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METABOLIZABLE ENERGY AND AMINO ACID BIOAVAILABILITY OF FIELD PEA SEEDS IN BROILERS DIETS

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Abstract

The aim of this study was to determine the apparent (AME) & true (TME_n) metabolizable energy as well as the crude protein (CP) & amino acid (AA) total tract digestibility (bioavailability) of broilers diets and field pea seeds (FPS) of the Greek cultivar "Olympos".

Forty eight broilers (24 male & 24 female) were used. Birds were placed in individual cages in vertical decks of a digestibility chamber and randomly allocated into 4 dietary treatments. Each group was comprised of 6 male and 6 female broilers. Birds consumed 80 g/d of either a typical commercial diet or the same diet in which 100, 200 or 300 g/kg had been substituted by ground FPS. The experiment lasted 15d (counting from the 28th d of age). The first 4 days needed for the adaptation of birds in cages, 7 days was the duration of preliminary period and 4 days needed for the excreta collection. The endogenous nitrogen losses were determined by using an indigestible marker and this procedure lasted 28h.

Final BW of birds was significantly ($P<0.05$) reduced only in the treatment containing the highest rate of FPS inclusion. Apparent and true CP bioavailability of the diets were not significantly affected by FPS inclusion up to the level of 300 g/kg. Apparent and true CP bioavailability of FPS showed a linear trend of reduction by increasing inclusion rates of FPS and this reduction was significant ($P<0.05$) at the level of 300 g/kg. Amino acid bioavailability, as determined by the difference between the untreated diet's one and that containing 100 g FPS/kg, remained at high levels (~0.80), with the exception of methionine and valine and was similar to AA mean. The means AME and TME_n of FPS were estimated equal to 10.8 and 11.0 MJ ME/kg, respectively.

FPS "Olympos" are a valuable energy and protein source for broilers and could be included in their diets up to a level of 200 g/kg, without any adverse effect on the diets digestibility and broilers performance, contributing to natural feed resources exploitation.

Keywords: field pea seeds "Olympos", broilers, metabolizable energy, crude protein & amino acid bioavailability.

Abbreviations: FPS, field pea seeds; SBM, soybean meal; AME, apparent metabolizable energy; TME_n, true metabolizable energy corrected to zero nitrogen retention; CP, crude protein; AA, amino acids; CTTD, coefficient of total tract digestibility (bioavailability); ANFs, antinutritional factors.

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1. Introduction

Since the use of animal origin proteins in poultry diets was banned in the European Union (EU) in 2000, the role of soybean meal (SBM) in poultry nutrition became of major importance (Christodoulou *et al*., 2006; Diaz *et al*., 2006; Palander *et al*., 2006; Garsen *et al*.,

2008). Legume seeds are a valuable alternative to SBM energy and protein source and for that reason are used to a large extent in poultry nutrition (Gatel, 1994). Field pea seeds (FPS) (*Pisum sativum* L.) are one of the world's most important grain legumes (FAO, 1993; Wiseman *et al*., 2003). However, their energy value and crude protein (CP) & amino acid (AA) bioavailability varies and can be significantly affected by a number of factors. Crude fibre (CF), content in tannins and trypsin inhibitors activity among them play an important role (Igbasan *et al*., 1997; Wiseman *et al*., 2003; Kluth *et al*., 2005; Palander *et al*., 2006). Crude protein quality is affected to a large extent by its AA bioavailability. Because post-ileal fermentation contributes in variable degrees to AA excretion, it has often been argued that digestibility measured at the terminal ileum is more suitable for feed evaluation than measurements of total excretion (Ten Doeschate *et al*., 1993; Ravindran *et al*., 1999; Kadim *et al*., 2002; Kluth *et al*., 2005). However, for practical purposes, crude protein quality of a single feedstuff seems to be estimated satisfactorily by using the classical total tract (excreta collection) digestibility. Also, the nutritive value of a poultry feed is expressed by its energy content. The mean energy values (AME & TME_n) of FPS were estimated for adult broilers from 10.1 to 11.6 and 11.4 to 12.9, respectively (Brenes *et al*., 1993; Igbasan *et al*., 1997).

Field pea seeds are used to a significant degree in poultry nutrition in EU countries (FAO, 1993). However, the variability between cultivars in energy content, nutrients, antinutritional factors (ANFs), as well as the limited information concerning domestic (local) cultivars, suggests the need for further investigation.

