EFFECTS OF WHOLE SOYBEANS ON GROWTH PERFORMANCE AND BODY FAT COMPOSITION IN RABBITS

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Abstract - An *in vivo* digestibility experiment was conducted using 24 hybrid strain rabbits allotted to three experimental groups. The animals were given a commercial feed (control diet, C), without whole soybeans (WSB), or diets with two levels of WSB (3 and 6%) substituting a corresponding level of wheat and soybean solvent extracted meals (25:75) to maintain a constant crude protein content of the diets. WSB had no effect on digestibility parameters of the diets, excepting for ether extract which increased in WSB diets. A slight tendency to increase DE was shown in diets with 6% WSB due to the high gross energy content of WSB.

A growth experiment was carried out using a total of 72 rabbits. At weaning the animals were divided into three groups of 24 rabbits each. The dietary treatment was the same as in the first trial. The addition of WSB did not influence growth rate, feed intake and feed efficiency of rabbits, or carcass characteristics. However, the fatty acid composition of body fats was affected by diet. In kidney and scapular fat, linoleic acid content increased in WSB groups (21.7% C group Vs 24.2; 3%WSB and 24.6%; 6% WSB; $P<0.04$ for kidney fat and 20.7% C group Vs 24.1; 3%WSB and 24.1%; 6% WSB; $P<0.05$ for scapular fat). Oleic acid showed an inverse trend to linoleic acid content. PUFA and PUFA/saturated fatty acid ratio showed a tendency to increase with increasing WSB level in the diets. The fatty acid patterns of muscular fat evidenced the same relationship found in separable fats, with a higher values of linoleic acid, PUFA and PUFA/saturated fatty acid ratio in 6%WSB group. Peroxidation of muscle fat (TBARS) also showed a slight tendency to increase with increasing WSB levels.

INTRODUCTION

Dietary energy level and its relationship with digestible protein greatly affects growing performance in the intensive rabbit system. However, a carbohydrates overload of the hindgut may occur in a diet with high starch level, resulting in a high percentage of digestive disorders and mortality. To reduce such risk, a part of energy requirements could be provided as fat, although the level of fat inclusion in commercial feed is limited by manufacturing technology (hardness and dust of pellets).

In the last twenty years, the use of whole soybeans (WSB), also called "heated-treated whole soybeans or soybeans full-fat", in animal feeding has increased. WSB have high oil and crude protein contents and, with respect to raw beans, were devoid of undesirable anti-nutritional factors by appropriate heated treatment methods. In monogastric animals, the fatty acid composition of fat tissue is greatly influenced by incidence and proportion of fatty acids present in the feed. The fatty acids in soyoil are predominately oleic (23-26%), linoleic (50-54%) and linolenic (7-9%) (DE SCHUTTER and MORRIS, 1990). As a result, it is not surprising to discover that the concentration of these fatty acids is increased in the carcass fat. Diets containing a high concentration of linoleic acid result in high concentration of linoleic acid in the adipose tissue of chickens (GUENTER *et al*, 1971). In pigs fed rations containing WSB, changes in fatty acid composition, particularly in linoleic acid, are believed to be responsible for the observed increase in fat softness (WOOD, 1984). This important aspect should also be considered in rabbit production for the enhanced quantity of meat utilised in the preparation of processing foods. Recently it was confirmed that fatty acid composition of the diet affects the composition of body fat depots (COBOS *et al.*, 1993 ; 1994 ; KESSLER and PALLAUF,1994 ;1995 ; OUHAYOUN *et al.*, 1987).

The aim of present experiment was to verify the effects of the addition of different levels of WSB to commercial feed on performances and body fat composition in growing rabbits.

