

Evaluation of Protein and Starch Digestibilities and Energy Value of Pelleted or Unpelleted Pea Seeds from Winter or Spring Cultivars in Adult and Young Chickens

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Protein and starch digestibilities and apparent metabolizable energy values corrected to zero nitrogen retention (AMEn) were measured for pea mature seeds (*Pisum sativum*) from winter (Frisson) and spring (Finale) cultivars in 3-week-old chickens and adult cockerels. Pea seeds were either ground or ground, pelleted, and then reground. For young birds compared to adults the AMEn values of peas were similar, the pea starch digestibility was slightly lower, and the apparent digestibility of pea proteins was slightly higher. AMEn values, starch digestibility, and apparent protein digestibility were in general higher for the spring cultivar than for the winter one. Pelleting compared to grinding of peas induced positive effects, with the main effects being on AMEn values and starch digestibility. A sample of excreta from young birds fed on unpelleted winter pea diet was fractionated into four fractions differing in particle size. Coarse particles (>0.5 mm) of excreta contained the major part (73%) of undigested starch.

INTRODUCTION

According to Conan and Carré (1989), nutritional quality of pea seeds given to chickens can vary greatly between cultivars. Moreover, it was previously shown (Carré et al., 1987) that steam pelleting could improve the nutritional quality of smooth pea seeds for adult cockerels. It is not known, however, whether the effect of pelleting on pea seeds depends on their original nutritional quality. The present experiment was designed to investigate the nutritional effect of pelleting on two samples of peas (from winter and spring cultivars) expected to differ in quality when unpelleted.

The effect of age of birds on digestibility of pea nutrients was also investigated because most previous studies investigating the effect of age were directed to mixed diets (Fisher and McNab, 1987), cereals (Sibbald et al., 1960; Mollah et al., 1983), or fats (Kussaibati et al., 1982) and not to leguminous seeds. In this study, the digestibility of pea nutrients was measured both in adult cockerels and in young chickens.

MATERIALS AND METHODS

Diets. Four pea diets were formulated by mixing equal amounts of basal fraction and pea seeds (Table I). The basal fraction consisted of corn, soybean meal, and soybean oil (Table I). The basal diet was made up from the basal fraction to which DL-methionine, salts, minerals, and vitamins were added. Similar levels of these latter components were introduced in both basal and pea diets. The smooth pea mature seeds (*Pisum sativum*) were either from the winter cultivar Frisson (diets WP) or from the spring cultivar Finale (diets SP) (Table I). The pea seeds were either ground (diets WP_{OP} and SP_{OP}) or ground, pelleted, and then reground (diets WP_{IP} and SP_{IP}) (Table I). Grinding was carried out by using a hammer mill fitted with a 2-mm sieve. Steam pelleting of ground peas was conducted by using small hole dies of 4-mm diameter and 30-mm length, with a flow rate of 2.47 (Frisson peas) or 2.85 kg/min (Finale peas). Temperatures

of peas were 75 °C for inlet and 81 (Frisson) or 76 °C (Finale) for outlet. Regrinding after pelleting was performed with a hammer mill fitted with a 2.5-mm sieve. The analysis of particle size distribution was measured as described previously (Lacassagne et al., 1988). The mean diameters (d_{50}) of pea particles were 0.435 (Frisson) and 0.434 mm (Finale) for unpelleted samples. For pelleted and reground samples the mean diameters were 0.412 (Frisson) and 0.507 mm (Finale). The geometric standard deviations were similar between samples, ranging from 2.2 to 2.4.

Experimental Procedures. The five experimental diets were given to 35 adult Rhode Island Red cockerels and to 70 male Shaver broiler chickens, 7 adult and 14 young birds per diet. Broiler chickens were 19 days old at the beginning of the balance period. Birds were placed in metal cages, with, respectively, one and two individuals per cage for adults and young birds. In addition, three young birds (Y₁₂₃) placed in a single cage received the WP_{OP} diet. The pooled excreta of these three birds were treated separately and were not taken into account in the statistical analyses (see below). Each cage was provided with feeder, drinker, and a plastic tray placed under each cage for excreta collection. Adults were housed in a ventilated room with 16 h of light/day. Conditions for young birds were the same, and in addition the room temperature was maintained at 24 °C.

