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EFFECTS OF RAW SOYBEAN SEEDS LOW IN ANTINUTRITIONAL FACTORS ON GROWTH PERFORMANCE, CARCASS QUALITY AND NITROGEN EXCRETION OF HEAVY PIGS IN AN ORGANIC FARM

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SUMMARY

Forty eight pigs were reared in outdoor pens (1.2 ha of land surface) from 80 to 180 kg of live weight. Two isoenergetic (ME = 14.6 MJ/kg DM) and isonitrogenous (CP=17.0% DM) feeds were compared; the first feed contained 10% of toasted full-fat soybean of conventional cultivars (TS), in the second one the toasted soybean was replaced by raw full-fat soybean low in antinutritional factors (RS). The pigs were blocked in 4 groups of 12 animals each and fed ad libitum. At 0, 43, 106 days on trial pigs were weighted, back-fat thickness was measured and blood samples were collected. At slaughter, samples of ham cover fat were collected to evaluate their acidic profile. The effects of feeds on growth performance, slaughter parameters, metabolic profile and nitrogen excretion (Nex) were studied. Nex was computed as difference between consumption and retention. No significant effects were observed on growth performance, carcass quality and metabolic profile, but with RS plasma urea concentration significantly decreased with respect to TS (5.33 vs. 6.67 mmol/l; P<0.01), reflecting a lower N digestibility of RS. Results indicated that 10% of low antinutritional factors raw soybean can be used on heavy pigs without relevant effects on performance and carcass quality. N in manure averaged 22.8 kg/pig place/year (assuming 3 cycles/year). Caution should be taken in outdoor pig systems since the land is uncropped and the risks of pollution are high.

Key-words: pigs, soybean, trypsin inhibitor activity, blood urea, nitrogen excretion

INTRODUCTION

The EC 1804/99 regulation defined a list of feeds admitted in organic animal production. Since the solvent extracted soybean meal is not included in the list, alternative protein sources must be used for diet formulation of growing pigs. Full fat soybeans is an interesting feed resource but raw seeds produced on farm is not usually directly used because of their content of antinutritional factors (ANF), such as the trypsin inhibitors Kunitz and Bowman-Birk compounds (TI) (Huisman, 1994). To overcome these drawbacks, some cultivars low in Kuniz factors were selected and tested on growing pigs obtaining different effects on growth performance (Snidaro et al., 2001; Herkelman et al., 1992). The aims of this experiment were: i) to compare the chemical composition and the TI activity of some commercial cultivars of soybean; ii) to verify the effects of a cultivar of soybean low in TI on the growth performance of pigs, on the metabolic profile and on some carcass traits; iii) to quantify N excretion as a possible source of environmental pollution.

MATERIAL AND METHODS

Raw seeds of 3 conventional soybean cultivars (Dekagib, Cresir, Brillante) and 2 cultivars selected for a low content of TI (Hilario, Aires) were sampled from the market and sub-samples were autoclaved, to simulate toasting, for 20 min at 110 °C (Herkelman et al., 1992).

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Seeds of the cultivar Hilario were cropped and stored in the same farm where the following trial on growing pigs was performed. The trial was carried out in an organic farm between October 2005 and January 2006. Forty eight pigs (commercial hybrid, PIC Italia) were divided in 4 groups of 12 head each balanced for live weight (80 kg) and sex. Each group was reared in outdoor pen of 3000 m^2. Two groups received a diet containing 10% of toasted full-fat soybean of a mix of conventional cultivars (TS). In the diet received by the other groups the toasted soybean was replaced by raw seeds of the home produced Hilario (SIS, Bologna, I) cultivar (RS). The two diets were isoenergetic (ME=14.6 MJ/kg DM) and isonitrogenous (CP=17.0 % DM) (Table 1). Feeds and water were freely available. The amount of feed distributed to each group was daily recorded. At 0, 43 and 106 days on trial pigs were weighted and the thickness of back-fat at the last rib (P2) was measured with a Renco lean-meter (Renco Corporation SpA, Minnesota, USA). At the same time blood samples were collected from 6 pigs randomly selected within each group. At 109 days of the trial all the pigs were slaughtered and carcass, kidneys and liver were weighted. Samples of ham cover fat (at level of Femoral Biceps) were also collected, at 24 h post mortem to evaluate their acidic profile.

