Use of semi-leafless peas (*Pisum sativum* L) with enzyme addition in diets for broilers

Erja Koivunen[1,6], Eija Talvio[2], Eija Valkonen[3], Tuomo Tupasela[4], Petra Tuunainen[1], Jarmo Valaja[5]

[1]Natural Resources Institute Finland (Luke), Green Technology, Pig, poultry and fur animal production, 31600 Jokioinen, Finland

[2]HKScan Finland Oy, P.O. Box 50, 20521 Turku, Finland

[3]Hankkija Oy, P.O. Box 390, 05801 Hyvinkää, Finland

[4]Natural Resources Institute Finland (Luke), New Business Opportunities, New Products, Services and Technologies, 31600 Jokioinen, Finland

[5]Department of Agricultural Sciences, P.O. Box 28, University of Helsinki, 00014, Finland

[6]Current address: Unibio A/S, Billedskærervej 8, 1, 5230 Odense M, Denmark

email: ek@unibiogroup.com

The aim was to study the effects of dietary pea inclusion and the addition of Avizyme 1200 -enzyme cocktail on broiler performance, intestinal viscosity and organoleptic quality of meat. The experimental design was a 4 × 2 factorial, the factors being dietary pea inclusion (0, 150, 300 and 450 g kg^{-1}) in the diets fed from day 9 to day 38 and the addition of Avizyme 1200 enzyme cocktail including amylase, protease and xylanase during the entire experiment. The growth of birds improved ($p \leq 0.018$) with pea inclusion in a cubic manner. Feed conversion ratio (FCR) of the birds fed on unsupplemented diets improved along pea inclusion, while pea inclusion impaired the FCR on the birds on enzyme cocktail supplemented diets ($p \leq 0.006$). The use of the enzyme cocktail improved the body weight, body weight gain and FCR of the broilers and decreased intestinal viscosity ($p < 0.001$). The organoleptic quality of meat was similar among treatments ($p > 0.05$). In conclusion, 450 g kg^{-1} peas can be used in the broiler grower diets without negative effects on the bird performance. The use of enzyme cocktail improves bird performance.

Key words: amylase, broiler, pea, protease, wheat, xylanase

Introduction

Imported soybean meal (SBM) is the main protein source used in poultry feeds in Europe. However, there is a strong interest in maximizing the use of locally produced vegetable protein sources, such as peas (*Pisum sativum* L.) as a substitute for imported SBM. Peas and other domestic legumes offer the possibility to improve self-sufficiency in protein-rich feedstuffs (Gatel 1994). They have an important role in the crop rotation particularly in organic farming due to their ability to fix nitrogen (Stoddard et al. 2009).

Comparing to SBM protein pea protein is rich in lysine, equal in threonine, and deficient in methionine, cysteine and tryptophan (Gatel 1994). A compared to cereals, they are good source of lysine but they contain low levels of methionine, cysteine and cereal and peas complement each other well (Gatel 1994). High level of unprocessed peas of 300 – 480 g kg^{-1} in a broiler diet has been demonstrated to support good production (Farrell et al. 1999, Laudadio and Tufarelli 2010, Dotas et al. 2014).

Pea supply both energy and protein in poultry diets (Rodrigues et al. 2012) and therefore replaces both cereal grains and SBM in the diets. Cereal grains contain non-starch polysaccharides, which produce viscous solutions, thereby increasing digesta viscosity, reducing nutrient utilization, and causing sticky dropping (Annison 1993), whereas peas depending on variety may contain anti-nutritional factors (ANF). The most harmful ANFs are trypsin- and protease inhibitors and tannins that cause adverse effects on protein digestion (Gatel 1994). Different combinations of hemicellulase, cellulose, xylanase (XLS), amylase (AMS), pectinase, β-glucanase, endoglucanase and protease (PRT) have been studied to improve the nutritive quality of cereal-pea diets (Brenes et al. 1993, Igbasan and Guenter 1996, Cowieson et al. 2003). However, there is a lack of research on the benefits of the addition of the combination XLS, AMS and PRT to wheat-pea based diets. To our knowledge, there is no previous study, where the effect of pea inclusion on organoleptic quality of the breast meat has been studied.

