high temperature applied, begin to unfold, and denature. In the presence of water, gelatinates are formed, which are conveyed into a roller mill. When rolled into flake-like shape, the product is transported into a belt dryer where the excessive moisture evaporates. After the drying process, crunchy flakes with a low moisture content are formed.

Figure 4 shows the general processing steps for generating protein-rich starting material from the extrusion starting with whole legumes (like pea, lentil, lupin, bean, *etc.*).

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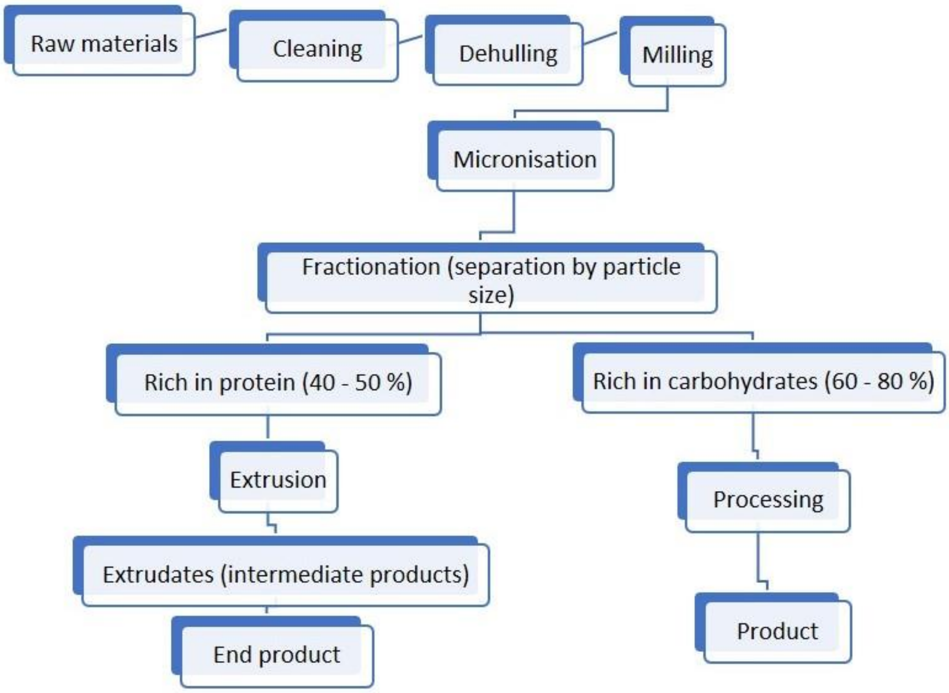

Figure 4. Processing steps for protein-rich extrudates from legumes.

3.2.2 *Production of legume protein powder*

Protein isolate from legumes like lupin is generated from the seeds, like in case of the sweet lupin. The next step to get the isolate is spray drying. The protein powder can be used in a variety of different products like drinks, sports nutrition, dressings, soups, meat products, ice cream and cake. Figure 5 describes the process for the production of protein isolate from legumes (Bremer, 1999).

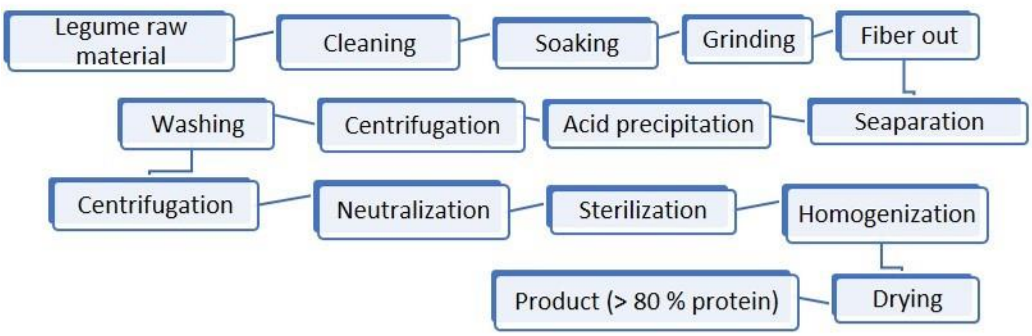

Figure 5. Processing steps to produce protein isolate from legumes.

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3.2.3 Spent Barley

The production of beer utilises cereals such as malted barley, wheat and oats that consist mainly of starch and protein. The beer making process (Figure 6) is interested in the starch as the greater the starch content the greater the potential yield of ethanol. In the processing step known as mashing, this starch is gelatinised, degraded into fermentable sugars and the sweet liquid that results, known as wort, is separated from the insoluble material. The wort is then boiled with hops to develop flavour before fermentation with yeast to produce the alcoholic and fizzy (from carbon dioxide) beverage we know as beer. The insoluble material, called, spent grain typically goes on to be used as feed.

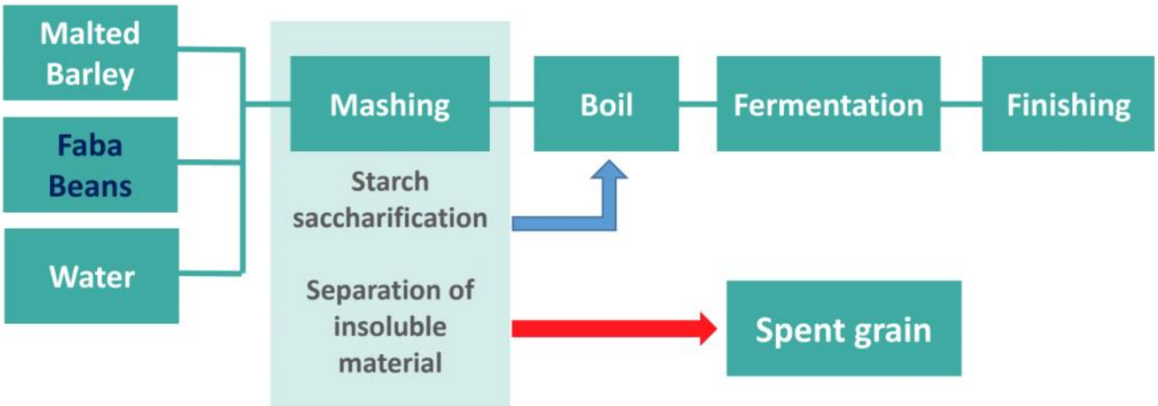

Figure 6. Processing of barley and beans for beer production and production of spent grain as a co-product.

Table 2. Nutritional composition of spent barley-bean tested in the project. If not quoted different values are presented as weight by kg dry matter. Adapted from Houdijk et al (2021)

Nutritional component	**g kg^{-1}**	
Dry Matter	966	
Crude Protein	257	
Acid-hydrolysis Ether Extract	57.4	
Acid Detergent Fibre	133	
Neutral Detergent Fibre	266	
Starch	152	
Sugars	111	
AME*(MJ kg^{-1} DM)	9.53	
Amino acids	**Total**	**SID****
Cystine	3.73	2.28
Aspartic acid	22.15	16.59
Methionine	3.31	2.45
Threonine	8.90	6.38
Serine	11.38	8.76
Glutamic acid	44.09	36.26
Glycine	10.14	6.96
Alanine	11.28	8.20
Valine	12.83	9.78
Iso-Leucine	10.56	8.11
Leucine	19.15	15.37
Tyrosine	6.93	5.41
Phenylalanine	11.80	9.38
Histidine	5.69	4.49
Lysine	11.28	8.73
Arginine	16.35	13.75
Proline	14.90	11.65
Tryptophan	2.48	1.76

*Apparent Metabolisable Energy predicted based on chemical composition.
**'Standardised ileal digestible' amino acid levels, determined in the project.

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4. Legume inclusion in diets

The potential of legume usage as whole-crop forage or in compound feed for cattle, sheep, pigs, poultry and fish (Ayadi, Rosentrate, & Muthukumar, 2012) has extensively been reviewed by Watson *et al.* (2017).

In aquafeeds, regional legumes are not only suitable to replace soybean protein, but also FM, which is not only considered an unsustainable but also an expensive resource. The amino acid and lipid profiles and the content of legumes are generally well-suited to formulated diets although the usual lack of essential amino acids in particular methionine is concerning in aquaculture diets. With the development of specialist supplementary amino acids (*e.g.,* Met-Met methionine supplement) many deficiencies can be overcome in formulated feeds. Based on the growing literature, a range of potentially optimal diets have been formulated for key poultry and aquaculture species (Whiteleg shrimp, Atlantic salmon, and European sea bass), extended with a case study of using legumes as SBM replacers for fattening pigs. A key attribute emerging from any monogastric feed formulations exploring greater use of legumes is the absolute requirement to ensure nutritionally balanced rations, with emphasis to overcome the generally reduced level and digestibility of sulphur-containing amino acids.