The aim of this study was to estimate the nutritive value of a Greek cultivar of FPS by determining their AME & TME_n, as well as their CP and AA bioavailability, in broilers diets at inclusion rates of 100, 200 and 300 g/kg.

2. Materials and methods

2.1. Field pea seeds "Olympos"

Ground FPS (screen diameter <3.0 mm) of the Greek cultivar "Olympos" were used in this study. FPS "Olympos" have a deep green color and their content in ANFs is relatively low (2.4 mg/kg DM for total phenols -tannins and non tannins- and 2.90 TIU/mg DM).

In Table 2.1.1 is shown the content of FPS "Olympos" in nutrients, amino acids, total phenols and specific protein inhibitors.

2.2. Experimental diets, birds, housing and design

Forty eight (48) Cobb-500 broilers (24 male & 24 female) were used. Birds were placed in individual cages in vertical decks of a digestibility chamber and randomly allocated into four dietary treatments. Each group was comprised of 6 male and 6 female broilers. Birds consumed 80 g/d of either a typical commercial wheat-corn-soybean meal diet (13.2 MJ ME/kg, 224 g CP/kg) or the same diet in which 100, 200 or 300 g had been substituted by ground FPS (C, P_{100}, P_{200} & P_{300}, respectively). The experiment lasted 15d (counting from the 28^{th} d of age). The first 4 days needed for the adaptation of birds in cages, 7 days was the duration of preliminary period and 4 days needed for the excreta collection. Fe_2O_3 (3 g/kg) was used as indigestible marker. The procedure for the endogenous nitrogen losses estimation lasted 28h and was divided in 4 sub-periods, as following: Allowance of diet with marker for 4h, fasting period of 16h, allowance of N-free diet for 4h and finally re-allowance of diet with marker for another 4h. The composition of N-free diet was the following: sucrose (815 g/kg), corn seed oil (100 g/kg), ground wheat straw (30 g/kg) and vitamins & trace minerals premix (55 g/kg). In Tables 2.2.1 & 2.2.2 are shown the composition of experimental C & P_{100} diets in amino acids and the calculated (NRC, 1994) and chemically determined (Weende) analysis of experimental digestibility diets, respectively.

2.3. Calculations and determinations

During the experimental 4d-period the feed intake per bird was recorded daily as well as the quantity of excreta. Daily excreta of each bird were being weighted and then dried in an oven at 60 ^{o}C. The total dried excreta quantity of each bird was being mixed, ground (screen diameter 1.00 mm) and placed in an air-seal glass vial. Then, representative samples were given for the determination of energy content by using a Gallenkamp, Autobomb, automatic adiabatic bomb calorimeter, as well as for crude protein determination by using a VELP

Table 2.1.1. Content of FPS "Olympos" in nutrients, AA, tannins and TIU.

	Content			
	g/kg DM	**% of DM**	**g/16g N**	**mg/kg DM**
Nutrients				
Crude protein	248			
Ether extract	19			
Crude fibre	66			
Amino acids				
Aspartic acid		2.8	11.6	
Glutamic acid		4.4	17.7	
Serine		1.1	4.6	
Glycine		1.1	4.3	
Threonine		0.9	3.5	
Arginine		2.3	9.3	
Valine		1.1	4.3	
Phenylalanine		1.2	4.9	
Isoleucine		0.8	3.5	
Leucine		1.8	7.3	
Lysine		1.8	7.1	
Cystine		0.4	1.6	
Methionine		0.2	0.8	
Alanine		0.9	3.9	
Other nitrogenous ingredients		3.6	14.6	
Phenols				
Total	2.39			
Non-tannins	1.62			
Tannins	0.77			
TIU				2.90

Table 2.2.1. Composition of experimental C & P_{100} diets in AA (g/kg).

	Diets	
	Control - C	**100 g FPS/kg - P_{100}**
Amino acids		
Aspartic acid	13.2	14.8
Glutamic acid	44.0	42.0
Serine	9.0	9.2
Glycine	7.0	7.2
Threonine	8.7	8.9
Arginine	10.4	11.4
Valine	7.5	7.7
Phenylalanine	9.0	9.2
Isoleucine	6.6	7.0
Leucine	15.1	14.2
Lysine	13.8	14.0
Cystine	4.9	4.9
Methionine	4.9	4.8
Alanine	9.0	9.6

Table 2.2.2. Calculated (NRC, 1994) and chemically determined (Weende) composition of experimental digestibility diets.

