



Effect of differing proportions of protein-rich common vetch seeds (*Vicia sativa* L. cultivar Slovena) in organic diets for broiler chickens

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Abstract The common vetch is a legume with low requirements for soil type and water availability and a good weed suppression together with an intercropping partner, thereby recommending itself for organic agriculture, especially in view of climate change. The use of the protein-rich seeds of common vetch for poultry, however, is limited due to anti-nutritional factors (ANF). This study was conducted to examine the use of common vetch seeds (cultivar: Slovena) in 100% organic diets for slow-growing broiler chickens. The common vetch seeds were found to be rich in protein and lysine (343 and 21.5 g/kg dry matter), and their vicine (5.69 g), convicine (0.75 g) and γ -glutamyl- β -cyanoalanine (6.9 g) levels were at the lower range of previous reports, while tannin levels (8.1 g total tannins/kg dry matter) were at the higher end. A vetch-free control and experimental diets containing 75, 100 and 125 g common vetch seeds/kg of diet were fed during the grower (day 29–49) and finisher (day 50–70) period of two consecutive fattening periods, to a total of 8 groups of 20 mixed-sex

broilers per treatment. No differences in feed intake, animal performance and welfare status were found, therefore including up to 125 g common vetch seeds/kg of diet can be recommended. Based on a comparison of the ANF contents with previous reports, higher inclusion rates should be possible, as negative effects can be expected at 350 g common vetch seeds/kg of diet, which is higher than necessary in diet formulation anyway.

Keywords Feed · Fattening · Anti-nutritional factors · Slow-growing broilers

Introduction

The common vetch (*Vicia sativa* L.) originates from the Mediterranean area but has long since spread all around the world (Francis et al. 2000). It is both cultivated as a catch crop as well as for the production of seeds, which are used as feed for animals and food for humans (Huang et al. 2017). Among its advantages are low requirements for soil type and water availability (White et al. 2005), but the common vetch needs an intercropping partner to ensure a stable crop stand for machine harvest. When intercropped with oat (*Avena sativa* L.), high-growing vetch varieties like cultivar Slovena have been found to produce grain yields of 5.3 tons dry matter/ha and suppress weed effectively, as has been shown in work conducted at our own institute (Rinke et al. 2022). With 206–393 g

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crude protein/kg dry matter, common vetch seeds (CVS) are very rich in protein and therefore could provide an attractive feed component for poultry diets (Huang et al. 2017), which is especially relevant in organic poultry feeding, where protein sources are scarce and expensive. The protein content of common vetch seeds is comparable to faba beans (*Vicia faba* L.) and is even higher than in field peas (*Pisum sativum* L.) (Witten et al. 2015). However, in contrast to other parts of the world, the common vetch is only rarely used as feed in central Europe today. The presence of anti-nutritional factors (ANF) in the seeds hinders their inclusion in diets. According to Ressler et al. (1997), the primary source of toxicity to chickens is the non-proteinogenic amino acid γ -glutamyl- β -cyanoalanine, a derivative of β -cyanoalanine. These cyanogenic compounds are toxic, and high inclusion rates of 300 g CVS/kg of diet can even lead to mortality in young chicks, as shown in the early work of Arscott and Harper (1963, 1964). In addition to cyanoalanine toxins, CVS also contain the pyrimidine glucosides vicine and convicine, which are found in faba beans as well and can trigger favism, a haemolytic disease, in humans (Enneking 1994). Furthermore, CVS contain tannins, which are widespread in leguminous plants and have been shown to improve gut health but at high doses can also negatively affect the performance and health of broiler chickens (Hidayat et al. 2021). To avoid possible negative effects on broiler health and performance, several studies have tested different proportions of CVS in broiler diets; these studies uniformly report that a dietary inclusion rate of 100 g untreated CVS/kg of diet did not lead to differences in body weight gain compared to the respective control (Sadeghi et al. 2011; Saki et al. 2008; Darre et al. 1998). The authors of several studies aiming at increasing the inclusion rates of common vetch seeds in poultry diets report success in detoxifying CVS by varying treatments, ranging from soaking in water with (Sadeghi et al. 2011) or without heat (Kaya et al. 2011) and, autoclaving (Farran et al. 1995; Harper and Arscott 1962) to germination and ensiling (Höhne et al. 2022). However, feeding untreated CVS remains the easiest option with regard to cost and effort and is therefore still of interest. Despite reports on cultivar differences with regard to the content of nutrients and ANF in CVS (Chowdhury et al. 2001; Larbi et al. 2011; Collins et al. 2002), studies on feeding CVS rarely report

