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Original article

Rumen digestion and intestinal nutrient flows in sheep consuming pea seeds: the effect of extrusion or chestnut tannin addition

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Abstract — Different treatments aimed at reducing rumen degradability of pea protein were evaluated by in situ and in vivo measurements of rumen and intestine digestion of proteins. Four fistulated sheep were used. Pea seed provided 50% of dietary crude protein (CP) and was used raw (RP), with chestnut tannin (RPT2, 20 g·kg^{-1} of pea CP; RPT3, 30 g·kg^{-1} of pea CP), and extruded (EP). Rumen in situ degradability of pea protein was decreased by extrusion (83.3% vs. 90.8%) but was not affected by tannin addition. In vivo tannin addition did not affect the organic matter (OM) and N apparent digestibility, at the level of the rumen, the intestine or the whole tract. Extrusion decreased the apparent digestion of OM in the rumen but increased it in the small intestine. Total tract OM digestibility was not affected. Duodenal flow of non-ammonia N (NAN) increased by 27% between the RP and EP diet. This increase was mainly related to an increase in non-microbial N flow. Small intestine NAN apparent digestibility was not affected by extrusion and the amount of NAN apparently digested in the small intestine increased by 23% between the RP and EP diet. A slight decrease in total tract N digestibility was observed with extrusion. The efficiency of microbial protein synthesis was increased by extrusion. Small intestine apparent digestion of amino acids was greater for EP than for RP, but the profile of apparently absorbed amino acids was not affected. This study showed that low doses of tannins (up to 30 g·kg^{-1} of pea CP, 15 g·kg^{-1} of dietary CP) were inefficient in decreasing protein rumen degradability, however, the extrusion treatment largely increased the nitrogenous value of the pea seeds.

pea / digestion / extrusion / tannin / ruminant

Résumé — **Dégradation ruminale et digestion intestinale de la graine de pois : effets de l'extrusion ou d'un ajout de tannins de châtaignier.** L'effet de différents traitements visant à diminuer la

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dégradabilité ruminale des protéines du pois a été évalué par des mesures in situ et in vivo de la digestion ruminale et intestinale. Quatre moutons fistulés ont été utilisés. La graine de pois constituait 50 % de la matière azotée totale (MAT) de la ration. Le pois a été distribué cru (RP), avec des tannins de châtaignier (RPT2, 2 % de la MAT du pois ; RPT3, 3 % de la MAT du pois) ou extrudé. La dégradabilité théorique du pois a diminué sous l'effet du traitement d'extrusion (83,3 % vs. 90,8 %), mais n'a pas été affectée par l'ajout de tannins. In vivo, l'addition de tannin n'a eu aucun effet sur les paramètres de la digestion de la matière organique (MO) et de l'N. L'extrusion a diminué la digestion apparente de la MO dans le rumen, mais celle-ci a augmenté dans l'intestin grêle. Ainsi la digestibilité de la MO dans l'ensemble du tube digestif n'a pas été modifiée. Le flux duodénal de NAN a augmenté de 27 % entre les régimes RP et EP. Cette augmentation s'explique essentiellement par une augmentation du flux de NAN non-microbien. La digestibilité intestinale apparente n'a pas été modifiée par l'extrusion et la quantité de NAN apparemment digérée dans l'intestin grêle a augmenté de 23 % entre les régimes RP et EP. Une légère diminution de la digestibilité totale de l'N a été observée avec le traitement d'extrusion. L'efficacité de la synthèse de protéines microbiennes a été supérieure avec le régime EP. La quantité d'acides aminés apparemment digérée dans l'intestin grêle a été plus élevée avec le régime EP qu'avec le régime RP, mais le profil des acides aminés apparemment absorbés n'a pas été modifié. Cette étude montre que des faibles doses de tannins (jusqu'à 3 % de la MAT du pois, ou 1,5 % de la MAT de la ration) sont inefficaces pour diminuer la dégradabilité ruminale de la graine de pois, par contre l'extrusion peut largement améliorer la valeur azotée de ce protéagineux.

pois / digestion / extrusion / tannin / ruminants

1. INTRODUCTION

In the context of the banning of the use of meat and bone meal and the request of consumers to avoid the use of genetically modified crops in animal feeding, European countries have to reconsider their strategy for protein supply. Thus, alternatives to imported soya-bean meal must be proposed. Pea seed, which contains 250 g·kg^{-1} dry matter (DM) of crude protein (CP), is a potentially interesting candidate. Indeed, the culture of leguminous seeds such as peas is best suited in the climatic conditions of the European countries. However, the high in situ rumen degradability of pea protein gives it a low nitrogenous value in the French feeding system for ruminants (PDI system [31]). Nevertheless production studies in lactating cows have shown that pea seeds can serve as a substitute for soya-bean meal (as the protein source) without an adverse effect on milk production [6, 7, 17, 26]. These data suggest that the nitrogenous value of pea seeds is underestimated in the PDI system. Even so, treatments that decrease the rumen protein degradability can help to increase the nitrogenous value of the pea seeds. Extrusion has been shown to reduce rumen degradability of the pea protein both in situ [32] and in vivo [13]. The addition of hydrolysable tannin to the seed could be an alternative way to reach this objective. Indeed, data obtained with low doses (about 4 to 20 g·kg^{-1} of feed CP), suggest that chestnut tannins are efficient in decreasing rumen protein degradability [9, 22].

The objective of the current experiment was to determine the effect of an extrusion treatment and of the addition of low doses of chestnut tannins on the in vivo protein rumen digestion and amino acid absorption in the small intestine of sheep.

