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Effect of peas and pea products in diets for broiler chickens with consideration of the intestinal microbiota

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Primary Audience: Nutritionists, Feed Manufacturers, Broiler Producers

SUMMARY

In addition to the whole white-flowered pea, pea protein concentrates and pea hulls can be utilized in animal nutrition. In particular, fermentable carbohydrates and fibers in peas and pea products seem to contribute to intestinal health and health maintenance in poultry, due to their prebiotic effect on the intestinal microbiota. This study was conducted to investigate the effect of different proportions of peas (**P**), pea protein concentrate (**PPC**) and pea hulls (**PH**) in complete feed mixtures for broilers on growth and slaughter performance as well as intestinal microbiota. Twenty diets with varying proportions of peas and pea products were fed to male broilers from d 1 to 34. Short-chain fatty acid analysis and 16S sequencing were used to examine the ileal and cecal microbiota for selected feeding groups. Overall, the attained fattening performances were at a high level. The use of peas and pea products did not affect body weight on d 34 or slaughter performance. The use of pea hulls up to 6% resulted in the highest overall feed intake and overall feed conversion ratio ($P < 0.001$). Microbiota composition and ileal bacterial metabolites were unchanged. Microbiota changes in the cecum were found between dietary treatments for several subdominant microbial genera that preferentially ferment carbohydrates. This study has shown that peas and pea products are well-suited as feedstuffs for feeding broilers when used appropriately. Furthermore, the intestinal microbiota responded with an increased abundance of nonpathogenic genera that may help maintain intestinal microbial homeostasis.

Key words: pea, pea protein concentrate, pea hull, broiler, intestinal microbiota, 16S-sequencing, short-chain fatty acids

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DESCRIPTION OF PROBLEM

The grain pea (*Pisum sativum*) is the most important grain legume in Germany, considering its area under cultivation (Bellof and Freitag, 2021). From a nutritional point of view, the white-flowered varieties are particularly suitable for poultry feeding and, if used appropriately, can contribute to a reduction of soybean imports (Bellof et al., 2020; Bellof and Specht, 2022). Several studies in chicken fattening show that the systematic use of moderate amounts of up to 30% of white-flowered pea in

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poultry diets have no adverse effects on growth and slaughter performance (Bellof and Freitag, 2021). In addition to the whole pea (**P**), pea protein concentrate (**PPC**) and pea hulls (**PH**) can also be utilized in animal nutrition. Nevertheless, there is no published data on specific amounts for PH and PPC in chicken fattening. However, the potential for the use of grain pea as feed depends on the livestock system. In poultry farming, feed-specific restrictions of peas result from comparatively low amounts of essential sulfur-containing amino acids and antinutritional secondary plant constituents. Among the value-determining ingredients of grain pea dietary fiber components, in addition to crude protein, can be considered significant for animal production (Quendt et al., 2022). Dietary fibers and secondary substances in peas and pea products can affect intestinal- and animal health; particularly the prebiotic effect of dietary fiber components on the intestinal microbiota seems to contribute to intestinal health and health maintenance in poultry (Dahl et al., 2012). The gut microbiota plays an important role in improving colonization resistance against for instance *Salmonella Typhimurium* - causing clinical diseases in animals and humans - regulating the immune system and promoting digestion and host metabolism. To improve the intestinal microbiota and microbiota composition, prebiotics in the form of indigestible complex carbohydrates are often supplemented in poultry production (Khan and Chousalkar, 2020). The indigestible carbohydrates, such as resistant starch or nonstarch polysaccharides found in peas and pea products, are broken down into lactate and short-chain fatty acids (**SCFA**) by bacterial fermentation in the hindgut (Pan and Yu, 2014). SCFAs can be used by enterocytes as a key substrate for energy production, inhibit the colonization of pathogens, enhance intestinal immune response, regulate mucin production and relieve intestinal inflammation (Liu et al., 2021). Thus, a healthy intestinal microbiota and gut is considered a net benefit to the chicken and a key contributor to poultry health and increased productivity (Oakley et al., 2014; Liu et al., 2021). In the context of reducing antibiotic use, targeted dietary fiber supplementation may have a beneficial influence on gastrointestinal microbiota and consequently its host (Liu et al., 2021).

The objective of the study was to investigate the influence of peas and in particular the less researched use of PPC and PH, on broiler fattening and slaughter performance. It was hypothesized that feeding peas and pea products might positively influence the intestinal microbiota of poultry. Since the positive effects of using peas in poultry diets are already known, this study is intended to contribute in particular to the question of whether these are attributable to its value-determining constituents (protein, starch, fiber) or whether it is the totality. For this reason, peas as well as PH and PPC will be tested.

MATERIALS AND METHODS

Experimental procedures and animal husbandry were in compliance with the provisions of the German Animal Welfare Act and were reviewed and approved by the Animal Welfare Officer of the Bavarian State Estate Kitzingen.

Animals and Experimental Design

The feeding trial and the carcass value test were conducted at the Experimental and Educational Center for Poultry Husbandry of the Bavarian State Estate Kitzingen (Kitzingen, Germany) from February to March 2022. A total of 800 male day-old Ross 308 broiler chickens were purchased from a local commercial hatchery (Brüterei Süd ZN, BWE-Brüterei Weser-Ems GmbH & Co KG, Regenstauf, Germany). The chicks were raised in a 2-phase system (starter phase P1: d 1−14; fattening phase P2: d 14−34). The day-old chicks were randomly allocated to 1 of 10 dietary treatments with 4 replicates and 20 broilers per replicate so that a similar average bird weight was achieved in every pen. Two out of 4 replicates were arranged in pairs because recording water consumption was possible for adjacent pens. One pen of the control variant was eliminated from the experiment by cause of a high deviation of the standard fattening performance of all individuals compared to the remaining pens. Due to high feed intake, a reduction to

approximately 12 to 14 animals per pen was carried out on d 28 to ensure the availability of sufficient quantities of complete feed until the end of the trial. For this, birds were weighed individually and the lightest and heaviest broilers were carried out to keep the mean of each pen constant.

Experimental Diets and Analyses

The feed composition of the conventional complete feed mixtures used was based on the recommendations of the breeding company for the Ross 308 genotype from Aviagen (2019). The AME_N contents of the feed mixtures were lowered by 2.5% in the starter phase P1 and by 5% in the mast phase P2 compared to these recommendations to reduce the amino acid concentration (reduced by 2.5% in P1 and 5% in P2; constant energy to amino acid ratio) of the mixtures and the use of feed fat. Ten isoenergetic and isonitrogenic diets with different proportions of peas and pea products were formulated per phase (P1; P2) (Tables 1 and 2). For better comparability, the mixing ratios of peas and pea products used were determined so that each experimental diet had comparable pea protein and pea fiber (NDF) contents. Based on the ingredient compositions for P, PPC and PH, the crude protein content of PPC was higher by a factor of 3 compared to P, while the NDF content of PH was higher by approximately a factor of 6 compared to P (PH additionally dependent on broiler age). The basis for the determination was the test rations P 20% and P 30%. To achieve equal proportions of pea protein and pea fiber (ingredient composition of the ration) in the remaining test rations, proportions of 6.5% and 10% were obtained for PPC and 3% to 6% for PH. While the isolated variants were intended to help clarify the influence of peas, pea protein and pea fiber, the combined variants with PPC and PH focused on the effect that can be achieved without the pea starch (especially the resistant starch). The aim of the combined variant with P and PH was to investigate the effects to be expected from additionally increased pea fiber content.