4.1 Practical legume inclusion

4.1.1 Poultry

Recent work explored the use of faba beans in starter broilers (day old to 21 days of age), based on nutritional information found in the literature. This showed that the inclusion of 20% faba beans in feeds offered as meals tended to reduce weight gain and increase feed conversion ratio (Olukosi *et al.*, 2019). The nutritional value data obtained from that study was subsequently used to demonstrate that even only 5% faba beans inclusion in the starter feed offered as meals reduced performance, both during the starter phase as well as through the whole production cycle (Houdijk & Walker, 2021). This strongly suggests that starter birds may be too sensitive for faba bean inclusion in their meals, though it cannot be excluded that had feeds been offered as a crumb, such outcomes could have been avoided. Nevertheless, it seems that grower and finisher rations might be more suitable to accommodate faba beans in their diets which would reduce reliance on SBM. The latter

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was the basis of a dose response experiment, where faba beans were included from 0 to 20% in pelleted grower and finisher rations. The data indicated that 15% inclusion is likely an upper limit, as increasing faba bean inclusion further reduced performance (Houdijk & Walker, 2021). This was in agreement with the aforementioned work where 20% faba beans were included in meals rather than pellets (Olukosi *et al.*, 2019). To confirm these outcomes, a larger scale trial was performed, where 672 birds across 48 experimental units were fed grower and finisher rations without and with 15% faba beans. The bird performance was identical (Houdijk & Walker, 2022). Since the basal ration was very much based on commercially relevant wheat – SBM rations, the faba bean ration presented below represents a good example of practical legume inclusion for broilers (Table 3).

Table 3. The ingredients (%) of legume-based rations for grower and finisher broilers demonstrating same performance as their soybean meal only counterparts.

	Grower		Finisher	
Ingredients (g kg^{-1})	**Control**	**Beans**	**Control**	**Beans**
Wheat	678.8	557.3	699.1	576.9
Soybean meal	247.0	207.7	228.0	189.8
Field beans	0.0	150.0	0.0000	150.
Soybean oil	41.8	52.4	42.3	53.3
Limestone	9.9	9.9	9.2	9.3
Mono Calcium Phosphate	7.4	7.4	6.5	5.9
Salt	1.6	1.6	1.7	1.9
Sodium bicarbonate	1.8	1.8	1.7	1.7
Vitamin/Mineral Premix	4.0	4.0	4.0	4.0
L-Lysine HCl	3.3	2.8	3.1	2.6
DL Methionine	3.0	3.3	2.8	3.1
L-Threonine	1.5	1.5	1.4	1.4
L-Tryptophan	0.0	0.2	0.0	0.1
Enzymes	0.2	0.2	0.2	0.2

Since feed intake did not differ between the different ‘control’ and plus ‘bean’ recipes (rations) and using the observed levels of feed intake over a standard feed phasing of grower and finisher as day 11 to 24, and 24 to 42, respectively, the above represents a reduction in SBM usage of 16.5% *per* bird finished. This arises from the much larger intake *per* bird finished during the finisher period than during the grower period, owing to a longer finisher feeding phase as well as greater feed intake *per* day. Note that the bean rations have different levels of supplementary amino acids. Scotland’s Rural

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College (SRUC) in-house digestibility data (Olukosi *et al.*, 2019) for faba beans showed the greater use of added DL-methionine especially, to overcome the relative scarcity of sulphur-containing amino acids, but also to achieve a small increase in L-threonine and -tryptophan to ensure rations are nutritionally balanced.

4.1.2 Aquaculture

Legumes or legume-based products can partly or fully replace unsustainable resources like soybean and FM depending on the aquaculture species being studied. The success of a replacement is dependent on the target species to be fed, the specific legume-crop or -co-product, and a well-balanced formulation.

AWI demonstrated that feeds produced with an incremental legume inclusion as a protein ingredient is best to determine the extent to which soybean and FM replacement with regional legumes is possible for 3 important aquaculture candidates relevant for European aquaculture, namely Whiteleg shrimp, Atlantic salmon, and European seabass without any losses in growth or health impairments in controlled feeding experiments (Weiss & Slater, 2020).

Shrimp diet with lupin

Lupin seed meal (*Lupinus angustifolius*) was tested as sustainable diet component for Whiteleg shrimp (*Litopenaeus vannamei*). As an example, for nutrient balancing we show the calculation of amino acids for these diets (Table 5). Controlled feeding experiments demonstrated successful inclusion of dehulled lupin seed meal in feeds for Whiteleg shrimp without adverse effects on survival, growth performance or metabolic parameters for inclusion rates of up to 100 g kg^{-1} feed (Table 4). An immune-stimulating effect of this diet was observed. Higher inclusion rates of 200 g kg^{-1} resulted in comparable growth, but a negative influence on the metabolic level could be detected. The inclusion of 300 g kg^{-1} resulted in reduced growth and cannot be recommended. For future diet developments, higher substitution rates might be achieved by supplementing a mix of lupin meal and other regional plants, such as faba bean. This might provide a more balanced nutritional supply and make use of the immuno-stimulating effect of moderate lupin inclusion rates. Additionally, further research is required to assess methods for lupin pre-treatment to enhance digestibility Weiss *et al.*, 2020).

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Table 4. Formulation for a practically tested and proved diet for the Whiteleg shrimp (*L. vannamei*) containing 10% (equivalent to 30% animal protein) of lupin meal (dehulled *L. angustifoluis*, cv. Boregine)

Shrimp diet with Lupin (*L. angustifulius*)	
Ingredient	**g/kg feed**
Fish meal	150
Fish oil	20
Shrimp meal	90
Soybean meal (CP 48%)	205
Wheat (CP 12%)	327
Lecithin - Soy (Lipid 70%)	20
Gluten (corn)	75
Lupin meal (dehulled)	100
Cholesterol	2
Methionin	3
Lysin	3
Vitamin und Mineral Premix	5

Table 5. Calculation for amino acid balancing / contribution in a practically tested diet for Whiteleg shrimp (*L. vannamei*) containing 30% lupin.

Amino acid calculation	g kg-1	ARG	HIS	ILE	LEU	LYS	MET	M+C	PHE	P+T	THR	TRY	VAL
Fish meal	150	3,53	1,25	2,37	4,05	4,06	1,32	1,76	2,44	5,26	2,53	1,71	3,04
Fish oil	20	0	0	0	0	0	0	0	0	0	0	0	0
Shrimp meal	90	4,08	1,14	2,46	4,28	4,27	1,15	1,3	2,76	3,1	2,46	1,8	2,9
Soybean meal	205	3,45	1,21	2,09	3,53	2,76	0,64	1,3	2,36	4,09	1,72	0,73	2,15
Wheat	322	0,59	0,28	0,39	0,79	0,35	0,16	0,4	0,53	0,88	0,35	0,13	0,51
Lecithin	20	0	0	0	0	0	0	0	0	0	0	0	0
Gluten (corn)	75	1,81	1,16	2,84	11,76	0,97	1,27	2,3	4,18	7,69	2,12	0,25	3,11
Lupin	100	2,57	0,85	3,23	1,77	1,16	0,34	0	1,12	0	0,99	0,18	1,47
Cholesterol	2	0	0	0	0	0	0	0	0	0	0	0	0
Methionin	3	0	0	0	0	0	99	100	0	0	0	0	0
Lysin	3	0,57	0	0,3	0,49	54,6	0,1	0,16	0	0	0,28	0,04	0,37
Vitamin und Mineral Premix	5	0	0	0	0	0	0	0	0	0	0	0	0
TiO2	5	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	1000	2,19	0,80	1,67	3,03	2,02	0,91	1,25	1,69	2,77	1,33	0,65	1,70
Required		1,95	0,78	0,98	1,66	2,05	0,98	1,33	1,13	2,05	1,37	0,21	1,33

Salmon diets with lupin, lupin protein concentrate, and faba bean protein concentrate

Nine experimental diets were tested with salmon in controlled feeding experiments. Two diets served as control: the FM diet with a high FM content to induce optimum growth, the soybean protein concentrate (SPC) diet as an example of a commercial diet with 350g soybean protein concentrate. Additional diets were formulated with legumes/legume products to meet the requirements (according to International Aquaculture Feed Formulation Database (IAFFD) and Food and Agriculture Organisation (FAO) of the United Nations), *S. salar* as post smolts in the grow out phase, in terms of energy content, protein and amino acid profile, lipid and fatty acid composition, vitamins, and minerals (Table 6). All diets were isonitrogenous and isocaloric, except for the lupin diet, due to the low protein content of the raw material. Lupin concentrate (LC) and faba bean

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concentrate (BC) diets were designed once as a conservative formula mixed with SPC (SPC+BC, SPC+BC) and once with a more forward approach without SPC.