2. MATERIALS AND METHODS

2.1. Animals, diets, and experimental design

Four mature Texel wethers (50 ± 2 kg BW) were used in a 4 × 4 Latin square design. The sheep were surgically fitted with a ruminal cannula (PVC; 60 mm o.d.), and T-shaped cannula with an oval barrel

Table I. Chemical composition of dietary ingredients (g·kg^{-1} DM unless otherwise stated).

	Hay	Raw pea	Extruded pea
Dry matter (DM, g·kg^{-1} wet weight)	893	889	909
Organic matter	911	964	963
Starch	-	445	441
Neutral-detergent fibre	626	106	110
Acid-detergent fibre	336	65	53
Crude protein	133	249	247

(silicone rubber, 327 mm^2 opening) in the proximal duodenum and distal ileum. A polyester surgical mesh (Mersutures TS55, Ethicon, Neuilly/Seine, France) connected to the barrel of the cannula was wrapped around the duodenum as described by Horigane et al. [18]. Surgical procedures and post-surgical care were conducted in accordance with national legislation on the care and use of laboratory animals. The sheep were housed in a room under continuous lighting with controlled temperature (18 to 22 °C). They were fed 1014 g DM·d^{-1} in two equal meals at 12-h intervals (09:00, 21:00 h). The four diets contained (% of DM) 66% chopped orchard grass hay and 34% concentrate based on pea seeds (*Pisum Sativum,* Solara). The chemical composition of the dietary ingredients is given in Table I. The diets were formulated in such a manner that pea seeds contributed approximately 50% of the CP in each diet. The diets differed according to the treatment applied to the pea seeds: raw pea with and without two levels of tannin addition, and extruded pea. Pea extrusion was performed on a single-screw extruder (18% H_2O at 185 °C, specific mechanical energy delivery = 102 Wh·kg^{-1}) after conditioning for 10 min at 130 °C. The tannin extract was obtained from the chestnut tree, it contained about 77% hydrolysable tannins (81% in the DM). The compositions (% of DM) of the four concentrates were as follows: Raw pea (RP): 99.45% raw pea and 0.55% vitamins and minerals; raw pea + tannin (2% of pea CP; RPT2): 98.79% raw pea, 0.66% tannin extract, and 0.55% vitamins and minerals; raw pea + tannin (3% of pea CP; RPT3): 98.46% raw pea, 0.99% tannin extract, and 0.55% vitamins and minerals; extruded pea (EP): 99.48% pea, 0.52% vitamins and minerals. In the four concentrates, the pea seed was ground (3-mm screen) before pelleting. The sheep had free access to water and a salt block. Each experimental period lasted 6 weeks. The sheep were allowed to adapt to the diet during the first 2 weeks of each period. During the last 4 wk of the period, feed intake was measured daily.

2.2. In situ incubation of pea

The ruminal degradability of the pea seeds in the different concentrates, was estimated by the in situ incubation technique, on four sheep receiving the diet containing the concentrate that was tested. Dacron bags (5 cm × 10 cm, 30 to 60 µm pore size; Ankom; Fairport; NY) with heat-sealed edges were used. The concentrates used in the different diets were coarsely rolled before filling the bags. Duplicate bags with approximately 3 g (DM basis) of concentrate were incubated in the rumen of each sheep for 2, 4, 8, 16, 24, or 48 h. All bags were introduced in the rumen before the morning meal (day 15). After removal from

the rumen, the bags were washed under cold tap water to remove particles adhering to the outside of the bags and were frozen (–18 °C). After thawing all the bags were machine-washed (3 × 5 min) in cold water, and dried (80 °C, 48 h, forced-air oven).

2.3. Site and extent of nutrient digestion in vivo

Solute and particle markers were ^{51}Cr-EDTA (25 μCi·d^{-1}/sheep), and ^{103}Ru-phenanthroline (6 μCi·d^{-1}/sheep), respectively. The two markers were infused continuously into the rumen, via separate tubes, at a rate of 100 mL·d^{-1}. Infusion started on day 22 with a priming dose (100 mL of the infused solution) and continued until the end of the digestive flow measurements (day 36).

Total tract digestibility and marker recovery were determined by the total collection of urine and faeces over 7 d (from day 27 to 33).

For the determination of microbial protein synthesis in the rumen, a continuous infusion of $(^{15}NH_4)_2SO_4$ (35 mg ^{15}N·d^{-1}/sheep) started day 31 (60 h before intestinal digesta sampling) and continued until the end of the digestive flow measurements.

During the last 3-d of marker infusion (day 34 to 36), 12 samples were taken from the duodenum and the ileum such that each 1 h interval of the 12 h feeding cycle was represented. Each duodenal (150 mL) and ileal (80 mL) digesta sample was immediately subsampled under thorough mixing. One fraction (40 mL) was kept frozen as whole digesta. A second one (40 mL) was squeezed dry through a nylon gauze (250 μm pore size). The filtrate and particulate matter fractions were frozen. For duodenal samples, the remaining fraction (70 mL) was frozen for bacterial separation. Fractions were pooled over sampling times per animal.

For bacterial separation, after thawing, duodenal samples were centrifuged at 800 × *g* for 10 min at 4 °C to remove feed particles. The supernatant was collected and centrifuged again at 800 × *g* for 10 min at 4 °C. The supernatant was then centrifuged at 27 000 × *g* for 20 min at 4 °C for precipitation of the bacteria. The bacterial pellet was washed with cold saline (9 g NaCl·L^{-1}), and centrifuged again. The bacterial pellet thus obtained was lyophilised.