Adult and young birds were respectively adapted to experimental diets for 66 and 72 h and then fasted for 23 and 17 h before the balance period. The balance period consisted of ad libitum feeding for 55 h followed by starvation for 23 h (adult) or 17 h (young). Excreta were collected daily during the balance period, immediately stored at -20 °C, freeze-dried, weighed, and ground (0.5 mm). Pooled excreta of the three young birds Y₁₂₃ fed on the WP_{OP} diet were immediately stored at 4 °C after each collection and treated 2 days after the end of the balance period as follows: excreta were weighed (672 g) and thoroughly mixed; 118.0 g of the mixture was freeze-dried, weighed, and ground (0.5 mm). Another sample (183.5 g) of the mixture was briefly defatted by suspending it in hexane for 30 min before filtering it through a glass crucible (porosity 4). This sample was then successively passed through 0.85-, 0.5-, and 0.25-mm screens with a flow of distilled water, so that four fractions were obtained which were defined by the following particle sizes >0.85 mm; >0.5 < 0.85 mm; >0.25 < 0.5 mm; <0.25 mm. The latter fraction comprised also the water-soluble fraction. The four fractions were freeze-dried, weighed, and ground (0.5 mm).

Gross energy of food and excreta was determined by using an

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Table I. Composition of Smooth Pea Seeds and Diets (All Values Are on Dry Matter Basis)

	winter smooth pea seeds (cv. Frisson)	spring smooth pea seeds (cv. Finale)	basal fraction	basal diet (B)	winter pea diets		spring pea diets	
					unpelleted peas (WP _{OP})	pelleted peas (WP _{IP})	unpelleted peas (SP _{OP})	pelleted peas (SP _{IP})
basal fraction, %				94.39	47.21	47.57	47.63	47.74
corn, %			61.15					
soybean meal, %			34.09					
soybean oil, %			4.76					
winter peas, %					47.14	46.76		
spring peas, %							46.70	46.58
DL-methionine, %				0.22	0.23	0.23	0.23	0.23
sodium chloride, %				0.45	0.45	0.45	0.45	0.45
calcium carbonate, %				1.68	1.69	1.70	1.70	1.70
dicalcium phosphate, %				2.25	2.26	2.27	2.27	2.28
mineral mixture, ^a %				0.11	0.11	0.11	0.11	0.11
vitamin mixture, ^a %				0.90	0.91	0.91	0.91	0.91
gross energy, ^b MJ/kg	18.25	18.42		19.06	18.30	18.28	18.33	18.29
crude protein, %	24.89	25.49		24.15	23.81	23.81	24.09	24.09
AMEn ^c (young), MJ/kg				13.12	11.75	12.53	12.08	12.58
AMEn (adult), MJ/kg				14.12	12.45	12.95	12.69	13.19
starch, %	46.31	48.06		43.31	43.49	43.48	44.30	44.29
water-insoluble cell wall, %	14.36	12.40		12.08	12.81	12.81	11.89	11.89
TUI ^d /mg								
without pelleting	9.72	3.47						
after pelleting	9.72	2.98						

^a Composition given by Lacassagne et al. (1988). ^b 1 MJ = 239 kcal. ^c Apparent metabolizable energy corrected to zero nitrogen retention. ^d Trypsin units inhibited.

Table II. Energy Value and Digestibility of Protein and Starch of Smooth Pea Seeds, in Young and Adult Cockerels [Means ($n = 7$) ± Standard Deviations]

		winter smooth pea seeds (cv. Frisson)		spring smooth pea seeds (cv. Finale)	
		ground	pelleted and reground	ground	pelleted and reground
AMEn, ^a MJ/kg of dry matter	young	10.86 ± 0.464	12.52 ± 0.284	11.56 ± 0.276	12.62 ± 0.439
	adult	11.28 ± 0.364	12.33 ± 0.251	11.77 ± 0.309	12.84 ± 0.155
starch digestibility, %	young	80.9 ± 2.37	95.7 ± 2.35	84.7 ± 1.26	95.0 ± 1.36
	adult	84.1 ± 2.93	96.3 ± 1.30	84.6 ± 1.55	96.9 ± 0.89
apparent protein digestibility, %	young	75.9 ± 4.79	80.8 ± 4.42	80.3 ± 3.85	83.9 ± 3.50
	adult	74.6 ± 4.34	71.9 ± 3.85	75.3 ± 3.35	81.7 ± 2.92

