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Effect of variety and environment on the contents of crude nutrients, lysine, methionine and cysteine in organically produced field peas (*Pisum sativum* L.) and field beans (*Vicia faba* L.)

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Abstract

The composition of organically produced field peas and field beans as a source of valuable protein is of special interest for the planned 100 % organic feeding regulations in organic farming. For this reason, the influence of environment and variety on the contents of crude nutrients and the amino acids lysine, methionine, and cysteine were examined over three years. Laboratory analyses were conducted using NIRS. Peas contained on average 21.9 g crude protein 100 g^{1} dry matter with 8.0 g lysine 100 g^{-1}, 1.0 g methionine 100 g^{-1}, and 1.4 g cysteine 100 g^{1}. In field beans 29.6 g crude protein 100 g^{-1} dry matter with 6.4 g lysine 100 g^{-1}, 0.7 g methionine 100 g^{1}, and 1.1 g cysteine 100 g^{1} were found. Significant differences between varieties were found for crude protein and ether extract in field peas, as well as for all components in field beans. Environmental factors and interactions also had influences on the composition of both legume species. Furthermore, significantly negative correlations were found between the content of crude protein and starch (r = -0.69), sugar (r = -0.47), lysine (r = 0.76), and methionine (r = -0.51) in field beans, as well as of crude protein and starch (r = -0.79), sugar (r = -0.55), lysine (r = -0.78), methionine (r = -0.61), and cysteine (r = -0.55) in field peas. The shifts in composition were often undirected and for that reason not predictable. Hence, it is recommended to analyze every batch before formulating a diet.

Keywords: *Nutrient composition, grain legumes, factors of influence, amino acids*

Zusammenfassung

Einfluss von Sorte und Umwelt auf die Gehalte an Rohnährstoffen, Lysin, Methionin und Cystein in ökologisch angebauten Futtererbsen (*Pisum sativum* L.) und Ackerbohnen (*Vicia faba* L.)

Die Gehalte an Rohnährstoffen und vor allem an limitierenden Aminosäuren von ökologisch erzeugten Futtererbsen und Ackerbohnen sind vor dem Hintergrund der 100 %-Biofütterung von Interesse. Um den Einfluss von Sorte und Umweltfaktoren auf die Rohnährstoff-, Lysin-, Cystein- und Methioningehalte zu untersuchen, wurden in drei Jahren Proben aus Sortenversuchen in Deutschland genommen und mittels NIRS analysiert. Im Mittel enthielten Futtererbsen 21,9 g und Ackerbohnen 29,6 g Rohprotein in 100 g Trockensubstanz. Das Rohprotein von Futtererbsen und Ackerbohnen enthielt je 100 g im Mittel 8,0 g Lysin, 1,0 g Methionin und 1,4 g Cystein bzw. 6,4 g Lysin, 0,7 g Methionin und 1,1 g Cystein. Der Faktor Sorte beeinflusste die Rohprotein- und Rohfettgehalte von Futtererbsen und deren Ernteerträge sowie alle Inhaltsstoffe von Ackerbohnen signifikant. Umweltbedingte Faktoren und Interaktionen zwischen den Faktoren hatten ebenfalls Effekte auf die Zusammensetzung beider Kulturarten. Der Rohproteingehalt war in Ackerbohnen signifikant negativ mit dem Stärke- (r = -0,69), Zucker- (r = -0,47), Lysin- (r = -0,76) und Methioningehalt (r = -0,51) und in Futtererbsen mit dem Stärke- (r = -0,79), Zucker- (r = -0,55), Lysin- (r = -0,78), Methionin- (r = -0,61) und Cysteingehalt (r = -0,55) korreliert (p < 0,01). Die Schwankungen der Inhaltsstoffgehalte sind oftmals ungerichtet und somit nicht vorhersagbar, sodass eine laboranalytische Untersuchung der Futtermittel vor jeder Rationsberechnung empfohlen wird.

Schlüsselwörter: *Nährstoffzusammensetzung, Körnerlegumінosen, Einflussfaktoren, Aminosäuren*

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1 Introduction

In animal production, a diet is targeted which matches the animals' requirements and hence increases the feed conversion ratio. Furthermore, the goal is the use of a combination of feedstuffs with an amino acid (AA) profile equal to that needed in monogastric metabolism (Boisen et al, 2000). In conclusion, knowledge of the composition of the available feedstuffs is required for precise diet formulations. The exact contents of crude nutrients (CN) and essential AAs in the available feedstuffs are particularly interesting. For economic reasons, table values are often used to get information on the amounts of valuable ingredients in feedstuffs rather than results from laboratory analyses.

Organic farming has to deal with limitations in diet formulation. Extraction meals cannot be used since they are produced using solvents. Oilcakes are used instead. They contain less crude protein (CP) and more ether extract (EE) in comparison to extraction meals, and can therefore not be fed in the same amounts. Some feed additives, like free AAs, are not allowed in animal feed either. Furthermore, the organic production capacity of some protein feedstuffs, like protein concentrates, is not sufficient. Nevertheless, it is intended to implement a 100 % organic feeding requirement in 2018. Until then, 5 % of special protein feedstuff components of agricultural origin can be produced conventionally (EC, 2007; EC, 2008; EU, 2014). The main non-organic protein feedstuffs used are potato protein for piglets and maize gluten for poultry. Moreover, the use of locally produced crops is intended. Due to restrictions in field management, the yield (De Ponti et al., 2012) and the CP content (Jansen and Seddig, 2007; Worthington, 2001) of organically produced crops can be lower than that of conventional farming. But they are main factors of the economic quality of crops.

Despite their contents of secondary plant metabolites (like vicin/convicin, tannin, and trypsin inhibitors), which limit their applicability in nutrition (Krupa, 2008; Muzquiz, 2004), grain legumes are important protein feedstuffs, especially in organic farming. Both the crop yield and the CN content of grain legumes are influenced by site of cultivation, harvest year and variety (Wang and Daun, 2004). Even in conventional farming, the amounts of the CN, mainly of carbohydrates and CP, can vary widely (Avola et al., 2009; Burstin et al., 2011; De Almeida Costa et al., 2006; Duc et al., 1999). The restrictions of organic farming could be considered to intensify those variations. These are reasons not to use the common data collected in conventional agriculture for diet formulation without questioning it. Information on the composition of organically produced crops and the impact of different influencing factors is not comprehensive. Accordingly, expanding the data basis can possibly improve the predictability of the information needed for formulating a ration.