The peas and pea products were from white-flowering varieties (Emsland Group, Emlichheim, Germany). The complete feed mixtures did not contain coccidiostats and were fed to the broilers in pelleted form (3 mm). The feed was produced in the mixing plant of the Bavarian State Research Center for Agriculture (Grub-Poing, Germany). Table 3 and 4 show the concentrations of nutrients in the analyzed diets (starter phase P1 and grower phase P2). The analysis of complete feeds was performed according to VDLUFA (2012) and AME_N contents were estimated according to the formula of WPSA (1984) for compound feeds.

Experimental Management

The animals were housed in 40 floor pens on straw concentrate pellets (5 m^2/pen). Water and feed were available for ad libitum consumption. Fresh water was provided through nipple drinkers. Feeding was carried out from d 1 to 7 via feed plates and from d 7 to 34 with feeders. The temperature gradually decreased from 33˚C at the time of housing to 21˚C on d 34. The light duration was reduced from d 1 (23 h light) to d 4 (18 h light) and was afterward kept constant at 18 h light and 6 h darkness. The broilers were vaccinated against Newcastle Disease and Infectious Bronchitis.

Data Collection (Growth Performance)

Animals were weighed on d 1 and 7 on a pen basis and individual body weights were recorded on d 14, 28, and 34. The total feed intake in each pen was recorded weekly to calculate the average feed intake and feed conversion ratio. The collected data for the performance were corrected using recorded animal losses. The animals that were removed on d 28 due to the reduction were reared externally and classified as disposals, not included in the losses. Two mortality rates are presented separately since the total number of animals changed on d 28: one up to d 28 before reduction and the other from d 28 after reduction. The average feed intake from d 14 to d 28 was calculated based on the number of animals on d 28 before reduction. From d 28 to d 34, the average feed intake was calculated by using the number of animals from d 28 after reduction. The sum of these corrected values represents

Table 1. Composition (in % for 88% DM) and targeted nutrient concentrations (in % and AME_N in MJ/kg for 88% DM) of the complete feed mixtures used in the starter phase P1 (d 1–14).

	Feeding group									
Feed components	1	2	3	4	5	6	7	8	9	10
	Control	P 20	P 30	PPC 6.5	PPC 10	PH 3/4.5	PH 4.5/6	P 20+ PH 3/4.5	PPC 10+ PH 3/4.5	PPC 10+ PH 4.5/6
Soybean meal (HP)	36.0	29.5	26.5	24.0	17.5	36.5	37.5	30.5	18.5	19.0
Pea		20.0	30.0					20.0		
Pea protein concentrate				6.50	10.0				10.0	10.0
Pea hull						3.00	4.50	3.00	3.00	4.50
Maize	31.3	17.3	10.0	38.8	42.8	25.9	22.4	11.3	36.9	34.1
Wheat	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Rapeseed oil	3.20	3.80	4.10	1.10	0.00	5.10	6.20	5.80	1.90	2.80
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Feed lime	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Monocalcium phosphate	1.30	1.30	1.30	1.40	1.50	1.30	1.30	1.30	1.50	1.50
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine HCl	0.27	0.17	0.1	0.22	0.19	0.27	0.24	0.14	0.17	0.16
DL-Methionine	0.36	0.40	0.42	0.40	0.42	0.38	0.38	0.41	0.43	0.43
L-Threonine	0.14	0.17	0.15	0.15	0.16	0.14	0.13	0.15	0.16	0.16
L-Valine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
EcoVit R[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Target content										
AME_N [3]						12.2				
Crude protein						22.4				
Lysine						1.40				
Methionine						0.55				

[1]Contents per kg: 800,000 I.U. vitamin A, 350,000 I.U. vitamin D, 5,000 mg vitamin E, 200 mg vitamin K3, 200 mg vitamin B1, 400 mg vitamin B6, 2,000 mcg vitamin B12, 6,500 mg niacinamide, 1,600 mg calcium D-pantothenate, 200 mg folic acid, 20,000 mcg biotin, 60,000 mg choline chloride, 4,000 mg iron, 600 mg copper, 5,000 mg zinc, 6,000 mg manganese, 100 mg iodine, 20 mg selenium.

[2]EcoVit R: riboflavin-rich straight feed based on *Ashbya gossypii* (Agrano GmbH & Co. KG, 79359 Riegel am Kaiserstuhl, Germany).

[3]The AME_N contents of the feed mixtures were lowered by 2.5% compared to the recommendations from Aviagen (2019) to reduce the amino acid concentration requirements and the use of feed fat (constant energy to amino acid ratio).

the average feed intake in P2. The average feed conversion ratio of P2 was calculated by using the corrected feed intake in P2 and the average body weight on d 34, as the reduction on d 28 had no effect on the average body weight.

On d 36, 3 birds closest to the average pen weight were selected from each pen (3 birds × 4 replications) for determining slaughter performance and for collecting data for the microbiological characteristics of the intestine. These animals were weighed, stunned, slaughtered by cutting the vein and defeathered in the slaughterhouse from the Bavarian State Estate Kitzingen. The weighing of thighs, breast and wings with skin and abdominal fat was done the following day after chilling the bodies.

Microbiological Characteristics of the Intestine (16S-rDNA, Short-Chain Fatty Acids)

To evaluate dietary effects on the intestinal microbiota, the control diet and feeding groups with the highest single amount of pea product (P 30; PPC 10; PH 4.5/6) were chosen to describe the impact of the different pea products without interference. For 16S-rDNA gene sequencing and determination of short-chain fatty acids, digesta from the ileum and cecum of the slaughtered animals were collected during the slaughtering process on d 36 (DNA sequencing: 3 birds per replicate; SCFA determination: 1 bird per replicate). The ileum was divided into equal thirds between Meckel's

Table 2. Composition (in % for 88% DM) and targeted nutrient concentrations (in % and AME_N in MJ/kg for 88% DM) of the complete feed mixtures used in the fattening phase P2 (d 14−34).