^a Apparent metabolizable energy values corrected to zero nitrogen retention.

adiabatic bomb calorimeter (Gallenkamp). Moisture and nitrogen contents were measured according to official European methods (BIPEA, 1976). Protein was measured in excreta according to the method of Terpstra and de Hart (1974). Starch was measured with the dimethyl sulfoxide (DMSO) procedure (Boehringer Mannheim, 1980) as described previously (Carré et al., 1987) with a slight modification: 2.8 mg of amyloglucosidase (EC 3.2.1.3.) from *Rhizopus mold* (Sigma A-7255, 10 units/mg) was used instead of the 20 mg of amyloglucosidase from *Aspergillus niger* (Boehringer Mannheim, W. Germany) to treat the suspension for 6 h. A blank value was run without sample for starch determination. The contents of the water-insoluble cell wall were measured as described previously (Carré and Brillouet, 1989). The number of trypsin units inhibited (TUI) per milligram was estimated according to the method of Kakade et al. (1974) modified by Valdebouze et al. (1980).

All chemical analyses performed on feeds were run in duplicate or triplicate. The apparent metabolizable energy values corrected to zero nitrogen retention (AMEn) were calculated as described by Hill and Anderson (1958). The AMEn and digestibility values assigned to the pea fractions were calculated by assuming additivity of values assigned to basal and pea fractions. It was assumed that methionine and vitamins provided 0.13 MJ of AMEn/kg of dry matter of diet. Standard deviations of values relative to pea fractions were calculated as described by Yoshida (1972).

Effects of age, cultivar, and pelleting on AMEn and digestibility values assigned to pea fractions were tested by a three-way analysis of variance (Snedecor, 1956). The statistical model used was

$$Y_{ijkl} = \mu + A_i + C_j + T_k + (AC)_{ij} + (AT)_{ik} + (CT)_{jk} + (ACT)_{ijk} + e_{ijkl}$$

where μ is the population mean, A is age ($i = 1, 2$), C is pea cultivars ($j = 1, 2$), T is technological treatment ($k = 1, 2$), and e is random error ($l = 1, 2, \dots, 7$ birds).

RESULTS

Effect of Age of Birds on Pea Energy and Nutrient Utilization. No significant effect of age was observed on the AMEn values of peas (Tables II and III). The pea starch digestibility tended to be slightly higher in adults than in young birds, but this significant effect resulted only from two pea samples, namely, the unpelleted winter peas and the pelleted spring peas (significant effect of the interaction age × cultivar × treatment, Table III). The apparent digestibility of pea proteins was significantly higher in young birds than in adults, with a more pronounced effect observed on pelleted winter peas and unpelleted spring peas (significant effect of the interaction age × cultivar × treatment, Table III).

Effect of Cultivars on Pea Energy and Nutrient Utilization. The AMEn values of the winter peas were in all cases lower than those of the spring peas with a mean difference of 0.45 MJ/kg of dry matter (Tables II and III). The significant effect of cultivar on pea starch digestibility was only due to the difference observed in young birds with unpelleted seeds (interaction age × cul-

Table III. Effects of Age, Cultivar, and Technological Treatment on Energy Value and Digestibility of Protein and Starch of Smooth Pea Seeds (Statistical Analysis of Table II)

	effects						
	age <i>F</i> ^a	cultivar <i>F</i>	technol treatment <i>F</i>	age × cultivar <i>F</i>	age × treatment <i>F</i>	cultivar × treatment <i>F</i>	age × cultivar × treatment <i>F</i>
AMEn	3.56 NS	25.95***	186.47***	0.35 NS	2.88 NS	2.73 NS	3.13 NS
starch digestibility	7.61**	4.70*	616.00***	0.94 NS	0.07 NS	4.88*	5.61*
apparent protein digestibility	17.16***	18.61***	8.64**	0.48 NS	1.28 NS	3.41 NS	6.21*