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The aim of this study was to study the effects of dietary pea inclusion and the supplementation of combination of XLS, AMS and PRT enzymes in wheat-pea based diets on broiler performance, intestinal viscosity and organoleptic quality of the breast meat. A further aim was to find an optimal inclusion level of white-flowered semi-leafless green smooth spring peas (cv. Karita) in diets for broilers.

Materials and methods

Birds and housing

A total of 2880 1-day-old Ross 508 broiler chickens were obtained from a hatchery and reared in 48 floor pens (2m × 2m) with peat litter and with 60 broilers in each. Birds were sexed and equal number of females and males were reared in each pen. Temperature, light, relative humidity and ventilation were controlled according to Ross Broiler Management Handbook (Aviagen 2014). During the first 2 days of the experiment, the temperature was 33°C, and it was lowered to 31 °C at the end of the first week. At the beginning of the second week, the temperature was reduced by 3 °C per week until 5 weeks of age, when it was 19 °C. Light was provided in 20-hour light (5 to 10 lux) and 4-hour dark cycles. The study was approved by the Local Ethical Committee for Animal experiments.

Experimental design and diets

The experimental design was a 4 × 2 factorial, the factors being dietary pea inclusion and the combination of XLS, AMS and PRT supplementation. The eight dietary treatments were fed to 6 replicates, with one pen per replicate. Dietary treatments were randomly allocated to the pens. A cereal-SBM based starter diets without peas were offered from day 1 to day 9. (Table 1). The unprocessed pea seeds were included at 0, 150, 300 and 450 g kg^{-1} of the grower diet from day 9 to day 38. A pea cultivar used was white-flowered semi-leafless green smooth spring pea seeds cv. Karita (Lantmännen SW 1995). The exogenous enzyme combination used in the diets (1 g kg^{-1} % in the diet) was Avizyme 1200 -enzyme (Danisco Animal Nutrition, Marlborough, UK) which contains XLS, AMS and PRT.

Table 1. Composition (g kg^{-1}) of experimental diets

	Starter diet, from d 1 to 9	Grower diets, from d 9 to 38 from d 9 to 38			
Pea inclusion, g kg^{-1}	0	0	150	300	450
Avizyme 1200 0,1%	–/+	–/+	–/+	–/+	–/+
Wheat	555	651	539	428	317
Oat	100	100	100	100	100
Soybean meal	272	200	157	113	700
Pea	–	–	150	300	450
Rapeseed oil	25.1	09.0	13.5	18.0	22.4
Monocalcium phosphate	19.5	16.0	16.0	15.9	15.8
Limestone	13.2	11.6	11.8	11.9	12.0
Salt	4.0	3.7	3.7	3.7	3.7
Mineral premix[1]	2.0	2.0	2.0	2.0	2.0
Vitamin premix[2]	2.0	2.0	2.0	2.0	2.0
DL-Methionine	1.6	1.1	1.4	1.6	1.9
L-Lysine	4.3	3.4	3.0	2.5	2.1
L-Threonine	1.1	0.6	0.7	0.8	0.9
L-Tryptophan	–	–	–	0.1	0.3

[1] = Provided per kilogram of the complete diet: Ca 0.63 g, iron 29.1 mg, copper 8.0 mg, manganese 50.3 mg, zinc 65.1 mg, iodine 0.51 mg, selenium 0.20 mg.