2.4. Soluble N fractions in the rumen

On the day of rumen content sampling (day 41), a pulse dose of CrEDTA (30 mL, 2.77 g Cr·L^{-1}), was introduced in the rumen via the cannula 15 min before the morning meal. Rumen content samples (100 mL) were collected at 08:30, 09:30, 10:30, 11:30, 13:30, 15:30, and 18:30 h. Rumen contents were immediately strained through nylon gauze (250 μm) to remove the larger particles from the feed, the pH of the filtrate was recorded, and the samples were rapidly placed on ice. The samples were centrifuged (27 000 × *g* for 20 min at 4 °C) to remove small feed particles, bacteria and protozoa. The supernatant was subsampled for Cr (10 mL, stored at –18 °C), total-N (10 mL, stored at –18 °C), ammonia-N and volatile fatty acids (VFA; 10 mL + 1 mL H_3PO_4 5% w/v, stored at –18 °C). On the day following CrEDTA injection, one rumen content sample was collected at 13:00 h, and centrifuged as previously described for Cr analysis.

2.5. Intestinal digestibility

In situ N intestinal digestibility of raw and extruded pea seeds was estimated subsequently in a separated experiment by the mobile nylon bag technique. Because the addition of tannins had no effect on rumen degradability this treatment was not tested. Three sheep from the main experiment were used. They were fed the RP diet.

Feeds (RP and EP) were incubated in the rumen as described for ruminal degradability measurements. Residues were dried at 60 °C for 72 h, and ground through a 1-mm sieve. They were then pooled per feed and incubation time. A sample, composed of cumulative undegraded feed fractions flowing from the rumen, was constituted as proposed by Beckers et al. [5]. Mobile nylon bags (18 mm × 18 mm, 48 μm pore size) were filled with 120 mg of this mixture and were closed by heat-sealing (2 sets of 12 bags/feed). After the morning meal, the bags were introduced into the duodenum of the sheep at a rate of one bag every 15 min (4 bags of each feed in each sheep per day). Prior to their introduction, the bags were incubated for 1.5 h at 39 °C in a solution of pepsin- (2 $g \cdot L^{-1}$, Merck 2000 FIP-UIG) HCl 0.01N (pH 2.7). The ileal cannula were opened 3 h after the beginning of the introduction and the effluent was checked for bag recovery every 30 min. The recovered bags were immediately rinsed lightly under cold tap water and stored at –20 °C. After thawing, all the bags were washed (5 × 2 min) by a standardised shaking in 1-L flasks containing 500 mL of warm water (39 °C), and dried (80 °C, 48 h, forced-air oven).

2.6. Laboratory analysis

The DM (105 °C for 24 h), OM (ash: 550 °C for 6 h), starch [12] and N (Kjeldahl method) content were determined for feeds (fed and refusals), duodenal and ileal whole digesta and filtrate, faeces, and bacteria. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined on feed according to Goering and Van Soest [15]. Ammonia [33] concentration was determined in duodenal and ileal whole digesta and filtrate. The concentration of the markers (^{103}Ru, ^{51}Cr) in each fraction of duodenal and ileal digesta, faeces, urine and infusion solutions were determined simultaneously with a gamma counter (Minaxy γ 5500, Packard, Rungis, France). Amino acid (AA) analysis of the feeds, duodenal and ileal whole digesta and filtrate, and bacteria was determined with a Beckman Autoanalyser (Model 6300, Beckman instruments, Palo Alto, CA) after acid hydrolysis with 6N HCl for 24 h at 115 °C. For sulphur AA determination, the samples were oxygenated before hydrolysis. Nitrogen enrichment (^{15}N) was determined on the bacteria and duodenal whole digesta and filtrate by mass spectrophotometry. Before ^{15}N enrichment determination, ammonia (NH_3) was displaced from duodenal samples by adding an equal volume of saturated sodium tetraborate and heating at 95 °C for 24 h. Rumen fluid was analysed for VFA by gas chromatography [19], total N (Kjeldhal method), and NH_3 N [33]. Soluble N in raw and extruded pea was determined according to the procedure of Vérité and Demarquilly [29].

2.7. Calculations and statistics

Non-NH_3 N (NAN) was calculated as the difference between total N and NH_3 N. The pool size and passage rate (kp) of the rumen liquid were calculated from the decrease against time in Cr concentration within the rumen liquid phase. Rumen outflow of soluble NAN was calculated as an average soluble NAN concentration in the rumen over the feeding cycle (mg $N \cdot L^{-1}$) × the liquid outflow rate ($L \cdot d^{-1}$).

The double marker technique [11] was used to estimate intestinal DM flow. The 'true digesta' was mathematically reconstituted combining filtrate and digesta; the mean reconstitution factor (R) among the samples was –0.06 ± 0.07. Bacterial N flow at the duodenum was estimated from the bacterial and the duodenal digesta ^{15}N enrichment. The apparent disappearance from the small intestine was calculated as the difference between duodenal and ileal flow.

Rumen effective degradability (ED) of pea N was estimated from the kinetics of in situ degradation of nitrogenous fractions [25] assuming the outflow rate of particulate matter from the rumen to be 0.06 h^{-1} [30].

Data were analysed by ANOVA using the GLM procedure of SAS [27], with the animal, period and diet as factors. When the treatment effect was significant ($P < 0.05$) the means were compared by the Duncan multiple-range test.