In regarding an observed negative correlation between the contents of CP and the essential AAs (Igbasan et al., 1996), it has to be considered that the selection of high CP contents can have a detrimental effect on the protein quality. The AA profile of the protein can change negatively due to higher increases of nonessential AAs, like proline and arginine, than of essential ones (Monti and Grillo, 1983; Partanen et al., 2001; Wang and Daun, 2004). This phenomenon can also occur under drought stress conditions, when even absolutely higher contents of nitrogen and sulfur can be detected in crops (Schumacher et al., 2011).

The organic production of soybeans, which are, due to their high protein content and -quality, the main protein feedstuff used in European countries, is not yet sufficient. Furthermore, it is desired to support local organic production without import of protein feedstuffs originating from Third World countries. Therefore, the importance of local grain legumes, like field peas and field beans, as valuable sources of protein is further increased. High lysine (Lys) contents, in comparison to grain cereals, make their AA profiles beneficial, especially for swine. In contrast, the contents of sulfur-containing AAs (SAAs), namely methionine (Met) and cysteine (Cys), are relatively low in field peas and field beans. In total, the SAAs can often be limiting in diets for poultry and, besides Lys, even in diets for swine (Boisen et al., 2000). As mentioned above, they are, in addition to tryptophan, also limiting in field peas and field beans (Emmert and Baker, 1997; Wang and Daun, 2004; Zdunczyk et al., 1997). Accordingly, it is favorable to harvest large quantities of grain legumes with high CP and total SAA contents. When compared with field beans, field peas contain lower amounts of CP and relatively higher amounts of SAAs (Schumacher et al., 2011). In addition to a variety of environmental factors, multiple genetic factors can also cause differences in the composition between varieties. Therefore, the measured contents of CN and of AAs in field peas and field beans also range widely (Kotlarz et al., 2011; Makkar et al., 1997; Nikolopoulou et al., 2007; Urbatzka et al., 2011).

Of particular benefit would be the extension of the data basis and further knowledge on influences of the variety and the environment (site and harvest year), as well as on relations between CN and AAs, which eventually would allow predictions. The predictability of those variations in the composition of crops would be of great interest for diet formulation.

The aim of the study was to examine whether the amounts of CN, Lys, Met, and Cys differ markedly in field pea and field bean varieties which are currently used under practical conditions on organic farms. Furthermore, the predictability of the strength of their reaction to genetic and environmental differences was questioned. Another issue was whether the maximization of CP content can be an adequate breeding objective for those grain legumes with regard to their protein quality for use as protein feedstuffs for monogastric animals.

2 Materials and methods

2.1 Sampling

Field pea (*Pisum sativum* L.) and field bean (*Vicia faba* L.) samples from field variety trials of various organically managed experimental sites in Germany were collected in the years 2011, 2012, and 2013. In total, 113 samples of 12 white-flowering spring field pea varieties and 105 spring field bean samples of 12 varieties were available for further analyses (Table 1).

Table 1
Characteristics of the field pea (*Pisum sativum*) and field bean (*Vicia faba*) varieties of the sample set

	Leaf-type		Flower-type	Vicin/ Convicin	Tannin
***Pisum sativum* L.**		***Vicia faba* L.**			
Abarth	s	Alexia	c	+	+
Alvesta	s	Bioro	c	+	+
Auckland	s	Divine	c	-	+
Casablanca	s	Espresso	c	+	+
La Mancha KWS	s	Fabelle	c	-	+
Mythic	s	Fanfare	c	+	+
Navarro	s	Fuego	c	+	+
Natura	n	Isabell	c	+	+
Protecta	s	Julia	c	+	+
Rebel	s	Pyramid	c	+	+
Respect	s	Taifun	w	+	+
Salamanca	s	Tangenta	w	+	-

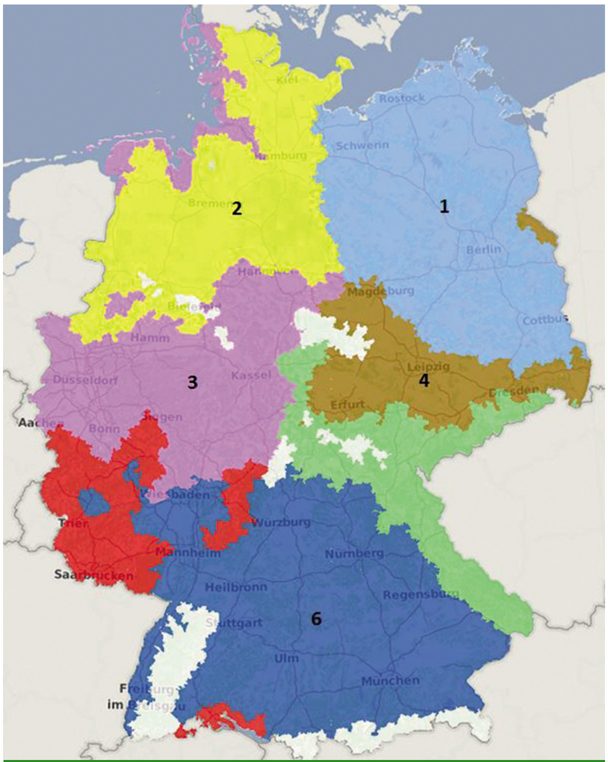
n = normal-leafed, s = semi-leafless, w = white-flowering, c = colored-flowering

Samples of field pea and field bean varieties originated from 13 trials per species. The trials took place in different parts of Germany and therefore in different organic agricultural production areas with homogenous climatic conditions (according to JKI, 2014; Figure 1).

Table 2
Numbers of varieties, trials, and cultivation sites of spring field pea (*Pisum sativum* L.) and spring field bean (*Vicia faba* L.) samples in three harvest years and in total

		Varieties	Trials	Sites	Regions	Total
***Pisum sativum* L.**	**Total**	**12**	**13**	**10**	**5**	**113**
	2011	8	2	2	2	15
	2012	9	4	4	2	34
	2013	11	7	7	3	64
***Vicia faba* L.**	**Total**	**12**	**13**	**8**	**3**	**105**
	2011	9	1	1	1	9
	2012	10	5	5	3	35
	2013	11	7	7	3	61

Field pea varieties were cultivated at ten sites of the Areas 1, 2, 3, 4, and 6. Field bean varieties grew at eight sites of the Areas 2, 3, and 6 (Table 2). In the field bean trials, 5 to 11 varieties, and in the field pea trials, 7 to 10 varieties were tested, respectively.