The feed ingredients were analyzed for chemical composition before the preparation of the experimental diets. The crude protein content of the grower diets was formulated to decrease with dietary pea inclusion, but the diets were formulated to be equal in the terms of energy (MJ kg^{-1}), methionine, lysine, threonine, tryptophan, calcium and available phosphorus (per MJ of AME). The diets were formulated on a total amino acid basis to meet the amino acid requirements of Ross broilers (Aviagen 2014). The energy value calculations (MJ per kg AME) were

based on the chemical analyses of the feed ingredients. Before feed mixing, the feed ingredients were ground in a roller mill (Gehl Company, West Bend, Wisconsis, USA). The mixed feeds were cold-pelleted (Amandus Kahl Laborpresse 1175, Germany). The birds were given the starter diets as 3mm pellets and then the grower diets as 4mm pellets. For 5 days before the birds were slaughtered, they received grower diets without coccidiostat. Feed and water were offered ad libitum.

Experimental and analytical procedures

Birds per replicate were group weighed (per replicate) at the beginning of the trial, at 9, 20, and 37 d of age. The feed intake was recorded throughout the trial, and the birds were fasted for 12 hours before slaughter at 38 d of age. Mortality and culling was recorded daily. The carcass weight of each pen was measured at a commercial slaughterhouse.

Samples were taken from the litter of each pen at the end of the experiment and analyzed for dry matter content. A rooster from each pen was selected at 20 and 30 d of age, one for ileal viscosity determination. Sampled birds were euthanized by cervical dislocation. The ileum was dissected from Meckel's diverticulum to the ileocaeco-colonic junction and digesta were quantitatively collected from the distal ileum (the latter half of ileum) for viscosity determination. Ileal digesta samples were centrifuged (12,000 x g, 3 min) and the viscosity was measured using a Brookfield DV-II+ Cone and Plate Programmable Viscometer (Brookfield Engineering Laboratories Inc., Middleboro, USA). The cone used was CPE-40.

One rooster from each pen was euthanized at 37 d of age to determine the weight and proportion of the breast meat and abdominal fat and for organoleptic evaluation of breast meat. Breast muscles and abdominal fat were removed and weighed. One breast from every sample broiler per pen was immediately frozen to –20 °C prior to evaluating the organoleptic quality of the meat. The organoleptic quality evaluation of the breast meat samples was performed using a seven point scale form, according to the instructions by the Natural Resources Institute Finland (Luke 2014). An expert panel of five people evaluated the flavour (1 dislike very much to like very much 7), tenderness (1 tough to tender 7) and juiciness (1 dry to juicy 7) of meat samples immediately after frying. The mean values of grades for each sample breast meat given by panelists were used in statistical analysis. Panelists were trained with pre-samples before tasting began. The sensory properties and scores of the pre-samples were discussed and agreed upon. Five samples per session were evaluated.

Feed samples were taken from every batch made and then pooled. The pooled samples were passed through a hammer mill fitted with a 1-mm mesh prior to analysis. Crude fat and ash contents were determined by standard methods (AOAC 1990, methods 942.05 and 920.39). Crude fiber content was determined with a modified method (AOAC 1990 method 962.09) using glass wool instead of a ceramic filters. The nitrogen content was analyzed using a Leco FP 428 nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA). The crude protein content was calculated by multiplying the nitrogen content by 6.25. The amino acid content (excluding tryptophan, which was not determined) was determined according to official EU procedures (EC 1998) using a Waters Finland MassTrak UPLC (Waters Corporation, Milford, MA, USA) with a application of UPLC Amino Acid Analysis Solution ®.

Statistical analyses

The data of production parameters, the weight and the proportion of breast and abdominal fat, ileal viscosity and litter dry matter was subjected to ANOVA using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). The following model was used: $Y_{ij} = \mu + t_i + \varepsilon_{ik}$, where Y_{ij} = observation, μ = the general mean, t_i = the effect of the treatment (i = 1, …,8), and ε_{ijk} = the experimental error term. The treatment effects were separated into seven orthogonal contrasts as follows: the linear, quadratic and cubic effect of dietary pea inclusion (P_L, P_Q, P_C), the effect of the supplementation of XLS, AMS and PRT (E) (unsupplemented diets vs. XLS, AMS and PRT supplemented diets), and the interactions of pea inclusion and the supplementation of XLS, AMS and PRT ($P_L \times E$, $P_Q \times E$, $P_C \times E$). Because the data of organoleptic quality of breast meat was not normally distributed (interpreted with the Shapiro-Wilk test for normality), it was analysed by the non-parametric Kruskall-Wallis test. In the current study, $p \leq 0.05$ was considered to be significant and $p < 0.10$ tended to be significant.

