EFFECT OF EXTRACT OF CHESTNUT WOOD INCLUSION (ENC®) IN NORMAL AND LOW PROTEIN AMMINOACID SUPPLEMENTED DIETS ON HEAVY BROILER RABBITS

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ABSTRACT

A chestnut (*Castanea sativa*) extract (ENC), rich in hydrolysable tannins, was evaluated in rabbit diets without antibiotics. A total of 72 five week-weaned rabbits were fed as follows: 1) control diet (C); 2) Aminoacid supplemented low protein diet (A); 3) diet A+0.45% ENC (TA); 4) diet C+0.45% ENC (TC). Feeding was modulated in two phases characterised by different levels of CP (DM basis): diet C first period 175 g/kg, second period 197 g/kg vs. diet A first period 145 g/kg, second period 159 g/kg. The ENC was extracted from chestnut wood by a heat and low-pressure treatment, and only the water-soluble fraction was kept and subsequently dehydrated. The product is commercially available as fine brown powder (92 to 95% dry matter) with a pure tannin content of 77% on a DM basis. The trial was carried out in full summer conditions. The productive traits were recorded each 14 days. ENC inclusion did not have any negative effects on the productive parameters, while low protein AA supplemented diets enhanced significantly (P<0.01) protein utilization. Over the whole period, ENC inclusion did not influence the daily feed intake, daily weight gain, feed conversion ratio and protein efficiency ratio, but reduced significantly (P<0.01) the mortality from 44% to 11% in group C and group TC fed high protein diets; while mortality was exactly the same (11%) in groups A and TA. The high level of protein can be suspected as being the cause of mortality.

Key words: Rabbit, Low protein diet, *Castanea sativa* natural extract addition.

INTRODUCTION

In 2006, the EU banned the use of antibiotics growth promoters (AGP) in animal feeding and the gastroenteric diseases of rabbits have significantly increased affecting mainly 35-50 day old rabbits. Many approaches have been attempted to control or prevent sub-clinical diseases and to optimize growth performances.

At present, the use of natural products in animal nutrition is of great interest. Due to the ever increasing knowledge of the chemical composition and biological activity of tannins, these compounds are no longer classified as univocally antinutritional (Mueller-Harvey, 2006). Condensed tannins have negative effects on animal feeding, but the fate of hydrolysable tannins is not well known. Recently some chestnut (*Castanea sativa*) extracts (ENC) rich in hydrolysable tannins, such as castalagin, has been successfully experimented for poultry feeding (Schiavone *et al.*, 2008). Maertens and Struklec (2006), using a tannin extract in rabbits diets, underlined a positive effect on growth performance, mortality and enteric pathologies. An *in vitro* antimicrobial activity of chestnut extract was observed by Li and Song (2004) on different bacterial strains, such as *E. coli, S. enteriditis, C. perfrigens.*

The ENC (supplied by Silva Extracts Italy) was extracted from chestnut wood by a heat and low-pressure treatment, and only the water-soluble fraction was kept and subsequently dehydrated. The product is commercially available as fine brown powder (92 to 95% dry matter) with a pure tannin content of 77% on a DM basis. The chemical composition of ENC batch used in the experiment was: water 2.9%; tannin 77.8%; non tannin 17.7%; insoluble 1.6%; crude fibre 0.24%; ashes 1.7%; pH 3.26 (10% solution). Tannin percentage was obtained by gravimetric analysis of vegetable tanning agents using Filter-Freiberg Hide Powder Method (Küntzel, 1954). This research aims to evaluate whether the ENC supplementation can improve the growth performance of rabbits fed normal protein or aminoacid supplemented low protein diets.

MATERIALS AND METHODS

Animals and experimental design

Seventy two five-week-old rabbits, obtained from the Italian ANCI selection scheme of *Macchiata Italiana* and *Bianca Italiana* streams (36 males and 36 females) were used. The rabbits, single housed in two-floor cages, were randomly allotted to four dietary groups and fed diets, without antibiotics, from the 1st to the 28th day and from the 29th to the 56th day, as reported in Table 1.

Table 1: Ingredients and chemical composition of the diets (g/kg DM)

Diets	C		A	
Periods	First period	Second period	First period	Second period
Ingredients (g/kg):				
Wheat milling	270	202	335.1	270
Alfalfa meal	213	154.5	250	104.2
Sunflower meal (30%CP)	150	173	10	140
Dried beet pulp	165	161	140	181
Wheat straw	70	40	130	90
Barley	30	80	40	-
Wheat bran	35	30	33.5	40
Sugar cane molasses	30	20	30	30
Soybean meal (44%CP)	10	60	-	8
Peas	10	40	-	-
Soybean oil	-	10	10	27
Corn	-	-	-	75
Vitamin-mineral premix[1]	16.7	28	16.7	28
DL-Methionine	0.3	-	1.6	1.4
L-Lysine	-	1.5	1.5	3.4
L-Threonine	-	-	1.2	1.5
L-Thryptophane	-	-	0.4	0.5
Chemical composition (g/kg DM):				
Dry matter	885	885	884	885
Crude protein	175	197	145	159
Ether extract	34	33	44	58
Crude Fibre	208	181	205	181
NDF	437	369	452	391
ADF	242	206	242	210
ADL	52	49	45	42
Starch	98	135	98	137
Lys – K	6.94	8.92	6.93	8.81
Met - M	3.24	3.50	3.84	4.12
Thr – T	6.16	6.90	6.18	6.88
Trp – W	2.32	2.49	2.28	2.44
Digestible energy (DE)[2] (MJ/kg DM)	10.71	11.71	10.74	11.74
Digestible protein/DE[2] (g/MJ)	11.47	11.78	9.45	9.49