Organic agricultural production area (as above)
1 Sandy areas in the north-east
2 Sandy areas in the north-west
3 Loamy areas in the west
4 Areas with loess in the east
6 Agricultural areas in the south and altitudes in the south-west

Figure 1
Organic agricultural production areas with homogenous climatic conditions in Germany (according to JKI, 2014) from which the samples originated

2.2 Laboratory analyses

The samples were dried for 48 hours at 60 °C and then stored in a cooling chamber at 8 °C. A part of each sample was either ground to pass through a 1.0 mm sieve for CN analyses or through a 0.5 mm sieve for AA analyzes. CP (Kjeldahl N*6.25), ash, EE, crude fiber (CF), starch, and sugar as well as the AAs Lys, Met, and Cys were analyzed with near infrared reflectance spectroscopy (NIRS). Spectral data for NIRS analyses were recorded using the Fourier-Transform NIR spectrometer (NIRLab N-200, Fa. Büchi, Essen) in the spectral range from 1000 to 2500 nm and then exported to the NIRCal chemometric software (Fa. Büchi, Essen). Subsequently, the contents of CN, Lys, Met, and Cys were predicted using existing calibrations. The calibrations for each ingredient were based on reference analyses of 430 pea and 315 bean samples as described by Aulrich et al. (2016).

2.3 Statistics

When available, crop yields were extracted from open access field trial results and attributed to the samples.

The normal distribution of the variable and the residuals were proved with histograms and the Shapiro-Wilk-Test. No transformation of the data was required. Statistical analyses for unbalanced data were conducted using proc MIXED with the REML estimation method and the Kenward-Roger-Estimation in SAS 9.4 (SAS® Institute Inc.) to observe differences in the least square means of the contents of CN and AAs between the field pea and field bean varieties. Furthermore, the covariance of the factors site and harvest year should be observed. Therefore, variety was set as fixed effect, while site and year as well as the two-sided interactions between the three factors (site*year, variety*site, variety*year) were initially set as random effects. A model optimization was done for each analyzed ingredient on the basis of the Bayesian information criterion (BIC) as a measure of the relative quality of the models. If the model showed a statistically significant influence of the fixed effect, a Tukey-test was added as post-hoc-test (Moll and Piepho, 2001). The macro %mult.sas was used to describe significant differences ($p < 0.05$) with letters (Piepho, 2012). Furthermore, Pearson correlation analyses (proc CORR, SAS 9.4) were performed to examine relations between the contents of the observed CN and AAs in field beans and field peas. In the following text, significant correlations are marked with ** ($p < 0.01$).

3 Results and discussion

3.1 Field peas (Pisum sativum L.*)*

High variations of the composition of field peas were observed. In addition, the relation of the SAAs to Lys varied (Table 3).

Table 3

Ranges and means with standard deviations (SD) of the contents of crude nutrients (g 100 g^{-1} dry matter), lysine, methionine and cysteine in organically produced field peas (*Pisum sativum* L.) (g 100 g^{-1} crude protein)

	Mean	SD	Min	Max
Dry Matter	92.82	0.57	91.43	94.19
Crude Protein	21.90	1.89	16.81	25.86
Lysine	8.02	0.36	6.90	8.99
Methionine	1.02	0.09	0.86	1.24
Cysteine	1.42	0.09	1.18	1.62
Methionine+Cysteine (SAA)	2.44	0.17	2.12	2.86
SAA:Lysine	0.30	0.02	0.27	0.33
Ether Extract	2.00	0.15	1.64	2.39
Crude Ash	3.12	0.18	2.64	3.48
Crude Fiber	6.58	0.32	5.82	7.33
Starch	52.54	1.61	47.80	56.54
Sugar	5.42	0.29	4.81	6.11

In pig nutrition a ratio of about 0.6 between SAAs and Lys is recommended as optimal (Boisen, 1997). Poultry need even more SAAs in their metabolism, for example for feather growth. Thus, the SAAs are limiting in field peas. Nevertheless, since the ratio of SAA:Lys is notably higher in cereals, the limiting AA in cereal-based diets is Lys. Therefore, field peas can contribute to a balanced AA profile in the diet.

Schumacher et al. (2011) reported even higher variations in the CP content of their organically produced field pea samples (18 39 g CP 100 g^{-1} DM) of various origins. In 50 varieties of forage, marrowfat, catch crop and winter peas available at the Norddeutsche Pflanzenzucht KG, the CP contents were higher, the Lys contents lower and the SAA contents identical to the present ones. Although the data was derived from analyses of conventionally produced crops, Evonik Industries (2010) published slightly lower values (AMINODat® 4.0) in comparison to the present data. They found lower mean Lys and Met contents in 18 field pea samples harvested in Germany. The conditions of cultivation and the analyzed varieties are unknown. DLG (DLG, 2006-2010) summarized higher mean contents of CP (n = 75), Met and Cys, but lower Lys contents (n = 46) in organically produced field peas. Wang and Daun (2004) found slightly lower Lys and higher SAA contents in conventionally produced field peas. Table values for CN derived from conventionally produced field peas (Staudacher and Potthast, 2014) differed partially from the results. For EE and starch they were lower than the observed values, whereas for sugar and ash they were higher. Other authors observed diverting results especially for CP and starch, as well as comparable results for EE, ash, and CF. High variations of the composition of field peas were found (Jezierny et al., 2011; Ravindran et al., 2010; Schumacher et al., 2009; Bastianelli et al., 1998) with regard to high differences in the selection of varieties. The present results were generally in agreement with those outcomes. Fluctuations could be additionally due to varying environmental influences.

Several studies confirmed that the variety of field peas has an influence on its CP content as well as on many component parts like other CN, AAs, minerals or trypsin inhibitors (Guillamón et al., 2008; Kotlarz et al., 2011; Wang et al., 2008; Canbolat et al., 2007).

In the present study, significant differences of the CP and EE contents were observed between field pea varieties (Table 4).