Feed components	Feeding group									
	1	2	3	4	5	6	7	8	9	10
	Control	P 20	P 30	PPC 6.5	PPC 10	PH 3/4.5	PH 4.5/6	P 20+ PH 3/4.5	PPC 10+ PH 3/4.5	PPC 10+ PH 4.5/6
Soybean meal (HP)	27.0	21.5	18.5	14.5	7.0	29.0	30.5	24.0	9.0	10.0
Pea		20.0	30.0					20.0		
Pea protein concentrate				6.50	10.0				10.0	10.0
Pea hull						4.50	6.00	4.50	4.50	6.00
Maize	36.1	26.3	24.2	39.1	33.5	31.8	38.1	26.5	29.7	31.5
Wheat	30.0	25.0	20.0	35.0	45.0	25.0	15.0	15.0	40.0	35.0
Rapeseed oil	3.00	3.40	3.50	0.90	0.40	5.80	6.50	6.20	2.90	3.60
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Feed lime	1.00	1.00	1.00	1.10	1.10	0.90	0.90	0.90	1.00	1.00
Monocalcium phosphate	1.00	1.00	1.00	1.00	1.00	1.10	1.10	1.10	1.00	1.00
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine HCl	0.24	0.11	0.06	0.19	0.18	0.20	0.17	0.06	0.14	0.12
DL-Methionine	0.29	0.32	0.35	0.32	0.34	0.30	0.30	0.33	0.35	0.36
L-Threonine	0.11	0.11	0.11	0.12	0.14	0.10	0.08	0.09	0.13	0.13
EcoVit R[2]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Acid mixture[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Target content										
AME_N[4]						12.5				
Crude protein						19.5				
Lysine						1.16				
Methionine						0.47				

[1]Contents per kg: 800,000 I.U. vitamin A, 350,000 I.U. vitamin D, 5,000 mg vitamin E, 200 mg vitamin K3, 200 mg vitamin B1, 400 mg vitamin B6, 2,000 mcg vitamin B12, 6,500 mg niacinamide, 1,600 mg calcium D-pantothenate, 200 mg folic acid, 20,000 mcg biotin, 60,000 mg choline chloride, 4,000 mg iron, 600 mg copper, 5,000 mg zinc, 6,000 mg manganese, 100 mg iodine, 20 mg selenium.

[2]EcoVit R: riboflavin-rich straight feed based on *Ashbya gossypii* (Agrano GmbH & Co. KG, 79359 Riegel am Kaiserstuhl, Germany).

[3]Contents per kg: 376,000 mg formic acid, 108,000 mg propionic acid, 46,000 mg lactic acid, 6,000 mg zitronic acid, 6,000 mg sorbic acid, Carrier: diatomaceous earth, vermiculite; the acid mixture was utilized to enhance the storability of the feed mixtures because of their high fat content and the extended time between production and feeding.

[4]The AME_N contents of the feed mixtures were lowered by 5% compared to the recommendations from Aviagen (2019) to reduce the amino acid concentration requirements and the use of feed fat (constant energy to amino acid ratio).

diverticulum and the ceca-colonic juncture. The middle third was used for DNA extraction. For cecal samples, 1 cecum was separated 1 cm before the confluence with the ileum. The digesta were carefully emptied by hand-stripping into sample containers, immediately placed on dry ice in styrofoam boxes and then frozen at −20˚C.

DNA extraction from the digesta was performed using the QIAamp Power- Fecal Pro DNA kit from Qiagen (Hilden, Germany) according to the protocol for experienced users; extracted DNA was stored short term at −20˚C until further processing. The variable V3 to V4 region of bacterial 16S rDNA was analyzed via sequencing by a commercial laboratory (LGC Genomics GmbH, Berlin, Germany) to characterize the intestinal microbiome. Sequence data were analyzed according to the methods outlined in Grześkowiak et al. (2019). Essentially, amplicon sequence variants of quality controlled, demultiplexed combined-read 16S rDNA sequences were used to analyze the bacterial composition in ileal and cecal samples with the QIIME2 pipeline. Results are given as relative abundance (%) of bacterial 16S-rDNA and as the ecological indices Richness (number of species), Evenness (uniformity of community) and Shannon index (measure of species diversity).

Table 3. Content of nutrients (analyzed; in g for 88% DM) and energy (calculated; AME_N MJ/kg) of the complete feed mixtures used in the starter phase P1 (d 1–14).

	Feeding group[1]									
	1	2	3	4	5	6	7	8	9	10
Item	Control	P 20	P 30	PPC 6.5	PPC 10	PH 3/4.5	PH 4.5/6	P 20+ PH 3/4.5	PPC 10+ PH 3/4.5	PPC 10+ PH 4.5/6
Dry matter	880	880	880	880	880	880	880	880	880	880
Ash	59.7	58.9	59.1	57.6	54.7	58.9	59.8	60.2	56.8	56.8
Crude protein	232	223	221	228	218	226	228	228	234	236
Crude fiber	30.3	31.4	37.4	21.5	25.4	51.0	56.8	54.3	39.2	51.9
$aNDF_{om}$	101	83.4	93.5	93.8	84.0	119	131	123	104	116
ADF_{om}	36.2	35.3	53.2	32.2	28.3	70.6	79.4	57.2	54.8	53.8
Crude fat	57.8	59.8	56.1	46.9	44.9	71.6	78.4	73.0	51.9	57.8
Starch	353	360	360	385	419	325	304	307	369	353
Sugar	48.0	47.1	46.3	42.0	36.1	47.1	47.0	47.4	36.2	36.2
AME_N [2]	12.1	12.2	12.0	12.1	12.4	12.0	11.9	11.8	12.0	11.9
Lysine	13.8	13.7	13.4	14.3	12.8	13.5	13.7	14.1	15.2	14.2
Methionine	6.36	6.47	6.10	6.15	6.64	6.57	6.86	6.51	6.26	6.75
Cysteine	3.62	3.43	3.25	3.42	3.03	3.53	3.43	3.45	3.33	3.33
Met+Cys	10.0	9.91	9.35	9.57	9.67	10.1	10.3	10.0	9.59	10.1
Threonine	9.30	9.12	9.15	9.18	8.69	9.42	9.41	9.67	9.89	9.40
Calcium	10.1	10.1	10.0	9.72	10.6	9.76	10.0	10.2	10.2	10.4
Phosphorus	8.03	7.55	7.53	7.91	8.30	7.75	7.55	7.89	8.61	8.57
Potassium	10.0	10.1	9.8	8.55	7.03	10.1	9.90	10.5	7.54	7.93

[1]P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).

[2]AME_N: calculated according to WPSA (1984).

Table 4. Content of nutrients (analyzed; in g for 88% DM) and energy (calculated; AME_N MJ/kg) of the complete feed mixtures used in the fattening phase P2 (d 14–34).