Table 6. Experimental diets formulated for *Salmo salar* post-smolt. FM – fish meal, SPC – soybean protein concentrate, L – lupin, LC – lupin concentrate, BC – faba bean concentrate. Diets in grey-text cannot be recommended for practical use.

Ingredient (g/kg)	FM	SPC	L	LC	BC	SPC + LC	SPC +BC	SPC +LC +BC	LC +BC
Fish meal	600	150	150	150	150	150	150	150	0
Soy protein concentrate (CP 65%)	0	350	0	0	0	150	150	150	0
Lupine meal (L. angustifolius, Boregine)	0	0	350	0	0	0	0	0	0
Lupine protein concentrate (CP 54%)	0	0	0	350	0	200	0	150	300
Faba bean protein conentrate (CP 66%)	0	0	0	0	350	0	200	150	300
Weizengluten	50	150	150	150	150	150	150	50	50
Wheat (CP 12%)	137,4	104,4	67,4	96,4	94,4	92,4	102,4	99,4	88,4
Fish oil	114	126	126	114	126	126	126	126	114
Canola oil	76	84	84	76	84	84	84	84	76
Threonin	0	0	8	9	3	4	1	3	9
Methionin	0	3	7	7	6	5	4	5	10
Lysin	0	0	25	15	4	6	0	0	15
Astaxanthin premix (8%)	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6
Monoammoniumphosphat	10	20	20	20	20	20	20	20	25
Vitamin Premix	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
Mineral Premix	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5
Ti02	5	5	5	5	5	5	5	5	5
Cholin	3	3	3	3	3	3	3	3	3

The growth performance after feeding the diets for 60 days indicate a comparable growth for all experimental diets except the LC+BC without FM (Figure 7). These results showed that fish performance is reduced with the low protein lupin (L) diet, and it can be assumed that this trend could become more pronounced with time. Metabolic imbalance caused by L and LC+BC feeds underline these findings. The results show that **SPC as a plant-based protein source can easily be replaced by other legume products. A replacement of SPC with locally produced LC and BC can be recommended.**

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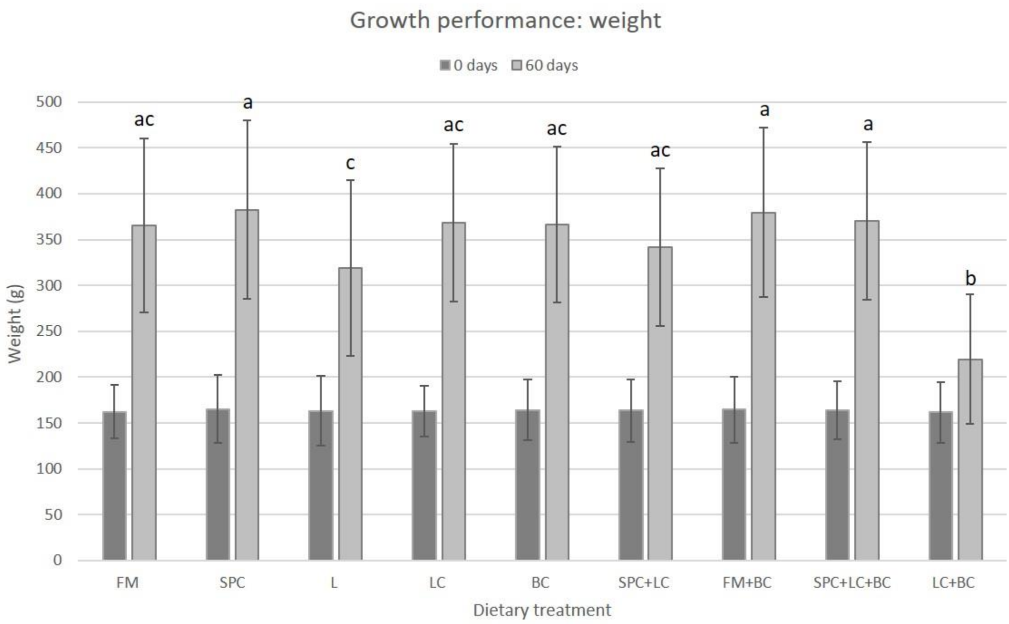

Figure 7: Growth (mean ± standard deviation) of *Salmo salar* fed experimental diets. FM – fish meal, SPC – soybean protein concentrate, L – lupin, LC – lupin concentrate, BC – faba bean concentrate, significant differences (ANOVA), are indicated by different letters.

Seabass (lupin, and fermented lupin)

Ten experimental diets (Table 7) containing different amounts of untreated and fermented lupin were tested with European seabass (*Dicentrarchus labrax*) juveniles. Two diets served as control: the FM diet offers an exaggerated surplus of FM to provide all nutrients for optimum growth, and the soy-based meal (SM) diet, which is comparable to a commercial seabass diet containing 15% soy. Additional diets were formulated with fermented and untreated lupin to meet the requirements of *D. labrax* juveniles, with regards to energy content, protein and amino acid profile, lipid, and fatty acid composition, vitamins, and minerals.

Table 7. Experimental diets formulated for European seabass (*Dicentrarchus labrax*). FM – Fish meal, SM – soybean meal, LM – lupin meal untreated, FLM – fermented lupin meal. Diets in grey-text can only be recommended to a limited extent.

Ingredient (g/kg)	FM	SM15	LM15	LM30	LM50	LM65	FLM15	FLM30	FLM50	FLM65
Fish meal	650	500	500	350	150	0	500	350	150	0
Fish oil	77	77	77	77	77	77	77	77	77	77
Lupin meal untreated	0	0	150	300	500	650	0	0	0	0
Lupin meal fermented	0	0	0	0	0	0	150	300	500	650
Soy	0	150	0	0	0	0	0	0	0	0
Wheat gluten	20	65	65	110	155	190	65	110	155	190
Wheat starch	177	147	147	117	87	52	147	117	87	52
Cellulose 10% of FM	65	50	50	35	20	20	50	35	20	20
Vitamin/Mineral-Premix	6	6	6	6	6	6	6	6	6	6
Marker (TiO2)	5	5	5	5	5	5	5	5	5	5

A set of fermented lupin meal was included to test the potential inhibiting effect of non-digestible and anti-nutritive substances (phytic acid and non-starch polysaccharides (NSP)) in lupin flour. The inhibiting substances were enzymatically digested in a fermentation process to increase the availability of phosphate and bivalent minerals, as well as digestible carbohydrates. As the results show (Figure 8), only small juvenile seabass (Fricke *et al.* 2021) are susceptible to the negative impact of any anti-nutritive substances. Fermentation of lupin therefore is recommended for fish weighing less than 30 g, and larger individuals are not affected by a pre-fermentation of lupin meal.

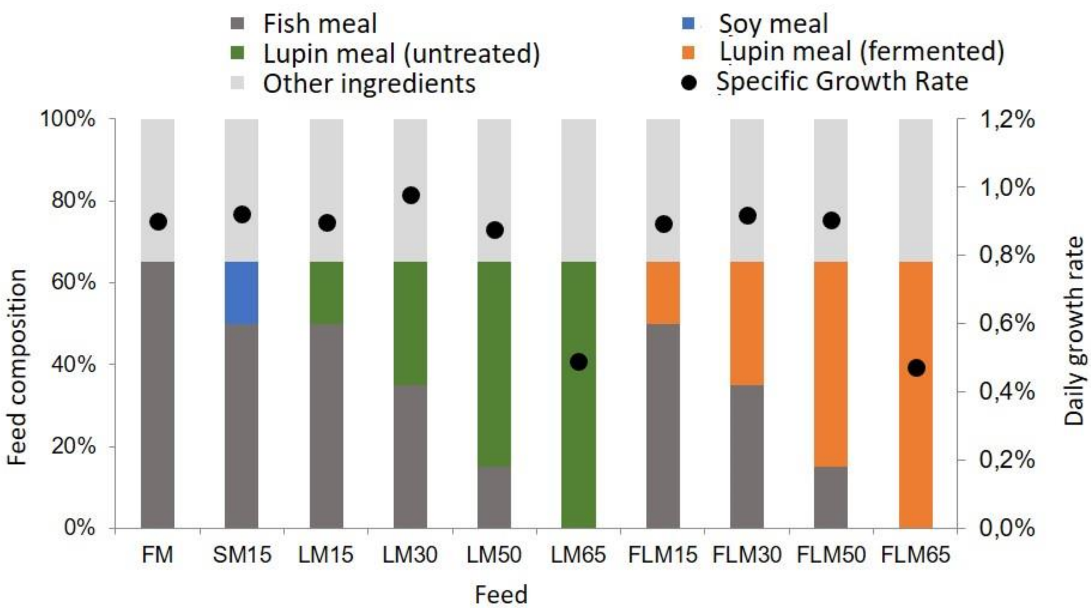

Figure 8. Main results of seabass fed different diets containing lupin: feed composition (bars), Specific Growth Rate (% *per* day, dots), FM – Fish meal, SM – soybean meal, LM – lupin meal untreated, FLM – fermented lupin meal.