^a *F* = variance ratio = mean square of sample means/mean square of individuals (Snedecor, 1956). Degrees of freedom were 1,48 for all *F* values. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Table IV. Distribution of Dry Matter, Starch, and Protein in Fractions of Excreta Differing in Particle Size, Determined in Pooled Excreta of Three Young Chickens (Y₁₂₃) Fed on the WP_{OP} Diet

	feed intake	total output ^a	output in fractionated defatted excreta; particle size of fractions			
			<0.25 mm	>0.25 < 0.5 mm	>0.5 < 0.85 mm	>0.85 mm
dry matter, g	467	171	80.0 (52) ^b	19.6 (13)	29.5 (19)	24.8 (16)
starch, g	203	21.1	2.9 (16)	2.0 (11)	7.1 (38)	6.4 (35)
protein, g	111	24.4	14.1 (70)	2.4 (12)	2.2 (11)	1.4 (7)

^a Determined from an unfractionated sample of excreta. ^b Within parentheses, the outputs observed in fractionated excreta are expressed as percent of the total output determined from the fractionated samples of excreta.

tivar × treatment), the lowest value being obtained with the winter peas (Tables II and III). The apparent digestibility of pea protein was lower with winter cultivar than with spring cultivar, but this effect was not always consistent (interaction age × cultivar × treatment): a marked effect of cultivar appeared with pelleted seeds in adult birds, whereas the protein digestibility values were very similar between cultivars with unpelleted seeds in adult birds.

The apparent digestibility values of protein and starch observed in the three young birds Y₁₂₃ fed on the WP_{OP} diet were in the range of values found in the seven other replicates, with values of 78.0 and 89.6%, respectively (Table IV). The major portions of dry matter and protein of the fractionated excreta were found in the smallest particles (<0.5 mm). In contrast, starch occurred mainly in the coarse particles (>0.5 mm) of the fractionated excreta.

Effect of Pelleting on Pea Energy and Nutrient Utilization. Pelleting induced a strong positive effect both on AMEn values and on starch digestibility of peas. The positive effect of pelleting was much less pronounced on the apparent digestibility of pea protein and was more consistent in young than in adult bird (Tables II and III). When the analysis of variance was applied on data obtained with young birds only, the effects of technological treatment and cultivar on the apparent digestibility of pea protein were similar, with respective *F* variance ratios (Snedecor, 1956) of 7.27 (*P* < 0.05) and 5.66 (*P* < 0.05). No effect of the interaction technological treatment × cultivar (*F* = 0.17) on the apparent digestibility of pea protein was observed in young birds.

DISCUSSION

The starch and protein contents of the winter pea seeds (Table I) were similar to the values previously found (46.91 and 23.65% of the dry matter, respectively) on seeds from the same cultivar (Carré et al., 1987). The content of water-insoluble cell wall (WICW) found in the spring pea seeds was close to the value (13.1% of the dry matter) determined in a previous study (Carré and Brillouet, 1986). Both starch and protein contents were higher in the spring pea

seeds than in the winter pea seeds. Correlatively, WICW content was lower in the spring pea seeds than in the winter pea seeds. The difference in WICW content between the two cultivars (1.96% of the dry matter) was very close to the difference in the sum of protein and starch contents between cultivars (2.35% of the dry matter), suggesting that the WICW parameter can give an accurate prediction of available nutrient (mainly starch and protein) content in pea seeds.

Digestibility of pea starch tended to be lower in young than in adult cockerels, but the difference was only pronounced with unpelleted winter smooth peas (Tables II and III). The apparent protein digestibility of peas was higher in young birds than in adults (Tables II and III): higher apparent protein digestibility has often been observed in young birds (Håkansson and Eriksson, 1974; Fonolla et al., 1981; Hassan and Delpech, 1986), but the reason for this remains unknown. Håkansson and Eriksson (1974) suggested that the proportion of fecal protein precipitated with metal ions could differ between young and adult birds. Thus, the differences could be an artifact. But, even if higher apparent protein digestibility in young birds is a fact, it is not known whether this is due to better utilization of dietary proteins or lower endogenous (or microbial) protein losses.

Higher AMEn values for spring seeds compared to winter seeds are not surprising since the latter contained less protein and starch. However, protein and starch digestibility was also lower with winter seeds, especially in young birds. It is not excluded that the lower protein digestibility observed with winter seeds was due to their higher level of TUI (Table I). However, it has been previously observed (Carré and Conan, 1989) that no significant correlation existed between pea TUI level and pea protein digestibility measured in young birds. Moreover, pelleting increased protein digestibility (Tables II and III) without noticeable change in TUI level (Table I).