Casablanca had on average the highest CP content, which was significantly different from Navarro, Natura, Auckland, Respect, Alvesta, and Abarth. Protecta had significantly higher CP contents than Respect, Alvesta, and Abarth. In addition, KWS La Mancha differed significantly from Auckland. The EE content was highest in Abarth and similarly high in Mythic, Navarro, Protecta, and Rebel. It was significantly lower in Alvesta, La Mancha KWS, and Salamanca. Salamanca had the lowest EE content, which was also significantly lower than in Auckland. The only normal-leafed variety Natura had relatively low ash and CF contents, which did not differ significantly from any of the semi leafless varieties, and performed on a medium level in all other parameters. There seems to be an inverse relationship between the CP content and the contents of starch, Lys, and Met between the varieties (Table 4). This might be due to genetic factors and/or to differences in the process of maturation (Flinn and Pate, 1968; Geervani and Devi, 1988).

Table 4

Least square means of crude nutrient contents (g 100 g^{-1} dry matter) and contents of the amino acids lysine, methionine, and cysteine (g 100 g^{-1} crude protein) with standard error (SE) and with letters to mark significant differences ($p < 0.05$) in various field pea (*Pisum sativum* L.) varieties

	Crude Protein	Ether Extract	Crude Ash	Crude Fiber	Starch	Sugar	Lysine	Methionine	Cysteine	SAA:Lysine
Abarth	20.77 d	2.00 a	3.00 d	6.68	53.59	5.46	8.15	1.01	1.42	0.30
SE	0.66	0.08	0.12	0.17	0.63	0.09	0.14	0.05	0.05	0.01
Alvesta	21.11 d	1.91 cd	3.04 bd	6.67	53.04	5.49	8.11	1.00	1.41	0.30
SE	0.61	0.08	0.12	0.17	0.58	0.08	0.13	0.05	0.05	0.01
Auckland	21.65 cd	1.96 ac	3.10 bc	6.74	52.87	5.42	8.01	0.99	1.38	0.29
SE	0.61	0.08	0.12	0.17	0.58	0.08	0.13	0.05	0.05	0.01
Casablanca	23.38 a	1.93 ad	3.09 abd	6.66	51.58	5.33	7.81	0.95	1.38	0.30
SE	0.71	0.09	0.12	0.18	0.67	0.10	0.16	0.05	0.05	0.01
KWS La Mancha	23.08 ab	1.90 cd	3.10 bc	6.64	51.68	5.41	7.91	0.97	1.41	0.30
SE	0.61	0.08	0.12	0.17	0.59	0.08	0.13	0.05	0.05	0.01
Mythic	22.39 ad	2.00 ab	3.10 abc	6.75	51.69	5.49	8.00	0.97	1.4	0.30
SE	0.84	0.09	0.12	0.18	0.77	0.11	0.18	0.06	0.06	0.01
Natura	21.69 bcd	1.91 bcd	3cd	6.41	52.84	5.39	8.00	0.99	1.38	0.30
SE	0.74	0.09	0.12	0.19	0.69	0.12	0.16	0.05	0.05	0.01
Navarro	21.88 bcd	1.99 ab	3.10 b	6.73	52.5	5.37	8.11	0.99	1.41	0.30
SE	0.61	0.08	0.12	0.17	0.58	0.08	0.13	0.05	0.05	0.01
Protecta	22.73 ac	1.98 ab	3.19 a	6.78	51.78	5.36	7.81	0.97	1.38	0.30
SE	0.66	0.08	0.12	0.17	0.63	0.09	0.14	0.05	0.05	0.01
Rebel	22.24 ad	1.99 ab	3.07 bd	6.61	52.16	5.40	7.91	0.97	1.38	0.30
SE	0.81	0.09	0.12	0.18	0.76	0.10	0.17	0.06	0.06	0.01
Respect	21.12 d	1.96 ad	3.05 bd	6.6	53.32	5.50	8.05	1.02	1.4	0.30
SE	0.66	0.09	0.12	0.18	0.62	0.10	0.15	0.05	0.05	0.01
Salamanca	22.00 ad	1.89 d	3.09 bc	6.59	52.25	5.43	8.01	0.99	1.39	0.30
SE	0.61	0.08	0.12	0.17	0.58	0.08	0.13	0.05	0.05	0.01

SAA = Methionine+Cysteine

Neither the other CN nor the AAs in the CP of field peas differed significantly between the varieties of the data set. Even Ravindran et al. (2010) did not find significant differences in the CN and AA contents between five pea cultivars cultivated in New Zealand. Canbolat et al. (2007) examined only slight but significant differences of the contents of CP, CA, and CF between white- and purple-flowered peas from two consecutive harvest years in Turkey. Furthermore, the testa color had an effect on the CN contents as brown colored peas did contain higher amounts of CP, CA, and CF but lower amounts of N-free extracts (carbohydrates). Moreover, Gronle et al. (2015) found a higher CP and CF content, but a lower EE, starch, and sugar content as well as a lower energetic value in a colored-flowering winter pea compared to a

Table 5

Estimated covariance of random factors on crude nutrients, some amino acids, and crop yield of field peas (*Pisum sativum* L.)

Parameter	Crude Protein	Ether Extract	Crude Ash	Crude Fiber	Starch	Sugar	Lysine	Methionine	Cysteine	SAA:Lys	Yield
site					1.869*	0.000	0.050			0.00002*	
year	0.00	0.017	0.037	0.060				0.006	0.005	0.00030	
year*site	2.53**	0.007*	0.009*	0.045*	0.168	0.049*	0.036	0.002*	0.003*		128.28**
year*variety	0.37*				0.293*		0.013	0.001*	0.001	0.00002*	
Site*variety											
Residual	0.57***	0.006***	0.008***	0.04***	0.436***	0.038***	0.043***	0.001***	0.002***	0.00002***	17.049***

Cells without a value show parameters removed during model selection by BIC; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

white-flowering winter pea. The size of a pea seed can impact the composition, particularly the CF contents (Bastianelli et al., 1998). However, in the present study only yellow, white-flowering peas were observed. Some further studies with various experimental set-ups did not reveal remarkable differences between varieties although single varieties showed significantly differing contents of some CN (Kotlarz et al., 2011; Wang et al., 2008). Nevertheless, the variability of those ingredients due to varietal characteristics influences diet formulation and should therefore be regarded.

Environmental factors are known to affect the nutrient composition of crops (Wang et al., 2010). For instance, the year can affect the amounts of CN (Kotlarz et al., 2011). Borreani et al. (2007) did not find any significant differences in the composition of mature crops of conventionally produced field peas between the observed harvest years and varieties. However, different environmental factors of year and site can interact and therefore have unpredictable effects on the composition of field peas (Nikolopoulou et al., 2007). In the present study, the random factor year*site had an effect in the selected models of all analyzed parameters (Table 5). Hence, it can be concluded that there is an interaction between the environmental effects, which leads to differences in the composition.