Results

Pea cultivar contained 222 g kg^{-1} DM crude protein, 24.1 g kg^{-1} DM crude fat and 54.4 g kg^{-1} DM crude fibre (not shown in the tables). Those values for SBM were 547, 29.4 and 43.5 g kg^{-1} DM (not shown in the tables). As designed, the (analyzed) crude protein content of the diets decreased with increased dietary pea inclusion (Table 2). The analyzed diet compositions were in line with the formulated values. The crude protein, lysine, methionine, cysteine and threonine contents of grower diets (g kg^{-1} DM basis) varied between 204–222, 11.7–13.2, 4.25–5.40, 3.60–4.69 and 7.75–9.85. The cystine, lysine and threonine contents were the highest for diets that included peas up to 150 g kg^{-1}. With enzyme unsupplemented diets, the methionine content was highest for diets including peas up to 150 g kg^{-1}, but for the enzyme supplemented diets the methionine content was similar among the experimental diets.

Table 2. Calculated and analyzed chemical composition of the experimental diets (g kg^{-1} DM), except AME and DM

	Starter diets, from d 1 to 9		Grower diets, from d 9 to d 38							
Pea inclusion, g kg^{-1}			0	150	300	450	0	150	300	450
Avizyme 1200 0,1%	–	+	–	–	–	–	+	+	+	+
Calculated composition										
AME, MJ kg^{-1}	13.6	13.6	13.5	13.5	13.5	13.5	13.5	13.5	13.5	13.5
Crude protein	242	242	219	211	203	195	219	211	203	195
Lysine	15.4	15.4	12.7	12.8	12.7	12.8	12.7	12.8	12.7	12.8
Methionine	5.55	5.55	4.65	4.69	4.62	4.66	4.65	4.69	4.62	4.66
Methionine + cysteine	9.95	9.95	8.79	8.56	8.22	7.98	8.79	8.56	8.22	7.98
Threonine	10.0	10.0	8.38	8.38	8.38	8.38	8.38	8.38	8.38	8.38
Tryptophan	2.96	2.96	2.62	2.43	2.34	2.37	2.62	2.43	2.34	2.37
Calcium	10.8	10.8	9.26	9.30	9.27	9.25	9.26	9.30	9.27	9.25
Phosphorus (available)	5.42	5.42	4.63	4.65	4.65	4.64	4.63	4.65	4.65	4.64
Analyzed composition										
DM, g kg^{-1}	876	875	877	874	875	873	880	878	878	873
Crude protein	249	257	213	221	209	204	222	220	212	205
Crude fat	57.6	56.7	37.6	43.3	49.4	53.3	38.7	46.7	46.5	50.9
Crude fiber	35.8	29.6	37.2	39.4	35.8	50.1	38.1	44.3	45.6	50.6
Ash	68.1	69.1	60.0	60.0	54.2	55.8	59.6	60.0	55.4	59.0
Alanine	10.2	10.1	8.31	8.97	8.28	8.44	8.60	8.78	8.44	8.51
Arginine	14.8	15.6	12.7	14.8	13.0	14.0	13.3	13.1	12.8	13.0
Aspartic acid	22.9	22.8	18.0	21.6	19.5	20.1	18.7	19.3	18.5	19.7
Cysteine	4.13	4.16	3.95	4.69	3.60	3.73	3.91	4.14	3.81	3.70
Glutamic acid	58.9	58.3	53.1	56.2	48.9	43.5	56.9	52.5	45.6	42.1
Glycine	10.3	10.3	9.00	9.73	8.80	8.81	9.23	8.89	8.62	8.53
Histidine	6.32	6.38	5.71	5.96	5.41	5.23	5.73	5.45	5.24	4.87
Isoleucine	10.4	9.81	7.75	9.20	7.90	8.30	8.31	8.27	7.52	8.01
Leucine	18.5	18.5	15.8	16.3	14.8	14.3	15.9	15.8	15.2	14.2
Lysine	14.2	15.0	11.7	13.2	12.7	12.9	11.9	12.7	12.3	12.7
Methionine	5.64	5.63	4.25	5.40	4.66	4.55	4.74	4.44	4.33	4.75
Phenylalanine	11.9	11.7	10.3	10.8	9.77	9.73	10.5	10.4	10.1	9.53
Proline	16.0	17.2	15.9	15.7	13.9	11.7	16.8	14.9	13.5	11.6
Serine	12.5	12.4	10.6	11.7	10.4	10.0	11.2	10.5	9.90	9.85
Treonine	9.85	9.72	7.92	9.09	8.27	8.16	8.26	8.37	7.75	8.15
Valine	11.9	10.6	9.01	9.60	8.56	8.22	9.34	8.56	8.29	7.83