3. RESULTS

Soluble N content of the pea seed was 73% of total N. It was slightly decreased by tannin addition (71 and 69% of total N for RPT2 and RPT3, respectively). With cooking-extrusion, it decreased to 21% of total N. The effects of the treatments on the characteristics of the N fraction of pea seeds are presented in Table II. The rumen in situ degradability of pea N was decreased by cooking-extrusion ($P < 0.05$) but not affected by tannin addition. Cooking-extrusion decreased ($P < 0.05$) the rapidly degraded fraction (a), but did not significantly affect the degradation rate (c) of the slowly degraded fraction (b).

Daily means of intra-ruminal traits are presented in Table III. Rumen VFA concentration and proportion, and pH were similar between the treatments. Total N in the rumen liquid phase was not affected by the treatments, however cooking-extrusion decreased NH_3 N concentration ($P < 0.01$) and increased soluble NAN concentration ($P < 0.05$). Soluble NAN also tended to increase with the highest level of tannins. Consequently, soluble NAN outflow from the rumen increased by about 1 g $N \cdot d^{-1}$ between RP and EP, and about 0.3 g $N \cdot d^{-1}$ between RP and RPT3.

The addition of tannins, irrespective of level, did not affect OM and N digestion along the digestive tract (Tabs. IV and V). Cooking-extrusion decreased rumen OM digestion ($P < 0.05$) but increased small intestinal OM digestion ($P < 0.05$). Total tract OM digestibility was not affected. Rumen starch digestibility was not affected by the treatments ($P > 0.10$) and was on average 87% (data not shown). Duodenal flow of NAN increased by 27% between RP and EP ($P < 0.05$). This increase was mainly related to an increase in non-microbial N flow ($P < 0.01$). Microbial N flow accounted for 69% and 62% of NAN flow to the duodenum with RP and EP respectively. Small intestine apparent NAN digestibility was

Table II. Effect of treatments on in situ degradation of pea N in the rumen.

	Diet[1]					
Item[2]	RP	EP	RPT2	RPT3	SEM[3]	*P*
a	71.0[a]	36.8[b]	74.8[a]	73.8[a]	9.0	0.043
b	28.5[b]	61.5[a]	24.3[b]	25.5[b]	9.0	0.045
c	0.14	0.26	0.10	0.09	0.06	0.209
ED, %	90.8[a]	83.3[b]	89.7[a]	88.8[a]	1.0	0.013

[1] RP = raw pea, EP = extruded pea, RPT2 and RPT3 = raw pea + 2 and 3% of tannins, respectively.
[2] a = the rapidly degraded fraction, b = slowly degraded fraction, c = rate of degradation of the b fraction (h^{-1}), ED = ruminal effective degradability (n = 4).
[3] SEM = standard error of the mean.

Table III. The effect of pea treatment on ruminal pH, total volatile fatty acid (VFA) concentration and molar proportions of individual VFA, ammonia N (NH_3 N) and soluble non-ammonia N (NAN) concentrations, and rumen turnover.

	Diet[1]					
Item	RP	EP	RPT2	RPT3	SEM[2]	*P*
pH	6.32	6.34	6.34	6.33	0.04	0.980
VFA, mM	112.5	102.2	102.9	99.6	2.69	0.332
Acetate, %	67.8	67.3	67.0	68.6	0.64	0.368
Propionate, %	17.9	18.8	16.9	16.3	0.54	0.159
Butyrate, %	10.5	10.6	11.9	11.6	0.31	0.108
Isobutyrate, %	1.21	1.29	1.40	1.18	0.05	0.622
Valerate, %	0.87	0.84	0.87	0.74	0.03	0.160
Isovalerate, %	1.38	1.23	1.61	1.16	0.10	0.490
Caproate, %	0.42	0.32	0.37	0.26	0.04	0.499
Soluble N, $g \cdot L^{-1}$	361	346	341	383	12.8	0.384
NH_3 N, $mg \cdot L^{-1}$	213[a]	131[b]	191[a]	208[a]	10.9	0.003
NAN, $mg \cdot L^{-1}$	148[b]	215[a]	150[b]	175[ab]	11.3	0.015
Rumen liquid						
Volume, L	5.4[b]	6.2[a]	5.4[b]	6.0[a]	0.20	0.019
Fractional passage rate, $\% \cdot h^{-1}$	8.38	8.34	9.01	8.15	0.35	0.665
Soluble NAN outflow, $g \cdot d^{-1}$	1.52[c]	2.57[a]	1.72[c]	2.00[b]	0.13	0.001

[1] RP = raw pea, EP = extruded pea, RPT2 and RPT3 = raw pea + 2 and 3% of tannins, respectively.
[2] SEM = standard error of the mean.

not affected by cooking-extrusion and the amount of NAN apparently digested in the small intestine increased by 23% between RP and EP. Nevertheless, a slight decrease in total tract N digestibility was observed with cooking-extrusion. The efficiency of microbial protein synthesis (expressed as a percentage of the OM apparently or truly digested in the rumen) was increased by cooking-extrusion ($P < 0.05$).

Amino acid compositions of dietary ingredients and duodenal bacteria are presented in Table VI. The effects of pea treatments on the duodenal flow of amino acids are presented in Table VII. Total amino acid flow to the duodenum increased by 33% between RP and EP. This increase was similar for all individual amino acids and no change in the amino acid profile was observed. Total amino acid flow to the duodenum was not affected by tannin addition, however a slight decrease in serine, proline, phenylalanine, lysine and histidine flow was observed between RP and RPT2 ($P < 0.05$). Small intestine apparent digestion of amino acids (total and individual) was increased ($P < 0.05$) by 31% between RP and EP (Tab. VIII), and no change in the profile of apparently absorbed amino acids was observed. The net disappearance of the amino acids from the small intestine were

Table IV. The effect of pea treatment on organic matter (OM) digestion in sheep (n = 4).