	Feeding group[1]									
	1	2	3	4	5	6	7	8	9	10
Item	Control	P 20	P 30	PPC 6.5	PPC 10	PH 3/4.5	PH 4.5/6	P 20+ PH 3/4.5	PPC 10+ PH 3/4.5	PPC 10+ PH 4.5/6
Dry matter	880	880	880	880	880	880	880	880	880	880
Ash	56.3	55.4	53.5	50.5	50.5	55.4	56.3	54.5	52.5	53.4
Crude protein	185	185	186	184	184	198	198	191	194	195
Crude fiber	24.3	27.2	29.2	23.3	18.4	53.4	57.2	57.4	46.7	49.5
$aNDF_{om}$	93.2	84.6	81.8	79.6	82.5	126	124	119	108	119
ADF_{om}	39.8	43.8	45.8	33.0	31.0	60.2	75.7	78.8	52.5	65.1
Crude fat	58.3	65.1	64.2	44.6	41.7	79.6	93.1	81.7	64.2	66.0
Starch	417	416	422	463	480	360	342	356	429	406
Sugar	47.6	46.7	46.7	39.8	34.0	47.6	46.6	45.7	29.2	29.1
AME_N [2]	12.4	12.6	12.8	12.6	12.7	12.4	12.6	12.3	12.7	12.4
Lysine	11.0	10.8	10.8	11.1	11.4	11.3	11.4	11.4	11.7	12.1
Methionine	5.15	5.45	5.74	5.43	6.11	5.34	5.34	5.64	5.54	6.12
Cysteine	3.01	2.92	2.82	2.72	2.81	2.91	2.91	2.82	2.72	2.91
Met+Cys	8.16	8.36	8.57	8.15	8.93	8.26	8.25	8.46	8.27	9.03
Threonine	7.58	7.58	7.50	7.47	7.66	7.67	7.66	7.58	7.58	8.06
Calcium	9.23	8.75	7.88	9.12	9.02	8.64	8.25	8.36	8.95	9.62
Phosphorus	6.80	6.71	6.04	6.79	6.79	7.09	6.50	6.71	6.61	6.60
Potassium	8.64	8.46	8.18	6.50	5.24	8.74	8.64	8.95	5.45	5.63

[1]P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).

[2]AME_N: calculated according to WPSA (1984).

SCFA were determined via gas chromatography (Agilent Technologies 6890N with autosampler G2614A and injection tower G2613A). Acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid and n-valeric acid were analyzed and reported in μmol/g and % mol per original substance, respectively. Digesta (300 mg) was mixed with 1 mL capronic acid solution (concentration) as an internal standard. Samples were shaken for 1 h (IKA, D-79219 Staufen, type: Vortex 3) and centrifuged at 21,100 $\times$ *g* for 10 min (Heraeus, D-6450 Hanau, type: Fresco 21). After the addition of oxalic acid (1%), extracts were analyzed by gas chromatography.

Statistical Analyses

Fattening and slaughter data were evaluated by a 1-factor ANOVA (GLM procedure) by using the SAS 9.4 program (SAS Institute Inc., Cary, NC). The pen was considered as the experimental unit for performance data, while individual animals were used to analyze slaughter data.

The following model was used for the investigation.

$$y_i = \mu + FG_i + e_i$$

where y_i = observation value of the nth animal, μ = overall mean, FG_i = fixed effect of feeding group, i = 1 to 10 for the investigation of the fattening and slaughter data; 1 to 4 (Control, P30, PPC10, PH4.5/6) for the investigation of the microbiological characteristics of the intestine, e_i = residual error.

Tukey's multiple comparison test was used to determine significant differences between mean scores. Results are expressed as LS-Means $\pm$ standard errors (**SE**). A P value of $\leq$0.05 was considered significant.

Non-normally distributed data (16S-rDNA, short-chain fatty acids) were analyzed with the Kruskal-Wallis test using SPSS 29.0 (IBM, Chicago, IL) at a significance level of $P \leq 0.05$. Where appropriate, a Mann-Whitney significance test was performed to detect significant differences between experimental groups. Alpha error accumulation in the multiple comparisons was neutralized by Bonferroni correction. A significance level of $P \leq 0.10$ was used for the adjustment of the multiple comparison with Bonferroni. The results are given as the median.

The Spearman correlation was used to establish relationships between ordinal variables by using SPSS 29.0 (IBM, Chicago, IL). The level of significance was set to $P \leq 0.05$. Additionally, dendrograms of correlation analysis of ileal Lactobacillus 16S data (average clustering method) were used to identify possible cluster formation for different fiber sources.

RESULTS AND DISCUSSION

Effect of Peas and Pea Products on Fattening and Slaughter Performance

Total mortality was 4.8% before animal reduction up to d 28 and 1.2% from d 28 to d 34. The average body weight of 43 g on the day of stabling was not different among dietary treatments (P = 0.773). The effects of peas and pea products on fattening performance are shown in Table 5. Feeding groups showed no differences in feed intake in the starter phase P1 ($P > 0.05$). In the fattening phase P2 and overall (P1+P2), animals of feeding groups PH 3/4.5, PH 4.5/6 and P 20+PH 3/4.5 had a higher feed intake than animals from control group, PPC 6.5 and PPC 10 ($P < 0.001$). The present feeding trial consistently showed a higher feed intake compared to the male performance curve according to Aviagen (2021). Differences in water consumption were observed among the feeding groups ($P < 0.001$). Animals in group P 20+PH 3/4.5 had higher water consumption (4.40 kg/animal) than animals in the PPC 10 (3.17 kg/animal) and PPC 10+PH 3/4.5 (3.28 kg/animal) feeding groups, which was related to the potassium content of the feed mixtures (Tables 3 and 4). On d 14, body weight of broilers of feeding groups PPC 6.5, PPC 10+PH 3/4.5 and PPC 10+PH 4.5/6 was higher than that of the birds from group P 30 (P = 0.001). At the end of the feeding trial (d 34) no differences in body weights were detected between the treatment groups ($P > 0.05$). All birds showed a distinctly higher body weight at d 34 compared to the reference data according to Aviagen (2021). In the starter phase P1, the

Table 5. Effect of peas and pea products on fattening performance (feed intake (FI) in g/animal, body weight (BW) in g/animal, feed conversion ratio (FCR) in kg feed/kg body weight gain) of broiler chickens from d 1 to 14 (starter phase P1), d 14 to 34 (mast phase P2) and d 1 to 34 (overall growth phase, P1+P2) (LS means and *standard error*).