AME = apparent metabolizable energy; DM = dry matter

Table 3. The effects of dietary pea inclusion level and enzyme supplementation on production performance. Values are means of 6 observations per treatment.

	treatment									*p*-values						
Pea inclusion, g kg^{-1}	0	150	300	450	0	150	300	450	SEM	PL	PQ	PC	E	PL × E	PQ × E	PC × E
Avizyme 1200 0,1%	–	–	–	–	+	+	+	+								
Body weight, g																
d 9	238	239	240	238	238	243	239	244	3.0	0.409	0.689	0.462	0.268	0.522	0.867	0.227
d 37	2263	2288	2267	2265	2313	2352	2300	2305	13.6	0.302	0.125	0.018	<0.001	0.506	0.865	0.330
Carcass weight, g	1457	1507	1471	1505	1531	1561	1540	1505	13.1	0.911	0.036	0.025	<0.001	0.018	0.204	0.169
Body weight gain, g d^{-1}																
from d 1 to d 9	198	198	200	197	197	203	199	203	2.9	0.431	0.638	0.498	0.280	0.448	0.848	0.254
from d 10 to d 37	2025	2049	2027	2026	2075	2108	2060	2061	13.8	0.232	0.151	0.028	<0.001	0.427	0.839	0.482
from d 1 to d 37	2223	2247	2226	2224	2272	2311	2259	2265	13.6	0.294	0.120	0.018	<0.001	0.518	0.868	0.339
Feed consumption, g d^{-1}																
from d 1 to d 9	25	25	25	25	25	25	25	25	0.3	0.669	0.598	0.781	0.253	0.278	0.816	0.447
from d 10 to d 37	131	129	129	130	128	130	128	131	1.0	0.855	0.166	0.485	0.478	0.148	0.689	0.073
from d 1 to d 37	104	103	103	103	102	103	102	104	0.8	0.714	0.318	0.510	0.240	0.162	0.696	0.146
FCR, g of feed/g growth																
from d 1 to d 9	1.15	1.14	1.14	1.13	1.13	1.12	1.13	1.12	0.013	0.424	0.900	0.577	0.061	0.718	0.736	0.694
from d 10 to d 37	1.85	1.79	1.82	1.83	1.77	1.75	1.77	1.82	0.011	0.105	<0.001	0.087	<0.001	0.006	0.947	0.218
from d 1 to d 37	1.79	1.73	1.76	1.77	1.71	1.69	1.71	1.75	0.009	0.144	<0.001	0.076	<0.001	0.006	0.894	0.272
FCR, g of feed/g carcass	2.65	2.52	2.60	2.56	2.49	2.46	2.48	2.57	0.022	0.984	0.002	0.048	<0.001	0.004	0.540	0.020

