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Fermentation and enzymatic treatment of pea for turkey nutrition

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ABSTRACT

The present study was conducted to investigate how fermentation (**FE**) and enzymatic treatment (**ET**) of pea affect standardized ileal digestibility (**SID**) of nutrients in diets with peas as the only protein source, and evaluate the consequences of inclusion of different pea products in turkey diets (100 g/kg) on growth performance and bird health. For FE process, *Pisum sativum* L. was mixed with water (1:1) containing 4.9×10^8 *Bacillus subtilis* and *licheniformis* spores/kg pea (BIOPLUS 2B®, Chr. Hansen, Denmark). The prepared dough was fermented for 48 h at 30 °C. For ET, the dough water contained three enzymes, AlphaGal™ (α-galactosidase – Kerry, USA), RONOZYME® ProAct and VP (protease and pectinases, respectively – DSM, Switzerland). The dough (500 g/kg DM) was incubated for 24 h at 30 °C. Both processes reduced α-galactosides, phytate, trypsin inhibitor activity and resistant starch in peas. For standardized ileal digestibility (**SID**) assay, 288 turkeys were assigned to 24 pens and received four experimental diets including native (**NP**), fermented (**FEP**) and enzymatically treated peas (**ETP**) as well as a N-free diet (all supplemented with vitamins and minerals). The ETP had better SID of protein, Glu, Phe and Val compared with FEP and NP. Enzymatic treatment of pea also improved standardized ileal digestibility of Ala, Gly, His, Ilu, Leu and Lys ($P \leq 0.05$), however digestibility of these nutrients in fermented pea were similar to other two types of pea ($P > 0.05$). Both processes drastically improved ileal digestibility of starch ($P \leq 0.05$). For performance trial, 960 turkeys were allocated into 60 pens and received 4 different diets consisted of a basal mash wheat-SBM diet (**CON**) and there experimental diets which were prepared by inclusion of each pea products NP (**NPD**), FEP (**FEPD**) and ETP (**ETPD**) in the basal diet at the rate of 100 g/kg. The experiment lasted 105 d. In general, in the most time periods of the performance trial, birds received ETPD or FEPD diets showed better growth performance than those fed NPD diet, while birds in ETPD group displayed similar performance to those fed CON diet. At the end of the trial, birds fed CON and ETPD diets had the best FCR and birds which received NPD diet had the worst one ($P \leq 0.05$). Birds in ETPD group showed the best footpad dermatitis score and turkeys in the NPD group had the worst score ($P \leq 0.05$). The footpad dermatitis scores for turkeys in CON and FEPD groups

Abbreviations: AA, amino acid; AID, apparent ileal digestibility; ANF, anti-nutritional factors; BWG, body weight gain; CF, crude fat; CON, control diet; CP, crude protein; DM, dry matter; FCR, feed conversion ratio; ET, enzymatic treatment; ETP, enzymatically treated pea; ETPD, enzymatically treated pea diet; FE, fermentation; FEP, fermented pea; FEPD, fermented pea diet; FI, feed intake; GIT, gastrointestinal tract; NP, native pea; NPD, native pea diet; NSP, non-starch polysaccharides; RE, raffinose equivalent; RS, resistant starch; RSM, rapeseed meal; SBM, soybean meal; SCFA, short chain fatty acids; SID, standardized ileal digestibility; SSF, solid state fermentation; TDF, total dietary fiber; TIA, trypsin inhibitor activity

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were identical and considerably different from those in ETPD and NPD groups ($P \leq 0.05$). In conclusion, both processes could improve the nutritional quality of pea by reduction in ANF and increasing ileal starch digestibility. Furthermore, ET process considerably improved SID of protein and AAs in pea. Inclusion of ETP in turkey diets (100 g/kg) demonstrated neither positive nor negative impact on growth performance, while it remarkably improved footpad dermatitis score. The present data shows the feasibility of these processes, particularly ET, for improving the nutritional quality of pea as a protein source for turkey diets.

1. Introduction

The most common plant protein source in poultry nutrition is soybean meal (**SBM**). Cultivation of soybean is limited in many part of the world due to the environmental conditions. That is why, globally, the majority of protein needs of poultry industry are covered by the import of soy products from few exporting countries. On the other hand, there is a growing environmental and ethical concern (e.g. the loss of ecologically important rainforests, contamination with genetically modified plants, etc.) against soy cultivation and import in some parts of the world like Europe. Therefore, domestic alternatives for SBM are urgently needed.

The interest of using peas, a traditional legume used for animal and human nutrition, as a protein source for poultry nutrition has been increasing (Nalle et al., 2011). Peas have relatively high crude protein (**CP**) contents and can provide considerable amount of energy due to their high starch contents. However, peas contain also variable amounts of anti-nutritional factors (**ANF**) such as α-galactosides, trypsin inhibitors (**TI**), resistant starch (**RS**), pectins, tannins, lectin, phytic acid and non-starch polysaccharides (**NSP**), which can significantly impair the digestive process especially in young poultry. This may lead to pancreatic hypertrophy, wet litter and footpad dermatitis as well as an increased need for supplementing sulphur-containing amino acids (**AA**) and finally poor bird performance (Martland, 1984; Igbasan and Guenter, 1996; Nalle et al., 2011; Frikha et al., 2013; Goodarzi Boroojeni et al., 2017). Using various processing methods, e.g. soaking, autoclaving, dehulling, and micronization followed by air classification tend to reduce the content of ANF in peas but are labor demanding and variable in efficacy and cost (Igbasan and Guenter, 1996; Laudadio et al., 2012; Frikha et al., 2013). Fermentation processes have been traditionally used for centuries in human food production. Fermentation (**FE**) of legumes can eliminate ANF e.g. tannins, trypsin inhibitors, α-galactosides and modify AA profile by microbial synthesis and breakdown (Ouoba et al., 2003; Ouoba et al., 2007; Gefrom et al., 2013). One of the major beneficial impacts of FE process on ANF seems to be through enzymatic degradation by fermenting bacteria. As a common approach, microbial enzymes are used as feed additives in poultry diets to inactivate or/and eliminate certain ANF and elevate availability of nutrients that were physically or chemically sequestered by ANF substances (Bedford, 2000). However, the optimum efficiency of these enzymes is limited. The retention time of feed in the gastrointestinal tract (**GIT**) of poultry is very short and, since the optimum pH of most of exogenous enzymes is between 4 and 6, the degradation activity of them is mainly limited to the proximal part (crop, proventriculus and gizzard) of the GIT (Svihus et al., 2002; Ravindran, 2013).