		Feeding group[1]											
		1	2	3	4	5	6	7	8	9	10		
Item		Control	P 20	P 30	PPC 6.5	PPC 10	PH 3/4.5	PH 4.5/6	P 20+ PH 3/4.5	PPC 10+ PH 3/4.5	PPC 10+ PH 4.5/6	*F* value	*P* value
FI	P1	578 ± *6.87*	582 ± *5.95*	582 ± *5.95*	597 ± *5.95*	579 ± *5.95*	589 ± *5.95*	574 ± *5.95*	591 ± *5.95*	584 ± *5.95*	595 ± *5.95*	1.57	0.170
	P2	2.928^{d} ± *78.1*	3.222^{abcd} ± *67.6*	3.111^{cd} ± *67.6*	3.056^{d} ± *67.6*	2.977^{d} ± *67.6*	3.492^{a} ± *67.6*	3.445^{ab} ± *67.6*	3.418^{abc} ± *67.6*	3.149^{bcd} ± *67.6*	3.181^{abcd} ± *67.6*	8.01	<0.001
	P1+P2	3.506^{d} ± *77.1*	3.804^{abcd} ± *66.8*	3.693^{cd} ± *66.8*	3.653^{d} ± *66.8*	3.556^{d} ± *66.8*	4.081^{a} ± *66.8*	4.019^{ab} ± *66.8*	4.009^{abc} ± *66.8*	3.733^{bcd} ± *66.8*	3.776^{abcd} ± *66.8*	8.28	<0.001
BW	D 0	42.8 ± *0.05*	42.8 ± *0.04*	42.8 ± *0.04*	42.9 ± *0.04*	42.9 ± *0.04*	42.8 ± *0.04*	42.9 ± *0.04*	42.8 ± *0.04*	42.9 ± *0.04*	42.9 ± *0.04*	0.62	0.773
	D 14	575^{abc} ± *8.19*	575^{abc} ± *7.09*	555^{c} ± *7.09*	598^{ab} ± *7.09*	571^{abc} ± *7.09*	578^{abc} ± *7.09*	564^{bc} ± *7.09*	571^{abc} ± *7.09*	603^{a} ± *7.09*	596^{ab} ± *7.09*	4.75	0.001
	D 34	2.503 ± *76.1*	2.702 ± *65.9*	2.622 ± *65.9*	2.655 ± *65.9*	2.648 ± *65.9*	2.651 ± *65.9*	2.558 ± *65.9*	2.659 ± *65.9*	2.814 ± *65.9*	2.784 ± *65.9*	1.83	0.105
FCR	P1	1.09^{abc} ± *0.012*	1.09^{abc} ± *0.011*	1.14^{a} ± *0.011*	1.08^{bc} ± *0.011*	1.10^{abc} ± *0.011*	1.10^{ab} ± *0.011*	1.10^{ab} ± *0.011*	1.12^{ab} ± *0.011*	1.04^{c} ± *0.011*	1.08^{bc} ± *0.011*	5.44	<0.001
	P2	1.53^{bc} ± *0.034*	1.51^{bc} ± *0.029*	1.50^{bc} ± *0.029*	1.49^{c} ± *0.029*	1.44^{c} ± *0.029*	1.68^{a} ± *0.029*	1.73^{a} ± *0.029*	1.64^{ab} ± *0.029*	1.42^{c} ± *0.029*	1.45^{c} ± *0.029*	13.42	<0.001
	P1+P2	1.43^{bc} ± *0.024*	1.43^{bc} ± *0.021*	1.43^{bc} ± *0.021*	1.40^{c} ± *0.021*	1.37^{c} ± *0.021*	1.56^{a} ± *0.021*	1.60^{a} ± *0.021*	1.53^{ab} ± *0.021*	1.35^{c} ± *0.021*	1.38^{c} ± *0.021*	17.87	<0.001

[1]P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).

[a−e]Means within a row without a common superscript differ significantly, $P \leq 0.05$.

feed conversion ratio (**FCR**) of birds from feeding groups P 30, PH 3/4.5, PH 4.5/6 and P 20 +PH 3/4.5 was higher than that of birds in PPC 10+PH 3/4.5 ($P < 0.001$). FCR in fattening phase P2 and overall was higher in groups PH 3/4.5, PH 4.5/6 and P 20+PH 3/4.5 and lower in the feeding groups PPC 6.5, PPC 10, PPC 10 +PH 3/4.5 and PPC 10+PH 4.5/6 ($P < 0.001$). The effects of peas and pea products on slaughter performance are shown in Table 6. Peas and pea products did not influence breast, thigh, wing and abdominal fat weight ($P > 0.05$).

Peas. The use of white-flowered peas of up to 30% in the complete feed mixtures did not affect fattening and slaughter performance compared to the control group with soybean meal. Many studies confirm these findings (Moschini et al., 2005; Diaz et al., 2006; Laudadio and Tufarelli, 2011; Dotas et al., 2014; Bellof and Freitag, 2021). Further, a positive effect of pea starch on performance, especially on feed efficiency has been described (Herwig et al., 2019; Janocha et al., 2022). These findings cannot be confirmed in the present study. In tendency, the results of the feeding experiment show a decline in growth performance with increasing pea proportions in the complete feed mixtures. It is possible that the starch digestibility of peas used in the present study was reduced as described by Czerwiński et al. (2010) and Herwig et al. (2019). Hence, this could have caused the reduced growth, especially at high levels of pea inclusion (Herwig et al., 2019).

Pea Hulls and Pea + Pea Hulls. The use of pea hulls (PH 3/4.5, PH 4.5/6) and the combination of peas and pea hulls (P 20+PH 3/4.5) led to a higher feed intake, while the body weight and slaughter performance were not affected. In addition, feeding groups PH 3/4.5 and PH 4.5/6 showed the significantly highest FCR in fattening phase P2 and overall. It is known that increasing levels of dietary fiber (particularly neutral detergent fiber) has a negative effect on the digestibility of nutrients and energy and results in a deterioration of the feed conversion ratio (Dahl et al., 2012; Jha and Leterme, 2012; Durst et al., 2021). The analyses of the complete feed mixtures show that the contents of crude fiber and cell wall constituents increased in pea hulls and pea plus pea

Table 6. Effect of peas and pea products on slaughter performance (% of slaughter weight) of broiler chickens on d 36[1] (LS means and *standard error*).

	Feeding group[2]											
	1	2	3	4	5	6	7	8	9	10		
Item	Control	P 20	P 30	PPC 6.5	PPC 10	PH 3/4.5	PH 4.5/6	P 20+ PH 3/4.5	PPC 10+ PH 3/4.5	PPC 10+ PH 4.5/6	*F* value	*P* value
Breast[3]	34.6 ± *0.553*	34.2 ± *0.479*	34.4 ± *0.479*	35.5 ± *0.479*	34.7 ± *0.479*	34.6 ± *0.479*	34.9 ± *0.479*	34.8 ± *0.479*	34.4 ± *0.479*	35.2 ± *0.479*	0.650	0.754
Thigh[3]	28.0 ± *0.340*	28.3 ± *0.295*	28.1 ± *0.295*	27.4 ± *0.295*	27.7 ± *0.295*	27.7 ± *0.295*	27.2 ± *0.295*	27.7 ± *0.295*	28.0 ± *0.295*	27.6 ± *0.295*	1.26	0.267
Wings[3]	*9.90 ± 0.231*	*9.83 ± 0.200*	*10.3 ± 0.200*	*10.0 ± 0.200*	*10.1 ± 0.200*	*10.2 ± 0.200*	*10.5 ± 0.200*	*10.1 ± 0.200*	*10.1 ± 0.200*	*9.96 ± 0.200*	0.830	0.589
Abdominal Fat	1.00 ± *0.091*	1.10 ± *0.080*	1.03 ± *0.080*	1.10 ± *0.080*	1.10 ± *0.080*	0.81 ± *0.080*	0.90 ± *0.080*	0.93 ± *0.080*	1.00 ± *0.080*	1.00 ± *0.080*	1.41	0.191

[1] $n = 12$ per dietary treatment (control group $n = 9$).
[2] P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).
[3] Breast, thigh, and wings with skin.

hull-containing diets without resulting in nutrient dilution due to targeted calculation. It is possible that the high concentrations of crude fiber and cell wall components in the complete feed mixtures negatively affected nutrient digestibility. Due to the high FCR and comparatively numeric low abdominal fat content of pea hull-treated birds, a negative influence on nutrient digestibility cannot be excluded. Possibly, the reduced energetic utilization led to a compensation mechanism that resulted in an increased feed intake (Bellof et al., 2005).