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Table 8. Results of the degree of impairment of metric, metabolic and enzymatic analyses in fish fed different percentages of lupin in experimental diets.

Lupin meal content	*30%*	*50%*	*65%*
Specific growth rate	normal	normal	impaired
Feed conversion rate	normal	normal	impaired
Proteolytic enzyme activity	normal	impaired	impaired
Lipolytic enzyme activity	normal	impaired	impaired
Hepato somatic index	normal	impaired	impaired

Growth experiments also revealed that lupin meal can be included up to 50% in the diet for *D. labrax* (Table 8), without negatively impairing growth. **An inclusion of 30% is strongly recommended as it does not negatively impact the metabolism on the broad examined levels.**

4.2 Theoretical inclusion: formulated diets

4.2.1 Poultry

In this project, the nutritional value of a potential new feed *i.e.,* dried spent barley-beans, was assessed with the emphasis on it being a potential new protein source for poultry. Thus, this product was assessed for its standardised ileal digestible (SID) amino acids levels, as described above (Houdijk *et al.* 2022). This provided the required SID lysine and methionine levels, which are key criteria for protein nutrition and pulse inclusion for poultry feeds, respectively. In addition, chemical analysis provided the estimated level of AME (apparent metabolizable energy), which is the key energy criterion for poultry feeds. These values were 8.73 g kg^{-1} for SID lysine, 2.45 g kg^{-1} for SID methionine and 9.53 MJ kg^{-1} for AME. Compared to intact beans, the lysine content and energy is lower but interestingly the methionine is larger. Typical SID lysine, SID methionine and AME values for intact beans, as used in the poultry trials described above, are 11.2, 1.2 and 10.4 MJ kg^{-1}, respectively.

Using theoretical rationing for finisher broilers, Table 9 shows the inclusion of dried spent barley-beans at 0, 5, 10 and 15%, as well as with 15% faba beans as described previously. The latter was used as a target, as it was shown to work out well in terms of broiler performance. Note that these theoretical rations have been formulated considering the contribution of spent barely-bean grains

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to mineral provision. Therefore, the emphasis is focus on its possible role for SID AA and AME provision.

Table 9. Broiler finisher ration recipes (ingredients shown in g/kg) based on either soybean meal (SBM, "control"), 15% inclusion of "faba beans", and theoretical ration recipes with gradual increases in the levels of "spent" barley-bean grains (5, 10 and 15 %) derived from brewing.

	Finisher rations				
Ingredients (g kg^{-1})	**Control SBM**	**Spent 5%**	**Spent 10%**	**Spent 15%**	**Faba Beans 15%**
Wheat	699.1	655.8	612.4	569.0	576.9
Soybean meal	228.0	215.3	202.5	189.8	189.8
Field beans	0.0	0.0	0.0	0.0	150.0
Spent barley-bean grains	0.0	50.0	100.0	150.0	0.0
Soybean oil	42.3	48.5	54.6	60.8	53.3
Limestone	9.2	9.3	9.3	9.3	9.3
Mono Calcium Phosphate	6.5	6.3	6.1	5.9	5.9
Salt	1.7	1.7	1.8	1.9	1.9
Sodium bicarbonate	1.7	1.7	1.7	1.7	1.7
Vitamin/Mineral Premix	4.0	4.0	4.0	4.0	4.0
L-Lysine HCl	3.1	3.1	3.2	3.2	2.6
DL Methionine	2.8	2.8	2.8	2.9	3.1
L-Threonine	1.4	1.4	1.3	1.3	1.3
L-Tryptophan	0.0	0.0	0.0	0.1	0.1
Enzymes	0.2	0.2	0.2	0.2	0.2
Calculated analysis (as fed) (g kg^{-1})					
Dry matter	882	888	893	899	887
Crude protein	188	189	191	193	188
Acid-hydrolysed ether extract	63.7	71.2	78.7	86.2	74.0
Neutral detergent fibre	79.5	87.5	95.6	103.6	86.7
Acid detergent fibre	29.6	34.2	38.9	43.6	39.6
SID Lysine	10.4	10.4	10.4	10.4	10.4
SID Methionine	5.2	5.2	5.2	5.2	5.2
SID Threonine	2.1	2.1	2.1	2.1	2.1
SID Tryptophan	7.2	7.2	7.2	7.2	7.2
AME	13.48	13.48	13.48	13.48	13.48

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The following observations were made.

- Relative to the SBM ration, the relatively low level of AME in the spent barley-beans required a greater inclusion level of oil to maintain ration energy levels at the required 13.48 MJ kg^{-1} AME. The elevated level of oil is significant but would not be a constraint for a broiler ration.
- The low levels of SID lysine compared to that available in faba beans led to a similar if not slightly greater inclusion of synthetic lysine, that was within acceptable ranges.
- Relative to the use of faba beans, the elevated levels of SID methionine in the spent barley-bean resulted in a reduction in supplemental DL-methionine. This therefore represents a saving on supplemental AA costs and input. Similar results were observed, though for a lower magnitude, for tryptophan.
- At inclusion levels greater than 5%, the spent barley-bean ration resulted in elevated levels of neutral detergent fibre, which are not digestible by the bird. The extent to which this fibre is fermentable in the bird would need to be established (total tract digestibility) as such elevated levels of fibre may result in an intake constraint. This would be consistent with the observation that ileal DM digestibility on semi-synthetic diet used to determine SID levels for this spent barley-bean was very low at <50%.
- The ratio of SID-AA to crude protein increases with elevated levels of spent barley-beans. This would suggest a significant amount of excess protein would be consumed, which would either be excreted *via* the faeces or post absorption in the form of uric acid *via* the urine. Either way, the elevated spent barley-bean rations would likely be associated with an increased N excretion, which unless compensated for by improved performance (*e.g.,* using enzymes), would not benefit the environmental footprint of broiler production.
- Although a performance trial using the above set of rations would be conclusive, these results suggest that dried spent barley-bean may not be a suitable feedstuff for poultry. In addition to these theoretical limitations, the commodity also needs to be dried, which requires resource input (both financially and environmentally) before it can be used in pelleted poultry diets. In addition to the high levels of fibre for spent barley-bean, its wet form following its primary production would not be a constraint as ruminant feed, since it could be fed as a higher protein brewer's grain.

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Thus, in conclusion, whilst the use of spent barley-bean grains as a protein source for broilers is arguable limited, the inclusion of 15% faba beans in nutritionally balanced grower and finisher rations is highly recommended as it does negatively impact growth performance.

4.2.2 Aquaculture

Based on our knowledge and practical experience AWI were able to theoretically formulate feed for Shrimp (Table 10), Salmon (Table 11) and Seabass (Table 12) containing legumes from the different biogeographical regions, to show the feasibility and give aquafarmers/small scale feed mills the possibility to produce their own feed based on a locally grown protein source as well as from residuals from food production. These diets are formulated circumspectly with moderate local legume inclusion rates to ensure optimum growth and health at a very low risk level. All diets are well balanced to accurately meet the requirements of the corresponding species to optimise nutrient provision and to minimise nutritional wastage. For salmon and seabass, AWI also included diets that completely exclude FM. Although all nutrients are well balanced, we cannot recommend these diets as in practice, these diets resulted in reduced growth performance.

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Shrimp

Table 10. Diets calculated to meet the requirements of *Litopenaeus vannamei* (Whiteleg shrimp) in the grow out phase. 10% of the diets is based on a legume product (lupin meal (L1), - flakes (L2), - protein concentrate (L3), faba bean meal (FB1), - protein concentrate (FB2), pea protein isolate, lentil).