the used cultivar, and information about the ANF is scarce as well. Therefore, the aim of our study was to investigate the effect of different proportions of common vetch seeds of cultivar Slovena in broiler diets, as well as to analyse the nutrients and ANF in the seeds of cultivar Slovena and the diets. If successful, common vetch seeds could provide a welcome alternative to other protein sources currently used in broiler feeding in central Europe. Therefore, the tested inclusion rates of 0, 75, 100 and 125 g CVS/kg of diet were based on the above-mentioned reports recommending a maximum of 100 g CVS/kg of diet, with the 125 g CVS/kg diet treatment being a moderate challenge. We included the CVS in 100% organic diets, which were fed to mixed-sex slow-growing broilers during the grower and finisher period.

Material and methods

The feeding trial was conducted at the experimental farm of Thünen Institute of Organic Farming (Westerau, Germany) in 2018. Animal husbandry followed the rules of EU Directive EC 834/2007 on organic agriculture (EC 2007), EU Directive 2010/63/EU on animal experiments (EC 2010) and the production guidelines of the German organic farming association Bioland (Bioland 2014). The trial was announced to the Schleswig Holstein Ministry of Energy Transition, Agriculture, Environment and Rural Areas on March 21, 2017 and acknowledged on April 24, 2017 (reference number V 241–23253/2017).

Cultivation of common vetch seeds

The common vetch (cultivar Slovena) used in the trial was grown in 2016 at the experimental farm of Thünen Institute of Organic Farming (20 m above sea level, 693 mm annual precipitation, 9.0 °C average temperature). The cultivar Slovena was bred by Saatbau Linz (Austria). It has a thousand seed weight of 50–70 g, and the light grey seeds have black seed hulls. The common vetch was intercropped with oats, and after harvest the seeds were separated from the oat grains using a sieving process.

Chemical analysis of nutrients and anti-nutritional factors

Feed analysis of the CVS and the experimental feeds was carried out at the laboratory of Thünen Institute of Organic Farming. Before analysis, samples were ground to pass a 1.0 mm sieve. Analysis of crude nutrients followed Commission Regulation 152/2009 (EC 2009), Annex III. According to method A, dry matter content was determined by oven-drying at 103 °C. Crude ash, crude fat, sugar, and starch were analysed according to methods M, H, J and L, respectively (EC 2009). Nitrogen content was measured using the Kjeldahl method (method C, EC 2009), and crude protein content was calculated by multiplying nitrogen content by 6.25. The energy value of the feed, given as apparent metabolizable nitrogen-corrected energy (AME_N), was calculated using the equation of WPSA (1984). Before analysis of amino acids, minerals, and ANF, the samples were further ground to pass a 0.5 mm sieve. Amino acid contents were analysed using a modified method following Commission Regulation 152/2009, Annex III, Method F (EC 2009). For a detailed description of the HPLC method see Witten et al. (2020). Following the official method 10.8.1.2 of VDLUFA (2012), minerals were analysed after microwave-assisted digestion. Phosphorus was measured photometrically according to Commission Regulation 152/2009, Annex III, method P (EC 2009), and calcium was determined by atomic absorption spectroscopy. The contents of vicine and convicine were determined using HPLC according to Pulkkinen et al. (2015) with some modifications. The identity of the analytes was confirmed using a modified LC–MS analysis (Aulrich 2023; personal communication). The cyanoalanine-toxins γ -glutamyl- β -cyanoalanine and β -cyanoalanine were analysed using HPLC and MS according to Thavarajah et al. (2012) with some changes (Aulrich, 2023; personal communication). The determination of the tannins was carried out according to the Folin-Ciocalteu method based on Makkar et al. (1993), and the results are presented as tannic acid equivalents. Table 1 shows the contents of nutrients and anti-nutritional factors in CVS.