Pea Protein Concentrate and Pea Protein Concentrate + Pea Hulls. The present study shows that the use of pea protein concentrate leads to an overall increase in the performance of broiler chickens. It should be emphasized that this body weight was numerically exceeded by the combination of pea protein concentrate and pea hulls (PPC 10+PH 3/4.5, PPC 10+PH 4.5/6). Based on the increased fattening performance, the results of the conducted feeding trial indicate a high protein quality and high amino acid digestibility of the pea protein concentrate used, which according to Bellof and Freitag (2021) are substantial for the protein supply of poultry.

Feeding the high-quality PPC, especially in combination with low amounts of PH (as previously described high amounts of PH possibly leads to a reduction in performance) resulting in an increased performance of broilers. The realized high performance can possibly explained by the combination of PPC and PH. Hetland et al. (2004) described that feed conversation ratio in poultry can be improved by structural components. In their study, an increase in starch digestibility of wheat components was noted by adding 10% fine cellulose powder to the feed. In addition, Hetland et al. (2004) describe that insoluble fibers decrease the passage rate in the gizzard. The extended residence time of the chymus in the gastrointestinal tract results in a longer exposure time of endogenous enzymes, thus improving the digestibility of nutrients (Velayudhan et al., 2019). In summary, the results indicate a high amino acid digestibility of the PPC. In addition, the structural components may lead to an improvement in the starch digestibility of wheat components. The effect of improved nutrient digestibility appears to be enhanced with moderate amounts of PH.

Use of Peas and Pea Products in Complete Feed Mixtures for Broilers

The results of the analysis and the composition of the complete feed mixtures show that peas and pea protein concentrate are well-suited as protein-providing feedstuffs for feeding broilers. The use of peas and in particular pea protein concentrate can reduce the proportion of soybean meal in the diet without having a negative impact on broiler performance and health. This can reduce the import of soy products, which is critically viewed according to Bellof et al. (2020). The feed-specific restrictions described by Quendt et al. (2022) due to the comparatively low content of essential, sulfur-containing amino acids of the pea in the broiler feed could be compensated in the present feeding trial by the use of free amino acids (DL-methionine). The feeding trial confirms the statement of Dadalt et al. (2016) that the biological value of pea protein can be adapted to the needs of monogastric animals by adding free amino acids. Due to their medium crude protein content, peas cannot be used as a single dietary protein source, but their high starch content also reduces the proportion of energy-containing feedstuffs in the complete feed mixtures such as corn and wheat. In a targeted combination, peas can also reduce the use of monoculture cereals in the rations. It not only provides an advantage for GMO-free feeding as suggested by Quendt et al. (2022) but also agronomic and managerial advantages (Bellof et al., 2020; Quendt et al., 2022). The use of pea hulls in the complete feed mixtures leads to increasing contents of crude fiber and cell wall components ($aNDF_{om}$, ADF_{om}). The finding of Weber et al. (2021) that pea hulls are well suited as a fiber-dense feed for fattening pigs can be confirmed for broiler feeding as long as pea hulls are used appropriately.

Effect of Peas and Pea Products on the Intestinal Microbiota

Ileal Microbiota. Ileal bacterial diversity was not significantly different between feeding

Table 7. Effect of pea, pea protein concentrate and pea hulls on ileal bacterial diversity and genera abundance in broiler chickens on d 36[1] (%) (median).

	Feeding group[2]				
Item	Control	P 30	PPC 10	PH 4.5/6	*P* value
Ecological indices					
Richness	18.5	21.5	18.0	18.0	0.299
Shannon index	1.73	1.69	1.70	2.06	0.662
Evenness	0.541	0.540	0.579	0.664	0.244
Genus					
Lactobacillus	99.6	99.2	99.8	99.9	0.731
Romboutsia	10.1	1.16	2.11	0.211	0.350
Unknown (family Lachnospiraceae)	0.292	0.102	0.654	0.024	0.587
Blautia	0.333	0.050	0.050	n.d.	0.390
Helicobacter	0.481	0.069	0.057	0.042	0.269

[1]$n = 12$ per dietary treatment.

[2]P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).

groups (Table 7). The ileal intestinal microbiota was dominated by lactobacilli with over 99% abundance in all feeding groups. Feeding peas and pea products containing diets increased lactobacilli dominance even further leading to the numerical displacement of the second dominant genus *Romboutsia* ($P > 0.05$). The same displacement effect was observed for subdominant genera like *Blautia* or an unknown *Lachnospiraceae* genus. The abundance of *Helicobacter* was numerically distinctly higher in the control than in the peas and pea products groups.

In an attempt to characterize the most dominant lactobacilli further, all *Lactobacillus* sequences were cropped on the amplicon sequence level and putative species names were assigned to high-quality sequences where possible. A total of 56 distinct *Lactobacillus* sequences were found in ileal samples. *Lactobacillus* diversity did not change significantly (Table 8), but numeric increases in Richness, Shannon index and Evenness were noted for peas and pea products containing diets. *L. aviarius* was the most dominant species in all feeding groups, however, the response of the dominant ileal lactobacilli differed according to diet displacement effects were recorded for a range of lactobacilli. Thus, *L. kitasatonis* numerically increased its abundance in PPC and PH treatment groups, while the unknown *Lactobacillus* 42 showed a numerically increase in abundance only in the PH group. The nearest neighbor dendrogram of ileal lactobacilli abundance shows that PH had the most influence on lactobacilli composition, while P and PPC were less influential (Figure 1). Furthermore, a Spearman correlation analysis of performance data with ileal 16S data of lactobacilli sequences resulted in significantly positive correlations for certain ileal subdominant lactobacilli and feed conversion ratio (data not shown) ($P \leq 0.05$).

Although significant results were not obtained in this study, the use of peas and pea products tended to modify the *Lactobacillus* composition in the ileum. PH were especially influential on ileal lactobacilli diversity as judged by dendrogram analysis. Sequence analysis generally showed an enhanced Richness, Shannon index and Evenness for ileal lactobacilli compared to the control diet for peas and pea products, where again PH displayed the highest changes. Derived from these results, it can be speculated that feeding peas and pea products enhance small intestinal lactobacilli that favor complex carbohydrates over simpler substrates such as starch. Due to the complex nature of the fiber substrates, it is only natural that a larger diversity of intestinal lactobacilli develops which in turn may act more resilient against pathogenic bacteria. Contrary to mammals, the small intestine is not as important for poultry health in terms of microbial infections, since the fast digesta transit times act as a defense system against intestinal pathogens (Patterson and Burkholder, 2003). However, resorption processes may be affected. Although a causal relationship could not be established with the correlation data at hand, positive

Table 8. Effect of pea, pea protein concentrate and pea hulls on ileal *Lactobacillus* diversity and *Lactobacillus* core abundance (>1%) in broiler chickens on d 36[1] (median).