Significant correlation between protein and starch digestibility in young birds for nonpelleted peas was previously observed (Conan and Carré, 1989). The present data are in good agreement with the latter correlation as shown in Figure 1, where data from the present study and

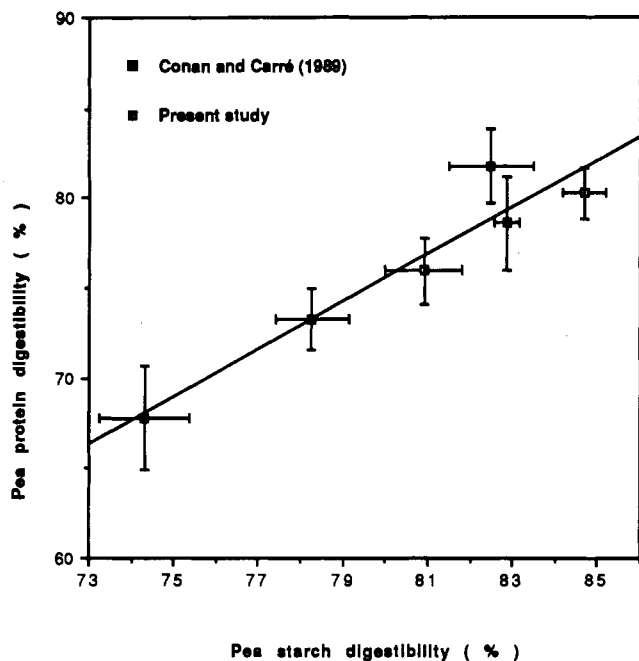


Figure 1. Relationship between the digestibility of starch and the digestibility of protein for unpeeled pea seeds in 3-week-old chickens (means and standard errors).

those from Conan and Carré (1989) are combined. This correlation ($R^2 = 0.886$, $P < 0.01$) suggests occurrence of a nonspecific factor able to act both on starch and on protein digestibility. Examination of starch distribution in excreta fractions differing in particle size (Table IV) strongly suggests nonaccessibility in coarse particles as the nonspecific factor. Protein did not display the same distribution compared to starch (Table IV) because of the superimposition of microbial proteins occurring in small particles (Parsons et al., 1982). Coarse particles of excreta (>0.5 mm) (Table IV) accounted for 6.65 and 3.24% of the ingested starch and protein, respectively. The latter percentages are roughly equivalent to the differences in starch and protein digestibility between basal diet and diet WP_{0P}.

Trypsin inhibitors and lectins are not likely to be the main limiting factors for pea utilization by poultry. It is probable that a low accessibility to nutrients due to strong cellular cohesion in pea cotyledons is a major limiting factor.

When data from the present experiment are pooled with those from Conan and Carré (1989), it appears that digestibility of protein and digestibility of starch from unpeeled peas for young birds are significantly correlated with the sum of protein and starch contents found in pea seeds (Figure 2). The values of R^2 for these correlations with protein and starch digestibilities were, respectively, 0.681 ($P < 0.05$) and 0.801 ($P < 0.05$). The sum of protein and starch contents is inversely correlated both with hull content and with cotyledon cell wall content (as reflected by NDF; Reichert and MacKenzie, 1982). Both hull content and cotyledon cell wall content may depend on some physical and histological features of the seeds such as seed size, cell size, cell filling, and cell wall thickness. It is possible that some histological features of the seeds influence characteristics of seed flours and affect nutrient availability. Beneficial effects of thermomechanical processes such as pelleting (Carré et al., 1987, and present work) or autoclaving (Conan and Carré, 1989) could be due to the breaking up of cell walls. These beneficial effects could also be due to the increase in the sensitivity of starch