However, these interacting effects are as unpredictable as the environmental alterations (like climatic conditions or possible alterations of the site). Additionally, year*variety affected the contents of CP, starch, and the AAs. This interaction was rather low and could not be found by others (Borreani et al., 2007; Kotlarz et al., 2011).

The crop yield of the field peas was on average 4.4 ± 1.2 t ha^{-1} and differed significantly between the examined varieties. Mythic had the highest (4.8 ± 3.7 t ha^{-1}) and Rebel the lowest (4.1 ± 3.5 t ha^{-1}) crop yield. Due to a high CP content, Mythic also had the highest CP yield per hectare. In consequence of low CP contents, Abarth showed the lowest CP yield per hectare. Additionally, there were differences between the varieties regarding the yields of AAs per hectare. The interaction between site and harvest year influenced the yields (Table 5). Hence, there is apparently an effect by the cultivation site, which might mainly be due to site-specific characteristics of the soil (plus soil fertility management, tillage, time of seeding and harvesting, row spacing, seed rate, pest and disease infestations, and further factors) combined with weather conditions and differences of the cultivation management connected to the harvest year. The present results indicate that individual varieties can have an improved AA profile and/or essential AA yield.

For diet formulation, the composition of the protein of harvested crops determines part of its quality. High amounts of limiting AAs can increase the value of a feedstuff. With regard to the amino acid gap it could be of increased interest to reward these characteristics instead of the CP yield in crops for use in monogastric nutrition. Thus, it is of interest to observe the changes in the AA profile with varying CP contents in the crops. The contents of the first-limiting AAs for swine and poultry, namely Lys, Met and Cys, were significantly negatively correlated with the CP content (Figure 2).

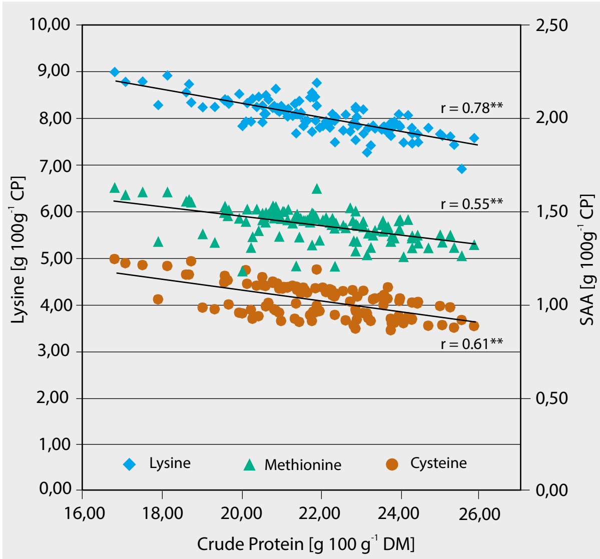


Figure 2
Correlation of lysine and the sulfur-containing amino acids (SAA), methionine and cysteine, with crude protein in field peas (*Pisum sativum* L.) with the coefficient of correlation (r, **= $p < 0.01$)

Wang and Daun (2004) also found a significant negative correlation between the contents of Lys and CP (r = -0.52**) in field peas. However, their analyses of 24 samples did not reveal a relationship between the amounts of the SAAs and CP. In contrast, a more or less strong negative correlation between the CP content and the amounts of SAA in the CP of field peas is described by other authors, who used more than 190 samples (Igbasan et al., 1996; Reichert and MacKenzie, 1982). Schumacher et al. (2011) found Lys and CP correlated with r = -0.50**, Cys and CP with r = -0.70** as well as SAA with r = -0.67** in 50 field pea samples from different varieties grown organically at two sites in the year 2007. These results indicate a negative relationship between the protein quality, more precisely the AA profile of the CP, and the CP quantity. Further investigations regarding the whole AA profile are required to gain comprehensive knowledge. The AAs in the profile, which account for those decreasing essential AAs, must be studied.

Moreover, there were correlations between different CNs (Table 6). A strong negative correlation of CP and starch was found with r = -0.79**. Actually, it is stated that starch accounts for variations in the CP content of field peas in the first place (Bastianelli et al., 1998; Holl and Vose, 1980; Reichert and MacKenzie, 1982; Wang et al., 2008). A reason for this negative correlation between starch and CP contents can be the higher increase of starch in comparison to CP in the maturation process of the seeds (Borreani et al., 2007). This change in the relationships between the ingredients during maturation could also be a possible reason for described correlations between other nutrients like CP and sugar. Correlations with partly varying strength have been found by different researchers. Reichert and MacKenzie (1982) also found negative correlations of starch, EE, and CF with CP. Nikolopoulou et al. (2007) published higher correlations between CP and ash and further correlations between ash and EE, ash and starch, as well as EE and starch than within the present data. They found CP and EE positively correlated in field peas. Bastianelli et al. (1998) found EE with CP, starch, and CF negatively correlated. Moreover, this inverse correlation between CP and EE was observed when drought stress occurred during the maturation of soybeans (Dornbos and Mullen, 1992). The correlation coefficients seem strongly dependent on the data basis. Hence, correlations are not suitable for predicting the composition of harvested crops due to a high variability of genetic and environmental influences on the maturation process (Gallardo et al., 2008; Gutierrez et al., 2007; Weber et al., 2005).

Table 6

Pearson correlation coefficients (n = 113, r > 0.30, p < 0.01) between crude nutrients (g 100 g^{-1} DM) of field peas (*Pisum sativum* L.)