Table 1. Formulation and chemical composition of the diets

Ingredients, % of feed	TS	RS	Chemical analysis	TS	RS
- Wheat middlings	40	40	- Dry matter, (DM)	89.44	89.91
- Barley grain	25	25	- Crude protein, % DM	16.87	17.11
- Toasted full-fat soybean	10	-	- Lysine, % DM	0.83	0.84
- Raw full-fat soybean	-	10	- Lipids, % DM	5.43	5.36
- Maize grain	10	10	- Crude fibre, % DM	6.31	6.17
- Alfalfa dehydrated hay	5	5	- Ash, % DM	8.10	7.89
- Pea seeds	5	5	- Linoleic acid, % DM	2.85	2.62
- Calcium carbonate	1.8	1.8	- ME, MJ/kg DM	14.6	14.6
- Dicalcium phosphate	0.6	0.6	- SFA, % Lipids	18.74	19.59
- Sodium chloride	0.3	0.3	- MUFA, % Lipids	19.86	19.71
- Yeast	1.3	1.3	- PUFA, % Lipids	61.40	60.70

TS: toasted soybean diet; RS: raw soybean diet; ME: metabolizable energy; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids;

Raw and autoclaved seeds, as well as the complete feeds (TS and RS), were analysed in duplicate for DM, CP, lipids, ash and crude fibre (AOAC, 2000). Lipids were extracted according to Folch et al. (1957) procedure and fatty acids were separated by gas chromatography (model 8000 serial Top, CE instruments). Urease activity was assessed by the method NGD B12 (1976). TI activity was determined as suggested by Krishnan (2001), in quadruplicate. Blood metabolic profile was performed with automatic equipment Hitachi 911 (Roche Diagnostics) following official procedures. Nitrogen (N) excretion was computed as difference between N consumption and N retention as suggested by ERM (2001). N excretion was expressed per pig produced, per pig place/year and per hectare of land. To compute N in manure, volatile N losses were assumed to be 25% of N excreted (ERM, 2001) and the number of pigs equivalent to 170 kg/year of N in manure was computed. Data were subjected to ANOVA (SAS, 1990). For growth performance, carcass traits and acidic profile of ham cover fat, the following model was run: $Y_{ijkl} = \mu + D_i + S_j + P_k(D_i) + e_{ijkl}$; where: Y_{ijkl} is the dependent variable; μ is the general mean; D_i is the diet effect (i=1,2); S_j is the sex effect (j=1,2); $P_k(D_i)$ is the effect of pen within diet (k=1,2) and e_{ijkl} is the residual error. Data of metabolic profile were expressed as differences between the individual values found at 43 and 106 days minus the values detected at the beginning of the trial (day 0). Then, these differences were analysed with the model: $Y_{ijkl} = \mu + D_i + C_j + A_k(D_i) + DC_{ij} + e_{ijkl}$; where: D_i is the diet effect (i=1,2); C_j is the effect of the period of sampling (j=1,2); $A_k(D_i)$ is the effect of the animal within diet (k=1,2…12); DC_{ij} is the interaction of DxC. Orthogonal contrasts were run to evaluate significant differences due to diets within each period of sampling.

RESULTS AND DISCUSSION

In Table 2 the chemical composition, the urease activity and the TI activity of 5 cultivars of soybean are given. The chemical composition showed by the soybean seeds of various origin was fairly homogeneous. Only the seeds of the home produced soybean cultivar, used for the experimental diets, showed lower lipids and higher crude fibre contents with respect to the other ones. These differences may depend on some specific farm pedo-climatic conditions of cropping. Autoclaving did not affect the chemical composition of soybean (values not given). The urease activity of raw seeds was about 2.2 ΔpH without differences among the cultivars. As a consequence of processing the ΔpH decreased to 0.05-0.20. These values can be considered acceptable, both with regard to the inactivation of the antinutritional factors and to regard the maintaining of good properties of solubility and digestibility of the feed protein (Qin et al., 1996). TI activities of Hilario and Aires raw seeds ranged between one-half and one-third to the values found for the raw conventional soybeans seeds (Cresir, Brillante, Dekagib). The toasted soybeans seeds showed some residual TI activity. The replacement of the 10% of raw soybean by an equal amount of toasted seeds did not cause significant differences on the average daily gain (ADG) and on feed conversion ratio (FCR) (Table 3). The values of Feed conversion ratio (FCR) observed for the two groups of pigs were quite high with respect to those commonly found in the practice, with pigs kept indoor fed restricted diets (around 3.6-3.8).