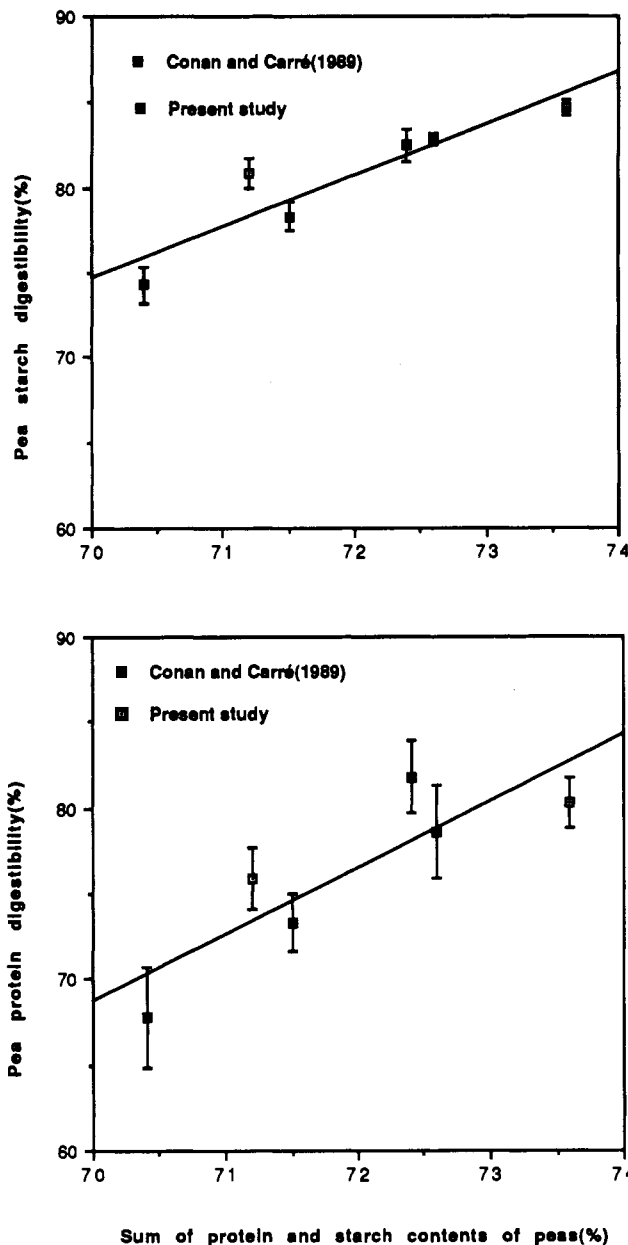


Figure 2. Digestibility of starch and protein for unpeeled pea seeds in 3-week-old chickens related to the sum of protein and starch contents of peas (means and standard errors).

and protein to enzyme degradation, thus reducing the consequences of low accessibility. In this field, it is pertinent to notice that Sugimoto (1980) observed pea starch granules are more resistant to pancreatin than those of corn.

Among the three factors investigated here, pelleting was the most effective for inducing change in pea AMEn and pea starch digestibility (Table III). An effect ($P < 0.01$) of cultivar on starch digestibility was observed with young birds for unpeeled peas. In contrast, this effect was not observed for pelleted peas. In a previous study involving adult cockerels (Carré et al., 1987), pea starch digestibility increased up to 96% with pelleting, a value very similar to that found in the present study (96.6%); however, the starch digestibility of unpeeled peas observed in the previous study (Carré et al., 1987) was 7% higher than in the present study. So, pelleting tends to reduce the variability of pea starch digestibility for poultry. If pelleted, peas would probably vary in their quality mainly because of variations in their nutrient contents and not because of variations in their nutrient digestibility. Fur-

ther research on the prediction of AMEn values of peas would be more meaningful using pelleted peas.

As an illustration of this, by use of the AMEn predicting equation based on gross energy, crude protein, and water-insoluble cell walls (Carré and Brillouet, 1989), the AMEn values of diets for adult cockerels were predicted accurately, except for diets containing unpelleted peas (WP_{OP} and SP_{OP}). For these two diets, the differences (0.88 MJ/kg) between predicted and measured AMEn values were outside the range of the residual confidence limit previously found (0.44 MJ/kg; Carré and Brillouet, 1989), whereas the differences for basal (0.08 MJ/kg) and pelleted pea diets (0.36 MJ/kg) were within the range of the residual confidence limit. The latter calculation also shows that the use of peas, if pelleted, leads to normal AMEn values for diets given to the chicken.

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