Wet litter caused by high amount of ANF in turkey diets is known to induce footpad dermatitis (Mayne, 2005; Shepherd and Fairchild, 2010). In the previous study, FE of pea with *Bacillus subtilis* and enzymatic treatment (**ET**) of pea with α-galactosidase, protease and pectinases could effectively reduce α-galactosides, phytate, trypsin inhibitor activity (**TIA**) and RS in peas (Goodarzi Boroojeni et al., 2017). Thus, it can be hypothesized that FE and ET can improve the nutritional quality of pea in turkey diets (by reduction in ANF) and through this improvement may reduce incidence of footpad dermatitis and positively impact nutrient digestibility of pea as well as feed efficiency. Furthermore, processed peas with reduced levels of ANF may be promising alternatives for partial replacement of SBM in turkey nutrition. Therefore, the present study was conducted to investigate how FE and ET of pea affect standardized ileal digestibility (SID) of nutrients in diets with peas as the only protein source, and evaluate the consequences of inclusion of different pea products in turkey diets (100 g/kg) on growth performance and bird health.

2. Experimental methods

The production procedures of processed pea products were according to the methods used by Goodarzi Boroojeni et al. (2017). A commercial batch of Madonna pea (*Pisum sativum L.)* was hammer milled (2 mm screen size) as full seeds (including hulls) to be used for the continuous production of pea dough (500 g/kg DM) during ET and FE.

2.1. Solid state fermentation

For the solid state fermentation (**SSF**) process, BIOPLUS 2B® (EU authorized probiotic to be used in turkey feed) consisting of *Bacillus licheniformis* and *Bacillus subtilis* in a 1:1 ratio (Chr. Hansen, Denmark) was used. The water of dough production was inoculated with the probiotic spores beforehand, resulting in final concentration of approximately 4.9×10^8 *Bacillus subtilis* and *licheniformis* spores/kg pea. The homogeneously mixed dough (500 g/kg DM), was pumped in internal cathodic protected (**ICP**) tanks and incubated in temperature (30 °C) controlled containers for 48 h.

2.2. Enzymatic treatment (pre-digestion)

For ET, three different commercial exogenous enzymes plus a mixture of organic acids (10 ml/kg pea dough; 8 ml lactic and 2 ml acetic acid) were added to the water of dough production beforehand. Added organic acids mixture resulted in a final pH of 4.5 in the dough (500 g/kg DM), inhibiting uncontrolled FE of pea by its associated microorganisms (spoilage) during 24 h incubation time at 30 °C. The applied commercial enzymes were 0.2 g/kg pea RONOZYME® ProAct (DSM, Switzerland) containing a protease (enzyme activity: 15000 u/kg pea), 0.2 g/kg pea RONOZYME® VP (DSM, Switzerland) containing a blend of β-glucanase and pectinases (enzyme activities: 10 u/kg pea) as well as 0.1 g/kg pea AlphaGal™ (Kerry EMEA, USA) containing an α-galactosidase (enzyme activity: 115 u/kg pea). Nutrients content and ANF composition of native pea (**NP**) and enzymatically treated peas (**ETP)** are the same as presented by Goodarzi Boroojeni et al. (2017) in the previous publication.

2.3. Drying and milling of processed pea

Right after the incubation time, the wet processed materials were simultaneously dried and ground by an Ultra-Rotor dryer mill (Ultra-Rotor Type U III a, Jäckering Mühlen- und Nährmittelwerke GmbH, Hamm, Germany). The Ultra-Rotor dryer mill, generating a very high turbulence and heated air (6000 m^3/h), simultaneously milled and dried the pea products in less than 3 s (throughput of 160 kg dry product per h). The maximum product temperature was below 75 °C. In total, seven samples from dried products were analyzed concerning their particle size via laser particle size analyzer (Type 930, Cilas S.A., Orléans, France). The mean particle size of the final products was approximately 55 ± 5.2 µm.

2.4. Animals and experimental design

The experiments, including a digestibility assay and a growth performance trial, were conducted in the experimental facilities of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn, Poland. All the experimental protocols were approved by the Local Animal Ethics Committee.

2.5. Digestibility assay

Standardized ileal digestibility of nutrients in all three pea products (native, fermented and enzymatically treated) were measured conducting the method described by Lemme et al. (2004) and Kozłowski et al. (2011). A N-free diet was used to determine basal (non-specific) ileal endogenous amino acid losses. The assay diets were based on glucose and the pea products. The diets contained 3 g TiO_2 (Sigma Aldrich, St. Louis, MO) per kg feed as an indigestible marker to allow for the determination of ileal digestibility coefficients. The compositions of the three experimental diets containing pea products and N-free diet are presented in Table 3.

One-d-old male turkeys (Hybrid Converter) were obtained from a local hatchery. The temperature, lighting and ventilation programs were according to the breeder's recommendations (Hybrid Turkeys, 2016). The birds received commercial turkey feed from d 1 to 77. On d 77 post hatching, two hundred and eighty eight birds within a narrow weight range (8.21 ± 0.14 kg) were randomly allocated into 24 pens (6 replicate-pens and 12 birds per pen). The birds fed the experimental diets for 7 d. All birds had access to the experimental diets and water ad libitum. On d 84 post hatching all birds were sacrificed after stunning by cervical dislocation. The ileum was dissected from Meckel's diverticulum to the ileo-caecal junction and the digesta was collected from the distal 2/3 for SID determinations. The digesta of all birds within each pen were pooled and immediately frozen (−80 °C) until further analysis. The pooled digesta was freeze dried before chemical analysis.