Item	Diet[1]				SEM[2]	*P*
	RP	EP	RPT2	RPT3		
OM intake, g·d^{-1}	928	923	922	927	4	0.700
OM apparently digested, g·d^{-1}						
Stomach	427^{a}	340^{b}	434^{a}	427^{a}	13.0	0.023
Small intestine	181^{b}	217^{a}	168^{b}	182^{b}	6.9	0.010
Large intestine	51	76	51	51	4.2	0.165
OM apparently digested, % of OM intake						
Stomach	46.0^{a}	36.8^{b}	47.0^{a}	46.1^{a}	1.36	0.023
Small intestine	19.5^{b}	23.5^{a}	18.2^{b}	19.7^{b}	0.78	0.011
Large intestine	5.5	8.2	5.6	5.5	0.45	0.155
Total tract	71.0	68.5	70.8	71.3	0.45	0.237

[1] RP = raw pea, EP = extruded pea, RPT2 and RPT3 = raw pea + 2 and 3% of tannins, respectively.
[2] SEM = standard error of the mean.

Table V. The effect of pea treatment on nitrogen digestion in sheep (n = 4).

Item[2]	Diet[1]				SEM[3]	*P*
	RP	EP	RPT2	RPT3		
N intake, g·d^{-1}	28.0	27.6	26.9	27.6	0.26	0.801
NAN at duodenum, g·d^{-1}	25.8^{b}	32.7^{a}	25.2^{b}	26.5^{b}	0.95	0.011
Microbial N, g·d^{-1}	17.8	20.3	17.8	18.9	0.50	0.396
Non microbial N, g·d^{-1}	8.0^{b}	12.5^{a}	7.4^{b}	7.6^{b}	0.64	0.004
NAN at ileum, g·d^{-1}	9.4^{b}	12.6^{a}	9.3^{b}	10.0^{b}	0.42	0.017
NAN apparently digested in the small intestine						
g·d^{-1}	16.4^{b}	20.1^{a}	15.9^{b}	16.5^{b}	0.65	0.019
% N intake	58.4^{b}	72.9^{a}	59.2^{b}	59.7^{b}	2.37	0.012
% NAN at duodenum	63.3	61.3	63.0	62.2	0.76	0.245
Apparent total tract N digestibility, %	72.5^{a}	66.8^{b}	72.2^{a}	72.8^{a}	0.76	0.013
Microbial protein synthesis						
g CP·kg^{-1} OMADR[3]	262^{b}	386^{a}	264^{b}	276^{b}	18.9	0.046
g CP·kg^{-1} OMTDR[4]	182^{b}	232^{a}	179^{b}	185^{b}	8.0	0.048

[1] RP = raw pea, EP = extruded pea, RPT2 and RPT3 = raw pea + 2 and 3% of tannins, respectively.
[2] NAN = non-ammonia N, OMADR = organic matter (OM) apparently digested in the rumen, OMTDR = OM truly digested in the rumen (OMADR corrected for microbial OM).
[3] SEM = standard error of the mean.

Table VI. Amino acid (AA) composition of pea, hay, and duodenal bacteria.

Item	Hay	Pea	Bacteria
		g·100 g^{-1} of AA	
Aspartic acid	10.15	11.84	11.47
Threonine	5.90	4.56	6.13
Serine	4.59	4.67	4.60
Glutamic acid	11.64	17.5	12.83
Proline	5.46	4.05	3.59
Glycine	5.74	4.26	5.37
Alanine	7.08	4.30	7.32
Valine	5.83	4.19	6.10
Cysteine	7.64	5.57	6.99
Methionine	2.60	1.59	2.29
Isoleucine	4.34	3.62	4.65
Leucine	8.69	7.12	7.70
Tyrosine	3.27	3.24	3.76
Phenylalanine	5.48	4.59	3.91
Lysine	5.06	7.11	6.79
Histidine	1.64	2.20	1.72
Arginine	4.88	9.58	4.79
Total AA (g·kg^{-1} DM)	116	249	333

similar with or without tannins, even if a small decrease was observed between RP and RPT2 for some amino acids (valine, phenylalanine, histidine). The apparent digestibility of amino acids in the small intestine was not affected by the treatments, and was on average 69%.

In situ intestinal digestibility of rumen undegraded pea N was 91% and 98% for RP and EP, respectively.

4. DISCUSSION

4.1. Effects of tannins

The effect of condensed tannins in ruminant nutrition has been widely studied (cf. review Barry and McNabb [3]).

Concentrations of total condensed tannins in temperate forages required to decrease rumen digestion of feed protein and increase the absorption of essential amino

Table VII. The effect of pea treatment on amino acid (AA) duodenal flow (g·d^{-1}).