MATERIAL AND METHODS

Experiment 1

Table 1 : Ingredients (%) and chemical composition (%DM) of diets and whole soybeans (WSB)

		WSB	Diet		
			C	3%WSB	6%WSB
WSB			0.00	3.00	6.00
%					
Wheat	"		17.00	16.20	15.40
Barley	"		18.00	18.00	18.00
Wheat bran	"		25.76	25.76	25.76
Soybean meal (44%CP)	"		9.00	6.80	4.60
Sunflower meal	"		8.00	8.00	8.00
Mixed hay (10%CP)	"		20.00	20.00	20.00
Mineral vitamin supplement [1]			2.24	2.24	2.24
Dry matter	%	88.4	91.4	91.1	90.5
Crude protein	%	38.5	18.2	18.2	17.8
DM					
Ether extract	"	20.4	2.8	3.2	3.8
Crude fibre	"	5.9	15.6	15.6	15.0
NDF	"	13.5	37.3	37.6	36.9
ADF	"	9.1	19.3	19.0	18.9
Ash	"	5.2	8.47	8.24	8.18
Gross energy	cal/KgDM	5580	4203	4233	4282
C14:0	% total	--	0.31	0.37	0.29
FA					
C16:0	"	12.2	19.5	17.6	16.2
C18:0	"	4.42	2.94	2.78	2.75
C18:1	"	21.3	17.6	18.0	18.6
C18:2	"	54.9	51.0	54.2	55.3
C18:3	"	7.26	8.61	7.04	6.85

[1]

A digestibility experiment was conducted using 24 female rabbits, belonging to a commercial hybrid strain (Provisal), housed at weaning (30-33d of age) in individual cages and allotted to three experimental groups. Animals were given *ad libitum* a pelleted commercial feed (control diet, C), without WSB, or diets with two levels of WSB (3 or 6%) substituting a corresponding level of wheat and soybean solvent extracted meals (25:75) to maintain a constant crude protein content of the diets. Diets were formulated to meet nutrients requirement for growing rabbits reared in intensive system (MAERTENS, 1992). The ingredients and chemical composition of diets and WSB are reported in Table 1.

The procedures for *in vivo* determination of total digestibility were adopted according to standard methods suggested by European Group on Rabbit Nutrition (EGRAN, 1995). After a preliminary adaptation period (from weaning to 50d of age), the performances of rabbits were detected (50-87 d of age), while a collection 5-d period (72-76d of age) was performed in order to determine the apparent digestibility coefficients (ADC) of main parameters (Table 2). Feed and faeces were analysed by AOAC (1990) standard methods. Ether extract was detected after acid hydrolysis. Gross energy content was determined by adiabatic calorimeter IKA 800.

Experiment 2

A growth experiment was carried out using a total of 72 female rabbits belonging to the same strain of first experiment. At weaning, performed at 33d of age, the animals were divided into 3 groups of 24 rabbits each The dietary treatment was the same as in the first trial. The animals were kept in a wire single-floor cage, in which a pair of rabbits was housed. The experiment lasted 51d until a final liveweight of approximately 2.6 kg was reached. During the trial, rabbits were weighed individually and two-cage feed intakes were recorded weekly to determine average daily gain, average daily feed intake and feed:gain ratio.

The rabbits were slaughtered at 91d of age, a week after the end of growing trial. The transport of the animals lasted approximately 2h, without apparent stress, and after the arrival at the abattoir the rabbits were slaughtered without any resting period. Slaughter procedures and criteria of carcass manipulation were as according to the guidelines suggested by BLASCO *et al.*, 1993. The commercial carcasses were chilled at 4°C and pH values were measured at 45min, 3 and 24h on *longissimus dorsi* and hindleg muscles (*biceps femoris* and *tensor fasciae latae*). After storage period, reference carcasses were detected and perirenal and scapular depot fats and *longissimus dorsi* muscle were dissected for gaschromatography analysis.