	Feeding group[2]				
Item	Control	P 30	PPC 10	PH 4.5/6	*P* value
Ecological indices					
Richness	10.5	18.0	14.5	13.5	0.154
Shannon index	0.92	1.51	1.57	1.71	0.149
Evenness	0.430	0.621	0.622	0.669	0.161
Species assignment					
Lactobacillus aviarius	32.0	34.3	25.4	19.1	0.233
Lactobacillus kitasatonis	6.34	11.2	18.3	14.9	0.709
Lactobacillus pontis	4.76	2.90	10.27	0.63	0.261
Unknown Lactobacillus 55	5.47	7.86	9.34	12.0	0.664
Unknown Lactobacillus 42	2.00	1.06	0.72	23.2	0.063
Unknown Lactobacillus 32	1.35	5.52	4.79	3.14	0.688
Unknown Lactobacillus 40	4.93[a]	1.04[b]	n.d.	0.307[b]	0.041
Unknown Lactobacillus 35	3.62	2.79	2.10	1.35	0.183
Lactobacillus vaginalis	1.62	1.09	1.60	2.64	0.098
Unknown Lactobacillus 34	0.942	1.01	1.52	2.03	0.759
Unknown Lactobacillus 13	2.16	2.31	1.44	2.48	0.502
Unknown Lactobacillus 18	4.04	0.876	1.88	1.17	0.210
Unknown Lactobacillus 10	2.32	3.24	3.31	2.30	0.348
Lactobacillus agilis	0.351	n.d.	n.d.	n.d.	n.e.
Lactobacillus reuteri	0.771	2.90	1.32	0.982	0.422
Lactobacillus oris	0.229	0.319	0.79	1.25	0.652
Unknown Lactobacillus 11	1.68	1.62	4.30	1.42	0.147
Unknown Lactobacillus 24	0.775	0.427	0.99	2.87	0.093
Unknown Lactobacillus 59	3.11	0.222	6.81	1.07	0.372
Unknown Lactobacillus 35	3.62	2.79	2.10	1.35	0.183
Unknown Lactobacillus 49	n.d.	0.212	9.98	0.533	0.895
Unknown Lactobacillus 31	0.932	0.394	0.846	3.76	0.054

[1]n = 12 per dietary treatment.

[2]P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).n.d. not detected, n.e. no evaluation possible.

[a,b]Means within a row without a common superscript differ significantly, $P \leq 0.05$.

correlations of certain lactobacilli to feed conversion may indicate an indirect positive influence on nutrient resorption and digestion. Other studies have shown that dietary supplementation of probiotics with *Lactobacillus* leads to improved body weight gain and FCR in broilers (Jin et al., 1998; Panda et al., 2005; Peng et al., 2016). Thus, similar effects may have been an effect in this study.

Cecal Microbiota. Cecal bacterial diversity was not significantly different between feeding groups, but PPC displayed the numerically

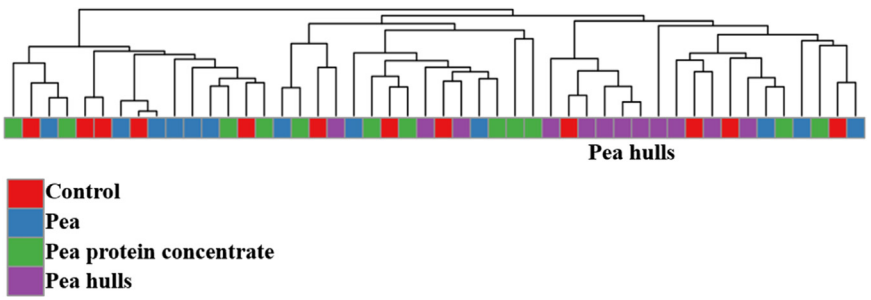

Figure 1. Nearest neighbor dendrogram of ileal lactobacilli abundance between control, pea, pea protein concentrate and pea hulls.

Table 9. Effect of pea, pea protein concentrate and pea hulls on cecal bacterial diversity and genera abundance in broiler chickens on d 36[1] (%) (median).

	Feeding group[2]				
Item	Control	P 30	PPC 10	PH 4.5/6	*P* value
Ecological indices					
Richness	177	178	183	190	0.699
Shannon index	4.19	4.33	4.24	4.48	0.182
Evenness	0.799	0.837	0.824	0.854	0.135
Genus					
Unknown (family Lachnospiraceae)	19.5	17.1	20.3	16.7	0.418
Faecalibacterium	16.2	14.4	13.3	10.5	0.311
Lactobacillus	8.44	10.4	11.5	8.76	0.828
Blautia	6.05	4.59	6.28	7.62	0.093
Ruminococcaceae_UCG-014	4.10	6.52	4.37	5.64	0.306
Fusicatenibacter	2.65	2.04	3.55	2.43	0.274
Subdoligranulum	2.68^{b}	2.42^{b}	5.90^{a}	4.28^{a}	0.042
Ruminococcaceae_UCG-005	1.94^{b}	3.88^{ab}	2.42^{b}	5.53^{a}	0.041
Campylobacter	0.226^{ab}	0.201^{b}	0.650^{a}	0.128^{b}	0.046
Shuttleworthia	0.516^{b}	0.350^{b}	0.254^{b}	0.665^{a}	0.005
Ruminococcaceae_UCG-004	0.175^{b}	0.172^{b}	0.173^{b}	0.289^{a}	0.007
Lachnospiraceae_UCG-010	0.092^{bc}	0.164^{a}	0.060^{c}	0.125^{ab}	0.014
Defluviitaleaceae_UCG-011	0.124^{ab}	0.130^{b}	0.140^{b}	0.255^{a}	0.016
Unknown (family Erysipelotrichaceae)	0.407^{ab}	0.171^{b}	0.124^{b}	0.370^{a}	0.039
Unknown (family Christensenellaceae)	0.058^{b}	0.157^{a}	0.103^{a}	0.049^{b}	0.040
Tyzzerella	0.332^{ab}	0.670^{a}	0.189^{b}	0.507^{a}	0.041
Erysipelatoclostridium	0.692	0.319	0.355	0.209	0.112

$^{a-c}$Means within a row without a common superscript differ significantly, $P \leq 0.05$; Adjustment for multiple comparison: Bonferroni, $P \leq 0.10$.

[1]$n = 12$ per dietary treatment.