	Treatments			
	C	**P_{100}**	**P_{200}**	**P_{300}**
Calculated composition (g/kg)				
Crude protein	226.2	225.4	223.6	222.4
Ether extract	66.7	62.6	58.4	53.4
Crude fibre	33.2	34.8	36.7	39.6
Ash	54.6	52.4	51.2	48.1
Ca	10.0	10.5	10.2	9.8
P (total)	6.9	6.9	7.0	6.7
Lysine	14.0	14.2	14.4	14.5
Methionine + cystine	10.0	9.8	10.2	10.4
ME (MJ/kg)	*13.17*	*12.97*	*12.76*	*12.55*
Chemical analysis (Weende)				
Dry matter	894.0	893.0	892.0	890.0
Crude protein	225.0	224.0	223.0	222.0
Ether extract	67.0	62.0	57.0	52.0
Crude fibre	32.0	35.0	37.0	40.0
Ash	55.0	52.0	50.0	47.0
Ca	10.0	9.8	9.7	9.4
P (total)	6.9	6.6	6.8	6.6

SCIENTIFICA UDK 132, SemiAutomatic Distillation Unit. The same calculations and determinations were being performed in ground FPS as well as in samples of the 4 experimental diets. The determination of amino acids in FPS, in diets C & P_{100} and in their derived excreta was performed by using an Eppendorf LC 3000 amino acid analyzer. Total phenols content of FPS was determined according to methods described by Terrill *et al*. (1990) and Makkar *et al*. (1993), whereas trypsin inhibitor activity according to analytical methods described by American Association of Cereal Chemists (1983).

Metabolizable energy (ME) of FPS was estimated by the method of difference. Crude protein and amino acids bioavailability were determined according to experimental procedure described by Bragg *et al*. (1969). Colorless excreta derived from N-free diet were used for the determination of endogenous nitrogen and amino acids.

The coefficient of apparent total tract digestibility of energy ($CATTD_{energy}$) of the diets was determined as:

$$CATTD_{energy} = \frac{GE_{diet} - GE_{excreta}}{GE_{diet}} \quad , \qquad \text{where GE: Gross Energy}$$

The coefficients of apparent total tract digestibility of CP and AA of the diets ($CATTD_{CP}$ and $CATTD_{AA}$) were determined by using similar equations. The coefficient of true total tract digestibility of energy ($CTTTD_{energy}$) of the diets was determined by the method of difference and by adding 4.1 kcal per each g of endogenous crude protein. The coefficients of true total tract digestibility of CP and AA ($CTTTD_{CP}$ and $CTTTD_{AA}$) were calculated as following:

$$CTTTD_{CP} = \frac{CP_{diet} - (CP_{ex} - CP_{en})}{CP_{diet}} , \qquad CTTTD_{AA} = \frac{AA_{diet} - (AA_{ex} - AA_{en})}{AA_{diet}}$$

where: CP_{diet}: crude protein in diet; CP_{ex}: crude protein in excreta; CP_{en}: endogenous crude protein

AA_{diet}: amino acids in diet; AA_{ex}: amino acids in excreta; AA_{en}: endogenous amino acids

The coefficients of apparent and true digestibility of energy, CP and AA of field pea seeds were calculated from those of diets by the method of difference.

2.4. Statistical analysis

All data obtained in this study were subjected into two factors factorial analysis (2×4, sex × diet) (Hinkle *et al*., 2003; SPSS rel. 15.0.0). For significant treatment effects or interactions, statistically significant differences among means were identified by using Duncan and Dunnett test. Differences between means were considered significant at $P<0.05$.

3. Results

Final BW of birds was significantly ($P<0.05$) reduced only in the treatment containing the highest rate of FPS inclusion (300 g/kg). Apparent and true (total tract) CP digestibility of the diets were not significantly affected by FPS inclusion up to the level of 300 g/kg (Table 3.1).

Table 3.1. Nitrogen balance[1] (g/bird/day) of experimental broilers diets.