SEM = standard error of mean; FCR = feed conversion ratio; PL = the linear effect of pea inclusion; PQ = the quadratic effect of pea inclusion; PC = the cubic effect of pea inclusion; E = the effect enzyme supplementation; PL × E = the interaction of the linear effect of dietary pea inclusion and enzyme supplementation; PQ × E = the interaction of the quadratic effect of dietary pea inclusion and enzyme supplementation; PC × E = the interaction of the cubic effect of dietary pea inclusion and enzyme supplementation

Table 4. The effects of dietary pea inclusion level and enzyme supplementation on the weight and proportion of breast and abdominal fat, ileal viscosity, litter dry matter content and mortality. Values are means of 6 observations per treatment (in case of breast/abdominal fat weight and proportion and ileal viscosity each observation is value of one bird per replicate).

	treatment									*p*-values					
Pea inclusion, g kg^{-1}	0	150	300	450	0	150	300	450	SEM	PL	PQ	PC	E	PL × E	PQ × E	PC × E
Avizyme 1200 0,1%	–	–	–	–	+	+	+	+								
Breast																
weight, g	494	563	520	535	595	574	562	529	20.8	0.320	0.273	0.295	0.013	0.031	0.478	0.124
proportion[1]	0.23	0.24	0.23	0.24	0.25	0.25	0.24	0.23	0.005	0.235	0.475	0.197	0.309	0.124	0.951	0.380
Abdominal fat																
weight, g	45	46	45	48	48	45	44	48	2.7	0.618	0.338	0.587	0.911	0.509	0.515	0.991
proportion[1]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.001	0.476	0.165	0.940	0.270	0.928	0.902	0.454
Ileal viscosity[2], cPs	10.2	7.3	7.2	6.8	3.5	3.9	3.5	3.3	0.90	0.053	0.466	0.753	<0.001	0.121	0.229	0.493
Ileal viscosity[3], cPs	6.6	6.6	5.9	5.7	5.0	4.5	4.4	4.5	0.47	0.092	0.816	0.760	<0.001	0.525	0.646	0.646
Litter dry matter[4], g kg^{-1}	396	400	385	383	368	401	407	398	11.9	0.582	0.159	0.587	0.777	0.048	0.299	0.780
Mortality[5], %	4.0	2.4	3.5	3.2	5.6	5.4	4.3	5.4	1.08	0.637	0.383	0.875	0.018	0.940	0.999	0.311

SEM = standard error of mean; PL = the linear effect of pea inclusion; PQ = the quadratic effect of pea inclusion; PC = the cubic effect of pea inclusion; E = the effect enzyme supplementation; PL × E = the interaction of the linear effect of dietary pea inclusion and enzyme supplementation; PQ × E = the interaction of the quadratic effect of dietary pea inclusion and enzyme supplementation; PC × E = the interaction of the cubic effect of dietary pea inclusion and enzyme supplementation; [1] = proportion of live weight; [2] = at 20 d of age; [3] = at 30 d of age; [4] = at 37 d of age; [5] = during the entire experiment

Feed consumption was similar among treatments ($p > 0.05$) during the starter and grower periods and the entire experiment (Table 3). Body weight, carcass weight and body weight gain were similar ($p > 0.05$) among treatments during the starter period. Birds´ body weight, carcass weight and body weight gain improved with pea inclusion level of 150 g kg^{-1} and declined by pea inclusion levels of 300 and 450 g kg^{-1} (cubic effect of pea inclusion, $p \leq 0.028$) compared to those of birds on control diet during the grower period and the entire experiment. However impairments in body weight, carcass weight and body weight gain were numerically mostly seen for the birds fed on pea inclusion of 450 g kg^{-1} and supplemented with XLS, AMS and PRT. Dietary pea inclusion improved FCR of the birds from the control to level of 150 g kg^{-1}, but declined with levels of 300 and 450 g kg^{-1} (cubic effect of pea inclusion, $p \leq 0{,}001$). However impairment of FCR was evident only for the birds fed pea inclusion of 450 g kg^{-1} supplemented with XLS, AMS and PRT.