Table 1 Analysed contents of nutrients and anti-nutritional factors in common vetch seeds (cultivar Slovena, harvest of 2016) fed in the feeding trial, g/kg dry matter unless stated otherwise

	Common vetch seeds
Dry matter ¹	886
AME _N (MJ/kg) ²	13.3
Crude protein	343
Lysine	21.5
Methionine	2.4
Cysteine	3.5
Threonine	11.1
Tryptophan	3.1
Crude fat	17.2
Crude starch	414
Crude sugar	32.7
Crude ash	46.3
Calcium	1.0
Phosphorus	4.0
Vicine	5.69
Convicine	0.75
γ -Glutamyl- β -Cyanoalanine	6.90
β -Cyanoalanine	0.03
Total tannins	8.10

¹ g/kg as fed

²AME_N=apparent metabolizable nitrogen-corrected energy, calculated according to WPSA (1984)

Birds and housing conditions

A total of 640 broilers (Hubbard JA 757) in two consecutive fattening periods (FPs) of 320 broilers each were kept for the experiment. The FPs started on April 9 and June 25, 2018, with the arrival of the day-old chicks. Prior to delivery, the chicks received vaccinations against Marek's disease and coccidiosis at the hatchery. During the four-week rearing period, the chicks were kept together in one group in a barn with an adjacent covered outdoor area. The experimental period was six weeks long and lasted from the 29th to the 70th day of life (short: day 29 to 70). At the beginning of the experimental period, the broilers were weighed individually, randomly split into 16 groups of 20 animals each and marked with coloured rings around the left shank indicating the experimental

treatment. The sex of the birds was determined later on the basis of their external anatomy and behaviour. In total, the diets containing 0, 75, 100 and 125 g CVS/kg diet were fed to 78 females and 80 males, 74 females and 86 males, 84 females and 74 males, and 68 females and 91 males, respectively. After grouping, each group of 20 birds was transferred to one of 16 replicate pens, located in four mobile barns placed on permanent grassland. The pens were bedded with straw and equipped with perches, nipple drinkers and a feed trough. Access to the adjacent green outdoor run (4 m² per bird) was granted from 10:00 am to dusk until the birds were 49 days old, and around the clock thereafter. For a drawing of the housing system, further details are provided by Höhne et al. (2022). At the age of 71 days, all broilers were slaughtered at a nearby slaughter facility.

Feeding

The broilers were fed in three feeding periods: starter (day 1–28), grower (day 29–49), and finisher (day 50–70). Feed and water were provided ad libitum, and all feeds were offered as 3 mm pellets. All feed mixtures were of 100% organic origin and were produced at the in-house feed mill shortly before the start of the feeding trial to ensure that only one batch of each feed mixture was fed in both FPs. The starter feed was based on feed components commonly used in organic broiler feeding in Germany, namely wheat, triticale, spring field peas and press cakes (soybean, sunflower and rapeseed). Following the European guidelines for organic agriculture, the press cakes derived from mechanical oil production without the use of solvents, and no synthetic amino acids were used to balance the amino acid profiles. During the experimental period, four feeding variants (FVs) were compared: a control feed without CVS, a feed including 75 g CVS/kg of diet, a feed including 100 g CVS/kg of diet, and a feed including 125 g CVS/kg of diet. The control diet included field peas, which were replaced by CVS in the experimental diets. None of the diets contained faba beans to avoid a possible confounding effect of those ANFs that are present in both faba beans and common vetch, e.g. vicine and convicine. The nutritional composition of the FVs is shown in Table 2.

Data collection and calculations

Prior to each FP, a sample of the CVS was collected for analysis. In each FP, two samples from each FV were collected during the starter, grower, and finisher period and combined into one pooled sample per FV and feeding period, resulting in a total of two pooled samples per FV and FP. All feed samples were analysed as described in Sect. "Chemical analysis of nutrients and anti-nutritional factors". All chicks were weighed individually on the day of arrival and once a week throughout the experiment. Average daily weight gain was calculated for each bird once a week. Once a week, feed refusals were weighed to calculate feed intake on a group basis. Feed conversion ratio (FCR) on a group basis was calculated by dividing feed intake by weight gain. After slaughter, the carcasses of all birds were weighed after overnight cooling and carcass yield was calculated. The weight and yield of the valuable cuts (breast fillet, legs) were obtained by cutting up five birds per replicate pen, resulting in 20 birds per FV. These birds were selected based on their body weight by choosing one closest to the mean and another four closest to the mean ± 1 and 2 standard deviations of the respective replicate pen. The same 20 broilers per FV received scores for such welfare indicators as hock burns, footpad lesions, and breast blisters at the slaughterhouse in accordance with the Welfare Quality Protocol (2009), where score "0" indicates an unimpaired state, "1" indicates minor changes, and "2" indicates major changes.