Muscle samples were carefully homogenised with 9 vols. of 0.14 M NaCl/10 mM, pH 6.5, in a Potter Elvehjem homogenise at 0-4°C. In homogeneous samples, lipid peroxidation was stimulated by iron salts. The reaction mixture (total volume: 1 ml) contained the same buffer used for the homogenisation, protein (1 mg), $FeCl_2$ at different concentration. After 1 hr of incubation at 37° C, lipid peroxidation was measured by the formation of

thiobarbituric acid reactive material (TBARS) according to a slightly modified procedure earlier described (BEUGE and AUST, 1978). The incubated samples received, prior to TBA reagent, 10 µl of 2% (w/v) butylated hydroxytoluene (BHT) ethanolic solution and 200 µ of 8% (w/v) sodium dodecyl sulphate. Then, 1.5 ml of a 20% (v/v) acetic acid solution, pH 3.5, and 1.5 ml of a 0.8% (w/v) TBA solution were added. The mixture were heated at 100° C for 15 min, cooled on ice and extracted with butanol. Protein was estimated after LOWRY *et al.*, 1951 using bovine serum albumin as standard. Total lipids were extracted from depot fats, and *longissimus dorsi* muscle according to BLIGH and DYER, 1959. The fatty acid composition was determined by GC using the method described by CHRISTOPHERSON and GLASS, 1969.

Statistical analyses were performed by LSMLMW procedure of HARVEY, 1987 using the following linear model:

$Y_{klm} = \mu + \gamma_k + \varepsilon_{klm}$; where Y_{klm}= exp. observation, μ= general mean, γ_k = effect of WSB level (k=3), ε_{klm} = residual exp. error.

Table 2 : Experiment 1: performance of rabbits, apparent digestibility and digestible nutrients content of diets

		Diet			SEM[1]	P-value	
		C	3%WBS	6%WBS			
No. of rabbits		8	8	8			
Initial weight (50d of age)	Kg	1.376	1.411	1.459	0.142	0.51	
Final weight (87d of age)	Kg	2.876	2.892	3.000	0.187	0.37	
Average daily gain	g	40.7	40.2	41.7	4.20	0.77	
Average daily feed intake	g	166.4	167.7	169.9	9.90	0.79	
Feed: gain ratio		4.13	4.19	4.09	0.332	0.82	
Apparent digestible coefficients:							
Dry matter	%	60.8	60.1	61.2	1.39	0.26	
Ether extract	"	69.9 B	81.8 A	84.5 A	6.01	0.01	**
Organic matter	"	63.4	62.1	63.3	1.33	0.10	
Crude protein	"	74.2	75.3	76.0	1.74	0.15	
Crude fibre	"	14.5	14.2	13.7	3.32	0.75	
NDF	"	31.0	30.5	33.0	2.72	0.18	
ADF	"	10.8	11.4	11.4	4.45	0.96	
Energy	"	61.7	61.6	62.7	1.36	0.22	
Digestible nutrients (as fed basis):							
Dry matter	g/Kg	556	548	554	12.6	0.38	
Ether extract	"	17.9 B	23.8 A	24.5 A	1.62	0.01	**
Organic matter	"	526	515	521	11.0	0.10	
Crude protein	"	123	125	122	2.98	0.16	
Crude fibre	"	21.3	20.2	18.9	4.68	0.58	
NDF	"	106	105	110	0.931	0.44	
ADF	"	19.1	19.7	19.5	7.69	0.99	
Energy	Kcal/Kg	2371	2376	2430	52.6	0.07	

[1] DF 21 ** A,B :P<0.01

RESULTS AND DISCUSSION

Experiment 1

Apparent Digestible Coefficients (ADC) of the diets are shown in Table 2. The diets containing WSB showed digestibility values similar and comparable to those of the basal diet for all the parameters, except for EE which increased significantly in WSB groups (P<0.001). ADC values of OM and gross energy averaged 62.9±1.30 and 62.0±1.36% respectively. However, due to higher gross energy content of WSB, DE of the diets showed a tendency to increase in 6%WSB group. A slight trend of CP digestibility, improved with the increasing of WSB level, was also noted. Similar results were reported by FERNANDEZ *et al.*, 1994. According to digestibility results, no significant differences were found between the groups regarding animal performances. At 87 days of age, the average liveweight of the rabbits was 2.923 ± 0.187 kg. During the whole period the average daily weight gain was normal (40.9 ± 4.20 g) for growing rabbits reared in intensive conditions and also the feed efficiency.