The following formula was used for apparent ileal digestibility (**AID**) calculation:

AID of nutrient = 1 − [(*concentration of marker in feed* × *concentration of nutrient in the ileum)/(concentration of marker in the ileum* × *concentration of nutrient in feed)]*

The SID of CP and AA in the pea product diets were calculated by correcting AID of CP and AA for basal endogenous (**BE**) CP and AA losses:

BE nutrient loss = concentration of nutrient in the ileum × *(concentration of marker in feed/concentration of marker in the ileum)*

SID of nutrient = AID of nutrient + [(BE nutrient loss, as g/kg DM intake)/(concentration of nutrient in the assay diet, as g/kg DM)]

2.6. Growth performance trial

Nine hundred and sixty one-d-old female turkeys (Hybrid Converter) were obtained from a local hatchery and, using completely randomized design, were assigned to 4 groups each with 15 replicate-pens (floor pens) and 16 birds per pen. The experimental diets consisted of a basal mash (produced in Agrocentrum feed mill, Kałęczyn, Poland) wheat-SBM diet (**CON**) and three experimental diets which formulated by inclusion of each pea products NP (**NPD**), Fermented (**FEPD**) and ETP (**ETPD**) in the basal diet at the rate of 100 g/kg. The experiment lasted 105 d and a four-phase feeding schedule was used with formulation of a pre-starter (d 1–28), a

starter (d 29–56), a grower (d 57–84) and a finisher (d 85–105) diet. The diets were formulated to be isocaloric and isonitrogenous for each phase and meet or exceed the recommendations of Hybrid Converter (Hybrid Turkeys, 2016). The compositions of the pre-starter (d 1–28), starter (d 29–56), grower (d 57–84) and finisher (d 85–105) experimental diets are shown in Table 4.

Body weights (**BW**) of the birds were recorded at d 1 and weekly during the experiment. Feed intake (**FI**) was recorded weekly and feed conversion ratio (**FCR**) was calculated. At the end of each experimental period, the fresh and clean (free from feathers, litter and feed) excreta from each pen was collected from plastic liners placed in the excreta collection trays (0.6 × 0.4 m) underneath each pen. Dry matter content of collected excreta from each pen was determined (Table 7).

At the end of the experiment footpad dermatitis scores for all birds were determined by trained personnel according to the method described by Hocking et al. (2008). The method is based on a subjective scoring system from 0 to 4; 0 representing no external signs of footpad dermatitis and 4 representing swelling footpad with more than a half covered by necrotic cells.

Table 1
Analyzed nutrient composition of pea products.

	NP[a,1,2]	FEP[1,3]	ETP[a,1,3]
Nutrient composition (g/kg DM)			
Crude fat	12.1	6.5	6.5
Crude protein	228	235.2	230
Starch	442	436.6	434
Ala	9.9	9.8	9.6
Arg	19.1	16.5	18.6
Asp	25.2	24	24.6
Cys	5.3	5.3	4.9
Glu	23.9	22.3	22.9
Gly	9.7	9.6	9.5
His	8.1	7.9	7.8
Ile	8.6	8.6	8.7
Leu	15.8	15.6	15.5
Lys	16.6	16	16.2
Met	2.6	2.8	2.4
Phe	10.9	10.8	11.1
Pro	9.9	10.6	9.1
Ser	11.9	11.2	11.4
Thr	9	8.9	8.7
Tyr	6.9	6.3	7.1
Val	9.9	9.9	9.9
Total AA	206	205	201
Minerals and trace elements			
Ca (g/kg DM)	0.73	0.89	0.82
P (g/kg DM)	3.17	3.24	3.24
K (g/kg DM)	7.6	8.13	8.16
Na (g/kg DM)	0.27	0.25	0.32
Mg (g/kg DM)	1.27	1.24	1.21
Zn (mg/kg DM)	23	25	28
Cu (mg/kg DM)	6	7	7
Fe (mg/kg DM)	42	48	58
Mn (mg/kg DM)	10	11	10
Bacterial metabolites (μmol/g as fed)			
Acetic acid	2.2	11	56.1
Propionic acid	ND[4]	ND	ND
i-Butyric acid[5]	ND	ND	ND
n-Butyric acid[5]	ND	ND	ND
i-Valeric acid[5]	0.8	4.3	1.4
n-Valeric acid[5]	14.8	8.6	23.9
L-Lactate	0.52	150	184
D-Lactate	0.05	141	2
Ammonium	0.05	24.9	1.7
pH of the products before drying	–	4.5 ± 0.09	4.5 ± 0.14

[1] NP = Native pea, FEP = Fermented pea, ETP = Enzymatically treated pea.
[2] Dry mater of native pea: 909 g/kg.
[3] Dry mater of fermented and enzymatically treated pea: 921 g/kg.
[4] ND: Not detected.
[5] Branched chain volatile fatty acids.
[a] They were also shown by Goodarzi Boroojeni et al. (2017).

2.7. Chemical analysis

Basic composition analysis were performed, using standard procedures (Naumann and Bassler, 2004). The AA analyses were carried out using a Biochrom 20 Plus amino acid analyzer (Amersham Pharmacia Biotech, Piscataway, USA) following standard method (VDLUFA, 2003). A commercial enzymatic test (Starch UV-Test, R-Biopharm, Darmstadt, Germany) was used to determine starch content (Naumann and Bassler, 2004). The P content was determined following the ammonium vanadate/molybdate method (Gericke and Kurmies, 1952). Other minerals were measured by applying an atomic absorption spectrophotometer (AAS vario®, Analytik Jena, Jena, Germany). The concentration of TiO_2 in feed and digesta was measured using the method described by Short et al. (1996). The method of Kakade et al. (1974) was used for TIA measurements. The AACC methods were employed to analyze RS (AACC 32-40.01, 2003) and total dietary fiber (**TDF**; AACC method 32-25 – Uppsala method). The soluble- and insoluble-NSP were measured by gas liquid chromatography (Perkin Elmer Autosystem XL) following Englyst and Cummings (1984) method. The α-galactosides concentration (in pea mainly raffinose, stachyose and verbascose) was measured applying enzymatic assay (raffinose/D-galactose Assay Kit, Megazyme International, Bray, Ireland) and is presented as raffinose equivalent (**RE**). Phytate content was determined using phytate assay kit (Megazyme International, Bray, Ireland).