Statistical analysis

All data were analyzed using a linear mixed model (proc GLIMMIX of SAS® 9.4, 2002–2012). The experimental unit for feed intake and FCR was the replicate pen (a group of 20 birds), while the experimental unit for live weight, daily weight gain, carcass traits, and welfare indicators was the individual broiler. The model for feed intake and FCR included the fixed factors FV, FP, and the week of age. The model for live weight, daily weight gain, carcass traits, and welfare indicators included the fixed factors FV, FP, sex, and the two-way interaction of FV x sex. The group nested within FV and FP was used as a random effect. P-values of less than 0.05 were interpreted as indicating significant differences.

Table 2 Composition and analysed contents of nutrients and anti-nutritional factors in the starter feed (day 1–28) and the feeding variants including 0, 75, 100 or 125 g common vetch

seeds (CVS)/kg of diet during the grower (day 29–49) and finisher period (day 50–70), g/kg dry matter unless stated otherwise

Ingredients	Starter	Grower, share of CVS in diet				Finisher, share of CVS in diet			
		0 g/kg	75 g/kg	100 g/kg	125 g/kg	0 g/kg	75 g/kg	100 g/kg	125 g/kg
Wheat	300	430	487	497	485	480	572	569	556
Triticale	100	-	-	-	-	-	-	-	-
CVS	-	-	75	100	125	-	75	100	125
Spring field peas	200	180	40	-	-	193	-	-	-
Soybean cake	200	180	170	170	155	135	130	121	100
Sunflower cake	50	25	25	25	30	25	20	20	20
Rapeseed cake	50	75	75	75	65	75	70	60	66
Wheat bran	-	26	40	50	50	27	60	50	50
Rice gluten	58	51	55	55	57	34	43	49	52
Minerals ¹	42	33	33	34	33	31	30	31	31
Nutrients and anti-nutritional factors (g/kg dry matter)									
AME _N (MJ/kg) ²	12.9	12.9	12.8	12.7	12.8	12.8	13.1	13.1	13.3
Crude protein	238	229	233	241	239	216	221	222	220
Lysine	12.8	12.3	12.3	12.5	12.3	12.2	12.1	12.4	12.7
Methionine	3.6	3.2	3.7	3.7	3.7	3.1	3.3	3.4	3.3
Cysteine	4.2	3.9	4.4	4.5	4.5	4.0	4.1	4.2	4.2
Threonine	8.9	8.6	8.5	8.9	8.6	8.2	8.1	8.3	8.3
Crude fat	47.3	43.7	46.0	46.0	45.3	39.8	50.5	47.0	48.2
Crude starch	407	425	415	401	411	449	437	448	452
Crude sugar	61.9	57.9	54.4	52.2	51.3	50.3	49.4	47.3	49.4
Crude ash	71.6	62.4	63.7	65.4	65.2	61.6	61.5	59.8	62.6
Calcium	14.8	10.5	10.6	10.7	11.0	10.9	10.4	10.6	11.2
Phosphorus	10.0	10.1	10.2	10.6	10.7	9.4	9.4	9.6	10.2
Vicine	0.09	0.06	0.46	0.62	0.74	0.20	0.47	0.61	0.71
Convicine	0.02	0.02	0.06	0.08	0.09	0.06	0.07	0.08	0.09
γ-Glutamyl-β- Cyanoalanine	0.00	0.01	0.34	0.49	0.54	0.03	0.32	0.47	0.56
β-Cyanoalanine	0.000	0.000	0.006	0.007	0.008	0.000	0.006	0.008	0.012
Total tannins	1.5	1.9	2.1	1.9	1.9	1.4	1.8	1.8	2.0

¹210 g Ca, 107 g P, 47 g Na, 7 g Mg, 450,000 iE vitamin A, 90,000 iE vitamin D3, 2265 mg vitamin E, 2500 mg Fe, 3340 mg Zn, 2670 mg Mn, 500 mg Cu, 26 mg I, 7 mg Se kg⁻¹

²AME_N = apparent metabolizable nitrogen-corrected energy, calculated according to WPSA (1984)

Results

Table 1 shows the contents of nutrients and anti-nutritional factors in CVS (cultivar Slovena, harvest 2016) that were used in the feeding trial. Performance traits of the broilers are summarised in Tables 3 and 4. During the grower period, statistical analysis showed a significant effect of the FV on daily weight gain, but none of the pairwise comparisons were found to be significant. During the finishing period, the

interaction between FV and sex was significant. However, this finding was not relevant because pairwise comparisons only showed higher daily weight gain in male broilers compared to females, albeit with higher differences between the sexes in the groups receiving 75 and 100 g CVS/kg of diet (37–38%) than in the groups with 0 and 125 g CVS/kg of diet (28–31%).