	Crude Protein	Ether Extract	Crude Ash	Crude Fiber	Starch	Sugar
Crude Protein	1	- 0.31	0.51	- 0.36	- 0.79	- 0.55
Ether Extract		1				
Crude Ash			1		- 0.35	
Crude Fiber				1	0.42	
Starch					1	
Sugar						1

The crop yield of the peas was positively correlated with the CP content (r = 0.50*) and the ash content (r = 0.38*) as well as negatively correlated with the starch content (r = -0.44*) and the CF content (r = -0.39*). However, it was observed to be negatively correlated with the CP content and positively correlated with the starch content (Al-Karaki and Ereifej, 1999; Tar'an et al., 2004). Those negative correlations between CP and yield are also described for other legumes (Baudoin and Maquet, 1999; Dahiya et al., 1977; Erskine et al., 1985; Malik et al., 2006; Malik et al., 2007). Nevertheless, Pandey and Gritton et al. (1975) found variable but mostly positive correlations between CP content and the yield of peas. Tar'an et al. (2004) observed the variation of crop yield and CP content accounted for by two different trait loci. They concluded that it is possible to breed field pea varieties combining high crop yields and high CP contents. Thus, it is possible that correlations between crop yield and composition are influenced by the observed varieties. Another possible explanation is that the crop yield of field peas is highly variable depending on factors of the cultivation sites and harvest years. A crop shortfall due to pests or weather phenomena does not have to affect the CP content equally and can therefore alter the outcomes of correlation analyses.

3.2 Field beans (*Vicia faba* L.)

The analyzed field beans contained on average 29.6 g CP 100 g^{-1} DM with 6.4 g Lys 100 g^{-1} CP, 0.7 g 100 g^{-1} Met, and 1.1 g 100 g^{-1} Cys. The relation of the SAA to Lys varied between 0.2 and 0.3 (Table 7), even more widely than in field peas, and was mostly less favorable than the ratio found in field peas.

Schumacher et al. (2011) found lower contents of CP and Lys in organically produced field beans. However, the contents of Met and Cys did not differ from the results in Table 7. Although their field bean samples also originated from different varieties (not all of them actually in use), sites and harvest years, they reported lower variations of the CP content. The data of AMINODat® 4.0 derived from analyses of 18 conventionally produced field bean samples from Germany (Evonik Industries, 2010) with unknown cultivation conditions and varieties, published lower CP and Lys contents as well as higher Cys contents. The mean content of CP in 59 organically produced field bean samples was higher in the online tables of the DLG (2006-2010). The samples contained less Lys while the average amounts of Met and Cys (43 samples) were similar to the present findings. Conventionally derived table values (Staudacher and Potthast, 2014) were lower for CP but higher for Lys, Met, and Cys. The table values for EE, starch, and CF were also lower, while sugar contents were higher. Taken as a whole, the observed composition agrees with the widely varying literature results for both conventionally and organically cultivated field beans.

Table 7

Ranges and means with standard deviations (SD) of the contents of crude nutrients (g 100 g^{-1} dry matter), lysine, methionine and cysteine in organically produced field beans (*Vicia faba* L.) (g 100 g^{-1} crude protein)

	Mean	SD	Min	Max
Dry Matter	92.65	0.81	90.42	93.94
Crude Protein	29.56	1.70	25.32	33.28
Lysine	6.44	0.35	5.59	7.52
Cysteine	1.09	0.09	0.90	1.28
Methionine	0.71	0.05	0.62	0.80
Methionine+Cysteine (SAA)	1.80	0.13	1.55	2.08
SAA:Lysine	0.28	0.03	0.22	0.34
Ether Extract	1.72	0.18	1.39	2.43
Crude Ash	4.00	0.42	3.41	5.67
Crude Fiber	9.66	0.62	8.61	11.79
Starch	42.99	2.01	35.40	47.39
Sugar	3.08	0.14	2.80	3.45

Just as for field peas, the genetic influence of the variety can also affect the composition of field beans (Ghaouti, 2007). The field bean variety had a significant effect on the content of all CNs and AAs (p < 0.05) (Table 8).

The varieties low in CP (Alexia, Espresso, Pyramid, and Fuego), showed significantly higher amounts of CF, Lys, and Met than the varieties high in CP (Bioro, Divine, Julia, and

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Table 8

Least square means of crude nutrient contents (g 100 g^{-1} dry matter) and contents of the amino acids lysine, methionine, and cysteine (g 100 g^{1} crude protein) with standard error (SE) and with letters to mark significant differences ($p < 0.05$) in various field bean (*Vicia faba* L.) varieties

	Crude Protein	Ether Extract	Crude Ash	Crude Fiber	Starch	Sugar	Lysine	Methionine	Cysteine	SAA:Lysine
Alexia	27.70 d	1.70 df	3.77 d	9.78 cd	44.73 a	2.97 f	6.8 a	0.73 ab	1.09 b	0.27 bd
SE	0.97	0.05	0.13	0.29	0.88	0.04	0.30	0.02	0.05	0.02
Bioro	29.86 ab	1.66 f	3.96 bc	9.48 ef	43.07 bcd	3.07 cde	6.61 e	0.69 fgh	1.04 def	0.26 cde
SE	0.96	0.05	0.12	0.29	0.87	0.04	0.30	0.02	0.05	0.02
Divine	29.95 ab	1.65 f	3.98 bc	9.48 ef	42.29 d	3.06 de	6.65 cde	0.68 h	1.04 e	0.26 e
SE	0.96	0.05	0.12	0.29	0.87	0.04	0.30	0.02	0.05	0.02
Espresso	27.85 d	1.74 cef	3.84 cd	10.08 ab	43.67 ac	3.15 bc	6.76 ad	0.73 ab	1.08 be	0.27 bc
SE	0.99	0.06	0.14	0.30	0.92	0.05	0.31	0.02	0.05	0.02
Fabelle	30.25 a	1.69ef	4.06 ab	9.24 g	42.71 cd	3.06 de	6.64 de	0.69 gh	1.05 cef	0.26 ce
SE	0.98	0.05	0.12	0.29	0.87	0.03	0.30	0.02	0.05	0.02
Fanfare	29.19 ad	1.74 de	3.98 bc	9.86 bc	42.82 bcd	3.08 bd	6.66 bcde	0.71 ce	1.07 bf	0.27 bc
SE	1.05	0.05	0.13	0.29	0.91	0.04	0.30	0.02	0.05	0.02
Fuego	28.33 cd	1.86 b	4.05 ab	10.14 a	42.87 cd	3.11 bd	6.64 de	0.71 cd	1.07 bc	0.27 b
SE	0.96	0.05	0.12	0.28	0.86	0.03	0.30	0.02	0.05	0.02
Isabell	29.33 ac	1.74 de	4.04 ac	9.63 de	43.08 bcd	3.07 cd	6.61 e	0.70 def	1.06 cef	0.27 bc
SE	0.96	0.05	0.12	0.28	0.86	0.03	0.30	0.02	0.05	0.02
Julia	30.04 ab	1.57 g	3.89 cd	9.39 fg	43.55 bc	3.01 ef	6.6 e	0.69 eg	1.04 ef	0.26 cde
SE	0.97	0.05	0.12	0.29	0.88	0.04	0.30	0.02	0.05	0.02
Pyramid	28.10 d	1.77 cd	4.20 a	10.10 a	43.07 bcd	3.14 b	6.78 ab	0.73 b	1.07 bcd	0.27 bc
SE	0.97	0.05	0.13	0.29	0.88	0.04	0.30	0.02	0.05	0.02
Taifun	28.54 bcd	1.95 a	4.15 ab	9.69 ce	44.86 a	3.11 bd	6.75 ad	0.75 a	1.14 a	0.28 a
SE	1.06	0.05	0.14	0.3	0.93	0.04	0.31	0.02	0.05	0.02
Tangenta	28.57 cd	1.83 bc	4.20 a	9.78 cd	43.97 ab	3.27 a	6.77 ac	0.72 bc	1.07 be	0.26 be
SE	1.00	0.05	0.13	0.29	0.9	0.04	0.30	0.02	0.05	0.02