2.8. Determination of bacterial metabolites

Measurement of short chain fatty acids (SCFA) was conducted by gas chromatography (Agilent Technologies 6890N coupled with an auto sampler G2614A and an auto injector G2613A; Santa Clara, CA, USA) following standard procedure (Schäfer, 1995). The column was an Agilent 19095N-123 HP-INNOWAX polyethylene glycol.

Ammonia was quantified using the Berthelot reaction assay. Following standard procedure, D- and L-lactate were measured by HPLC, using an Agilent 1100 system with a Phenomenex C18 (4.0 × 2.0 mm) guard column followed by a Phenomenex Chirex 3126 (D)-penicillamine column (150 × 4.6 mm) (Agilent Technologies). The UV detector wavelength was 253 nm and the column temperature was 35 °C. The carrier was $CuSO_4$ in a gradient from 0.5 to 2.5 mmol/L with a flow rate of 1 mL/min at 35 °C, and the injection volume was 20 µL. The concentration of bacterial metabolites in the pea products is presented in Table 1.

2.9. Statistical analysis

Data were subjected to ANOVA using the GLM procedure of SPSS 19.0 (SPSS Inc., Chicago, IL). Treatment means were separated by the Tukey least significant difference post hoc test at $P \leq 0.05$ statistical level. Replicate-pen was the experimental unit for all variables measured.

3. Results

3.1. Influence of technological processing on nutrients and anti-nutritional factors of the pea products

The effects of FE and ET on nutrients and ANF composition of pea products are shown in Tables 1 and 2, respectively. Except a reduction in crude fat (**CF**) and a negligible increase in CP, no other considerable changes for the main nutrients like AA and starch were detected after processing of NP (Table 1). Both processes led to a minor increase in the concentration of P, Ca, K, Fe and partially

Table 2
Anti-nutritional factors of pea products (mean ± SD).

Anti-nutritional factors	Fermentation (FE)			Enzymatic treatment (ET)[a]	
	NP[c,1]	FEP[1]	Alteration by FE[2]	ETP[c,1]	Alteration by ET[c,2]
Insoluble-NSP[3] (g/100 g DM)[a]	11.3 ± 0.10	9.1 ± 0.17	−19.3%	9.9 ± 0.11	−12.6%
Soluble-NSP[3] (g/100 g DM)[a]	0.91 ± 0.02	1.07 ± 0.02	+17.6%	1.01 ± 0.01	+11.0%
Resistant starch (g/100 g DM)[a]	3.25 ± 0.02	0.78 ± 0.00	−76.0%	0.81 ± 0.00	−75.1%
Total dietary fibre[4] (g/100 g DM)[a]	18.1 ± 0.11	14.0 ± 0.11	−22.5%	14.8 ± 0.12	−18.0%
Trypsin inhibitor activity (TIU[5] mg/g DM)[a]	0.67 ± 0.01	0.54 ± 0.01	−19.4%	0.50 ± 0.01	−25.4%
Raffinose equivalents (mol/g DM)[b]	97.9 ± 4.4	40.0 ± 13.4	−59.1%	48.9 ± 10.5	−50.0%
Phytic acid (g/100 g DM)[b]	0.92 ± 0.02	0.77 ± 0.02	−16.8%	0.47 ± 0.01	−49.2%

[1] NP = Native pea, FEP = Fermented pea, ETP = Enzymatically treated pea.
[2] Compared with native pea.
[3] Non starch polysaccharides.
[4] Cellulose, hemicellulose, lignin, gums, modified celluloses, mucilages, ligosaccharides, and pectins and associated minor substances, such as waxes, cutin and suberin.
[5] Trypsin inhibitor activity unit.
[a] Data were obtained from duplicate measurements and are stated as mean value ± standard deviation.
[b] Data were obtained from triplicate measurements and are stated as mean value ± standard deviation.
[c] They were also shown by Goodarzi Boroojeni et al. (2017).

Table 3
Ingredients (g/kg, unless noted) and analyzed nutrient composition of turkey diets[a] for digestibility trial.

Ingredients (g/kg)	NP[b]	FEP[b]	ETP[b]	N-Free
Pea products	927.7	927.7	927.7	–
Maize starch	–	–	–	186.2
Soya oil	30.0	30.0	30.0	50.0
$NaHCO_3$	1.0	1.0	1.0	20.0
NaCl	2.4	2.4	2.4	2.0
KCl	–	–	–	12.0
MgO	–	–	–	2.0
Choline chloride	–	–	–	3.0
Limestone	17.1	17.1	17.1	13.0
MCP[b,*]/DCP[b,**]	19.0[*]	19.0[*]	19.0[*]	19.0[**]
Cellulose (Solkafloc)	–	–	–	50.0
TiO_2[c]	0.3	0.3	0.3	0.3
Glucose	–	–	–	640.0
Premix[d]	2.5	2.5	2.5	2.5
Analyzed Nutrient Composition (g/kg)				
Crude protein	191.7	199.2	192.8	9.06
Starch	314.3	354.9	344.7	364.4
Ala	8.44	8.32	8.12	8.12
Arg	15.63	13.67	15.06	0.00
Glu	20.18	20.03	19.11	0.71
Gly	8.13	8.12	7.80	0.19
His	6.52	6.43	6.15	0.19
Ilu	7.80	7.95	7.62	0.16
Leu	13.35	13.40	12.98	0.39
Lys	13.82	13.16	13.07	0.35
Phe	9.42	9.41	9.33	0.00
Pro	8.90	7.86	8.10	0.56
Ser	9.48	9.26	9.26	0.29
Thr	7.68	7.57	7.24	0.16
Val	8.82	8.95	8.48	0.37

[a] As-fed basis.
[b] NP = Native pea, FEP = Fermented pea, ETP = Enzymatically treated pea, MCP = Monocalcium phosphate, DCP = Dicalcium phosphate.
[c] Indigestible marker (Sigma Aldrich, St. Louis, MO).
[d] Content per kg premix: 3,840,000 IU Vitamin A; 1,920,000 IU Vitamin D_3; 24,000 mg Vitamin E; 1200 mg Vitamin K_3; 800 mg Vitamin B_1; 4800 mg Vitamin B_2; 2000 mg Vitamin B_6; 10 mg Vitamin B_{12}; 1000 mg Folic acid; 9200 mg Pantotenic acid; 34,000 mg Nicotinic acid; 150 mg Biotin; 48,000 mg Manganese; 48,000 mg Zinc;16,000 mg Iron; 10,000 mg Cooper; 800 mg Iodine; 120 mg Selenium.