Table 2. Chemical composition, urease activity and trypsin inhibitor (TI) activity of raw or toasted (110 °C for 20 min) seeds of different commercial cultivars of soybean

	Conventional cultivars:			Low contents of ANF cultivars:		
	Dekagib	Cresir	Brillante	Hilario	Aires	Hilario [1]
Dry matter (DM)	85.56	86.51	86.28	86.34	85.76	90.28
Crude Protein, % DM	41.57	40.56	41.70	40.09	40.78	39.35
Lipids, % DM	19.39	20.47	19.44	19.63	19.94	14.74
Crude fibre, % DM	6.33	7.18	6.27	7.27	6.02	11.03
Ash, % DM	5.12	5.04	4.98	4.98	5.26	5.44
SFA, % Lipids	16.92	16.91	16.15	16.85	16.30	15.69
MUFA, % Lipids	25.29	28.15	28.73	24.57	22.38	22.27
PUFA, % Lipids	57.79	54.94	55.13	58.59	61.32	62.04
Urease activity, ΔpH						
- Raw seeds	2.19	2.10	2.25	2.26	2.12	2.17
- Autoclaved seeds	0.13	0.20	0.18	0.15	0.05	0.19
TI activity, % inactivation/μg protein						
- Raw seeds	54	85	94	37	28	38
- Toasted seeds	11	24	3	9	17	1

[1] Soybean self produced in the farm and used to formulate the experimental diet "Raw Soybean" (RS)

Table 3. Effects of the experimental diets (Toasted Soybean "TS", Raw Soybean "RS") on growth parameters and on some carcass traits of heavy pigs at slaughter (109 days)

	Diet		*P*			MSE
Item	TS	RS	Diet	Sex	Pen (Diet)	
Initial live weight, kg	82	82				10
Final live weight , kg	180	181				17
Average daily gain, kg/d	0.925	0.934				0.163
Feed intake, kg/d	4.240	4.420				n.d.
Feed conversion ratio, kg/kg	4.591	4.737				n.d.
Initial backfat thickness (P2), mm	11.4	11.2		**		1.7
Final backfat thickness, (P2), mm	24.1	23.0				4.0
Carcass traits:						
- Carcass, kg	145.3	146.5				15.1
- Cold dressing, %	80.6	80.7			*	0.016
- Kidneys, g	282	279		*		82
- Liver, g	2397	2380		**		295
- Linoleic acid, % of ham cover fat	21.8	22.0				1.54

** $P<0.01$; * $P<0.05$;

It can be observed that in this experiment the pigs were fed *ad libitum* and kept outdoor during a winter season which was very cold. Since the average backfat thickness (P2 = 23-24 mm) was quite similar to that found in the practice, the high FCR were probably due to a high energy expenditure for thermoregulation and to the high amounts of feed wasted because of the wet environment conditions.
No effects due to the diets were observed on the carcass traits, on the organs weights and on the linoleic acid content of cover fat. On the average, the linoleic acid content of the fat covering the ham exceed the recommended value (below 15%) required for high quality dry crude ham of PDO Parma. Because of its content of oil, the whole fat soybean seeds in the fattening period must be considered with care, in the long seasoned products. The urea content of blood was significantly lower (P<0.05) in the pigs fed the RS diet with respect to those receiving TS, both for the periods 0-43 and 0-106 days (Table 4). This result likely reflected a lower digestibility of the dietary protein for the feed containing the raw soybean. The serum values of complement proteins increased over time (P<0.01). Higher values were found for the TS group with respect to the RS one, particularly at the last collection time (130 *vs.* 59 CH_{50}/100 µl; P<0.01). However alterations of the health status were not confirmed by any changes of serum protein profile, of erythropoietic parameters and of leukogram (data not shown). All these parameters were within the normal range of variability observed for pigs (Kaneko, 1989; Ubaldi et al., 1982). The metabolic enzymes, aspartate amino transferase (AST) and creatine chinase (CK), also increased over time (P<0.01), but, again, no effects due to the diets were observed.

Table 4. Changes of metabolic profile: effects due to the diets and the periods

Item	0 d	Period 0-43 day		Period 0-106 day		*P*		MSE	*P*
		TS	RS	TS	RS	Diet	Period		
Urea, mmol/l	4.13	$+2.66^{A}$	$+1.12^{B}$	$+2.42^{A}$	$+1.28^{B}$	**		0.83	*
Complement proteins									
- CH_{50}/100 µl	46.2	$+28.3^{A}$	-1.1^{A}	$+74.2^{B}$	$+12.9^{A}$	**	**	29.7	**
Metabolic enzymes									
- AST, U/l	16.7	+5.3	+5.1	+13.9	+11.2		**	7.13	*
- GGT, U/l	37.0	-7.8^{B}	-5.9^{B}	-6.3^{B}	$+1.9^{A}$		**	4.80	**
- CK, U/l	753	-157^{B}	$+166^{AB}$	$+167^{AB}$	$+591^{A}$		**	383	**