SAA = Methionine+Cysteine

Fabelle). Even the Cys contents were on average higher in varieties with low CP content. Furthermore, varieties with average contents of CP, like Taifun or Tangenta, had significantly the highest EE, ash, starch, and sugar contents as well as high amounts of Lys, Met, and Cys. Jezierny et al. (2011) also examined the composition of Divine, Espresso, and Fuego. They found different amounts of some CN in each variety. Divine was harvested in 2005 and had equal CP, lower EE and ash but higher starch and sugar contents than the present findings. Espresso and Fuego were harvested in 2004 and contained more CP but less starch and sugar. Even the contents of ash and EE were lower in Fuego harvested in 2004. These differences are probably dependent on the cultivation conditions. Duc et al. (1999) found no influence of the "vicin-gene" on the composition of field beans. However, the "tannin-gene" seemed to lead to higher CP- and lower CF-contents. In the present sample, the tannin-free variety Tangenta performed on a medium level and the vicin-free varieties Divine and Fabelle had high CP contents as well as low CF contents. These findings support the suggestion that factors other than varietal effects might be considerably high. Furthermore, it is possible that twelve years of breeding contributed to the altered composition with regard to this comparison. Taken as a whole, the results indicate that the choice of the field bean variety can most likely be of benefit for diet formulation for monogastrics. Further breeding strategies might be able to use those genetic differences to their advantage.

In addition to genetic influences, the environment can affect the composition of field beans, too (Römer, 1998). Just as in field peas, the factor year*site was found to affect all ingredients in the individual statistical models (Table 9). The combination of the environmental factors seems to be important with regard to the composition of crops. Furthermore, variety*site influenced the contents of CP and starch.

Remarkable is the fact that field beans, which were harvested in 2011 on Location 56_6, had lowest average contents of CP as well as very low Cys-contents, medium Met-, and the highest Lys-contents. Hence, the relationships between Cys:Met as well as SAA:Lys were particularly low. Even the contents of CF, ash, sugar, and starch were high in comparison and the content of EE was low. No causes for these effects could be determined for certain. However, the generative development of those field beans was quickened due

Table 9

Estimated covariance of random factors on crude nutrients, some amino acids, and crop yield of field beans (*Vicia faba* L.)

Parameter	Crude Protein	Ether Extract	Crude Ash	Crude Fiber	Starch	Sugar	Lysine	Methionine	Cysteine	SAA:Lys	Yield
site											
year	1.48			0.12	0.67	0.00	0.25	0.0013	0.0057	0.0011	46.77
year*site	1.40*	0.02**	0.15**	0.22*	3.19*	0.01**	0.04*	0.0005*	0.0041*	0.0001*	89.12*
variety*site	0.23*				0.23**						14.95*
year*variety	0.11				0.10						
Residual	0.25	0.01***	0.03***	0.04***	0.22**	0.005***	0.01***	0.0002***	0.0011***	0.0001***	12.37***

Cells without a value show parameters removed during model selection by BIC; * p < 0.05, ** p < 0.01, *** p < 0.001

to a dry period until May. Nevertheless, crop yields of about 5 t ha^{-1} were reached after a slow maturation. It is possible that drought and heat had detrimental effects on the nodulation and symbiosis in the early stages of development, respectively on N-fixation, and therefore on the CP contents (Hungria and Vargas, 2000) but there were no studies in the temperate zone.

No significant differences were observed for the crop yield of field bean varieties. It ranged between 3.9 ± 0.6 t ha^{-1} (Tangenta) and 5.3 ± 0.7 t ha^{-1} (Fanfare). The random factor year*site affected the crop yield. Furthermore, effects of site*variety and year led to the model with the lowest BIC (Table 9). The CP yield did not differ significantly between varieties. In conclusion, the sum of environmental influences strongly affected the yield of the field beans in the data set, independent of the variety.

In field beans, Lys and Met showed high negative correlations with CP. However, the Cys content was not correlated with the CP content of this species (Figure 3).

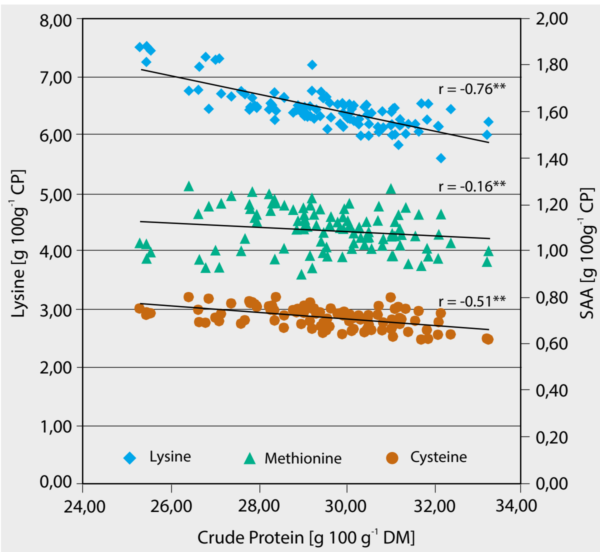

Figure 3

Correlation of lysine and the sulfur-containing amino acids (SAA), methionine and cysteine, with crude protein in field beans (*Vicia faba* L.) with the coefficient of correlation (r, **= p < 0.01)

A negative correlation between the CP content and the amounts of SAA in the CP of field beans has already been described (Gatel, 1994; Monti and Grillo, 1983). Schumacher et al. (2011), for example, found Met and CP correlated with r = -0.83**, Cys and CP correlated with r = -0.44^{ns} as well as SAA and CP correlated with r = -0.61**. In addition, Lys and CP had a strong negative correlation. Thus, also in field beans, a negative relationship between the quality and the quantity of the protein was found.