Item	Diet[1]				SEM[2]	*P*
	RP	EP	RPT2	RPT3		
Total flows						
Aspartic acid	15.41^{b}	21.03^{a}	14.59^{b}	15.70^{b}	0.252	0.001
Threonine	7.97^{b}	10.38^{a}	7.68^{b}	8.27^{b}	0.130	0.003
Serine	6.74^{bc}	8.71^{a}	6.50^{c}	7.02^{b}	0.066	0.001
Glutamic acid	18.48^{b}	25.18^{a}	17.20^{b}	18.79^{b}	0.291	0.001
Proline	5.72^{bc}	7.67^{a}	5.16^{c}	5.82^{b}	0.109	0.002
Glycine	7.46^{b}	9.76^{a}	7.03^{b}	7.50^{b}	0.107	0.001
Alanine	9.50^{b}	12.65^{a}	9.33^{b}	9.90^{b}	0.117	0.001
Valine	8.78^{b}	10.99^{a}	8.22^{b}	8.68^{b}	0.137	0.003
Cysteine	8.49^{b}	12.02^{a}	8.47^{b}	8.96^{b}	0.264	0.016
Methionine	2.74^{b}	3.51^{a}	2.63^{b}	2.90^{b}	0.049	0.005
Isoleucine	6.71^{b}	8.89^{a}	6.25^{b}	6.79^{b}	0.178	0.013
Leucine	11.92^{b}	15.61^{a}	11.06^{b}	11.97^{b}	0.208	0.003
Tyrosine	5.47^{b}	7.25^{a}	4.91^{b}	5.41^{b}	0.158	0.016
Phenylalanine	7.12^{b}	9.27^{a}	6.26^{c}	6.90^{b}	0.105	0.001
Lysine	9.23^{bc}	11.72^{a}	8.81^{c}	9.71^{b}	0.133	0.003
Histidine	2.79^{b}	3.64^{a}	2.47^{c}	2.73^{b}	0.038	0.001
Arginine	7.18^{b}	9.84^{a}	6.70^{b}	7.17^{b}	0.146	0.002
Total AA	141.7^{b}	188.1^{a}	133.3^{b}	144.2^{b}	1.847	0.001
Essential AA	65.7^{bc}	86.0^{a}	61.9^{c}	66.9^{b}	0.759	0.001
Bacterial						
Total AA	90.2	102.6	90.2	95.7	2.358	0.394
Essential AA	41.7	47.4	41.7	44.3	1.100	0.403
Dietary + endogenous						
Total AA	51.5^{b}	85.6^{a}	43.1^{b}	48.5^{b}	3.184	0.018
Essential AA	24.0^{b}	38.6^{a}	20.1^{b}	22.7^{b}	1.323	0.015

[1] RP = raw pea, EP = extruded pea, RPT2 and RPT3 = raw pea + 2 and 3% of tannins, respectively.
[2] SEM = standard error of the mean.

acids from the small intestine range from 30 to 40 g·kg^{-1} DM (about 100 to 150 g·kg^{-1} CP). Low doses of condensed tannins (1 to 2 g·kg^{-1} DM) appeared inefficient in modifying these parameters of N digestion; however, from 5 g·kg^{-1} DM, condensed tannins may prevent bloat in ruminants grazing forage [3].

The use of hydrolysable tannins to reduce rumen protein degradability is less

Table VIII. The effect of pea treatment on amino acid (AA) apparent disappearance from the small intestine (g·d^{-1}).

	Diet[1]					
Item	RP	EP	RPT2	RPT3	SEM[2]	*P*
Aspartic acid	11.03 [b]	14.02 [a]	10.23 [b]	10.87 [b]	0.241	0.011
Threonine	5.08 [b]	6.77 [a]	4.84 [b]	5.04 [b]	0.161	0.032
Serine	4.43 [b]	5.69 [a]	4.16 [b]	4.34 [b]	0.107	0.017
Glutamic acid	12.92 [b]	17.13 [a]	11.78 [b]	12.62 [b]	0.264	0.004
Proline	3.55 [b]	5.00 [a]	3.08 [b]	3.50 [b]	0.113	0.009
Glycine	5.07 [b]	6.57 [a]	4.66 [b]	4.86 [b]	0.111	0.007
Alanine	6.31 [b]	8.42 [a]	6.00 [b]	6.24 [b]	0.147	0.007
Valine	6.45 [b]	7.98 [a]	5.80 [c]	6.06 [bc]	0.110	0.004
Cysteine	5.06 [b]	7.30 [a]	4.70 [b]	4.84 [b]	0.202	0.020
Methionine	1.86 [b]	2.30 [a]	1.68 [b]	1.90 [b]	0.048	0.029
Isoleucine	5.03 [b]	6.81 [a]	4.54 [b]	4.95 [b]	0.179	0.026
Leucine	8.38 [b]	11.18 [a]	7.61 [b]	8.10 [b]	0.181	0.004
Tyrosine	4.20 [b]	5.67 [a]	3.69 [b]	4.02 [b]	0.151	0.027
Phenylalanine	5.31 [b]	7.04 [a]	4.56 [c]	4.96 [bc]	0.094	0.001
Lysine	7.22 [b]	8.98 [a]	6.69 [b]	7.30 [b]	0.141	0.012
Histidine	2.03 [b]	2.65 [a]	1.78 [c]	1.94 [bc]	0.045	0.005
Arginine	5.70 [b]	7.90 [a]	5.23 [b]	5.45 [b]	0.155	0.006
Total AA	99.64 [b]	131.39 [a]	91.03 [b]	96.99 [b]	1.730	0.002
Essential AA	46.43 [b]	61.00 [a]	42.20 [c]	45.09 [b]	0.584	0.001

[1] RP = raw pea, EP = extruded pea, RPT2 and RPT3 = raw pea + 2 and 3% of tannins, respectively.
[2] SEM = standard error of the mean.

documented. With tannic acid, Driedger and Hatfield [10] observed in vitro a linear decrease in protein breakdown when added to soya-bean meal (SBM) at up to 100 g·kg^{-1} (about 200 g·kg^{-1} CP); beyond this level the effect of the addition of tannins became less significant. Similarly, using the in situ technique, Hervas et al. [16] observed a linear decrease of rumen N degradability of SBM by adding tannic acid up to 130 g·kg^{-1}, leading, at this level, to a 40% decrease in N degradability. In accordance with Driedger and Hatfield [10], beyond this level, the effect of tannin addition was less effective. Furthermore, above the dose of 100 g of tannins per kg of SBM, intestinal digestion was decreased.