		Treatments				SE	P
		C	P_{100}	P_{200}	P_{300}		
Parameters							
Number of birds[2] (n)		12	12	12	12	-	-
Feed intake (g/bird/day)		80	80	80	80	-	-
Dietary nitrogen (N)		2.880	2.870	2.850	2.840	-	-
N in excreta	M	0.483^{a}	0.488^{a}	0.507^{ab}	0.540^{b}	0.024	*
	F	0.529^{a}	0.534^{a}	0.553^{ab}	0.584^{b}	0.036	*
	T	**0.506^{a}**	**0.510^{a}**	**0.503^{a}**	**0.562^{b}**	**0.022**	*
Endogenous N	M	0.057^{a}	0.063^{ab}	0.072^{b}	0.073^{b}	0.014	*
	F	0.063^{a}	0.067^{a}	0.075^{b}	0.072^{b}	0.008	*
	T	**0.060^{a}**	**0.065^{ab}**	**0.073^{b}**	**0.072^{b}**	**0.007**	*
$CATTD_{CP}$[3]	M	0.832	0.830	0.822	0.810	0.020	NS
	F	0.816	0.814	0.806	0.794	0.022	NS
	T	**0.824**	**0.826**	**0.814**	**0.802**	**0.020**	**NS**
$CTTTD_{CP}$[4]	M	0.852	0.851	0.847	0.835	0.018	NS
	F	0.838	0.836	0.832	0.819	0.017	NS
	T	**0.845**	**0.843**	**0.839**	**0.827**	**0.016**	**NS**

[1] Means and standard error.
[2] Total number of birds per treatment (6 male, 6 female).
M: Male, F: Female, T: Total.
[3] $CATTD_{CP}$: Coefficient of apparent total tract digestibility of CP.
[4] $CTTTD_{CP}$: Coefficient of true total tract digestibility of CP.
[a, b] Means in the same row sharing a different superscript are significantly different ($P<0.05$).

Endogenous N showed a significant increase ($P<0.05$) by increasing rates of FPS inclusion (Table 3.2). Apparent and true (total tract) CP digestibility of FPS showed a linear trend of reduction by increasing inclusion rates of FPS and this reduction was significant ($P<0.05$) at the level of 300 g/kg. This linear tendency of reduction was detected in both male and female chicks. In all cases the mean values of coefficients of digestibility were higher but not significantly different in male than those in female chicks. No significant interactions were detected between diet × sex concerning $CATTD_{CP}$ and $CTTTD_{CP}$ of FPS.

In Table 3.3 is shown the apparent and true total tract digestibility (bioavailability) of AA of FPS as calculated by the difference from the respective values of diets C and P_{100}.

From this table it can be concluded that apparent and true bioavailability of AA remained at high levels (~0.80) with the exception of methionine and valine and was similar to AA mean.

Table 3.2. Nitrogen balance[1] (g/bird/day) of FPS in broilers diets.

Parameters		FPS inclusion (g/kg) 100	200	300	SE	P
Number of birds[2] (n)		12	12	12	-	-
Feed intake (g/bird/day)		8.00	16.00	24.00	-	-
Dietary nitrogen (N)		0.287	0.573	0.860	-	-
N in excreta	M	0.053^{a}	0.121^{b}	0.202^{c}	0.024	*
	F	0.058^{a}	0.130^{b}	0.214^{c}	0.028	*
	T	**0.055^{a}**	**0.125^{b}**	**0.208^{c}**	**0.020**	*
Endogenous N	M	0.012^{a}	0.026^{b}	0.032^{c}	0.002	*
	F	0.011^{a}	0.024^{b}	0.030^{c}	0.003	*
	T	**0.011^{a}**	**0.025^{b}**	**0.031^{c}**	**0.002**	*
$CATTD_{CP}$[3]	M	0.815^{a}	0.788^{ab}	0.765^{b}	0.028	*
	F	0.798^{a}	0.772^{ab}	0.751^{b}	0.024	*
	T	**0.806^{a}**	**0.780^{ab}**	**0.758^{b}**	**0.026**	*
$CTTTD_{CP}$[4]	M	0.857^{a}	0.834^{ab}	0.802^{b}	0.027	*
	F	0.836^{a}	0.815^{ab}	0.786^{b}	0.021	*
	T	**0.846^{a}**	**0.825^{ab}**	**0.794^{b}**	**0.022**	*

[1] Means and standard error.
[2] Total number of birds per treatment (6 male, 6 female).
M: Male, F: Female, T: Total.
[3] $CATTD_{CP}$: Coefficient of apparent total tract digestibility of CP.
[4] $CTTTD_{CP}$: Coefficient of true total tract digestibility of CP.
[a, b, c] Means in the same row sharing a different superscript are significantly different (P<0.05).