** *P*<0.01; * *P* <0.05; ^{A,B} P<0.01;

Some indications about the N balance are given in Table 5. From the calculation it can be seen that a production of N in manure of about 7.5 kg can be expected for a single pig produced, under the specific condition of rearing adopted. Taking into account a potential number of production cycles of 3, the expected amount of N in manure per pig place is 22.8 kg/year. This value is much more higher than that of 12.1 kg which can be calculated from the EC 1804/99 regulation, in which it is assumed that 170 kg of N in manure are equivalent to 14 pig places. Thus, if in the farm an average pig load of 14 pig/ha are accepted by the Official Control Organism for organic production, the N load could be about the double to that required to comply the limit of 170 kg N in manure/year/ha. In the practice, lower level of crude protein and hence of soybean in the diet can be applied for pigs in the range of live weight from 80 to 180 kg.

Table 5. Nitrogen balance

	Nitrogen balance per:		
	pig produced	pig place/year	hectare of farm land/year [1]
Rounds / year [2]	-	3.02	3.02
Pig load, n./ha/year	-	-	14
- N consumption, kg	12.5	37.8	530
- N retention, kg	2.5	7.5	104
- N excretion, kg	10.0	30.4	425
- N in manure, kg [3]	7.5	22.8	319

[1] The EC 1804/99 regulation indicated that the number of pig places equivalent to 170 kg of N in manure is 14. [2]Computed assuming 15 days between two consecutive rounds of pig production. [3]Nitrogen in manure was computed assuming that 25% of total N excreted was lost in the atmosphere (ERM, 2001)

CONCLUSION

In conclusion, raw seeds of soybean cultivars with low contents in Kunitz compounds showed an average trypsin inhibitory activity which was about half to that observed for raw seeds of other conventional cultivars. Raw soybean from cultivars low in Kunitz factors can be usefully included in the diets of finishing pigs (80 to 170 kg LW) up to 10% of complete feed, without negative effects on growth performance, carcass quality and health status. However, in the practice, lower levels of soybean inclusions are required for the lean growth of finishing heavy pigs and for maintaining a low degree of unsaturation of body fat. Thus, low amount of raw soybean in the diets of heavy pigs can represent a interesting protein source for organic farms. Caution should be taken in outdoor pig systems since the land is not cropped and the risks of pollution can be high.

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REFERENCES

1. Association of Official Analytical Chemists (2000): Official methods of analysis of AOAC international. 17th ed., Dr W. Horwitz ed., Washington, DC.
2. ERM (2001): Livestock manures. European commission DG environment – D1, Brussels.
3. Folch, J., Lees, M., Sloane Stanley, G.H. (1957): A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry, 226:497-509.
4. Huisman, J. (1994): Antinutritional factors (ANFs) in feedstuffs used in pig diets. 3rd international feed production conference: 115-163.
5. Herkelman, K.L., Cromwell, G.L., Stably, T.S., Pfeiffert, T.W., Knabe, D.A., (1992): Apparent digestibility of amino acids in raw and heated conventional and low-trypsin-inhibitor soybeans for pigs. Journal of Animal Science, 70:818-826.
6. Kaneko, J.J. (1989): Clinical Biochemistry of Domestic Animals 4thEd. Academic Press, NY.
7. Krishnan, H.B. (2001): Characterization of a soybean (Glycine max (L.) Merr.) mutant with reduced levels of Kunitz trypsin inhibitor. Plant Science, 160:979-986.
8. NGD (1976): Norme Grassi e Derivati. Stazione sperimentale per le industrie degli oli e dei grassi. 3°ed. Milano.
9. Qin, G., Elst, E.R., ter Bosch, M.W., van der Poe1, A.F.B. (1996): Thermal processing of whole soya beans: Studies on the inactivation of antinutritional factors and effects on ileal digestibility in piglets. Animal Feed Science and Technology, 57:313-324.
10. SAS (1990): SAS user's guide: statistics. SAS Institute Inc., Cary, North Carolina.
11. Snidaro, M., Signor, M., Baret, R., Cadel, G., Danelon, I. (2001): Buoni risultati con l'impiego di soia a basso contenuto di fattori antinutrizionali. Informatore agrario, 12:55-58.
12. Ubaldi, A., Corbella, E., Montanari, P., (1982): Diagnostica Chimico - Clinica Veterinaria, Casa Editrice Ambrosiana, Milano.

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