The ability of chestnut tannins (hydrolysable tannins) to protect feed protein against rumen degradation was first studied by Zelter et al. [34]. The data reported showed that, in vitro, 80 g of tannins per kg of SBM blocked microbial protein degradation, and that the effectiveness of tannins was related to feed protein nature. Mathieu and Jouany [22] investigated low doses of tannins (< 80 g·kg^{-1}) in vitro, and observed that the fermentability of SBM nitrogen

exponentially decreased as tannin levels increased from 0 to 53 g of tannins per kg SBM. Optimum levels appeared to be about 10 g·kg^{-1} DM (20 g·kg^{-1} CP). Conversely, the in situ technique [9] showed that rumen degradability of the milled peanut cake (MPC) was not modified by the addition of tannins at up to 25 g·kg^{-1} DM (50 g·kg^{-1} CP), and a linear decrease was observed between 25 to 100 g·kg^{-1} DM. As reported for tannic acid (see above), at 100 g·kg^{-1} DM inclusion, the intestinal digestibility of feed nitrogen was significantly reduced. The discrepancy between the optimal dose of tannins that have to be used to protect proteins against degradation observed in vitro [22] and in situ [9] could originate from the characteristics of each technique. However the differences in the effectiveness of tannins according to protein source could also explain the difference in reported optimal doses. Indeed Zelter et al. [34] showed that the minimal level of chestnut tannins which blocks MPC protein breakdown was twice that of the SBM protein. In the present study, 2 levels of chestnut tannins close to the optimal dose observed in vitro [22] were chosen, because it has been observed in vivo that the addition of a very low level of chestnut tannins (4 g·kg^{-1} of dietary CP) are sufficient to affect the rumen digestion of nitrogen [9]. With these levels of tannins (20 and 30 g·kg^{-1} of pea CP), in agreement with previous observations on MPC [9], the in situ rumen degradability was not affected but N solubility of the treated pea tended to decrease (–5% with RPT3). Tannins in the present work accounted for 10 and 15 g·kg^{-1} of dietary CP. Rumen concentration and outflow of NAN in the liquid phase increased with the highest level of tannins suggesting a slight decrease in protein degradation rate. However, OM and NAN flows to the duodenum and the ileum were not significantly affected by tannin addition. These data contrast with those of Decruyenaere et al. [9] who reported in steers a 10% increase in duodenal NAN flow with only 4 g of tannin per kg of the diet CP (grass silage). Here again an interaction between feed protein characteristics and tannin effectiveness may be involved. Nevertheless, in the present study the addition of low levels of tannins did not decrease the in situ degradability of the pea and did not significantly affect the N digestion of the diet.

4.2. Effects of extrusion

Pea N solubility was severely decreased (–70%) by cooking-extrusion. The in situ rumen N degradability was also decreased but to a lower extent (–10%). With the exception of Petit et al. [26], a decrease in rumen pea N degradability in response to extrusion was generally reported [2, 32].

Cooking-extrusion of pea seeds produced a 45% increase in soluble NAN concentration in the rumen fluid, and decreased rumen NH3 N concentration, suggesting that pea proteins were less readily degraded, and accumulate in the rumen. This observation contrasts with the data of Aufrère et al. [2], who observed a decrease in soluble NAN outflow from the rumen after pea extrusion (Baccara variety).

Focant et al. [13] reported that in heifers, pea extrusion increased the duodenal flow of NAN, but only 25% was linked to feed N flow increase and 75% was from a higher bacterial N flow. In the present study cooking-extrusion also increased duodenal flow of NAN, but 65% of the increase resulted from non-microbial N flow. Nevertheless in both studies the efficiency of bacterial protein synthesis was increased by the extrusion treatment. It can be speculated that this effect was related to a slow down in the pea protein degradation rate induced by the extrusion, which led to a more constant availability of nitrogen for microbial synthesis. Indeed, not only was the average concentration of soluble NAN in the rumen higher for the extruded than for the raw pea, but also this soluble NAN concentration was nearly constant throughout the feeding cycle with