Further correlations were even found in field beans. The contents of starch and sugar were, for example, negatively correlated with the CP content (Table 10). Duc et al. (1999) found an equal relationship of starch and CP within 74 genotypes of faba beans (r = -0.67**). The maturation process might account for this correlation in field beans just as it does in field peas (Borreani et al., 2007; Holl and Vose, 1980). EE, ash and CF decreased with increasing starch contents and were positively correlated with each other. Furthermore, light correlations were found between the sugar content and the contents of ash and CF (Table 10). Those correlations are not described consistently in the literature. Therefore, influences of parameters within the trials, like the varieties, the harvest years with their climatic factors and the locations with different soil characteristics, tillage, time of seeding and harvesting, row spacing, seed rate, and further factors, might be very high.

Table 10

Pearson correlation coefficients (n = 105, r > 0.30, p < 0.01) between crude nutrients (g 100 g^{-1} dry matter) of field beans (*Vicia faba* L.)

	Crude Protein	Ether Extract	Crude Ash	Crude Fiber	Starch	Sugar
Crude Protein	1				-0.69	-0,47
Ether Extract		1	0,57	0,39	-0.34	
Crude Ash			1	0,56	-0,43	0,41
Crude Fiber				1	-0,37	0,39
Starch					1	
Sugar						1

A correlation of r = 0.55** between crop yield and starch content could be observed in spring faba beans of the present study. Furthermore, the crop yield was also slightly negatively correlated with the amounts of SAA (Cys: r = -0.40**, Met: r = -0.36**). Al-Karaki and Ereifej (1999) found the crop yield of field peas to be positively correlated with the starch content, which equals the present findings in field beans. Nevertheless, crop yields are strongly influenced by environmental factors and can therefore vary depending on harvest year and cultivation site. These influences can affect the correlation coefficients, especially in an unbalanced dataset.

In the present study, as well as in earlier studies (Schumacher et al., 2011; Wang and Daun, 2004; Wang et al., 2008), both grain legume species showed variations in their composition. Regarding the present results as well as the results of Schumacher et al. (2011), the reactions of the amount of Met in the CP seem to be more stable and dependent on the CP contents than the reactions of Cys in the seeds of field beans. The range of the Met content is smaller than the range of the Cys content. Statistically significant correlations between Met and CP content could be found repeatedly, while there were no correlations between Cys and CP content in the studies.

Results of variety trials on certain cultivation sites in one year indicate that varieties of both observed species react within their genetic possibilities depending on the environmental factors like soil, climate, and cultivation. The interaction of both genetic and environmental factors at a single site in one year is of interest. In multiannual trials, genetically indicated varietal differences can be derived from repetitive observations under different environmental conditions (site, weather conditions, cultivation management). However, varietal differences are more obvious when the variance of environmental differences is reduced. Therefore, homogenous climatic areas, and subsequently cultivation areas for different crop species, were identified as a basis to improve recommendations for varieties of crop species (Graf et al., 2009). Based on the knowledge that varieties can show differences in their development, crop yield, and even quality parameters depending on the cultivation system, there is also a separation into organic and conventional variety trials. Every time a crop is cultivated, there are many different expressions of environmental effects as well as various interactions between those effects (precipitation, tillage, fertilization…), which can affect further plant development. Those influences cannot be overlooked. Therefore, a precise estimation of the influence of environmental factors on variations of crop yield, CP content or other parameters is not possible. Nevertheless, in the present study it was possible to show that varietal differences in the nutrient composition of field peas and field beans occur regardless of environmental effects related to site and year. However, since there is an undirected interference between different environmental conditions and varietal characteristics, the result of the plant development under different conditions is not yet predictable. Hence, it is recommended to analyze every batch before formulating a diet.

Since a negative correlation between the CP content and the limiting AA, Lys, Met, and Cys (Monti and Grillo, 1983; Partanen et al., 2001; Wang and Daun, 2004) was confirmed, the use of varieties with high CP contents or a cultivation management that leads to high CP contents might also be disadvantageous for diet formulation. Therefore it is aimed to improve the SAA contents in field beans and field peas. The fertilization with sulfur can have a positive impact on the quantities of SAA in the crops (Scherer, 2001). Beside sulfur-fertilization, the use of breeding strategies to improve both CP content and protein quality is another relevant possibility in the production of grain legumes as feedstuffs. Regarding successes in soybeans (Imsande, 2001), the breeding of Met- or SAA-enriched varieties of native grain legumes seems promising (Schumacher et al., 2009). These findings are supported by the identification of genes, which control the amounts of CP and SAA in legume seeds (Gallardo et al., 2008).

4 Conclusions

Both genetic and environmental factors can have an influence on the contents of CN, Lys, Cys, and Met in the seeds of field peas and field beans and also on the crop yield. The possibility to make predictions for the nutrient composition of these crops is very limited due to unforeseeable combinatory effects of variety, site (cultivation), and climate (year). It is yet not possible to make predictions with regard to the influence of those combined environmental factors. The effect of the variety is the most certain factor of influence, and is more or less affected by other parameters. Most of the currently used varieties, which were part of the study, did not differ significantly, but the choice of the variety can be essential with regard to the amount of quality-determining constituents.

Some correlations could be confirmed by the present data. In general, the negative correlation of the Lys-, Met-, and Cys content with the CP content was proved. However, the strength of the correlation was not equal in comparison with other studies and seemed to be dependent on the database and its influencing factors. The cultivation of varieties with higher CP contents might decrease the amounts of essential AAs in the CP. This is not desired for grain legumes, which are used to feed monogastric animals. Therefore, breeding for higher protein contents alone is not adequate. An improvement in the protein quality to meet the requirements of monogastric animals must be the focus of attention rather than protein yield. Since the direction of described variations is not predictable, it is clearly recommended to analyze every batch before diet formulation to match the requirements of monogastric animals. Especially in organic farming, the use of this information has the potential to improve the feeding system. Further research is required regarding the protein quality of crops and the influencing factors.

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