Daily weight gain from hatching to slaughter (not shown in Table 3) was calculated using the average weight of the day-old chicks and did not differ

Table 3 Live weight, daily weight gain, feed intake and feed conversion ratio during the grower (day 29–49), finisher (day 50–70) and the total experimental period (day 29–70; LS-

Means of the interaction of FV x sex, or FV, respectively) in relation to the feeding variants (FV) including 0, 75, 100 or 125 g common vetch seeds (CVS)/kg of diet

	Share of CVS in diet								P values			SEM ¹
	0 g/kg		75 g/kg		100 g/kg		125 g/kg		FV	sex	FV x sex	
	female	male	female	male	female	male	female	male				
Number of animals	78	80	74	86	84	74	68	91				
Live weight (g) ²												
Day 29 of age	610	670	610	686	607	684	617	672	0.888	<0.001	0.638	9.6–11.2
Day 50 of age	1531	1739	1551	1769	1589	1851	1628	1829	0.058	<0.001	0.648	27.0–27.1
Day 71 of age	2542	3032	2560	3165	2598	3210	2613	3192	0.130	<0.001	0.274	43.6–47.5
Average daily weight gain (g) ²												
Grower period	43.8	51.0	44.8	51.6	46.6	56.0	48.2	54.8	0.037	<0.001	0.322	1.66
Finisher period	48.1 ^a	61.6 ^b	48.1 ^a	66.4 ^b	47.5 ^a	65.4 ^b	48.5 ^a	63.7 ^b	0.498	<0.001	0.017	1.22–1.31
Total period	46.0	56.3	46.4	59.0	47.1	60.8	47.6	59.7	0.096	<0.001	0.081	1.17–1.18
Number of replicate pens	8		8		8		8					
Average daily feed intake (g) ³												
Grower period	99		98		97		90		0.088			2.9
Finisher period	147		151		148		151		0.607			2.5
Total period	123		124		123		121		0.561			1.9
Feed conversion ratio (kg/kg) ³												
Grower period	2.1		2.0		2.0		1.7		0.099			1.69–2.10
Finisher period	2.6		2.5		2.6		2.5		0.924			1.02
Total period	2.3		2.2		2.3		2.1		0.122			1.04

¹Standard error of the mean of FV x sex (²) or FV (³), if given as a range then because of uneven data distribution²Experimental unit: individual animal³Experimental unit: replicate pen**Table 4** Carcass traits in relation to the feeding variants (FV) including 0, 75, 100 or 125 g common vetch seeds (CVS)/kg of diet. (LS-Means of the interaction of FV x sex)

Carcass traits ²	Share of CVS in diet								P values			SEM ¹
	0 g/kg		75 g/kg		100 g/kg		125 g/kg		FV	sex	FV x sex	
	female	male	female	male	female	male	female	male				
Number of animals	78	80	74	86	84	73	68	91				
Final live weight (g)	2542	3032	2560	3165	2598	3210	2614	3192	0.130	<0.001	0.274	43.6–47.5
Chilled carcass weight (g)	1669	1997	1685	2078	1719	2110	1749	2079	0.145	<0.001	0.401	31.6–34.8
Carcass yield (g/100 g)	65.5	65.7	65.7	65.6	66.1	65.8	66.7	65.2	0.828		0.177 0.226	0.43–0.50
Number of animals	17	23	20	20	26	13	19	20				
Breast fillet weight (g)	444	466	453	502	483	525	464	505	0.111	<0.001	0.776	14.1–18.6
Breast fillet yield (g/100 g)	26.2	23.9	26.5	24.0	26.8	24.4	26.9	24.1	0.657	<0.001	0.905	0.38–0.48
Leg weight (g)	574	691	575	742	601	743	583	725	0.067	<0.001	0.370	12.0–17.0
Leg yield (g/100 g)	33.9	35.9	33.5	35.7	33.3	35.4	33.8	35.3	0.304	<0.001	0.571	0.25–0.34

¹Standard error of the mean of FV x sex, given as a range because of uneven data distribution²Experimental unit: individual animal

between the FV (39, 40, 41 and 41 g, when diets including 0, 75, 100 and 125 g CVS/kg of diet were fed, $p=0.133$). There was a significant effect of sex on live weight, with male birds reaching a 22% higher final live weight (3150 g) than female birds (2578 g). Accordingly, daily weight gain was also higher in male broilers. On average, male and female broilers grew 44 and 36 g per day from hatching to slaughter, respectively ($p < 0.001$).