The proportion of breast muscle and the weight and the proportion of abdominal fat of the birds on experimental diets were similar ($p > 0.05$) among treatments (Table 4). Pea inclusion tended to decrease ($p \leq 0.092$) intestinal viscosity of the birds fed on experimental diets in a linear manner at 20 and 30 day of age.

The supplementation of XLS, AMS and PRT tended to improve ($p = 0.061$) FCR during the starter period. The final body weight (day 37), carcass weight, body weight gain and FCR values improved ($p \leq 0.001$) by XLS, AMS and PRT supplementation during the grower period and the entire experiment. Enzyme supplementation decreased ($p = 0.001$) intestinal viscosity of birds at age 20 and 30 days. Enzyme supplementation increased mortality ($p = 0.018$). Litter dry matter content was similar ($p > 0.05$) among treatments. The organoleptic quality of breast meat did not differ ($p > 0.05$) among treatments (Table 5).

Table 5. The effects of dietary pea inclusion level and enzyme supplementation on the tenderness, juiciness and flavour of breast muscle1. Values are means of 6 observations per treatment (each observation is value of one bird per replicate).

	treatment									*p*-value
Pea inclusion, g kg^{-1}	0	150	300	450	0	150	300	450	SEM	
Avizyme 1200 0,1%	–	–	–	–	+	+	+	+		
Tenderness	4.8	4.8	4.8	4.8	4.1	4.9	4.5	5.3	0.25	0.169
Juiciness	4.5	4.5	4.5	4.7	4.2	4.6	4.5	4.8	0.17	0.312
Flavour	4.6	4.8	4.7	4.7	4.3	4.9	4.4	4.7	0.19	0.689

SEM = standard error of mean; [1] = a seven point scale form (tenderness, 1 tough to tender 7; juiciness, 1 dry to juicy 7; flavour, 1 dislike very much to like very much 7)

Discussion

The chemical composition of pea and experimental diets

The chemical composition was in line with that presented by Gatel and Grosjean (1990) and Gatel (1994). The crude protein content was comparable to previous reports where the same pea variety was used (Partanen et al. 2001, Partanen et al. 2006). Cystine, lysine, methionine (only in case of enzyme unsupplemented diets) and threonine contents were the highest for diets that included peas up to 150 g kg^{-1}, which had a beneficial effect on production performance. The major limitation of the study is that the composition of dietary fibre was not determined and starch content in feed ingredients used was not analyzed. These measurements would have help us confirm that the positive effect of enzyme combination was due to degradation of backbone substituent of wheat discussed later. For the limitation of study, the crude protein and amino acid contents of diet changed among diets, and the effects of pea inclusion level cannot be fully separated from those differences.

Effect of pea inclusion

Growth performance results interpreted without addition of enzyme cocktail were comparable to previous studies, where pea inclusions between 100–480 g kg^{-1} had no negative effect on broilers' body weight and feed consumption (Igbasan and Guenter 1996, Laudadio and Tufarelli 2010, Dotas et al. 2014). Good production performance of birds in all treatments and consistent feed intake suggest that the pea cultivar studied did not appear to contain harmful levels of ANF. This confirms the results by Smulikowska et al. (2001), who reported that the role of ANF in modern pea cultivars is found to be less important. However growth and FCR depressing effects of peas might be alleviated through provision of DL-methionine and L-lysine, as shown previously by Igbasan and Guenter (1996). The diets (enzyme cocktail unsupplemented and supplemented diets) including peas up to 150 g kg^{-1} supported better body weight and body weight gain compared to other diets. Moreover, in case of enzyme unsupplemented diets the diet including peas 150 g kg^{-1} supported better FCR compared to other diets.