Zn. The ETP had higher Na content compared with other pea products.

As expected, the pH value of the ETP did not vary with time (4.5 ± 0.14). Due to the FE process, the concentration of acetic acid, L-lactate, D-lactate and ammonium in fermented pea (**FEP**) were remarkably higher than NP, which led to reduction in pH from 6.38 down to 4.5 ± 0.09 within 48 h incubation time.

Enzymatic treatment and fermentation of pea caused a slight increase in S-NSP (11.0% and 17.6%, respectively) and a slight reduction in I-NSP (12.6% and 19.3%, respectively) and TDF (18.0% and 22.5%, respectively), with reductions being more pronounced for the FEP. Furthermore, a remarkable decrease in RS was caused by FE and ET (more than 75% reduction).

The ETP had approximately 25% (0.50 TIU mg/g) lower TIA than the NP (0.67 TIU mg/g), while this reduction was 19.4% for FEP (0.54 TIU mg/g). The RE content of pea was reduced by 59.1% in the FEP (from 97.87 mol/g down to 40.03 mol/g) and by 50.0% in the ETP (down to 48.9 mol/g). Enzymatic treatment degraded 49% of total phytate in NP, whereas the reduction by FE process was only of 16.8% (0.92 g phytate/100 g NP).

3.2. Standardized ileal nutrient digestibility

The SID of nutrients is presented in Table 5. The ETP had better ($P \leq 0.05$) SID of CP, Glu, Phe and Val (0.97, 0.98, 0.98 and 0.96) compared with FEP (0.92, 0.94, 0.93 and 0.91) and NP (0.91, 0.93, 0.92 and 0.89). Enzymatic treatment of pea also ameliorated ($P \leq 0.05$) standardized ileal digestibility of Ala, Gly, His, Ilu, Leu and Lys (0.97, 0.96, 0.98, 0.97, 0.98 and 0.98 vs. 0.90, 0.92, 0.92, 0.90, 0.91 and 0.92), however standardized ileal digestibility of these nutrients in fermented pea were similar to other two types of pea ($P > 0.05$). Enzymatic treatment and fermentation of NP remarkably improved ileal digestibility of starch ($P \leq 0.05$).

3.3. Turkey growth performance and health

Performance variables are presented in Table 6. At the end of the pre-starter period (d 1–28), NPD and FEPD groups had the highest daily body weight gain (**dBWG**) and CON group had the lowest ($P \leq 0.05$), while birds received NPD diet had the highest

Table 4
Ingredients (g/kg, unless noted) and calculated nutrient composition of turkey diets[a] for growth performance trial.

Experimental groups[b]	CON	NPD	FEPD	ETPD	CON	NPD	FEPD	ETPD	CON	NPD	FEPD	ETPD	CON	NPD	FEPD	ETPD
Experimental period	D 1–28				D 29–56				D 57–84				D 85–105			
Ingredients (g/kg)																
Wheat	473.2	405.8	408.3	406.7	502.5	435.1	437.5	436.0	608.7	541.3	543.8	542.2	683.9	616.4	618.9	617.4
Soybean meal	458.7	429.9	427.5	429.1	409.3	380.5	378.1	379.7	299.0	270.2	267.8	269.4	231.2	202.4	200.0	201.6
Pea products[b]	–	100	100	100	–	100	100	100	–	100	100	100	–	100	100	100
Soybean oil	17.89	14.64	14.29	14.54	43.66	40.41	40.07	40.31	52.91	49.67	49.32	49.57	52.80	49.56	49.21	49.46
$NaSO_4$	1.50	1.50	1.50	1.50	2.25	2.25	2.25	2.25	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
NaCl	1.99	2.01	2.01	2.01	2.31	2.33	2.33	2.33	1.35	1.37	1.37	1.37	1.40	1.42	1.43	1.43
Limestone	16.15	16.21	16.23	16.22	12.13	12.19	12.20	12.20	13.81	13.87	13.89	13.88	12.02	12.08	12.10	12.09
MCP	17.40	17.57	17.58	17.58	15.44	15.60	15.62	15.61	12.23	12.40	12.41	12.40	9.28	9.45	9.46	9.45
DL-Met	3.01	3.00	3.03	3.01	2.44	2.43	2.46	2.44	1.75	1.73	1.77	1.75	1.23	1.21	1.24	1.22
L-Lys	4.25	3.58	3.73	3.54	4.23	3.57	3.72	3.53	3.87	3.20	3.36	3.16	2.86	2.19	2.35	2.15
L-Thr	0.83	0.72	0.78	0.72	0.68	0.58	0.64	0.58	0.74	0.64	0.70	0.64	0.25	0.15	0.21	0.15
Premix[c,d]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.00	4.00	4.00	4.00	3.50	3.50	3.50	3.50
Ronozyme® P[e]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated Nutrient Composition (g/kg, unless noted)																
Crude protein	265	265	265	265	245	245	245	245	205	205	205	205	180	180	180	180
AME (kcal/kg)	2700	2700	2700	2700	2900	2900	2900	2900	3050	3050	3050	3050	3100	3100	3100	3100
Dig. Met + Cys	10.2	10.2	10.2	10.2	9.2	9.2	9.2	9.2	7.7	7.7	7.7	7.7	6.7	6.7	6.7	6.7
Dig. Lys	15.6	15.6	15.6	15.6	14.5	14.5	14.5	14.5	11.9	11.9	11.9	11.9	9.7	9.7	9.7	9.7
Dig. Thr	9.2	9.2	9.2	9.2	8.4	8.4	8.4	8.4	7.1	7.1	7.1	7.1	5.8	5.8	5.8	5.8
Ca	12.0	12.0	12.0	12.0	10.5	10.5	10.5	10.5	9.5	9.5	9.5	9.5	8.0	8.0	8.0	8.0
P total	7.9	7.9	7.9	7.9	7.3	7.3	7.3	7.3	6.4	6.4	6.4	6.4	5.6	5.6	5.6	5.6
P available	5.5	5.5	5.5	5.5	5.0	5.0	5.0	5.0	4.2	4.2	4.2	4.2	3.5	3.5	3.5	3.5
Na	1.5	1.5	1.5	1.5	1.3	1.3	1.3	1.3	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2