None of the carcass traits were affected by the FV (Table 4). Again, the effect of sex was significant on all carcass traits except carcass yield, which ranged from 65.5 to 66.7%.

Regarding the welfare indicators, no differences between the FVs were found (Fig. 1). An unimpaired state (score “0”) was recorded for the majority of the broilers, with 84.3, 93.7 and 91.1% scoring “0” for breast blisters ($p=0.106$), hock burns ($p=0.354$) and foot pad lesions ($p=0.224$), respectively. There was a significant effect of sex on all welfare indicators, however, fewer male broilers than female broilers received a score “0” (76.4 vs. 92.8% for breast blisters, 90.0 vs. 97.5% for hock burns and 88.2 vs. 94.4% for foot pad lesions, data not shown).

Discussion

This study was conducted in order to test the effect of different proportions of common vetch seeds (CVS) of cultivar Slovena in 100% organic diets for mixed-sex slow-growing broiler chicken during the grower (day 29–49) and finisher period (day 50–70). The

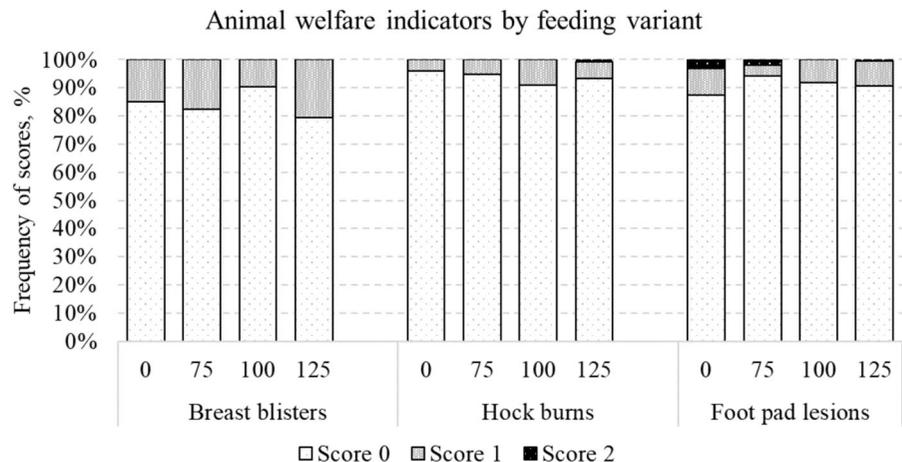
seeds of cultivar Slovena were found to be rich in protein and lysine, and at an inclusion rate of up to 125 g/kg of diet did not affect feed intake, animal performance and welfare.

Nutrient composition of cultivar Slovena

Several authors report differences between common vetch cultivars with regard to their contents of nutrients and ANF (Chowdhury et al. 2001; Larbi et al. 2011; Collins et al. 2002). Still, the literature on CVS as animal feed rarely provides details on the used cultivar. Without naming cultivars, Huang et al. (2017) provide the ranges of the most relevant nutrients and essential amino acids as 206–393 g crude protein, 8.9–38.0 g crude fat, 15.4–20.1 g lysine, 1.4–4.9 g methionine, 0.4–5.1 g cystine, 6.1–12.1 g threonine, and 2.3–3.1 g tryptophan/kg dry matter. Our cultivar Slovena from the harvest of 2016 was in the upper part of the range given by Huang et al. (2017) for crude protein and crude fat (343 and 17.2 g/kg dry matter, respectively) and even exceeded the maximum values reported for lysine and tryptophan (21.5 and 3.1 g/kg dry matter, respectively). Contents of methionine and cysteine in Slovena (2.4 and 3.5 g/kg dry matter, respectively), however, were in the lower part of the range given by Huang et al. (2017), and the content of threonine (11.1 g/kg dry matter) was in the upper part of the reported range.