Those improvements are potentially due to higher essential amino acid content of those diets. The latest also agree with the results in previous studies where green or light coloured pea seeds or pea meal used in comparable inclusions improved FCR (Igbasan and Guenter 1996).

However, in diets supplemented with XLS, AMS and PRT pea inclusion of 450 g kg^{-1} impaired the FCR of birds in line with the results by Cowieson et al. (2003). Otherwise FCR was unchanged by pea inclusion as in the findings of Dandanell Daveby et al. (1998), Laudadio and Tufarelli (2010) and Dotas et al. (2014). The complex results found in FCR by the addition of XLS, AMS and PRT with increasing pea inclusion is likely due to differences in crude protein and amino acid contents as well as differences in carbohydrate fraction of diets (Cowieson et al. 2003).

We found that pea inclusion tended to decrease intestinal viscosity of the birds fed on experimental diets, but this is explained by the effect of decreasing content of wheat in the diet. The result from intestinal viscosity tests agree with the findings by Farrell et al. (1999), who have demonstrated that among field peas, faba beans, chick peas and sweet lupins, only lupins increase intestinal viscosity. The result in unchanged mortality among pea diets agree with the findings of Laudadio and Tufarelli (2010) and Dotas et al. (2014), who reported no difference in mortality between the control group and groups offered diets with peas up to 480 g kg^{-1}.

Our results in the organoleptic quality test for breast meat agree with the results of Mc Neill et al. (2004), who found that the inclusion of field peas up to 200 g kg^{-1} had no effect on flavour of breast meat. To our knowledge no research has been carried out on the effect of pea inclusion on tenderness and juiciness of breast meat.

The effect of enzyme addition

Improvements in production performance with the addition of XLS, AMS and PRT were most likely associated with the more intensive hydrolyzation of wheat starch and fibre carbohydrates than those of peas. Even though the effects of XLS, AMS and PRT could not be distinguished, we assume that the improvement in bird performance was more likely due to AMS and XLS than that of PRT as evidenced by the earlier literature (Longstaff and Mc Nab 1987, Brenes et al. 1993, Igbasan and Guenter 1996, Cowieson et al. 2003). Reduced intestinal viscosity by enzyme supplementation supports this suggestion.

Cowieson et al. (2003) have previously suggested that the presence of XLS could be responsible for some of the improvements in weight gain and FCR through a reduction in the anti-nutritive effects associated with wheat arabinoxylans, whereas the addition of XLS (Brenes et al. 2003, Cowieson et al. 2003) AMS (Brenes et al. 2003) or PRT (Igbasan and Guenter 1996) into pea diets have not produced positive response in the growth of birds or the positive effects have been depending on the pea cultivar used. Arabinoxylans in wheat have been shown to reduce chick performance (Annison 1993). Peas have studied to contain minimal amount of arabinoxylans (Bach-Knudsen 1997, Glada 1998), but contain relatively high levels of cellulose and pectic polysaccharides (Bach-Knudsen 1997). This complex polysaccharide mixture in peas degrades particularly by cellulase and AMS (Longstaff and Mc Nab 1987), which indicates that the nutrient availability from peas may be improved by AMS. Our results indicate that the addition of AMS improves the degradation of the starch in wheat and pea, while the addition of XLS leads to break up of arabinoxylans, which are the major cell wall polysaccharides in wheat. Enzyme supplementation increased slightly the mortality of birds. However, this is not substantial finding and in general mortality was low in all feeding treatments.

In conclusion, when diets are balanced with regards to their amino acid content 450 g kg^{-1} semi-leafless peas can be used in broiler grower diets based on wheat and SBM. However, the crude protein and amino acid contents of diet changed with the pea inclusion level used, hence the effects of pea inclusion level cannot be separated from diet crude protein and amino acid contents. The use of enzyme combination of XLS, AMS and PRT enhances the nutritive value of wheat-pea diets. The beneficial effects of this are however more likely a result of the effects of the enzymes on the backbone substituent in wheat.

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