[1]Premix provided per kg of diet: vitamin A, 20,000 IU; vitamin D_3, 2,000 IU; vitamin E acetate, 40 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0,02 mg; vitamin PP, 51,5 mg; vitamin K_3, 2,6 mg; biotin, 0,5 mg; Fe, 200 mg; Cu, 24 mg; Mn, 40 mg; Co, 4 mg; I, 1,5 mg; Zn, 100 mg; Se, 0.1 mg
[2]Based on Villamide *et al*. (1998)

Diets were and characterised by different level of CP (DM basis): diet C first period 175 g/kg, second period 197 g/kg vs. diet A first period 145 g/kg, second period 159 g/kg. The rabbits were fed *ad libitum* as follows: 1) Control diet (C) 2). Aminoacid supplemented low protein diet (A); 3) diet C + 0.45% ENC (TC); 4) diet A + 0.45% ENC (TA). Trial was carried out in full summer conditions, from August 10th to October 15th 2007, and temperature was higher than 25°C until the end of September.

Individual live weight and feed intake were controlled at 14, 28, 42 and 56 days of trial until the age of 91 days, when the rabbits reached the market size for the Piedmont choice of heavy broiler products with a body weight of around 2.8 kg. Daily feed intake (DFI), daily weight gain (DWG), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated at the end of each period and at the end of the trial. The status of health of the animals and mortality were controlled daily.

Statistical Analysis

The data were at first analyzed using GLM procedure of SPSS (1999) considering the initial body weight (IBW) as covariate and diets, breed and sex as fixed factors. Due to the lack of significance of the main factors, interactive and covariate effects of IBW the statistical analysis was carried out using one way ANOVA and Fisher's exact test for mortality. Differences among the diets were evaluated by the Tukey test. Statistical significance was accepted at P<0.05.

RESULTS AND DISCUSSION

The level of ENC inclusion in the rabbit diets was set at 0.45%, a slightly lower value than that in the previous report in growing rabbits by Maertens and Struklec (2006) but somewhat higher than the current ENC levels utilized in commercial poultry feeding to obtain an evident reduction in faeces moisture (Baricco, 2007, pers. comm.). During the first two weeks of the trial the feeding intake showed significant differences amongst the treatments: the DFI in rabbits fed diet A was significantly (P<0.05) lower than the other three groups (76.3 g vs. 85.9; 87.9 and 87.6 for group C, TC and TA, respectively). Consequently, the DWG in diet A was significantly (P<0.05) lower than the other three diets (30.1 g vs. 35.5; 37.0 and 36.7 for groups C, TC and TA, respectively). During this period, ENC inclusion improved growth performance for the low protein AA supplemented diet, while no difference was observed for the control diet. The significant differences for DFI and DWG, observed during the first two weeks, disappeared from the 14th to the 56th day, while PER instead showed a significantly more favourable value (P<0.05) from day 14th until day 28th in the group fed diet TA (3.29 vs. 2.67; 2.74 and 2.96 for treatment C, TC and A, respectively). Irrespectively of addition of ENC to the diet, from day 28th to the end of trial, the rabbits fed the low protein content diet showed significantly (P<0.05) higher values of PER than the groups fed C and TC diets. Positive effect of diets supplemented with ENC in the starting phases of poultry feeding has been observed by Schiavone *et al*. (2008).

Table 2 summarizes the productive traits observed during the two periods (1-28 and 29-56 days) of feeding: the performances of the whole period are also reported. Data concerning the results of the first period showed a similar trend to that observed in the first 14 days. The PER in group TA was significantly higher than the values observed in the C, A and TC groups. In spite of a similar initial body weight (IBW), diet A reached a lower final body weight in the first period than the rabbits fed diet C while the rabbits receiving diets TA and TC were in an intermediate position.

During the second period the rabbits of group A showed a compensatory growth and, at the end of the experiment, there were no longer any significant differences in the final body weight (FBW). PER was significantly influenced by the diets during both the first and second periods and showed similar trends in both periods. The FCR was only influenced during the second period with group A being better than group C. From an overall point of view, DFI, DWG and FCR were not affected by the diets; due to the lower content of CP, diets A and TA showed significantly higher PER than C and TC with the values of diets supplemented with tannins insignificantly higher than those without tannins.