As part of the research work on common vetch at Thünen Institute, we also analysed seed samples from cultivar Slovena harvested in 2018 and 2019. Interestingly, the harvest from 2016 showed the highest

Fig. 1 Animal welfare indicators in relation to the feeding variants including 0, 75, 100 or 125 g common vetch seeds/kg of diet (0, 75, 100, 125; % of animals with the respective score; score 0 = unimpaired state, score 1 = minor changes, score 2 = major changes)



values of crude protein (343 vs. 330 g/kg dry matter), crude fat (17.2 vs. 13.9 and 14.2 g/kg dry matter), lysine (21.5 vs. 18.3 and 16.5 g/kg dry matter), methionine (2.4 vs. 2.0 and 2.2 g/kg dry matter), and cysteine (3.5 vs. 3.0 and 3.1 g/kg dry matter), while contents of threonine (11.1 vs. 10.4 and 11.4 g/kg dry matter) and tryptophan (3.1 vs. 3.0 and 3.3 g/kg dry matter) varied. Judging from these three vegetation periods, most nutrient contents of the 2016 harvest were above-average.

To summarise, Slovena seems to be a common vetch cultivar richer in crude protein, crude fat, lysine, and tryptophan than other cultivars, but low in sulfur-containing amino acids, which does not make it the ideal cultivar for inclusion in diets for broiler chicken.

Animal performance

The overall level of daily weight gain observed in our study (39–41 g from hatching to slaughter) is in agreement with an earlier feeding trial at our institute, in which we offered CVS in raw or treated form to broiler chickens with similar voluntary intake (38–41 g, Höhne et al. 2022).

In the literature on feeding CVS to broiler chicken, several authors report that a dietary inclusion rate of 100 g untreated CVS/kg of diet did not lead to differences in body weight gain compared to the respective control (Sadeghi et al. 2011; Saki et al. 2008; Darre et al. 1998). Higher inclusion rates of 200 g CVS/kg of diet already reduced daily weight gain significantly in the studies of Sadeghi et al. (2011) and Saki et al. (2008), but not in Farran et al. (2002), who only found a negative effect on growth when including 400 g CVS/kg of diet. All mentioned studies were conducted under floor husbandry conditions and conventional feeding, including the use of synthetic methionine to balance the amino acid profile. Also, the birds had a higher growth potential than in our study, and birds were male except for the study of Sadeghi et al. (2011), which, like our study, used mixed-sex birds. Cultivar info is only provided by Darre et al. (1998), who mention that the used variety was thought to be related to Willamette. In our study, we found no effect of including up to 125 g CVS cultivar Slovena/kg of diet in grower and finisher diets for mixed-sex slow-growing broiler chicken on feed intake, performance, and animal welfare. This finding is in agreement with

the above-mentioned reports, although husbandry conditions and slaughter age differed considerably. The significant effect of sex on various parameters of performance in our study was as expected, and the lack of a significant interaction between dietary treatment and sex indicates that both sexes were equally not affected by including CVS in the diet.

Anti-nutritional factors in CVS and their effect on animal performance

In their early work on the toxicity of CVS for young chicks, Arscott and Harper found 100% mortality after an average survival time of 12.9 (Arscott and Harper 1963) and 13.3 days (Arscott and Harper 1964) when including 300 g CVS/kg of diet. In line with these findings, Farran et al. (2001) showed 100% mortality after 5.1 days when including 600 g untreated CVS/kg in diets for chicks, with the unnamed cultivar containing 3.6 g β -cyanoalanine, 7.91 g vicine, and 1.47 g convicine. In another short feeding trial with inclusion rates of 0, 200, 400, and 600 g untreated CVS/kg (unnamed cultivar containing 5.3 g β -cyanoalanine, 7.31 g vicine, and 0.8 g convicine) in diets fed to male broilers between day 7 and 21, Farran et al. (2002) found that no adverse effects on survival and growth were caused by the 200 g CVS/kg of diet treatment. The contents of vicine (5.69 g/kg dry matter) and convicine (0.75 g/kg dry matter) found in our cultivar Slovena were slightly lower than those reported by Farran et al. (2001, 2002) and also by Pitz et al. (1980, 7.5 g vicine, 0.8 g convicine). The vicine and convicine content in the diet including 125 g CVS/kg of diet was 0.74 and 0.09 in the grower and 0.71 and 0.09 g kg⁻¹ dry matter in the finisher period, respectively. These values are lower than those of the 200 g CVS/kg diet of Farran et al. (2002, 1.29 g vicine and 0.14 g convicine/kg of diet) and also lower than in a diet including 200 g faba beans/kg of diet (0.95 g vicine, 0.43 g convicine/kg of diet) fed to male broilers by Nolte et al. (2020), which in both cases did not affect animal performance. Therefore, our finding that including up to 125 g CVS of cultivar Slovena/kg of diet does not impair animal performance is in line with these reports.