Ingredient (g/kg)		FM	L1	L2	L3	FB1	FB2	Pea	Lentil
Fish meal		250	150	150	150	150	150	150	150
Fish oil		20	20	20	20	20	20	20	20
Shrimp meal (C. crangon)		90	90	90	90	90	90	90	90
Soybean meal (48% CP)		205	205	155	105	205	100	50	140
Wheat (12% CP)		403	327	381	431	331	436	486	396
Lecithin - Soy (70%)		20	20	20	20	20	20	20	20
Gluten (corn)		0	75	75	75	75	75	75	75
Lupin Meal		0	100	0	0	0	0	0	0
Lupin Flakes		0	0	100	0	0	0	0	0
Lupin protein concentrate (54% CP)		0	0	0	100	0	0	0	0
Faba bean meal-dehulled seed		0	0	0	0	100	0	0	0
Faba protein conentrate (66% CP)		0	0	0	0	0	100	0	0
Pea protein Isolat		0	0	0	0	0	0	100	0
Lentils (whole seeds)		0	0	0	0	0	0	0	100
Cholesterol		2	2	2	2	2	2	2	2
Methionin		5	3	2	2	2	2	2	2
Lysin		0	3	0	0	0	0	0	0
Vitamin and Mineral Premix		5	5	5	5	5	5	5	5
Calculated composition	**req.**								
DM%	90	89,6	90,0	89,5	90,4	80,9	89,8	90,3	80,9
Ash%	8	7,9	6,7	6,5	6,1	6,7	6,0	5,9	6,6
GE MJ/kg		18,4	18,5	18,5	18,2	16,8	18,6	18,8	16,7
DE MJ/kg	13,1	14,5	14,6	14,4	14,5	13,3	14,9	13,1	13,2
CP%	35	37,7	37,4	36,6	36,6	38,3	37,5	37,4	35,2
Dig CP%		33,7	33,8	29,5	33,3	31,2	34,0	26,1	29,0
Lipid%	8	7,6	7,8	7,2	8,0	7,3	7,5	7,9	7,3
Fibre%		2,4	4,2	2,3	3,7	2,6	3,0	2,1	3,1

Salmon

Table 11. Diets calculated to meet the requirements of *Salmo salar* (Atlantic salmon) post smolt. 35% or 20% of the diets is based on an alternative legume product (fish meal (FM), soybean meal (SM), lupin meal (LM1), - flakes (LM2), - protein concentrate (LM3), faba bean meal (FB1), - protein concentrate (FB2), pea protein isolate, lentil).

Ingredient (g/kg)		FM	SM	LM1	LM2	LM3	FB1	FB2	Pea	Lentil	SM + LM3	SM +FB2	SM +LM3 +FB2	LM3 +FB2
Fish meal		600	150	150	150	150	150	150	150	150	150	150	150	0
Fish oil		170	170	170	170	170	170	170	170	170	170	170	170	170
Soy concentrate (65% CP)		0	350	0	0	0	0	0	0	0	150	150	150	0
Lupin Meal		0	0	350	0	0	0	0	0	0	0	0	0	0
Lupin Flakes		0	0	0	350	0	0	0	0	0	0	0	0	0
Lupin protein concentrate (54% CP)		0	0	0	0	350	0	0	0	0	200	0	200	300
Faba bean meal-dehulled seed		0	0	0	0	0	350	0	0	0	0	200	0	0
Faba protein conentrate (66% CP)		0	0	0	0	0	0	350	0	0	0	0	200	300
Pea protein Isolat		0	0	0	0	0	0	0	350	0	0	0	0	0
Lentils (whole seeds)		0	0	0	0	0	0	0	0	350	0	0	0	0
Wheat (12 CP)		160	175	5	30	130	25	180	270	5	155	100	98	110
Gluten (wheat)		40	115	295	270	170	275	120	30	295	145	200	2	90
Astaxanthin premix (8%)		5	5	5	5	5	5	5	5	5	5	5	5	5
Monoammoniumphosphat		4	4	4	4	4	4	4	4	4	4	4	4	4
Choline		3	3	3	3	3	3	3	3	3	3	3	3	3
Methionin		5	5	5	5	5	5	5	5	5	5	5	5	5
Binder (inert)		6	6	6	6	6	6	6	6	6	6	6	6	6
Vitamin und Mineral Premix		7	7	7	7	7	7	7	7	7	7	7	7	7
Calculated composition	**req.**													
DM%		91,04	91,89	91,98	90,05	92,55	59,97	90,30	91,78	60,03	92,25	73,62	91,63	91,55
Ash%		10,21	5,36	4,20	4,46	4,17	4,28	4,06	4,64	5,04	4,68	4,75	5,17	2,44
GE MJ/kg	20	22,63	20,94	21,06	21,02	20,24	14,87	21,74	22,70	14,88	20,53	17,46	21,17	20,57
DE MJ/kg	19	20,29	17,53	18,38	18,05	18,04	13,87	19,50	12,98	13,94	17,80	15,40	18,54	18,46
CP%	45	46,23	46,41	42,99	45,59	45,09	45,51	45,47	45,55	44,42	45,27	45,17	46,40	45,37
Dig CP%	38	42,38	43,81	41,25	31,25	43,36	31,59	43,12	14,96	32,95	43,17	36,10	43,84	43,74
Lipid%	20	22,92	19,78	22,33	20,30	23,05	20,46	21,05	22,35	20,83	21,63	20,12	21,61	22,12

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Seabass

Table 12. Diets calculated to meet the requirements of *Dicentrarchus labrax* (European sea bass) in the grow out phase. 30% or 20% of the diets is based on an alternative legume product ((fish meal (FM), soybean meal (SM), lupin meal (LM1), - flakes (LM2), - protein concentrate (LM3), faba bean meal (FB1), - protein concentrate (FB2), pea protein isolate, lentil).

Ingredient (g/kg)		FM	SM	LM1	LM2	LM3	FB1	FB2	Pea	Lentil	SM + LM3	SM +FB2	SM +LM3 +FB2	LM3 +FB2
Fish meal		650	500	350	350	350	350	350	350	350	350	350	350	0
Fish oil		110	110	110	110	110	110	110	110	110	110	110	110	110
Soy meal		0	150	0	0	0	0	0	0	0	150	150	150	0
Lupin meal		0	0	300	0	0	0	0	0	0	0	0	0	0
Lupin Flakes		0	0	0	300	0	0	0	0	0	0	0	0	0
Lupin protein concentrate (54% CP)		0	0	0	0	300	0	0	0	0	200	0	150	300
Faba bean meal		0	0	0	0	0	300	0	0	0	0	0	0	0
Faba protein conentrate (66% CP)		0	0	0	0	0	0	300	0	0	0	200	150	300
Pea protein Isolat		0	0	0	0	0	0	0	300	0	0	0	0	0
Lentils (whole seeds)		0	0	0	0	0	0	0	0	300	0	0	0	0
Wheat (12% CP)		160	100	0	25	95	30	135	210	5	60	85	40	105
Weizengluten		60	120	220	190	125	190	85	10	215	110	85	30	165
Methionin		5	5	5	5	5	5	5	5	5	5	5	5	5
Binder (inert)		5	5	5	5	5	5	5	5	5	5	5	5	5
Vitamin und Mineral Premix		10	10	10	10	10	10	10	10	10	10	10	10	10
Calculated composition	**req.**													
DM%	90	90,47	90,61	91,22	89,51	91,62	63,70	89,65	90,83	63,81	91,42	90,11	90,81	90,94
Ash%	12	11,23	8,82	7,41	7,67	7,36	7,48	7,26	7,74	8,13	7,05	6,98	7,28	2,65
GE MJ/kg	24	21,69	21,40	20,73	20,69	19,94	15,38	21,18	21,93	15,42	20,55	21,38	21,10	19,31
DE MJ/kg	19	19,22	18,61	18,00	17,65	17,52	14,01	18,69	12,93	14,16	17,90	18,69	18,52	16,97
CP%	50	51,23	49,96	49,27	50,82	50,80	50,21	50,86	50,21	50,10	50,04	50,21	50,80	50,10
Dig CP%		47,06	45,82	46,46	37,17	48,07	36,99	47,63	22,82	38,96	46,53	46,37	46,96	48,47
Lipid%	17	17,45	19,35	17,31	15,56	17,83	15,60	16,06	17,05	15,99	19,91	18,74	19,64	16,35