Experiment 2

Table 3 : Experiment 2: productive performance of growing rabbits

		Diet			SEM	P-value
		C	3%WBS	6%WBS		
N° of rabbits		23	23	21		
Initial weight	Kg	0.843	0.858	0.820	0.089(1)	0.38
Final weight	Kg	2.600	2.663	2.664	0.210(1)	0.51
Average daily gain	g					
0-14		38.8	37.6	36.9	10.0(1)	0.82
15-28		36.0	37.3	37.2	5.62(1)	0.67
29-51		31.1	33.2	35.1	6.00(1)	0.10
0-51		34.4	35.4	36.1	3.68(1)	0.32
Average daily feed intake g						
0-14		91.5	92.0	95.7	8.67(2)	0.64
15-28		133.9	137.8	130.9	8.55(2)	0.39
29-51		145.0	150.1	156.5	9.25(2)	0.13
0-51		128.3	131.8	133.9	6.56(2)	0.35
Feed : gain ratio						
0-14		2.35	2.39	2.60	0.359(2)	0.44
15-28		3.73	3.72	3.53	0.323(2)	0.46
29-51		4.66	4.64	4.40	0.426(2)	0.51
0-51		3.73	3.74	3.67	0.196(2)	0.79

(1) Referred to single rabbit. DF 64 (2) Referred to subgroup of 4 rabbits. DF 15

Table 4 : Carcass characteristics and pH values of some muscles

		Diet			SEM(1)	P-value
		C	3%WSB	6%WSB		
No. of rabbits		16	16	16		
Liveweight at slaughter Kg		2.818	2.823	2.920	0.171	0.18
Hot carcass weight	Kg	1.717	1.701	1.761	0.097	0.21
Dressing percentage	%	60.9	60.3	60.3	1.24	0.31
Chilling losses	%	2.70	2.61	2.62	0.180	0.60
Reference carcass (2) c.c.	%	83.5	83.1	83.1	1.49	0.82
pH values:						
- 45 min after slaughter						
longissimus dorsi		6.78	6.93	6.89	0.199	0.11
biceps femoris		6.81	6.91	6.84	0.140	0.16
tensor fasciae latae		6.86	6.93	6.88	0.170	0.50
- 3 hr after slaughter						
longissimus dorsi		6.45	6.47	6.37	0.230	0.77
biceps femoris		6.38	6.29	6.32	0.207	0.38
tensor fasciae latae		6.32	6.23	6.30	0.237	0.47
- 24 hr after slaughter						
longissimus dorsi		5.83	5.80	5.82	0.143	0.78
biceps femoris		5.77	5.76	5.79	0.122	0.82
tensor fasciae latae		5.80	5.80	5.77	0.123	0.62

(1) DF 45 (2) c.c.: chilled commercial carcass. Reference carcass: chilled c.c. without head, thorax organs, liver and kidneys

The rabbits showed a satisfactory health condition, with no relevant mortality rates, equal to 4.2; 4.2 e 12.6% in the C, 3 and 6% WSB groups respectively. The technological quality of the feed was good for the C and 3% WSB groups, while the feed containing 6% of WSB displayed less hardness and more dusty.

The WSB level did not influence the growth performances in the three groups (Table 3). Weight gain (g/d) and feed efficiency resulted similar in the three groups and averaged 35.3±3.68 and 3.71±0.20, respectively. Nevertheless, a slight tendency to the improvement in the growth performances of the rabbits receiving 6% WSB appeared during the last two trial weeks. The results of this study confirm those generally reported in previous works (PARTRIDGE *et al.*, 1986; SANTOMA *et al.*, 1987; FERNANDEZ and FRAGA, 1992) where different types of dietary fat did not enhance growth performance of rabbits.