[a] As-fed basis.
[b] CON = Control diet without pea products, NPD = Contained 100 g/kg native pea, FEPD = Contained 100 g/kg fermented pea, ETPD = Contained 100 g/kg enzymatically treated pea.
[c] Content per kg premix for weeks 1–8: 5,000,000 IU Vitamin A; 1,330,000 IU Vitamin D_3; 670,000 IU Vitamin D_3 HyD; 40,000 mg Vitamin E; 1600 mg Vitamin K_3; 1800 mg Vitamin B_1; 6000 mg Vitamin B_2; 2000 mg Vitamin B_6; 16 mg Vitamin B_{12}; 1400 mg Folic acid; 11,200 mg Pantotenic acid; 44,000 mg Nicotinic acid; 150 mg Biotin; 64,000 mg Manganese; 64,000 mg Zinc; 32,000 mg Iron; 10,000 mg Cooper; 1000 mg Iodine; 120 mg Selenium.
[d] Content per kg premix for weeks 9–15: 3,840,000 IU Vitamin A; 1,920,000 IU Vitamin D_3; 24,000 mg Vitamin E; 1200 mg Vitamin K_3; 800 mg Vitamin B_1; 4800 mg Vitamin B_2; 2000 mg Vitamin B_6; 10 mg Vitamin B_{12}; 1000 mg Folic acid; 9200 mg Pantotenic acid; 34,000 mg Nicotinic acid; 150 mg Biotin; 48,000 mg Manganese; 48,000 mg Zinc;16,000 mg Iron; 10,000 mg Cooper; 800 mg Iodine; 120 mg Selenium.
[e] Ronozyme® P5000 (DSM, Switzerland).

Table 5
Standardized ileal nutrient (unless noted) digestibility (mean[1] values) of pea products for turkeys (84 d old).

Experimental groups[2]	NP	FEP	ETP	SEM	*P* value
Crude protein	0.91[b]	0.92[b]	0.97[a]	0.010	0.018
Starch[3]	0.85[b]	0.94[a]	0.95[a]	1.183	< 0.001
Ala	0.90[b]	0.92[ab]	0.97[a]	0.012	0.029
Arg	0.96	0.97	0.99	0.006	0.058
Glu	0.93[b]	0.94[b]	0.98[a]	0.008	0.014
Gly	0.92[b]	0.92[ab]	0.96[a]	0.009	0.038
His	0.92[b]	0.95[ab]	0.98[a]	0.010	0.020
Ilu	0.90[b]	0.93[ab]	0.97[a]	0.012	0.008
Leu	0.91[b]	0.94[ab]	0.98[a]	0.010	0.014
Lys	0.92[b]	0.94[ab]	0.98[a]	0.009	0.025
Phe	0.92[b]	0.93[b]	0.98[a]	0.010	0.011
Pro	0.95	0.97	0.97	0.005	0.212
Ser	0.93	0.93	0.97	0.008	0.083
Thr	0.91	0.90	0.94	0.009	0.134
Val	0.89[b]	0.91[b]	0.96[a]	0.011	0.009

[a,b]Means of each main factors with different superscripts in a row differ significantly ($P \leq 0.05$).
[1] Data are means of 6 replicate pens. Pooled digesta of 12 birds per pen.
[2] NP = Native pea, FEP = Fermented pea, ETP = Enzymatically treated pea, SEM = Pooled standard of mean.
[3] Ileal starch digestibility.

daily feed intake (**dFI**). Birds fed FEPD diet had higher dFI compared with CON group ($P \leq 0.05$). Birds in the ETPD group had similar dFI to CON and FEPD groups ($P > 0.05$). Turkeys in FEPD group showed better FCR compared with CON and ETPD groups ($P \leq 0.05$).

Table 6
Effect of the experimental diets on performance variables (mean[1] values).

Experimental groups[2]		CON	NPD	FEPD	ETPD	SEM	*P* value
D 1–28	dBWG (g)	38.8^{c}	41.3^{a}	40.7^{a}	39.8^{b}	0.20	0.001
	dFI (g)	53.1^{c}	55.7^{a}	54.5^{b}	54.1^{bc}	0.24	0.001
	FCR	1.37^{a}	1.35^{ab}	1.34^{b}	1.37^{a}	0.004	0.022
D 29–56	dBWG (g)	100.9^{b}	104.1^{ab}	107.3^{a}	107.1^{a}	0.93	0.044
	dFI (g)	168.7^{b}	178.5^{a}	181.1^{a}	176.7^{ab}	1.52	0.021
	FCR	1.66^{c}	1.72^{a}	1.69^{b}	1.65^{c}	0.005	0.001
D 57–84	dBWG (g)	135.2^{b}	133.3^{bc}	132.0^{c}	139.8^{a}	0.61	0.001
	dFI (g)	310.6	319.1	309.8	319.6	1.79	0.085
	FCR	2.27^{b}	2.40^{a}	2.35^{a}	2.25^{b}	0.012	0.001
D 85–105	dBWG (g)	131.0^{a}	128.9^{a}	127.4^{ab}	122.7^{b}	0.97	0.015
	dFI (g)	406.9	409.2	405.1	408.3	2.80	0.962
	FCR	3.11^{c}	3.23^{ab}	3.20^{bc}	3.31^{a}	0.018	0.001
D 1–105	dBWG (g)	99.5	100.1	100.0	101.0	0.32	0.452
	dFI (g)	246.1	239	238.3	240.1	1.95	0.485
	FCR	2.21^{c}	2.28^{a}	2.25^{b}	2.21^{c}	0.007	0.001

[a,b]Means of each main factors with different superscripts in a row differ significantly ($P \leq 0.05$).
[1]Data are means of 15 replicate pens with 16 birds per pen.
[2]CON = Control wheat-soybean diet without pea products, NPD = Wheat-soybean diet with 100 g/kg native pea, FEPD = Wheat-soybean diet with 100 g/kg fermented pea, ETPD = Wheat-soybean diet with 100 g/kg enzymatically treated pea, SEM = Pooled standard error of mean.
[3]FCR = feed conversion ratio (g of feed intake/g of body weight gain), dFI = daily feed intake (g), dBWG = daily body weight gain (g).