4.3 Other animal production systems

As for the poultry industry, European pig production also relies heavily on SBM, which likewise increases concerns about food security, sustainability, and environmental impact. Greater use of home-grown peas and faba beans could reduce such reliance. Following a series of small-scale studies that demonstrated under carefully controlled conditions the complete replacement of SBM in experimental rations without impacting on growth performance and carcass measures (Smith *et al.,* 2013; White *et al.,* 2015). Effective translation into more complex commercial rations was demonstrated using a series of large-scale field trials (Houdijk *et al.*, 2013a). In the case study here, faba beans (var Fuego) or peas (var Prophet) were included at 300 g/kg in commercially formulated test diets without any SBM (Table 13). Control diets contained SBM at 98.0 and 47.6 g/kg for grower (35-60 kg) and finisher (60-110 kg) pig, respectively (Table 13). The pelleted feeds were formulated to be iso-energetic (9.75 and 9.30 MJ net energy *per* kg for growers and finishers respectively), have the same standardised ileal digestible lysine content (9.5 and 8.8 g/kg), and meet the minimum requirements of other amino acids (BSAS, 2003) by modifying the inclusion of pure amino acids. Wheat, barley, biscuit meal, wheat feed, fat and macro minerals were allowed to float, whilst rapeseed meal, distillers' dark grains with solubles (DDGS) and other ingredients were kept constant. Diet was fed to a total of 1230 mixed sex American Hampshire × Landrace/Large White pigs of ~35 kg, housed on slats (10 pens *per* diet; 11 pigs *per* pen), or on straw (10 pens *per* diet, of which there were

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8 with 25 pigs and 2 with 50 pigs). Mean pig body weight gain and feed conversion ratio (as feed intake divided over body weight gain) are presented here for the combined grower-finisher period. In the absence of housing x diet interactions on performance (P>0.45), pigs performed the same between the rations; across the housing types, pigs on SBM, peas and faba bean rations grew on average 906, 924 and 915 g/day (SEM 10 g/d; P=0.36), at an averaged feed conversion of 2.68, 2.66 and 2.67 (SEM 0.01; P=0.80), respectively. Farm manager comments were favourable, stating normal pig cleanliness, performance, feed handling and carcass gradings across diets. This and other large-scale commercial demonstration trials (Houdijk et al 2013b) verify that feeding pea- or faba bean-based diets is unlikely to affect pig performance, indicating peas and faba beans are viable home-grown alternatives to SBM for grower and finisher pigs. The increased capacity of peas and beans to replace SBM in pig production systems compared to the aforementioned in poultry is to a large extent due to pig rations using greater levels of non-SBM protein sources to meet AA requirements (*e.g.*, rapeseed meal, DDGS *etc.*). **Thus, in conclusion, the inclusion of 30% faba beans or peas in nutritionally balanced grower and finisher rations for pigs is strongly recommended as it does not seem to negatively impact growth performance or carcass characteristics.**

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Table 13. Grower and finisher ration recipes (ingredients shown in g/kg) based on either soybean meal (SBM, "control"), 30% inclusion of "peas" or "faba beans" for growing pigs from 30 kg to slaughter.

	Grower			Finisher		
Ingredients (g kg^{-1})	**SBM**	**Peas**	**Faba beans**	**SBM**	**Peas**	**Faba beans**
Wheat	322	146	271	257	100	101
Barley	250	250	150	250	240	250
Soya bean meal	98	0	0	47.6	0	0
Peas	0	300	0	0	300	0
Beans	0	0	300	0	0	300
Biscuit meal	80	80	80	56.0	44.8	78
Wheat feed	25.8	0	0	150	79	35.3
Rapeseed meal	110	110	81	125	125	125
DDGS[a]	75	75	75	75	75	75
Limestone	12.2	10.0	10.2	13.2	13.3	12.8
DCP	0.2	0	0	0	0	0
Salt	2.7	0.55	2.8	2.4	2.4	2.2
Sodium Bicarbonate	0	3.0	0	0	0	0
Lysine	6.6	5.1	5.9	6.8	3.0	3.3
Methionine	1.0	1.8	2.3	0.86	1.2	1.3
Threonine	1.3	1.6	1.8	1.2	0.92	0.86
Fat	3.0	4.66	6.9	3.0	3.0	3.0
Tryptophan	0.0	0.4	0.47	0.0	0.18	0.22
Vit-Min premix	2.0	2.0	2.0	2.0	2.0	2.0
Rouxmol[b]	10	10	10	10	10	10
Phytase	0.1	0.1	0.1	0	0	0
Valine	0.0	0.14	0.15	0	0	0

[a] Distiller's dark grans with solubles.

[b] Molasses based mixture to improve pellet quality.

5. Inclusion limits, causes, and potential solutions

Many apparent barriers discourage feed producers from using locally produced legumes for compound feed production. As with many alternative proteins and other diet ingredients the primary consideration is economic, but other concerns are much more nuanced and are dealt with in detail in the following sub-sections. For many of these problems, there are simple potential solutions, others require further research and development of transitional pathways for crop production and processing.

5.1 Manufacturing issues

One of the difficulties is the relative imprecise classification of the raw substances (in contrast to wheat flours with its type numbers). Therefore, diet extrusion parameters must be adapted for each extrusion step. Generally, the extrusion of legume isolates and concentrates is relatively new so that there is still a lot of uncertainties to the process itself. Most of the time, protein isolates from legumes, which are very fine powders are not easy to convey in the extrusion process so that the throughput is relatively low. Hence, experience can assist in overcoming such concerns and of course scale is essential. When legumes move beyond small batches to large volumes and standardised mixtures for blending exist, many of these problems will dissipate. See also below in relation to variations in overall quality, protein content *etc.*

5.2 Availability of legumes

Legumes crop species span large range of diverse types, and include pea, field bean, lupin, soybean, alfalfa, clover, and vetch. Looking at 2019 cultivated areas in just Germany, we find that grain legumes in account: field pea, 75,000 ha; field bean, 50,000 ha; soybean, 28,000 ha; and lupin, 21,000 ha. Therefore, there are no issues with availability, although prices can be high and vary with the season. Compared with FM prices by weight, the cost of legumes is relatively low. Hence, strong economic benefits can be gained by replacing FM by legumes. Here the economic consideration is however a comparison to the inclusion of extremely low-cost soy. Any convincing argument in favour of local legumes over soybean must be based on either higher possible FM replacement levels with local legumes, improved fish performance with local legumes or local and regional improved saleability of legume feeds and the resulting animal / fish products (BDP, 2021).

5.3 Quality fluctuations

Due to varying quality parameters in the legumes, extrusion parameters must be adapted constantly. This is a necessary but time-consuming task to ensure constant quality in the final product. Where scales increase and stores of sufficient size are available, the legume grains can be present to feed producers at sufficient and consistent quantities and qualities as to allow their effective blending. As the point of production, and to aid with this process, grain-grading and protein determination are standard practices for mainly larger scale grain producers, and therefore aggregators may need to fulfil this requirement for smaller producers.

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5.4 Anti-nutritional substances

Plants typically synthesise a number of secondary metabolites as part of their protection against attack by herbivores, insects, and pathogens, or as a means of survival under adverse growing conditions (Khokhar & Apenten, 2003). If animals or humans consume these plants, particularly as a significant proportion of their diet, such compounds may cause adverse physiological effects. That is, the nutritional value of grain legumes does not only depend solely on the nutrient content but also on the amount of antinutritional and/or toxic factors (Betancur-Ancona *et al.*, 2012). Presented here is an aggregated information about the most relevant antinutritional substances in the presented legumes. The following table summarises the common anti-nutritive substances in legumes.

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Table 14. Anti-nutritional ingredients in legume according to Jeroch *et al.* (1993).

Substance group	Chemical compound	Effect	Occurrence
Phenol derivatives	Tannins	Reduced feed intake, inhibition of proteolytic enzymes, decreased protein digestibility	Field beans, peas
Proteins	Lectins	Coagulation of the erythrocytes, impairment of the body's own defence mechanisms	Broad beans, peas, lupins
	Protease inhibitors	trypsin-inhibiting effect, pancreatic hypertrophy and plasia, growth depression	Field beans, peas, lupins
Glucosides	Vicin, Convicin, (pyrimidine glucosides)	Disturbance of lipid metabolism, reduced egg production and single egg mass, depression of fertilization and hatching capacity	Field beans, vetches
	a -Galactoside 1		Lupins, broad beans, peas
	Cyanogenic Glucosides	Symptoms of poisoning from released hydrocyanic acid	Sweet peas
Alkaloids	Sparteine, Lupinin, Lupanine, Hydroxylupanine, Angustifolin	Liver damage, respiratory paralysis, reduced feed intake	Bitter lupins, only traces in sweet lupins
Antivitamins		Decreased activity of niacin	Broad beans

5.4.1 Field Beans

Normally, all field beans can be used to feed ruminants. But when feeding monogastric animals, one needs to check the content of tannins and certain alkaloids. A high content of tannins would have negative effects on the growth of pigs and poultry.