Table 3.3. Amino acids bioavailability of FPS.

Amino acids	Bioavailability Apparent	True
Aspartic acid	85.0	89.2
Glutamic acid	88.2	91.4
Serine	83.2	87.4
Glycine	85.0	89.0
Threonine	83.2	86.5
Arginine	88.6	92.8
Valine	43.6	54.5
Phenylalanine	82.9	87.7
Isoleucine	79.8	89.5
Leucine	85.8	90.6
Lysine	83.8	87.4
Cystine	78.5	85.4
Methionine	72.6	78.5
Alanine	82.0	88.5

The calculations and estimations concerning apparent and true metabolizable energy values of experimental diets and FPS (AME and TME_n, respectively) are shown in Table 3.4. From this table it can be conducted that AME of the diets showed a linear decrease which was significant in diets P_{200} & P_{300} as compared to P_{100} ones. This result was detected in both sexes as well as for the total of birds. The AME of FPS was significantly ($P<0.05$) affected by the rate of FPS inclusion and varied from 10.50 ± 0.13 to 11.21 ± 0.21 MJ/kg (mean 10.83 ± 0.17 MJ/kg). The TME_n values of FPS varied from 10.63 ± 0.23 to 11.36 ± 0.16 MJ/kg (mean 10.98 ± 0.20 MJ/kg) and were significantly affected by the rate of FPS inclusion. The mean AME and TME_n values were higher in male than female chicks. No significant interactions were observed between diet × sex.

Table 3.4. Apparent (AME) and true (TME_n) metabolizable energy[1] (kcal/kg) of experimental diets and FPS.

		Treatments				SE	P
		C	**P_{100}**	**P_{200}**	**P_{300}**		
Parameters							
Total number of birds[2] (n)		12	12	12	12	-	-
AME of diet	M	3113^{a}	3073^{ab}	3010^{b}	2938^{c}	42	*
	F	3081^{a}	3038^{ab}	2975^{b}	2914^{c}	38	*
	T	**3097^{a}**	**3055^{ab}**	**2993^{b}**	**2926^{c}**	**36**	*
AME of FPS	M		2710^{a}	2598^{b}	2530^{c}	45	*
	F		2650^{a}	2551^{b}	2490^{c}	42	*
	T		**2680^{a}**	**2575^{b}**	**2510^{c}**	**40**	*
			$\bar{x}$ = 2588 kcal or 10.83 MJ/kg				
TME_n of FPS	M		2748^{a}	2638^{b}	2564^{c}	58	*
	F		2685^{a}	2590^{b}	2522^{c}	62	*
	T		**2716^{a}**	**2614^{b}**	**2543^{c}**	**48**	*
			$\bar{x}$ = 2624 kcal or 10.98 MJ/kg				

[1] Means and standard error.
[2] Each group was consisted of 12 birds (6 male, 6 female).
M: Male, F: Female, T: Total.
[a, b, c] Means in the same row sharing a different superscript are significantly different ($P<0.05$).

4. Discussion

The results of this study concerning the $CATTD_{CP}$ values of FPS (mean 0.782) are in general agreement with those of other researchers who estimated values from 0.785 in adult layers (Gruhn & Zander, 1990) to 0.810 (Brenes *et al*., 1993) or slightly higher, as 0.816 (Dotas, 2006).

The mean values of apparent and true AA bioavailability of FPS determined in this study were 0.80 and 0.85 respectively and this result is in accordance to those of Castell *et al*. (1996) and Igbasan and Guenter (1996a & 1996b) who report mean values of AA apparent digestibility of FPS cultivars with deep green color equal to 0.79.

Our results concerning AME and TME_n values of FPS are in general agreement with those suggested in other researches (Reinchert & MacKenzie, 1982; Würzner *et al*., 1988; Carré *et al*., 1991; Brenes *et al*., 1993; Igbasan *et al*., 1997) in which the calculated values of AME varied from 10.23 to 11.59 MJ/kg.

5. Conclusion

Field pea seeds of the Greek cultivar "Olympos" are a valuable energy and protein source for broilers and could be included in their diets up to a level of 200 g/kg without any

adverse effects on the diets digestibility and broilers performance, contributing to natural feed resources exploitation and sustainable development.

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