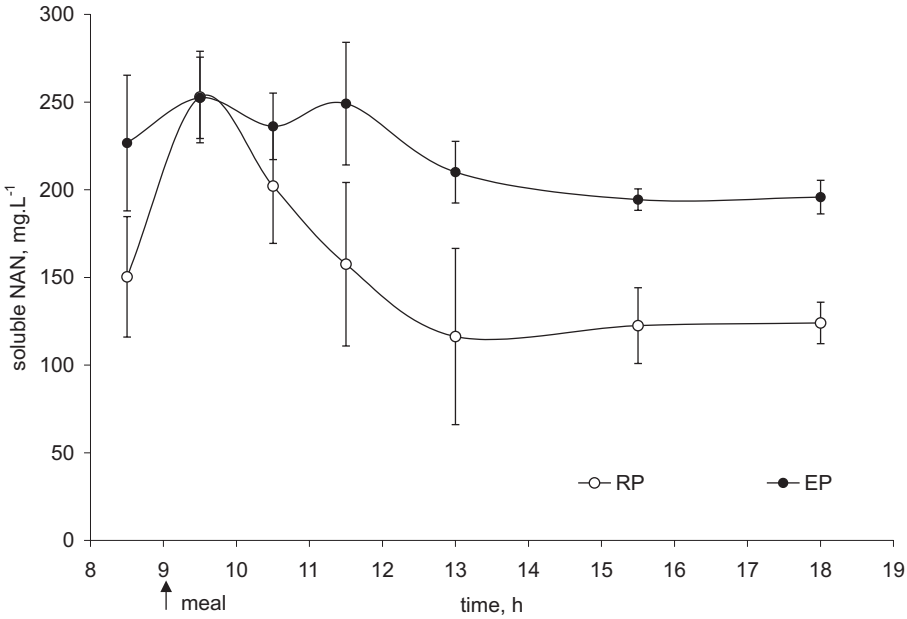

Figure 1. The effect of pea treatment on soluble non-ammonia N (NAN) concentration in the rumen fluid of sheep (n = 4) fed raw pea (RP) or extruded pea (EP).

the extruded pea, conversely to what was observed with the raw pea (Fig. 1).

Assuming an in vivo rumen degradability of hay of 73% [23], and an endogenous flow of N at the duodenum of 1.5 $g \cdot d^{-1}$, in vivo rumen degradability of pea N was estimated to be 80 and 47% for the raw and extruded pea respectively. These values were lower than those obtained in situ (90.8 and 83.3% for raw and extruded respectively). This discrepancy could be explained by pea protein losses through the nylon bags. Indeed it was shown that these losses of particulate N is high for the raw pea compared to other feedstuffs [24]. Furthermore, some soluble pea proteins (or large peptides) could transiently survive in the rumen [28] and be washed out into the duodenum with the liquid phase. The large difference that was observed between the in situ and in vivo measurement of extruded pea N degradability is difficult to explain. However, this suggests that the in situ technique is not adapted to test the effect of treatments on pea seed degradability. Indeed, with these seeds, N disappearance from the bags seems mainly related to losses of particulate and soluble N, and therefore does not provide accurate information on the resistance of proteins to rumen degradation.

Cooking-extrusion of pea produced an overall increase in the amino acid flow to the duodenum. However, the increase in the proportion of dietary N in NAN flow was not large enough to alter the AA profile at the duodenal level.

Intestinal digestibility of the rumen undegraded N of raw pea (91%) observed in the present experiment is in agreement with Frydrich [14] and Masoero et al. [21] (both reported a value of 92%), but higher than that retained by Verité et al. [30] in the PDI system (protein digested in the small intestine). It was greater for extruded pea (98 vs.

91%). This finding concurs with observations on cottonseeds [1] and lupin seeds [8].

4.3. Nitrogenous value of pea seed

The PDIA value (dietary protein digested in the small intestine [31]) of raw and extruded pea, can be calculated from the in vivo rumen degradability of pea N, and the in situ intestinal digestibility of pea N observed in the present work.

The PDIA value of raw pea thus obtained was higher than that retained in the PDI system (45 vs. 23 $g \cdot kg^{-1}$ DM). This underestimation originates both from an overestimation of rumen degradability of pea N by the in situ technique, and from an underestimation of intestinal digestibility of rumen undegraded pea N. Cooking-extrusion raised the PDIA value of pea up to 128 $g \cdot kg^{-1}$ DM. When it is expressed as a percentage of the CP content of the feed, the PDIA value of the extruded pea was higher than that calculated for soya-bean meal in the PDI system (50 vs. 38%). As for PDIA, the PDIN value (PDIA plus protein digested in the small intestine supplied by microbial protein from the rumen-degraded protein [31]) can be calculated from the experimental set; it was 160 and 195 $g \cdot kg^{-1}$ DM for raw and extruded peas respectively. Using the microbial protein synthesis efficiency observed in the present work (174 and 207 g $CP \cdot kg^{-1}$ of fermentable organic matter, with RP and EP), the PDIE value (PDIA plus protein digested in the small intestine supplied by microbial protein from the rumen fermented OM [31]) was 129 and 230 $g \cdot kg^{-1}$ DM for the raw and extruded peas, respectively. Both the PDIN and PDIE value of the raw pea in the present experiment (160 and 129 $g \cdot kg^{-1}$ DM) were higher than those reported in the PDI system (155 and 100 $g \cdot kg^{-1}$ DM) but concur with those estimated by Cabon et al. [6] in a production trial (165 and 135 $g \cdot kg^{-1}$ DM).

These data strongly suggest that the nitrogenous value of pea seeds is actually underestimated in the PDI system. The extent of this underestimation could be even greater with other varieties of peas such as Baccara or Madria, for which lower rumen N degradability has been reported [2, 20]. Furthermore, in the present experiment pea was ground (3-mm screen) and pelleted, generating a small size of particles, which probably enhances the rumen degradability of the pea [4, 20]. Indeed, in production trials suggesting that the pea can serve as a substitute for SBM, pea was simply cracked [17, 26].

5. CONCLUSION

This study showed that the in situ technique overestimates the rumen degradability of the pea seed. Nevertheless this degradability remains high and strategies must be proposed to improve the nitrogenous value of this leguminous seed. The low doses of tannins tested in the present study were inefficient in decreasing the pea rumen degradability. However, because of the easiness of tannin use, such treatments should not be abandoned and higher levels of tannin addition should be tested. The extrusion treatment was very efficient in protecting pea protein against rumen degradation. It raised the nitrogenous value of the pea seed to a level comparable to that of soya-bean meal.

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