5.4.2 Peas

Thanks to breeding, anti-nutritive substances are usually no longer a concern in peas.

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5.4.3 Lupins

Blue, white, and yellow lupins may contain alkaloids that are neurotoxic to humans and animals. Through breeding, "sweet" varieties with a reduced alkaloid content are available. Otherwise, the seeds must be de-bittered by soaking or heating. Several enzymatic treatments as well as physical treatments are available to reduce anti-nutritive substances in legumes. However, recent studies have shown that any anti-nutritive effect is negligible in fish once they are adult, and that suppression of enzymes such as trypsin in juvenile fish may be compensated for by upregulation of other enzymatic pathways (Fricke, 2016).

5.4.4 Soybean

Soybeans contain lectins and trypsin inhibitors. These make raw soybeans unsuitable for human and animal consumption. However, these substances can be deactivated by toasting or heating (Wendling, 2021).

5.4.5 Reduction of ANS

ANS (anti-nutritional substances) are substances contained in food/feed which decrease the bioavailability of other micro- and macronutrients. Phytic acid for example forms complexes with divalent metal cations like Fe^{2+} or Zn^{2+}, which leads to a reduction of absorption of these cations. To reduce the concentration of ANS in food/feed there are different methods like soaking or fermentation. The content of phytic acid can be reduced by cooking or soaking. Soaking increases the hydration level of the fruit which leads to an activation of endogenous enzymes like the phytases. This enzyme is able to reduce phytic acid. Fermentation also influence the phytase because a pH is generated which is optimal for phytase activity. In addition, for example, the fermentation of millet for 12-24h reduces the levels of protease inhibitors. Protease inhibitors can also be decreased through cooking or soaking. A combination of cooking and soaking increase the reduction.

Furthermore, the separation of bran layer for example through milling is a method that affect ANS level. The concentration of tannin is decreased but there is also a reduction of valuable nutrients. To reduce tannin levels, it is possible to ferment the product.

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6. Economic consideration

The economic considerations of legume inclusion in animal feed, in particular feeds for fish and poultry, are often assumed to be purely numeric. However, our experience and recent studies show that considerations about lupin and other alternative proteins can be more nuanced and regional, or balanced against considerations of the processor's preferences and/or concerns regarding customers' acceptance (Mulazzani *et al.*, 2021).

From a direct economic perspective legume must be: a) much cheaper than FM; and b) either cheaper than SBM or offer significant advantages over soybean meal. These advantages can be:

1) potential for a greater percentage of FM replacement with local legumes (shown in seabass diets and potentially salmon diets); and,
2) improved performance of the feed animals with local legumes-based diet as opposed to soybean, or improved saleability of products within a local or regional context through either the avoidance of soybean or the inclusion of locally sourced legumes.

In all discussions with suppliers of seed and purchasers of legumes a strong positive desire in favour increasing grain legume production in the European Union was expressed. However, the limited scale and relatively high cost of European-grown legume grains in comparison to the imported soybean was also expressed. In addition, it was also believed by stakeholders in feed production that a significant quantity of legumes well suited to formulated feeds produced in the European Union are intended for direct human consumption. That is, the feed market must also compete with the rising demand for home-grown legumes used as food. Though with increasing production for food, there a likelihood that the percentage of these legume grains rejected as substandard for human consumption will also increase, and so become directly available at lower prices for feed use. Nevertheless, all three aspects must be accounted and require a significant stimulus to expand uptake of locally produced legume grains for use as feed.

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7. Stakeholder acceptance - Market potential of legumes in the fish feed industry

The results of feeding trials show that regionally grown legumes are a sustainable and cost-effective alternative to FM- and/or soybean-based products, with great potential for use in aquaculture, though also for pigs and poultry too. Whether relevant players in the German feed industry also recognise this potential was investigated in a socio-economic analysis regarding the application of lupin in compound feed, a grain legume species whose use is advocated in a recent report commissioned by AWI. For this purpose, relevant stakeholders from research, associations, feed manufacturing, authorities, NGOs, and other groups were interviewed. More than 63% of the respondents favoured supplementing current protein sources in animal feed with regionally produced lupins. However, they also acknowledged disadvantages in the use of lupins as a raw material. These disadvantages could be offset by improved sustainability of production, and improvement of the raw material itself, which appears to be very important to the majority (80%) of the respondents.

On the question of how lupin products can be used practically for feedstuffs, positive assessments were expressed, and possible chains of action identified. However, clearly mentioned were exclusion criteria, such as a lack of consistent supply on the market, yield instability, and the low quality of lupins grown in Germany. As a result, many feed manufacturers prefer to follow established sourcing structures of soy.

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Appendix: Background to the TRUE-Project

Executive Summary

TRUE's perspective is that the scientific knowledge, capacities, and societal desire for legume supported systems exist, but that practical co-innovation to realise transition paths have yet to be achieved. TRUE presents 9 Work Packages (WPs) supported by an *Intercontinental Scientific Advisory Board*. Collectively, these elements present a strategic and gender-balanced work-plan through which the role of legumes in determining '*three pillars of sustainabilit*y' – '*environment*', '*economics',* and '*society'* - may be best resolved. TRUE realises a genuine multi-actor approach, the basis for which are three *Regional Clusters* managed by WP1 ('*Knowledge Exchange and Communication',* University of Hohenheim, Germany), that span the main pedo-climatic regions of Europe, designated here as *Continental*, *Mediterranean* and *Atlantic,* and facilitate the alignment of stakeholders' knowledge across a suite of 24 Case Studies. The Case Studies are managed by partners within WPs 2-4 comprising '*Case Studies'* (incorporating the project database and *Data Management Plan*), '*Nutrition and Product Development'*, and '*Markets and Consumers'.* These are led by the Agricultural University of Athens (Greece), Universidade Catolica Portuguesa (Portugal) and the Institute for Food Studies & Agro-Industrial Development (Denmark), respectively. This combination of reflective dialogue (WP1), and novel legume-based approaches (WP2-4) will supply hitherto unparalleled datasets for the '*sustainability WPs*', WPs 5-7 for '*Environment*', '*Economics'* and '*Policy and Governance'.* These are led by greenhouse gas specialists at Trinity College Dublin (Ireland; in close partnership with LCA specialists at Bangor University, UK), Scotland's Rural College (in close partnership with University of Hohenheim), and the Environmental and Social Science Research Group (Hungary), in association with Coventry University, UK), respectively. These *Pillar WPs* use progressive statistical, mathematical and policy modelling approaches to characterise current legume supported systems and identify those management strategies which may achieve sustainable states. *A key feature* is that TRUE will identify key *Sustainable Development Indicators* (SDIs) for legume-supported systems, and thresholds (or goals) to which each SDI should aim. Data from the *foundation WPs* (1-4), to and between the *Pillar WPs* (5-7), will be resolved by WP8, '*Transition Design',* using machine-learning approaches (*e.g. Knowledge Discovery in Databases*), allied with *DEX* (*Decision Expert*) methodology to enable the mapping of existing knowledge and experiences. Co-ordination is managed by a team of highly experienced senior staff and project managers based in The Agroecology Group, a Sub-group of Ecological Sciences within The James Hutton Institute.

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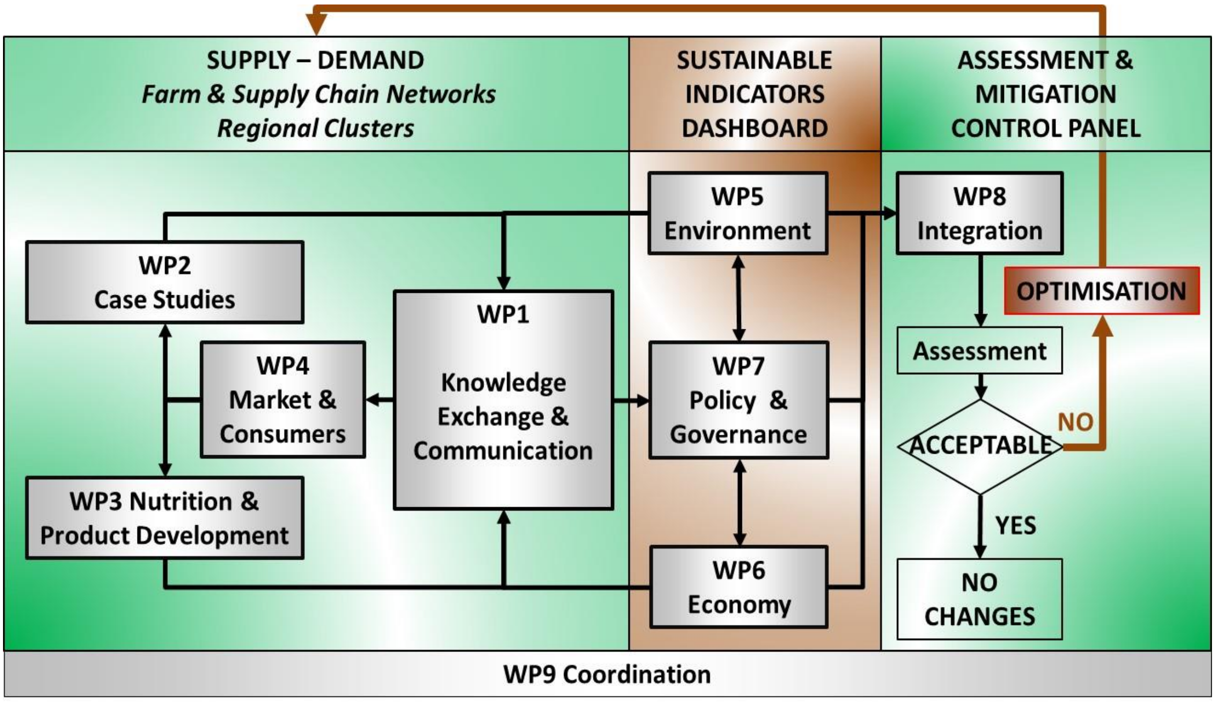
Work-package structure