The non-proteinogenic amino acid γ -glutamyl- β -cyanoalanine has been named as the primary source of toxicity to chickens (Ressler et al. 1997). The level of γ -glutamyl- β -cyanoalanine found in cultivar

Slovena (6.9 g/kg dry matter) was between those reported in previous feeding trials with chicks by Darre et al. (1998, 5 g/kg) and Ressler et al. (1997, 7.4 g/kg), both using a variety thought to be *Willamette* or related to it. In a study testing CVS as feed for pigs, Collins et al. (2002) reported γ -glutamyl- β -cyanoalanine levels of 9–12, about 13 and less than 7 g/kg for the cultivars *Blanchefleur*, *Languedoc*, and *Morava*, respectively. Compared with these reports, *Slovena* seems to be a cultivar with a γ -glutamyl- β -cyanoalanine content on the lower end of the range. Chowdhury et al. (2001) included the CVS cultivars *Jericho White* and *Blanchefleur* at different inclusion rates in diets for 3-week-old chicks (sex not specified) and found no negative effect on growth rate when the γ -glutamyl- β -cyanoalanine content in the diet was lower than 2.5 g/kg. The γ -glutamyl- β -cyanoalanine content of the diet including 125 g CVS cultivar *Slovena*/kg of diet in our study was 0.54 in the grower and 0.56 g/kg dry matter in the finisher period, which is considerably lower than the 2.5 g/kg threshold found by Chowdhury et al. (2001), and the lack of treatment effect in our study therefore confirms their findings.

The total content of tannins in our cultivar *Slovena* was 8.1 g/kg dry matter, which is at the upper end of the range given by Aletor et al. (1994) for various *Vicia sativa* lines (0.28–8.27 g tannins/kg of diet, measured as vanillin-HCl catechin equivalents) and higher than the values given by Parissi et al. (2022) for seven *Vicia sativa* cultivars (4.3–5.2 g total hydrolyzed tannins/kg). While a recent meta-analysis of tannins in broiler diets found a negative effect of an increased tannin intake on growth (Hidayat et al. 2021), the tannin content in our diet including 125 g CVS/kg of diet was only 1.9 in the grower and 2.0 g/kg dry matter in the finisher period, which can be considered low and therefore did not affect animal performance.

While it is promising that no adverse effects of including up to 125 g CVS/kg diet were found in our study, recommendations for upper levels of ANF contents are of interest for the future use of CVS. The scarce literature reporting both ANF contents and negative effects on broiler growth can be summarised as follows: Chowdhury et al. (2001) observed depressed growth in broilers at the age of 3 weeks when the γ -glutamyl- β -cyanoalanine content of the diet exceeded 2.5 g/kg. For broilers of the same age

(day 14–21), Farran et al. (2002) reported a negative effect on daily weight gain when the diet contained 1.88 g β -cyanoalanine, 2.58 g vicine and 0.29 g convicine/kg diet. For tannins, Hidayat et al. (2021) report that weight gain of 42-day-old broilers was decreased at levels starting at 2.8 g/kg diet.

Conclusion

The seeds of the common vetch cultivar *Slovena* were found to be rich in crude protein and lysine, with vicine, convicine and γ -glutamyl- β -cyanoalanine levels at the lower end of the range and tannin levels at the higher end of the range reported previously for CVS. Including up to 125 g CVS/kg in the organic diets of mixed-sex slow-growing broiler chickens did not affect feed intake, animal performance, and welfare indicators at all. Based on a comparison of the contents of the above-mentioned ANF in the diet with previous reports, negative effects of including CVS of cultivar *Slovena* in broiler diets could be expected at an inclusion rate of 35%, a level which is not relevant for practical diet formulation anyway. It can therefore be concluded that the use of untreated CVS of cultivar *Slovena* in broiler diets can be recommended, at inclusion rates supporting a balanced diet. Further research should focus on possible differences between cultivars, both regarding their amino acid profile, and differences in ANF contents.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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