Table 7
Effect of the experimental diets on DM of excreta and footpad dermatitis score (mean[1] values).

Experimental groups[2]	CON	NPD	FEPD	ETPD	SEM	*P* value
Excreta DM on d 28 (%)	20.6	21.2	20.6	19.9	0.27	0.395
Excreta DM on d 56 (%)	22.0	20.3	20.2	20.6	0.47	0.525
Excreta DM on d 84 (%)	21.3	20.7	21.4	20.4	0.27	0.537
Excreta DM on d 105 (%)	21.9	20.7	20.7	20.5	0.22	0.078
Footpad Dermatitis Score	1.37^{b}	1.94^{c}	1.52^{b}	0.36^{a}	0.057	0.001

[a,b]Means of each main factors with different superscripts in a row differ significantly ($P \leq 0.05$).
[1] Data are means of 15 replicate pens with 16 birds per pen.
[2] CON = Control wheat-soybean diet without pea products, NPD = Wheat-soybean diet with 100 g/kg native pea, FEPD = Wheat-soybean diet with 100 g/kg fermented pea, ETPD = Wheat-soybean diet with 100 g/kg enzymatically treated pea, SEM = Pooled standard error of mean.

For the starter period (d 29–56), birds fed diets with processed pea had better dBWG compared with those fed CON diet ($P \leq 0.05$), whilst birds fed NPD and FEPD diets had better dFI than those in CON group. In this experimental period, CON and ETPD groups displayed better FCR compared with other two groups and NPD group had the worst FCR ($P \leq 0.05$).

Birds fed ETPD diet had the best dBWG and, together with CON group, had better FCR compared with two other groups during the grower (d 57–84) period ($P \leq 0.05$). Birds in FEPD group gained less weight compared with those in CON and ETPD groups while NPD group had similar dBWG to CON and FEPD groups. For the finisher (d 85–105) period, birds fed CON and NPD diets showed better dBWG compared with those fed ETPD diet. Turkeys fed diet without pea products had better FCR compared with NPD and ETPD groups and FCR of birds in ETPD group was worse than those in FEPD group ($P \leq 0.05$). At the end of the experiment, CON and ETPD groups displayed the best FCR and birds received NPD diet showed the worst one ($P \leq 0.05$), while there were no differences in dBWG of birds in different experimental groups ($P > 0.05$). The growth performance data of the grower (d 57–84), finisher (d 85–105) and whole grow-out (d 1–105) periods showed no differences in dFI ($P > 0.05$).

There were no differences in excreta DM of turkeys received different experimental diets on d 28, 56, 84 and 105, although a trend observed on d 105 with numerically reduced DM in groups fed pea products, particularly ETPD group ($P \leq 0.10$). Birds in ETPD group had the best footpad dermatitis score and turkey in the NPD group showed the worst score ($P \leq 0.05$). The footpad dermatitis scores for turkeys in CON and FEPD groups were identical and considerably different from those in ETPD and NPD groups ($P \leq 0.05$). The data is presented in Table 7.

4. Discussion

Due to the technical relevance and feasibility of FE process for improving nutritional quality of plant derived feed ingredients e.g. legumes, this type of processing has been used for decades by food and feed industry. Exogenous enzymes, mainly with bacterial origin, are routinely used in poultry diets to improve the nutritional quality of feed by overcoming the negative impacts of ANF substances. The main limiting factors for optimal enzyme functionality in poultry nutrition were reported to be the short passage rate

of feed, and the variable pH which these enzymes encounter in different segments of the GIT (Adeola and Cowieson, 2011).

The native and enzymatically treated peas used in the present study were from the same batches of products, which have been used in the previous broiler study conducted by Goodarzi Boroojeni et al. (2017). In this study, yellow pea was fermented by *Bacillus subtilis* (GalliPro®, EU authorized probiotic to be used in broiler feed), while in the present study the same yellow pea was fermented by a mixture of *Bacillus licheniformis* and *Bacillus subtilis* (BIOPLUS 2B®, EU authorized probiotic to be used in turkey feed). Comparing the results of these two studies shows that FE processing of pea by a mixture of *Bacillus licheniformis* and *Bacillus subtilis* could cause more reduction in insoluble-NSP (−19.7% vs. −1.9%), and TDF (−22.5% vs. −10.8%) compared with when pea was fermented using only *Bacillus subtilis*. For TIA in pea, it seems to be other way around and the magnitude of reduction was considerably higher when pea was fermented by only *Bacillus subtilis* (−65.7% vs. −19.4%). Reduction in phytate content (−16.8%) of fermented pea was similar to what has been observed in the previous broiler study with using only *Bacillus subtilis* (−16.4%). The observed alterations in phytate, RE, I-NSP and TDF in the present and previous studies can be explained by activation of microbial enzymes (from native flora of pea as well as *Bacillus licheniformis* and *Bacillus subtilis*) and pea's endogenous enzymes. Furthermore, the acidic pH-value during both type of processes might have induced an increase in activity of endogenous amylase and phytase (Selle et al., 2000; Adeola and Cowieson, 2011). Similar to the previous broiler study, FE process could not modify the AA content of the peas (except a slight increase in CP). Regarding CF (Table 1) and RS (Table 2) in pea products, FE process by a mixture of *Bacillus licheniformis* and *subtilis* reduced CF (−46%) and RS (−76%) content to the same extent as FE by only *Bacillus subtilis* (−46% and −78%, respectively) and ET (−46% and −75%, respectively) did in the previous study (Goodarzi Boroojeni et al., 2017). This outcome may confirm that the CF reduction in both fermented products as well as enzymatically treated pea was due to the endogenous lipase activity of pea itself. This is in agreement with other report showing a distinct reduction of CF concentration in fermented soybean due to an increase in lipase activity of its native flora during FE processing (Ruiz-Teran and Owens, 1996). In both, the present and previous studies, the reason for observed minor increases in the concentration of analyzed minerals by FE and ET is unclear. These types of processes in lab scale did not lead to any changes in ash content of peas (unpublished data). Given the volume of water used for dough production, these minor increases might be justified by mineral content of the water in the production site and also wear of metallic pieces in the dryer used for drying the products (Goodarzi Boroojeni et al., 2017). It has been discussed before that the wear of metallic pieces in hydrothermal processing machines can cause an increase in mineral content of hydrothermally processed feed (Goodarzi Boroojeni et al., 2016).