Table 2: Growth performance of rabbits (n=72, mean±SE)

	Control C	A	TA	TC
First period (1-28 d)				
IBW (g)	878.5±36.03	825.2±31.87	785.4±47.34	826.0±58.49
Daily feed intake (g)	103.2 ±1.66	92.9±2.34	96.7±3.58	100.1±2.84
Daily weight gain (g)	35.7±1.66	30.3±1.33	35.70±1.36	35.0±1.36
FCR	2.90±0.06	3.10±0.11	2.70±0.09	2.90±0.10
PER	2.2 ^{a} ±0.05	2.5 ^{a}±0.07	2. 9 ^{b}±0.09	2.3 ^{a}±0.09
FBW of 1^{st} period (g)	1875.9^{b}±74.26	1688.9 ^{a} ±57.08	1765.4 ^{ab}±52.95	1735.4 ^{ab}±68.87
Mortality (%)	22.2^{A}	5.5^{B}	0 ^{B}	5.5 ^{B}
Second period (29-56 d)				
IBW of 2^{nd} period (g)	1875.9 ^{b}±74.26	1688.9 ^{a} ±57.08	1765.4 ^{ab}±52.95	1735.4 ^{ab}±68.87
Daily feed intake (g)	135.5±6.62	136.9±4.23	143.3±6.32	139.8±6.93
Daily weight gain (g)	30.9±2.12	35.6±1.17	35.7±1.61	34.8±0.91
FCR	4.5 ^{b}±0.19	3.9 ^{a}±0.08	4.0 ^{ab}±0.11	4.0 ^{ab}±0.11
PER	1.3 ^{a}±0.05	1.8 ^{b}±0.04	1.7 ^{b}±0.05	1.5 ^{a}±0.07
FBW (g)	2725.9±116.23	2653.5±72.33	2800.7±79.87	2727.2±87.70
Mortality (%)	28.6	5.9	11.1	5.9
Whole period (1-56 d)				
Daily feed intake (g)	117.0±4.32	114.8±2.93	118.8±3.84	118.6±4.11
Daily weight gain (g)	32.4±1.39	32.5±0.87	35.7±0.84	35.3±1.33
FCR	3.6±0.08	3.5±0.09	3.3±0.05	3.4±0.07
PER	1.7 ^{a}±0.03	2.1 ^{b}±0.04	2.2 ^{b}±0.04	1.8 ^{a}±0.07
Mortality (%)[1]	44.4 ^{A}	11.1 ^{B}	11.1 ^{B}	11.1 ^{B}

IBW: initial body weight; FBW: final body weight; FCR: feed conversion ratio; PER: protein efficiency ratio
a, b different letters in the same row had significant difference ($P<0.05$)
[1]A, B difference at level of $P<0.01$ (Fisher's exact test)

ENC inclusion did not have any negative effects on any the compared parameters. No toxicity in the animal was observed in the experiment which is in accordance with the probable existence of a threshold-limit (Al-Mamary *et al*., 2001).

The results concerning mortality are interesting. The mortality observed in diets A, TA and TC was the same (11.1%) but significantly lower ($P<0.01$) than in the group C (44.4%). The use of ENC seems to reduce mortality and, from this point of view, it is known that in the human medicine, tannins from various plants extracts act to prevent or dissociate the colonization of intestinal parasites, bacteria, protozoa and viruses (Lewis and Elvin-Lewis, 2003). In our trial, the autopsies on all animals that died showed evidence of unspecific enteropathy but no signs of ERE were observed. Unspecific enteropathy can be induced by diets that are high in crude protein and the antibiotic prevention is performed throughout the world (Falcão *et al*., 2007; Maertens, 2007). It is possible to suppose that, in rabbits fed diet high in protein, ENC can modulate gut pH and prevent the development of the microflora that causes enteropathy and in agreement with Maertens and Struklec (2006), protein overload in the gut can be avoided. In the first period, ENC inclusion increased the PER of diet TA than that of A.

At the end of experiment, irrespectively of the protein level and even though no statistical differences were evident, the ENC supplemented diets showed a positive tendency for PER. The potential effect of hydrolysable tannins on protein utilization can be hypothesized. Furthermore, the final results showed aminoacid supplementation, which is lower than those suggested by Adamson and Fisher (1973), could satisfy the nutrient requirement of rabbits. Protein level can be further decreased and aminoacid supplementation can increase the protein utilization and reduce nitrogen excretion, which is important from an environmental point of view.

CONCLUSIONS

The inclusion of ENC in diets at a level of 0.45% has been confirmed as a natural alternative candidate to antibiotic growth promoters which is able to improve the health and welfare of rabbits. No negative effect on the growing performance of the rabbit was evident. ENC seems able to reduce the frequency of unspecific enteropathy, probably due to the use of diets high in protein, and the consequent mortality in high risk conditions due to summer period. These first results are in agreement with those of Maertens and Struklec (2006) but more research is necessary to evaluate the effectiveness of this plant extract.

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