Carcass weight and slaughter yield were comparable among the groups (Table 4). The liveweight values are referred to animals slaughtered at 13 weeks of age. Such values are 200 g higher than that previously reported as final liveweight in growth trial. The carcass weights averaged 1.726±0.097kg, a rather heavy weight that is currently very much on demand in the meat processing industry.

The muscular pH values did not evidence significant differences among the groups for the three muscles considered. The pH values decreased from 6.87 at 45 min, to 6.35 after 3 h, and to 5.79

after 24 h *post-mortem* (Table 4). Moreover, ultimate pH values did not differ between *longissimus dorsi* and hindleg muscles according to the results reported by OUHAYOUN and DELMAS (1988) ; BLASCO and PILES (1990). Other authors found higher final pH in the muscles of the hindleg with respect to *longissimus dorsi* (PARIGI-BINI *et al.*, 1992 ; BERNARDINI BATTAGLINI *et al.*, 1995).

Concerning perirenal and scapular fats (Tables 5 and 6), linoleic acid content increased in WSB groups (21.7% C group Vs 24.2; 3%WSB and 24.6%; 6% WSB; $P<0.04$ for kidney fat and 20.7% C group vs. 24.1; 3%WSB and 24.1%; 6% WSB; $P<0.05$ for scapular fat) while the oleic acid content decreased, PUFA and PUFA/saturated fatty acids ratio showed a tendency to increase with increasing of WSB in diets. As regard to muscular total lipids (Table 7), similar relationships to those found in depot fats were evidenced. Linoleic acid content was higher in WSB groups (24.1 C group vs 25.8 3%WSB and 26.2 6%WSB; $P<0.05$), while oleic acid and total saturated fatty acids showed a decrease in 3% WSB group. PUFA and PUFA/saturated fatty acids increased with 6%WSB in the diet.

Table 5 : Fatty acid composition of kidney fat

		Diet			SEM[1]	P-value
		C	3%WSB	6%WSB		
N° of analysis		6	6	6		
C14:0	% total FA	3.70	3.22	3.37	0.330	0.06
C16:0	"	31.6	30.5	30.6	1.61	0.43
C16:1	"	6.05	5.70	6.00	1.04	0.82
C18:0	"	6.80	6.57	6.22	0.639	0.32
C18:1	"	27.4	26.7	26.1	0.970	0.10
C18:2	"	21.7 [b]	24.2 [a]	24.6 [a]	1.88	0.04 *
C18:3	"	3.20	3.09	3.05	0.381	0.77
Total saturated FA	"	42.3	40.3	40.2	1.95	0.14
Total PUFA	"	24.9	27.3	27.6	2.12	0.12
Unsaturation index (UI)[2]		86.2	90.1	90.0	3.69	0.16
PUFA/saturated FA		0.59 [b]	0.68 [a]	0.69 [a]	0.072	0.05 *

[1] DF 15 [2] The unsaturation index (UI) is defined by $\sum m_i \cdot n_i$, where m_i is the percentage and n_i the number of double bonds, taking into account all fatty acids with two or more double bounds. * a,b:$P<0.05$

Table 6 : Fatty acid composition of scapular fat

		Diet			SEM[1]	P-value
		C	3%WSB	6%WSB		
N° of analysis		6	6	6		
C14:0	% total FA	3.86	3.54	3.60	0.430	0.42
C16:0	"	30.9	29.7	30.9	2.38	0.59
C16:1	"	6.57	5.19	6.12	1.09	0.11
C18:0	"	7.06	6.88	6.74	1.11	0.89
C18:1	"	28.1 [A]	27.3 [A]	25.7 [B]	1.18	0.01 **
C18:2	"	20.7 [b]	24.1 [a]	24.1 [a]	2.51	0.05 *
C18:3	"	3.04	3.06	3.07	0.491	0.99
Total saturated FA	"	41.8	40.1	41.3	3.02	0.61
Total PUFA	"	23.8	27.2	27.2	2.89	0.07
Unsaturation index		85.3	89.9	89.0	6.05	0.39
PUFA/saturated FA		0.58	0.69	0.66	0.107	0.21