[2]P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).

highest richness and Shannon index (Table 9). The effect of pea-containing diets on the cecal microbiota was limited to changes in mostly subdominant genera (<1%) with the exceptions of the genus *Subdoligranulum* and the *Ruminococcaceae* UCG-005. The cecal microbiota was dominated by an unknown genus of the family *Lachnospiraceae, Faecalibacterium*, and *Lactobacillus*, followed by *Blautia, Ruminococcaceae*_UCG-014, *Fusicatenibacter*, and *Subdoligranulum*. Dietary effects for *Subdoligranulum* were limited to diets with PPC or PH, but not peas. Conversely, *Ruminococcaceae* UCG-005 abundance was only enhanced in the P and PH group. Interestingly, P or PH reduced *Campylobacter* abundance, but not PPC. However, the pea products did not exhibit a significant difference in comparison to the control group. In addition, peas and pea products strongly reduced *Erysipelatoclostridium* abundance in some broilers, but no significant effects were observed.

Peas and pea products did not modify the abundance of the dominant cecal microbiota, but several subdominant genera were affected. Furthermore, differences in the relative abundances of *Campylobacter* were also evident for P and PH, while the gastric pathogen *Helicobacter* in the ileum and *Erysipelatoclostridium* in the cecum showed lower abundances in diets containing peas and pea products. The mentioned genera are either pathogenic or can become opportunistic pathogens as noted for *Erysipelatoclostridium* (Khan and Chousalkar, 2020). The increase of pathogenic genera is the result of dysbiotic microbiota and dysbiosis can trigger diseases (Stecher, 2015). The results of

Table 10. Effect of pea, pea protein concentrate, and pea hulls on short-chain fatty acids, branched-chain fatty acids (BCFA), and total fatty acids (μmol/g and % mol of original substance) in the digesta of the ileum and cecum of broiler chickens on d 36[1] (median).

	Ileum					Cecum				
	Feeding group[2]					Feeding group[2]				
Item	Control	P 30	PPC 10	PH 4.5/6	*P* value	Control	P 30	PPC 10	PH 4.5/6	*P* value
μmol/g										
Acetic acid	1.26	0.976	2.17	1.96	0.529	56.1	70.3	73.4	57.4	0.906
Propionic acid	0.032	0.023	0.040	0.016	0.239	4.28	11.64	9.67	7.69	0.785
iso-Butyric acid	0.008	0.015	0.000	0.007	0.733	1.44	1.60	1.21	1.10	0.587
n-Butyric acid	0.019	0.000	0.020	0.026	0.447	13.2	23.3	26.2	22.2	0.579
iso-Valeric acid	0.131	0.039	0.133	0.077	0.116	1.61	1.65	1.15	1.32	0.288
n-Valeric acid	0.004	0.004	0.000	0.004	0.699	1.54	1.88	1.86	1.49	0.723
BCFA	0.139	0.056	0.146	0.105	0.396	3.03	3.26	2.36	2.51	0.702
Total fatty acids	1.42	1.07	2.38	2.19	0.392	84.6	114.0	115.4	86.5	0.617
% mol										
Acetate	86.3	90.5	91.5	89.4	0.839	72.0	64.8	65.2	67.1	0.734
Propionate	2.74	2.61	1.57	0.745	0.196	5.92	10.16	9.55	8.97	0.885
iso-Butyrate	0.410	1.54	0.000	0.215	0.543	1.87	1.51	1.31	1.03	0.752
n-Butyrate	1.24	0.000	0.838	0.941	0.259	15.7	20.2	22.6	20.4	0.582
iso-Valeriate	8.92	0.984	6.27	2.25	0.774	1.96	1.47	1.05	1.31	0.310
n-Valeriate	0.205	0.526	0.000	0.107	0.488	1.93	1.53	1.60	1.74	0.723
BCFA	9.96	2.52	6.27	4.32	0.736	3.83	2.98	2.54	2.34	0.533

[1]$n = 4$ per dietary treatment.

[2]P (pea), PPC (pea protein concentrate), PH (pea hull) with indication of the amount used in the complete feed mixtures (in %, based on the original substance).

this study support the statement by Khan and Chousalkar (2020) that the use of prebiotic supplementation, here in the form of pea dietary fibers, adapts the intestinal microbiota toward a more diverse carbohydrate fermentation. This is also indicated by cecal SCFA concentrations, especially the increase in n-butyrate.

Short-Chain Fatty Acids. Ileal short-chain fatty acid analysis showed no significant differences between feeding groups (Table 10). The inclusion of peas and pea products increased SCFA in the cecum numerically (Table 10), but significant changes were not found. Noteworthy was the increased propionate concentration in the pea diet as well as increased n-butyrate concentrations in all pea product diets.

The results for the influence of peas on the SCFA concentration in the ileum are similar to the study by Czerwiński et al. (2010). In contrast, an influence of peas and their amount of starch could be found on cecum contents by Czerwiński et al. (2010) and Herwig et al. (2020). There is still a need for research into the use of PPC and PH. Numerically, the conducted study showed a higher total fatty acid content in the cecum and ileum and higher n-butyrate content in the cecum in the peas and pea products treated groups as well as an increased cecal propionate with diets containing peas. Many studies have shown that SCFAs, which are produced by bacterial fermentation of dietary fiber components, play a major role in the modulation of intestinal health in poultry (Pan and Yu, 2014; Liu et al., 2021). According to Liu et al. (2021), SCFA may promote the proliferation of beneficial bacteria such as bifidobacteria in the hindgut and lactobacilli in the small intestine, while n-butyrate is known to be used by intestinal epithelial cells with energy-sparing and stabilizing effects on intestinal tissues (Hamer et al., 2008).

Overall, the results of the present study indicate that peas and pea products might have positive effects on the intestinal microbiota in broiler chickens. To confirm this, further studies should be carried out.

CONCLUSIONS AND APPLICATIONS

1. Adequate dietary use of P and PPC led to a considerable reduction in the proportion of

required soybean meal; P also reduced the proportion of energy-supply feed in complete feed mixtures without compromising the performance of the broilers. The use of PH increased the crude fiber and cell wall components in complete feed mixtures.

2. The use of P in proportions up to 30% had no negative effects on broiler performance. The effect of feeding PH up to 6% and the combination of P with PH led to an overall high feed intake, while the use of PH up to 6% also resulted in the overall highest FCR. Diets containing PPC up to 10% and the combination of PPC with PH led to an overall increase in broiler performance.
3. Diets containing peas and pea products had no negative effect on the slaughter performance of broilers.
4. Peas and pea-containing diets had no effect on short-chain fatty acids concentrations in the digesta of the ileum and cecum and the relative abundances of the ileal microbiome. The effect on the cecal microbiota was limited to changes in mostly subdominant genera. Pea products tended to reduce the abundance of some pathogenic bacteria and conversely, increased the abundance of commensal bacterial genera.

DISCLOSURES

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gerhard Bellof reports a relationship with Union for the Promotion of Oil and Protein Crops that includes: nonfinancial support.

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