In the present study, FE and ET of peas drastically increased ileal digestibility of starch. The considerable improvement in ileal starch digestibility might be explained by the reduction in RS content as well as starch swelling and gelatinization that might have happened during processing (incubation in a moist environment) and drying (Goodarzi Boroojeni et al., 2017). Enzymatically treated pea showed higher SID for most of AAs compared with NP, while these values for FEP were mainly in between two other types of peas. The increases in SID of AAs by ET process could be explained by observed reduction in ANF as well as degradation of complex material, leading to better availability of AAs in the ETP. The more pronounce improvements in SID of AAs by ET process compared with FE process, may also be explained by the extra time enzymes have had during digestion process in the GIT of turkeys. In the previous broiler study, 10, 20 and 30% of the required CP of broiler diets were supplied by either NP, FEP or ETP (Goodarzi Boroojeni et al., 2017). The diets were fed to broilers for 35 d. There were no remarkable differences in AID of CP, AAs and minerals for broiler received diets containing these three types of pea products (Goodarzi Boroojeni et al., 2017).

In general, in the most time periods of the growth performance trial, birds received ETPD or FEPD diets showed better growth performance than those fed NPD diet, while birds in ETPD group displayed similar growth performance to those fed diets with only SBM (CON group). At the end of the trial (d 105), birds fed CON and ETPD diets had the best FCR and birds offered NPD diet had the worst one ($P \leq 0.05$). It has been shown before that broilers fed diets containing FEP and ETP had lower FI after d 21 compared with those fed diets containing NP ($P \leq 0.05$), whereas broilers fed diet containing ETP had the best FCR compared with other two groups for the grower (d 22–35) and whole grow-out (d 1–35) experimental periods (Goodarzi Boroojeni et al., 2017). In the current study, the lower concentrations of ANF and better nutrient digestibility for ETP seem to be the main reasons for the superior growth performance of birds received ETPD diets. When barley was treated with a mixture of protease, xylanase, and β-glucanase and then used as a substitute for native barley in broiler diets, it could remarkably improve BWG and FCR in broiler chicks (Svihus et al., 1997). In contrast, replacement of native brewers' spent grains by enzymatically treated ones (with xylanase) in broiler diets had no beneficial impact on BWG and FCR (Denstadli et al., 2010). Broiler chickens received fermented SBM (with *Aspergillus oryzae*) as a full replacement for native SBM displayed better FI, BWG and FCR (Feng et al., 2007). Feeding broilers with diets containing 10% fermented RSM (with *Lactobacillus fermentum, Enterococcus faecium, Saccharomyces cerevisiae* and *Bacillus subtilis*) improved BWG and FCR compared with when birds fed diets containing 10% native RSM (Chiang et al., 2010).

Footpad dermatitis is a very common incidence in turkey farms using high amount of SBM. Footpad dermatitis, in addition to affecting the animal welfare status, has a considerable negative impact on farmer's income due to the downgrades and condemnations of saleable turkey paws (Shepherd and Fairchild, 2010). The most well-known reason for footpad dermatitis is wet litter and one the main causes for wet litter in turkey production is high amount of ANF, particularly NSP, in the diets (Mayne, 2005; Shepherd and Fairchild, 2010). In the present study, there were no differences in DM content of excreta thus, the observed improvement in footpad dermatitis score of ETPD group seems to be not because of improvement in litter quality. A study showed that adding NSP-degrading enzymes to maize-SBM broiler diets could lead to a lower incidence of mild footpad dermatitis with no effect on the moisture level of litter (Nagaraj et al., 2007a). It has been also reported that some of the sticky NSPs (not specified) in the ingredients of poultry feed, primarily SBM, could be caustic and contribute to footpad dermatitis (Hess et al., 2004; Shepherd and Fairchild, 2010). Therefore, it can be speculated that the added enzymes during ET process, on top of improving nutritional quality and nutrient digestibility of pea, had an extra counteracting impact (mainly acting in the turkey GIT) on ANF, especially NSPs, of the whole ETPD diet, which might

have caused reduction in concentration of certain caustic NSPs of the complete diet. On the other hand, it has been discussed that, besides NSPs, several other factors such as level and source of dietary protein can affect the incidence of footpad dermatitis in broilers (Nagaraj et al., 2007b). Therefore, observed improvements in SID of protein and AAs by enzymatic treatment can be another reason for better footpad dermatitis score of turkeys fed ETPD diet.

It is noteworthy that although applied processes, especially ET process, led to considerable improvements in nutrient digestibility and, subsequently growth performance and footpad dermatitis score, but these improvements did not seem to compensate for production costs of the prototype pea products (FEP and ETP). Thus, in order to practically use ETP and FEP as partial replacements for SBM in poultry feed, these processes need to be optimized (from engineering and practical perspectives) and become competitive and cost effective in large scale production setups.

5. Conclusion

Both of applied processes could modify the nutritional quality of pea by reduction in ANF and enhancing ileal starch digestibility. Moreover, ET process drastically improved SID of CP and AAs in pea. Inclusion of ETP (100 g/kg) in turkey diets demonstrated neither positive nor negative impact on growth performance, while it remarkably improved footpad dermatitis score and animal welfare. Improvement in SID of protein and AAs in pea by ET process, might be the main reason for lower incidence of footpad dermatitis in turkeys fed ETPD diet. The present data shows the feasibility of these processes, particularly enzymatic treatment, for improving the nutritional quality of pea as a protein source for turkey nutrition and as a partial replacement for SBM.

Conflict of interest

The authors have no conflicts of interest to declare.

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