[1] DF 15 * a,b:$P<0.05$ ** A,B:$P<0.01$;

Table 7 : Lipid peroxidation (TBARS) and fatty acid composition of total lipids in *longissimus dorsi*

		Diet			SEM[1]	P-value	
		C	3%WSB	6%WSB			
N° of analysis		8	8	9			
Total lipids	mg/g	11.6	10.6	11.9	2.67	0.66	
Protein	"	191.8	181.6	207.7	24.6	0.28	
TBARS	nmol/mg prot	0.91	1.44	1.80	0.639	0.08	
C14:0	% total FA	2.46 A	1.87 B	1.80 B	0.238	0.01	**
C16:0	"	27.2	27.8	26.6	0.881	0.07	
C16:1	"	4.10	3.66	3.45	1.03	0.48	
C18:0	"	6.79	6.18	6.09	0.858	0.27	
C18:1	"	24.6	22.8	23.1	1.45	0.07	
C18:2	"	24.1 b	25.8 a	26.2 a	1.62	0.05	*
C18:3	"	1.56	1.48	1.41	0.605	0.88	
C20:4	"	6.71	6.65	7.25	0.681	0.21	
C22:4+C22:5+C22:6	"	4.29	3.94	4.04	0.619	0.58	
Total saturated FA	"	35.9 A	35.8 A	34.6 B	0.87	0.01	**
Total PUFA	"	37.0 b	37.1 b	38.9 a	1.58	0.05	*
Unsaturation index		127.4	127.5	130.9	4.38	0.25	
PUFA/saturated FA		1.03 b	1.06 b	1.12 a	0.060	0.02	*

[1] DF 22 * a,b:P<0.05 ** A,B:P<0.01

The turnover of fatty acids in adipose tissue depends on the energy status of the animal. (ENSER, 1984). The majority of animals fed *ad libitum* are probably always in a positive energy balance and hence do not have to rely on stored fat to supply their energy needs. The type of fatty acids synthetized is regulated, in part, by the type and quantity of fatty acids absorbed by the intestine, so that the latter are the main regulators of the composition of adipose tissue. Saturated fatty acids and their glycerides may be poorly absorbed and may have a limiting effect on fatty acid synthesis, whereas vegetables oils which are well absorbed may be active acids(ENSER, 1984). Nevertheless, FERNANDEZ et al., 1994 found a digestibility of saturated acids from 22 to 83% in growing rabbits fed diets with different fats.

The change in linoleic acid was in agreement with previous reports (KESSLER and PALLAUF, 1994;1995; COBOS *et al.*, 1993) that found a close correspondence between the fatty acids of dietary soyoil and the fatty acid profile in fat tissues of growing rabbits. As reported also in other works (PARIGI-BINI et al., 1992; KESSLER and PALLAUF, 1994;1995), the fatty acid composition of muscle tissue was rather comparable to that of deposit adipose tissue. The differences observed could be attributed to the more active participation of phospholipids in the total lipids of the muscle tissue (OUHAYOUN *et al.*, 1985; CAMBERO *et al.*, 1991). Moreover, the presence of long chain polyunsaturated fatty acids C20:4; C22:4; C22:5 and C22:6 affected the Unsaturation Index that appears to be higher in muscle tissue than depot fats. In present research, polyunsaturated C20-C22 fatty acids composition was not influenced by dietary treatments. The TBARS values of total lipids extracted from *longissimus dorsi*, in spite of the high variability and low mean, seem to confirm the tendency of meats produced with diets containing WSB to be more susceptible to peroxidation and hence more difficult to preserve in time. In the future this aspect will have to be considered more closely in the light of the demands of the meat processing industry.

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