The flow of information and knowledge in TRUE, from the definition of the 24 Case Studies (left), quantification of sustainability (centre) and synthesis and decision support (right).



Project partners

No	Participant organisation name (and acronym)	Country	Organisation Type
1 (C*)	The James Hutton Institute (JHI)	UK	RTO
2	Coventry University (CU)	UK	University
3	Stockbridge Technology Centre (STC)	UK	SME
4	Scotland's Rural College (SRUC)	UK	HEI
5	Kenya Forestry Research Institute (KEFRI)	Kenya	RTO
6	Universidade Catolica Portuguesa (UCP)	Portugal	University
7	Universitat Hohenheim (UHOH)	Germany	University
8	Agricultural University of Athens (AUA)	Greece	University
9	IFAU APS (IFAU)	Denmark	SME
11	Bangor University (BU)	UK	University
12	Trinity College Dublin (TCD)	Ireland	University
13	Processors and Growers Research Organisation (PGRO)	UK	SME
14	Institut Jozef Stefan (JSI)	Slovenia	HEI
15	IGV Institut Fur Getreideverarbeitung Gmbh (IGV)	Germany	Commercial SME
16	ESSRG Kft (ESSRG)	Hungary	SME
17	Agri Kulti Kft (AK)	Hungary	SME
18	Alfred-Wegener-Institut (AWI)	Germany	RTO
19	Slow Food Deutschland e.V. (SF)	Germany	Social Enterprise
20	Arbikie Distilling Ltd (ADL)	UK	SME
21	Agriculture and Food Development Authority (TEAG)	Ireland	RTO
22	Sociedade Agrícola do Freixo do Meio, Lda (FDM)	Portugal	SME
23	Eurest -Sociedade Europeia De Restaurantes Lda (EUR)	Portugal	Commercial Enterprise
24	Solintagro SL (SOL)	Spain	SME
25	Public Institution Development of the Međimurje County (PIRED)	Croatia	Development Agency

*Coordinating institution

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Objectives

Objective 1: Facilitate knowledge exchange (UHOH, WP1)

- *Develop a blueprint for co-production of knowledge*

Objective 2: Identify factors that contribute to successful transitions (AUA, WP2)

- *Relevant and meaningful Sustainable Development Indicators (SDIs)*

Objective 3: Develop novel food and non-food uses (UCP, WP3)

- *Develop appropriate food and feed products for regions/cropping systems*

Objective 4: Investigate international markets and trade (IFAU, WP4)

- *Publish guidelines of legume consumption for employment and economic growth*
- *EU infrastructure-map for processing and trading*

Objective 5: Inventory data on the environmental intensity of production (TCD, WP5)

- Life Cycle Analyses (LCA) -novel legumes rotations and diet change

Objective 6: Economic performance - different cropping systems (SRUC & UHOH, WP6)

- *Accounting yield and price risks of legume-based cropping systems*

Objective 7: Enable policies, legislation and regulatory systems (ESSRG, WP7)

- *EU-policy linkages (on nutrition) to inform product development/uptake*

Objective 8: Develop decision support tools: growers to policymakers (JSI, WP8)

- *User-friendly decision support tools to harmonise sustainability pillars*

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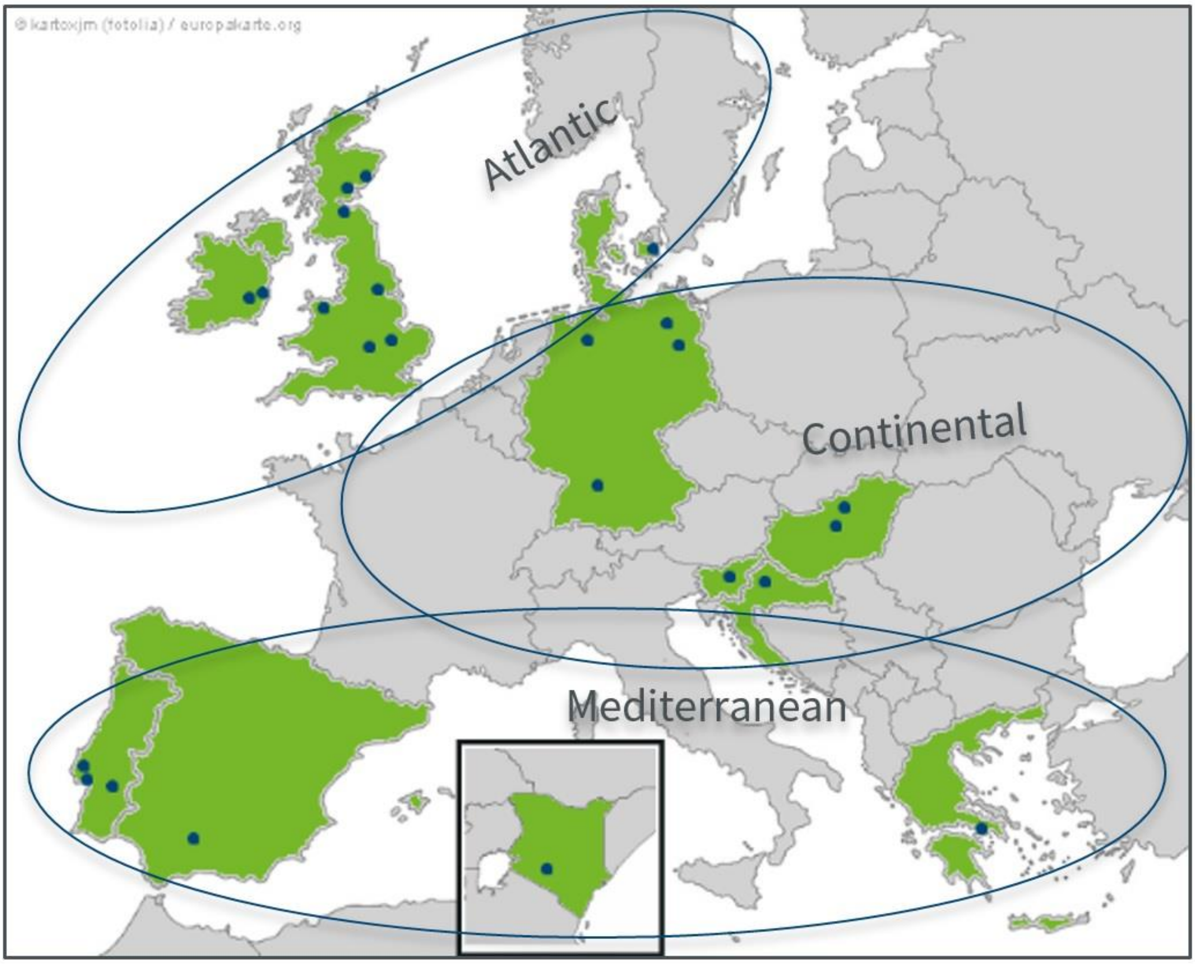
Legume Innovation Networks



Knowledge Exchange and Communication (WP1) events include three TRUE European Legume Innovation Networks (E-LINs), and these engage multi-stakeholders in a series of focused workshops. The E-LINs span three major biogeographical regions of Europe illustrated above within the ellipsoids for Continental, Mediterranean and Atlantic zones.

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